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XXXVI Ciclo

Suscettibilità dei grani antichi ad insetti in postraccolta:
interazioni allelochimiche e caratteristiche tecnologiche

Tutor: Prof. Giacinto Salvatore Germinara
Giacinto Salvatore Germinara
Co-Tutor: Dr. Pasquale De Vita
Candidato: Ilaria D'Isita

A mio marito, Alfonso,

ai tuoi occhi vispi

ai nostri sogni di vita insieme.

Ad Alfredo e Andrea,

preziosi doni

amore senza fine.

Riassunto

Gli insetti infestanti arrecano notevoli danni ai cereali conservati, stimati tra il 9% nei Paesi industrializzati e il 50% nei Paesi in via di sviluppo. Il controllo di tali infestanti è reso particolarmente complesso dalle crescenti limitazioni all'uso degli insetticidi chimici di sintesi e dallo sviluppo di resistenze ai pochi principi attivi attualmente ammessi. L'individuazione di varietà e di genotipi di cereali resistenti o tolleranti agli attacchi di insetti in postraccolta, eventualmente da utilizzare anche come fonti di resistenza in programmi di miglioramento genetico, e lo studio delle interazioni semiochimiche insetto-pianta ospite sono tra i principali ed i più innovativi ambiti di ricerca per lo sviluppo di mezzi di controllo sostenibile in alternativa agli insetticidi di sintesi. La selezione della pianta ospite da parte degli insetti fitofagi è un complesso processo che si articola nelle fasi di ricerca e di accettazione dell'ospite. Nel caso degli insetti infestanti i cereali conservati, numerosi studi hanno evidenziato che Composti Organici Volatili (COVs) emessi della pianta svolgono un ruolo chiave nella fase di ricerca dell'ospite, mentre le caratteristiche fisico-chimiche delle cariossidi sono fondamentali nella fase di accettazione del substrato.

Il crescente interesse dei consumatori per cibi sani e sicuri e per i sapori della tradizione ha portato, negli ultimi anni, ad una riscoperta dei così detti "grani antichi" e di alcuni genotipi a cariosside colorata, per l'elevato contenuto di antocianine, la cui coltivazione rappresenta una potenziale fonte di reddito per gli agricoltori, soprattutto nelle aree marginali, oltre che a costituire una preziosa risorsa di biodiversità. La spesso richiamata rusticità di tali genotipi induce ad ipotizzare una minore attrattività/suscettibilità verso insetti infestanti in postraccolta; tuttavia, tali interazioni risultano ancora poco o per nulla studiate.

Obiettivo principale della tesi di dottorato è stato quello di approfondire le conoscenze sulle interazioni tra *Sitophilus granarius* (L.) e *Rhyzopertha dominica* (F.), tra i principali infestanti primari dei cereali in postraccolta, e "grani antichi" con l'obiettivo di identificare genotipi resistenti o meno suscettibili, con buone caratteristiche tecnologiche e nutrizionali, potenzialmente utili in programmi di miglioramento genetico, e come possibili fonti di composti bioattivi (attrattivi, repellenti, fagodeterrenti) da impiegare per lo sviluppo di strategie di controllo integrato innovative e a basso impatto. A tal fine sono stati condotti saggi di suscettibilità, studi

comportamentali con diversi tipi di olfattometro, microestrazione in fase solida dei COVs dallo spazio di testa delle cariossidi dei diversi genotipi di frumento (HS-SPME) e loro identificazione mediante gascromatografia abbinata a spettrometria (GC-MS).

In tale prospettiva, è stata studiata l'accettazione e l'utilizzazione di una varietà di frumento a cariosside colorata da parte degli adulti di *S. granarius*, attraverso la determinazione di indici di fagodeterrenza e nutrizionali, e contribuito ad uno studio che ha valutato la capacità di utilizzo di diversi tipi di derrate da parte di *R. dominica*.

Considerata l'importanza che l'approccio integrato riveste nella protezione delle derrate dagli attacchi degli insetti infestanti, le attività del dottorato hanno riguardato anche la valutazione dell'attività insetticida 1) di diversi estratti di luppolo verso gli adulti di *S. granarius* 2) di una zeolite di origine cubana e di una terra di diatomee contro gli adulti di *Acanthoscelides obtectus* (Say), importante infestante dei legumi.

Infine, l'acquisizione delle tecniche di indagine per lo studio delle interazioni semiochimiche insetto-pianta ospite hanno permesso di fornire un contributo significativo a due studi riguardanti rispettivamente la risposta olfattiva di adulti di *Stegobium paniceum* (L.) ai COVs di piante officinali cinesi e quella di *S. oryzae* (L.) ai COVs di diverse varietà di riso, al fine di identificare semiochimici di tali specie.

In generale, le attività di ricerca svolte durante il dottorato hanno permesso di chiarire il diverso grado di attrattività/suscettibilità di grani antichi, frumenti colorati e altri substrati alimentari verso *S. granarius* e/o *R. dominica*, di definire le differenze tra i profili di composti volatili e alcuni parametri chimico-fisici dei genotipi studiati e di ipotizzarne il possibile ruolo nel determinare i diversi livelli di attrattività/suscettibilità riscontrati. Risultati molto promettenti sono stati ottenuti anche per quanto riguarda la valutazione dell'attività insetticida di estratti vegetali e polveri inerti. I diversi estratti di luppolo hanno mostrato principalmente attività tossica per contatto e repellenza verso gli adulti di *S. granarius* confermata dalla presenza negli estratti di sostanze volatili in grado di stimolare il sistema olfattivo dell'insetto. Lo studio dell'attività insetticida delle polveri inerti ha evidenziato un'elevata efficacia della zeolite cubana, risultata comparabile a quella già nota per le terre di diatomee. Gli studi sulla risposta olfattiva degli adulti di *S. paniceum* e *S. oryzae* verso gli odori di substrati ospiti hanno permesso di identificare alcuni composti volatili ad azione cairomonale.

Nel complesso, i risultati conseguiti durante il dottorato, oltre ad ampliare le conoscenze sulle caratteristiche chimiche, fisiche e nutrizionali e sull’attrattività e suscettibilità dei grani antichi, frumenti colorati e altri substrati verso infestanti del postraccolta, appaiono interessanti per futuri programmi di miglioramento genetico finalizzati allo sviluppo di genotipi di frumento resistenti o poco suscettibili. I risultati degli studi sulle interazioni semiochimiche e sull’efficacia insetticida di estratti vegetali e di polveri inerti, inoltre, possono contribuire allo sviluppo di strategie innovative di controllo a basso impatto.

Abstract

Postharvest wheat losses due to pest attacks are estimated to be from 9% to 50% in developing countries of the global annual production. Control of stored-product insect pests is very difficult due to the increasing restrictions on the use of synthetic insecticides and the developing of insect resistance to the few eligible insecticides. The identification of resistant or low-susceptible wheat varieties also useful in breeding programs and the study of semiochemical interactions among insect pests and host plants are among the main and most innovative research areas for developing sustainable control means towards stored-product insect pests as alternative to synthetic insecticides. Host plant selection by phytophagous insects is a complex process consisting in the phases of host finding and host acceptance. For storage insects, several studies showed that Plant Volatile Organic Compounds (VOCs) play a pivotal biological role in plant-insect interactions. As well as, the physicochemical properties of the kernel grains are fundamental in food acceptance causing different susceptibility levels of grains.

The increasing consumers’ request for a wholesome, traditional, and sustainable food supply has led to a rediscovery of the so-called “ancient wheats” and of some pigmented genotypes, rich in anthocyanin. Growing these wheat genotypes could represent a source of income for farmers, in particular in agriculturally marginal areas, and is also a way to safeguard these precious genetic resources. The rusticity of these genotypes leads to the hypothesis of a lower attractiveness/susceptibility to storage pests; however, little is known about interactions among ancient wheats or pigmented

genotypes and stored-product insect pests. The PhD thesis was mainly focused on deepening the knowledge on feeding behaviour of primary pests *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) on ancient wheats to identify 1) genotypes resistant or less-susceptible with good technological and nutritional performance, potentially useful in breeding programs, and 2) source of bioactive compounds (attractant, repellent, deterrent compounds) useful to develop innovative and low-impact semiochemical-based control strategies. To this end, susceptibility and behavioural bioassays with different olfactometers were carried out. Furthermore, the VOCs emitted by the wheat kernels of genotypes studied in susceptibility and behavioural bioassays were identified and quantified using the headspace solid-phase micro-extraction (HS-SPME) technique and gas chromatography coupled with mass spectrometry (GC-MS). Additionally, host acceptance and utilisation of a pigmented wheat genotype by *S. granarius* adults and of different stored products by *R. dominica* adults were evaluated.

In the framework of the IPM approaches to control stored-product pests, during the PhD period, studies were carried out also to evaluate the insecticidal activity of different wild hop extracts against *S. granarius* adults, and of a Cuban zeolite and a diatomaceous earth against *Acanthoscelides obtectus* (Say) adults, one of the most serious pests of stored legumes worldwide. The acquired techniques allowed to provide a significant contribution to two studies concerning respectively the olfactory response of *Stegobium paniceum* (L.) adults to VOCs from Chinese medicinal plants and of *S. oryzae* (L.) to VOCs from different rice varieties, in order to identify semiochemicals of these species.

Research activities carried out during the PhD period allowed to 1) clarify the susceptibility/attractiveness degree of ancient wheats, pigmented genotypes, and other food substrates towards *S. granarius* and/or *R. dominica*, to 2) define differences among VOCs profiles of genotypes studied, and to 3) identify some kernel physicochemical proprieties involved in resistance mechanisms of grains to primary pests. The insecticidal activity of plant extracts and inert dusts also provided promising results. The different hop extracts showed mainly contact toxicity and repellence towards *S. granarius* adults due to the presence in the extracts of VOCs able to stimulate insect olfactory system. The study on the insecticidal activity of inert dusts highlighted a high efficacy of Cuban zeolite, comparable to that already known for diatomaceous earths. Furthermore, studies on the olfactory response of adults of *S. paniceum* and *S. oryzae* towards the odours of host substrates allowed to identify some volatile compounds with kairomonal action.

Overall, the findings of the present PhD thesis provide a significant contribution to growing scientific knowledge on the attractiveness and susceptibility degree of ancient wheats, pigmented genotypes, and other food substrates to storage insects and their physicochemical and nutritional properties. Furthermore, present results are interesting for further breeding programs aimed at develop genotypes resistant or less susceptible towards storage pests. As well as, studies on semiochemical interactions and insecticidal activities of plant extracts and inert dusts can contribute to the development of innovative low-impact control strategies.

Thesis outline

Chapter 1 - Introduction

The introductive chapter focuses on the actual global importance of wheat and on the rediscovery of wheat biodiversity, including landraces, ancient and old wheat varieties, and pigmented wheat genotypes. Furthermore, the several damages caused by stored-product insect pests are discussed and the most important traits of the biology and ecology of *Sitophilus granarius* (L.) (Coleoptera, Curculionidae) and *Rhyzopertha dominica* (Coleoptera, Bostrichidae), two of the most damaging primary pests worldwide, are reported.

Chapter 2 - Host plant selection by phytophagous insects

The Chapter 2 analyses the host plant selection process by phytophagous insects. The main chemical and physical plant properties involved in the behavioural response of host location, acceptance or rejection by insects are discussed. In particular, the pivotal biological role of Volatile Organic Compounds (VOCs) in host finding by storage pests is highlighted.

Chapter 3 - Sustainable control means of stored-product insect pests

In this chapter, the urgent need of sustainable control means in Integrated Pest Management (IPM) strategies of stored-product insect pests is highlighted. A dept analysis of semiochemical-based control means, resistant wheat varieties, botanicals, and inert dusts as suitable low-impact alternative to synthetic insecticides for controlling storage insect pests is reported.

Chapter 4 - Aim of the thesis

The objectives of the PhD thesis are described in a specific chapter. Briefly, the present thesis mainly focused on deepening the knowledge on the attractiveness/susceptibility of ancient and old wheat species to the primary pests *S. granarius* and *R. dominica* with the aim of identify 1) genotypes resistant or less-susceptible with good technological and nutritional performance, potentially useful in breeding programs, and 2) sources of bioactive compounds useful to develop semiochemical-based control means. Furthermore, host acceptance and utilisation of a pigmented wheat genotype by *S. granarius* adults was investigated, as well as, the utilisation of different stored products by *R. dominica* adults. Considering the current IPM approaches to control stored-product insect pests, further studies were carried out to evaluate 1) the olfactory responses of *Stegobium paniceum* (L.) (Coleoptera, Anobiidae) and *Sitophilus oryzae* (L.) (Coleoptera, Curculionidae) adults to VOCs respectively from Chinese medicinal plants and different rice cultivars, to provide a basis for further studies aiming at developing semiochemical-based control means, and 2) the insecticidal activity of different wild hop extracts, and of a Cuban zeolite and a diatomaceous earth against *Acantoscelides obtectus* (Say) (Coleoptera, Bruchidae) adults.

Chapter 5 - Susceptibility of old and modern wheat genotypes to *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.)

This chapter is based on the published paper by D'Isita *et al.* (2024) in Journal of Stored Products Research, 106, 102265. In this study, the susceptibility of three old genotypes (Senatore Cappelli, old Saragolla, and Dauno III) and four modern wheat varieties (Mec, Ofanto, Svevo, and Faridur) towards *S. granarius* and *R. dominica* was evaluated. All wheat varieties were susceptible to attacks by both species, however some significant differences among them were recorded. Moreover, the study highlighted that the wheat susceptibility to stored grain pests is the result of different physicochemical features and their interactions. Among them, kernel hardness seems to be the first barrier to *S. granarius* and *R. dominica* attacks. In addition, lesser susceptible varieties to both species showed a higher protein content. Furthermore, wheat varieties with a higher thousand-kernel mass were more susceptible to the lesser grain borer, whilst it did not influence the susceptibility to the granary weevil. Due to the increasing interest of consumers and farmers in old grains, the study offers new knowledge useful for a more rational postharvest management of these cereal resources.

Chapter 6 - Behavioural responses of *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) to odours of old and modern wheat genotypes

This chapter is based on the paper by D'Isita *et al.* (2024) accepted for publication in Journal of Stored Products Research. In this study, the VOCs profiles of old genotypes, Senatore Cappelli, old Saragolla, Dauno III, and modern wheat varieties, Mec, Ofanto, Svevo, and Faridur, were characterized by headspace - solid phase microextraction (HD-SPME) and gaschromatography coupled with mass spectrometry (GC-MS) and their attractiveness towards *S. granarius* and *R. dominica* adults was evaluated in two-choice pitfall bioassays and Y-tube olfactometer, respectively. *Sitophilus granarius* adults were significantly attracted to odours of all varieties, with females being significantly more attracted by Faridur, Ofanto, Mec, and old Saragolla compared to Svevo. *Rhyzopertha dominica* adults exhibited a significant olfactory preference for the odours of Faridur, Mec, and old Saragolla. The olfactory preferences of both species could be related to differences emerged in varietal VOCs profiles. The study offers new knowledge for further investigation testing VOCs of the most and the lowest attractive varieties to identify effective attractants or repellents towards *S. granarius* and *R. dominica*.

Chapter 7 - Susceptibility of ancient wheats to *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) and their allelochemical interactions

This chapter is based on paper D'Isita *et al.* submitted for publication to Grain & Oil Science and Technology. In this study, the susceptibility level and the attractiveness of improved emmer (Padre Pio) and spelt (Benedetto) varieties towards *S. granarius* and *R. dominica* was evaluated in laboratory bioassays. Furthermore, the VOCs profiles of the hulled ancient wheats studied were characterized by headspace - solid phase microextraction (HD-SPME) and gaschromatography coupled with mass spectrometry (GC-MS). Regarding the susceptibility degree of hulled wheat species, glumes and glumelles represented a physical barrier against *S. granarius,* but not against *R. dominica*. Avoiding the glumes protectant effect, the kernel hardness and protein content represented the main factors involved in resistance mechanisms to *R. dominica* and *S. granarius*, respectively. Considering the behavioural responses to hulled emmer and spelt, *S. granarius* were effectively attracted by both ancient wheat species, with a significant preference of both sexes for the emmer Padre Pio. By contrast, odours from emmer and spelt varieties did not elicit a significant attraction in *R. dominica* adults. The possible reasons for differences in susceptibility and attractiveness are discussed.

Chapter 8 - Acceptance and utilisation efficiency of a purple durum wheat genotype by *Sitophilus granarius* (L.)

This chapter is based on the published paper by D'Isita *et al.* (2023) in Scientific Reports, 13(1), 14246. In this study, the susceptibility of an anthocyanin-rich purple durum wheat genotype (T1303) to the granary weevil was evaluated in comparison with two yellow durum (Ofanto) and bread (Mec) wheat varieties. The feeding response and food utilisation efficiency by adult insects was also investigated by calculating nutritional indices in whole flour disk bioassays. Different levels of susceptibility to granary weevil emerged among genotypes tested. Results provide evidence for the antifeedant and toxic effects of anthocyanins present in the T1303 pericarp against the granary weevil. Overall, the study contributes new insights into the mechanisms of host acceptance and food utilisation by *S. granarius* and would be useful to identify antifeedant flavonoids as well as to develop varietal resistance-based strategies against this pest.

Chapter 9 - Population development of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) on different stored products

This chapter is based on the published paper by Ren *et al.* (2023) in Entomological Research, 53(10), 359-366. In this study, host utilisation of different stored products (angelica, jujube, maize, rice, soybean, and wheat) by *R. dominica* adults was evaluated considering the development, survival, reproduction, and life table parameters. Overall, results indicate that wheat was the most suitable stored product, whereas angelica was the least suitable, for the feeding, development, and population increase of *R. dominica*. These findings provide basic information about the occurrence trends and characteristics of *R. dominica* that will be useful for the control of this pest on different stored products. The physicochemical properties of angelica should be further explored for potential application in the control or integrated management of *R. dominica*.

Chapter 10 - Electrophysiological and behavioural responses of *Stegobium paniceum* to volatile compounds from Chinese medicinal plant materials

This chapter is based on the published paper by Cao *et al*. (2022) in Pest Management Science, 78(8), 3697-3703. In this study, the olfactory responses of *S. paniceum* to the most abundant volatile components of some drugstore attractant CMPMs such as *Panax notoginseng*, *Angelica sinensis*, *Gastrodia elata* and *Peucedanum praeruptorum*, namely falcarinol, 3-n-butylphthalide, p-cresol and α-pinene, respectively, were studied by electroantennography (EAG) and behavioural bioassays in six- and four-arm olfactometers. The results indicated that the four volatiles of CMPMs are perceived by the peripheral olfactory system of *S. paniceum* adults and can individually elicit a positive chemotaxis in *S. paniceum* adults confirming the role of chemical cues in host plant detection and selection by this pest. Furthermore, these findings provide a basis for further studies aiming at developing semiochemical-based strategies against this storage-beetle pest.

Chapter 11 - Attraction of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) to the semiochemical volatiles of stored rice materials

This chapter is based on the published paper by Cao *et al*. (2024) in Journal of Pest Science, 97(1), 73-85. In this study, in order to explore the influence of stored cereal volatiles on the behaviour of *S. oryzae*, the olfactory responses of adult rice weevils to the volatiles of different rice cultivars [Red brown rice (RBR), Daohuaxiangmi (DHXM), Baishuigongmi (BSGM), Yashuixinmi (YSXM), and white glutinous rice (WGR)] were studied using electroantennography (EAG) and behavioural bioassays in different types of olfactometers. Briefly, the results indicated that the volatiles of the preferred rice cultivar (RBR) were perceived by the peripheral olfactory system of *S. oryzae* adults and individually elicited positive chemotaxis. These findings offer new insights into the mechanism of host preferences of stored-grain pests and provide a basis for developing novel monitoring and control tools against *S. oryzae*.

Chapter 12 - Bioactivity of wild hop extracts against the granary weevil, *Sitophilus granarius* (L.)

This chapter is based on the published paper by *Paventi et al*. (2021) in Insects, 12(6), 564. This study investigated the bioactivity of methanol, acetone, and *n*-hexane hop flower extracts against *S. granarius* adults by evaluating toxic (contact, inhalation, and ingestion), repellent, antifeedant, and nutritional effects, as well as, their anticholinesterase activity and olfactory sensitivity. Obtained results show that hop crude extracts could represent a valid tool for *S. granarius* control.

Chapter 13 - Insecticidal activity of a diatomaceous earth and a natural Cuban zeolite against adults of the bean weevil, *Acanthoscelides obtectus*

This chapter is based on the paper by D'Isita *et al*. (2024) accepted for publication in Acta Horticulturae. The present study aimed at evaluating the insecticidal activity of a diatomaceous earth (DE) and a natural Cuban zeolite against *A. obtectus* on chickpea and lentil seeds. Overall, both inert dusts were effective in controlling *A. obtectus* at lower doses tested and in short exposure-times, with the DE showing a faster insecticidal activity.

Chapter 14 - General discussion and conclusion

The main findings obtained in the various research activities involved in the PhD thesis are discussed in a concluding chapter. Furthermore, future prospective of the acquired knowledge are reported.

1. Introduction

1.1 The importance of wheat

Cereal grains were the first agricultural attempts by early man and provide more food energy worldwide than any other type of crops (Sarwar *et al.*, 2013). The high adaptability to different environments, the long shelf life, and the easy transportability of the grain are the main characteristics that have allowed their diffusion and cultivation in almost half of the Earth's surface. For this reason, cereals can be considered the most important group of plants of agricultural interest in the world and staple food crops (Nuovo, 2013). Among cereals, wheat is one of the most important global primary crops with strong consumer's demand (Taylor and Koo, 2015; Igrejas and Branlard, 2020) and a production of more than 700 thousand tons in 2021 worldwide (FAO, 2023). Wheat is cultivated in nearly all regions of the world, from the equator to temperate lands (as high as latitudes 60°N and 44°S) and at altitudes as high as 3000 m above sea level (Pena-Bautista *et al.*, 2017). Asia and Europe are the two regions with the highest share of world wheat production (Le Gouis *et al.*, 2020). The total production in Asia is around 340 thousand tons with the main producer as China, India, and Russian federation (FAO, 2023). In Europe the total production is of around 300 thousand tons (FAO, 2023). In this continent, the main producer of soft wheat are France, Germany, Poland, and Romania (Eurostat, 2024). Whilst, the majority of durum wheat in Europe and in the Mediterranean Basin is produced in Italy (Xynias *et al.*, 2020; Eurostat, 2024). The durum wheat is grown mainly in the Southern Italy, in particular Apulia region, with a total production of almost 8 million of quintals of grain (Istat, 2023). The leading role of Italy in durum wheat production is partly attributable to the economic importance of the pasta industry in this country, which has promoted the intense breeding work carried out in Italy since the beginning of the 20th century (Mefleh *et al.*, 2019). The populations or cultivars of wheat plants grown nowadays are very different from their wild progenitors and from domesticated and cultivated populations (Mefleh *et al.*, 2019). Indeed, a continuous genetic selection over the centuries were done on wheat populations. A first kind of selection was involuntarily done by farmers that choose the best plants and seeds to grow in the following season. Then, a voluntary selection was carried out and continues by breeders. Wheat breeders firstly focused on characteristics associated to grain yield like lodging resistance, frost resistance, disease resistance of roots, stems and leaves, and grain yield components (Igrejas and Branlard, 2020). Thus, whilst the total land area given over to bread and durum wheat in the world increased by only 6.8% from 204 million of hectares to 218 million of hectares between 1961 and 2013, the world production increased by 321% from 222 million tons to 713 million tons (FAO, 2014). This genetic advance linked with the agronomic techniques, particularly improved during the Green Revolution, made wheat an essential crop to humankind (Igrejas and Branlard, 2020).

1.2 Ancient wheats, old wheat varieties, and landraces

It is reported that before to become settled, nomadic peoples ate wild cereals (Davenport, 1945). The basis of food production was the domestication of wild progenitors (Fuller and Colledge, 2008). Domestication is changes to the plant probably involuntarily due to an "unconscious selection" (Charles Darwin; 1883; Zohary, 1969; Harlan *et al*., 1973; Hillman and Davies, 1990a, b). Indeed, people did not set out to domesticate plants, but to manipulate productivity through cultivation. Therefore, the "domestication syndrome" can be defined as a set of morphological and physiological characters that differ between domesticated crops and their wild ancestors (Harlan *et al*., 1973; Hawkes, 1983; Zohary and Hopf, 2000; Gepts, 2004). It should be noted that the domestication syndrome differs for different kinds of crops. In the case of wheat, the traits modified due to the domestication were loss of spike shattering at maturity to prevent seed loss at harvesting, reduction or loss of glumes and awns, and conversion of hulled kernels into free-threshing forms or naked grains, increase in seed size, and reduction in tiller numbers (Evans, 1993; Dubcovsky and Dvorak, 2007; Shewry, 2009). Wheat is an autogamous species whose natural population comprises a mixture of several different pure lines. When cultivated, these genetically heterogeneous populations evolve under the pressure of farmers and natural selection in a specific environment, giving rise to the so-called "landraces" (Simmonds, 1979).

The term "ancient wheat" generally refers to the wheats cultivated by ancient civilizations following their domestication (Mefleh *et al*., 2019). The hulled wheats

Triticum monococcum L., *T. turgidum* ssp. *dicoccum* Schubler, and *T. aestivum* ssp. *spelta* L. (einkorn, emmer, and spelt, respectively), together commonly known as "Farro", are among the most ancient cereal crops of the Mediterranean basin to be domesticated (Perrino *et al*., 1993; Buerli, 2006; Dinu *et al*., 2008) and contributed significantly to the phylogenesis of modern wheats (Arzani, 2011). Einkorn, the first domesticated wheat (Weiss and Zohary, 2011), was cultivated in 10,000 years BC in the Fertile Crescent zone, a limited area in the Middle-East between the Tigris and the Euphrates. At the same time and in the same area, hunter-gatherers begun to cultivate the wild emmer (Dinu *et al*., 2018). The emmer wheat is the ancient durum wheat that represented the transition from the wild tetraploid spp. *dicoccoides* (wild emmer wheat) to durum wheat (Salamini *et al*., 2002). From Fertile Crescent zone, emmer later spread to Asia, Europe, in particular in Italy, and Africa (Matsuoka, 2001; Salamini *et al*., 2002; Dubcovsky and Dvorak, 2007; Arzani, 2011; Bordoni *et al*., 2016). Emmer was the most widespread wheat species during the Neolithic and Bronze Age periods and was a staple food for the Babylonians, Assyrians, and Egyptians, who were the first to make oven-baked bread (Mefleh *et al*., 2019). Later, spelt wheat, and progressively free-threshing wheat (*Triticum turgidum* ssp. *durum* Desf.) and bread wheat (*Triticum aestivum* L.) spread out from the Mediterranean Basin towards eastern, then western Europe, around 4,000 and 3,500 BC, respectively (Bonjean, 2016). Thus, the emmer cultivation started to diminish, and, in the beginning of the 20th century, it had been almost completely substituted by the derived free-threshing species, durum and bread wheats (Mefleh *et al*., 2019).

Italy is one of the richest European areas in biodiversity also in plant communities, such as spermatophytes, the super division which also includes cereals (Domina and Marzio, 2018). Over the last two decades, several studies have been published on Italian ancient wheats, old wheat varieties, and landraces (Piergiovanni, 2013). Until the beginning of the 20th century, landraces were the only types of emmer and durum wheat cultivars being grown (Mefleh *et al*., 2019). Italy was rich in durum wheat landraces, mainly in the south and on the islands (Careddu *et al*., 2023). Most of them belonged to the Mediterraneum type and were tall (up to 180 cm), prone to lodging, and late in flowering (Dib *et al*., 1992). Furthermore, other landraces arrived in Italy from the Near-East and North Africa, including some Syriacum types, which were shorter and earlier in flowering (Dib *et al.*, 1992). Landraces were evolved and adapted to the agroecosystem of the Southern Italy characterized by high levels of complexity and genetic diversity (Careddu *et al.*, 2023). In this context, wheat landraces were part of the rural economy aimed at satisfying the needs of the families by means of different animals and crops. Italy was the first country within the Mediterranean basin to begin “conscious” durum wheat breeding (Mefleh *et al.*, 2019). The cultivar Senatore Cappelli, released in 1915 by the renowned breeder Nazareno Strampelli, was the most popular variety of this period widely used in breeding programs (Scarascia Mugnozza, 2005), with excellent organoleptic characteristics still appreciated by the consumer (Fanelli *et al.*, 2023). In recent years, the increasing consumers’ request for a wholesome, traditional, and sustainable food supply is contributing to a rediscovery of the Italian cereal biodiversity, especially in the South of Italy, and is giving to landraces, ancient and old wheat genotypes a new chance of survival (Arzani, 2011; Nuovo, 2013). Nowadays, there is no accurate clarification, but it is usually accepted that old wheats are genotypes remained unchanged over the last hundred years (Boukid *et al.*, 2018). By contrast, modern wheat includes genotypes generated in the second half of the 20th century, during the Green-Revolution (Khush, 2001). In general, landraces, ancient and old wheat genotypes are characterized by a low productivity compared with modern ones (Giunta *et al.*, 2007), on the other hand, they showed a greater adaptability to adverse soil and climatic conditions (Perrino, 1993; D’Antuono, 1994; D’Antuono and Bravi, 1996). They cannot benefit from high sowing rates and high nitrogen fertilization rates due to their susceptibility to lodging (Giunta *et al.*, 2007) and they are generally later in flowering than modern varieties (Motzo *et al.*, 2007). Their low nitrogen requirements make them a valid choice for rotation with legumes (Sandras *et al.*, 2016). These proprieties make these genotypes suitable to low-input systems, where the higher yield potential of modern cultivars is not fully expressed (Mefleh *et al.*, 2019), or where organic farming methods are adopted because of either low soil fertility and/or the conservative attitude of the farmers (Sandras *et al.*, 2016). Thus, also considering the great market interest, growing these varieties could represent a source of income for farmers, especially in agriculturally marginal areas (Stagnari *et al.*, 2008). Based on the total economic value approach, old wheat genotypes should not only be considered commercially in monetary terms, but the social and cultural values (Karabak and Kan, 2021). As well as, in modern agriculture, growing old wheat genotypes rich in their genetic variability could be a way to contribute to agricultural diversification (Padulosi *et al.*, 1996) and safeguard these valuable genetic resources useful also for breeding purposes (Mefleh *et al.*, 2019; Karabak and Kan, 2021). Nowadays, the most common commercially available ancient wheat species are einkorn, emmer, khorasan and spelt (Di Francesco *et al.*, 2021). Additionally, there are several old genotypes of both *T. aestivum* and *T. durum* cultivated from the mid-1800s to the beginning of the 20th century (before the "Green Revolution") including landraces of durum wheat, such as Russello, Senatore Cappelli, Dauno, Saragolla antica, Timilia or Tumminia, and "soft" wheat, such as Gentil Rosso, Maiorca, Sieve, Solina, and Verna (Di Francesco *et al.*, 2021).

1.3 Pigmented wheat genotypes

The increasing interest for the benefits of bioactive compounds in food to improve health and prevent disease, have prompted plant breeders to increment the levels of these compounds in crops, including cereals (Gani *et al.*, 2012). Some bioactive compounds are specific of cereals, for example, γ-oryzanol in rice, avenanthramides and saponins in oat, β-glucans in oat and barley, alkylresorcinols in rye (Escribano-Bailón *et al.*, 2004; Gani *et al.*, 2012; Ficco *et al.*, 2014 a). Cereal seed coats have been reported to contain a variety of flavonoids (Collins, 1987) and these have been found to have significant effects on the quality of cereal grains (McCallum and Walzer, 1990). The presence of anthocyanins in coloured rice, corn, barley, buckwheat, sorghum, millet, bread and durum wheat has been extensively reported in previous studies (Escribano-Bailón *et al.*, 2004; Hosseinian *et al.*, 2008; Abdel-Aal *et al.*, 2010; Ficco *et al.*, 2014 a). Anthocyanins are able to scavenge free radicals that can cause oxidative stress in human cells (Shipp and Abdel-Aal, 2010). Thus, increasing attention has been paid to these compounds for their health benefits as dietary antioxidants (Knievel *et al.*, 2009; Abdel-Aal *et al.*, 2010). Anthocyanins accumulation in wheat kernels is an extremely rare trait which has a high frequency in Ethiopian wheat germoplasm and was reported for the first time in some tetraploid wheats (Zeven, 1991). The anthocyanins content was identified and quantified in various blue, purple, and red bread wheats (Abdel-Aal *et al.*, 2010) and varied from

7.1 to 211.9 μg/g (Ficco *et al.*, 2014 b). The most common anthocyanin in purple wheat is cyanidin 3-O-glucoside (Cy-3-Glc), followed by peonidin 3-O-glucoside (Pn-3-Glc); whereas delphinidin 3-O-glucoside (Dp-3-Glc) is the most abundant anthocyanin in blue wheat (Abdel-Aal and Hucl, 2003; Escribano-Bailón *et al.*, 2004). Furthermore, the purple pigment is localized to the grain pericarp (outer layer), whereas the blue pigment is localized to the grain aleurone layer (Zeven, 1991). Pigmented wheat genotypes that carry genes for purple pericarp or for blue aleurone controlling grain anthocyanin pigmentation (Knievel *et al.*, 2009; Ficco *et al.*, 2014 b) are very interesting due to the high nutritional value conferred to the final products (Ficco *et al.*, 2014 b; Ficco *et al.*, 2016; Ficco *et al.*, 2020). Furthermore, phenolic compounds, including flavonoids, are involved in plant resistance to abiotic and biotic stress (Drewnowski and Gomez-Carneros, 2000). Thus, these genotypes could be an interesting source of biodiversity also useful as a potential reservoir of resistance genes towards insect pests that could be transferred to commercial varieties (Germinara *et al.*, 2019; D'Isita *et al.*, 2023).

1.4 The stored-product insect pests

Insect pests are a common and serious increasingly issue of stored food, processed products and packed commodities globally (Phillips and Throne, 2010; Rajendran, 2020 and references therein). Postharvest wheat losses due to pest attacks are estimated to be about 10-15% of the global annual production (Rajendran, 2002; Neethirajan *et al.*, 2007) and, in some developing countries, they can reach 50% of the total harvest (Fornal *et al.*, 2007). This percentage of damage can be varying annually in dependence of climatic conditions, storage facilities and pest control measures adopted (Rajendran, 2002; Neethirajan *et al.*, 2007; Fornal *et al.*, 2007; Rajendran, 2020 and references therein; Tadesse, 2020 a, b). Severe quantitative and qualitative damages worldwide are linked to insect feeding activity, alteration of nutritional and aesthetic value of commodities, and off-odours. Insects contribute to food contamination with silk threads, body fragments, *hastisetae*, excrement and chemical secretions (Rajendran, 2020 and references therein; Lemic *et al.*, 2020 and reference therein). Furthermore, storage pests increase the moisture content of grains and spread mycotoxigenic fungi favouring the production of mycotoxins during storage (Rajendran, 2002; Magan *et al.*, 2003).

Storage insect pests may be divided into primary pests, if are able to attack whole, sound and undamaged seeds, or secondary pests, that can attack just seeds already damaged mechanically or by primary insect pests. More than 1,000 insect species infest stored products belonging to the orders of Thysanura, Dictyoptera, Orthoptera, Dermaptera, Psocoptera, Hemiptera, Coleoptera, Lepidoptera, Hymenoptera, and Diptera (Imura, 1990). However, the orders of Coleoptera and Lepidoptera contain the most economically important and harmful stored-product pests (Khare, 1994; Shankar and Abrol, 2012). In particular, about 600 stored-product pest species belonging to more than 30 families of the Coleoptera, as well as Lepidoptera includes about 75 pest species from more than 10 families (Imura, 1990). In general, grain damaged by Lepidoptera are only done by the larvae, whereas in the case of Coleoptera, both larvae and adults feed on grain, thus both stages are responsible for the damage.

1.4.1 Biology and ecology of *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.)

In Coleoptera order, the granary weevil, *S. granarius*, and the lesser grain borer, *R. dominica*, are major primary pests of stored cereals, causing severe quantitative and qualitative damages worldwide (Trematerra and Throne, 2012; Majeed *et al.*, 2015; Lemic *et al.*, 2020). *Sitophilus granarius* is a typical member of the family Curculionidae with other two species of this genus, *S. oryzae* and *S. zeamais* (Motschulsky). Whereas *S. oryzae* and *S. zeamais* are closely related and their morphological identification is very difficult (Devi *et al.*, 2017), *S. granarius* adults can be distinguished from these two species due to their uniformly in colour polished chestnut-brown or reddish-brown to shiny black and the fused elytra and atrophied hindwings, which make them unable to fly (Longstaff, 1981). Infestations by *S. granarius* are related to the origin of grain storage and could be the result of a complex co-evolutionary process of the domestication and storage of grain by humans in Neolithic (Plarre, 2013 a). Nowadays, *S. granarius* is present worldwide (Adler *et al.*, 2022), it is considered a completely domesticated pest and its survival depends on the permanent availability of stored grain (Levinson and Levinson, 1994). The granary weevil feeds mainly on cereal grains such as wheat, barley, rye, oats, corn, rice, millet, and derived products (Schwartz and Burkholder, 1991; Bell, 2014). Male beetles produce aggregation pheromones attractive to both sexes, so-called sitophilure, 1-ethylpropyl-(2S,3R)-3-hydroxy-2-methylpentanoate (Faustini *et al*,. 1982; Phillips *et al*., 1989; Chambers *et al*., 1996). The male-produced aggregation pheromone carries the sexes together in the presence of food ensuring a selective advantage for females that perceive the signal useful to find mating, feeding, and oviposition sites and also for males to aggregate for food and mates (Faustini *et al*., 1982). Female of *S. granarius* bore into the kernels and lay generally a single egg within the grain endosperm; then, close the hole with a gelatinous substance that rapidly hardens (Niewiada *et al*., 2005). Following the egg stage, four larval instars were observed that complete their development feeding into the grain kernels until the adult emergence.

Rhyzopertha dominica is a member of the family Bostrichidae, known as auger beetles or powderpost beetles. Bostrichids are reddish-brown to dark-brown, vary in sizes, are elongated, cylindrical in cross-section, and their head is invisible when viewed from above. The insects live mainly in dead and dried wood and are pests of timber (Potter, 1935; Fisher, 1950; Mathew, 1987; Ivie, 2002 a, b). *Rhyzopertha dominica* is frequent in forest habitats and in grain storage environments. Previous studies suggest that the adults are able to fly between agricultural and non-agricultural landscapes (Edde *et al*., 2005; Mahroof and Phillips, 2007; Jia *et al*., 2008; Mahroof *et al*., 2010). The origin of *R. dominica* is not so clear, probably come from the Indian subcontinent, the region where are focused the many species of bostrichids (Chittenden, 1911; Schwardt, 1933; Potter, 1935), and then were largely distributed due to the transport of grain (Doane, 1919; Chûjô, 1958). Nowadays, *R. dominica* is widely distributed around the world (Potter, 1935; Chûjô, 1958) and is a primary pest of stored grain in warmer regions between latitude 40 °N and latitude 40 °S of the equator (Potter, 1935). The lesser grain borer can cause economic losses to a wide range of stored products, but achieves its maximum reproductive success on dry grains, particularly on wheat (Potter, 1935; Bashir, 2002; Edde and Phillips, 2006; Naseri and Majd-Marani, 2022; Ren *et al*., 2023). Mating in *R. dominica* may occur within 24 h of eclosion (Thompson, 1966). Similar to *S. granarius*, male beetles produce aggregation pheromones, dominicalure-1, (S)-(+)-1-methylbutyl (E)-2-methyl-2-pentenoate, and dominicalure-2, (S)-(+)-1-methylbutyl (E)-2,4-dimethyl-2-pentenoate, attractive to both sexes (Williams *et al*., 1981). *Rhyzopertha dominica* females require more than one mating to fertilize all the eggs produced during her lifetime (Barnes and Groove, 1916; Potter, 1935; Thompson, 1966). Differently from *S. granarius*, eggs of *R. dominica* are deposit by females in cluster on the surface of grain kernels or singly among the frass produced by insects (Edde, 2012). In a day, one *R. dominica* can deposit from 33 (Thompson, 1966) to 45 eggs (Howe, 1950). Thus, in a lifetime one female is capable of laying between 207 eggs and 586 eggs in dependence of temperature, humidity, and physical properties of adult food (Barnes and Groove, 1916; Birch, 1945; Howe, 1950; Thompson, 1966). After egg hatching, there are on average four instars (Potter, 1935; Howe, 1950; Thompson, 1966). The first instar is campodeiform, with mandibulate mouthparts (Potter, 1935) and moves rapidly into the grains (Winterbottom, 1922). Larvae feed into the kernels where remain it pupates and emerges as an adult (Winterbottom, 1922; Potter, 1935). Finally, the newly adults emerge from the kernel by chewing through the outer grain layer. Grains infested by *R. dominica* have a characteristic sweetish odour due to the male-produced aggregation pheromones (Khorramshahi and Burkholder, 1981). Adult feeding activities produce large amounts of frass (Breese, 1960) that contain larvae exuviae, excrement, fragments of insects which compromise the quality of the infested grain (Sanchez-Marinez *et al.*, 1997; Seitz and Ram, 2004; Park *et al.*, 2008). Both larvae and adults feed on kernel germ and endosperm and are able to reduce wheat kernels to the pericarp (Winterbottom, 1922; Campbell and Sinha, 1976).

2. Host plant selection by phytophagous insects

Host plant selection by phytophagous insects is a complex process that consists of two different phases, host finding and host acceptance (Thorsteinson, 1958). This process is mediated by the integration of numerous sensory inputs, including olfactory, visual and gustatory cues, within the insect central nervous system (Bruce *et al.*, 2005). Thus, both chemical and physical characteristics determine the behavioural response of acceptance or rejection of plants by insects (Bernays and Chapman, 2007). Generally, at some life stage phytophagous insects move to find their host plant; some insects simply remain on the plant where they or their parents developed, other are able to disperse also hundreds of kilometers (Finch, 1986).

In the host plant finding, insects perceive some plant characteristics at a distance and "choose" according to these impressions (Visser, 1986). In this context, Plant Volatile Organic Compounds (VOCs) play a pivotal biological role in plant-insect interactions, since they are the first chemicals detected by insects useful to identify host plants for feeding, mating and oviposition and also to avoid unsuitable habitats and hosts (Dickens, 1984; Visser, 1986; Agelopoulos *et al*., 1999; Reddy and Guerrero, 2004; Bernays and Chapman, 2007). Plant volatiles have been defined as the "invisible players" in the plant-insect co-evolution, involved in different plant-mediated tri-trophic interactions within the ecosystem (Binyameen *et al*., 2021). Plant VOCs belonging to the category of allelochemicals, compounds emitted by one species that modify the physiology or behaviour of another species (Nordlund and Lewis, 1976). Allelochemicals are classified by the ecological benefit or detriment they infer on the recipient: those that attract pest species are defined as kairomones, those that repel pest species are defined allomones (Dicke and Sabelis, 1988). Plant volatiles can act as kairomones attracting insect pests. Whereas, certain plants have evolved counter strategies to defend themselves against insects, including the emission of repellent VOCs that act as allomones (Cook *et al*., 2007). The plant VOCs include a wide variety of short chain alcohols, aldehydes, ketones, esters, aromatic phenols, and lactones, as well as mono- and sesquiterpenes (Bernays and Chapman, 2007). The smaller molecular weight compounds tend to be the most volatile and reach measurable concentrations at greater distances from the plant; thus, these are of most importance in attraction to the host plant from a distance (Bernays and Chapman, 2007). Blend composition and ratio of plant volatiles are crucial in host finding by herbivores insects (Bruce *et al*., 2005 and references therein; Bruce and Pickett, 2011). In field, insects are able to detect not just blends but also ratios of volatiles to determine their host plant, particularly if blends are composed of ubiquitous compounds produced by many plants in a habitat (Bruce *et al*., 2005). This formidable capability of insects to recognize the correct blend and ratio, not in clean air, but against a background of bioactive components constantly emitted by non-host plants is mediated by olfactory receptor neurons (ORNs), located primarily on the insect antenna. Indeed, ORNs convert the chemical signal into an electrical signal that inputs directly to the central nervous system (Hansson, 2002). If the majority of peripheral receptors of phytophagous insects detect compounds that are not unique to their host plants, the ratio of volatiles emitted by the plant becomes a vital component of the olfactory signal. Also stored-product insects use chemical cues to find host plants (Visser, 1986; Stadler, 1992) and behavioural responses to plant VOCs have been investigated for many pest species (Trematerra *et al*., 2000; Germinara *et al*., 2008; Ukeh *et al*., 2010; Ndomo-Moualeu *et al*., 2015; Cao *et al*., 2024 a, b). The host finding by S. *granarius* could be considered a complex process based on the balance of positive and negative grain odour stimuli (Germinara *et al*., 2008). *Sitophilus granarius* adults are able to perceive a wide range of volatile compounds (Germinara *et al*., 2002) emitted by grains of several cereals (Maga, 1978; Zhou *et al.*, 1999) and are attracted to the odour blends of commercial wheat with yellow kernels (Levinson e Kanaujia, 1981; Germinara *et al*., 2007). Whilst, in behavioural bioassays, *S. granarius* adults did not exhibit a preferential orientation toward the odours of pigmented wheat genotypes. Probably due to a higher presence some short-chain repellent aldehydes in the VOCs profiles of pigmented wheats (Germinara *et al*., 2019). Also Piesik and Wenda-Piesik (2015), reported the behavioural responses of granary weevil to different blends of cereal volatiles. In this case, adults of *S. granarius* were attracted to specific concentrations of a blend with aliphatic alcohols, a blend with aromatics, and a blend with aliphatic alcohols, aliphatic aldehydes, aliphatic ketones, aromatics. Whereas, no attraction was found for blends consisting of aliphatic aldehydes and aliphatic ketones. These observations are in accordance with previous studies where some aldehydes and aliphatic ketones identified from cereal grains showed repellent effects against granary weevil adults (Germinara *et al*., 2008, 2012).

Olfactory cues are important also for the lesser grain borer adults to discriminate different kinds of food substrates (Edde and Philips, 2006; Cao *et al*., 2024 b). During the host finding process, *R. dominica* uses plant VOCs to discriminate suitable and unsuitable plants, with a strong olfactory preference for wheat substrates which provided the maximum reproductive success (Potter, 1935; Bashir, 2002; Edde and Phillips, 2006; Cao *et al*., 2024 b). However, the way *R. dominica* responds to plant volatiles during host finding is not fully understood (Edde *et al*., 2012 and references therein). Further studies are needed the better understand the role of single or blends of VOCs in host finding process.

Once an insect has arrived on a plant it is faced with the decision of whether to accept it. The host plant acceptance is defined as the propensity of insects to accept a potential host for feed and oviposition activities (Miller and Strickler, 1984; Jaenike, 1990; Schäpers *et al.*, 2017). The host acceptance mainly depends on behavioural responses of insects to non-volatile chemical and physical features of the plants (Bernays and Chapman 2007). Generally, this phase involves contact with the cuticle surface when the gustatory receptors come into play (Chapman, 2003). Indeed, gustation has a key role in food acceptance or rejection by phytophagous insects, considering that feeding by phytophagous insects is governed by the balance of phagostimulatory and deterrent inputs (Chapman, 2003). Thus, a positive reaction initiates feeding or oviposition. The reaction model is slightly different for stored-grain insects compared with those feeding on green plants (Nawrot *et al.*, 2010). Indeed, unlike living plant tissues, kernels do not possess biological response systems such as secondary defensive compounds that are active against insects. Thus, their chemical composition and nutritional value for insects are fairly stable during the storage period (Nawrot *et al.*, 2010). In this context, the physicochemical properties of the kernel grains play a pivotal role in food acceptance and influence the demographic performance of insect pests (Throne *et al.*, 2000).

3. Sustainable control means of stored-product insect pests

For many decades, control of stored-product insect pests has been mainly based on the use of synthetic insecticides e.g., phosphine and methyl bromide (Taylor, 1994; Hagstrum and Phillips, 2017). However, it is well know that synthetic pesticides can cause adverse effects in human's health, ranging from acute to chronic impacts (Mostafalou and Abdollahi, 2013; Tuet *et al.*, 2021), and in environment, contaminating soil, water, turf and damaging non target organisms (Handford *et al.*, 2015; Tudi *et al.*, 2021). Thus, mainly in developed countries, legislation limits on the use of pesticides are increasing (Handford *et al.*, 2015). For example, the methyl bromide has been phased out with exceptions for critical uses because it depletes the ozone layer (Taylor, 1994; Fields and White, 2002). Worldwide fast developing of resistant in major storage pest species to key fumigant phosphine are reported (Lorini *et al.*, 2007; Song *et al.*, 2011; Boyer *et al.*, 2012; Kocak *et al.*, 2015; Afful *et al.*, 2017; Nayak *et al*., 2020; Sakka *et al*., 2022). Additionally, the endophytic development of immature stages of some species, such as *S. granarius* and *R. dominica*, causes hidden grain infestation difficult to detect and manage (Rotundo *et al*., 2000; Edde, 2012 and references therein). In this context, the control of storage pests is very difficult and there is a global interest in the research of low-impact control tools useful in Integrated Pest Management (IPM) strategies (Fields and White, 2002; Germinara *et al*., 2007; Rajendran and Sriranjini, 2008; Germinara *et al*., 2012; Germinara *et al*., 2015; Rotundo *et al*., 2019; Chaudhari *et al*., 2021; Urrutia *et al*., 2021). Integrated Pest Management is a holistic system that integrates control across types of tactics and commodities to manage pests in a cost-effective way below economic injury levels (Deguine *et al*., 2021). One way to diversify IPM programs is by investing in developing and use of semiochemical-based control means, resistant wheat varieties, essential oils (EOs), and inert dusts that are considered a suitable low-impact alternative to synthetic insecticides to control storage pests (Cox, 2004 and references therein; De Cristofaro *et al*., 2010; Phillips and Throne, 2010; Abd El-Aziz, 2011; Germinara *et al*., 2007 2012, 2015; Cao *et al*., 2024 a; Throne *et al*., 2000; Golob, 1997; Eroglu, 2014; De Smedt *et al*., 2015; Zeni *et al*., 2021; Trdan *et al*., 2008; Pavela, 2015, 2016; Ntalli *et al*., 2021; Chaudhari *et al*., 2021).

3.1 Semiochemical-based control tools

Semiochemicals are natural low-molecular-weight signaling chemicals that mediate the interactions between organisms of the same (pheromones) or of different (allelochemicals) species (Law and Regnier, 1971; Nordlund and Lewis, 1976; Dicke and Sabelis, 1988). Various pest control strategies used in IPM programs are based on semiochemicals, including indirect control means, such as monitoring, and direct control means, such as mass trapping, attract and kill, lure and kill, mating disruption, and push-pull strategy (Tremblay e Rotundo, 1980; Rotundo and Germinara, 2015). Semiochemicals due to their natural origin, high specificity, and low toxicity towards mammals are considered a safe alternative to chemical insecticides. Thus, to protect stored products, a considerable attention has been paid to the use of semiochemical-based technology (Fields and White, 2002; Phillips and Throne, 2010; Trematerra, 2012; Plarre, 2013 b) with considerable progress (Germinara and Savoldelli, 2023).

To date, the main pheromone components of the major stored-product pests have been identified (Plarre, 2013 b). There are two main types of pheromone: sex pheromones, usually produced by the female to attract male, and the aggregation pheromones, which are primarily produced by the males and attract both sexes. Sex pheromones can be found in species where the adult life expectancy is shorter than one month, as is the case for all stored-product pest moths and a few beetles in the Anobiidae, Bruchidae, Dermestidae and Cerambycidae families (Burkholder, 1982, 1990; Allison *et al*., 2004). Aggregation pheromones have greater biological relevancy than only for facilitating mating (Plarre and Vanderwel, 1999; Wertheim *et al*., 2005). They appear in species where the adult individual survives in general for longer than one month, as Curculionidae, Cucujidae and Bostrichidae (Burkholder; 1982, 1990). Much of the behaviour of insect pests of stored products is associated also with the search for food and egg-laying sites (Cox and Collins, 2002). This behaviour is modified by allelochemicals (kairomones and allomones) produced by host and non-host plants (Cox, 2004). The kairomones often enhance the attraction of aggregation pheromones acting in a synergistic way with it (Plarre, 2013 b; Faustini *et al*,. 1982; Edde and Phillips, 2006).

The most successful implementation of behaviorally-based strategies to control storage pests has been through the adoption of semiochemical-based monitoring and mating disruption at food facilities (Morrison III *et al*., 2021). Monitoring pest population in food storage and facilities allows an early detection of pests and optimizes spatial and temporal control strategies, enhancing the efficacy of treatments (Phillips, 1997). Moreover, pheromone traps applied outdoors can provide information of infestation sources and infestation pressures from neighbouring buildings and landscapes (Plarre, 2013 b).

Other than monitoring, different semiochemical-based direct control tools have been evaluated towards different species of stored-product insect pests, with different level of success (Morrison III *et al*., 2021 and references therein).

Attract and kill

In attract and kill pests are attracted by pheromones or other semiochemicals to a specific source, where they come into contact with an insecticide or with some entomopathogenic microorganism. This technique allows to reduce the quantity of insecticides to be used in food storage and facilities, reducing the risk of food contamination. Positive results have been reported using attract and kill in mills and facilities against *Ephestia kuehniella* Zeller, *Cadra cautella* (Walker), in Italy, against *Lasioderma serricorne* (F.) in Hawaii (Trematerra and Capizzi, 1991; Pierce, 1994; Trematerra, 1995; 1997; Süss *et al*., 1999), and against *Plodia interpunctella* (Hubner) in the United States (Nansen and Phillips, 2002; 2004; Campos and Phillips, 2010; 2014).

Mass trapping

The mass trapping aims to reduce pest population level in the environment. To this end, properly traps are placed in strategic positions to remove a sufficiently high proportion of individuals from the pest population to achieve the required level of protection. The technique is mainly used for the control of moth pests of stored products and promising results were reported in stored facilities to control *C. cautella*, *P. interpunctella, E. kuehniella*, and also *L. serricorne* (Fleurat-Lessard *et al*., 1986; Trematerra, 1990; Buchelos and Levinson, 1993; Pierce, 1994; Trematerra 1994; Süss *et al*., 1996; Carvalho and Mexia, 2002; Carvalho *et al*., 2006; Trematerra and Gentile, 2010). Traps are generally activated using sex pheromones, however the mass trapping efficacy could be improved by using kairomones, that attract both sexes of pests (Phillips *et al*., 1993; Likhayo and Hodges, 2000).

Even if attract and kill and mass trapping have been investigated in various contexts for different species, little adoption of both tactics is reported in the food industry (Mohandass *et al*., 2007).

Push-pull strategy

Push-pull strategy uses a combination of behaviour-modifying stimuli to manipulate the distribution and abundance of insect pests and/or natural enemies. In this strategy, the pests are repelled or deterred away from the protected resource (push) by using stimuli that mask host apparency, repellent, or deterrent. Simultaneously, pests are attracted (pull) using highly attractive stimuli, to other areas facilitating their control.

To the best of our knowledge, has been no work with push-pull strategies in stored products. Only one paper was published to control a stored-product pest, the lesser mealworm, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae), but in poultry houses (Hassemer *et al*., 2019).

Mating disruption

Mating disruption involves the release of high concentration of synthetic sex pheromone in the environment to interferes with the mate finding process impacting on pest population dynamics. The use of mating disruption has been much more successful in stored sector, with commercial products available and high effectiveness (Morrison III *et al*., 2021). Several studies report the application of mating disruption, both in the laboratory and in the field trails, towards storage pests, particularly Lepidoptera, such as *P. interpunctella*, *C. cautella*, *E. kuehniella*, *Sitotroga cerealella* (Olivier) (Sower and Witmer, 1977; Vick *et al*., 1978; Hodges *et al*., 1984; Prevett *et al*., 1989; Mafra-Neto and Baker, 1996; Süss *et al*., 1999; Burks *et al*., 2011; Ryne *et al*., 2001; Shani and Clearwater, 2001; Fadamiro and Baker, 2002; Ryne *et al*., 2006; Ryne *et al*., 2007; Sieminska *et al*., 2009; Pease and Storm, 2010; Savoldelli and Süss, 2010; Trematerra *et al*., 2011; Burks and Kuenen, 2012; Trematerra and Savoldelli, 2013; Trematerra and Spina, 2013; Trematerra *et al*., 2013; Athanassiou *et al*., 2016; Trematerra *et al*., 2017). Indeed, mating disruption may primarily target moths because adults are short-lived, whereas many stored product beetles are long-lived, presenting more opportunities for finding mates and thwarting mating disruption technology (Gerken and Campbell, 2018; Amoah *et al*., 2019). However, despite limitations of mating disruption with longer lived species, there has been significant progress made with *L. serricorne* (Levinson and Levinson, 1999; Mahroof and Phillips, 2014; Amoah *et al*., 2019).

Overall, 58 pheromones are known for stored product insects (45 for Coleoptera and 13 for Lepidoptera) (Maille *et al.*, 2020), even if commercially available lures only attract 27 species (Maille *et al.*, 2020) and there is a lack of semiochemical-based strategies application in food and storage facilities (Morrison III *et al*., 2021). Morrison III *et al*. (2021), suggest that this lack is linked to technological, commercialization, logistical, and cultural challenges distinguished in low, moderate, and critical severity.

A challenge for mating disruption and mass trapping may be the regular immigration of insects into food facilities. Indeed, high immigration from surrounding areas may significantly decrease the efficacy, in particular of mating disruption (Régnière *et al.*, 2019). However, one of the key advantages of deploying mating disruption in food facilities is that most spaces are enclosed, reducing immigration relative to other systems. Regarding the possible development of push-pull system toward storage pests, one of the critical challenges is identify and testing repellent compounds that do not transfer off-odours into the commodities to protect.

One of the most important critical challenges for the efficacy of semiochemical-based control tools is the ubiquitous presence of already attractive food cues in facilities. The background odours in an area may change behaviour exhibited by insects that perceive a particular volatile (Webster *et al.*, 2010). More importantly, this challenge means that potential stimuli need to be even more attractive or be present in concentrations that are more attractive than the primary food source to be effectively used in behaviorally-based strategies at food facilities.

In conclusion, further studies are required to identify more effective novel stimuli useful to modify storage insect behaviour. Besides, large-scale studies are needed to develop and set up semiochemical-based control strategies in food and storage facilities. Furthermore, could be also important to evaluate behaviorally-based strategies in addition to other low-impact control tools, such as heat treatments or aeration, in order to provide multiple hurdles against pest infestation (Morrison III *et al.*, 2020). While there is still much work to do in developing behaviorally-based strategies for stored products, it is clear that these strategies have much potential to control storage pests and protect commodities in alternative to chemical control tools.

3.2 Resistant wheat varieties

Plant resistance to insect pests is the set of heritable characteristics by which a plant reduces the possibility of being successfully used as a host by a phytophagous (Beck, 1965). The development of varietal resistance to insect attacks during grain storage is a passive control technology and a promising alternative to chemical insecticides that could play a very important role in IPM strategies (Throne *et al.*, 2000). Even if, production yields and disease resistance have represented the main objectives of genetic improvement of wheat, whilst the resistance to attacks of storage pests have received little attention in breeding programs because generally not evaluated prior to release of commercial varieties (Fortier *et al.*, 1982; Throne *et al.*, 2000).

From an in-depth analysis of the literature, most evaluations of varietal resistance in cereals have been conducted using no-choice bioassays in which adult insects are confined with samples of each tested variety. Generally, the most common parameters used to compare varieties are total progeny production (Horber, 1983) and insect development time (Dobie, 1974). These parameters are used to calculate the Susceptibility Index (S.I.) = (log. F)/D x 100, where F is the total number of F1 progeny and D is the median development period (Dobie, 1974). According Dobie (1974), this index can be used to classified varieties tested in resistant (0-3), moderately resistant (4-7), susceptible (8-10) and highly susceptible (≥ 11). Other parameters to evaluate varietal resistance are the percentage of insect mortality, adult offspring per female, oviposition rate, weight loss percentage by insect infestation, and number of insect damaged kernels (IDK).

Varieties that express resistance have physical or biochemical attributes that modify behavioural responses (xenobiosis) or that adversely affect development or survival of the pest insect species through metabolic aberrations (antibiosis) (Throne *et al.*, 2000). Thus, to determine resistance mechanisms, insect biological parameters measured could be associated with measured physical or biochemical properties of kernels through statistical correlation (Throne *et al.*, 2000).

It was intensively reported that kernel hardness, grain weight and size, protein, carbohydrate, lipids, gluten, or phenol content can influence the demographic performance of storage insect pests, causing different susceptibility levels of grains (Kućerová and Stejskal, 1984; Warchalewski *et al.*, 2002; Nawrot *et al.*, 2006, 2010; Mebarkia *et al.*, 2010; Gałęcki *et al.*, 2019; Arthur *et al.*, 2020; D'Isita *et al.*, 2023, 2024; Ren *et al.*, 2023). These features can affect pest species differently; below is a briefly summary on the influence of the main physicochemical characteristics of kernels on the degree of wheat susceptibility towards *S. granarius* and *R. dominica*.

The roughness of kernel surface, the thickness of the outer grain layers and folds, and the distance between fold or crevice walls which reflect the mechanical properties of the kernels influence the degree of susceptibility to infestation by *S. granarius* (Nawrot *et al.*, 2010). Indeed, the infestation by insects increases exponentially with the width of the valleys between folds, by contrast the thickness of the seed coat represent a barrier to granary weevil oviposition and feeding (Nawrot *et al.*, 2010). The grain hardness positively contributes to wheat resistance against weevils (McCain *et al.*, 1964; Dobie, 1974; Saad *et al.*, 2018). Kernel hardness negatively influences the ability of *Sitophilus* weevils to penetrate and oviposit into the grain kernel, reducing progeny production and damage (McGaughey *et al.*, 1990; Nawrot *et al.*, 2006; Suleiman *et al.*, 2015; Antunes *et al.*, 2016; Kalsa *et al.*, 2019; Željko *et al.*, 2020). Furthermore, higher kernel hardness levels have been linked also with lesser susceptibility to attacks by *R. dominica* (Amos *et al.*,1986; Toews *et al.*, 2000; Arthur *et al.*, 2020; D'Isita *et al.*, 2024).

Considering chemical grain proprieties, a high protein content is a possible factor responsible for wheat susceptibility to *R. dominica* (Khokhar and Gupta, 1974), probably because a protein-rich substrate is more nutritious for the growth and development of the lesser grain borer progeny (Arthur *et al.*, 2020). Besides, protein content could help in the prediction of the abundance of *R. dominica* progeny (Amos *et al.*, 1986). By contrast, no effects of protein content on the susceptibility of some rice (Astuti *et al.*, 2013) and wheat varieties (Toews *et al.*, 2000) to *R. dominica* were found. Regarding *S. granarius*, low protein content was considered as a possible factor of wheat varietal resistance (Nawrot *et al.*, 2006; Mebarkia *et al.*, 2010).

Some plant secondary compounds, called feeding deterrents, may inhibit or deter insect feeding (Bernays and Chapman, 2007). Kordan *et al.* (2019), showed that a greater presence of phenolics in wheat grain determines a higher adult mortality and a reduction of insect fitness and damage. Furthermore, a pigmented wheat genotypes rich in anthocyanins was less susceptible to *S. granarius* compared with two commercial wheat varieties and induced antifeedant, deterrent and toxic effects against *S. granarius* adults (D'Isita *et al.*, 2023).

In conclusion, effects of physicochemical commonly associated with wheat resistance to stored-product insect pests are not clear-cut (Throne *et al.*, 2000). Wheat susceptibility to stored grain pests probably is the result of different physicochemical features and their interactions (Throne *et al.*, 2000; Nawrot *et al.*, 2010; D'Isita *et al.*, 2024). Additional studies are needed to find potential sources of resistance and to elucidate the resistance mechanisms among wheat varieties to storage pests, including *S. granarius* and *R. dominica*. Indeed, the identification of the sources and mechanisms of host plant resistance to specific insect species is the first step toward the design of good assays and for the breeding of new varieties (Stoner and Shelton, 1988; Jyoti *et al.*, 2001).

3.3 Botanicals

Botanicals (powders, extracts, and non-volatile oils) and essential oils (EOs) of plant origin, rich in bioactive compounds, are considered sustainable alternatives to synthetic insecticides to control storage pests (Trdan *et al.*, 2008; Pavela, 2015; Ntalli *et al.*, 2021; Chaudhari *et al.*, 2021). In particular, EOs are nonpersistent in water and soils (Isman, 2000), present reduced onset of pest resistance (Regnault-Roger *et al.*, 2012), do not form residues in the environment and food (Karabörklü and Ayvaz, 2023), may act as attractant for natural enemies (Hatt *et al.*, 2019), and are relatively cost-effective (Isman, 2000; 2011; Campolo *et al.*, 2018; Giunti *et al.*, 2021; Benelli and Pavela, 2018 a, b). Furthermore, many EOs showed low toxicity on non-target vertebrates, including aquatic ones and mammals (Regnault-Roger *et al.*, 2012; Pavela and Benelli, 2016).

Essential oils are secondary metabolites synthesized by different plant organs (flowers, herbs, leaves, fruit, seeds, etc.) involved in plant defence against biotic and abiotic stresses and signaling processes, including the attraction of pollinators and natural enemies (Smith *et al.*, 2006; Walters, 2010; Pavela, 2015; Zuzarte and Salgueiro, 2015; Hatt *et al.*, 2019). Plant species (over 17,000) that produce essential oils, called aromatic plants, are distributed worldwide and belong to a limited number of botanical families: Asteraceae, Cupressaceae, Lamiaceae, Lauraceae, Rutaceae, Myrtaceae, Piperaceae, and Poaceae (Bruneton, 1999; Svoboda and Greenaway, 2003). Essential oils are complex blends of aromatic (alcohols, aldehydes, phenols, methoxy compounds, and methylene dioxy derivatives) and aliphatic compounds (lactones, alcohols, acids, acyclic esters or aldehydes, coumarins, and compounds bearing nitrogen and sulfur), terpenoids (isoprenoids, phenols, alcohols, ketones, aldehydes, acids, esters, and ethers), and hydrocarbon terpenes (isoprenes, monoterpenes, sesquiterpenes, hemiterpenes, diterpenes, triterpenes, and tetraterpenes (Karabörklü and Ayvaz, 2023). In particular, monoterpenes and sesquiterpenes synthesized in the cytoplasm and plastids generally are the main compounds present in EOs (Hüsnü and Buchbauer, 2015; Zebec *et al*., 2016). Essential oils and their fractions have various biological properties, such as insecticidal, bactericidal, virucidal, and fungicidal activities (Bakkali *et al*., 2008).

Stored product sector seems to be a perfect candidate for the development of new EO-based pest control strategies (Campolo *et al*., 2018). Even if, a huge number of research studies aimed at assessing the insecticidal activity of EOs against crop pests as well as against disease vectors (Isman *et al*., 2011; Pavela, 2015; Benelli and Pavela, 2018 a, b), but less attention has been paid to stored-product pests (Campolo *et al*., 2018). In this context, the insecticidal activity of EOs were most studied against Coleoptera, in particular against *Tribolium castaneum* (Herbst) and *S. oryzae*, followed by Lepidoptera, in which *P. interpunctella* and *E. kuehniella* represented the species most studied (Campolo *et al*., 2018).

Essential Oils proved to have significant efficiency against storage pests through ingestion, contact toxicity, oviposition inhibition, fumigant, repellent, and antifeedant activities (Stefanazzi *et al*., 2006; Werdin-González *et al*., 2008; Fabres and Xavier-Filho, 2014; Campolo *et al*., 2014 a, b; Kanda *et al*., 2017; Liang et al., 2017; Giunti *et al*., 2019, 2021; Paventi *et al*., 2020, 2021; Kavallieratos *et al*., 2022 a, b) in dependence of the species (Kavallieratos *et al*., 2022 a). Generally, the main components of the EOs characterize their biological activity (Campolo *et al*., 2018). For example, the efficacy of Sweet Orange EO (SO-EO) seems to be associated to the content of monoterpenes and particularly to the main component, limonene (Ibrahim *et al*., 2001; Malacrinò *et al*., 2016). Indeed, SO-EO presented the highest amount of limonene among *Citrus* EOs, as well as the highest toxicity against insect pests (Campolo *et al*., 2014 a, b; Chaieb *et al*., 2018). Some EOs components exhibited pronounced effectiveness on storage pests and the activity of compounds varies depending on their structure (Karabörklü and Ayvaz, 2023). Karabörklü and Ayvaz (2023), reviewed the effectiveness of EOs components in stored-product pests management. Briefly, eucalyptol, camphor, linalool, eugenol, limonene, terpinen-4-ol, menthone, and anethole have the most effective insecticidal activity compared with some synthetic chemicals in fumigant and contact toxicity tests. Similarly, eugenol, eucalyptol, anethole, camphor, and linalool showed significant repellent activity.

Moreover, limonene, linalool, eugenol, isoeugenol, methyleugenol, and sparteine caused significant feeding deterrence. Besides, some components such as eucalyptol, eugenol, camphor, isoeugenol, methyleugenol, α-pinene, β-caryophyllene, limonene, α-bisabolol, linalool, terpinen-4-ol, and geraniol have prevented the development stages and decreased the life span of stored-product pests. Components such as eucalyptol, eugenol, camphor, anethole, α-pinene, β-caryophyllene, limonene, and linalool displayed a significant reduction in the fertility of stored-product pests.

Mode of action (MOA) of EOs in insect is not clearly understood (Karabörklü and Ayvaz, 2023). Essential oils are supposed to act as neuro-insecticides (Jankowska *et al.*, 2017). Several research studies showed neurotoxic actions of EOs, causing insect paralysis followed by death (Jankowska *et al.*, 2017 and references therein). Besides, EOs are able to interfere with basic metabolic, biochemical, and physiological functions of insect pests and may also produce histological modifications (Osman *et al.*, 2016). On this basis, it is possible to suggest that the EOs has a broad spectrum insecticidal activity probably attributable to the characteristics of these plant extracts, which are composed by numerous different compounds operating via several MOA toward insect species. This EOs' MOA at multiple levels reduce the probability of insect resistance developing (Gutiérrez *et al.*, 2009).

Essential oil-based pesticides may present some concerns related to their physicochemical characteristics (Giunti *et al.*, 2021). Standardization of the chemical composition could be a criticism of EO-based pesticides (Do *et al.*, 2015; Thompson *et al.*, 2003). Indeed, considerable variation in chemical composition of EO can be related to several factors, as plant variety, season and climatic conditions, harvesting time, and plant processing (Thompson *et al.*, 2003; Fabroni *et al.*, 2012; Benelli and Pavela, 2018 b). Furthermore, other actual obstacles to their application under real conditions are the high volatility, the scarce water solubility, and the rapid degradation of the EOs (Moretti *et al.*, 2002). Recently, to overcome these problematics, nanotechnologies have been proposed to increase the stability and solubility of EOs (Kah *et al.*, 2013; Pavela, 2016) by encapsulating them inside nanoparticles (Werdin-González *et al.*, 2014; Campolo *et al.*, 2017; Giunti *et al.*, 2023) or formulating them as nano-emulsions (Golden *et al.*, 2018; Giunti *et al.*, 2019; Pavoni *et al.*, 2019). These techniques showed promising results also in the control of stored-product pests

(Hashem *et al.*, 2018; Giunti *et al.*, 2018, 2019, 2021; Di Palermo *et al.*, 2021; Kavallieratos *et al.*, 2022 a, b). Furthermore, the persistence of EO-based insecticides and repellent could be enhanced also through repellent food packaging used to prevent damages by stored-product beetles (Benelli and Pavela, 2018 a).

Despite the promising results, few authorised commercial EO-based insecticide formulations are available on the market (Campolo *et al.*, 2018). Future research studies on the MOA of the EOs against insects and EO-based insecticide formulations are needed to develop effective EO-based insecticides (Campolo *et al.*, 2018). Furthermore, according with previous studies, it will be also interesting to evaluate EOs in combination with other low-impact control tools, such as inert dusts, in order to find mixture with potential synergistic toxic activity useful to control storage pests (Yang *et al.*, 2010; Campolo *et al.*, 2014 b; Ziaee *et al.*, 2014; Lampiri *et al.*, 2022).

3.4 Inert dusts

In recent years, the research interest on the use of inert dusts in protection as sustainable control tools is increased (Eroglu, 2014; De Smedt *et al.*, 2015). Inert dusts do not release chemical residues in food and demonstrated a low mammalian toxicity (Subramanyam *et al.*, 1994). In USA zeolites and diatomaceous earths are rated by the Food and Drug Administration as GRAS (Generally Recognized As Safe) for human consumption and are also registered as animal feed additives (Banks and Fields, 1995; EFSA 2013 a, b).

Insecticidal activity of different inert dusts has been found against common storage pest species, such as *S. zeamais*, *R. dominica*, *S. oryzae*, *T. castaneum*, *L. serricorne*, *Tribolium confusum* Jacquelin du Val, *Callosobruchus maculatus* (Fabricius), *Meligethes* spp. (Haryadi *et al.*, 1994; Pezzutti *et al.*, 1979; Athanassiou *et al.*, 2005; Kljajic *et al.*, 2008, 2010a, 2010b, 2011; Andrić *et al.*, 2012; Daniel *et al.*, 2013; Eroglu, 2014; Rumbos *et al.*, 2016; Lü *et al.*, 2017; Eroglu *et al.*, 2019). However, to the best of our knowledge, the research on inert dusts as sustainable tools to control storage insect pests was mainly focused on diatomaceous earths, kaolin, sand, and various nanomaterials (Golob, 1997; Stadler *et al.*, 2012; Islam *et al.*, 2010; Athanassiou *et al.*, 2013; Campolo *et al.*, 2014 a, b; Bougherra-Nehaoua *et al.*, 2015; Bohinc *et al.*, 2020; Zhanda *et al.*, 2020). Whilst, less information is available on the insecticidal effect of other dusts, such as zeolites and attapulgite (Germinara *et al*., 2019; Lampiri *et al*., 2022).

The efficacy of inert dusts depends on different parameters such as insect species (Korunić *et al*., 1998; Fields and Korunić 2000) and development stage (Vayias and Athanassiou, 2004), particle size distribution (Korunić, 1998, Subramanyam and Roesli, 2000, Vayias and Stephou, 2009), geographical origin (Vayias and Stephou, 2009), air and substrate moisture content, temperature, and duration of insect exposure to the formulations used (Fields and Korunić, 2000; Arthur and Puterka2002; Athanassiou *et al*., 2005; Kavallieratos *et al*., 2005; Kljajić *et al*., 2010 a, b). Thus, before to use an inert dust to control harmful stored-product insect pests, studies are needed to carefully investigated its efficacy as insecticide (Germinara *et al*., 2019 b).

There are different theories about the MOA of these materials (Zeni *et al*., 2021); it seems that the insect mortality is induced by desiccation destructing the cuticle waxy layer and the abrasion is a complementary action (Golob, 1997). Due to their physical properties and MOA, generally inert dusts slower acting (Maceljski and Korunic, 1972), thus should be considered a preventive measure against storage pests (Germinara *et al*., 2019 b).

Furthermore, previous researches showed that when used together, the EOs could enhance the insecticidal efficacy of inert dusts also reducing the costs of the management of stored grain pests (Islam *et al*., 2010; Athanassiou *et al*., 2013; Campolo *et al*., 2014 a, b; Bougherra-Nehaoua *et al*., 2015). Thus, could be important to evaluate inert dusts in combination with EO, in order to provide multiple strategies to manage pest infestation during storage.

4. Aim of the thesis

Most studies have been focused on interaction among storage insect pests and modern commercial granaries. Whilst, relatively few studies have been made taking into account ancient and old wheats (Trematerra and Gentile, 2002; Almaši and Poslončec, 2014 and reference therein; Gałęcki *et al.*, 2019), which are considered source of income for farmers, especially in agriculturally marginal areas, and valuable genetic resources. In this context, the thesis was mainly focused on deepening the knowledge on the attractiveness and susceptibility ancient and old wheat species to the two primary pests *S. granarius* and *R. dominica* on with the aim to identify 1) genotypes resistant or less-susceptible with good technological and nutritional performance, potentially useful in breeding programs, and 2) source of bioactive compounds useful to develop semiochemical-based control tools towards both species.

Additionally, in order to expand knowledge on storage insects and host plant interactions, host acceptance and utilisation of a pigmented wheat genotype by *S. granarius* adults and of different stored products by *R. dominica* adults were also evaluated.

In the framework of current IPM approaches to control stored-product insect pests, multidisciplinary activities were also planned, conceived, and conducted. In particular, studies were carried out to evaluate 1) the olfactory responses of *S. paniceum* and *S. oryzae* adults to VOCs respectively from Chinese medicinal plants and different rice cultivars were assessed, to provide a basis for further studies aiming at developing semiochemical-based strategies, and 2) the insecticidal activity of different wild hop extracts against *S. granarius* adults, and of a Cuban zeolite and a diatomaceous earth against *A. obtectus* adults.

5. Susceptibility of old and modern wheat genotypes to *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.)

Ilaria D'Isita[1*], Onofrio Marco Pistillo[1], Antonella Marta Di Palma[1], Pasquale De Vita[2], Giacinto Salvatore Germinara[1]. *Journal of Stored Products Research, 2024, 106, 102265.*

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[1] Department of Agricultural Sciences, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Via Napoli 25, 71122 Foggia, Italy

[2] CREA Research Centre for Cereal and Industrial Crops, 71122 Foggia, Italy

*Corresponding author

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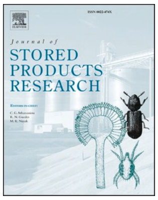

Susceptibility of old and modern wheat genotypes to *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.)

Ilaria D'Isita [a,*], Onofrio Marco Pistillo [a], Antonella Marta Di Palma [a], Pasquale De Vita [b], Giacinto Salvatore Germinara [a]

[a] *Department of Agricultural Sciences, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Via Napoli 25, 71122, Foggia, Italy*
[b] *CREA Research Centre for Cereal and Industrial Crops, 71122, Foggia, Italy*

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ABSTRACT

The granary weevil, *Sitophilus granarius* (L.), and the lesser grain borer, *Rhyzopertha dominica* (F.), are among the major primary pests of stored cereals worldwide. The development of resistant wheat varieties to insect attacks during postharvest is a potential alternative to chemical insecticides. The rediscovery of old wheat genotypes could represent a source of income for farmers, especially in agriculturally marginal areas, and it is also a way to safeguard these valuable genetic resources, useful for breeding purposes. In this study, the susceptibility of three old genotypes (Senatore Cappelli, old Saragolla, and Dauno III) and four modern wheat varieties (Mec, Ofanto, Svevo, and Faridur) towards *S. granarius* and *R. dominica* was evaluated. All wheat varieties were susceptible to attacks by both species, however some significant differences among them were recorded. Using a scale 0 to ≥11, in which 0 is resistance and ≥11 highly susceptible, the Susceptibility Index (S.I.) of Faridur (14.9) to *S. granarius* was significantly higher than those of Dauno III (11.1) and Senatore Cappelli (10.5), whereas the S.I. of Faridur (12.6) to *R. dominica* was significantly higher than those of Dauno III (11.2) and Ofanto (11.0). Wheat susceptibility to stored grain pests probably is the result of different physicochemical features and their interactions. Among them, kernel hardness seems to be the first barrier to *S. granarius* and *R. dominica* attacks. In addition, lesser susceptible varieties to both species showed a higher protein content. Furthermore, wheat varieties with a higher thousand-kernel mass were more susceptible to the lesser grain borer, whilst it did not influence the susceptibility to the granary weevil. Due to the increasing interest of consumers and farmers in old grains, the present study offers new knowledge useful for a more rational postharvest management of these cereal resources.

1. Introduction

Insect pests are a common and serious issue of stored food, processed products and packed commodities (Rajendran, 2020 and references therein). Losses caused by stored-products insect pests are estimated about 10–15% of the annual production (Evans, 1987; Rajendran, 2002; Neethirajan et al., 2007). The granary weevil, *Sitophilus granarius* (L.) (Coleoptera, Curculionidae), and the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera, Bostrichidae), are among the major primary pests of stored cereals, causing severe quantitative and qualitative damages worldwide (Trematerra and Throne, 2012; Majeed et al., 2015; Lemic et al., 2020). *Sitophilus granarius* feeds mainly on stored cereals and derived products (Bell, 2014). Females bore into the kernels and lay eggs within the grain endosperm, closing the hole with a gelatinous substance that rapidly hardens (Niewiada et al., 2005). Instead, *R. dominica* is a member of the family Bostrichidae that are pests of timber (Potter, 1935; Mathew, 1987; Ivie 2002; Edde, 2012) but achieves its maximum reproductive success on dry grains, especially on wheat (Potter, 1935; Bashir, 2002; Edde and Phillips, 2006). Females deposit eggs outside of grain kernels on the surface or among the frass produced by insects and the first instar moves into the grains (Edde, 2012). Larvae and pupae of both species develop inside kernels causing hidden grain infestation (Rotundo et al., 2000; Edde, 2012).

For many decades, control of stored-product insect pests has been mainly based on the use of synthetic insecticides e.g., phosphine and methyl bromide (Taylor, 1994; Hagstrum and Phillips, 2017). However, it is well known that synthetic pesticides can cause adverse effects in human's health, ranging from acute to chronic impacts (Mostafalou and Abdollahi, 2013; Tuet et al., 2021), and in environment, contaminating soil, water, turf and damaging non target organisms (Handford et al.,

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* Corresponding author.
E-mail address: ilaria.disita@unifg.it (I. D'Isita).

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2015; Tudi et al., 2021). Thus, mainly in developed countries, legislation limits on the use of pesticides are increasing (Handford et al., 2015), for example the methyl bromide has been phased out with exceptions for critical uses because it depletes the ozone layer (Taylor, 1994; Fields and White, 2002). Furthermore, worldwide fast developing of resistant in major storage pest species to key fumigant phosphine are reported (Lorini et al., 2007; Song et al., 2011; Boyer et al., 2012; Kocak et al., 2015; Afful et al., 2018; Nayak et al., 2020; Sakka et al., 2022). In this context, the control of storage pests is very difficult and there is a global interest in the research of low-impact control tools (Fields and White, 2002; Germinara et al., 2007, 2012, 2015; Rajendran and Sriranjini, 2008; Rotundo et al., 2019; Chaudhari et al., 2021; Urrutia et al., 2021).

The development of varietal resistance to insect attacks during grain storage is a passive control technology alternative to chemical insecticides that could play a very important role in pest management strategies (Throne et al., 2000; Lemic et al., 2020). Production yields and disease resistance have represented the main objectives of genetic improvement of wheat, whilst the resistance to attacks of storage pests has received little attention in breeding programs (Lemic et al., 2020) because not evaluated prior to release of commercial varieties (Fortier et al., 1982; Throne et al., 2000).

In recent years, the increasing consumers' request for healthy and typical local products is contributing to a rediscovery of the Italian cereal biodiversity, especially in the South of Italy, and is giving to old wheat genotypes a new chance of survival (Nuovo, 2013). Landraces and old cultivars are characterized by a low productivity compared with modern ones (Giunta et al., 2007), on the other hand, they showed a greater adaptability to adverse soil and climatic conditions (Perrino, 1993; D'Antuono, 1994; D'Antuono and Bravi, 1996). These proprieties make old genotypes suitable for low-input systems, where the higher yield potentially of modern cultivars is not fully expressed (Mefleh et al., 2019). In this context, growing old varieties could represent a source of income for farmers, especially in marginal areas (Stagnari et al., 2008), and it is also a way to safeguard these valuable genetic resources, useful for breeding purposes.

To the best of our knowledge, little is known about the susceptibility degree of old genotypes to stored-insect pests, particularly to *S. granarius* and *R. dominica*. In order to identify genotypes potentially useful in breeding programs, in this work the susceptibility of three old genotypes (Senatore Cappelli, old Saragolla, Dauno III) originating from Southern Italy and four modern wheat varieties (Mec, Ofanto, Svevo, Faridur) towards the primary stored-cereal pests *S. granarius* and *R. dominica* was evaluated.

2. Materials and methods

2.1. Insects

Insect populations of *S. granarius* and *R. dominica* used in the experiments started from specimens collected in wheat storage facilities of the Apulia region reared in the dark at 25 ± 2 °C and 28 ± 2 °C, respectively, 60 ± 5% relative humidity (r.h.) on durum wheat kernels (var. Simeto) in cylindrical glass containers (Ø 15 × 15 cm) covered with a fine mesh net (0.5 mm). Eighth generation of both species was used for the experiments.

2.2. Plant materials

Seven wheat genotypes named Mec, Senatore Cappelli, old Saragolla, Dauno III, Ofanto, Svevo, and Faridur were grown in a single field trial during the 2020-21 growing season at Foggia, Italy (41°28′N, 15°32′E; 75 m a.s.l.). The evaluated genotypes included bread and durum wheat varieties representing different origin and breeding periods. Mec is an Italian bread wheat variety used as a susceptible control based on preliminary studies. The old Saragolla, Dauno III and Senatore Cappelli are representative landrace and old varieties of durum wheat cultivated between 1900 and 1940, while Ofanto and Svevo are two modern varieties released in 1988 and 1996, respectively. Furthermore, another variety of durum wheat, derived from a specific breeding program, was added. Faridur is the first Italian soft kernel durum wheat variety, released in 2020 and derived from an introgression program to transfer the puroindoline genes from hexaploid wheat into durum wheat (Morris et al., 2011; Morris and Fuerst, 2015). As the recipient variety was Svevo, Faridur could represent a very useful genetic material to verify the effect of kernel hardness on the postharvest insect attack, since the two varieties share the same genetic background. Recently, Faridur was evaluated for its technological quality by Pasqualone et al. (2023). After harvest, grain samples were stored at low temperatures (4 ± 1 °C) until the time of the study.

2.3. Physicochemical measurements of grain samples

Kernel hardness was performed on 300-kernels sample by the Perten SKCS 4100 (Springfield, IL, USA) according to the AACCI method 55-31 (AACCI, 2010). The experiments were repeated independently three times, and kernel samples were collected at different times. The higher the value of the hardness index (Ha), the harder the grain. Thousand kernel weight (TKW, g) was assessed in three samples of 100 g of the mechanically harvested grain per variety. Protein content (PC) (N × 5.7, dry weight, AACC method 46-13.0160) was determined by micro-Kjeldahl. Gluten index (%, AACC method 38-12.0260) was measured using an automatic gluten washing apparatus (Glutomatic) followed by centrifugation. Yellow index (L*, a*, b*) was carried out by means of the reflectance colorimeter Chroma Meter CM-600d (Konica Minolta Sensing, Osaka, Japan). Five replications were carried out.

2.4. Susceptibility tests

Susceptibility tests with intact kernels were performed using unsexed *S. granarius* (30-day-old) and *R. dominica* (15-day-old) adults. After frozen treatment, uninfested kernel samples (60 g) of each variety were placed in cylindrical glass containers (Ø 9 × 14.5 cm) closed by screw caps and conditioned for 7 days at 25 ± 2 °C and 60 ± 5% r.h. After that, wheat samples were infested with 12 adults of *S. granarius* and *R. dominica* and kept at 25 ± 2 °C and 28 ± 2 °C respectively, 60 ± 5% r.h., and L0:D24 photoperiod. For each wheat variety and insect species, 5 replicates were set up. After the oviposition period (15 days), parental insects were removed, sexed and the number of dead specimens in each replicate was recorded. For each wheat genotype, the following parameters were calculated: (1) total number of F1 progeny, monitored by removing and counting newly emerged adults every 3 days, until no adults emerged for five consecutive days (Shazali, 1987); (2) median development period (D), estimated as the time, expressed in days, from the middle of the oviposition period to the emergence of 50% of the F1 generation (Dobie, 1974); (3) percentage of mortality during the oviposition period; (4) number of adult offspring per female; (5) Susceptibility Index (S.I.), calculated as S.I. = (log. F)/D x 100, where F is the total number of F1 progeny and D is the median development period (Dobie 1974), used to classified varieties tested in resistant (0–3), moderately resistant (4–7), susceptible (8–10) and highly susceptible (≥11).

2.5. Statistical analysis

Data were submitted to Shapiro-Wilk's test to verify normal distribution, to Levene's test for homogeneity of variance, and then to the analysis of variance (ANOVA) followed by Tukey's HSD test for mean comparisons. Linear regression analysis was performed to evaluate the correlation between *S. granarius* and *R. dominica* biological parameters and grain quality traits. Statistical analyses were performed with SPSS (Statistical Package for the Social Sciences) v.18 for Windows (SPSS Inc., Chicago, IL).

3. Results

3.1. Physicochemical characteristics of wheat varieties

Characteristics of the wheat grain used for experiments are summarised in Table 1. The bread wheat variety Mec showed the lowest values of TKW (30.2 g) and a low value of Ha (44.7), being a variety with medium/soft kernels. As expected, the durum wheat varieties showed high Ha values, which are typical for the hard kernel wheat category. Faridur, a soft kernel variety, confirmed the lowest Ha values of the grain (26.3), the distinctive feature for which it was selected. All durum wheat varieties showed TKW values higher than that of the bread wheat variety Mec. Among them, the old variety Senatore Cappelli showed the highest values (49.5 g).

The protein content was highly variable, ranging from 11.8% (Mec) to 16.7% (Faridur). Faridur had a protein content value that was significantly slightly higher ($F = 215.90$; $df = 6$; $P < 0.001$) than the Svevo variety, with a difference of about 1 percentage point. Among the old varieties, Senatore Cappelli showed the highest values (15.1%), while the old Saragolla the lowest (12.4%). The gluten index was also highly variable, with values ranging from 26.5 (Dauno III) to 79.3 (Svevo). The lowest values were recorded from the old durum wheat varieties, while the highest value was recorded from the Svevo variety (79.3). The average gluten index values of Ofanto and Faridur were 46.6 and 53.9, indicating moderate gluten strength. The yellow index values of the semolina were low for all tested varieties, with the exception of durum wheat Svevo, specifically selected for this trait, much appreciated by pasta consumers, as well as its strong gluten.

3.2. Susceptibility tests

During the oviposition period, no significant differences emerged in the mean mortality rate of *S. granarius* ($F = 2.01$; $df = 6$; $P = 0.104$) and *R. dominica* ($F = 1.87$; $df = 6$; $P = 0.122$) adults reared on different wheat genotypes (Tables 2 and 3). By contrast, in both species significant differences were found for the other biological parameters with a large range in the number of F1 progeny and a smaller range for median development time and the S.I.

Table 1
Grain quality traits of wheat samples used in the present study.

Genotype	Grain quality traits				
	TKW[a] (g)	PC[b] (% d.m.)	Ha[c]	Gluten index	Yellow index
	(Mean ± S.E.)	(Mean ± S.E.)	(Mean ± S.E.)	(Mean ± S.E.)	(Mean ± S.E.)
Bread wheat					
Mec*	30.2 ± 0.7 e	11.8 ± 0.1 e	44.7 ± 0.7 d	67.8 ± 1.8 b	16.6 ± 0.1 e
Durum wheat					
Ofanto*	40.9 ± 0.7 d	15.3 ± 0.1 b	88.0 ± 0.7 bc	46.6 ± 1.8 c	19.8 ± 0.1 b
Svevo*	45.3 ± 0.7 bc	15.3 ± 0.1 b	90.0 ± 0.7 ab	79.3 ± 1.8 a	25.3 ± 0.1 a
Faridur*	46.7 ± 0.7 ab	16.7 ± 0.1 a	26.3 ± 0.7 e	53.9 ± 1.8 c	19.4 ± 0.1 b
Dauno III**	40.6 ± 0.7 d	14.6 ± 0.1 c	90.0 ± 0.7 ab	26.5 ± 1.8 e	18.4 ± 0.1 c
Old Saragolla**	42.6 ± 0.7 cd	12.4 ± 0.1 d	86.0 ± 0.7 c	34.7 ± 1.8 de	16.2 ± 0.1 e
Senatore Cappelli**	49.5 ± 0.7 a	15.1 ± 0.1 bc	92.0 ± 0.7 a	36.0 ± 1.8 d	17.6 ± 0.1 d
F	73.96	215.90	1292.52	117. 67	968.90
df	6	6	6	6	6
P	<0.001	<0.001	<0.001	<0.001	<0.001

In the same column, values with different letters are significantly different at $P < 0.001$.
*Modern wheat varieties; ** Old wheat varieties.
[a] : Thousand-kernel weight.
[b] : Protein content.
[c] : Kernel hardness.

In the details, for *S. granarius*, significant differences were found among all the varieties tested regarding the number of F1 progeny ($F = 4.72$; $df = 6$; $P = 0.003$) and offspring per female ($F = 8.01$; $df = 6$; $P < 0.001$) (Table 2). In particular, the offspring per female from the old durum wheat varieties Senatore Cappelli (13.2) and Dauno III (14.7) was significantly lower than those obtained from the modern bread variety Mec (44.0), and durum wheat varieties Ofanto (47.7) and Faridur (38.9). The largest median development period of *S. granarius* was recorded in the old variety Dauno III (42.1 days), that was significantly higher ($F = 11.91$; $df = 6$; $P < 0.001$) than those recorded for the modern Faridur (37.5 days) and Svevo varieties (38.1 days) (Table 2). The S.I. of different varieties varied from 10.5 to 14.9 and those of Senatore Cappelli (10.5) and Dauno III (11.1) old varieties were significantly lower ($F = 4.75$; $df = 6$; $P = 0.003$) than that of the Faridur (14.9) modern variety (Table 2).

Similar results were observed in the case of *R. dominica* (Table 3). Among all wheat varieties, significant differences were found both in the number of F1 progeny ($F = 3.89$; $df = 6$; $P = 0.006$) and offspring per female ($F = 2.71$; $df = 6$; $P = 0.033$). The number of the F1 progeny emerged from the modern durum wheat variety Faridur (539.6) was significantly higher than that recorded for the old variety Dauno III (274.6) (Table 3). The number of the F1 progeny per female emerged from the Faridur (105.8) and Svevo (107.7) modern durum wheat varieties was significantly higher than those observed for the Ofanto (47.7) and Dauno III (56.0) (Table 3). The development period of *R. dominica* reared on the Senatore Cappelli (52.9 days) old variety was significantly longer ($F = 9.43$; $df = 6$; $P < 0.001$) compared to Mec (48.9 days) and Faridur (49.7 days) modern varieties and to old Saragolla (51.0 days) and Dauno III (50.0 days) old varieties. The S.I. of different varieties ranged from 11.0 to 12.6 (Table 3) where the Ofanto (11.0 ± 0.40) and Dauno III (11.1) old varieties being significantly lower ($F = 3.07$; $df = 6$; $P = 0.020$) than which of the Faridur (12.6) modern variety (Table 3). Results of linear regression analyses are reported in Tables 4 and 5. In the case of *S. granarius*, a significant inverse correlation between kernel hardness and F1 progeny production ($R^2 = 0.76$; $P = 0.049$) and S.I. ($R^2 = 0.76$; $P = 0.047$) were found. As regard *R. dominica*, no significant correlations were found between kernel features and biological parameters.

4. Discussion

Pest management during grain storage is crucial for both the food security and food safety of wheat products. Traditional approaches to pest control often involve the use of chemical insecticides, raising concerns about environmental impact and human health. In this context, developing resistant or less susceptible wheat varieties could represent an excellent low-cost and eco-friendly strategy for preserving the integrity of wheat during storage as an alternative to chemical insecticides (Throne et al., 2000; Keba and Sori, 2013). In the present study, we evaluated the degree of susceptibility of a set of durum wheat varieties to *S. granarius* and *R. dominica* with the aim of identifying genotypes or kernel traits useful for planning specific breeding programs. For this purpose, we compared old and new durum wheat genotypes to understand whether the genetic modifications introduced with the selection of new varieties influenced the response to postharvest insect pests. Furthermore, we evaluated some physicochemical characteristics of the different wheat varieties to highlight their possible effects on the degree of susceptibility to storage insects.

According the Dobie's classification (1974), all wheat varieties tested were susceptible or high susceptible to *S. granarius* and *R. dominica* attacks; however, among some of them, significant differences were found. The S.I. of the soft durum wheat Faridur to *S. granarius* was significantly higher compared to those of Dauno III and Senatore

Table 2
Percentage of mortality during the oviposition period, number of F1 progeny, offspring produced by a parental female and the median development period of *S. granarius* adults reared on modern wheat (Mec, Ofanto, Svevo, Faridur) and old wheat (Dauno III, old Saragolla, Senatore Cappelli) varieties.

Genotype	Biological parameters measured				
	Mortality	F1 progeny	Offspring/female	Development period	Susceptibility Index
	(%) (Mean ± S.E.)	(n.) (Mean ± S.E.)	(n.) (Mean ± S.E.)	(days) (Mean ± S.E.)	(Mean ± S.E.)
Bread wheat					
Mec*	8.3 ± 4.8 a	213.7 ± 26.9 ab	44.0 ± 4.2 bc	41.0 ± 0.6 c	13.0 ± 0.4 ab
Durum wheat					
Ofanto*	11.1 ± 5. 6 a	213.0 ± 6.1 ab	47.7 ± 7.1 c	40.7 ± 0.4 c	13.2 ± 0.2 ab
Svevo*	13.3 ± 4.2 a	139.2 ± 35.3 ab	25.8 ± 7.0 ab	38.1 ± 0.8 ab	12.6 ± 1.0 ab
Faridur*	3.3 ± 2.0 a	270.8 ± 25.4 b	38.9 ± 3.4 bc	37.5 ± 0.3 a	14.9 ± 0.3 b
Dauno III **	12.7 ± 4.8 a	116.2 ± 21.3 a	14.7 ± 3.0 a	42.1 ± 0.5 c	11.1 ± 0.5 a
Old Saragolla **	1.7 ± 1. 7 a	222.6 ± 47.7 ab	33.0 ± 4.1 abc	40.1 ± 0.1 bc	13.2 ± 0.7 ab
Senatore Cappelli**	3.3 ± 2.0 a	87.2 ± 24.1 a	13.2 ± 2.7 a	40.9 ± 0.4 c	10.5 ± 0.7 a
F	2.01	4.72	8.01	11.91	4.75
df	6	6	6	6	6
P	0.104	0.003	<0.001	<0.001	0.003

In the same column, values with different letters are significantly different at $P < 0.05$ and $P < 0.001$.

[a] * Modern wheat varieties; ** Old wheat varieties.

Table 3
Percentage of mortality during the oviposition period, number of F1 progeny, offspring produced by a parental female and the median development period of *R. dominica* adults on modern wheat (Mec, Ofanto, Svevo, Faridur) and old wheat (Dauno III, old Saragolla, Senatore Cappelli) varieties.

Genotype	Biological parameters measured				
	Mortality	F1 progeny	Offspring/female	Development period	Susceptibility Index
	(%) (Mean ± S.E.)	(n.) (Mean ± S.E.)	(n.) (Mean ± S.E.)	(days) (Mean ± S.E.)	(Mean ± S.E.)
Bread wheat					
Mec*	0.0 ± 0.0 a	310.0 ± 54.7 ab	68. 9 ± 14.3 ab	48.9 ± 0.8 a	11.6 ± 0.5 ab
Durum wheat					
Ofanto*	1.7 ± 1.7 a	297.2 ± 59.5 ab	47.7 ± 9.3 a	51.3 ± 0.4 bc	11.0 ± 0.4 a
Svevo *	5.0 ± 3.3 a	520.2 ± 80.0 ab	107.7 ± 16.7 b	51.1 ± 0.3 bc	12.1 ± 0.3 ab
Faridur *	18.3 ± 4.9 a	539.6 ± 52.8 b	105. 8 ± 20.9 b	49.7 ± 0.2 ab	12.6 ± 0.2 b
Dauno III **	3.3 ± 3.3 a	274.6 ± 39.8 a	56.0 ± 12.3 a	50.0 ± 0.3 ab	11.1 ± 0.3 a
Old Saragolla**	13.3 ± 6.2 a	340.4 ± 50.9 ab	64.0 ± 13.7 ab	51.0 ± 0.2 b	11.3 ± 0.3 ab
Senatore Cappelli**	0.00 ± 0.0 a	464.2 ± 52.3 ab	85.5 ± 11.0 ab	52. 9 ± 0.2 c	11.6 ± 0.2 ab
F	1.87	3.89	2.71	9.43	3.07
Df	6	6	6	6	6
P	0.122	0.006	0.033	<0.001	0.020

In the same column, values with different letters are significantly different at $P < 0.05$ and $P < 0.001$.
*Modern wheat varieties; ** Old wheat varieties.

Table 4
Linear regression analyses among kernels grain quality and biological parameters measured for *S. granarius*.

S. granarius		Biological parameters measured				
		Mortality	F1 progeny	Offspring/female	Development period	Susceptibility Index
Grain quality traits	Ha	0.30	−0.76	−0.60	0.44	−0.76
		$P = 0.516$	$P = 0.049$	$P = 0.153$	$P = 0.319$	$P = 0.047$
	TKW	−0.31	−0.29	−0.52	−0.43	−0.16
		$P = 0.500$	$P = 0.524$	$P = 0.236$	$P = 0.339$	$P = 0.735$
	PC	0.13	−0.08	−0.18	−0.50	0.11
		$P = 0.786$	$P = 0.871$	$P = 0.694$	$P = 0.255$	$P = 0.816$
	Gluten index	0.33	0.24	0.42	−0.60	0.42
		$P = 0.473$	$P = 0.612$	$P = 0.351$	$P = 0.153$	$P = 0.350$
	Yellow index	0.61	−0.19	−0.08	−0.58	0.08
		$P = 0.146$	$P = 0.689$	$P = 0.870$	$P = 0.174$	$P = 0.857$

Cappelli old varieties. These differences resulted from the higher number of F1 progeny and the shorter development time recorded for the granary weevils on Faridur. In the same way, in susceptibility tests with *R. dominica*, the S.I. of the Faridur variety was significantly higher than those observed for Dauno III and the modern Ofanto variety because of the higher number of F1 progeny recorded on Faridur.

The Faridur variety showed the lowest value of kernel hardness compared to the other above-mentioned varieties. Considering the significant inverse correlation between kernel hardness and S.I. to *S. granarius*, the lower kernel hardness of Faridur variety could partially explain its higher degree of susceptibility to granary weevils. The grain hardness is one of the most measured parameters, also highly heritable (Pomeranz et al., 1985, 1988; Toews et al., 2000), which positively contributes to wheat resistance against weevils (McCain et al., 1964;

Table 5

Linear regression analyses among kernels grain quality and biological parameters measured for *R dominica*.

R. dominica		Biological parameters measured				
		Mortality	F1 progeny	Offspring/female	Development period	Susceptibility Index
Grain quality traits	Ha	−0.46 $P = 0.298$	−0.25 $P = 0.596$	−0.31 $P = 0.498$	0.70 $P = 0.080$	−0.60 $P = 0.153$
	TKW	0.35 $P = 0.438$	0.68 $P = 0.090$	0.51 $P = 0.242$	0.72 $P = 0.068$	0.36 $P = 0.433$
	PC	0.27 $P = 0.563$	0.61 $P = 0.142$	0.48 $P = 0.279$	0.31 $P = 0.500$	0.44 $P = 0.318$
	Gluten index	−0.05 $P = 0.919$	0.45 $P = 0.315$	0.57 $P = 0.185$	−0.29 $P = 0.526$	0.55 $P = 0.201$
	Yellow index	0.00 $P = 0.992$	0.53 $P = 0.216$	0.55 $P = 0.203$	0.16 $P = 0.733$	0.40 $P = 0.381$

Dobie, 1974; Saad et al., 2018). Indeed, according to previous studies, kernel hardness negatively influences the ability of *Sitophilus* weevils to penetrate and oviposit into the grain kernel, reducing progeny production and damage (McGaughey et al.,1990; Nawrot et al., 2006; Suleiman et al., 2015; Antunes et al., 2016; Kalsa et al., 2019; Željko et al., 2020). Furthermore, some kernel physical features, such as hardness, have been linked also with susceptibility to attacks of *R. dominica* (Amos et al., 1986; Toews et al., 2000; Arthur et al., 2020); however no significant correlation was found between kernel hardness and S.I. in this study. The possible role of kernel hardness in the wheat susceptibility to *S. granarius* is also suggested by the lower S.I. of soft kernel wheat Faridur compared to the recipient Svevo variety, genetically differing only for genes regulating kernel hardness. The major role in grain hardness is the degree of adhesion between the storage protein matrix and starch granules rather than the hardness of the individual seed components (Glenn et al., 1991). Moreover, studies on biomechanical properties confirmed that the interaction between starch granules and the protein matrix has a significant impact on kernel texture (Chichiti et al., 2015). Scanning electron microscopic analyses revealed that vitreous kernels exhibited coherent and continuous structures with starch granules tightly embedded within the protein matrix (Dexter and Edwards, 1998; Baasandorj et al., 2016). In contrast, a less compact structure and physically discontinuous protein matrix were observed for white and opaque kernels (Dexter and Edwards, 1998; Turnbull and Rahman, 2002).

In addition to kernel hardness, susceptibility of wheat varieties to insect pests has also been related to other physical and chemical features, such as kernel weight and size, protein, carbohydrate, lipids, gluten, or phenol content (Kućerová and Stejskal, 1994; Toews et al., 2000; Warchalewski et al., 2002; Nawrot et al., 2006, 2010; Mebarkia et al., 2010; Gałęcki et al., 2019; Arthur et al., 2020; D'Isita et al., 2023). In this study, for both species no significant correlations were found among the protein content, thousand-kernel weight, gluten and yellow indices and the biological parameters studied, however some trends were observed. In particular, as regards *R. dominica*, the Faridur variety showed a higher thousand-kernel mass compared to Ofanto and Dauno III, suggesting a possible direct relation between this kernel feature and susceptibility to the lesser grain borer; by contrast, a negative correlation between wheat kernel size and susceptibility to the same pest has been previously reported by Toews et al. (2000). Similar values of thousand-kernel mass were measured for Faridur and Senatore Cappelli, respectively the most and the least susceptible varieties to *S. granarius*, thus suggesting that kernel size and weight did not influence the susceptibility to the granary weevil of the studied wheat varieties. In previous studies, wheat varieties characterized by a low thousand-kernel mass and small kernels were more resistant to *S. granarius* and *S. oryzae*, respectively (Nawrot et al., 2006; Campbell, 2002); on the contrary, maize varieties with large kernels were more resistant to *S. zeamais* than varieties with smaller grains (Gudrups et al., 2001).

Among the wheat varieties tested, the Faridur variety showed a significantly higher protein content compared to Ofanto, Senatore Cappelli, and Dauno III, suggesting a possible direct relation between protein content and wheat susceptibility to both *S. granarius* and *R. dominica*. High protein content has also been suggested as a possible factor responsible for wheat susceptibility to *R. dominica* (Khokhar and Gupta, 1974), probably because a protein-rich substrate is more nutritious for the growth and development of the lesser grain borer progeny (Arthur et al., 2020). Besides, protein content could help in the prediction of the abundance of *R. dominica* progeny (Amos et al., 1986). By contrast, no effects of protein content on the susceptibility of some rice (Astuti et al., 2013) and wheat varieties (Toews et al., 2000) to *R. dominica* were found. Finally, low protein content was considered as a possible factor of wheat varietal resistance to *S. granarius* (Nawrot et al., 2006; Mebarkia et al. 2010). The wheat varieties considered in this study showed significant differences in the yellow index indicating differences in concentrations of carotenoid pigments. In particular, Svevo showed a medium-high value while all the other varieties had rather low average yellow index values. Grain weight loss by *S. granarius* resulted positively correlated to total carotenoids, lutein, and zeaxanthin content (Kordan et al., 2019). By contrast, a study conducted by Stathers et al. (2020), highlighted how the infestation level of some stored insect pests, including *S. zeamais*, was largely independent of the carotenoid content in corn kernels. More recently, Benson et al. (2023) suggested a potential role of beta-carotene in influencing the feeding preference of *S. zeamais* on corn. However, considering that in bread and durum wheat beta-carotene is present in traces while the dominant carotenoid is lutein (Garcia Molina et al., 2021), any further study should concern the effect of lutein on the behaviour of postharvest insects.

The gluten index of Faridur, the most susceptible variety to *S. granarius*, was significantly higher compared to the least susceptible ones, Dauno III and Senatore Cappelli. In previous studies, a significant positive correlation was observed among percentage of grain damaged by *S. oryzae* and content of gluten (Aly and Ali, 2019). By contrast, Nietupski et al. (2006) showed that some protein groups such as glutenins could be impeded the development of *S. granarius*. In the case of *R. dominica*, Faridur and Ofanto, the most and the least susceptible varieties respectively, showed similar value of gluten index, suggesting that this index did not influence the varietal susceptibility to the lesser grain borer. Overall, wheat susceptibility to stored grain pests probably is the result of different physicochemical features and their interactions. Among them, kernel hardness seems to be the first barrier for the oviposition of *S. granarius* and the penetration of *R. dominica* larvae inside wheat kernels. However, other kernel features like protein content, indirectly associated with kernel hardness, might influence wheat grains susceptibility to attacks of *S. granarius* and *R. dominica.* To better understand the interactions between the physicochemical characteristics of the wheat varieties and the susceptibility to postharvest pests, it will be necessary to develop specific genetic materials (i.e., Near-Isogenic Lines) for each single kernel trait. In this work, for the first time the comparison for resistance to postharvest insects was reported between two durum wheat varieties (Faridur and Svevo) that shared the same genetic background, with the except of the kernel hardness.

Moreover, considering the high susceptibility of the varieties tested, it is necessary to investigate further durum wheat varieties including landraces and old genotypes to identify genetic materials with a lower index of susceptibility to *S. granarius* and *R. dominica* useful in breeding program.

In conclusion, considering that the determination of the susceptibility level of wheat varieties to stored-product insect pests is a crucial point in Integrated Pest Management (IPM) (Toews et al., 2000; Nawrot et al., 2006) this study represents, to the best of our knowledge, the first one for the old durum wheat varieties investigated. Thus, in light of the increasing interest of consumers and farmers in old grains, the present study offers new knowledge useful for a more rational postharvest management of these cereal resources.

CRediT authorship contribution statement

Ilaria D'Isita: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Onofrio Marco Pistillo:** Investigation, Formal analysis. **Antonella Marta Di Palma:** Writing – review & editing. **Pasquale De Vita:** Writing – review & editing, Resources, Investigation, Formal analysis, Conceptualization. **Giacinto Salvatore Germinara:** Writing – review & editing, Supervision, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ilaria D'Isita reports financial support was provided by Italian Ministry of University and Research (Ph.D. scholarship - code number DOT13YISJ8). Giacinto Salvatore Germinara reports financial support was provided by Italian Ministry of University and Research (PRIN project 2022 Prot. 202282ZTPL). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Afful, E., Elliott, B., Nayak, M.K., Phillips, T.W., 2018. Phosphine resistance in North American field populations of the lesser grain borer, Rhyzopertha Dominica (Coleoptera: Bostrichidae). J. Econ. Entomol. 111 (1), 463–469.

Aly, M.F., Ali, A.M., 2019. Evaluation of certain Egyptian wheat cultivars against rice weevil, Sitophilus oryzae (L.) (Coleoptera: Curculionidae) using biochemical and biophysical traits. J. Phytopathol. Pest Manag. 54–65.

Amos, T.G., Semple, R.L., Williams, P., 1986. Multiplication of Some Stored Grain Insects on Varieties of Wheat. General and Applied Entomology: the Journal of the Entomological Society of New South Wales, vol. 18, pp. 48–52.

Antunes, C., Mendes, R., Lima, A., Barros, G., Fields, P., Da Costa, L.B., et al., 2016. Resistance of rice varieties to the stored-product insect, Sitophilus zeamais (Coleoptera: Curculionidae). J. Econ. Entomol. 109 (1), 445–453.

Arthur, F.H., Bean, S.R., Smolensky, D., Cox, S., Lin, H.H., Peiris, K.H.S., Peterson, J., 2020. Development of Rhyzopertha Dominica (Coleoptera: Bostrychidae) on sorghum: quality characteristics and varietal susceptibility. J. Stored Prod. Res. 87, 101569.

Astuti, L.P., Mudjiono, G., Rasminah, S.C., Rahardjo, B.T., 2013. Susceptibility of milled rice varieties to the lesser grain borer (Rhyzopertha Dominica, F). J. Agric. Sci. 5 (2), 145.

Bashir, T., 2002. Reproduction of Rhyzopertha Dominica (F.) (Coleoptera: Bostrichidae) on different host-grains. Pakistan J. Biol. Sci. 5, 91–93.

Bell, C.H., 2014. Food safety assurance systems: infestation management in food production premises. Encyclopedia Food Saf. 4, 189–200.

Baasandorj, T., Ohm, J.B., Simsek, S., 2016. Effects of kernel vitreousness and protein level on protein molecular weight distribution, milling quality, and breadmaking quality in hard red spring wheat. Cereal Chem. 93 (4), 426–434.

Benson, G.A., Oyetunde, O.A., Adeboye, K.A., Asebioge, O.O., Joda, A.O., Oyetunde, A. K., Adeshina, G.A., 2023. An assessment of the relationship between seed nutritional components and resistance of maize to Sitophilus zeamais. Cereal Res. Commun. 1–9.

Boyer, S., Zhang, H., Lempérière, G., 2012. A review of control methods and resistance mechanisms in stored-product insects. Bull. Entomol. Res. 102 (2), 213–229.

Campbell, J.F., 2002. Influence of seed size on exploitation by the rice weevil, Sitophilus oryzae. J. Insect Behav. 15, 429–445.

Chaudhari, A.K., Singh, V.K., Kedia, A., Das, S., Dubey, N.K., 2021. Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: prospects and retrospects. Environ. Sci. Pollut. Control Ser. 28, 18918–18940.

Chichiti, E., George, M., Delenne, J.Y., Lullien-Pellerin, V., 2015. Changes in the starch-protein interface depending on common wheat grain hardness revealed using atomic force microscopy. Plant Sci. 239, 1–8.

D'Antuono, L.F., 1994. Obsolete wheats in Italy: an overview on cultivation, use and perspectives for their conservation. In: Report of the IPGRI Workshop on Conservation and Use of Underutilized Mediterranean Species. IPGRI, Rome, Italy, pp. 41–48.

D'Antuono, L.F., Bravi, R., 1996. The hulled wheat industry: present developments and impact on genetic resources conservation. In: Padulosi, S. (Ed.), 1996. Hulled Wheats: Proceedings of the First International Workshop on Hulled Wheats, 21-22 July 1995, Castelvecchio Pascoli, Tuscany, Italy. Bioversity International (vol. 4).

D'Isita, I., Di Palma, A.M., De Vita, P., Germinara, G.S., 2023. Acceptance and utilization efficiency of a purple durum wheat genotype by Sitophilus granarius (L). Sci. Rep. 13 (1), 14246.

Dexter, J.E., Edwards, N.M., 1998. The Implications of Frequently Encountered Grading Factors on the Processing Quality of Durum Wheat. Association of Operative Millers (AOM), pp. 7165–7171.

Dobie, P., 1974. The laboratory assessment of the inherent susceptibility of maize varieties to postharvest infestation by Sitophilus zeamais Motsch.(Coleoptera, Curculionidae). J. Stored Prod. Res. 10 (3–4), 183–197.

Edde, P.A., Phillips, T.W., 2006. Potential host affinities for the lesser grain borer, Rhyzopertha Dominica: behavioral responses to host odors and pheromones and reproductive ability on non-grain hosts. Entomol. Exp. Appl. 119 (3), 255–263.

Edde, P.A., 2012. A review of the biology and control of Rhyzopertha Dominica (F.) the lesser grain borer. J. Stored Prod. Res. 48, 1–18.

Evans, D.E., 1987. Stored products. In: Burrn, Aj, Coaker, T.H., Jepson, P.C. (Eds.), Integrated Pest Management. Academic Press, London, pp. 425–461.

Fields, P.G., White, N.D., 2002. Alternatives to methyl bromide treatments for stored-product and quarantine insects. Annu. Rev. Entomol. 47 (1), 331–359.

Fortier, G., Arnason, J.T., Lambert, J.D.H., McNeill, J., Nozzolillo, C., Philogene, B.J.R., 1982. Local and improved corns (Zea mays) in small farm agriculture in Belize, CA; their taxonomy, productivity, and resistance to Sitophilus zeamais. Phytoprotection 63 (2), 68–78.

Gałęcki, R., Bakuła, T., Wojtacki, M., Żuk-Gołaszewska, K., 2019. Susceptibility of ancient wheat species to storage pests Sitophilus granarius and Tribolium confusum. J. Stored Prod. Res. 83, 117–122.

Garcia Molina, M.D., Botticella, E., Beleggia, R., Palombieri, S., De Vita, P., Masci, S., et al., 2021. Enrichment of provitamin A content in durum wheat grain by suppressing β-carotene hydroxylase 1 genes with a TILLING approach. Theor. Appl. Genet. 134, 4013–4024.

Germinara, G.S., Rotundo, G., De Cristofaro, A., 2007. Repellence and fumigant toxicity of propionic acid against adults of Sitophilus granarius (L.) and S. oryzae (L). J. Stored Prod. Res. 43 (3), 229–233.

Germinara, G.S., De Cristofaro, A., Rotundo, G., 2012. Bioactivity of short-chain aliphatic ketones against adults of the granary weevil, Sitophilus granarius (L). Pest Manag. Sci. 68, 371–377.

Germinara, G.S., De Cristofaro, A., Rotundo, G., 2015. Repellents effectively disrupt the olfactory orientation of Sitophilus granarius to wheat kernels. J. Pest. Sci. 88 (4), 675–684.

Giunta, F., Motzo, R., Pruneddu, G., 2007. Trends since 1900 in the yield potential of Italian-bred durum wheat cultivars. Eur. J. Agron. 27 (1), 12–24.

Glenn, G.M., Younce, F.L., Pitts, M.J., 1991. Fundamental physical properties characterizing the hardness of wheat endosperm. J. Cereal. Sci. 13 (2), 179–194.

Gudrups, I., Floyd, S., Kling, J.G., Bosque-Perez, N.A., Orchard, J.E., 2001. A comparison of two methods of assessment of maize varietal resistance to the maize weevil, Sitophilus zeamais Motschulsky, and the influence of kernel hardness and size on susceptibility. J. Stored Prod. Res. 37 (3), 287–302.

Hagstrum, D.W., Phillips, T.W., 2017. Evolution of stored-product entomology: protecting the world food supply. Annu. Rev. Entomol. 62, 379–397.

Handford, C.E., Elliott, C.T., Campbell, K., 2015. A review of the global pesticide legislation and the scale of challenge in reaching the global harmonization of food safety standards. Integrated Environ. Assess. Manag. 11 (4), 525–536.

Ivie, M.A., 2002. Family 69. Bostrichidae. In: Arnett Jr, R.H., Thomas, M.C., Skelley, P.E., Frank, J.H. (Eds.), American Beetles, Polyphaga: Scarabaeoidae through Curculionoidea. CRC Press, Boca Raton, pp. 233–244 (2).

Kalsa, K.K., Subramanyam, B., Demissie, G., Mahroof, R., Worku, A., Gabbiye, N., et al., 2019. Susceptibility of Ethiopian wheat varieties to granary weevil and rice weevil infestation at optimal and sub-optimal temperatures. J. Stored Prod. Res. 83, 267–274.

Keba, T., Sori, W., 2013. Differential resistance of maize varieties to maize weevil (Sitophilus zeamais Motschulsky) (Coleoptera: Curculionidae) under laboratory conditions. J. Entomol. 10, 1–12.

Khokhar, D.S., Gupta, D.S., 1974. Relative resistance of some varieties of wheat to Sitophilus oryzae (L.) and Rhizopertha Dominica (F.) at different temperatures. Bullet. Grain Technol. 12 (3), 117–123.

Kocak, E., Schlipalius, D., Kaur, R., Tuck, A., Ebert, P., Collins, P., Yilmaz, A., 2015. Determining phosphine resistance in rust red flour beetle, Tribolium castaneum (Herbst.) (Coleoptera: tenebrionidae) populations from Turkey. Turkish J. Entomol. 39, 129–136.

Kordan, B., Skrajda-Brdak, M., Tańska, M., Konopka, I., Cabaj, R., Załuski, D., 2019. Phenolic and lipophilic compounds of wheat grain as factors affecting susceptibility to infestation by granary weevil (Sitophilus granarius L.). J. Appl. Bot. Food Qual. 92, 64–72.

Kućerová, Z., Stejskal, V., 1994. Susceptibility of wheat cultivar to postharvest losses caused by Sitophilus granarias (L.) (Coleoptera: Curculionidae)/Attraktivität von Weizensorten für Sitophilus granarius (Coleoptera: Curculionidae) und die dadurch verursachten Nachernteverluste. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection 641–648.

Lemic, D., Mikac, K.M., Genda, M., Jukić, Ž., Pajač Živković, I., 2020. Durum wheat cultivars express different level of resistance to granary weevil, Sitophilus Granarius (Coleoptera; Curculionidae) infestation. Insects 11 (6), 343.

Lorini, I., Collins, P.J., Daglish, G.J., Nayak, M.K., Pavic, H., 2007. Detection and characterisation of strong resistance to phosphine in Brazilian Rhyzopertha Dominica (F.) (Coleoptera: Bostrychidae). Pest Manag. Sci.: Form Pestic. Sci. 63 (4), 358–364.

Majeed, M.Z., Mehmood, T., Javed, M., Sellami, F., Riaz, M.A., Afzal, M., 2015. Biology and management of stored products' insect pest Rhyzopertha Dominica (Fab.) (Coleoptera: Bostrichidae). Int. J. Biosci. 7 (5), 78–93.

Mathew, G., 1987. Insect borers of commercially important stored timber in state of Kerala, India. J. Stored Prod. Res. 23, 185–190.

McCain, F.S., Eden, W.G., Singh, D.N., 1964. A technique for selecting for rice weevil resistance in corn in the laboratory 1. Crop Sci. 4 (1), 109–110.

McGaughey, W.H., Speirs, R.D., Martin, C.R., 1990. Susceptibility of classes of wheat grown in the United States to stored-grain insects. J. Econ. Entomol. 83 (3), 1122–1127.

Mebarkia, A., Rahbé, Y., Guechi, A., Bouras, A., Makhlouf, M., 2010. Susceptibility of twelve soft wheat varieties (Triticum aestivum) to Sitophilus granarius (L.) (Coleoptera: Curculionidae). Agric. Biol. J. N. Am. 1, 571–578.

Mefleh, M., Conte, P., Fadda, C., Giunta, F., Piga, A., Hassoun, G., Motzo, R., 2019. From ancient to old and modern durum wheat varieties: interaction among cultivar traits, management, and technological quality. J. Sci. Food Agric. 99 (5), 2059–2067.

Morris, C.F., Simeone, M.C., King, G.E., Lafiandra, D., 2011. Transfer of soft kernel texture from Triticum aestivum to durum wheat, Triticum turgidum ssp. durum. Crop Sci. 51 (1), 114–122.

Morris, C.F., Fuerst, E.P., 2015. Quality characteristics of soft kernel durum; a new cereal crop. In: Advances in Wheat Genetics: from Genome to Field: Proceedings of the 12th International Wheat Genetics Symposium. Springer Japan, pp. 275–278.

Mostafalou, S., Abdollahi, M., 2013. Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. Toxicol. Appl. Pharmacol. 268 (2), 157–177.

Nawrot, J., Warchalewski, J.R., Piasecka-Kwiatkowska, D., Niewiada, A., Gawlak, M., Grundas, S.T., Fornal, J., 2006. The effect of some biochemical and technological properties of wheat grain on granary weevil (Sitophilus granarius L.) (Coleoptera: Curculionidae) development. Proc. 9th Int. Work. Conf.Stor.Product Protect. 15, 400–407.

Nawrot, J., Gawlak, M., Szafranek, J., Szafranek, B., Synak, E., Warchalewski, J.R., et al., 2010. The effect of wheat grain composition, cuticular lipids and kernel surface microstructure on feeding, egg-laying, and the development of the granary weevil, Sitophilus granarius (L). J. Stored Prod. Res. 46 (2), 133–141.

Nayak, M.K., Daglish, G.J., Phillips, T.W., Ebert, P.R., 2020. Resistance to the fumigant phosphine and its management in insect pests of stored products: a global perspective. Annu. Rev. Entomol. 65, 333–350.

Neethirajan, S., Karunakaran, C., Jayas, D.S., White, N.D.G., 2007. Detection techniques for stored-product insects in grain. Food Control 18 (2), 157–162.

Niewiada, A., Nawrot, J., Szafranek, J., Szafranek, B., Synak, E., Jeleń, H., Wąsowicz, E., 2005. Some factors affecting egg-laying of the granary weevil (Sitophilus granarius L). J. Stored Prod. Res. 41 (5), 544–555.

Nietupski, M., Ciepielewska, D., Fornal, Ł., 2006. Wpływ Źróżnicowania chemicznego białek w Ziarnie Wybranych Odmian Pszenicy Na Rozwój Szkodników Magazynowych. Prog. Plant Prot./Postępy W Ochr. Roślin 46, 420–423.

Nuovo, P., 2013. Evoluzione delle varietà di grano, della tecnica molitoria e panificatoria. Industrie Grafiche Pacini Editore.

Pasqualone, A., Palombieri, S., Koksel, H., Summo, C., De Vita, P., Sestili, F., 2023. Milling performance and bread-making aptitude of the new soft kernel durum wheat variety Faridur. Int. J. Food Sci. Technol. 58 (1), 268–278.

Perrino, P., 1993. The farro: an ancient crop to renew. Agricoltura 21, 9–15.

Pomeranz, Y., Peterson, C.J., Mattern, P.J., 1985. Grown under Widely different climatic conditions. Cereal Chem. 62 (6), 463–467.

Rajendran, S., 2002. Postharvest pest losses. In: Pimentel, D. (Ed.), Encyclopedia of Pest Management. Marcel Dekker, Inc, New York, pp. 654–656.

Rajendran, S., Sriranjini, V., 2008. Plant products as fumigants for stored-product insect control. J. Stored Prod. Res. 44 (2), 126–135.

Rajendran, S., 2020. Insect pest management in stored products. Outlooks Pest Manag. 31 (1), 24–35.

Rotundo, G., Germinara, G.S., De Cristofaro, A., 2000. Immuno-osmophoretic technique for detecting Sitophilus granarius (L.) infestations in wheat. J. Stored Prod. Res. 36 (2), 153–160.

Rotundo, G., Paventi, G., Barberio, A., De Cristofaro, A., Notardonato, I., Russo, M.V., Germinara, G.S., 2019. Biological activity of Dittrichia viscosa (L.) Greuter extracts against adult Sitophilus granarius (L.) (Coleoptera, Curculionidae) and identification of active compounds. Sci. Rep. 9 (1), 6429. https://doi.org/10.1038/s41598-019-42886-4.

Saad, A.S., Tayeb, E.H.M., El-Shazli, M.M., Baheeg, S.A., 2018. Susceptibility of certain Egyptian and imported wheat cultivars to infestation by Sitophilus oryzae and Rhyzopertha Dominica. Arch. Phytopathol. Plant Protect. 51 (1–2), 14–29.

Sakka, M.K., Jagadeesan, R., Nayak, M.K., Athanassiou, C.G., 2022. Insecticidal effect of heat treatment in commercial flour and rice mills for the control of phosphine-resistant insect pests. J. Stored Prod. Res. 99, 102023.

Shazali, M.E.H., 1987. Weight loss caused by development of Sitophilus oryzae (L.) and Sitotroga cerealella (Oliv.) in sorghum grains of two size classes. J. Stored Prod. Res. 23 (4), 233–238.

Song, X., Wang, P., Zhang, H., 2011. Phosphine resistance in Rhyzopertha Dominica (Fabricius) (Coleoptera: Bostrichidae) from different geographical populations in China. Afr. J. Biotechnol. 10 (72), 16367–16373.

Stagnari, F., Codianni, P., Pisante, 2008. Agronomic and kernel quality of ancient wheats grown in central and southern Italy. Cereal Res. Commun. 36 (2), 313–326.

Stathers, T.E., Arnold, S.E., Rumney, C.J., Hopson, C., 2020. Measuring the nutritional cost of insect infestation of stored maize and cowpea. Food Secur. 12, 285–308.

Suleiman, R., Rosentrater, K.A., Bern, C.J., 2015. Evaluation of maize weevils Sitophilus zeamais Motschulsky infestation on seven varieties of maize. J. Stored Prod. Res. 64, 97–102.

Taylor, R.W.D., 1994. Methyl bromide-Is there any future for this noteworthy fumigant? J. Stored Prod. Res. 30 (4), 253–260.

Trematerra, P., Throne, J., 2012. Insect and Mite Pests of Durum Wheat. Durum Wheat, Chemistry and Technology, second ed. AACC International Inc., St. Paul, MN, USA, pp. 73–83.

Throne, J.E., Baker, J.E., Messina, F.J., Karl, J.K., Howard, J.A., 2000. Varietal resistance. In: Subramanyam, B., Hagstrum, D.W. (Eds.), Alternatives to Pesticides in Stored-Product IPM. Kluwer Academic, Massachusetts, pp. 165–192.

Toews, M.D., Cuperus, G.W., Phillips, T.W., 2000. Susceptibility of eight US wheat cultivars to infestation by Rhyzopertha Dominica (Coleoptera: Bostrichidae). Environ. Entomol. 29 (2), 250–255.

Tudi, M., Daniel Ruan, H., Wang, L., Lyu, J., Sadler, R., Connell, D., et al., 2021. Agriculture development, pesticide application and its impact on the environment. Int. J. Environ. Res. Publ. Health 18 (3), 1112.

Tuet, W.Y., Pierce, S.A., Racine, M.C., Stone, S., Pueblo, E., Dukes, A., et al., 2021. Cardiopulmonary effects of phosphine poisoning: a preliminary evaluation of milrinone. Toxicol. Appl. Pharmacol. 427, 115652.

Turnbull, K.M., Rahman, S., 2002. Endosperm texture in wheat. J. Cereal. Sci. 36 (3), 327–337.

Urrutia, R.I., Yeguerman, C., Jesser, E., Gutierrez, V.S., Volpe, M.A., González, J.O.W., 2021. Sunflower seed hulls waste as a novel source of insecticidal product: Pyrolysis bio-oil bioactivity on insect pests of stored grains and products. J. Clean. Prod. 287, 125000.

Warchalewski, J.R., Gralik, J., Winiecki, Z., Nawrot, J., Piasecka-Kwiatkowska, D., 2002. The effect of wheat α-amylase inhibitors incorporated into wheat-based artificial diets on development of Sitophilus granarius L., Tribolium confusum Duv., and Ephestia kuehniella Zell. J. Appl. Entomol. 126 (4), 161–168.

Željko, J., Matković, A., Liška, A., i Jukić, K., 2020. Resistance of different wheat cultivars to granary weevil (Sitophilus granarius L). Glasnik Zaštite Bilja 43 (5), 34–41.

6. Behavioural responses of *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) to odours of old and modern wheat genotypes

Ilaria D'Isita[1*], Onofrio Marco Pistillo[1], Federica Lo Muzio[1], Sandra Pati[1], Antonella Marta Di Palma[1], Pasquale De Vita[2], Giacinto Salvatore Germinara[1]. *Journal of Stored Products Research, 2024, 109, 102433.*

[1] Department of Agricultural Sciences, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Via Napoli 25, 71122 Foggia, Italy.
[2] CREA Research Centre for Cereal and Industrial Crops, 71122 Foggia, Italy.

*Corresponding author

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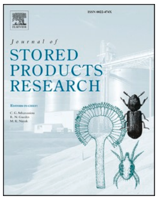

Behavioural responses of *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) to odours of old and modern wheat genotypes

Ilaria D'Isita [a,*], Onofrio Marco Pistillo [a], Federica Lo Muzio [a], Sandra Pati [a], Antonella Marta Di Palma [a], Pasquale De Vita [b], Giacinto Salvatore Germinara [a]

[a] *Department of Agricultural Sciences, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Via Napoli 25, 71122, Foggia, Italy*
[b] *CREA Research Centre for Cereal and Industrial Crops, 71122, Foggia, Italy*

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ABSTRACT

Sitophilus granarius (L.) and *Rhyzopertha dominica* (F.) are among the major stored-product pests worldwide. The identification of low or un-attractive wheat genotypes toward storage pests and their Volatile Organic Compounds (VOCs) profile is useful in breeding programs and in characterization of behaviourally active compounds suitable in developing semiochemical-based control means. Old wheat cultivars are genetic resources useful for breeding purposes. In this study, the VOC profiles of old genotypes, Senatore Cappelli, old Saragolla, Dauno III, and modern wheat varieties, Mec, Ofanto, Svevo, and Faridur, were characterized by headspace - solid phase microextraction (HD-SPME) and gaschromatography coupled with mass spectrometry (GC-MS) and their attractiveness towards *S. granarius* and *R. dominica* adults was evaluated in two-choice pitfall bioassays and Y-tube olfactometer, respectively. *Sitophilus granarius* adults were significantly attracted to odours of all varieties, with females being significantly more attracted by Faridur, Ofanto, Mec, and old Saragolla compared to Svevo. *Rhyzopertha dominica* males and females exhibited a significant olfactory preference for the odours of Faridur, Mec, and old Saragolla. The olfactory preferences of both species could be related to differences emerged in varietal VOC profiles. The most attractive variety for both species, Faridur, was rich in alcohols, alkanes, and terpenes. Old Saragolla, Ofanto, and Mec were rich in alcohols and organic acids. Dauno III was the richest variety in alcohols and lactones, and the poorest in aldehydes and terpenes compared to other varieties. Svevo showed the lowest amounts of alcohols and the highest value of aldehydes; Senatore Cappelli was rich in aldehydes, organic acids, and alkanes. The possible effects of the differences in VOC profiles on the olfactory preferences of insects are discussed. The study offers new knowledge for further investigation testing VOCs of the most and the lowest attractive varieties to identify effective attractants or strong repellents towards *S. granarius* and *R. dominica*.

1. Introduction

Wheat is one of the most important global primary crops essential to humankind with strong consumer's demand and a production of more than 700 thousand tons in 2021 worldwide (Taylor, 1994; Igrejas and Branlard, 2020; FAO, 2023). Insect pests are a serious issue of stored wheat causing postharvest losses of global annual production ranking from around 10% in developed countries to 50% in developing ones, varying according to climatic conditions, storage facilities and pest control measures adopted (Rajendran, 2002; Neethirajan et al., 2007; Fornal et al., 2007; Rajendran, 2020 and references therein; Tadesse, 2020). Stored grains are damaged by different insect pests, including the granary weevil, *Sitophilus granarius* (L.) (Coleoptera, Curculionidae), and the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera, Bostrichidae). These pests are among the major primary pests of stored cereals leading to quantitative and qualitative losses during storage (Trematerra and Throne, 2012; Majeed et al., 2015; Lemic et al., 2020). Control of storage insects is very difficult due to the increasing legislation limits on the use of pesticides, mainly in developed countries, aimed to reduce environmental and health risks (Handford et al., 2015) and the fast resistance development in target species (Lorini et al., 2007; Song et al., 2011; Boyer et al., 2012; Kocak et al., 2015; Afful et al., 2018; Nayak et al., 2020; Sakka et al., 2022). Furthermore, the early detection of insect infestations in stored grain is essential to apply timely and

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* Corresponding author.
E-mail address: ilaria.disita@unifg.it (I. D'Isita).

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effectively pest management strategies. *Sitophilus granarius* females are able to lay eggs within the grain endosperm (Niewiada et al., 2005). Instead, *R. dominica* females deposit eggs on the surface of kernels or among the frass produced by insects, then the first instar moves into the grains (Edde, 2012). In both species, larvae and pupae develop inside kernels causing hidden grain infestation difficult to be detected (Rotundo et al., 2000; Edde, 2012, Cai et al., 2022a). In this contest, the development of sustainable control tool to manage storage pests is urgently needed (Fields and White, 2002; Germinara et al., 2007; Rajendran and Sriranjini, 2008; Germinara et al., 2012; Adhikary et al., 2014, 2015, 2016; Germinara et al., 2015; Rotundo et al., 2000; Chaudhari et al., 2021; Urrutia et al., 2021).

Semiochemicals are low-molecular-weight signaling chemicals that mediate the interactions between organisms of the same or of different species (Law and Regnier, 1971; Nordlund and Lewis, 1976; Dicke and Sabelis, 1988). Plant Volatile Organic Compounds (VOCs) play a pivotal biological role in plant-insect interactions, since they are the first chemicals detected by insects useful to identify host plants for feeding, mating and oviposition and also to avoid unsuitable habitats and hosts (Dickens, 1984; Visser, 1986; Agelopoulos et al., 1999; Reddy and Guerrero, 2004; Bernays and Chapman, 2007; Piesik et al., 2020). In fact, the granary weevil adults perceive and respond behaviourally (Germinara et al., 2002, 2008) to a wide range of cereal VOCs (Maga, 1978; Zhou et al., 1999) and are attracted to the odours emitted by commercial wheat (Levinson and Kanaujia, 1981; Germinara et al., 2007, 2019). In the same way olfactory cues are important for the lesser grain borer adults to discriminate different kinds of food substrates (Edde and Phillips, 2006; Cao et al., 2024).

The development of semiochemical-based control means toward stored-product pests has showed their possible alternatives to synthetic insecticides (Cox, 2004 and references therein; De Cristofaro et al., 2010; Phillips and Throne, 2010; Abd El-Aziz, 2011; Germinara et al., 2007, 2011, 2012, 2015; Cai et al., 2022b; Cao et al., 2023); thus several studies were aimed to identify, from storage products, chemical volatiles attractants or repellents applicable as direct and indirect control means (Germinara et al., 2012, 2015, 2019, 2019b; Cao et al., 2023, 2024). Furthermore, the use of resistant wheat varieties is considered a preventive agronomic control tools according to the Integrated Pest Management (IPM) approach (Throne et al., 2000; Keba and Sori, 2013); accordingly the identification of wheat varieties, rich in repellent compounds able to modify the insect behaviour, could represent an innovative goal for breeding programs (Germinara et al., 2019).

In recent years, landraces and old cultivars have received a great market interest and thus could represent a source of income for farmers, especially in marginal areas (Perrino, 1993; Stagnari et al., 2008; Ficco et al., 2019; Mefleh et al., 2019). Furthermore, in modern agriculture, growing old wheat genotypes rich in their genetic variability could be a way to safeguard these valuable genetic resources for breeding purposes (Mefleh et al., 2019; D'Isita et al., 2024). To the best of our knowledge, little is known about the behavioural responses of *S. granarius* and *R. dominica* adults to odours emitted by the kernels of old wheat genotypes. In order to identify source of bioactive compounds useful to develop semiochemical-based control tools, the VOC profiles and the olfactory response of three old genotypes (Senatore Cappelli, old Saragolla, Dauno III) and four modern wheat varieties (Mec, Ofanto, Svevo, Faridur) towards the primary stored-cereal pests *S. granarius* and *R. dominica* was here evaluated.

2. Materials and methods

2.1. Insects

Adults of *S. granarius* and *R. dominica* were collected in wheat storage facilities of the Apulia region and reared in the dark at 25 ± 2 °C and 28 ± 2 °C, respectively, 60 ± 5% relative humidity (r.h.) for several generations on durum wheat kernels (var. Simeto) in cylindrical glass containers (Ø 15 × 15 cm) covered with a fine mesh net (0.5 mm). For bioassays, the eighth generation of both species was used.

2.2. Plant materials

The wheat kernel genotypes used in this study, Senatore Cappelli, old Saragolla, Dauno III, Mec, Ofanto, Svevo, and Faridur, were provided by the CREA Research Center for Cereal and Industrial Crops, Italy and grown in a single field trial during 2020–21 at Foggia, Italy (41°28′N, 119 15°32′E; 75 m a.s.l.). In particular, the durum wheat kernels were collected from an old landrace Saragolla, two old varieties, released in 1914 (Dauno III) and 1915 (Senatore Cappelli), and three modern varieties, named Ofanto (1990), Svevo (1996), and Faridur (2020). Additionally, the bread wheat variety Mec, released in 1974, was included as a control for its high attractiveness shown in previous studies (Germinara et al., 2019). Faridur derived from an introgression program to transfer the puroindoline genes from hexaploid wheat into durum wheat using Svevo as the recipient variety (Morris et al., 2011; Morris and Fuerst, 2015). Thus, durum wheat varieties Faridur and Svevo shared the same genetic background except for the kernel hardness. The seven genotypes were characterized in a previous study for the level of susceptibility to *S. granarius* and *R. dominica* (D'Isita et al., 2024).

2.3. Two-choice pitfall bioassays

To assess the behavioural response of *S. granarius* adults (3 to 4-week-old) to the kernel odours, two-choice pitfall bioassays were carried out according to previous studies (Phillips et al., 1993; Pike et al., 1994; Germinara et al., 2008).

Briefly, the test arena was a steel container (Ø 30 cm × h 7 cm) covered with filter paper (Whatman No. 1) to facilitate insect movements (Pike et al., 1994) and with two diametrically opposed holes (Ø 3 cm) located 3 cm from the side wall. Glass flasks (500 ml), left empty or containing the stimulus (200 g of uninfested intact kernels of each genotype), were positioned under each hole and the inside surfaces of their necks were coated with mineral oil to prevent captured insects from returning to the arena. Unsexed insects ($n = 20$) deprived of food for at least 4 h were placed under an inverted Petri dish (Ø 3 cm × h 1,2 cm) at the center of the test arena and allowed 30 min to acclimate. Then, insects were released for 3 h and given a choice between control (air) and stimulus. During the bioassay, the arena was covered with a steel lid to prevent insects from escaping. Insects were only used once and, at the end of each experiment, the insects that chose the stimulus, the control or those that did not choose any of them were counted and sexed. Experiments were carried out between 09:00 a.m. and 4:00 p.m. in the dark at 25 ± 2 °C and 60 ± 5% r.h. and 10 replicates for each variety were performed. In each trial, a response index (RI) was calculated by using $RI = [(T - C)/Tot] \times 100$, where T is the number of insects responding to the treatment, C is the number of insects responding to the control, and Tot is the total number of insects released (Phillips et al., 1993). Positive and significant RI values indicate attraction to the stimulus, while negative and significant RIs indicate repellence.

2.4. Y-tube olfactometer bioassays

The behavioural response of *R. dominica* adults (3-week-old) to the kernel odours was assessed in a still-air Y-tube olfactometer with a rectangular section (stem 3.5 cm long, each arm 6 cm long at 75 °C angle, stem and arms 1 cm wide and 0.7 cm high) in Z-PLA pro material (Zortrax S.A., Olsztyn, Poland), properly designed for this purpose and manufactured using a 3D printer (Zortrax S.A., Olsztyn, Poland). Each arm of the Y-tube terminated with an odour chamber (2 cm long, 2 cm wide, 2 cm high), whose floor was, therefore, 1.3 cm deeper than that of the base and arms. This prevented the insects from perceiving the stimuli. During the experiments, the base and vertical walls of the olfactometer were covered with filter paper (Whatman No. 1) and closed at the top with a glass plate (18 × 18 cm). Two-way comparisons were made using uninfested intact kernels (3 g) of each variety as stimulus *versus* control air. Before each experiment, the olfactometer was conditioned for 10 min. After that, individual insects starved for at least 2 h were released at the open end of the Y-tube stem. A choice was recorded when the insect moved 1 cm up an arm of the Y-tube and remained beyond the decision line (marked on both arms) for more than 30 s. The time spent by test insects in each arm was also recorded. After 5 individuals were tested, the filter paper and stimulus were renewed. Treatments and control between arms were switched to avoid position bias. After experiments, the sex of the insects tested was determined by observing their genitalia with a stereomicroscope. For each test stimulus, at least 70 beetles, used only once, were tested. Experiments lasted 10 min and were carried out between 09:00 a.m. and 4:00 p.m. in the dark at 28 ± 2 °C and 60 ± 5% r.h.

2.5. *Volatile organic compounds (VOCs) analysis*

The VOCs emitted by the wheat kernels of different varieties tested in behavioural bioassays were identified and quantified using the headspace solid-phase micro-extraction (HS-SPME) technique (Beleggia et al., 2009; Germinara et al., 2019). Wheat kernels were stored at a low temperature (4 ± 1 °C) in glass containers until use. SPME headspace extracts were obtained by exposure for 24 h of SPME fibers (Supelco Co., Bellefonte, PA, USA), coated with either 50/30 μm of divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS), into the headspace of a 20-mL glass vial containing 3 g of each sample and closed by a cap with a PTFE/Silicon septa (Supelco, Co., Bellefonte, PA). During SPME headspace sampling, the vial was maintained at 30 ± 0.1 °C in a water bath. The fibers were conditioned before use by heating them in the injection port of the gas chromatography (GC) at 270 °C for 30 min, according to the manufacturer's instructions. A fiber cleaning step of 10 min at the conditioning temperature with the split valve opened was performed in the GC injector after every chromatographic run to remove any absorbed residue. Furthermore, before each acquisition, a blank test was made under the same experimental conditions to avoid possible impurities. Each sampling was performed in triplicate. Gas chromatography combined with mass-spectrometry (GC-MS) analyses were performed using an Agilent 7890B series gas chromatograph (Agilent Technologies, Milan, Italy) coupled with an Agilent 5977A mass selective detector (MSD) equipped with an HP-5MS capillary column (30 m × 0.25 mm ID, 0.5 μm film thickness, J&W Scientific Inc., Folsom, CA, USA). The VOCs were thermally desorbed at 250 °C for 4 min in splitless mode with a programmed temperature from 40 °C to 250 °C at 5 °C/min and a final holding time of 10 min. Helium was used as the carrier gas at a constant flow rate of 1.25 mL/min. Spectra were recorded in the electron impact mode (ionization energy, 70 eV) in a range of 15–550 amu at 2.9 scans/s. The VOCs identification was reached by comparison of their mass spectra with those of the data system library (NIST11, $p > 90\%$) and, when necessary, by comparing retention times (R.T.) and mass spectra with those of commercially available standards purchased from Sigma-Aldrich Inc. (Milan, Italy). A mixture of a continuous series of straight-chain hydrocarbons, C5-C40 (Alkane Standard Solution C5-C40, Sigma Aldrich, Milan, Italy), was injected into an HP-5MS column under the same conditions previously described to obtain the Linear Retention Indices (RIs) (Van Den Dool and Kratz, 1963). The relative abundance of each compound was calculated using the integrated peak area data from the GC-MS trace.

2.6. *Statistical analysis*

Data were submitted to Shapiro-Wilk's test, Levene's test, and then to the analysis of variances (ANOVA) followed by Tukey's HSD test for mean comparisons.

In the two-choice pitfall bioassays, the significance of the mean RIs in each treatment was evaluated by the Student's *t*-test for paired comparisons (Phillips et al., 1993). Furthermore, the number of males and females was analyzed by paired sample *t*-test and for each experiment, the sex ratio percentage was calculated. In Y-tube olfactometer bioassays, the significance of differences between the number of *R. dominica* adults choosing the treatment or control arm of the olfactometer were compared using $\chi2$ test. The differences between the time spent by insects in each arm were analyzed by paired sample *t*-test. Statistical analyses were performed with SPSS (Statistical Package for the Social Sciences) v.18 for Windows (SPSS Inc., Chicago, IL).

The unsupervised method of Hierarchical Cluster Analysis (HCA), useful to group various samples based on their similarity without knowledge about the number of clusters to be established (Tufariello et al., 2023), was run with the aim of grouping the varieties tested in dependence of chemical classes identified in the VOCs profile and the mean percentages of VOCs GC peak areas.

3. Results

3.1. *Behavioural response of S. granarius in the two-choice pitfall bioassays*

The mean sex ratio percentage was in a range between 49.1 ± 2.0 for Mec and 54.5 ± 4.0 for Svevo, without significant differences in the number of males and females of insects tested (Tables 1 and 2). The granary weevil adults showed positive and significant ($P < 0.001$; Student's *t*-test) RIs that indicate actual attraction in response to the odours of all genotypes studied (Tables 1 and 2). The RI registered for *S. granarius* males ranged between 50.71 ± 10.1 (Dauno III) and 77.91 ± 8.0 (Faridur), without significant differences ($F = 1.939$; $df = 6$; $P = 0.090$) among varieties tested (Table 1). Instead, for females the RI ranged between 34.02 ± 9.1 (Svevo) and 77.55 ± 5.5 (Faridur). The RI of Svevo (34.0 ± 9.1) was significantly lower compared to those calculated for old Saragolla (68.73 ± 4.1), Mec (71.87 ± 5.8), Ofanto (73.43 ± 8.3) and Faridur (77.55 ± 5.5) (Table 2).

3.2. *Behavioural response of R. dominica in Y-tube olfactometer bioassays*

In Y-tube behavioural bioassays, *R. dominica* males and females presented with intact kernels *vs.* clean-air control exhibited significant preference for Faridur (male: $\chi2 = 7.111$, $df = 1$, $P = 0.008$; female: $\chi2 = 5.121$, $df = 1$, $P = 0.024$), Mec (male: $\chi2 = 3.846$, $df = 1$, $P = 0.050$; female: $\chi2 = 5.233$, $df = 1$, $P = 0.022$), and old Saragolla (male: $\chi2 = 5.121$, $df = 1$, $P = 0.024$; female: $\chi2 = 54.172$, $df = 1$, $P = 0.041$). In addition, for the same varieties, both sexes also spent significantly more time in the treatment arm (Tables 3 and 4). On the other hand, odours from the modern varieties Ofanto and Svevo and the old varieties Senatore Cappelli and Dauno III did not elicit a significant attraction in *R. dominica* adults evaluated either as first choice and time spent in the treatment arm when clean air was the alternative.

3.3. *Volatile organic compounds (VOCs) analysis*

A total of 27 VOCs were identified in the headspace of the varieties studied including: 9 alcohols, 7 aldehydes, 4 alkanes, 2 terpenes, 1 organic acid, 1 benzene derivative, 1 lactone, 1 ketone, and 1 other compound. The percentages of the compounds detected, expressed as relative abundance, are reported in Table 5.

According to the HCA run to group the varieties studied in dependence on chemical classes, two clusters were observed (Fig. 1). The first cluster included Senatore Cappelli and Svevo together with Faridur and Mec, the second one contained Ofanto and old Saragolla plus Dauno III (Fig. 1). The HCA run to cluster varieties according to the mean percentages of VOCs GC peak areas showed two clusters (Fig. 2). The first one consisted of old Saragolla and Ofanto plus Mec and Dauno III and the second one contained Faridur and Svevo together with Senatore

Table 1
Behavioural responses in two-choice pitfall bioassays of *S. granarius* males to odours emitted by kernels (200 g) of modern wheat (Mec, Ofanto, Svevo, Faridur) and old wheat (Dauno III, old Saragolla, Senatore Cappelli) varieties *vs.* control air.

Genotype	Males (Mean ± S.E.)	Kernel odours (Mean ± S.E.)	Control air (Mean ± S.E.)	Student's *t*-test		Response index (Mean ± S.E.)
				t value	*P* value	
Bread wheat						
Mec	9.25 ± 0.4 a	6.86 ± 0.5	0.43 ± 0.2	10.510	<0.001	70.37 ± 7.01 a
Durum wheat						
Ofanto	9.22 ± 0.6 a	7.67 ± 0.6	1.00 ± 0.2	9.177	<0.001	71.43 ± 5.4 a
Svevo	11.10 ± 0.8 a	8.70 ± 0.8	2.30 ± 0.4	6.532	<0.001	57.52 ± 7.01 a
Faridur	10.50 ± 0.5 a	9.10 ± 0.5	1.00 ± 0.4	9.847	<0.001	77.91 ± 8.02 a
Dauno III	10.56 ± 0.6 a	7.56 ± 0.6	2.44 ± 0.5	5.303	0.001	50.71 ± 10.1 a
Old Saragolla	10.20 ± 0.6 a	7.70 ± 0.5	1.70 ± 0.4	11.163	<0.001	61.36 ± 6.8 a
Senatore Cappelli	10.67 ± 0.8 a	7.89 ± 0.6	2.56 ± 0.4	7.761	<0.001	51.35 ± 7.6 a

In the same column, values with different letters are significantly different at $P < 0.05$.

Table 2
Behavioural responses of *S. granarius* females to odours emitted by kernels (200 g) of modern wheat (Mec, Ofanto, Svevo, Faridur) and old wheat (Dauno III, old Saragolla, Senatore Cappelli) varieties, in two-choice pitfall bioassays.

Genotype	Females (Mean ± S.E.)	Kernel odours (Mean ± S.E.)	Control air (Mean ± S.E.)	Student's *t*-test		Response index (Mean ± S.E.)
				t value	*P* value	
Bread wheat						
Mec	9.63 ± 0.5 a	7.14 ± 0.3	0.80 ± 0.2	8.000	<0.001	71.87 ± 5.8 b
Durum wheat						
Ofanto	8.78 ± 0.4 a	7.33 ± 0.4	1.00 ± 0.4	9.216	<0.001	73.43 ± 8.3 b
Svevo	9.30 ± 0.8 a	6.10 ± 0.6	3.20 ± 0.5	3.934	0.003	34.02 ± 9.1 a
Faridur	9.50 ± 0.4 a	8.40 ± 0.5	1.00 ± 0.3	11.045	<0.001	77.55 ± 5.5 b
Dauno III	9.11 ± 0.5 a	7.22 ± 0.6	1.78 ± 0.4	6.519	<0.001	59.69 ± 9.01 ab
Old Saragolla	8.90 ± 0.6 a	7.10 ± 0.5	1.00 ± 0.2	11.597	<0.001	68.73 ± 4.1 b
Senatore Cappelli	9.33 ± 0.7 a	7.44 ± 0.4	1.78 ± 0.4	10.251	<0.001	63.22 ± 7.5 ab

In the same column, values with different letters are significantly different at $P < 0.05$.

Table 3
Response of *R. dominica* males in a Y-tube olfactometer to different odour sources emitted by kernels (3 g) of modern wheat (Mec, Ofanto, Svevo, Faridur) and old wheat (Dauno III, old Saragolla, Senatore Cappelli) varieties.

Genotype	N[a]	First choice (Mean)			Number of minutes spent in arm (mean ± S.E.)			
		Treated[b]	X^2	*P* value	Treated	Control	*t* value	*P* value
Bread wheat								
Mec	26(26)	0.69	3.846	0.050	5.01 ± 0.7	1.77 ± 0.6	2.569	0.017
Durum wheat								
Ofanto	32(29)	0.55	0.310	0.577	2.97 ± 0.6	2.94 ± 0.7	0.027	0.979
Svevo	39(39)	0.62	2.077	0.150	4.06 ± 0.6	2.69 ± 0.6	1.202	0.237
Faridur	36(36)	0.72	7.111	0.008	5.09 ± 0.6	2.22 ± 0.6	2.397	0.022
Dauno III	47(46)	0.50	0	1.000	3.67 ± 0.6	3.28 ± 0.6	0.362	0.719
Old Saragolla	36(33)	0.70	5.121	0.024	3.97 ± 0.6	1.65 ± 0.5	2.350	0.025
Senatore Cappelli	38(37)	0.46	0.243	0.622	3.46 ± 0.1	3.43 ± 0.1	0.028	0.977

[a] Total sample size (*N*, number of individuals that made a choice in parentheses).
[b] Proportion of individuals (of those that made a choice) that chose the treated arm first.

Table 4
Response of *R. dominica* female in a Y-tube olfactometer to different odour sources emitted by kernels (3 g) of modern wheat (Mec, Ofanto, Svevo, Faridur) and old wheat (Dauno III, old Saragolla, Senatore Cappelli) varieties.

Genotype	N[a]	First choice (Mean)			Number of minutes spent in arm (mean ± S.E.)			
		Treated[b]	X^2	*P* value	Treated	Control	*t* value	*P* value
Bread wheat								
Mec	44(43)	0.67	5.233	0.022	4.31 ± 0.5	2.04 ± 0.5	2.348	0.024
Durum wheat								
Ofanto	38(33)	0.58	0.758	0.384	3.56 ± 0.6	2.63 ± 0.6	0.827	0.414
Svevo	31(29)	0.52	0.034	0.853	3.59 ± 0.7	3.29 ± 0.7	0.221	0.827
Faridur	34(33)	0.70	5.121	0.024	4.36 ± 0.6	1.74 ± 0.5	2.461	0.019
Dauno III	23(22)	0.59	0.727	0.394	3.74 ± 0.8	2.32 ± 0.7	1.050	0.305
Old Saragolla	34(29)	0.69	4.172	0.041	4.15 ± 0.6	1.62 ± 0.5	2.249	0.021
Senatore Cappelli	32(29)	0.48	0.034	0.853	2.94 ± 0.6	3.44 ± 0.7	−0.397	0.694

[a] Total sample size (*N*, number of individuals that made a choice in parentheses).
[b] Proportion of individuals (of those that made a choice) that chose the treated arm first.

Table 5
VOCs levels detected in the head-space of modern wheat (Mec, Ofanto, Svevo, Faridur) and old wheat (Dauno III, old Saragolla, Senatore Cappelli) varieties.

Compounds	R.T.	RI Lit[a]	RI Exp[b]	Area (%) (Mean ± S.E.)						
				Mec	Ofanto	Svevo	Faridur	Senatore Cappelli	Old Saragolla	Dauno III
Alcohols										
1-Pentanol	4.79	766	765	0.9 ± 0.1	1.0 ± 0.1	2.8 ± 0.1	2.9 ± 0.2	1.3 ± 0.01	1.7 ± 0.2	1.1 ± 0.1
2,3-Butanediol	5.08	802	802	1.6 ± 0.2	4.3 ± 0.3	2.7 ± 0.4	2.0 ± 0.2	2.5 ± 0.5	9.4 ± 0.5	16.2 ± 2.5
1-Hexanol	7.16	867	867	1.0 ± 0.2	1.7 ± 0.2	2.4 ± 0.3	3.8 ± 1.2	1.3 ± 0.1	1.6 ± 0.2	1.8 ± 0.3
1-Heptanol	10.16	969	943	0.4 ± 0.0	0.5 ± 0.1	0.9 ± 0.02	0.8 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	–
1-Hexanol, 2-ethyl-	11.99	1015	999	10.1 ± 1.9	5.7 ± 0.6	4.1 ± 0.1	4.5 ± 0.1	4.7 ± 0.3	3.9 ± 0.6	3.4 ± 0.2
Benzyl Alcohol	12.20	1036	1005	6.1 ± 0.4	6.8 ± 0.2	1.7 ± 0.2	3.0 ± 0.3	4.4 ± 1.0	3.8 ± 0.5	10.9 ± 1.0
1-Octanol	13.27	1038	1039	1.3 ± 0.1	1.1 ± 0.1	1.5 ± 0.1	1.5 ± 0.0	1.1 ± 0.1	1.2 ± 0.1	0.4 ± 0.0
Phenylethyl Alcohol	14.65	1109	1082	3.3 ± 0.2	3.9 ± 0.2	1.0 ± 0.01	1.7 ± 0.1	1.8 ± 0.5	3.3 ± 0.5	3.1 ± 0.4
1-Nonanol	16.26	1169	1134	1.5 ± 0.1	1.8 ± 0.1	1.3 ± 0.1	2.2 ± 0.01	1.5 ± 0.2	2.3 ± 0.3	1.0 ± 0.01
Total alcohols				26.2 ± 1.8	27.0 ± 0.5	18.4 ± 0.1	22.4 ± 0.2	19.1 ± 1.8	27.7 ± 1.5	37.9 ± 1.7
Aldehydes										
Hexanal	5.47	806	803	3.7 ± 0.9	4.4 ± 0.6	18.5 ± 1.0	8.8 ± 1.0	10.0 ± 0.5	8.2 ± 1.0	1.7 ± 0.3
Heptanal	8.11	894	901	0.8 ± 0.1	0.4 ± 0.04	0.9 ± 0.03	0.6 ± 0.1	0.7 ± 0.02	0.8 ± 0.1	–
Benzaldehyde	9.97	928	937	4.4 ± 0.4	4.3 ± 0.3	1.5 ± 0.1	2.9 ± 0.2	3.5 ± 0.9	1.7 ± 0.4	5.5 ± 0.2
Octanal	11.22	1002	975	0.9 ± 0.1	0.4 ± 0.04	0.8 ± 0.03	0.6 ± 0.02	0.5 ± 0.01	0.5 ± 0.1	–
Nonanal	14.31	1089	1071	3.9 ± 0.6	1.5 ± 0.04	3.5 ± 0.3	2.2 ± 0.4	1.7 ± 0.2	1.9 ± 0.6	1.8 ± 0.5
Decanal	17.27	1207	1168	4.1 ± 0.4	2.2 ± 0.3	3.3 ± 0.2	2.3 ± 0.5	3.4 ± 0.2	4.1 ± 1.0	0.8 ± 0.1
Dodecanal	22.66	1405	1364	1.0 ± 0.1	0.8 ± 0.1	1.1 ± 0.1	0.3 ± 0.01	2.9 ± 0.1	0.8 ± 0.2	0.4 ± 0.0
Total aldehydes				18.7 ± 0.2	14.0 ± 1.1	29.6 ± 0.3	17.7 ± 0.3	22.6 ± 1.6	18.0 ± 0.7	10.2 ± 0.2
Alkanes										
Tridecane	19.82	1300	1256	2.1 ± 0.3	3.3 ± 0.2	2.3 ± 0.1	3.3 ± 0.1	3.5 ± 0.6	1.2 ± 0.1	1.1 ± 0.1
Tetradecane	22.40	1400	1354	2.7 ± 0.3	3.6 ± 0.6	14.7 ± 1.2	18.4 ± 2.2	11.3 ± 0.8	2.4 ± 0.01	2.9 ± 0.2
Dodecane	17.07	1200	1161	1.2 ± 0.2	0.8 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	5.1 ± 0.9	0.5 ± 0.04	1.5 ± 0.1
Octadecane	31.40	1794	1745	1.4 ± 0.1	0.9 ± 0.2	–	0.1 ± 0.01	0.4 ± 0.1	1.5 ± 0.8	–
Total alkanes				7.4 ± 0.5	8.6 ± 1.0	18.3 ± 0.5	23.1 ± 1.1	20.4 ± 2.3	5.6 ± 0.9	5.5 ± 0.1
Terpenes										
Limonene	12.06	1030	1001	3.6 ± 0.5	0.8 ± 0.05	2.5 ± 0.2	8.2 ± 0.5	1.8 ± 0.2	1.5 ± 0.2	0.6 ± 0.01
Isobornyl acetate	19.65	1280	1250	0.6 ± 0.1	0.4 ± 0.1	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	0.3 ± 0.02	0.3 ± 0.01
Total terpenes				4.3 ± 0.5	1.2 ± 0.1	2.6 ± 0.1	8.4 ± 0.5	1.9 ± 0.2	1.8 ± 0.2	0.9 ± 0.01
Organic acids										
Acetic Acid	2.89	633	623	19.4 ± 4.1	32.5 ± 2.7	22.4 ± 2.5	17.3 ± 3.5	25.3 ± 2.4	33.9 ± 1.4	21.9 ± 2.0
Total organic acids				19.4 ± 4.1	32.5 ± 2.7	22.4 ± 2.5	17.3 ± 3.5	25.3 ± 2.4	33.9 ± 1.4	21.9 ± 2.0
Benzene derivatives										
p-Xylene	7.27	888	881	11.8 ± 1.9	1.2 ± 0.2	1.3 ± 0.1	2.0 ± 0.5	1.8 ± 0.2	2.1 ± 0.3	1.4 ± 0.1
Total benzene derivatives				11.8 ± 1.9	1.2 ± 0.2	1.3 ± 0.1	2.0 ± 0.5	1.8 ± 0.2	2.1 ± 0.3	1.4 ± 0.1
Lactones										
Butyrolactone	8.52	915	917	3.1 ± 0.8	8.2 ± 0.7	4.3 ± 0.2	5.0 ± 0.1	4.2 ± 0.1	5.5 ± 1.0	17.4 ± 0.5
Total lactones				3.1 ± 0.8	8.2 ± 0.7	4.3 ± 0.2	5.0 ± 0.1	4.2 ± 0.1	5.5 ± 1.0	17.4 ± 0.5
Ketones										
5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	23.77	1421	1408	1.3 ± 0.2	0.9 ± 0.1	0.7 ± 0.1	1.5 ± 0.1	1.2 ± 0.03	1.0 ± 0.05	1.4 ± 0.1
Total ketones				1.3 ± 0.2	0.9 ± 0.1	0.7 ± 0.1	1.5 ± 0.1	1.2 ± 0.03	1.0 ± 0.05	1.4 0.1
Others										
Phenol	10.49	995	953	7.9 ± 0.8	6.4 ± 0.3	2.4 ± 0.5	2.6 ± 0.3	3.5 ± 1.1	4.2 ± 0.5	3.5 ± 0.1
Total others				7.9 ± 0.8	6.4 ± 0.3	2.4 ± 0.5	2.6 ± 0.3	3.5 ± 1.1	4.2 ± 0.5	3.5 ± 0.1

[a] RI_{Lit} = linear retention index from the literature.

[b] RI_{Exp} = determined linear retention index against mixture of n-alkanes (C5–C40) on HP-5MS column.

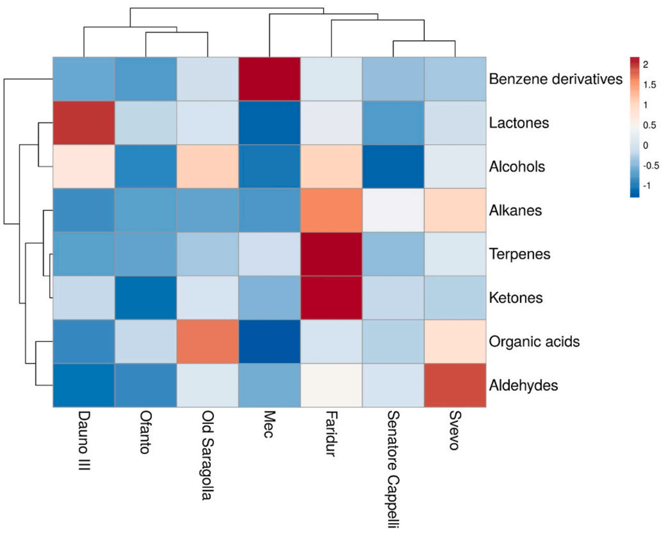

Fig. 1. Dendrogram of Hierarchical Cluster Analysis (HCA) run to group the varieties tested in dependence of chemical classes identified in the VOCs profile.

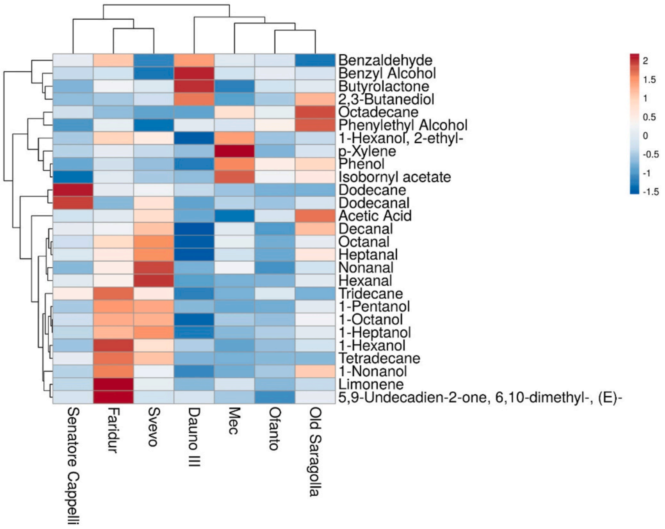

Fig. 2. Dendrogram of Hierarchical Cluster Analysis (HCA) run to group the varieties tested in dependence of the mean percentages of VOCs GC peak areas.

Cappelli (Fig. 2). In particular, in Dauno III VOCs profile the highest values of alcohols (37.9 ± 1.7) and lactones (17.4 ± 0.5), and the lowest values of aldehydes (10.2 ± 0.2) and terpenes (0.9 ± 0), compared to the other varieties, were recorded. The most abundant alcohols were 2,3-butanediol (16.2 ± 2.5) and benzyl alcohol (10.9 ± 1.0), whilst, lactones were represented only by butyrolactone (17.4 ± 0.5).

The most represented chemical classes, in Faridur VOCs profile, were alkanes (23.1 ± 1.1) and alcohols (22.4 ± 0.2). Besides, compared to the other varieties studied, Faridur showed the highest presence of alkanes and terpenes (8.4 ± 0.5) (Table 5). The most abundant alcohols were 1-hexanol, 2-ethyl- (4.5 ± 0.1), 1-hexanol (3.8 ± 1.2), benzyl alcohol (3.0 ± 0.3), and 1-pentanol (2.9 ± 0.2), while tetradecane (18.4 ± 2.2) and limonene (8.2 ± 0.5) were the most abundant alkane and terpene, respectively. By contrast, in Svevo VOCs profile the lowest amount of alcohols (18.4 ± 0.1) and the highest value of aldehydes (29.6 ± 0.3), mostly represented by hexanal (18.5 ± 1.0), were recorded (Table 5). Similarly, aldehydes (22.6 ± 1.6) were among the most represented chemical classes in Senatore Cappelli VOCs profile along with organic acid (25 ± 2), and alkanes (20.4 ± 2.3). Also in Senatore Cappelli VOCs profile, hexanal (10.0 ± 0.5) was the most abundant aldehyde, while alkanes were mostly represented by tetradecane (11.3 ± 0.8).

In old Saragolla and Ofanto, the most represented chemical classes were organic acids (old Saragolla: 33.9 ± 1.4; Ofanto: 32.5 ± 2.7), and alcohols (old Saragolla: 27.7 ± 1.5; Ofanto: 27.0 ± 0.5), mostly represented by 2,3-butanediol (9.4 ± 0.5) and benzyl alcohol (6.8 ± 0.2), respectively. Alcohols (26.2 ± 1.8), in particular 1-hexanol, 2-ethyl- (10.1 ± 1.9), and organic acids (19.4 ± 4.1) were the most represented chemical classes also in Mec VOCs profile. Moreover, in this variety the highest value of benzene derivates, only represented by *p*-Xylene (11.8 ± 1.9), was recorded compared to the other varieties.

4. Discussion

Control of stored-product insect pests for many years was mostly based on the use of synthetic insecticides, in particular phosphine and methyl bromide (Taylor, 1994; Hagstrum and Phillips, 2017). However, the repeated use of chemicals has resulted in the "3 R" problems of Resurgence, Resistance, and Residual effects with negative consequences on human health, environment and no target organism (Parkash et al., 2023). The present study aimed to identify potential source of bioactive compounds and un-attractive genotypes to be used in future breeding programs.

The granary weevil adults showed positive and significant RIs indicating an effective attraction in response to odours of all varieties tested. However, among some of them, significant differences were recorded. For both sexes of *S. granarius*, the Faridur modern variety showed the higher RI. Analysis of variance did not highlight significant differences in males. Whilst, in females the RIs to Faridur, Ofanto, Mec, and old Saragolla were significantly higher than that to Svevo variety. These observations are in accordance with results from a previous study that investigated the antennal sensitivity of *S. granarius* males and females to a wide range of cereal volatiles by electroantennography (Germinara et al., 2002). In that study, the cluster analysis found a greater number of EAG response groups in females than in males. The greater ability of females to discern odours of different food substrates could be associated with a higher need to carefully select sites not only for feeding but also for mating and oviposition (Germinara et al., 2002).

In the case of *R. dominica*, both sexes exhibited a significant preference for Faridur, Mec, and old Saragolla kernels. On the other hand, odours from the modern varieties Ofanto and Svevo and the old varieties Senatore Cappelli and Dauno III did not elicit a significant attraction in *R. dominica* beetles. It is notable that in the no-choice susceptibility tests, Faridur variety, characterized by a lower kernel hardness, showed a greater susceptibility to both *S. granarius* and *R. dominica* compared to all the varieties tested in this study (D'Isita et al., 2024).

Most of the VOCs identified in the present study were already found by other authors (Maga, 1978; Zhou et al., 1999; Mattiolo et al., 2017; Germinara et al., 2019; De Flaviis et al., 2021, 2022, 2023; Buśko et al., 2010). Among the seven genotypes, some differences in the VOCs profile regarding the relative abundance of chemical classes and compounds were recorded; however, according to the HCA analysis, it was not possible to discriminate the old and modern varieties studied by volatile profile, contrary to a previous study (De Flaviis et al., 2023). Faridur, the most susceptible and attractive variety for both species, was rich in alcohols, alkanes and terpenes. In old Saragolla, Ofanto and Mec the most represented chemical classes were alcohols and organic acids. Dauno III was the richest variety in alcohols and lactones, and the poorest in aldehydes and terpenes content compared to other varieties. Whilst, Svevo showed the lowest amount of alcohols and the highest value of aldehydes, Senatore Cappelli VOCs were rich in aldehydes but also in organic acids and alkanes.

In the present study, the olfactory preferences recorded among varieties for both species could be associated with differences in types and concentrations of volatile components and may be affected by synergistic effects of several compounds (Bruce et al., 2005; Najar-Rodriguez et al., 2010; Webster et al., 2010; Bruce and Pickett, 2011; Cha et al., 2011). Different cereals VOCs, particularly some alcohols and short-chain aliphatic aldehydes, are detected by antennal sensilla of *S. granarius* and elicit significant behavioural responses (Maga 1978; Zhou et al., 1999; Germinara et al., 2002, 2008, 2019; Piesik and Wenda-Piesik, 2015; Trematerra et al., 2021; Cao et al., 2023). Among alcohols, 1-hexanol acted as a repellent, instead, 1-pentanol showed a dual activity as attractive at lower concentrations and repellent at higher ones (Germinara et al., 2008). 1-octanol was reported as an egg-laying stimulant for *S. granarius* females (Niewiada et al., 2005). Furthermore, 2-ethyl-hexanol is considered a potential attractant for another important storage insect species, *Callosobruchus maculatus* (F.) (Coleoptera, Curculionidae) (Ajayi et al., 2015). By contrast, aldehydes could disrupt the granary weevil orientation to kernels of attractive commercial wheat varieties (Germinara et al., 2015) and could also inhibit the granary weevil orientation to pigmented wheat genotypes (Germinara et al., 2019). In particular, hexanal and heptanal showed a strong repellent effect against adult *S. granarius* adults (Germinara et al., 2008). As a consequence, the host finding by S. *granarius* could be considered a complex process based on the balance of positive and negative odour stimuli (Germinara et al., 2008). Moreover, Piesik and Wenda-Piesik (2015) showed that *S. granarius* adults were not attracted by a blend of aliphatic aldehydes emitted by cereal grains. In this regard, the higher content of aliphatic aldehydes in the VOCs profile of the Svevo variety could at least partly explain their lower attractiveness towards granary weevil adults.

The grain hardness reflects the degree of adhesion between the starch granules and the protein matrix (Swan et al., 2006). It is notable that Faridur, the most attractive variety to granary weevil, and Svevo, the lowest attractive one, genetically differ only for puroindoline (*Pina* and P*inb*) genes regulating kernel hardness (Pasqualone et al., 2023). In this study, the two genotypes differed significantly for some classes of compounds. This strongly suggests that the cohesive forces between molecules of the matrix influence the production of VOCs.

During the host finding process, also *R. dominica* uses olfactory cues to discriminate between volatiles from suitable and unsuitable plants, with a strong olfactory preference for wheat substrates which provided the maximum reproductive success (Potter, 1935; Bashir, 2002; Edde and Phillips, 2006; Cao et al., 2024). However, to the best of our knowledge, little is known about the role of chemical classes or specific VOCs on the orientation of Bostrichidae, and in particular of *R. dominica* to host-plant.

In conclusion, this study is the first attempt to evaluate attractiveness of old wheat varieties towards *S. granarius* and *R. dominica*; moreover, it provides new knowledge useful for further investigation testing, in behavioral bioassays, the VOCs of the most and the lowest attractive varieties. In fact, defining the biological activity of these VOCs could offer new insights into the mechanism of host finding by both pests. From a practical perspective, this information would be useful to identify bioactive compounds valuable in the development of effective attractants for semiochemical-based control means to enhance the performance of aggregation pheromone-based lures (Chambers, 1990; Dowdy et al., 1993; Dissanayaka et al., 2020; Morrison III et al., 2023) or strong repellents deterring host finding by pests (Germinara et al., 2008, 2012). Furthermore, clarifying the possible role of VOCs in the mechanisms involved in semiochemical interactions between host-plant and postharvest insect pests, is crucial for new breeding programs aiming to develop new varieties producing high levels of repellents that increase the storability of wheat grains.

CRediT authorship contribution statement

Ilaria D'Isita: Writing – original draft, Investigation, Formal analysis, Conceptualization. **Onofrio Marco Pistillo:** Investigation, Formal analysis. **Federica Lo Muzio:** Investigation, Formal analysis. **Sandra Pati:** Writing – review & editing, Formal analysis. **Antonella Marta Di Palma:** Writing – review & editing. **Pasquale De Vita:** Writing – review & editing, Resources, Investigation, Formal analysis, Conceptualization. **Giacinto Salvatore Germinara:** Writing – review & editing, Supervision, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Ilaria D'Isita reports financial support was provided by Italian Ministry of University and Research (Ph.D. scholarship - code number DOT13YISJ8). Giacinto Salvatore Germinara reports financial support was provided by Italian Ministry of University and Research (PRIN project 2022 Prot. 202282ZTPL). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

Abd El-Aziz, S.E., 2011. Control strategies of stored product pests. J. Entomol. 8 (2), 101–122.

Adhikary, P., Mukherjee, A., Barik, A., 2014. Role of surface wax alkanes from *Lathyrus sativus* L. seeds for attraction of *Callosobruchus maculatus* (F.) (Coleoptera: bruchidae). J. Stored Prod. Res. 59, 113–119. https://doi.org/10.1016/j.jspr.2014.06.005.

Adhikary, P., Mukherjee, A., Barik, A., 2015. Attraction of *Callosobruchus maculatus* (F.) (Coleoptera: bruchidae) to four varieties of *Lathyrus sativus* L. seed volatiles. Bull. Entomol. Res. 105 (2), 187–201. https://doi:10.1017/S000748531400087X.

Adhikary, P., Mukherjee, A., Barik, A., 2016. Free fatty acids from Lathyrus sativus seed coats acting as short-range attractants to *Callosobruchus maculatus* (F.) (Coleoptera: bruchidae). J. Stored Prod. Res. 67, 56–62. https://doi.org/10.1016/j.jspr.2016.01.005.

Afful, E., Elliott, B., Nayak, M.K., Phillips, T.W., 2018. Phosphine resistance in North American field populations of the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae). J. Econ. Entomol. 111 (1), 463–469. https://doi.org/10.1093/jee/tox284.

Agelopoulos, N., Birkett, M.A., Hick, A.J., Hooper, A.M., Pickett, J.A., Pow, E.M., Woodcock, C.M., 1999. Exploiting semiochemicals in insect control. J. Pestic. Sci. 55 (3), 225–235. https://doi.org/10.1002/(SICI)1096-9063(199903)55:3<225::AID-PS887>3.0.CO;2-7.

Ajayi, O.E., Balusu, R., Morawo, T.O., Zebelo, S., Fadamiro, H., 2015. Semiochemical modulation of host preference of *Callosobruchus maculatus* on legume seeds. J. Stored Prod. Res. 63, 31–37. https://doi.org/10.1016/j.jspr.2015.05.003.

Bashir, T., 2002. Reproduction of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) on different host-grains. Pakistan J. Biol. Sci. 5, 91–93.

Beleggia, R., Platani, C., Spano, G., Monteleone, M., Cattivelli, L., 2009. Metabolic profiling and analysis of volatile composition of durum wheat semolina and pasta. J. Cereal. Sci. 49 (2), 301–309. https://doi.org/10.1016/j.jcs.2008.12.002.

Bernays, E.A., Chapman, R.F., 2007. Host-plant Selection by Phytophagous Insects, 2. Springer, Science & Business Media, New York, pp. 14–54.

Boyer, S., Zhang, H., Lempérière, G., 2012. A review of control methods and resistance mechanisms in stored-product insects. Bull. Entomol. Res. 102 (2), 213–229. https://doi.org/10.1017/S0007485311000654.

Bruce, T.J., Pickett, J.A., 2011. Perception of plant volatile blends by herbivorous insects-finding the right mix. Phytochemistry 72, 1605–1611. https://doi.org/10.1016/j.phytochem.2011.04.011.

Bruce, T.J., Wadhams, L.J., Woodcock, C.M., 2005. Insect host location: a volatile situation. Trends Plant Sci. 10, 269–274. https://doi.org/10.1016/j.tplants.2005.04.003.

Buśko, M., Jeleń, H., Góral, T., Chmielewski, J., Stuper, K., Szwajkowska-Michałek, L., et al., 2010. Volatile metabolites in various cereal grains. Food Addit. Contam. 27 (11), 1574–1581. https://doi.org/10.1080/19440049.2010.506600.

Cai, L., Macfadyen, S., Hua, B., Xu, W., Ren, Y., 2022b. The correlation between volatile compounds emitted from *Sitophilus granarius* (L.) and its electrophysiological and behavioral responses. Insects 13 (5), 478. https://doi.org/10.3390/insects13050478.

Cai, L., Macfadyen, S., Hua, B., Zhang, H., Xu, W., Ren, Y., 2022a. Identification of biomarker volatile organic compounds released by three stored-grain insect pests in wheat. Molecules 27 (6), 1963. https://doi.org/10.3390/molecules27061963.

Cao, Y., Hu, Q., Huang, L., Athanassiou, C.G., Maggi, F., D'Isita, I., et al., 2023. Attraction of *Sitophilus oryzae* (L.)(Coleoptera: Curculionidae) to the semiochemical volatiles of stored rice materials. J. Pest. Sci. 1–13. https://doi.org/10.1021/acs.jafc.3c04530.

Cao, Y., Jian, L., Athanassiou, C.G., Yang, Y., Hu, Q., Zhang, X., et al., 2024. Behavioral responses of *Rhyzopertha dominica* (F.) to volatiles of different stored grains. J. Stored Prod. Res. 105, 102235. https://doi.org/10.1016/j.jspr.2023.102235.

Cha, D.H., Linn Jr, C.E., Teal, P.E., Zhang, A., Roelofs, W.L., Loeb, G.M., 2011. Eavesdropping on plant volatiles by a specialist moth: significance of ratio and concentration. PLoS One 6 (2), e17033. https://doi.org/10.1371/journal.pone.0017033.

Chambers, J., 1990. Overview on stored-product insect pheromones and food attractants. J. Kans. Entomol. Soc. 490–499.

Chaudhari, A.K., Singh, V.K., Kedia, A., Das, S., Dubey, N.K., 2021. Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: prospects and retrospects. Environ. Sci. Pollut. Control Ser. 28, 18918–18940. https://doi.org/10.1007/s11356-021-12841-w.

Cox, P.D., 2004. Potential for using semiochemicals to protect stored products from insect infestation. J. Stored Prod. Res. 40 (1), 1–25. https://doi.org/10.1016/S0022-474X(02)00078-4.

De Cristofaro, A., Anfora, G., Germinara, G.S., Ioriatti, C., Mazzoni, V., Rotundo, G., 2010. Cell responding to pheromone components and plant volatiles could affect semiochemical based control strategies of insect pests in agricultural ecosystems. IOBC-WPRS Bull. 54, 410.

De Flaviis, R., Mutarutwa, D., Sacchetti, G., Mastrocola, D., 2022. Could environmental effect overcome genetic?. A chemometric study on wheat volatiles fingerprint. Food Chem. 372, 131236. https://doi.org/10.1016/j.foodchem.2021.131236.

De Flaviis, R., Sacchetti, G., Mastrocola, D., 2021. Wheat classification according to its origin by an implemented volatile organic compounds analysis. Food Chem. 341, 128217. https://doi.org/10.1016/j.foodchem.2020.128217.

De Flaviis, R., Santarelli, V., Sacchetti, G., Mastrocola, D., 2023. Heritage and modern wheat varieties discrimination by volatiles profiling. Is it a matter of flavor? Food Chem. 401, 134142. https://doi.org/10.1016/j.foodchem.2022.134142.

Dicke, M., Sabelis, M.W., 1988. Infochemical terminology: based on cost-benefit analysis rather than origin of compounds? Funct. Ecol. 131–139. https://doi.org/10.2307/2389687.

Dickens, J.C., 1984. Olfaction in the boll weevil, *Anthonomus grandis* Boh.(Coleoptera: Curculionidae): electroantennogram studies. J. Chem. Ecol. 10 (12), 1759–1785. https://doi.org/10.1007/BF00987360.

D'Isita, I., Pistillo, O.M., Di Palma, A.M., De Vita, P., Germinara, G.S., 2024. Susceptibility of old and modern wheat genotypes to *Sitophilus granarius* (L.) and *Rhyzoperta dominica* (F.). J. Stored Prod. Res. 106, 102265. https://doi.org/10.1016/j.jspr.2024.102265.

Dissanayaka, D.M.S.K., Sammani, A.M.P., Wijayaratne, L.K.W., Rajapakse, R.H.S., Hettiarachchi, S., Morrison, I.I.I.W.R., 2020. Effects of aggregation pheromone concentration and distance on the trapping of *Rhyzopertha dominica* (F.)(Coleoptera: bostrychidae) adults. J. Stored Prod. Res. 88, 101657. https://doi.org/10.1016/j.jspr.2020.101657.

Dowdy, A.K., Howard, R.W., Seitz, L.M., McGaughey, W.H., 1993. Response of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) to its aggregation pheromone and wheat volatiles. Environ. Entomol. 22 (5), 965–970. https://doi.org/10.1093/ee/22.5.965.

Edde, P.A., Phillips, T.W., 2006. Potential host affinities for the lesser grain borer, *Rhyzopertha dominica*: behavioral responses to host odors and pheromones and reproductive ability on non-grain hosts. Entomol. Exp. Appl. 119 (3), 255–263. https://doi.org/10.1111/j.1570-7458.2006.00417.x.

Edde, P.A., 2012. A review of the biology and control of *Rhyzopertha dominica* (F.) the lesser grain borer. J. Stored Prod. Res. 48, 1–18. https://doi.org/10.1016/j.jspr.2011.08.007.

FAO, 2023. World Food and Agriculture - Statistical Yearbook 2023. https://doi.org/10.4060/cc8166en. Rome.

Ficco, D.B.M., Prandi, B., Amaretti, A., Anfelli, I., Leonardi, A., Raimondi, S., et al., 2019. Comparison of gluten peptides and potential prebiotic carbohydrates in old and modern Triticum turgidum ssp. genotypes. Food Research International 120, 568–576. https://doi.org/10.1016/j.foodres.2018.11.007.

Fields, P.G., White, N.D., 2002. Alternatives to methyl bromide treatments for stored-product and quarantine insects. Annu. Rev. Entomol. 47 (1), 331–359. https://doi.org/10.1146/annurev.ento.47.091201.145217.

Fornal, J., Jeliński, T., Sadowska, J., Grundas, S., Nawrot, J., Niewiada, A., et al., 2007. Detection of granary weevil *Sitophilus granarius* (L.) eggs and internal stages in wheat grain using soft X-ray and image analysis. J. Stored Prod. Res. 43 (2), 142–148. https://doi.org/10.1016/j.jspr.2006.02.003.

Germinara, G.S., De Cristofaro, A., Rotundo, G., 2012. Bioactivity of short-chain aliphatic ketones against adults of the granary weevil, *Sitophilus granarius* (L.). Pest Manag. Sci. 68, 371–377. https://doi.org/10.1002/ps.2272.

Germinara, G.S., De Cristofaro, A., Rotundo, G., 2015. Repellents effectively disrupt the olfactory orientation of *Sitophilus granarius* to wheat kernels. J. Pest. Sci. 88 (4), 675–684. https://doi.org/10.1007/s10340-015-0674-y.

Germinara, G.S., Beleggia, R., Fragasso, M., Pistillo, M.O., De Vita, P., 2019. Kernel volatiles of some pigmented wheats do not elicit a preferential orientation in *Sitophilus granarius* adults. J. Pest. Sci. 92 (2), 653–664. https://doi.org/10.1007/s10340-018-1035-4.

Germinara, G.S., De Cristofaro, A., Rotundo, G., 2008. Behavioral responses of adult *Sitophilus granarius* to individual cereal volatiles. J. Chem. Ecol. 34, 523–529. https://doi.org/10.1007/s10886-008-9454-y.

Germinara, G.S., Rotundo, G., De Cristofaro, A., Giacometti, R., 2002. Risposte elettroantennografiche di *Sitophilus granarius* (L.) e *S. zeamais* Motschulsky a sostanze volatili dei cereali. Tec. Molit. 53, 27–34.

Germinara, G.S., Rotundo, G., De Cristofaro, A., 2007. Repellence and fumigant toxicity of propionic acid against adults of *Sitophilus granarius* (L.) and *S. oryzae* (L.). J. Stored Prod. Res. 43 (3), 229–233. https://doi.org/10.1016/j.jspr.2006.06.002.

Hagstrum, D.W., Phillips, T.W., 2017. Evolution of stored-product entomology: protecting the world food supply. Annu. Rev. Entomol. 62, 379–397. https://doi.org/10.1146/annurev-ento-031616-035146.

Handford, C.E., Elliott, C.T., Campbell, K., 2015. A review of the global pesticide legislation and the scale of challenge in reaching the global harmonization of food safety standards. Integrated Environ. Assess. Manag. 11 (4), 525–536. https://doi.org/10.1002/ieam.1635.

Igrejas, G., Branlard, G., 2020. The Importance of Wheat. Wheat Quality for Improving Processing and Human Health, pp. 1–7. https://doi.org/10.1007/978-3-030-34163-3_1.

Keba, T., Sori, W., 2013. Differential resistance of maize varieties to maize weevil (*Sitophilus zeamais* Motschulsky) (Coleoptera: Curculionidae) under laboratory conditions. J. Entomol. 10, 1–12. https://doi.org/10.3923/je.2013.1.12.

Kocak, E., Schlipalius, D., Kaur, R., Tuck, A., Ebert, P., Collins, P., Yilmaz, A., 2015. Determining phosphine resistance in rust red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: tenebrionidae) populations from Turkey. Turkish J. Entomol. 39, 129–136. https://doi.org/10.16970/ted.17464.

Law, J.H., Regnier, F.E., 1971. Pheromones. Annu. Rev. Biochem. 40, 533–548.

Lemic, D., Mikac, K.M., Genda, M., Jukić, Ž., Pajač Živković, I., 2020. Durum wheat cultivars express different level of resistance to granary weevil, *Sitophilus Granarius* (Coleoptera; Curculionidae) infestation. Insects 11 (6), 343. https://doi.org/10.3390/insects11060343.

Levinson, H.Z., Kanaujia, K.R., 1981. Phagostimulatory responses of male and female Sitophilus granarius L. to newly harvested and stored wheat grains. https://doi.org/10.1007/BF01047166.

Lorini, I., Collins, P.J., Daglish, G.J., Nayak, M.K., Pavic, H., 2007. Detection and characterisation of strong resistance to phosphine in Brazilian *Rhyzopertha dominica* (F.) (Coleoptera: bostrychidae). Pest Manag. Sci.: formerly Pesticide Science 63 (4), 358–364. https://doi.org/10.1002/ps.1344.

Maga, J.A., 1978. Cereal volatiles, a review. J. Agric. Food Chem. 26 (1), 175–178. https://doi.org/10.1021/jf60215a055.

Majeed, M.Z., Mehmood, T., Javed, M., Sellami, F., Riaz, M.A., Afzal, M., 2015. Biology and management of stored products' insect pest *Rhyzopertha dominica* (Fab.) (Coleoptera: Bostrichidae). Int. J. Biosci. 7 (5), 78–93. https://doi.org/10.12692/ijb/7.5.78-93.

Mattiolo, E., Licciardello, F., Lombardo, G.M., Muratore, G., Anastasi, U., 2017. Volatile profiling of durum wheat kernels by HS–SPME/GC–MS. European Food Research and Technology 243, 147–155. https://doi.org/10.1007/s00217-016-2731-z.

Mefleh, M., Conte, P., Fadda, C., Giunta, F., Piga, A., Hassoun, G., Motzo, R., 2019. From ancient to old and modern durum wheat varieties: interaction among cultivar traits, management, and technological quality. J. Sci. Food Agric. 99 (5), 2059–2067. https://doi.org/10.1002/jsfa.9388.

Morris, C.F., Fuerst, E.P., 2015. Quality characteristics of soft kernel durum; a new cereal crop. In: Advances in Wheat Genetics: from Genome to Field: Proceedings of the 12th International Wheat Genetics Symposium. Springer, Japan, pp. 275–278.

Morris, C.F., Simeone, M.C., King, G.E., Lafiandra, D., 2011. Transfer of soft kernel texture from *Triticum aestivum* to durum wheat, *Triticum turgidum* ssp. durum. Crop Sci. 51 (1), 114–122. https://doi.org/10.2135/cropsci2010.05.0306.

Morrison III, W.R., Agrafioti, P., Domingue, M.J., Scheff, D.S., Lampiri, E., Gourgouta, M., et al., 2023. Comparison of different traps and attractants in 3 food processing facilities in Greece on the capture of stored product insects. J. Econ. Entomol. 116 (4), 1432–1446. https://doi.org/10.1093/jee/toad107.

Najar-Rodriguez, A.J., Galizia, C.G., Stierle, J., Dorn, S., 2010. Behavioral and neurophysiological responses of an insect to changing ratios of constituents in host plant-derived volatile mixtures. J. Exp. Biol. 213 (19), 3388–3397. https://doi.org/10.1242/jeb.046284.

Nayak, M.K., Daglish, G.J., Phillips, T.W., Ebert, P.R., 2020. Resistance to the fumigant phosphine and its management in insect pests of stored products: a global perspective. Annu. Rev. Entomol. 65, 333–350. https://doi.org/10.1146/annurev-ento-011019-025047.

Neethirajan, S., Karunakaran, C., Jayas, D.S., White, N.D.G., 2007. Detection techniques for stored-product insects in grain. Food Control 18 (2), 157–162. https://doi.org/10.1016/j.foodcont.2005.09.008.

Niewiada, A., Nawrot, J., Szafranek, J., Szafranek, B., Synak, E., Jeleń, H., Wąsowicz, E., 2005. Some factors affecting egg-laying of the granary weevil (*Sitophilus granarius*

L.). J. Stored Prod. Res. 41 (5), 544–555. https://doi.org/10.1016/j.jspr.2004.11.001.

Nordlund, D.A., Lewis, W.J., 1976. Terminology of chemical releasing stimuli in intraspecific and interspecific interactions. J. Chem. Ecol. 2, 211–220. https://doi.org/10.1007/BF00987744.

Parkash, J., Rajat, A.K., Jeevan, B.G., 2023. Integrated pest management: a comprehensive overview. Adv. Entomol. 3.

Pasqualone, A., Palombieri, S., Koksel, H., Summo, C., De Vita, P., Sestili, F., 2023. Milling performance and bread-making aptitude of the new soft kernel durum wheat variety Faridur. Int. J. Food Sci. Technol. 58 (1), 268–278. https://doi.org/10.1111/ijfs.16200.

Perrino, P., 1993. The farro: an ancient crop to renew. Agricoltura 21, 9–15.

Phillips, T.W., Throne, J.E., 2010. Biorational approaches to managing stored-product insects. Annu. Rev. Entomol. 55, 375–397. https://doi.org/10.1146/annurev.ento.54.110807.090451.

Phillips, T.W., Jiang, X.L., Burkholder, W.E., Phillips, J.K., Tran, H.Q., 1993. Behavioral responses to food volatiles by two species of stored-product Coleoptera, *Sitophilus oryzae* (Curculionidae) and *Tribolium castaneum* (Tenebrionidae). J. Chem. Ecol. 19, 723–734. https://doi.org/10.1007/BF00985004.

Piesik, D., Wenda-Piesik, A., 2015. *Sitophilus granarius* responses to blends of five groups of cereal kernels and one group of plant volatiles. J. Stored Prod. Res. 62, 36–39. https://doi.org/10.1016/j.jspr.2015.03.007.

Piesik, D., Bocianowski, J., Sendel, S., Krawczyk, K., Kotwica, K., 2020. Beetle orientation responses of *Gastrophysa viridula* and *Gastrophysa polygoni* (Coleoptera: chrysomelidae) to a blend of synthetic volatile organic compounds. Environ. Entomol. 49 (5), 1071–1076. https://doi.org/10.1093/ee/nvaa082.

Pike, V., Smith, J.L., White, R.D., Hall, D.R., 1994. Studies of responses of stored-products pests, *Prostephanus truncatus* (Horn) and *Sitophilus zeamais Motsch*., to food volatiles. In: Proceedings of the 6th International Working Conference on Stored-Product Protection. CAB International, Wallingford, UK, pp. 566–569.

Potter, C., 1935. The Biology and Distribution of *Rhyzopertha dominica* (FAB.), 83. Transactions of the Royal Entomological Society of, London, pp. 449–482.

Rajendran, S., 2002. Postharvest pest losses. In: Pimentel, D. (Ed.), Encyclopedia of Pest Management. Marcel Dekker, Inc., New York, pp. 654–656.

Rajendran, S., 2020. Insect pest management in stored products. Outlooks Pest Manag. 31 (1), 24–35. https://doi.org/10.1564/v31_feb_05.

Rajendran, S., Sriranjini, V., 2008. Plant products as fumigants for stored-product insect control. J. Stored Prod. Res. 44 (2), 126–135. https://doi.org/10.1016/j.jspr.2007.08.003.

Reddy, G.V.P., Guerrero, A., 2004. Interactions of insect pheromones and plant semiochemicals. Trends Plant Sci. 9, 253–261. https://doi.org/10.1016/j.tplants.2004.03.009.

Rotundo, G., Germinara, G.S., De Cristofaro, A., 2000. Immuno-osmophoretic technique for detecting *Sitophilus granarius* (L.) infestations in wheat. J. Stored Prod. Res. 36 (2), 153–160. https://doi.org/10.1016/S0022-474X(99)00033-8.

Sakka, M.K., Jagadeesan, R., Nayak, M.K., Athanassiou, C.G., 2022. Insecticidal effect of heat treatment in commercial flour and rice mills for the control of phosphine-resistant insect pests. J. Stored Prod. Res. 99, 102023. https://doi.org/10.1016/j.jspr.2022.102023.

Song, X., Wang, P., Zhang, H., 2011. Phosphine resistance in *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) from different geographical populations in China. Afr. J. Biotechnol. 10 (72), 16367–16373. https://doi.org/10.5897/AJB11.1101.

Stagnari, F., Codianni, P., Pisante, 2008. Agronomic and kernel quality of ancient wheats grown in central and southern Italy. Cereal Res. Commun. 36 (2), 313–326. https://doi.org/10.1556/crc.36.2008.2.11.

Swan, C.G., Meyer, F.D., Hogg, A.C., Martin, J.M., Giroux, M.J., 2006. Puroindoline B limits binding of puroindoline A to starch and grain softness. Crop Sci. 46 (4), 1656–1665. https://doi.org/10.2135/cropsci2005.06-0135.

Tadesse, M., 2020. Post-harvest loss of stored grain, its causes and reduction strategies. Food Sci. Qual. Manag. 96, 26–35.

Taylor, R.W.D., 1994. Methyl bromide-Is there any future for this noteworthy fumigant? J. Stored Prod. Res. 30 (4), 253–260. https://doi.org/10.1016/S0022-474X(94)90317-4.

Throne, J.E., Baker, J.E., Messina, F.J., Karl, J.K., Howard, J.A., 2000. Varietal resistance. In: Subramanyam, B., Hagstrum, D.W. (Eds.), Alternatives to Pesticides in Stored-Product IPM. Kluwer Academic, Massachusetts, pp. 165–192.

Trematerra, P., Throne, J., 2012. Insect and mite pests of durum wheat. In: Durum Wheat, Chemistry and Technology, second ed. AACC International Inc., St. Paul, MN, USA, pp. 73–83.

Trematerra, P., Pistillo, O.M., Germinara, G.S., Colacci, M., 2021. Bioactivity of cereal- and legume-based macaroni pasta volatiles to adult *Sitophilus granarius* (L.). Insects 12 (9), 765. https://doi.org/10.3390/insects12090765.

Tufariello, M., Palombi, L., Rizzuti, A., Musio, B., Capozzi, V., Gallo, V., et al., 2023. Volatile and chemical profiles of Bombino sparkling wines produced with autochthonous yeast strains. Food Control 145, 109462. https://doi.org/10.1016/j.foodcont.2022.109462.

Urrutia, R.I., Yeguerman, C., Jesser, E., Gutierrez, V.S., Volpe, M.A., González, J.O.W., 2021. Sunflower seed hulls waste as a novel source of insecticidal product: pyrolysis bio-oil bioactivity on insect pests of stored grains and products. J. Clean. Prod. 287, 125000. https://doi.org/10.1016/j.jclepro.2020.125000.

Van Den Dool, H., Kratz, P.D., 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal of chromatography.

Visser, J.H., 1986. Host odor perception in phytophagous insects. Annu. Rev. Entomol. 31 (1), 121–144. https://doi.org/10.1146/annurev.en.31.010186.001005.

Webster, B., Gezan, S., Bruce, T., Hardie, J., Pickett, J., 2010. Between plant and diurnal variation in quantities and ratios of volatile compounds emitted by Vicia faba plants. Phytochemistry 71 (1), 81–89. https://doi.org/10.1016/j.phytochem.2009.09.029.

Zhou, M., Robards, K., Glennie-Holmes, M., Helliwell, S., 1999. Analysis of volatile compounds and their contribution to flavor in cereals. J. Agric. Food Chem. 47 (10), 3941–3953. https://doi.org/10.1021/jf990428l.

7. Susceptibility of ancient wheats to *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) and their allelochemical interactions

Ilaria D'Isita[1*], Onofrio Marco Pistillo[1], Sandra Pati[1], Antonella Di Palma[1], Pasquale De Vita[2], Giacinto Salvatore Germinara[1]. *Submitted for publication to Grain & Oil Science and Technology.*

[1] Department of Agricultural Sciences, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Via Napoli 25, 71122 Foggia, Italy.
[2] CREA Research Centre for Cereal and Industrial Crops, 71122 Foggia, Italy.

*Corresponding author

Susceptibility of ancient wheats to *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) and their allelochemical interactions

Ilaria D'Isita[1*], Onofrio Marco Pistillo[1], Sandra Pati[1], Antonella Di Palma[1], Pasquale De Vita[2], Giacinto Salvatore Germinara[1].

[1] Department of Agricultural Sciences, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Via Napoli 25, 71122 Foggia, Italy.

[2] CREA Research Centre for Cereal and Industrial Crops, 71122 Foggia, Italy.

Corresponding author
Ilaria D'Isita
ilaria.disita@unifg.it

Abstract

The need for a traditional and sustainable food supply resulted in a renewed interest in ancient wheats providing an additional source of income for farmers, in particular in agriculturally marginal areas. Infestations of ancient wheats by different storage insect species, including two of the major primary pests, *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) are previously reported. Most studies have been focused on the interaction among storage insect pests and modern commercial granaries, whilst relatively few have been made take into account ancient wheat species. With the aim of deepening the knowledge on feeding behaviour of storage pests on ancient wheats, in this study the susceptibility level and the attractiveness of improved emmer (Padre Pio) and spelt (Benedetto) varieties towards *S. granarius* and *R. dominica* was evaluated in laboratory bioassays. Furthermore, the VOCs profiles of the hulled ancient wheats studied were characterized by headspace - solid phase microextraction (HD-SPME) and gaschromatography coupled with mass spectrometry (GC-MS). Results obtained showed that glumes and glumelles represented a physical barrier against *S. granarius,* but not against *R. dominica*. Avoiding the glumes protectant effect, kernel hardness and protein content represented the main factors involved in resistance mechanisms to *R. dominica* and *S. granarius*, respectively. Considering the behavioural responses to hulled emmer and spelt, *S. granarius* were effective attracted by both ancient wheat species, with a significant preference of both sex for the emmer Padre Pio. By contrast, odours from emmer and spelt varieties did not elicit a significant attraction in *R. dominica* adults. The possible reasons of differences in susceptibility and attractiveness are discussed. This study deepens knowledge on interaction among *R. dominica* and *S.*

granarius and emmer and spelt varieties and offers new information useful for a more rational postharvest management of these cereals, in particular in agriculturally marginal areas.

Keywords: granary weevil, lesser grain borer, wheat resistance, susceptibility tests, Volatile Organic Compounds, Two-choice pitfall bioassays, Y-tube olfactometer bioassays.

Introduction

The hulled wheats *Triticum monococcum* L., T. *dicoccum* Schubler, and *T. spelta* L. (einkorn, emmer and spelt), commonly known as "Farro", are among the most ancient cereal crops of the Mediterranean basin to be domesticated (Perrino *et al*., 1991; Dinu *et al*., 2018; Buerli, 2006) and contributed significantly to the phylogenesis of modern wheats (Arzani, 2011). Farro were a staple food for a long time until they fell into disuse (Arzani, 2011). Nowadays, the need for a wholesome, traditional, and sustainable food supply resulted in a renewed interest in ancient wheats providing an additional source of income for farmers, in particular in agriculturally marginal areas (Arzani, 2011). Indeed, their low nitrogen requirements and the high adaptability to adverse soil and climatic conditions (Perrino, 1993; D'Antuono, 1994; D'Antuono and Bravi, 1996) make ancient cereals suitable to low-input agricultural systems (Mefleh *et al*., 2019). Furthermore, the increasing demand for ancient wheat products also contributing to agricultural diversification (Padulosi *et al*., 1996) and safeguard the precious biodiversity of these populations (Mefleh *et al*., 2019). Currently, Farro are grown in marginal areas of several European countries, such as Italy, former Yugoslavia, Spain and Turkey (Trematerra and Gentile, 2002). In Italy, Farro representing a deeply rooted in popular tradition (Arzani, 2011) and is cultivated mainly in the central south of the peninsula, both as animal and human food (D'Antuono e Pavoni, 1993; Perrino *et al*., 1981, 1984, 1991). For Farro, generally no particular control means against stored product insect pests are adopted (Trematerra and Gentile, 2002). However, a study carried out in Central-Southern Italy revealed infestations of Farro by different storage insect species, including two of the major primary pests of stored cereals, the granary weevil, *Sitophilus granarius* (L.) (Coleoptera, Curculionidae) and the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera, Bostrichidae). Infestations by *S. granarius* are related to the origin of grain storage and could be the result of a complex co-evolutionary process of the domestication and storage of grain by humans in Neolithic (Plarre, 2013). The granary weevil feeds mainly on cereal grains such as wheat, barley, rye, oats, corn, rice, millet, and derived products (Schwartz & Burkholder, 1991; Bell, 2014). Instead, the lesser grain borer can cause economic losses to a wide range of stored products, but achieves its maximum reproductive success on dry grains, in particular on wheat (Potter, 1935; Bashir, 2002; Edde and Phillips, 2006; Naseri and Majd-Marani, 2022; Ren *et al*., 2023).

Females of *S. granarius* lay eggs within the grain endosperm (Niewiada *et al*., 2005), whilst, *R. dominica* females deposit eggs on the surface of kernels or among the frass produced by insects and the first instar moves into the grains (Edde, 2012). The complete development of immature stages of both species take place inside kernels causing hidden grain infestation difficult to detect and manage (Rotundo *et al*., 2000; Edde, 2012). Insect pests are a common and serious issue of stored food causing severe quantitative and qualitative damages worldwide due to feeding activity, alteration of nutritional value and contamination of commodities with insect bodies, excrement and microrganism such as mycotoxigenic fungi (Campbell and Sinha, 1976; Rajendran, 2002; Magan *et al*., 2003; Majeed *et al*., 2015; Lemic 2020 and reference therein). By contrast, due to the legislation limits on chemical insecticides, in particular in developing counties (Handford *et al*., 2015), the developing of resistant strains of target insects (Lorini *et al*., 2007; Song *et al*., 2011; Boyer *et al*., 2012; Kocak *et al*., 2015; Afful *et al*., 2017; Nayak *et al*., 2020; Sakka *et al*., 2022), and risks due to pesticides residues (Mostafalou and Abdollahi, 2013; Handford *et al*., 2015; Tuet *et al*., 2021; Tudi *et al*., 2021), the control of storage pests is very difficult. Thus, there is a global interest in the research and development of low-impact control tools (Field and White, 2002; Germinara *et al*., 2007; Rajendran and Sriranjini, 2008; Chaudhari *et al*., 2021; Urrutia *et al*., 2021).

Food finding and food acceptance are the two phases of food plant selection by phytophagous insects (Thorsteinson, 1958). The acceptance or rejection of plant by insects is mediated by the plant physicochemical proprieties (Bernays and Chapman, 2007). In particular, Plant Volatile Organic Compounds (VOCs) play a focal biological role in the identification of host plants by insects for feeding, mating, oviposition and also to avoid unsuitable habitats (Dickens, 1984; Visser, 1986; Agelopoulos *et al.* 1999; Reddy and Guerrero, 2004; Bernays and Chapman, 2007). Both *S. granarius* and *R. dominica* adults use olfactory cues to discriminate different kinds of food substrates (Germinara *et al*., 2007, 2019; Edde and Philips, 2006; Cao *et al*., 2022, 2024). Grain physical and chemical features, such as hardness, weight and size, protein, carbohydrate, lipids, gluten, or phenol content can influence demographic performance of storage insect pests, including *S. granarius* and *R. dominica*, causing different susceptibility levels of grains (Kućerová and Stejskal, 1984; Toews *et al*., 2000; Warchalewski *et al*., 2002; Nawrot *et al*., 2006, 2010; Mebarkia *et al*., 2010; Gałęcki *et al*., 2019; Arthur *et al*., 2020; D'Isita *et al*., 2023, 2024; Ren *et al*., 2023). Most studies have been focused on interaction among storage insect pests and modern commercial granaries, whilst relatively few have been made take into account ancient hulled wheat (Trematerra and Gentile, 2002; Almaši and Poslončec, 2014 and reference therein; Gałęcki *et al*., 2019). With the aim of deepening the knowledge on feeding behaviour of stored product insect pests on ancient hulled wheat species, in the present study the susceptibility level and the attractiveness of improved emmer (Padre Pio) and spelt (Benedetto) varieties towards *S. granarius* and *R. dominica* was evaluated in laboratory bioassays. Furthermore, the VOCs profiles of the ancient wheats studied were characterized.

Materials and methods

Insects

Sitophilus granarius and *R. dominica* populations started from infested wheat kernels collected in storage facilities in the Apulia region (Southern Italy). Insect laboratory colonies were reared on durum wheat (var. Simeto) in cylindrical glass containers (Ø 15 × 15 cm) covered with a fine mesh net (0.5 mm) to allow air exchange, and maintained in controlled conditions (25±2°C for *S. granarius*, 28±2°C for *R. dominica*, dark, 60±5% relative humidity). Specimens of the eighth generation were used for laboratory bioassays.

Plant materials

The evaluated genotypes included the emmer Padre Pio variety (*T. turgidum* subsp. *dicoccum*) and the spelt Benedetto variety (*T. aestivum* subsp. *spelta*). Padre Pio variety is an improved variety of emmer wheat, released in Italy in 2016 and selected from a cross between a local emmer wheat population called Molise and Simeto, a commercial durum wheat variety. It is an early cycle variety, medium-tall in size, with good resistance to lodging and the main fungal diseases, particularly suitable for marginal environments. Excellent technological performance for pasta making, with hulled grain at harvest. Instead, Benedetto variety is an improved spelt wheat released in Italy in 2010. It was derived from an interspecific cross between an old spelt wheat "Altgold rotkorn" and a bread wheat variety Centauro. Benedetto is a mid-late, medium-tall spelt with good quality grains, especially suitable for organic farming. Genotypes used were grown in a single field trial during the 2020-21 growing season at Foggia, Italy (41°28′N, 15°32′E; 75 m a.s.l.). In susceptibility bioassays, the modern durum wheat variety, Ofanto, and the modern bread wheat variety, Mec, were used as susceptible controls based on previous study (D'Isita *et al*., 2024). After harvest, spelt and emmer samples were dehulled and cleaned with a laboratory seed cleaner (Samatec-Roeber, Bad Oeynhausen, Germany) in order to remove hulls, straw, and damaged kernels. Grain samples were stored at low temperatures (4±1°C) until the time of the study.

Physicochemical measurements of grain samples

Moisture of whole grains was determined using the single-stage air oven (Termostabil k3, Cavalo S.R.L., Milan, Italy) method (ASAE 2003). This method utilizes whole grain dried for 18 h at 130°C to determine the moisture content. Thousand-kernel weight (TKW) was calculated from the mean weight of three sets of 500 grains per genotype. Protein content (PC) was determined by nitrogen combustion analysis according to Approved Method 46-30 (AACC International 2010) using a Dumas nitrogen analyzer (Leco Corp, St. Joseph, MI). The Single Kernel Characterization System 4100 (SKCS) (Perten Instruments, North America, Inc., Springfield, IL, USA) was used to characterize kernels hardness (Ha) using a sample of 300 kernels (Method 55-31) (AACC, 2010). The experiments were repeated independently three times, and kernel samples were collected at different times. The higher the value of the hardness index (Ha), the harder the grain. Gluten index (%, AACC method 38-12.0260) was measured using an automatic gluten washing apparatus (Glutomatic) followed by centrifugation.

Susceptibility tests

To assess the susceptibility of plant materials to *S. granarius* and *R. dominica*, laboratory bioassays were carried out according D'Isita *et al.*, 2024. Considering the potential effect of the glumes as physical barrier to storage insect pests, the susceptibility tests were carried out using both hulled and de-hulled grains of Padre Pio and Benedetto varieties. Briefly, 60 g of uninfested grains hulled or de-hulled of each genotypes were weight and kept in cylindrical plastic containers (Ø 9 x 14.5 cm) closed by screw caps and conditioned for 7 days at 25±2°C and 60±5% r.h. For grain infestation, 12 unsexed adults of *S. granarius* (30-day-old) and *R. dominica* (15-day-old) were used. These insects were kept on grains at 25±2°C for *S. granarius* and 28±2°C for *R. dominica*, 60±5% r.h., in the dark for 15 days for mating and oviposition. After this oviposition period, the adult insects of each replicate were removed, sexed, and the number of dead specimens was recorded. The grains were then returned to the plastic containers for subsequent evaluation of the F1 progeny. For each insect species and genotypes 5 replicates were set up. For the evaluation of the total number of F1 progeny, the grains were observed every 3 days, removing and counting newly emerged adults until no adults emerged for 5 consecutive days (Shazali, 1987). Furthermore, the median development period (D), estimated as the time (days) from the middle of the oviposition period to the emergence of 50% of the F1 generation (Dobie, 1974) was evaluated. The total number of F1 progeny (F) and the median development period (D) were used to calculate the Susceptibility Index (S.I.) = (log. F)/D x 100 according Dobie 1974, useful to classified genotypes tested in resistant (0-3), moderately resistant (4-

7), susceptible (8-10) and highly susceptible (≥ 11). Finally, the percentage of mortality during the oviposition period and the number of adult offspring per female were also evaluated.

Two-choice pitfall bioassays

Two-choice pitfall bioassays were set up to assess the behavioural response of *S. granarius* adults (3 to 4-week-old) to the emmer and spelt varieties odours (Phillips *et al.*, 1993; Pike *et al.*, 1994; Germinara *et al.*, 2008). A steel container (Ø 30 cm × h 7 cm) was used as test arena and was covered with filter paper (Whatman No. 1) to allow insect activities (Pike *et al.*, 1994). The test arena was opportunely drilled 3 cm from the side wall making two holes (Ø 3 cm) diametrically opposed. Under each hole, glass flasks (500 ml) empty or containing the stimulus (200 g of uninfested hulled kernels of each genotypes) were positioned. The inside surfaces of their necks were coated with mineral oil to prevent insects from returning to arena. Insect used for experiments were maintained without food for at least 4 h. Twenty insects of mixed sex were put at the center of the test arena under an inverted Petri dish (Ø 3 cm × h 1,2 cm) and left 30 min to acclimate prior to release. Thus, insects were given choice between control (air) and stimulus (emmer or spelt). To prevent insects from escaping, the test arena was closed with a steel lid. Finally, after 3 h, insects fell in the stimulus or in the control flask and insects remained in the test arena were counted and sexed. Tests were carried out in the dark at 25±2°C and 60±5% r.h. between 09:00 A.M. and 4:00 P.M. Insects were used once and for each variety 10 replicates were set up. A response index (RI) was calculated for each replicate as follow: $RI = [(T - C) / Tot] \times 100$, with T as the number of insects responding to the stimulus, C as the number of insects responding to the control, and Tot as the total number of insects (Phillips *et al.*, 1993). Attraction to the stimulus is indicated by positive values of RI, by contrast negative values indicate repellence.

Y-tube olfactometer bioassays

To assess behavioural response of *R. dominica* adults (3-week-old) to plant materials tested, laboratory bioassays in a still-air Y-tube olfactometer (stem 3.5 cm long, each arm 6 cm long at 75°C angle, stem and arms 1 cm wide and 0.7 cm high) were carried out. Each arm of the two-choice olfactometer terminated with an odour chamber (2 cm long, 2 cm wide, 2 cm high) empty (air, control) or containing the stimulus (3 g of uninfested hulled kernels of each genotypes). To avoid insect from seeing stimuli, the olfactometer arms floor was above the odour chambers floor. Before each trial, the apparatus was conditioned for 10 minutes. The base and vertical walls of the olfactometer were covered with filter paper (Whatman No. 1) to facilitate insect movements. During the experiments, the olfactometer was closed with an inverted glass plate (18 x 18 cm). In static conditions, individual insects were released at the open end of the Y-tube stem and presented with the control air and the stimulus. A choice was recorded when the insect moved and remained beyond the decision line (1 cm up an arm of the Y-tube) for more than 30 sec. Furthermore, the time spent by test insects in each arm was recorded. After each trial lasted 10 min insects were sexed observing genitalia with a stereomicroscope. Treatments and control between arms were switched to avoid position bias. The filter paper and stimulus after 5 specimens were renewed. For each emmer, 70 beetles used once starved for at least 2 h were tested. Tests were carried out between 09:00 A.M. and 4:00 P.M. in the dark at 28±2°C and 60±5% r.h.

Volatile organic compounds (VOCs) analysis

Grain samples used for VOCs characterization were stored at low temperature (4±1°C) in glass containers until the analysis. The VOCs emitted by the hulled kernels of Padre Pio and Benedetto varieties were identified and quantified using the headspace solid-phase micro-extraction (HS-SPME) technique and gaschromatography coupled with mass spectrometry (GC-MS) (Beleggia *et al*., 2009, Germinara *et al*., 2019). During SPME headspace sampling, each sample (3 g) was placed in vials (20 mL) with caps in PTFE/Silicon septa (Supelco, Co., Bellefonte, PA) and maintained at 30 ± 0.1 °C in a water bath. The SPME fibers (Supelco Co., Bellefonte, PA, USA) used for extraction, coated with either 50/30 μm of divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS), were inserted into the vial headspace for 24 h. Before use, fibers were conditioned agree with manufacturer's instructions in the injection port of the gas chromatography (GC) at 270 ◦C for 30 min. To remove possible residues, fibers were left 10 min at the conditioning temperature with the split valve opened in the GC injector after every chromatographic run. As well as, a blank test was made under the same experimental conditions before each acquisition. For each genotype, 3 replicates were performed. Analysis were carried out using an Agilent 7890B series gas chromatograph (Agilent Technologies, Milan, Italy) coupled with an Agilent 5977A mass selective detector (MSD) equipped with an HP-5MS capillary column (30 m × 0.25 mm ID, 0.5 μm film thickness, J&W Scientific Inc., Folsom, CA, USA). The VOCs were thermally desorbed in splitless mode at 250 °C for 4 min with a programmed temperature from 40 °C to 250 °C at 5 °C/min and a final holding time of 10 min. Helium was the carrier gas used at a constant flow rate (1.25 mL/min). Spectra were recorded in the electron impact mode (ionization energy, 70 eV) in a range of 15-550 amu at 2.9 scans/s. The VOCs identification was obtained by comparison of their mass spectra with those of the data system library (NIST11, $p > 90\%$). A mixture of a continuous series of straight-chain hydrocarbons, C5-C40 (Alkane

Standard Solution C5-C40, Sigma Aldrich, Milan, Italy) was analysed under the same conditions to calculate the Linear Retention Indices (RIs) (Van Den Dool and Kratz, 1963). The relative abundance of each compound was calculated using the integrated peak area data from the GC-MS trace.

Statistical analysis

In susceptibility bioassays, data were submitted to Shapiro-Wilk's test to verify normal distribution, to Levene's test for homogeneity of variance, and then to the analysis of variances (ANOVA) followed by Tukey's HSD test for mean comparisons. Furthermore, linear regression analysis was performed to evaluate the correlation between *S. granarius* and *R. dominica* biological parameters and grain quality traits.

In the two-choice pitfall bioassays, the significance of the mean RIs in each treatment was evaluated by the Student's *t* test for paired comparisons (Phillips *et al*,. 1993). Furthermore, the number of *S. granarius* males and females was analysed by paired sample *t*-test and for each experiment, the sex ratio percentage was calculated. Finally, the mean values of RI for male and female were compared using by paired sample *t*-test. In Y-tube olfactometer bioassays, the significance of differences between the number of *R. dominica* adults choosing the treatment or control arm of the olfactometer were compared using $\chi 2$ test. The differences between the time spent by insects in each arm were analysed by paired sample *t*-test. Statistical analyses were performed with SPSS (Statistical Package for the Social Sciences) v.18 for Windows (SPSS Inc., Chicago, IL).

Results

Physicochemical measurements of grain samples

The average values of the main qualitative and technological parameters determined on the wheat kernel samples immediately after harvesting are reported in Table 1. The bread wheat variety (Mec) used as a control showed the lowest values for all traits evaluated. The Padre Pio emmer variety showed the highest average TKW and PC value among the materials tested; the Ha trait was also very high for this variety and comparable with that of the Ofanto durum wheat variety. On the contrary, Spelta showed a good protein content of the grain and the highest GI value. These reflect the breeding objectives followed for the development of emmer wheat Padre Pio (De Vita *et al*., 2006) and spelt wheat Benedetto (Koutroubas *et al*., 2014). Generally, the local varieties of spelt and emmer wheat are characterized by poor technological value of the storage proteins, and this makes them unsuitable for making pasta and/or bread (De Vita *et al*., 2007; Beleggia *et al*., 2021). Padre Pio and Benedetto were improved varieties obtained by interspecific crossing with modern wheat varieties with high technological value. The results reported in this study confirm the breeding strategy adopted for the development of modern emmer and spelt varieties with good grain quality (De Vita *et al*., 2006; Koutroubas *et al*., 2014).

Susceptibility tests

In the case of *S. granarius*, during the oviposition period the mean mortality percentage of hulled emmer Padre Pio (80.00 ± 12.53) and hulled spelt Benedetto (63.33 ± 11.37) was significantly higher ($F = 9.372$; $df = 5$; $P < 0.001$) than that recorded for both controls, Ofanto (2.78 ± 2.78) and Mec (2.78 ± 2.78) wheat varieties (Table 2). Both the total number of F1 progeny and offspring per female recorded for hulled and de-hulled Padre Pio and Benedetto varieties were significantly lower (F1 progeny: $F = 88.319$; $df = 5$; $P < 0.001$; offspring per female: $F = 57.663$; $df = 5$; $P < 0.001$) compared to those recorded for Ofanto and Mec (Table 2). In particular, no progeny emerged from hulled Padre Pio variety (Table 2). Differences emerged in the total number of F1 progeny resulting in significant ($F = 17.796$; $df = 4$; $P < 0.001$) differences in the S.I. calculated. In the detail, the S.I. of different varieties varied from 2.98 (hulled Benedetto) to 14.18 (Ofanto), and those of hulled (2.98) and de-hulled (7.44) Benedetto and de-hulled Padre Pio (6.31) were significantly lower than that of Mec (13.41) and Ofanto (14.18) wheat varieties (Table 2). For hulled Padre Pio, it was not possible to calculate S.I. due to the complete absence of progeny.

For *R. dominica*, the mean mortality percentage during the oviposition period of hulled (58.33 ± 13.18) and de-hulled (61.67 ± 14.34) Benedetto was significantly (F 5.241=; $df = 5$; $P = 0.003$) higher than that recorded for the bread wheat variety, Mec (0) (Table 3). Among all wheat varieties, significant differences were found both in the number of F1 progeny ($F = 6.723$; $df = 5$; $P = 0.001$) and offspring per female ($F = 3.946$; $df = 5$; $P = 0.012$). The number of the F1 progeny emerged from the hulled emmer Padre Pio (42.20 ± 5.22) and the hulled spelt Benedetto (44.20 ± 6.87) was significantly lower than that recorded for the bread wheat variety Mec (319.33 ± 3.84) (Table 3). The number of the offspring per female emerged from hulled Benedetto (14.10 ± 4.21) was significantly lower than those observed for the Ofanto (48.92 ± 8.39) and Mec (65.48 ± 7.09) (Table 3). For *R. dominica*, significant differences were found also for the median development period and S.I. with small range of variation. In particular, the median development period of hulled Benedetto (46.85 ± 0.67) was significantly higher ($F = 4.017$; $df = 5$; $P = 0.011$) of that recorded for Mec variety (42.03 ± 0.19) (Table 3). The S.I. ranged from 7.98 (Benedetto hulled) to 13.72 (Mec) and the S.I. calculated for both hulled Padre Pio (8.29) and Benedetto (7.98) was significantly lower ($F = 14.474$; $df = 5$; $P < 0.001$) compared to de-hulled Padre Pio (11.79), Ofanto (11.51) and Mec (13.72) (Table 3). Linear regression analyses results are reported in Tables 4 and 5. In the case of *S. granarius*, a significant inverse correlation between protein content and F1 progeny ($R^2 = 0.946$; $P = 0.054$), offspring per female ($R^2 = 0.967$; $P = 0.033$), and S.I. ($R^2 = 0.980$; $P = 0.020$) were found (Table 4). For *R. dominica*, a significant direct correlation emerged between kernel hardness and median development period ($R^2 = 0.980$; $P = 0.020$) (Table 5).

Behavioural response of *S. granarius* in the two-choice pitfall bioassays

The mean sex ratio percentage calculated was of 51.70 ± 2.18 for Padre Pio and 53.89 ± 4.88 for Benedetto, without significant differences in the number of males and females tested (Table 6 and 7). The granary weevil adults showed positive and significant ($P < 0.001$; Student's *t* test) RIs resulting in an actual attraction in response to the odours of both Padre Pio and Benedetto varieties (Table 6 and 7). For *S. granarius* males, the RI registered for the emmer Padre Pio (67.40 ± 6.56) was significantly higher ($F = 13.797$; df = 1; $P = 0.002$) compared to that of the spelt Benedetto (31.19 ± 7.13) (Table 6). Similar results were recorded for females, with the RI for the emmer Padre Pio (69.32 ± 4.30) significantly higher ($F = 5.584$ df = 1; $P = 0.033$) compared to that of the spelt Benedetto (43.72 ± 11.06) (Table 7).

Behavioural response of *R. dominica* in Y-tube olfactometer bioassays

In Y-tube behavioural bioassays, odours from the emmer Padre Pio and the spelt Benedetto did not elicit a significant attraction in both *R. dominica* males and females evaluated either as first choice and time spent in the treatment arm when clean air was the alternative (Table 8 and 9).

Volatile organic compounds (VOCs) analysis

A total of 50 VOCs were identified in the headspace of the varieties studied including 15 aldehydes, 9 alcohols, 3 benzene derivatives, 8 ketone, 6 alkanes, 2 alkenes, 2 terpenes, 2 organic acid, 2 other compounds, and 1 lactone. The percentages of the compounds detected from hulled Padre Pio and Benedetto are expressed as relative abundance and reported in Table 10. In the emmer Padre Pio VOCs profile, the most represented chemical classes were aldehydes (38.7 ± 0.1), followed by alcohols (14.3 ± 0.1) and ketones (10.9 ± 0.1). The most abundant aldehydes were hexanal (7.7 ± 0.3) and nonanal (7.3 ± 0.3), 1-pentanol (3.6 ± 0.01) was the most abundant alcohol, and 2-tridecanone (2.9 ± 0.05) e 2-pentadecanone (2.2 ± 0.2) were the most abundant ketones (Table 10). A similar VOCs profile was recorded for the spelt Benedetto with some slight differences. In particular, the most represented chemical classes in Benedetto VOCs profile were aldehydes (39.1 ± 0.1), slightly higher compared to Padre Pio VOCs profile, organic acids (19.8 ± 0.2) and alcohols (16.7 ± 0.1). The most present aldehydes were nonanal (7.0 ± 0.7), followed by hexanal (6.1 ± 0.7) and pentanal (6.0

± 0.02). Among the aldehydes, butanal,2-methyl (6.0 ± 0.02) was recorded exclusively in Benedetto VOCs profile. The most abundant alcohols were 1-pentanol (3.7 ± 0.3) and 1-butanol (3.8 ± 0.1), the latter exclusively found in Benedetto VOCs profile. Finally, the most abundant organic acid was the acetic acid (17.3 ± 0.2).

Discussion

The increasing interest in the traditional and sustainable food supply chain is given to ancient hulled wheats a new chance of survival, in particular in agriculturally marginal areas where these cereals are particularly suitable. Considering the lack of knowledge on ancient wheats and storage pests interactions, in the present study the susceptibility degree of hulled and de-hulled emmer *cv.* Padre Pio and the spelt *cv.* Benedetto towards *S. granarius* and *R. dominica* was evaluated also take into account possible correlations with some grain physicochemical parameters.

In susceptibility bioassays with *S. granarius*, significantly differences emerged between the susceptible controls, Mec and Ofanto, and both ancient wheats tested. According the Dobie's classification (1974), durum and bread wheat varieties, Mec and Ofanto, confirmed their high susceptible degree (D'Isita *et al.*, 2024), whilst, hulled Benedetto, with a S.I. about 3, and hulled Padre Pio, with no progeny emerged during the experiments, were classified as resistant. Furthermore, both de-hulled Padre Pio and Benedetto resulted moderately resistant, with a S.I. lesser than 8. Keep in mind that the S.I. is based on the F1 progeny and the median development period, these differences could be linked mostly to the large variation in total F1 progeny among genotypes. In fact, lower values of total progeny and, thus, of offspring per female for both hulled and de-hulled ancient grains compared to Mec and Ofanto were recorded. On the other hand, no significant differences emerged for median development time, showing that probably the physicochemical properties of these grains did not affect the *S. granarius* development period and its biological cycle. Even if, slightly higher values of development period of insects emerged from spelt Benedetto compared to the other genotypes were found. Food quality can significantly affect the population development of phytophagous insects (Ren *et al.*, 2023) and can also be evaluated based on insect life expectancy (Almaši and Poslončec, 2014). Almaši and Poslončec (2014) found that *Tribolium confusum* du Val develop in longer time on spelt than on common wheat kernels, but beetles persisted much longer with low adult mortality rate. Instead, in our study the mortality of *S. granarius* adults reared on hulled Padre Pio and Benedetto was significantly higher compared to the same de-hulled varieties and to susceptible controls. Our results are in accordance with previous studies which concluded that glumes and glumelles could represent a natural protection against attacks of some stored insect pest species (Kordan *et al*., 2007; Almaši *et al*., 2010; Bodroža-Solarov *et al*., 2010). In particular, Kordan *et al*. (2007) demonstrated that spikelets offered a physical barrier against *S. granarius* during the storage. As well as, *S. oryzae* (L.) is not able to feed, reproduce and develop on hulled kernels, thus hull has a total protective effect against this species (Almaši *et al*., 2010; Bodroža-Solarov *et al*., 2010). Avoiding the glumes protectant effect, the moderately resistant of de-hulled Padre Pio and Benedetto to *S. granarius* attacks seems to be related to their higher protein content. Indeed, a significant inverse correlation between protein content and F1 progeny, offspring per female, and S.I. were found. The resistance of the grains to storage insect pest attacks is mediated by different cues, which are environmental conditions and chemical and physical grain proprieties more or less genetically heritable (Georget *et al*. 2008; Ronga *et al*. 2020). It has been reported that the high proteins contents in the grains can cause antibiosis and antixenosis effects in insect pests (Júnior *et al*., 2008; Nhamucho *et al*., 2017; Lanzanova *et al*., 2021). In particular, wheat and maize varieties with high protein content resulted as resistant to *Sitophilus* spp. (Kućerová and Stejskal, 1994; Nawrot *et al*., 2006; Nhamucho *et al*., 2017; Kordan *et al*., 2023). By contrast, Mebarkia *et al*., (2010) suggested a possible direct relation between protein content and wheat susceptibility to *S. granarius*. In this context, it is notable that grain proprieties involved in varietal susceptibility to stored-product insect pests can act in isolation or in combination, thus find a key parameter is very difficult (Lanzanova *et al*., 2021).

In the case of *R. dominica*, all genotypes tested were susceptible or high susceptible to *R. dominica* attacks, with some differences among them. High level of adult mortality on hulled Padre Pio and hulled and de-hulled Benedetto compared with susceptible controls Mec and Ofanto were recorded. Hulled Benedetto showed the significantly lower S.I. value resulted from the lowest F1 progeny and the longest median development period. In general, the F1 progeny emerged from hulled Padre Pio and Benedetto was lower compared with both susceptible controls, but higher respect to that recorded for *S. granarius*, indicating a greater ability of lesser grain borer to develop even in presence of glumes. It has been reported that storage insect pests do not develop equally on different species and varieties of cereals (Almaši and Poslončec, 2014; Chougourou *et al*., 2013; Borzoui and Naseri, 2016; Golizadeh and Abedi, 2017; Ren *et al*., 2023). Almaši *et al*. (2010) and Bodroža-Solarov *et al*. (2010) studied the development, survival and reproduction of *S. oryzae* and *R. dominica* on hulled and de-hulled spelt wheat. In accordance with our findings, the progeny of *R. dominica* maintained on hulled spelt was smaller than those on de-hulled kernels; however, different than *S. oryzae*, *R. dominica* normally developed on both hulled and de-hulled spelt kernels (Almaši *et al*., 2010; Bodroža-Solarov *et al*., 2010). Considering de-hulled kernels, a significant direct correlation emerged between kernel hardness and median development period of *R. dominica*. Arthur *et al*., (2020) showed that softer kernel varieties resulted more susceptible to *R. dominica* attacks, since the female lays an egg on the outside of a grain kernel, and the neonate larva must penetrate the kernel to feed and complete development to the adult stage. However, results in the scientific literature can be conflicting. Fang *et al*. (2002) found no relationship between kernel hardness and susceptibility to *R. dominica*. As well as, Toewes *et al*. (2000) reporting no correlation between kernel hardness and *R. dominica* progeny production. According to previous studies, kernel hardness negatively influences also the ability of *Sitophilus* weevils, including *S. granarius*, to penetrate and oviposit into the grain kernels, reducing progeny production and damage (McGaughey *et al*.,1990; Nawrot *et al*., 2006; Suleiman *et al*., 2015; Antunes *et al*., 2016; Kalsa *et al*., 2019; Željko *et al*., 2020; D'Isita *et al*., 2024). In our study, the correlation was found among some *S. granarius* biological parameters and protein content, and not with kernel hardness. However, proteins, texture, and grain hardness could affect resistance in interconnected ways (Mwlolo *et al*., 2013). Overall, wheat susceptibility to stored grain pests probably is the resultant of different physicochemical features and their interactions. Among them, our findings highlight the role of protein content and kernel hardness in *S. granarius* and *R. dominica* interactions with emmer and spelt varieties studied.

To deep knowledge on the food finding interaction among hulled grains and *S. granarius* and *R. dominica*, the VOCs profile of above-mentioned varieties and their attractiveness towards adults of both species was also studied. The granary weevil adults showed positive and significant RIs indicating an effective attraction in response to odours of the all varieties tested. In particular, for both sex, RI registered for the emmer Padre Pio was significantly higher to that of the spelt Benedetto. By contrast, odours from emmer and spelt varieties did not elicit a significant attraction in *R. dominica* adults. It has been reported that *S. granarius* is able to perceive a wide range of cereals VOCs, and some of these elicit significant behavioural responses (Germinara *et al*., 2002, 2008, 2019; Piesik and Wenda-Piesik, 2015; Trematerra *et al*., 2021; Cao *et al*., 2023). The host finding by *S. granarius* could be considered a complex process (Piesik and Wenda-Piesik, 2015) based on the balance of positive and negative odour stimuli (Germinara *et al*., 2008). Piesik and Wenda-Piesik (2015), reported the behavioural responses of granary weevil to different blends of cereal volatiles. Adults of *S. granarius* were attracted to specific concentrations of a blend with aliphatic alcohols, a blend with aromatics, and a blend with aliphatic alcohols, aliphatic aldehydes, aliphatic ketones, aromatics. Whereas, no attraction was found for blends consisting of aliphatic aldehydes and aliphatic ketones. These results are in accordance with previous studies. Indeed, some aldehydes, such as hexanal and heptanal, and aliphatic ketones, including 2-pentanone, 2-hexanone, 2-heptanone and 2,3-

butanedione, identified from cereal grains showed repellent effects against granary weevil adults (Germinara *et al*., 2008, 2012). Also *R. dominica* uses olfactory cues in host-finding process to discriminate suitable and unsuitable plants, with a strong olfactory preference for wheat (Cao *et al*., 2024). However, to the best of our knowledge little is known about behavioural responses of *R. dominica* to cereal chemical classes or specific VOCs.

In the present study, most of the VOCs identified in emmer and spelt were already found in cereal kernels and products (Maga, 1978; Zhou *et al*., 1999; Beleggia *et al*., 2009; Busko *et al*., 2010; Mattiolo *et al*., 2017; Germinara *et al*., 2019; De Flaviis *et al*., 2021; 2022; 2023; Trematerra *et al*., 2021). The emmer Padre Pio, the most attractive grain to *S. granarius* adults, showed a slightly lesser amount of total aldehydes and an higher total content of ketones compared to spelt Benedetto. As well as, Benedetto showed an higher total content of alcohols and organic acids compared to Padre Pio emmer. However, overall the two VOCs profile resulted quite similar. Thus, probably the olfactory preferences of *S. granarius* for emmer Padre Pio could be linked to differences in VOCs emissions among the two substrates potential caused by differences in kernels and glumes characteristics. Further studies are in progress to better understand the VOCs emission of hulled and de-hulled kernels and to clarify the possible role of chemical compounds mainly present in both VOCs profile towards *S. granarius* and *R. dominica* adults.

In conclusion, this study deepens knowledge on interaction among *R. dominica* and *S. granarius* and emmer and spelt varieties. Considering the crescent interest in ancient grains, the present study offers new information also useful for a more rational postharvest management of these cereals, in particular in agriculturally marginal areas. Furthermore, determining the physicochemical parameters of host plants involving in varietal susceptibility degree is the first step required for the breeding of new varieties resistant to insect pests (Stoner and Shelton 1988; Jyoti *et al*., 2001). As well as, study the host and non-host plant VOCs could be valuable to identify bioactive chemical compounds useful to develop semiochemical-based control tools of storage pests (Germinara *et al*., 2012, 2019; Cai *et al*., 2022; Cao *et al*., 2023, 2024) or to identify cereal varieties, rich in repellent compounds able to modify the insect behaviour useful in innovative breeding programs (Germinara *et al*., 2019).

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References

Afful, E., Elliott, B., Nayak, M. K., & Phillips, T. W., 2018. Phosphine resistance in North American field populations of the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Journal of Economic Entomology, 111(1), 463-469.

Agelopoulos N., Birkett M. A., Hick A. J., Hooper A. M., Pickett J. A., Pow E. M., Woodcock C. M., 1999. Exploiting semiochemicals in insect control. Journal of Pesticide Science, 55(3), 225-235.

Almaši, R., & Poslončec, D. I., 2014. Reproduction of confused flour beetle *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae) on common and spelt wheat and their products. Pesticides and Phytomedicine/Pesticidi i fitomedicina, 29(3), 197-204.

Almasi, R., Bodroza-Solarov, M., & Posloncec, D., 2010. Development of rice weevils (*Sitophilus oryzae* L.) and lesser grain borers (*Rhizopertha dominica* F.) on kernels and spikes of spelt wheat. Savremena poljoprivreda, 59 (1-2), 92-98.

Antunes, C., Mendes, R., Lima, A., Barros, G., Fields, P., Da Costa, L.B., ... and Carvalho, M.O., 2016. Resistance of rice varieties to the stored-product insect, *Sitophilus zeamais* (Coleoptera: Curculionidae). Journal of economic entomology, 109(1), 445-453.

Arthur, F.H., Bean, S.R., Smolensky, D., Cox, S., Lin, H.H., Peiris, K.H.S., and Peterson, J., 2020. Development of *Rhyzopertha dominica* (Coleoptera: Bostrychidae) on sorghum: Quality characteristics and varietal susceptibility. Journal of Stored Products Research, 87, 101569.

Arzani, A., 2019. Emmer (Triticum turgidum ssp. dicoccum) flour and bread. In Flour and breads and their fortification in health and disease prevention, ed. by Preedy VR, Watson RR and Patel VB. (2011). Academic Press, London, 542.

Bashir T., 2002. Reproduction of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) on different host-grains. Pakistan Journal of Biological Sciences, 5, 91-93.

Beleggia, R., Omranian, N., Holtz, Y., Gioia, T., Fiorani, F., Nigro, F. M., ... & Papa, R., 2021. Comparative analysis based on transcriptomics and metabolomics data reveal differences between emmer and durum wheat in response to nitrogen starvation. International journal of molecular sciences, 22(9), 4790.

Beleggia, R., Platani, C., Spano, G., Monteleone, M., & Cattivelli, L., 2009. Metabolic profiling and analysis of volatile composition of durum wheat semolina and pasta. Journal of cereal science, 49(2), 301-309.

Bell, C. H., 2014. Food safety assurance systems: Infestation Management in Food Production Premises. Encyclopedia of Food Safety, 4, 189-200.

Bernays E. A., Chapman R. F., 2007. Host-plant selection by phytophagous insects. Springer, Science & Business Media, New York. 2, pp. 14-54.

Bodroža-Solarov, M., Almaši, R., Poslončec, D., Filipčev, B., & Šimurina, O., 2010. Protective effect of hulls Triticum aestivum ssp. spelta against insect infestation during storage. 2nd Workshop Feed-to-Food FP7 REGPOT-3. XIV International Symposium feed technology, Proceedings. Novi Sad, Serbia, 19-21.

Borzoui, E., Naseri, B., 2016. Wheat cultivars affecting life history and digestive amylolytic activity of *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae). Bulletin of Entomological Research 106: 464-473.

Boyer, S., Zhang, H., & Lempérière, G., 2012. A review of control methods and resistance mechanisms in stored-product insects. Bulletin of entomological research, 102(2), 213-229.

Buerli, M., 2006. Farro in Italy: A Desk-study by Markus Buerli. Global Facilitation Unit for Underutilized Species (GFU), Rome, 20.

Buśko, M., Jeleń, H., Góral, T., Chmielewski, J., Stuper, K., Szwajkowska-Michałek, L., ... & Perkowski, J., 2010. Volatile metabolites in various cereal grains. Food Additives and contaminants, 27(11), 1574-1581.

Cai, L., Macfadyen, S., Hua, B., Xu, W., & Ren, Y., 2022. The correlation between volatile compounds emitted from *Sitophilus granarius* (L.) and its electrophysiological and behavioral responses. Insects, 13(5), 478.

Campbell, A., & Sinha, R. N., 1976. Damage of wheat by feeding of some stored product beetles. Journal of Economic Entomology, 69(1), 11-13.

Cao, Y., Hu, Q., Huang, L., Athanassiou, C. G., Maggi, F., D'Isita, I., ... & Li, C., 2023. Attraction of *Sitophilus oryzae* (L.)(Coleoptera: Curculionidae) to the semiochemical volatiles of stored rice materials. Journal of Pest Science, 1-13.

Cao, Y., Jian, L., Athanassiou, C. G., Yang, Y., Hu, Q., Zhang, X., ... & Maggi, F., 2024. Behavioral responses of *Rhyzopertha dominica* (F.) to volatiles of different stored grains. Journal of Stored Products Research, 105, 102235.

Chaudhari, A.K., Singh, V.K., Kedia, A., Das, S., and Dubey, N.K., 2021. Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: prospects and retrospects. Environmental Science and Pollution Research, 28, 18918-18940.

Chougourou, D.C., Togola, A., Nwilene, F.E. *et al*., 2013. Susceptibility of some rice varieties to the lesser grain borer, *Rhyzopertha dominica* Fab. (Coleoptera: Bostrichidae) in Benin. Journal of Applied Science, 13, 173-177.

D'antuono, L. F., & Pavoni, A., 1993. Phenology and grain growth of *Triticum dicoccum* and *T. monococcum* from Italy. Biodiversity and wheat improvement, 273-286.

D'Antuono, L.F. and Bravi, R., 1996. The hulled wheat industry: present developments and impact on genetic resources conservation. In: Padulosi, S., 1996. Hulled Wheats: Proceedings of the First International Workshop on Hulled Wheats, 21-22 July 1995, Castelvecchio Pascoli, Tuscany, Italy (Vol. 4). Bioversity International.

D'Antuono, L.F., 1994. Obsolete wheats in Italy: an overview on cultivation, use and perspectives for their conservation. In Report of the IPGRI Workshop on Conservation and Use of Underutilized Mediterranean Species. Rome, Italy: IPGRI, 41-48.

D'Isita, I., Di Palma, A.M., De Vita, P., and Germinara, G.S., 2023. Acceptance and utilization efficiency of a purple durum wheat genotype by *Sitophilus granarius* (L.). Scientific Reports, 13(1), 14246.

D'Isita, I., Pistillo, O. M., Di Palma, A. M., De Vita, P., & Germinara, G. S., 2024. Susceptibility of old and modern wheat genotypes to *Sitophilus granarius* (L.) and *Rhyzoperta dominica* (F.). Journal of Stored Products Research, 106, 102265.

De Flaviis, R., Mutarutwa, D., Sacchetti, G., & Mastrocola, D., 2022. Could environmental effect overcome genetic?. A chemometric study on wheat volatiles fingerprint. Food Chemistry, 372, 131236.

De Flaviis, R., Sacchetti, G., & Mastrocola, D., 2021. Wheat classification according to its origin by an implemented volatile organic compounds analysis. Food chemistry, 341, 128217.

De Flaviis, R., Santarelli, V., Sacchetti, G., & Mastrocola, D., 2023. Heritage and modern wheat varieties discrimination by volatiles profiling. Is it a matter of flavor?. Food Chemistry, 401, 134142.

De Vita, P., Mastrangelo, A. M., Codianni, P., & Fornara, M., 2007. Bio-agronomic evaluation of old and modern wheat, spelt and emmer genotypes for low-input farming in Mediterranean environment. Italian Journal of Agronomy, 2(3), 291-302.

De Vita, P., Riefolo, C., Codianni, P., Cattivelli, L., & Fares, C., 2006. Agronomic and qualitative traits of *T. turgidum* ssp. *dicoccum* genotypes cultivated in Italy. Euphytica, *150*, 195-205.

Dickens J. C., 1984. Olfaction in the boll weevil, *Anthonomus grandis* Boh.(Coleoptera: Curculionidae): Electroantennogram studies. Journal of Chemical Ecology, 10(12), 1759-1785.

Dinu, M., Whittaker, A., Pagliai, G., Benedettelli, S., & Sofi, F., 2018. Ancient wheat species and human health: Biochemical and clinical implications. The Journal of nutritional biochemistry, 52, 1-9.

Dobie, P., 1974. The laboratory assessment of the inherent susceptibility of maize varieties to postharvest infestation by *Sitophilus zeamais* Motsch.(Coleoptera, Curculionidae). Journal of Stored Products Research, 10(3-4), 183-197.

Edde, P. A., & Phillips, T. W., 2006. Potential host affinities for the lesser grain borer, *Rhyzopertha dominica*: behavioral responses to host odors and pheromones and reproductive ability on non-grain hosts. Entomologia Experimentalis et Applicata, 119(3), 255-263.

Edde, P. A., 2012. A review of the biology and control of *Rhyzopertha dominica* (F.) the lesser grain borer. Journal of Stored Products Research, 48, 1-18.

Fields, P.G., and White, N. D., 2002. Alternatives to methyl bromide treatments for stored-product and quarantine insects. Annual review of entomology, 47(1), 331-359.

Gałęcki, R., Bakuła, T., Wojtacki, M., and Żuk-Gołaszewska, K., 2019. Susceptibility of ancient wheat species to storage pests *Sitophilus granarius* and *Tribolium confusum*. Journal of Stored Products Research, 83, 117-122.

Georget, D. M., Underwood-Toscano, C., Powers, S. J., Shewry, P. R., & Belton, P. S., 2008. Effect of variety and environmental factors on gluten proteins: An analytical, spectroscopic, and rheological study. Journal of agricultural and food chemistry, 56(4), 1172-1179.

Germinara G.S., De Cristofaro A., Rotundo G., 2012. Bioactivity of short-chain aliphatic ketones against adults of the granary weevil, *Sitophilus granarius* (L.). Pest Management Science, 68, 371-377.

Germinara, G. S., Beleggia, R., Fragasso, M., Pistillo, M. O., & De Vita, P., 2019. Kernel volatiles of some pigmented wheats do not elicit a preferential orientation in *Sitophilus granarius* adults. Journal of pest science, 92(2), 653-664.

Germinara, G. S., De Cristofaro, A., & Rotundo, G., 2008. Behavioral responses of adult *Sitophilus granarius* to individual cereal volatiles. Journal of Chemical Ecology, 34, 523-529.

Germinara, G. S., Rotundo, G., De Cristofaro, A., & Giacometti, R., 2002. Risposte elettroantennografiche di *Sitophilus granarius* (L.) e *S. zeamais* Motschulsky a sostanze volatili dei cereali. Tecnica Molitoria, 53, 27-34.

Germinara, G.S., Rotundo, G., and De Cristofaro, A., 2007. Repellence and fumigant toxicity of propionic acid against adults of *Sitophilus granarius* (L.) and *S. oryzae* (L.). Journal of Stored Products Research, 43(3), 229-233.

Golizadeh, A., Abedi, Z., 2017. Feeding performance and life table parameters of Khapra Beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) on various barley cultivars. Bulletin of Entomological Research, 107, 689-698.

Handford, C. E., Elliott, C. T., & Campbell, K., 2015. A review of the global pesticide legislation and the scale of challenge in reaching the global harmonization of food safety standards. Integrated environmental assessment and management, 11(4), 525-536.

Júnior, A. L. M., Vilarinho, A. A., de Paiva, W. R. S. C., & dos Santos Barreto, H. C., 2008. Resistência de híbridos de milho ao ataque de *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) em condições de armazenamento. Revista Acadêmica Ciência Animal, 6(1), 45-50.

Jyoti, J. L., Shelton, A. M., & Earle, E. D., 2001. Identifying sources and mechanisms of resistance in crucifers for control of cabbage maggot (Diptera: Anthomyiidae). Journal of Economic Entomology, 94(4), 942-949.

Kalsa, K.K., Subramanyam, B., Demissie, G., Mahroof, R., Worku, A., Gabbiye, N., ... and Abay, F., 2019. Susceptibility of Ethiopian wheat varieties to granary weevil and rice weevil infestation at optimal and sub-optimal temperatures. Journal of Stored Products Research, 83, 267-274.

Kocak, E., Schlipalius, D., Kaur, R., Tuck, A., Ebert, P., Collins, P., & Yilmaz, A., 2015. Determining phosphine resistance in rust red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) populations from Turkey. Turkish Journal of Entomology, 39, 129-136.

Kordan, B., Laszczak-Dawid, A., Nietupski, M., & Zuk-Golaszewska, K., 2007. Wplyw formy przechowywania pszenicy orkisz [*Triticum spelta* L.] na rozwoj wolka zbozowego [*Sitophilus granarius* L.]. Progress in Plant Protection, 47(1), 263-266.

Kordan, B., Nietupski, M., Ludwiczak, E., Gabryś, B., & Cabaj, R., 2023. Selected cultivar-specific parameters of wheat grain as factors influencing intensity of development of grain weevil *Sitophilus granarius* (L.). Agriculture, 13(8), 1492.

Koutroubas, S. D., Fotiadis, S., Damalas, C. A., & Papageorgiou, M., 2014. Grain-filling patterns and nitrogen utilization efficiency of spelt (*Triticum spelta*) under Mediterranean conditions. The Journal of Agricultural Science, 152(5), 716-730.

Kućerová, Z., and Stejskal, V., 1994. Susceptibility of wheat cultivar to postharvest losses caused by *Sitophilus granarias* (L.) (Coleoptera: Curculionidae)/Attraktivität von Weizensorten für *Sitophilus granarius* (Coleoptera: Curculionidae) und die dadurch verursachten Nachernteverluste. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection, 641-648.

Lanzanova, C., Agape, C., Castorina, G., Balconi, C., Alfieri, M., Locatelli, D. P., ... & Limonta, L., 2021. Are variations in kernel-related morphometric and chemical parameters correlated with differences in Sitophilus oryzae attack in maize?. Seed Science and Technology, 49(2), 93-105.

Lemic, D., Mikac, K. M., Genda, M., Jukić, Ž., & Pajač Živković, I. 2020. Durum wheat cultivars express different level of resistance to granary weevil, *Sitophilus Granarius* (Coleoptera; Curculionidae) infestation. Insects, 11(6), 343.

Lorini, I., Collins, P. J., Daglish, G. J., Nayak, M. K., & Pavic, H., 2007. Detection and characterisation of strong resistance to phosphine in Brazilian *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Pest Management Science: formerly Pesticide Science, 63(4), 358-364.

Maga, J. A., 1978. Cereal volatiles, a review. Journal of Agricultural and Food Chemistry, 26(1), 175-178.

Magan, N., Hope, R., Cairns, V., & Aldred, D., 2003. Post-harvest fungal ecology: impact of fungal growth and mycotoxin accumulation in stored grain. Epidemiology of Mycotoxin Producing Fungi: Under the aegis of COST Action 835 'Agriculturally Important Toxigenic Fungi 1998–2003', 723-730.

Majeed, M. Z., Mehmood, T., Javed, M., Sellami, F., Riaz, M. A., & Afzal, M., 2015. Biology and management of stored products' insect pest *Rhyzopertha dominica* (Fab.) (Coleoptera: Bostrichidae). International Journal of Biosciences, 7(5), 78-93.

Mattiolo, E., Licciardello, F., Lombardo, G. M., Muratore, G., & Anastasi, U., 2017. Volatile profiling of durum wheat kernels by HS–SPME/GC–MS. European Food Research and Technology, 243, 147-155.

McGaughey, W.H., Speirs, R.D., and Martin, C.R., 1990. Susceptibility of classes of wheat grown in the United States to stored-grain insects. Journal of Economic Entomology, 83(3), 1122-1127.

Mebarkia, A., Rahbé, Y., Guechi, A., Bouras, A., and Makhlouf, M., 2010. Susceptibility of twelve soft wheat varieties (*Triticum aestivum*) to *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Agriculture and Biology Journal of North America 1, 571-578.

Mefleh, M., Conte, P., Fadda, C., Giunta, F., Piga, A., Hassoun, G., and Motzo, R., 2019. From ancient to old and modern durum wheat varieties: Interaction among cultivar traits, management, and technological quality. Journal of the Science of Food and Agriculture, 99(5), 2059-2067.

Mostafalou, S., & Abdollahi, M., 2013. Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. Toxicology and applied pharmacology, 268(2), 157-177.

Naseri, B., & Majd-Marani, S., 2022. Different cereal grains affect demographic traits and digestive enzyme activity of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). Journal of Stored Products Research, 95, 101898.

Nawrot, J., Gawlak, M., Szafranek, J., Szafranek, B., Synak, E., Warchalewski, J.R., ... and Fornal, J., 2010. The effect of wheat grain composition, cuticular lipids and kernel surface microstructure on feeding, egg-laying, and the development of the granary weevil, *Sitophilus granarius* (L.). Journal of Stored Products Research, 46(2), 133-141.

Nawrot, J., Warchalewski, J.R., Piasecka-Kwiatkowska, D., Niewiada, A., Gawlak, M., Grundas, S.T., and Fornal, J., 2006. The effect of some biochemical and technological properties of wheat grain on granary weevil (*Sitophilus granarius* L.) (Coleoptera: Curculionidae) development. In Proceedings of the 9th International Working Conference on Stored Product Protection 15, pp. 400-407.

Nayak, M. K., Daglish, G. J., Phillips, T. W., & Ebert, P. R., 2020. Resistance to the fumigant phosphine and its management in insect pests of stored products: a global perspective. Annual Review of Entomology, 65, 333-350.

Nhamucho, E., Mugo, S., Gohole, L., Tefera, T., Kinyua, M., & Mulima, E., 2017. Resistance of selected Mozambican local and improved maize genotypes to maize weevil, *Sitophilus zeamais* (Motschulsky). Journal of Stored Products Research, 73, 115-124.

Niewiada, A., Nawrot, J., Szafranek, J., Szafranek, B., Synak, E., Jeleń, H., & Wąsowicz, E., 2005. Some factors affecting egg-laying of the granary weevil (*Sitophilus granarius* L.). Journal of Stored Products Research, 41(5), 544-555.

Padulosi, S., Hammer, K. & Heller, J. 1996: Hulled wheats. Proc. First Int. Workshop on Hulled Wheats, 21-22 July 1995, Castelvecchio Pascoli, Italy. Int. Plant Genetic Res. Inst., Rome, 1-262.

Perrino, P., 1993. The farro: an ancient crop to renew. Agricoltura, 21, 9-15.

Perrino, P., Hammer, K., Hanelt, P., 1981. Report of travels to South Italy 1980 for the collection of indigenous material of cultivated plants. Kulturpflanze, 29, 433-442.

Perrino, P., Hammer, K., Hanelt, P., 1984. Collection of land-races of cultivated plants in South Italy 1983. Kulturpflanze, 32, 207-216.

Perrino, P., Infantino, S., Laghetti, G., Volpe, N., & Dimarzio, A., 1991. Valutazione e selezione di farro in ambienti marginali dell'Appennino molisano. Informatore Agrario, 47.

Phillips, T. W., Jiang, X. L., Burkholder, W. E., Phillips, J. K., & Tran, H. Q., 1993. Behavioral responses to food volatiles by two species of stored-product Coleoptera, *Sitophilus oryzae* (Curculionidae) and *Tribolium castaneum* (Tenebrionidae). Journal of Chemical Ecology, 19, 723-734.

Piesik, D., & Wenda-Piesik, A., 2015. *Sitophilus granarius* responses to blends of five groups of cereal kernels and one group of plant volatiles. Journal of Stored Products Research, 62, 36-39.

Pike, V., Smith, J. L., White, R. D., & Hall, D. R., 1994. Studies of responses of stored-products pests, *Prostephanus truncatus* (Horn) and *Sitophilus zeamais Motsch*., to food volatiles.

In Proceedings of the 6th International Working Conference on Stored-Product Protection, CAB International, Wallingford, UK, pp. 566-569.

Plarre, R., 2013. An attempt to reconstruct the natural and cultural history of the granary weevil, *Sitophilus granarius* (Coleoptera: Curculionidae). European Journal of Entomology, 107(1), 1-11.

Potter, C., 1935. The biology and distribution of *Rhyzopertha dominica* (FAB.). Transactions of the Royal Entomological Society of London, 83, 449-482.

Rajendran, S., 2002. Postharvest pest losses. In: Pimentel, D. (Ed.), Encyclopedia of Pest Management. Marcel Dekker, Inc., New York, pp. 654-656.

Rajendran, S., and Sriranjini, V., 2008. Plant products as fumigants for stored-product insect control. Journal of stored products Research, 44(2), 126-135.

Reddy, G. V. P. & Guerrero, A., 2004. Interactions of insect pheromones and plant semiochemicals. Trends Plant Science, 9, 253-261.

Ren, Y., Wang, T., Wang, C., D'Isita, I., Hu, Q., Germinara, G. S., & Cao, Y., 2023. Population development of *Rhyzopertha dominica* (F.)(Coleoptera: Bostrichidae) on different stored products. Entomological Research, 53(10), 359-366.

Ren, Y., Wang, T., Wang, C., D'Isita, I., Hu, Q., Germinara, G. S., & Cao, Y. (2023). Population development of Rhyzopertha dominica (F.)(Coleoptera: Bostrichidae) on different stored products. *Entomological Research*, *53*(10), 359-366.

Ronga, D., Laviano, L., Catellani, M., Milc, J., Prandi, B., Boukid, F., ... & Francia, E., 2020. Influence of environmental and genetic factors on content of toxic and immunogenic wheat gluten peptides. European Journal of Agronomy, 118, 126091.

Rotundo, G., Germinara, G. S., & De Cristofaro, A., 2000. Immuno-osmophoretic technique for detecting *Sitophilus granarius* (L.) infestations in wheat. Journal of Stored Products Research, 36(2), 153-160.

Sakka, M. K., Jagadeesan, R., Nayak, M. K., & Athanassiou, C. G., 2022. Insecticidal effect of heat treatment in commercial flour and rice mills for the control of phosphine-resistant insect pests. Journal of Stored Products Research, 99, 102023.

Schwartz, B. E., & Burkholder, W. E., 1991. Development of the granary weevil (Coleoptera: Curculionidae) on barley, corn, oats, rice, and wheat. Journal of Economic Entomology, 84(3), 1047-1052.

Shazali, M.E.H., 1987. Weight loss caused by development of *Sitophilus oryzae* (L.) and *Sitotroga cerealella* (Oliv.) in sorghum grains of two size classes. Journal of stored products research, 23(4), 233-238.

Song, X., Wang, P., & Zhang, H., 2011. Phosphine resistance in *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) from different geographical populations in China. African Journal of Biotechnology, 10(72), 16367-16373.

Stoner, K. A., & Shelton, A. M. (1988). Role of non-preference in the resistance of cabbage varieties to the onion thrips (Thysanoptera: Thripidae). Journal of Economic Entomology, 81(4), 1062-1067.

Suleiman, R., Rosentrater, K.A., and Bern, C.J., 2015. Evaluation of maize weevils *Sitophilus zeamais* Motschulsky infestation on seven varieties of maize. Journal of Stored Products Research, 64, 97-102.

Thorsteinson A. J., 1958. The chemotactic influence of plant constituents on feeding by phytophagous insects. Entomologia Experimentalis et Applicata, 1(1), 23-27.

Toews, M.D., Cuperus, G.W., and Phillips, T.W., 2000. Susceptibility of eight US wheat cultivars to infestation by *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Environmental Entomology, 29(2), 250-255.

Toews, M.D., Cuperus, G.W., and Phillips, T.W., 2000. Susceptibility of eight US wheat cultivars to infestation by *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Environmental Entomology, 29(2), 250-255.

Trematerra, P., & Gentile, P., 2002. Stored insect pests in traditional cultivated hulled wheat crop areas of Central-Southern Italy with emphasis on *Sitotroga cerealella* (Olivier). IOBC WPRS BULLETIN, 25(3), 27-32.

Trematerra, P., Pistillo, O. M., Germinara, G. S., & Colacci, M., 2021. Bioactivity of cereal-and legume-based macaroni pasta volatiles to adult *Sitophilus granarius* (L.). Insects, 12(9), 765.

Tudi, M., Daniel Ruan, H., Wang, L., Lyu, J., Sadler, R., Connell, D., ... & Phung, D. T., 2021. Agriculture development, pesticide application and its impact on the environment. International journal of environmental research and public health, 18(3), 1112.

Tuet, W. Y., Pierce, S. A., Racine, M. C., Stone, S., Pueblo, E., Dukes, A., ... & Wong, B., 2021. Cardiopulmonary effects of phosphine poisoning: A preliminary evaluation of milrinone. Toxicology and Applied Pharmacology, 427, 115652.

Urrutia, R.I., Yeguerman, C., Jesser, E., Gutierrez, V.S., Volpe, M.A., and González, J.O.W., 2021. Sunflower seed hulls waste as a novel source of insecticidal product: Pyrolysis bio-oil bioactivity on insect pests of stored grains and products. Journal of Cleaner Production, 287, 125000.

Van Den Dool, H. A. N. D., & Kratz, P. D. (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal of chromatography, 11, 463.

Visser J. H., 1986. Host odor perception in phytophagous insects. Annual Review of Entomology, 31(1), 121-144.

Warchalewski, J.R., Gralik, J., Winiecki, Z., Nawrot, J., and Piasecka-Kwiatkowska, D., 2002. The effect of wheat α-amylase inhibitors incorporated into wheat-based artificial diets on development of *Sitophilus granarius* L., *Tribolium confusum* Duv., and *Ephestia kuehniella* Zell. Journal of Applied Entomology, 126(4), 161-168.

Željko J., Matković A., Liška A., i Jukić K., 2020. Resistance of different wheat cultivars to granary weevil (*Sitophilus granarius* L.). Glasnik Zaštite Bilja, 43(5), 34-41.

Zhou, M., Robards, K., Glennie-Holmes, M., & Helliwell, S., 1999. Analysis of volatile compounds and their contribution to flavor in cereals. Journal of agricultural and food chemistry, 47(10), 3941-3953.

Table 1. Grain quality traits of *T. aestivum* (Mec), *T. durum* (Ofanto), and de-hulled *T. dicoccum* (Padre Pio) and *T. spelta* (Benedetto).

Genotype	TKW[a] (g) (Mean ± S.E.)	PC[b] (% d.m.) (Mean ± S.E.)	Ha[c] (Mean ± S.E.)	Gluten index (Mean ± S.E.)
T. aestivum				
Mec	30.2 ± 0.72 c	11.8 ± 0.12 c	44.7 ± 0.75 d	67.8 ± 1.76 c
T. durum				
Ofanto	39.9 ± 0.42 b	12.1 ± 0.19 bc	89.0 ± 0.55 a	45.6 ± 1.66 d
T. dicoccum				
Padre Pio	45.8 ± 0.77 a	14.5 ± 0.15 a	85.9 ± 0.88 ab	70.5 ± 1.15 bc
T. spelta				
Benedetto	37.4 ± 0.66 b	13.8 ± 0.11 ab	55.6 ± 0.76 c	81.7 ± 1.44 a
F	64.612	125.641	975.613	84.827
df	4	4	4	4
P	< 0.001	< 0.001	< 0.001	< 0.001

In the same column, values with different letters are significantly different at $P < 0.05$.
[a]: Thousand-kernel weight; [b]: Protein content; [c]: Kernel hardness.

Table 2. Percentage of mortality during the oviposition period, number of F1 progeny, offspring produced by a parental female and the median development period of *S. granarius* adults reared on *T. aestivum* (Mec), *T. durum* (Ofanto), and hulled and de-hulled *T. dicoccum* (Padre Pio) and *T. spelta* (Benedetto).

Genotype	Mortality (%) (Mean ± S.E.)	F1 progeny (n.) (Mean ± S.E.)	Offspring/female (n.) (Mean ± S.E.)	Development period (days) (Mean ± S.E.)	Susceptibility Index (Mean ± S.E.)
T. aestivum					
Mec	2.78 ± 2.78 a	174.00 ± 20.78 b	53.17 ± 8.45 c	38.40 ± 0.42 a	13.41 ± 0.46 b
T. durum					
Ofanto	2.78 ± 2.78 a	214.00 ± 8.08 b	37.89 ± 1.25 b	37.85 ± 0.35 a	14.18 ± 0.23 b
T. dicoccum					
Padre Pio hulled	80.00 ± 12.53 c	0 a	0 a	-	-
Padre Pio de-hulled	53.33 ± 13.33 bc	21.80 ± 12.15 a	4.20 ± 2.46 a	38.92 ± 1.07 a	6.31 ± 1.41 a
T. spelta					
Benedetto hulled	63.33 ± 11.37 c	1.60 ± 0.81 a	0.27 ± 0.13 a	41.54 ± 3.06 a	2.98 ± 0.08 a
Benedetto de-hulled	13.33 ± 5.65 ab	24.80 ± 7.72 a	5.27 ± 1.83 a	40.57 ± 1.29 a	7.44 ± 0.76 a
F	9.372	88.319	57.663	1.159	17.796
df	5	5	5	5	4
P	< 0.001	< 0.001	< 0.001	0.373	< 0.001

In the same column, values with different letters are significantly different at $P < 0.05$.

Table 3. Percentage of mortality during the oviposition period, number of F1 progeny, offspring produced by a parental female and the median development period of *R. dominica* adults reared on *T. aestivum* (Mec), *T. durum* (Ofanto), and hulled and de-hulled *T. dicoccum* (Padre Pio) and *T. spelta* (Benedetto).

Genotype	Mortality (%) (Mean ± S.E.)	F1 progeny (n.) (Mean ± S.E.)	Offspring/female (n.) (Mean ± S.E.)	Development period (days) (Mean ± S.E.)	Susceptibility Index (Mean ± S.E.)
T. aestivum					
Mec	0 a	319.33 ± 3.84 c	65.48 ± 7.09 b	42.03 ± 0.19 a	13.72 ± 0.05 d
T. durum					
Ofanto	25.00 ± 12.73 ab	159.00 ± 18.33 abc	48.92 ± 8.39 b	43.93 ± 0.43 ab	11.51 ± 0.14 cd
T. dicoccum					
Padre Pio hulled	48.33 ± 14.77 ab	42.20 ± 5.22 a	17.19 ± 7.75 ab	44.81 ± 1.03 ab	8.29 ± 0.31 ab
Padre Pio de-hulled	11.67 ± 6.24 ab	212.00 ± 58.24 bc	34.24 ± 7.04 ab	44.31 ± 0.74 ab	11.79 ± 0.79 cd
T. spelta					
Benedetto hulled	58.33 ± 13.18 b	44.20 ± 6.87 a	14.10 ± 4.21 a	46.85 ± 0.67 b	7.98 ± 0.36 a
Benedetto de-hulled	61.67 ± 14.34 b	90.00 ± 7.14 ab	28.48 ± 6.84 ab	42.60 ± 1.03 a	10.57 ± 0.40 bc
F	5.241	6.723	3.946	4.017	14.474
df	5	5	5	5	5
P	0.003	0.001	0.012	0.011	< 0.001

In the same column, values with different letters are significantly different at $P < 0.05$.

Table 4. Linear regression analyses among grain quality and biological parameters measured for *S. granarius*.

S. granarius		Biological parameters measured				
		Mortality	F1 progeny	Offspring/female	Development period	Susceptibility Index
Grain quality traits	Ha	0.457	0.026	0.319	0.398	0.101
		P = 0.543	*P* = 0.974	*P* = 0.681	*P* = 0.602	*P* = 0.899
	TKW	0.790	0.484	0.732	0.045	0.593
		P = 0.210	*P* = 0.516	*P* = 0.268	*P* = 0.955	*P* = 0.407
	PC	0.861	0.946	0.967	0.630	0.980
		P = 0.139	*P* = 0.054	*P* = 0.033	*P* = 0.370	*P* = 0.020
	Gluten index	0.338	0.825	0.557	0.878	0.746
		P = 0.662	*P* = 0.175	*P* = 0.443	*P* = 0.122	*P* = 0.254

Table 5. Linear regression analyses among grain quality and biological parameters measured for *R dominica*.

R. dominica		Biological parameters measured				
		Mortality	F1 progeny	Offspring/female	Development period	Susceptibility Index
Grain quality traits	Ha	0.316	0.320	0.348	0.980	0.413
		P = 0.684	*P* = 0.680	*P* = 0.652	*P* = 0.020	*P* = 0.587
	TKW	0.053	0.449	0.703	0.933	0.585
		P = 0.947	*P* = 0.551	*P* = 0.297	*P* = 0.067	*P* = 0.415
	PC	0.548	0.462	0.883	0.456	0.590
		P = 0.452	*P* = 0.538	*P* = 0.117	*P* = 0.544	*P* = 0.410
	Gluten index	0.743	0.140	0.466	0.438	0.169
		P = 0.257	*P* = 0.860	*P* = 0.534	*P* = 0.562	*P* = 0.831

Table 6. Behavioural responses in two-choice pitfall bioassays of *S. granarius* male to odours emitted by kernels (200 g) of hulled *T. dicoccum* (Padre Pio) and *T. spelta* (Benedetto) *vs.* control air.

Genotype	Males	Kernel odours	Control air	Student's *t* test		Response index
	(Mean ± S.E.)	(Mean ± S.E.)	(Mean ± S.E.)	*t* value	*P* value	(Mean ± S.E.)
T. dicoccum						
Padre Pio	10.11 ± 0.45	8.11 ± 0.45	1.33 ± 0.33	10.956	< 0.001	67.40 ± 6.56 a
T. spelta						
Benedetto	10.25 ± 0.62	6.13 ± 0.61	2.50 ± 0.33	4.963	0.002	31.19 ± 7.13 b

In the same column, values with different letters are significantly different at $P < 0.05$.

Table 7. Behavioural responses in two-choice pitfall bioassays of *S. granarius* female to odours emitted by kernels (200 g) of hulled *T. dicoccum* (Padre Pio) and *T. spelta* (Benedetto) *vs.* control air.

Genotype	Females	Kernel odours	Control air	Student's *t* test		Response index
	(Mean ± S.E.)	(Mean ± S.E.)	(Mean ± S.E.)	*t* value	*P* value	(Mean ± S.E.)
T. dicoccum						
Padre Pio	9.44 ± 0.44	7.78 ± 0.43	1.22 ± 0.28	13.031	< 0.001	69.32 ± 4.30 a
T. spelta						
Benedetto	9.0 ± 0.93	6.25 ± 0.75	1.88 ± 0.58	4.034	0.005	43.72 ± 11.06 b

In the same column, values with different letters are significantly different at $P < 0.05$.

Table 8. Response of *R. dominica* male in a Y-tube olfactometer to different odour sources emitted by kernels (3 g) of hulled *T. dicoccum* (Padre Pio) and *T. spelta* (Benedetto) *vs.* control air.

Genotype	N[a]	First choice (Mean ± S.E.)			Number of minutes spent in arm (mean ± SE)			
		Treated[b]	X^2	*P* value	Treated	Control	*t* value	*P* value
T. dicoccum								
Padre Pio	37(36)	0.36	2.778	0.096	2.88 ± 0.7	3.19 ± 0.5	-0.276	0.784
T. spelta								
Benedetto	31(29)	0.48	0.034	0.853	2.75 ± 0.6	3.15 ± 0.7	-0.338	0.738

[a]Total sample size (*N*, number of individuals that made a choice in parentheses); [b] Proportion of individuals (of those that made a choice) that chose the treated arm first.

Table 9. Response of *R. dominica* female in a Y-tube olfactometer to different odour sources emitted by kernels (3 g) of hulled *T. dicoccum* (Padre Pio) and *T. spelta* (Benedetto) *vs.* control air.

Genotype	N[a]	First choice (Mean ± S.E.)			Number of minutes spent in arm (mean ± SE)			
		Treated[b]	X^2	*P* value	Treated	Control	*t* value	*P* value
T. dicoccum								
Padre Pio	33(30)	0.53	0.133	0.715	3.37 ± 0.7	2.85 ± 0.6	0.431	0.669
T. spelta								
Benedetto	39(33)	0.58	0.758	0.384	3.21 ± 0.6	2.76 ± 0.6	0.425	0.673

[a]Total sample size (*N*, number of individuals that made a choice in parentheses); [b] Proportion of individuals (of those that made a choice) that chose the treated arm first.

Table 10. VOCs levels detected in the head-space of hulled *T. dicoccum* (cv. Padre Pio) and *T. spelta* (cv. Benedetto).

Compounds	R.T.	RI Lit[1]	RI Exp[2]	Area (%) (Mean ± E.S.)	
				T. dicoccum Padre Pio	*T. spelta* Benedetto
Alcohols					
1-Butanol	3.634	668	677	-	3.8 ± 0.1
1-Pentanol	4.901	766	765	3.6 ± 0.01	3.7 ± 0.3
1-Hexanol	7,564	867	867	2.1 ± 0.1	1.6 ± 0.03
1-Heptanol	10.880	969	943	1.1 ± 0.04	1.1 ± 0.02
1-Hexanol, 2-ethyl-	12.891	1015	1003	1.3 ± 0.05	1.1 ± 0.1
1-Octanol	14.172	1060	1054	1.7 ± 0.05	1.3 ± 0.2
2-Nonanol	15.110	1098	1083	2.0 ± 0.2	1.8 ± 0.02
Phenylethyl Alcohol	15.598	1110	1103	1. 3± 0.1	1.5 ± 0.1
1-Nonanol	17.224	1169	1166	1.3 ± 0.02	1.0 ± 0.04
Total alcohols				14.3 ± 0.1	16.7 ± 0.1
Aldehydes					
Butanal, 3-methil	3.415	649	665	4.4 ± 0.2	3.6 ± 0.1
Butanal, 2-methyl	3.481	659	669	-	4.2 ± 0.01
Pentanal	3.834	698	709	5.2 ± 0.2	6.0 ± 0.02
Hexanal	5.675	800	803	7.7 ± 0.3	6.1 ± 0.7
2-Hexanal	7.156	805	813	1.1 ± 0.1	0.9 ± 0.03
Heptanal	8.631	894	901	1.9 ± 0.1	2.5 ± 0.1
Benzaldehyde	10.661	928	940	1.5 ± 0.02	2.1 ± 0.1
Octanal	11.990	1002	999	1.7 ± 0.1	1.4 ± 0.04
Phenylacetaldehyde	13.392	1043	1038	1.2 ± 0.01	1.0 ± 0.02
2-Octenal	13.794	1051	1051	1.2 ± 0.04	0.7 ± 0.03
Nonanal	15.269	1100	1094	7.3 ± 0.3	7.0 ± 0.7
2-Nonenal (E)-	16.951	1159	1146	1.0 ± 0.05	0.6 ± 0.1
Decanal	18.298	1207	1200	2.3 ± 0.1	1.2 ± 0.1
Undecanal	21.084	1308	1300	1.1 ± 0.1	0.8 ± 0.04
Tetradecanal	26.192	1606	1583	1.2 ± 0.1	0.9 ± 0.02
Total aldehydes				38.7 ± 0.1	39.1 ± 0.1
Alkanes					
Dodecane	18.079	1200	1191	0.8 ± 0.03	0.7 ± 0.03
Tridecane	20.871	1300	1273	1.5 ± 0.05	1.1 ± 0.1
Tetradecane	23.449	1400	1384	1.3 ± 0.04	0.8 ± 0.03
Pentadecane	30.647	1703	1699	1.0 ± 0.1	0.8 ± 0.1
Nonadecane	34.445	1899	1889	1.3 ± 0.4	0.6 ± 0.01
Eicosane	36.346	2000	1999	0.6 ± 0.02	0.5 ± 0.02

Total alkanes				6.5 ± 0.1	4.6 ± 0.05
Alkenes					
1-Dodecene	17.835	1187	1178	0.9 ± 0.1	-
1-Tetradecene	23.254	1389	1374	0.8 ± 0.01	0.6 ± 0.05
Total alkenes				1.8 ± 0.04	0.6 ± 0.02
Benzene derivatives					
Toluene	4.999	773	775	3.6 ± 0.3	3.4 ± 0.3
Benzene, ethyl-	7.443	851	847	0.9 ± 0.1	0.9 ± 0.04
p-Xylene	7.696	888	881	1.7 ± 0.1	1.7 ± 0.02
Total benzene derivatives				6.3 ± 0.2	6.0 ± 0.1
Ketones					
2-Heptanone	8.290	890	883	1.0 ± 0.1	0.7 ± 0.01
3-Octen-2-one	13.197	1038	1027	0.9 ± 0.03	0.6 ± 0.1
2-Nonanone	14.873	1079	1068	1.2 ± 0.04	0.7 ± 0.02
2-Undecanone	20.724	1273	1265	0.9 ± 0.1	0.7 ± 0.1
5,9-Undecadien-2-one, 6,10-dimethyl-, (E)-	24.820	1421	1408	1.2 ± 0.1	0.8 ± 0.1
2-Tridecanone	25.856	1494	1467	2.9 ± 0.05	1.6 ± 0.01
2-Pentadecanone	30.387	1698	1684	2.2 ± 0.2	0.9 ± 0.1
2-Heptadecanone	34.530	1900	1896	0.6 ± 0.1	0.6 ± 0.01
Total ketones				10.9 ± 0.1	6.8 ± 0.1
Lactones					
Butyrolactone	9.094	915	917	1.3 ± 0.01	0.9 ± 0.1
Total lactones				1.3 ± 0.01	0.9 ± 0.1
Organic acids					
Acetic Acid	2.962	633	623	11.5 ± 0.5	17.3 ± 0.2
Hexanoic acid	11.398	970	964	2.9 ± 0.04	2.5 ± 0.3
Total organic acids				14.4 ± 0.3	19.8 ± 0.2
Others					
Phenol	11.207	995	953	2.6 ± 0.2	2.1 ± 0.3
Furan, 2-pentyl	11.642	987	981	1.5 ± 0.1	1.4 ± 0.01
Total others				4.0 ± 0.1	3.5 ± 0.1
Terpenes					
α-pinene	9.759	917	931	0.9 ± 0.01	0.8 ± 0.02
Limonene	12.886	1030	1021	1.0 ± 0.1	1.2 ± 0.02
Total terpenes				1.8 ± 0.04	2.0 ± 0.02

^{1}RI$_{\text{Lit}}$= linear retention index from the literature; ^{2} RI$_{\text{Exp}}$= determined linear retention index against mixture of n-alkanes (C5–C40) on HP-5MS column

CRediT author statement

Ilaria D'Isita: Conceptualization, Investigation, Formal analysis, Writing - Original Draft.

Onofrio Marco Pistillo: Investigation, Formal analysis.

Sandra Pati: Formal analysis, Writing - Review & Editing.

Antonella Marta Di Palma: Writing - Review & Editing.

Pasquale De Vita: Conceptualization, Resources, Investigation, Formal analysis, Writing - Review & Editing.

Giacinto Salvatore Germinara: Conceptualization, Supervision, Formal analysis, Writing - Review & Editing.

8. Acceptance and utilization efficiency of a purple durum wheat genotype by *Sitophilus granarius* (L.)

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[1] Department of Agricultural Sciences, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Via Napoli 25, 71122 Foggia, Italy
[2] CREA Research Centre for Cereal and Industrial Crops, 71122 Foggia, Italy

*Corresponding author

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Acceptance and utilization efficiency of a purple durum wheat genotype by *Sitophilus granarius* (L.)

Ilaria D'Isita[1], Antonella Marta Di Palma[1], Pasquale De Vita[2] & Giacinto Salvatore Germinara[1]✉

The granary weevil (*Sitophilus granarius* L.) is a major primary pest of stored cereals throughout the world. Among the major classes of plant secondary metabolites, flavonoids can affect insect feeding behaviour and their growth rate. In this study, the susceptibility of an anthocyanin-rich purple durum wheat genotype (T1303) to the granary weevil was evaluated in comparison with two yellow durum (Ofanto) and bread (Mec) wheat varieties. The feeding response and food utilisation efficiency by adult insects was also investigated by calculating nutritional indices in whole flour disk bioassays. Different levels of susceptibility to granary weevil emerged among genotypes tested. The mean food consumption by an insect, F1 progeny, and female parental offspring calculated for the T1303 genotype were significantly lower than those of yellow kernel wheat varieties. Moreover, T1303 genotype induced deterrence in the adult insects as demonstrated by the positive values of the food deterrence index. Besides, relative grow rate and efficiency conversion of ingested food indices were negative for T1303 and positive for both yellow wheat varieties indicating respectively a decrease and an increase of insect body weight during the bioassays. Finally, a higher mortality rate was recorded for insects fed on T1303 flour disks compared to disks obtained from yellow wheat varieties. These results provide evidence for the antifeedant and toxic effects of anthocyanins present in the T1303 pericarp against the granary weevil. Overall, this study contributes new insights into the mechanisms of host acceptance and food utilization by *S. granarius* and would be useful to identify antifeedant flavonoids as well as to develop varietal resistance-based strategies against this pest.

Wheat is one of the most important food crops to human populations consumed worldwide[1]. Food security will depend on the ability to increase productivity while limiting losses during both cultivation[2] and postharvest[3]. Insect pests during product storage are an increasingly global problem[4]. In fact, postharvest wheat losses, due to pest attacks, are estimated to be about 10–15% of the global annual production[5,6] and, in some developing countries, they can reach 50% of the total harvest[7].

The granary weevil, *Sitophilus granarius* (L.) (Coleoptera, Curculionidae), is one of the most damaging primary pests of stored cereals worldwide. It can attack intact kernels and causes both severe quantitative and qualitative losses[5,8–10] due to larvae and adults feeding and commodities contamination with exuviae, excrements and mycotoxins that may result from insect-promoted fungal growth during storage[5,8–13].

The endophytic development of immature stages, the stringent legislation on the use of synthetic pesticides and the increasing consumer demand for safer food make the control of granary weevil very difficult[4,14]. Thus, sustainable control means as alternatives to chemical inputs during cereal storage are urgently needed[13,15]. Possible alternative control methods include the use of botanicals powders, extracts, and essential oils (EOs) of plant origin[13,16–23], semiochemicals[12,14,24], inert powder[25–31] and resistant varieties[32].

Food plant selection by phytophagous insects consists of food finding and food acceptance[33]. Volatile organic compounds (VOCs) play an important role in host finding because they are the first chemicals detected and used by insects to distinguish between suitable habitats and substrates and unsuitable ones[34–37]. The host-plant acceptance mainly depends on behavioural responses of insects to non-volatile plant chemical and physical features[37].

[1]Department of Agricultural Sciences, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Via Napoli 25, 71122 Foggia, Italy. [2]CREA Research Centre for Cereal and Industrial Crops, 71122 Foggia, Italy. ✉email: giacinto.germinara@unifg.it

Sitophilus granarius adults are able to perceive a wide range of volatile compounds[38] emitted by grains of several cereals[39,40] and are attracted to the odour blend of commercial wheat with yellow kernels[41,42]. Moreover, plant secondary metabolites are involved in insect-plant interactions from habitat selection to host acceptance[37,43–45].

Major class of secondary metabolites are flavonoids, including anthocyanins, which represent about 5–10% of the known secondary products in plants[46] and are significant cues in host recognition and host acceptance[43,44,47].

Several studies showed the dual nature of flavonoids as insect feeding stimulants or deterrents. Hamamura et al.[48] found sitosterol and the flavonol isoquercitrin as biting factors for the silkworm, *Bombyx mori* L. Moreover, flavonoids could have insect feeding stimulant activity[49–53]. By contrast, many flavonoids are deterrents to insects, may be deleterious if ingested and detrimental to their growth[37,44]. Flavonoids have also been implicated as plant compounds that stimulate or deter the female oviposition[43,44].

The increasing interest in the health benefits of flavonoids, has prompted plant breeders to increment the levels of these compounds in crops[54]. In this context, pigmented wheat genotypes that carry purple genes controlling anthocyanin pigmentation on grain pericarp are very interesting[55–57], due to the high nutritional value conferred to the final products[57–59].

In a previous study, marked differences in VOCs profile emerged within pigmented and yellow kernel of durum and bread wheat genotypes and adults of *S. granarius* did not exhibit a preferential orientation toward the odours of pigmented wheat genotypes during behavioural bioassays[60].

The aim of the present study was to investigate the influence of wheat anthocyanin pigments in host acceptance and utilization by *S. granarius* adults. To this end, intact kernel susceptibility tests and flour disks bioassays were carried out with two commercial yellow wheat varieties and a pigmented wheat genotype with a purple pericarp.

Materials and methods

Insects. *Sitophilus granarius* were reared for several generations on whole durum wheat kernels (var. Simeto) in cylindrical glass containers (Ø 15 × 15 cm) covered with a fine mesh net (0.5 mm). Colonies were maintained in the dark at 25 ± 2 °C and 60 ± 5% relative humidity. Mixed sexes 30-day-old adult beetles were used for intact kernel susceptibility tests and flour disks bioassays.

Plant materials and grain quality assessment. Three wheat genotypes were chosen for this study, including the bread wheat (*Triticum aestivum* L. subsp. *aestivum*) variety "Mec" (Marzotto/Combine) and two durum wheat [(*Triticum turgidum* L. subsp. *durum* (Desf.)] genotypes: "Ofanto" (Adamello/Appulo), an élite variety with high grain yield and wide adaptability to the Mediterranean basin[61], and a purple durum wheat genotype, "T1303" (USDA code PI 352395) with high levels of anthocyanins in the grain. The three wheat genotypes were grown simultaneously in a replicated (n = 10) field trial carried out at the CREA-CI of Foggia, Italy (41°28′ N, 15°32′ E; 75 m a.s.l.), on a clay-loam soil (Typic Chromoxerert) during the 2019–2020 growing season, using standard agronomic practices. The harvested wheat genotypes were analysed to determine the main qualitative and technological parameters and stored at low temperature (4 ± 1 °C) until needed for biological tests. Moisture of whole grains was determined using the single-stage air oven (Termostabil k3, Cavallo S.R.L., Milan, Italy) method (ASAE 2003). This method utilizes whole grain dried for 18 h at 130 °C to determine the moisture content. Thousand-kernel weight (TKW) was calculated from the mean weight of three sets of 500 grains per each wheat genotype. Protein content (PC) was determined by nitrogen combustion analysis according to Approved Method 46–30 (AACC International 2010) using a Dumas nitrogen analyzer (Leco Corp, St. Joseph, MI). The Single Kernel Characterization System 4100 (SKCS) (Perten Instruments, North America, Inc., Springfield, IL, USA) was used to characterize kernels hardness (Ha) using a sample of 300 kernels (Method 55-31) (AACC, 2010). A grain sample with 800 g of each sample were also milled by a Bona 4RB mill (Bona, Monza, Italy) after tempering according to their hardness. The flour obtained was used to perform alveograph test, according to ICC-Standard no. 121 (ICC, 1992). The variables of alveograph deformation energy (W) and curve configuration ratio [P/L-relation between dough tenacity (P) and extensibility (L)] were measured.

Total anthocyanin content (TAC) was evaluated using a colorimetric method with different pH solutions as reported by Ficco et al.[57]. Briefly, two aliquots of the supernatants extracted (750 µL) were put into different tubes and diluted (1:2, v/v) with either potassium chloride buffer (0.03 M KCl), for pH 1.00, or sodium acetate buffer (0.4 M $CH_3CO_2Na{\cdot}3H_2O$), for pH 4.50. The resulting samples were incubated for 30 min at room temperature in the dark and then filtered with 0.45 µm regenerated cellulose syringe filters. The absorbances of the samples at 520 nm were measured against distilled water as the blank. Total anthocyanin content was corrected for the dry matter and is expressed as Cy-3-Glc equivalents as micrograms per gram of dry matter.

In order to ensure the absence of live insects inside the wheat kernels to be used for biological bioassays, samples were frozen at − 20 °C for 72 h before the experiments.

Susceptibility tests. Susceptibility tests with intact kernels were performed on wheat samples conditioned for 7 days at 25 ± 2 °C, 60 ± 5% r.h. after frozen treatment. For each wheat genotype, not infested kernel samples (60 g) placed in cylindrical glass containers (Ø 9 × 14.5 cm) were infested with 12 *S. granarius* adults of mixed sexes. Containers were closed by screw caps and maintained in the incubator (Memmert GmbH + Co. KG IN 110 plus, Schwabach, Germania) at controlled conditions of photoperiod (L 0 : D 24), temperature (25 ± 2 °C), and relative humidity (60 ± 5%). For each wheat genotype there were 5 replicates. After 15 days exposure, insects were removed, sexed and the number of dead insects in each replicate was recorded. The F1 progeny was monitored by removing and counting newly emerged adults every 3 days. The experiment was terminated when no adults emerged for five consecutive days[62].

For each wheat genotype, the following parameters were calculated: (1) total number of F1 progeny; (2) median development period (D), estimated as the time, expressed in days, from the middle of the oviposition period to the emergence of 50% of the F1 generation[63]; (3) percentage of mortality during the oviposition period; (4) number of adult offspring per female; (5) percentage of weight loss $= Wi–Wf/Wi \times 100$ were $Wi =$ Initial dry weight and $Wf =$ Final dry weight[64]; (6) food consumption by an insect.

Flour disks bioassays. For each genotype, whole wheat flour was prepared by milling kernel samples (20 g) using a Tecator Cyclotec 1093 (International PBI, Milano, Italy) laboratory mill (1 mm screen-60 mesh). A sample (2.5 g) of each wheat flour was uniformly suspended in distilled water (8 mL) and stirred by a magnetic stirrer (MS-H280-PRO, DLAB Scientific Co., Beijing, China).

To obtain flour disks to be used in feeding bioassays, aliquots (200 µL) of suspension were dropped onto holes (Ø 1 cm, height 3 mm) of a rectangular support (15 cm × 15 cm) designed for this purpose and manufactured using a 3D printer (Zortrax S.A., Olsztyn, Poland). Then, the support was placed in a fume cupboard for 7 h until solid flour disks were obtained. Initial humidity of flour disks was stabilized overnight at 25 ± 2 °C in an airtight glass desiccator using a NaCl solution[65] that generated 60 ± 5% r.h.

In a pre-weighed glass vial (22 mL) two flour disks and 5 group-weighed weevil adults were introduced. Each vial was then re-weighed using an analytical balance (AS R2 PLUS series, Radwag Headquarters, Radom, Poland) and maintained in the incubator (Memmert GmbH + Co. KG IN 110 plus, Schwabach, Germania) at controlled conditions of photoperiod (L 0 : D 24), temperature (25 ± 2 °C), and relative humidity (60 ± 5%) for 6 days. For each genotype 5 replicates were set up. After 6 days, the glass vials with flour disks and alive insects were weighed again and the number of dead insects was recorded.

Six days after the experiment start, the adult weevil mortality rate (%) was calculated. Moreover, the following nutritional indices were calculated: Relative Consumption Rate $(RCR) = D/(B \times day)$, where $D =$ biomass ingested (mg)/No. of live insects on the sixth day; Relative Growth Rate $(RGR) = (A - B)/(B \times day)$, where $A =$ mean weight (mg) of live insects on the sixth day, $B =$ initial mean weight (mg) of insects; Efficiency Conversion of Ingested Food $(ECI) = (RGR/RCR) \times 100$; Feeding Deterrence Index (FDI) $(\%) = [(C - T)/C] \times 100$, where $C =$ consumption of control disks (Mec or Ofanto) and $T =$ consumption of disks of the genotype considered (T1303)[66,67].

Statistical analysis. Data were submitted to analysis of variance (ANOVA) followed by Tukey's HSD test for mean comparisons. Before ANOVA, data were submitted to Shapiro–Wilk's test to verify the normal distribution of data and to Levene's test to assess the homogeneity of variances. Statistical analyses were performed with SPSS (Statistical Package for the Social Sciences) v.18 for Windows (SPSS Inc., Chicago, IL).

Plants materials. Plant materials used in the present study are compliant with the local and national guidelines.

Ethics approval. This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent. Informed consent was obtained from all individual participants included in the study.

Results

Qualitative and technological parameters. The average values of the main qualitative and technological parameters determined on the wheat kernel samples immediately after harvesting are reported in Table 1. The average moisture values were the same for all the three wheat genotypes analyzed, while the other parameters showed statistically significant differences (TKW: $F = 8.254$ $df = 2$ $P = 0.019$; PC: $F = 19.397$ $df = 2$ $P = 0.002$; Ha: $F = 3309.80$ $df = 2$ $P = 0.000$; W: $F = 1393.00$ $df = 2$ $P = 0.000$; P/L: $F = 28.231$ $df = 2$ $P = 0.000$). The commercial durum wheat variety (Ofanto) showed the highest TKW and Ha values but its PC (13.5 ± 0.5%) was significantly lower than those of the other genotypes. The bread wheat variety Mec showed the lowest values of Ha, being a variety with medium/soft kernels, and the most suitable alveographic parameters for making bread (high value of W and low of P/L). The T1303 genotype was the only one with the presence of anthocyanins in the grain

Genotype (kernel color)	Moisture (%) (Mean ± S.E.)	TKW[a] (g) (Mean ± S.E.)	PC[b] (% d.m.) (Mean ± S.E.)	Ha[c]	TAC[d] (mg/kg) (Mean ± S.E.)	W-value[e] (10^{-4} Joule) (Mean ± S.E.)	P/L[f] (%) (Mean ± S.E.)
Bread wheat							
Mec (yellow)	10.3 ± 0.4 a	40.0 ± 1.2 a	16.1 ± 0.5 b	M = 44 ± 4 a	nd	190 ± 8 c	0.6 ± 0.02 a
Durum wheat							
Ofanto (yellow)	9.6 ± 0.4 a	46.0 ± 1.3 b	13.5 ± 0.5 a	H = 88 ± 3 b	nd	92 ± 6 b	1.2 ± 0.03 b
T1303 (purple)	10.4 ± 0.3 a	41.0 ± 1.5 a	15.4 ± 0.8 b	H = 85 ± 3 b	40.2 ± 0.03	45 ± 5 a	0.9 ± 0.04 ab

Table 1. Phenotypic characterization of the yellow varieties (Mec, Ofanto) and the pigmented genotype (T1303) evaluated in this study. In the same column, values with different letters are significantly different at $P < 0.05$. [a]Thousand-kernel weight. [b]Protein content. [c]Kernels hardness. [d]Total anthocyanin content. [e]Alveograph deformation energy. [f]Curve configuration ratio.

(40.2 ± 0.03 mg/kg), a high protein content (15.4 ± 0.8%), similar to Mec (16.1 ± 0.5%), and a Ha value (85 ± 3) similar to that of the Ofanto variety (88 ± 3).

Susceptibility tests. During the oviposition period, the mean mortality rate of *S. granarius* adults kept on the purple kernel genotype T1303 (16.7 ± 4.8%) was higher than those observed for the yellow wheat varieties, even if not significantly different ($F = 0.700$; $df = 2$; $P = 0.533$) (Table 2).

The number of F1 progeny emerged from the different genotypes showed significant differences ($F = 23.572$; $df = 2$; $P = 0.001$). The mean number of adults emerged from T1303 (67.0 ± 11.9) kernel samples was significantly lower than those obtained from Mec (213.7 ± 27.0) ($P < 0.05$; Tukey test) and Ofanto (213.0 ± 6.1) ($P < 0.05$; Tukey test) samples. This resulted in significant differences ($F = 18.455$; $df = 2$; $P = 0.003$) among the offspring originated by one parental female on different wheat genotypes (Table 2). By contrast, the median development period of *S. granarius* emerged from the purple wheat genotype T1303 (42.1 ± 0.7) was the highest but not significantly different ($F = 1.767$; $df = 2$; $P = 0.249$) compared to the yellow Mec (41.0 ± 0.6) and Ofanto (40.7 ± 0.4) varieties (Table 2).

The mean percentage of weight loss after the F1 emergence, corrected for changes in moisture content, showed significant differences ($F = 150.272$; $df = 2$; $P < 0.001$) among different genotypes (Table 3). The mean percentage of weight loss recorded for T1303 (1.46 ± 0.31%) was significantly lower ($P < 0.05$) than those observed for Mec (6.75 ± 0.31%) and Ofanto (7.02 ± 0.04%) varieties (Table 3). As a consequence, the mean food consumption by an insect emerged from the T1303 genotype (12.97 ± 0.44 mg) was significantly lower ($F = 13.835$; $df = 2$; $P = 0.006$) ($P < 0.05$; Tukey test) than those recorded for Mec (19.37 ± 1.57 mg) and Ofanto (19.82 ± 0.71 mg) varieties (Table 3).

Flour disk bioassays. The mean RCR value of insects fed with flour disks obtained from the purple wheat genotype T1303 (0.057 ± 0.004 mg/mg/day) was lower than that of insects fed on disks of yellow wheat varieties, even if not significantly different ($F = 1.095$; $df = 2$; $P = 0.366$) (Table 4).

Significant differences were found among the mean RGR and ECI indices calculated for different wheat genotypes ($F = 43.943$; $df = 2$; $P < 0.01$; $F = 65.863$; $df = 2$; $P < 0.01$, respectively). The mean RGR on flour disks from the T1303 purple genotype was negative (− 0.025 ± 0.004 mg/mg/day) and significantly lower ($P < 0.05$; Tukey test) than the positive RGR calculated for Mec and Ofanto (0.020 ± 0.003 and 0.023 ± 0.005 mg/mg/day, respectively) varieties (Table 4), indicating respectively a significant decrease and increase of the insect body weight during the experiment. As a consequence, the value of ECI index of T1303 flour disks (− 44.7 ± 6.2) was negative and significantly lower ($P < 0.05$; Tukey tests) than those of Mec (30.7 ± 4.2) and Ofanto (36.9 ± 6.2) flour disks (Table 4). Positive FDI indices were calculated for T1303 flour disks using the flour disk consumption of Mec (39.72 ± 4.60) or Ofanto (32.20 ± 5.12) as the control, indicating feeding deterrence (Table 4).

Six days after the experiment start, no mortality was observed for insects fed on Mec and Ofanto flour disks whereas a significant ($F = 36.000$; $df = 2$; $P < 0.001$) (P = 0.05; Tukey test) mean mortality rate (24.0 ± 4.0) was induced in insects fed on T1303 flour disks (Table 4).

Genotype (kernel color)	Adult mortality (%) (Mean ± S.E.)	F1 progeny (n.) (Mean ± S.E.)	Offspring/female (n.) (Mean ± S.E.)	Development period (days) (Mean ± S.E.)
Bread wheat				
Mec (yellow)	8.33 ± 4.81 a	213.67 ± 26.99 b	44.02 ± 4.23 b	40.99 ± 0.57 a
Durum wheat				
Ofanto (yellow)	11.11 ± 5.56 a	213.00 ± 6.11 b	47.67 ± 7.09 b	40.68 ± 0.41a
T1303 (purple)	16.66 ± 4.81 a	67.00 ± 11.93 a	10.47 ± 0.39 a	42.12 ± 0.67 a

Table 2. Percentage of mortality during the oviposition period, number of F1 progeny, offspring produced by a parental female and the median development period of *S. granarius* adults reared on yellow varieties (Mec, Ofanto) and pigmented genotype (T1303). In the same column, values with different letters are significantly different at $P < 0.05$.

Genotype (kernel color)	Final weight (mg) (Mean ± S.E.)	Weight loss % (Mean ± S.E.)	Food consumption by an insect (mg) (Mean ± S.E.)
Bread wheat			
Mec (yellow)	56.01 ± 0.19 a	6.75 ± 0.31 b	19.37 ± 1.57 b
Durum wheat			
Ofanto (yellow)	55.84 ± 0.04 a	7.02 ± 0.04 b	19.82 ± 0.71 b
T1303 (purple)	59.18 ± 0.21 b	1.46 ± 0.31 a	12.97 ± 0.44 a

Table 3. Damage by *S. granarius* on yellow (Mec, Ofanto) and pigmented (T1303) wheat genotypes. In the same column, values with different letters are significantly different at $P < 0.05$.

Genotype (kernel color)	Mortality (%) (mean ± S.E.)	FDI (%) (mean ± S.E.)		RCR (mg/mg/day) (mean ± S.E.)	RGR (mg/mg/day) (mean ± S.E.)	ECI (%) (mean ± S.E.)
		Mec	Ofanto			
Bread wheat						
Mec (yellow)	0 a	–		0.065 ± 0.002 a	0.020 ± 0.003 b	30.7 ± 4.2 b
Durum wheat						
Ofanto (yellow)	0 a		–	0.062 ± 0.005 a	0.023 ± 0.005 b	36.9 ± 6.2 b
T1303 (purple)	24.0 ± 4.0 b	39.7 ± 4.6	32.2 ± 5.1	0.057 ± 0.004 a	− 0.025 ± 0.004 a	− 44.7 ± 6.2 a

Table 4. Mortality, feeding deterrent index (FDI), relative consumption rate (RCR), relative growth rate (RGR), and efficiency conversion of ingested food (ECI) of *S. granarius* adults fed for six days on flour disks obtained from yellow bread (Mec) and durum (Ofanto) wheat varieties and pigmented wheat genotype (T1303). In the same column, values with different letters are significantly different at $P < 0.05$.

Discussion

Development of resistant wheat varieties to insect attacks during wheat grain storage is one of the most promising low-impact alternatives to insecticides in the management of stored grain pests[32]. Therefore, several studies aimed to develop antifeedant-based control means[44] as well as to identify new possible sources of resistance to the stored-product pests useful in breeding programs[68–71]. For instance, the results of bioassay using transgenic wheat plants, containing a modified avidin gene, challenged with granary weevil revealed 100% mortality of the insects showing high levels of resistance[72].

Pigmented wheat genotypes are characterized by a high antioxidant activity as well as large variations in the quality and composition of anthocyanins, that imparts purple, red or blue pigmentation in wheat[57]. Anthocyanins are involved in different kind of animal-plant interactions, which include the attraction of pollinators and frugivores and the repellence of herbivores and parasites[73].

The interest in the anthocyanins content of pigmented cereals has increased due to their benefit on the human health as nutraceutical ingredients and functional foods[74–76].

Early studies showed that polyphenol-rich pericarp purple corn extracts, containing anthocyanins, have several negative effects on the growth, development and fitness of different stages of *Manduca sexta* (L.) and *Spodoptera frugiperda* (JE Smith), suggesting their suitability as biopesticides[77–79]. By contrast, another study suggested that there are no differences in susceptibility to insect attacks during storage between white and red wheat varieties[80].

In the present study, susceptibility bioassays revealed a significant reduction in the total number of F1 progeny and female parental offspring emerged from purple wheat genotype compared with yellow wheat varieties. Moreover, insects fed on pigmented grains consumed less food substrate than those reared on yellow varieties, which resulted in a significantly lower damage. These results strongly suggested a different level of susceptibility among the purple genotype and yellow varieties studied.

It is known that host acceptance by stored cereal pests depends on both physical and chemical properties of grain kernels. Indeed, the susceptibility of various wheat cultivars to insect pests has been related to physical kernel features, such as water content, hardness and diameter of kernel, thousand-kernel weight, vitreosity[81,82] and content of some chemicals, such as protein or gluten, total lipids and cuticular lipids[83–88] that are function of genetic and environmental factors[89,90]. Besides, the role of pericarp cell wall components in cereal weevil resistance has also been one of the aspects studied in the past on various cereals[91,92]. However, several studies have shown that the different varietal response to external stress was much more likely caused by complex interactions between structural factors (proteins and polysaccharides) and phytochemicals present in the grain[93–96].

In our study, anthocyanins were contained exclusively in the pigmented genotype T1303 whereas qualitative and technological parameters of T1303 were similar to those of at least one of the two yellow wheat varieties. In fact, the pigmented T1303 genotype showed a thousand-kernel weight value and protein content similar to the yellow Mec variety and hardness values similar to the Ofanto variety.

In this contest, marked differences registered in population dynamics of *S. granarius* among yellow and purple kernel genotypes appeared to be associated with differences in the content of phenolic compounds, particularly anthocyanins. This is consistent with Kordan et al.[84], which showed that a greater presence of phenolics in wheat grain determines a higher adult mortality and a reduction of insect fitness and damage.

Thus, considering the possible role of pericarp anthocyanins in the susceptibility of T1303, Mec and Ofanto to *S. granarius*, flour disk bioassays, using whole flour of each genotype, were set up to definitively overcome the influence of physical features.

However, although small differences in the amounts of flour disk ingested, different food conversion efficiency were found among the purple and yellow genotypes. Indeed, RGR and ECI values were positive for the Mec and Ofanto varieties and negative for T1303 indicating respectively an increase and a decrease of insect body weight during the experiments. Besides, the positive FDI values calculated for T1303 using Mec or Ofanto as controls indicated actual feeding deterrence of the T1303 genotype. Lastly, after six-day exposure, the mortality percentages of *S. granarius* adults fed on T1303 wheat flour disks were significantly higher than those observed for insects fed on yellow wheat varieties.

On the whole, our results strongly suggested that the anthocyanins accumulated in the pericarp of T1303 kernels, evenly distributed in the whole flour used for flour disks preparation, determined antifeedant, deterrent and toxic effects against granary weevil adults that may explain the differences in susceptibility observed.

Certainly, a more thorough investigation will have to be conducted on other plant' secondary metabolites that could interfere with the metabolism of the insect[96].

In this context, our results pave the way to better understand the biological activity of the phenolic fraction of T1303 pericarp extracts and to identify their bioactive components. Finally, flour disk bioassays appear very promising for further wheat genetic investigation because they could be well suited to high-throughput analyses required by -omics approaches and, to accelerate the transfer of genes or genetic regions associated with resistance in a modern breeding program through marker-assisted selection. From a practical point of view, our results strongly suggest that purple wheat genotypes could be exploited in breeding programs to improve wheat resistance to the attacks of post-harvest stored pests, contributing to the alternative control options.

Data availability

All data generated or analysed during this study are included in this published article.

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References

1. Igrejas, G. & Branlard, G. The importance of wheat. In *Wheat Quality For Improving Processing And Human Health* 1–7 (Springer, Cham, 2020). https://doi.org/10.1007/978-3-030-34163-3.
2. Curtis, T. & Halford, N. G. Food security: The challenge of increasing wheat yield and the importance of not compromising food safety. *Ann. Appl. Biol.* **164**(3), 354–372. https://doi.org/10.1111/aab.12108 (2014).
3. Kumar D, Kalita P (2017) Reducing postharvest losses during storage of grain crops to strengthen food security in developing countries. Foods, 6(1): 8. In. Bortolini L. de O.F., Sartori M.R., Elias M.C., Guedes R.N.C., Fonseca R.G. da, Scussel V.M. (eds) Proceedings of the 9th international working conference on stored-product protection, ABRAPOS, Passo Fundo, pp 400–407. https://doi.org/10.3390/foods6010008
4. Phillips, T. W. & Throne, J. E. Biorational approaches to managing stored-product insects. *Annu. Rev. Entomol.* **55**, 375–397. https://doi.org/10.1146/annurev.ento.54.110807.090451 (2010).
5. Rajendran, S. Postharvest pest losses. In *Encyclopedia of Pest Management* (ed. Pimentel, D.) 654–656 (Marcel Dekker Inc., 2002).
6. Neethirajan, S., Karunakaran, C., Jayas, D. S. & White, N. D. G. Detection techniques for stored-product insects in grain. *Food Control* **18**(2), 157–162. https://doi.org/10.1016/j.foodcont.2005.09.008 (2007).
7. Fornal, J. *et al.* Detection of granary weevil *Sitophilus granarius* (L.) eggs and internal stages in wheat grain using soft X-ray and image analysis. *J. Stored Prod. Res.* **43**(2), 142–148. https://doi.org/10.1016/j.jspr.2006.02.003 (2007).
8. Sauer, D. B., Storey, C. L. & Walker, D. E. Fungal populations in U.S. farm-stored grain and their relationship to moisture, storage time, regions, and insect infestation. *Phytopathology* **74**(9), 1050–1053 (1984).
9. Magan, N., Hope, R., Cairns, V. & Aldred, D. Postharvest fungal ecology: Impact of fungal growth and mycotoxin accumulation in stored grain. *Eur. J. Plant Pathol.* **109**, 723–730. https://doi.org/10.1007/978-94-017-1452-5_7 (2003).
10. Plarre, R. An attempt to reconstruct the natural and cultural history of the granary weevil, *Sitophilus granarius* (Coleoptera: Curculionidae). *Eur. J. Entomol.* **107**(1), 1–11. https://doi.org/10.14411/eje.2010.001 (2010).
11. Dobie, P. & Kilminster, A. M. The susceptibility of triticale to postharvest infestation by *Sitophilus zeamais* Motschulsky, *Sitophilus oryzae* (L.) and *Sitophilus granarius* (L.). *J. Stored Prod. Res.* **14**(2–3), 87–93. https://doi.org/10.1016/0022-474X(78)90003-6 (1978).
12. Germinara, G. S., De Cristofaro, A. & Rotundo, G. Repellents effectively disrupt the olfactory orientation of *Sitophilus granarius* to wheat kernels. *J. Pest. Sci.* **88**(4), 675–684. https://doi.org/10.1007/s10340-015-0674-y (2015).
13. Germinara, G. S. *et al.* Bioactivities of *Lavandula angustifolia* essential oil against the stored grain pest *Sitophilus granarius*. *Bull. Insectol.* **70**(1), 129–138 (2017).
14. Germinara, G. S., De Cristofaro, A. & Rotundo, G. Behavioral responses of adult *Sitophilus granarius* to individual cereal volatiles. *J. Chem. Ecol.* **34**(4), 523–529. https://doi.org/10.1007/s10886-008-9454-y (2008).
15. Fields, P. G. & White, N. D. G. Alternatives to methyl bromide treatments for stored-product and quarantine insects. *Annu. Rev. Entomol.* **47**, 331–359 (2002).
16. Isman, M. B. Plant essential oils for pest and disease management. *Crop. Prot.* **19**(8–10), 603–608. https://doi.org/10.1016/S0261-2194(00)00079-X (2000).
17. Isman, M. B. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* **51**, 45–66. https://doi.org/10.1146/annurev.ento.51.110104.151146 (2006).
18. Tripathi, K. A., Upadhyay, S., Bhuiyan, M. & Bhattacharya, P. R. A review on prospects of essential oils as biopesticide in insect-pest management. *JPP* **1**, 52–63 (2009).
19. Isman, M. B., Miresmailli, S. & Machial, C. Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. *Phytochem. Rev.* **10**, 197–204. https://doi.org/10.1007/s11101-010-9170-4 (2011).
20. Pavela, R. History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects: A review. *Plant Prot. Sci.* **52**(4), 229–241. https://doi.org/10.17221/31/2016-PPS (2016).
21. Chaudhari, A. K., Singh, V. K., Kedia, A., Das, S. & Dubey, N. K. Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: Prospects and retrospects. *Environ. Sci. Pollut. Res.* **28**, 18918–18940. https://doi.org/10.1007/s11356-021-12841-w (2021).
22. Ntalli, N., Skourti, A., Nika, E. P., Boukouvala, M. C. & Kavallieratos, N. G. Five natural compounds of botanical origin as wheat protectants against adults and larvae of *Tenebrio molitor* L. and trogoderma granarium everts. *Environ. Sci. Pollut. Res.* **28**, 42763–42775. https://doi.org/10.1007/s11356-021-13592-4 (2021).
23. Paventi, G., Rotundo, G., Pistillo, M., D'Isita, I. & Germinara, G. S. Bioactivity of wild hop extracts against the granary weevil, *Sitophilus granarius* (L.). *Insects* **12**(6), 564. https://doi.org/10.3390/insects12060564 (2021).
24. Cao, Y. *et al.* Attraction of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) to the semiochemical volatiles of stored rice materials. *J. Pest. Sci.* https://doi.org/10.1007/s10340-023-01616-6 (2023).
25. Athanassiou, C. G. *et al.* Insecticidal efficacy of diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) on stored wheat: Influence of dose rate, temperature and exposure interval. *J. Stored Prod. Res.* **41**(1), 47–55. https://doi.org/10.1016/j.jspr.2003.12.001 (2005).
26. Kljajić, P. *et al.* Laboratory assessment of insecticidal effectiveness of natural zeolite and diatomaceous earth formulations against three stored-product beetle pests. *J. Stored Prod. Res.* **46**(1), 1–6. https://doi.org/10.1016/j.jspr.2009.07.001 (2010).
27. Kljajić, P., Andrić, G., Adamović, M. & Pražić-Golić, M. Possibilities of application of natural zeolites in stored wheat grain protection against pest insects. *J. Process Energy Agric.* **15**(1), 12–16 (2011).

28. Andrić, G. G. *et al.* Insecticidal potential of natural zeolite and diatomaceous earth formulations against rice weevil (Coleoptera: Curculionidae) and red flour beetle (Coleoptera: Tenebrionidae). *J. Econ. Entomol.* **105**(2), 670–678. https://doi.org/10.1603/EC11243 (2012).
29. Eroglu, N. A review: Insecticidal potential of Zeolite (Clinoptilolite), toxicity ratings and general properties of Turkish Zeolites. *IWCSPP* https://doi.org/10.14455/DOA.res.2014.116 (2014).
30. Rumbos, C. I., Sakka, M., Berillis, P. & Athanassiou, C. G. Insecticidal potential of zeolite formulations against three stored-grain insects, particle size effect, adherence to kernels and influence on test weight of grains. *J. Stored Prod. Res.* **68**, 93–101. https://doi.org/10.1016/j.jspr.2016.05.003 (2016).
31. Eroglu, N., Sakka, M. K., Emekci, M. & Athanassiou, C. G. Effects of zeolite formulations on the mortality and progeny production of *Sitophilus oryzae* and *Oryzaephilus surinamensis* at different temperature and relative humidity levels. *J. Stored Prod. Res.* **81**, 40–45. https://doi.org/10.1016/j.jspr.2018.11.004 (2019).
32. Throne, J. E., Baker, J. E., Messina, F. J., Karl, J. K. & Howard, J. A. Varietal resistance. In *Alternatives to Pesticides in Stored-Product IPM* (eds Subramanyam, B. & Hagstrum, D. W.) 165–192 (Kluwer Academic, 2000).
33. Thorsteinson, A. J. The chemotactic influence of plant constituents on feeding by phytophagous insects. *Entomol. Exp. Appl.* **1**(1), 23–27. https://doi.org/10.1111/j.1570-7458.1958.tb00005.x (1958).
34. Dickens, J. C. Olfaction in the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae): Electroantennogram studies. *J. Chem. Ecol.* **10**(12), 1759–1785. https://doi.org/10.1007/BF00987360 (1984).
35. Visser, J. H. Host odor perception in phytophagous insects. *Annu. Rev. Entomol.* **31**(1), 121–144 (1986).
36. Agelopoulos, N. *et al.* Exploiting semiochemicals in insect control. *Pest. Sci.* **55**(3), 225–235. https://doi.org/10.1002/(SICI)1096-9063(199903)55:3%3c225::AID-PS887%3e3.0.CO;2-7 (1999).
37. Bernays, E. A. & Chapman, R. F. *Host-Plant Selection by Phytophagous Insects* (Springer, 2007).
38. Germinara, G. S., Rotundo, G., Cristofaro, A. D. & Giacometti, R. Electroantennographic responses of *Sitophilus granarius* (L.) and *S. zeamais* Motschulsky to cereal volatiles. *Tecnica Molitoria* **53**(1), 37–34 (2002).
39. Maga, J. A. Cereal volatiles: A review. *J. Agric. Food Chem.* **26**, 175–178. https://doi.org/10.1094/CCHEM.1997.74.2.91 (1978).
40. Zhou, M., Robards, K., Glennie-Holmes, M. & Helliwell, S. Analysis of volatiles compounds and their contribution to flavor in cereals. *J. Agric. Food Chem.* **47**, 3941–3953. https://doi.org/10.1021/jf990428l (1999).
41. Levinson, H. Z. & Kanaujia, K. R. Phagostimulatory responses of male and female *Sitophilus granarius* to newly harvested and stored wheat grains. *Sci. Nat.* **68**, 44 (1981).
42. Germinara, G. S., Rotundo, G. & De Cristofaro, A. Repellence and fumigant toxicity of propionic acid against adults of *Sitophilus granarius* (L.) and *S. oryzae* (L.). *J. Stored Prod. Res.* **43**(3), 229–233. https://doi.org/10.1016/j.jspr.2006.06.002 (2007).
43. Simmonds, M. S. Importance of flavonoids in insect–plant interactions: Feeding and oviposition. *Phytochemistry* **56**(3), 245–252. https://doi.org/10.1016/S0031-9422(00)00453-2 (2001).
44. Harborne, J. B. & Grayer, R. J. Flavonoids and insects. In *The Flavonoids: Advances in Research Since 1986* 589–618 (Routledge, 2017).
45. Erb, M. & Kliebenstein, D. J. Plant secondary metabolites as defenses, regulators, and primary metabolites: The blurred functional trichotomy. *Plant Physiol.* **184**(1), 39–52. https://doi.org/10.1104/pp.20.00433 (2020).
46. Salunke, B. K., Kotkar, H. M., Mendki, P. S., Upasani, S. M. & Maheshwari, V. L. Efficacy of flavonoids in controlling *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae), a postharvest pest of grain legumes. *J. Crop Prot.* **24**(10), 888–893. https://doi.org/10.1016/j.cropro.2005.01.013 (2005).
47. Harborne, J. Flavonoid Pigments. In *Herbivores* (eds Rosenthal, G. A. & Janzen, D. H.) 619–656 (Academic Press, 1979).
48. Hamamura, Y. *et al.* Food selection by silkworm larvae. *Nature* **194**, 754–755 (1962).
49. Nielsen, J. K., Larsen, L. M. & Sørensen, H. Host plant selection of the horseradish flea beetle *Phyllotreta armoraciae* (Coleoptera: Chrysomelidae): Identification of two flavonol glycosides stimulating feeding in combination with glucosinolates. *Entomol. Exp. Appl.* **26**(1), 40–48. https://doi.org/10.1111/j.1570-7458.1979.tb02895.x (1979).
50. Besson, E. *et al.* C-glycosylflavones from *Oryza sativa*. *Phytochem* **24**(5), 1061–1064. https://doi.org/10.1016/S0031-9422(00)83183-0 (1985).
51. Klingauf, F. Die Wirkung des Glucosids Phlorizin auf das Wirtswahlverhalten von *Rhopalosiphum insertum* (Walk.) und *Aphis pomi* De Geer (Homoptera: Aphididae). *Zeitschrift für Angew Entomol.* **68**(1–4), 41–55. https://doi.org/10.1111/j.1439-0418.1971.tb03119.x (1971).
52. Bernays, E. A. Relationship between deterrence and toxicity of plant secondary compounds for the grasshopper *Schistocerca americana*. *J. Chem. Ecol.* **17**(12), 2519–2526. https://doi.org/10.1007/BF00994599 (1991).
53. de Boer, G. & Hanson, F. E. Feeding responses to solanaceous allelochemicals by larvae of the tobacco hornworm, *Manduca sexta*. *Entomol. Exp. Appl.* **45**(2), 123–131. https://doi.org/10.1111/j.1570-7458.1987.tb01071.x (1987).
54. Galili, G. New insights into the regulation and functional significance of lysine metabolism in plants. *Annu. Rev. Plan Biol.* **53**(1), 27–43 (2002).
55. Abdel-Aal, E. S. M. & Rabalski, I. Bioactive compounds and their antioxidant capacity in selected primitive and modern wheat species. *Open Agric.* https://doi.org/10.2174/1874331500802010007 (2008).
56. Knievel, E., Abdel-Aal, E. S. M., Rabalski, I. & Nakamura, T. Grain color development and the inheritance of high anthocyanin blue aleurone and purple pericarp in spring wheat (*Triticum aestivum* L.). *J. Cereal. Sci.* **50**, 113–120. https://doi.org/10.1016/j.jcs.2009.03.007 (2009).
57. Ficco, D. B. *et al.* Genetic variability in anthocyanin composition and nutritional properties of blue, purple, and red bread (*Triticum aestivum* L.) and durum (*Triticum turgidum* L. ssp. turgidum convar. durum) wheats. *J. Agric. Food Chem.* **62**(34), 8686–8695. https://doi.org/10.1021/jf5003683 (2014).
58. Ficco, D. B. M. *et al.* Use of purple durum wheat to produce naturally functional fresh and dry pasta. *Food Chem.* **205**, 187–195. https://doi.org/10.1016/j.foodchem.2016.03.014 (2016).
59. Ficco, D. B. M. *et al.* Effects of grain debranning on bioactive compounds, antioxidant capacity and essential and toxic trace elements in purple durum wheats. *LWT* **118**, 108734. https://doi.org/10.1016/j.lwt.2019.108734 (2020).
60. Germinara, G. S., Beleggia, R., Fragasso, M., Pistillo, M. O. & De Vita, P. Kernel volatiles of some pigmented wheats do not elicit a preferential orientation in *Sitophilus granarius* adults. *J. Pest Sci.* **92**(2), 653–664. https://doi.org/10.1007/s10340-018-1035-4 (2019).
61. De Leonardis, A. M. *et al.* Durum wheat genes up-regulated in the early phases of cold stress are modulated by drought in a developmental and genotype dependent manner. *Plant Sci.* **172**(5), 1005–1016. https://doi.org/10.1016/j.plantsci.2007.02.002 (2007).
62. Shazali, M. E. H. Weight loss caused by development of *Sitophilus oryzae* (L.) and *Sitotroga cerealella* (Oliv) in sorghum grains of two size classes. *J Stored Prod Res* **23**(4), 233–238. https://doi.org/10.1016/0022-474X(87)90007-5 (1987).
63. Dobie,. The laboratory assessment of the inherent susceptibility of maize varieties to postharvest infestation by Sitophilus zeamais Motsch (Coleoptera, Curculionidae). *J Stored prod res* **10**(3–4), 183–197. https://doi.org/10.1016/0022-474X(74)90006-X (1974).
64. Reed, C. The precision and accuracy of the standard volume weight method of estimating dry weight losses in wheat, grain sorghum and maize, and a comparison with the thousand grain mass method in wheat containing fine material. *J. Stored Prod. Res.* **23**(4), 223–231. https://doi.org/10.1016/0022-474X(87)90006-3 (1987).
65. Greenspan,. Humidity fixed points of binary saturated aqueous solutions. *J. Res. Natl. Inst.* **81**(1), 89–96. https://doi.org/10.6028/jres.081A.011 (1977).

66. Farrar, R. R., Barbour, J. D. & Kennedy, G. G. Quantifying food consumption and growth in insects. *Ann. Entomol. Soc. Am.* **82**(5), 593–598. https://doi.org/10.1093/aesa/82.5.593 (1989).
67. Huang, Y. & Ho, S. H. Toxicity and antifeedant activities of cinnamaldehyde against the grain storage insects, *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. *J. Stored Prod. Res.* **34**(1), 11–17. https://doi.org/10.1016/S0022-474X(97)00038-6 (1998).
68. Keneni, G. *et al.* Breeding food legumes for resistance to storage insect pests: Potential and limitations. *Sustainability* **3**(9), 1399–1415. https://doi.org/10.3390/su3091399 (2011).
69. Munyiri, S. W., Mugo, S. N., Otim, M., Mwololo, J. K. & Okori, P. Mechanisms and sources of resistance in tropical maize inbred lines to *Chilo partellus* stem borers. *J. Agric. Sci.* **5**(7), 51–60 (2013).
70. Nwosu, L. C. Chemical bases for maize grain resistance to infestation and damage by the maize weevil, *Sitophilus zeamais* Motschulsky. *J. Stored Prod Res.* **69**, 41–50. https://doi.org/10.1016/j.jspr.2016.06.001 (2016).
71. Locatelli, D. P., Castorina, G., Sangiorgio, S., Consonni, G. & Limonta, L. Susceptibility of maize genotypes to *Rhyzopertha dominica* (F.). *JDPD* **126**(6), 509–515. https://doi.org/10.1007/s41348-019-00250-8 (2019).
72. Abouseadaa, H. H. *et al.* Development of transgenic wheat (*Triticum aestivum* L.) expressing avidin gene conferring resistance to stored product insects. *BMC Plant Biol.* **15**(1), 1–8. https://doi.org/10.1186/s12870-015-0570-x (2015).
73. Lev-Yadun S, Gould KS (2008) Role of anthocyanins in plant defense. ACNs 22–28. https://doi.org/10.1007/978-0-387-77335-3_2
74. Choi, Y., Jeong, H. S. & Lee, J. Antioxidant activity of methanolic extracts from some grains consumed in Korea. *Food Chem.* **103**(1), 130–138. https://doi.org/10.1016/j.foodchem.2006.08.004 (2007).
75. Asem, I. D., Imotomba, R. K., Mazumder, P. B. & Laishram, J. M. Anthocyanin content in the black scented rice (Chakhao): Its impact on human health and plant defense. *Symbiosis* **66**(1), 47–54. https://doi.org/10.1007/s13199-015-0329-z (2015).
76. Iannucci, A., Suriano, S., Cancellaro, S. & Trono, D. Anthocyanin profile and main antioxidants in pigmented wheat grains and related millstream fractions. *Cereal Chem.* **99**(6), 1282–1295. https://doi.org/10.1002/cche.10591 (2022).
77. Tayal, M., Somavat, P., Rodriguez, I., Martinez, L. & Kariyat, R. Cascading effects of polyphenol-rich purple corn pericarp extract on pupal, adult, and offspring of tobacco hornworm (*Manduca sexta* L.). *Commun. Integr.. Biol* **13**(1), 43–53. https://doi.org/10.1080/19420889.2020.1735223 (2020).
78. Tayal, M. *et al.* Polyphenol-rich purple corn pericarp extract adversely impacts herbivore growth and development. *Insects* **11**(2), 98. https://doi.org/10.3390/insects11020098 (2020).
79. Singh, S. & Kariyat, R. R. Exposure to polyphenol-rich purple corn pericarp extract restricts fall armyworm (*Spodoptera frugiperda*) growth. *Behaviour* **15**(9), 1784545. https://doi.org/10.1080/15592324.2020.1784545 (2020).
80. White, N. D., Demianyk, C. J. & Fields, P. G. Effects of red versus white wheat bran on rate of growth and feeding of some stored-product beetles. *Can. J. Plant Sci.* **80**(3), 661–663. https://doi.org/10.1146/annurev.en.31.010186.001005 (2000).
81. Nawrot J, Warchalewski JR, Piasecka-Kwiatkowska D, Niewiada A, Gawlak M, Grundas ST, Fornal J (2006) The effect of some biochemical and technological properties of wheat grain on granary weevil (*Sitophilus granarius* L.) (Coleoptera: Curculionidae) development. In *Proceedings of the 9th International Working Conference on Stored Product Protection* (Vol 15), 400–407.
82. Fourar-Belaifa, R., Fleurat-Lessard, F. & Bouznad, Z. A systemic approach to qualitative changes in the stored-wheat ecosystem: Prediction of deterioration risks in unsafe storage conditions in relation to relative humidity level, infestation by *Sitophilus oryzae* (L.), and wheat variety. *J. Stored Prod. Res.* **47**(1), 48–61. https://doi.org/10.1016/j.jspr.2010.09.002 (2011).
83. Kućerová Z, Stejskal V (1994) Susceptibility of wheat cultivar to postharvest losses caused by *Sitophilus granarias* (L.) (Coleoptera: Curculionidae)/Attraktivität von Weizensorten für *Sitophilus granarius* (Coleoptera: Curculionidae) und die dadurch verursachten Nachernteverluste. JPDP 641–648.
84. Kordan, B. *et al.* Phenolic and lipophilic compounds of wheat grain as factors affecting susceptibility to infestation by granary weevil (*Sitophilus granarius* L.). *JABFQ* **92**, 64–72. https://doi.org/10.5073/JABFQ.2019.092.009 (2019).
85. Nawrot, J. Podstawy do zwalczania wołka zbożowego (*Sitophilus granarius* L.) (Coleoptera: Curculionidae) przy użyciu naturalnych związków chemicznych wpływających na zachowanie się chrząszczy. *Prace Nauk. Instytut Ochrony Roślin* **24**, 174–197 (1983).
86. Niewiada, A. *et al.* Some factors affecting egg-laying of the granary weevil (*Sitophilus granarius* L.). *J. Stored Prod. Res.* **41**(5), 544–555. https://doi.org/10.1016/j.jspr.2004.11.001 (2005).
87. Mebarkia, A., Yvan Rahbé, A., Guechi, A. & Bouras, M. M. Susceptibility of twelve soft wheat varieties (*Triticum aestivum*) to *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). *ABJNA* **1**(4), 571–578 (2010).
88. Nawrot, J., Gawlak, M., Szafranek, J. & Fornal, J. The effect of wheat grain composition, cuticular lipids and kernel surface microstructure on feeding, egg-laying, and the development of the granary weevil, *Sitophilus granarius* (L.). *J. Stored Prod. Res.* **46**, 133–141. https://doi.org/10.1016/j.jspr.2010.02.001 (2010).
89. Georget, D. M., Underwood-Toscano, C., Powers, S. J., Shewry, P. R. & Belton, P. S. Effect of variety and environmental factors on gluten proteins: An analytical, spectroscopic, and rheological study. *J. Agric. Food Chem.* **56**(4), 1172–1179. https://doi.org/10.1021/jf072443t (2008).
90. Ronga, D. *et al.* Influence of environmental and genetic factors on content of toxic and immunogenic wheat gluten peptides. *Eur. J. Agron.* **118**, 126091. https://doi.org/10.1016/j.eja.2020.126091 (2020).
91. García-Lara, S. *et al.* The role of pericarp cell wall components in maize weevil resistance. *Crop Sci.* **44**(5), 1546–1552. https://doi.org/10.2135/cropsci2004.1546 (2004).
92. Santiago, R., Barros-Rios, J. & Malvar, R. A. Impact of cell wall composition on maize resistance to pests and diseases. *Int. J. Mol. Sci.* **14**(4), 6960–6980. https://doi.org/10.3390/ijms14046960 (2013).
93. Akpodiete, O. N., Lale, N. E. S., Umeozor, O. C. & Zakka, U. Role of physical characteristics of the seed on the stability of resistance of maize varieties to maize weevil (*Sitophilus zeamais* Motschulsky). *IOSR J. Environ. Sci. Toxicol.* **9**(2), 60–66. https://doi.org/10.9790/2402-09226066 (2015).
94. Arnason, J. T. *et al.* Role of phenolics in resistance of maize grain to the stored grain insects, *Prostephanus truncatus* (Horn) and *Sitophilus zeamais* (Motsch.). *J. Stored Prod. Res.* **28**(2), 119–126. https://doi.org/10.1016/0022-474X(92)90019-M (1992).
95. Saulnier, L. & Thibault, J. F. Ferulic acid and diferulic acids as components of sugar-beet pectins and maize bran heteroxylans. *J. Sci. Food Agric.* **79**(3), 396–402. https://doi.org/10.1002/(SICI)1097-0010(19990301)79:3%3c396::AID-JSFA262%3e3.0.CO;2-B (1999).
96. Abdel-Aal, E. S. M., Hucl, F. W., Sosulski, R., Gillott, C. & Pietrzak, L. Screening spring wheat for midge resistance in relation to ferulic acid content. *J. Agric. Food Chem.* **49**(8), 3559–3566. https://doi.org/10.1021/jf010027h (2001).

Author contributions

I.D.I., P.D.V. and G.S.G. conceived and designed research. P.D.V. provided the plant materials and performed grain quality assessment. I.D.I. conducted the susceptibility tests and the flour disks bioassays. I.D.I., A.M.D.P., P.D.V. and G.S.G. analysed data. All authors wrote, read and approved the manuscript. The authors accepted that the paper is submitted for publication in the *Scientific Reports* and report that this paper has not been published or accepted for publication in another journal, and it is not under consideration at another journal.

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Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to G.S.G.

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9. Population development of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) on different stored products

Yanling Ren[1], Tao Wang[1], Chun Wang[2], Ilaria D'Isita[3], Qiqi Hu[2], Giacinto Salvatore Germinara[3]* and Yu Cao[2*]. *Entomological Research, 2023, 53(10), 359-366.*
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[1] Guizhou Light Industry Technical College, Guiyang, China
[2] Guizhou Provincial Key Laboratory for Rare Animal and Economic Insect of the Mountainous Region, Key Laboratory of Surveillance and Management of Invasive Alien Species in Guizhou Education Department, Department of Biology and Engineering of Environment, Guiyang, University, Guiyang, China
[3] Department of Agricultural Sciences, Food, Natural Resources and Engineering, University of Foggia, Foggia, Italy

*Corresponding author

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RESEARCH PAPER

Population development of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) on different stored products

Yanling REN[1,†], Tao WANG[1,†], Chun WANG[2], Ilaria D'ISITA[3], Qiqi HU[2], Giacinto Salvatore GERMINARA[3] and Yu CAO[2]

[1] Guizhou Light Industry Technical College, Guiyang, China
[2] Guizhou Provincial Key Laboratory for Rare Animal and Economic Insect of the Mountainous Region, Key Laboratory of Surveillance and Management of Invasive Alien Species in Guizhou Education Department, Department of Biology and Engineering of Environment, Guiyang University, Guiyang, China
[3] Department of Agricultural Sciences, Food, Natural Resources and Engineering, University of Foggia, Foggia, Italy

Correspondence
Giacinto Salvatore Germinara, Dipartimento di Scienze Agrarie, Alimenti, Risorse Naturali e Ingegneria (DAFNE), Università di Foggia, Via Napoli, 25, 71122 Foggia FG, Italy.
Email: giacinto.germinara@unifg.it

Yu Cao, Guizhou Provincial Key Laboratory for Rare Animal and Economic Insect of the Mountainous Region, Key Laboratory of Surveillance and Management of Invasive Alien Species in Guizhou Education Department, Department of Biology and Engineering of Environment, Guiyang University, HPQ7+M78, Shanxi Rd, Yunyan District, Guiyang 550003, China.
Email: yucaosuccess@126.com

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[†] Yanling Ren and Tao Wang contributed equally to this work.

Abstract

The lesser grain borer, *Rhyzopertha dominica*, is an important pest of various stored products around the world. In this study, the development, survival, reproduction, and life table parameters of *R. dominica* were investigated on six stored products (angelica, jujube, maize, rice, soybean, and wheat). The developmental time of the immature stage of *R. dominica* was shortest on wheat (40.20 days) and longest on angelica (67.04 days). The survival rate of the immature stage was highest on wheat (76.33%) and lowest on angelica (41.00%). The fecundity level of *R. dominica* was highest on wheat (246.05 eggs/female) and lowest on angelica (69.38 eggs/female). The net reproductive rate (R_0) and intrinsic rate of increase (r_m) of *R. dominica* differed significantly among the six stored products. The highest R_0 of *R. dominica* was on wheat (68.50), followed by rice (41.28), maize (32.32), soybean (27.17), jujube (23.16), and angelica (20.18); the r_m values showed a similar trend, with values of 0.059, 0.046, 0.042, 0.039, 0.036, and 0.033, respectively. Our results indicate that wheat was the most suitable stored product, whereas angelica was the least suitable, for the feeding, development, and population increase of *R. dominica*. These findings provide basic information about the occurrence trends and characteristics of *R. dominica* that will be useful for the control of this pest on different stored products. The physicochemical properties of angelica should be further explored for potential application in the control or integrated management of *R. dominica*.

Key words: lesser grain borer, life history, population development, stored product, suitable host

Introduction

Rhyzopertha dominica F (Coleoptera: Bostrichidae) is an extremely destructive insect pest of stored grains globally, especially in temperate and tropical areas (Edde 2012). It infests fresh and processed food resources produced from 53 plant species in 31 families (Buonocore *et al*. 2017; Dissanayaka *et al*. 2020), and has also been recorded on a variety of raw and processed food products, including cereals and pulses (Sinha & Watters 1985; Cinco-Moroyoqui *et al*. 2006). It feeds on crops such as legumes, tubers, and bulbs (Rees 2004), products of medical importance (Dissanayaka *et al*. 2020; Pappathi *et al*. 2021), and animal feed (Wijayaratne *et al*. 2019). Therefore, *R. dominica* can cause damage and economic losses to a wide range of stored products.

A common way to control stored product pests is to use gaseous chemical insecticides, such as phosphine (Bajracharya *et al*. 2016; Collins *et al*. 2017), and broad-spectrum organophosphorus insecticides (Rumbos *et al*. 2016; Kavallieratos *et al*. 2017; Paudyal *et al*. 2017) or sprays (Furlong *et al*. 2013; Miliordos *et al*. 2017). However, these chemical control methods can lead to problems, such as the development of insecticide resistance in pests and residues that are toxic to humans, animals, and non-target organisms (Finkelman *et al*. 2006; Rossi *et al*. 2010; Nayak *et al*. 2020). Therefore, it is necessary to minimize the use of chemical insecticides and search for alternative methods to control pests in stored products. In addition to this general problem, it should be noted that the control of *R. dominica* with insecticides is even more difficult because of the endophytic development of the larval and pupal stages.

The use of host plants that do not support the growth and reproductive performance of insects is one of the most economical tools to minimize the losses caused by pests (Lawrence & Koundal 2002; Parde *et al*. 2010). This may be related to certain resistance factors in host plants, e.g. physicochemical properties, and the inability of pests to adapt to these factors (Rahimi-Alangi & Bandani 2013; Ebadollahi & Borzoui 2019).

Different wheat varieties show varying degrees of resistance to *R. dominica* (Watts & Dunkel 2003), and therefore stored wheat exposed to insect pests is infested to a lesser or greater extent, showing variable degrees of kernel damage. This means that different stored products may reasonably affect the demographic performance of storage insect pests. In this study, the development, survival, oviposition rate, and other fitness-related characteristics of *R. dominica* on different stored products (angelica, jujube, maize, rice, soybean, and wheat) were investigated, and the population development and dynamics of *R. dominica* were assessed according to life table parameters. These results provide basic data that will be useful for predicting the occurrence of *R. dominica* on different stored products, for developing strategies to control this pest on economically important stored goods, and for further research to identify plant resistance factors against this pest.

Materials and methods

Insect rearing

Rhyzopertha dominica has been reared at the Guizhou Provincial Key Laboratory for Rare Animal and Economic Insect of the Mountainous Region, Guiyang University, China, since 2019. Before the experiments, the beetles were maintained on sorghum in 5-L glass jars at 30 ± 1°C, 60% ± 5% relative humidity, and a 8 h light : 16 h dark (L:D = 8:16) photoperiod, as reported by Li *et al*. (2009) and Wang *et al*. (2022).

Stored products

Six different stored cereal products, i.e. angelica (*Angelicae sinensis*), jujube (*Ziziphus jujuba* Mill. var. Jinsi No. 4), maize (*Zea mays* L. var. Xinzhongyu No. 667), rice (*Oryza sativa* L. var. Yixiangyou No. 2115), soybean (*Glycine max* L. var. Qiandou No. 12), and wheat (*Triticum aestivum* L. var. Guinong No. 9) (with moisture contents of 13%–15%, 20%–25%, 13%–14%, 12%–14%, 12%–13%, and 12%–13%, respectively) were purchased from the Guiyang Grain Commodity Market (Guiyang City, Guizhou Province, China), and were kept without pesticides. None of the materials was infested with other pests.

Development and survival of immature stages of *R. dominica*

Groups of 150 *R. dominica* (males and females) were placed into 2.5-L glass jars to oviposit for 24 h. Each jar contained 20.0 g of one of the six stored products. After ovipositing, the beetles were removed, and newly laid eggs were collected from the culture (50 eggs for each stored material). The eggs were carefully transferred into Petri dishes (60 mm in diameter, 15 mm in height) containing 10.0 g of each stored material (Naseri & Majd-Marani 2022). All eggs were checked daily, and the duration and survival rate of immature stages (egg hatching, larval, and pupal periods) were recorded. Three replicates were analyzed for each stored material.

Fecundity

Newly emerged male and female beetles from different stored materials in the above experiment were used to assess fecundity. All adult beetles were coupled and each pair (1 female : 1 male) was transferred into a single Petri dish (6 mm in diameter, 15 mm in height) containing 20.0 g of uninfested stored product for ovipositing. The adults were transferred daily to new Petri dishes containing fresh stored product, and all products were checked daily to assess the egg-laying. Fecundity (number of eggs laid during the reproductive time) was recorded for each individual until death. The offspring were reared to adulthood for sex determination and to establish the tertiary sex ratio of the offspring. If any male beetle died earlier than its mate, a replacement was supplied from the mass rearing colony. These reproduction assays were conducted with three replicates of

20–25 pairs of beetles on each stored product (with a total of 60–75 pairs per product).

Life table parameters

Based on the survivorship and reproductive data, life tables for *R. dominica* were constructed according to the methods of Carey (1993) and Naseri and Majd-Marani (2022). The life table parameters were calculated as follows: (i) net reproductive rate $R_0 = \sum l_x m_x$; (ii) intrinsic rate of increase $r_m = \frac{\ln R_0}{T}$; (iii) finite rate of increase $\lambda = e^{r_m}$; (iv) mean generation time $T = \sum x l_x m_x / R_0$; and (v) population doubling time $DT = \frac{\ln 2}{r_m}$, where x represents the time interval in units per day, l_x represents the population survival rate of *R. dominica* during the time x, which is the survival probability for a specific time, and m_x represents the average number of female offspring produced by each female beetle.

Statistical analysis

Data were analyzed using SPSS 22.0 (SPSS, Chicago, IL, USA). One-way analyses of variance followed by Tukey's honestly significant difference (HSD) test were used to detect significant differences ($P < 0.05$) in developmental time, survival rate, fecundity, and life table parameters of *R. dominica* among the six stored products.

Results

Development

Rhyzopertha dominica exhibited significant differences in the developmental periods for eggs ($F_{5,12} = 156.84$, $P < 0.01$), larvae ($F_{5,12} = 509.24$, $P < 0.01$), and pupae ($F_{5,12} = 155.90$, $P < 0.01$) among the six stored products (Table 1). There were also significant differences in the duration of development from egg to adult ($F_{5,12} = 499.59$, $P < 0.01$) among the six stored products. The duration of development from egg to adult was 40.20 days on wheat, 48.05 days on rice, 55.20 days on maize, 59.97 days on soybean, 63.64 days on jujube, and 67.04 days on angelica.

Survival

The survival rate of the immature stage (egg to adult) of *R. dominica* ($F_{5,12} = 533.56$, $P < 0.01$) differed significantly among the six stored products (Fig. 1). The highest survival rate of the immature stage of *R. dominica* was 76.33% on wheat, followed by 65.33% on rice, 54.33% on maize,

Table 1 Developmental time of *Rhyzopertha dominica* raised on different stored products

Diet	Egg incubation	Larval period	Pupal period	Immature period
Wheat	5.85 ± 0.17^{d}	30.31 ± 0.85^{f}	4.90 ± 0.14^{e}	40.20 ± 0.37^{f}
Rice	6.62 ± 0.33^{c}	35.36 ± 0.42^{e}	5.73 ± 0.21^{d}	48.05 ± 0.29^{e}
Maize	8.29 ± 0.22^{b}	40.25 ± 0.53^{d}	6.57 ± 0.21^{c}	55.20 ± 0.20^{d}
Soybean	8.80 ± 0.20^{ab}	42.80 ± 0.30^{c}	8.14 ± 0.20^{b}	59.97 ± 0.81^{c}
Jujube	9.06 ± 0.06^{a}	45.65 ± 0.43^{b}	8.64 ± 0.07^{ab}	63.64 ± 0.20^{b}
Angelica	9.34 ± 0.08^{a}	48.47 ± 0.38^{a}	9.04 ± 0.08^{a}	67.04 ± 0.10^{a}

Data are expressed as mean ± standard error (SE). Different superscript letters in the same column indicate significant differences (one-way analysis of variance followed by Tukey's HSD test, $P < 0.05$).

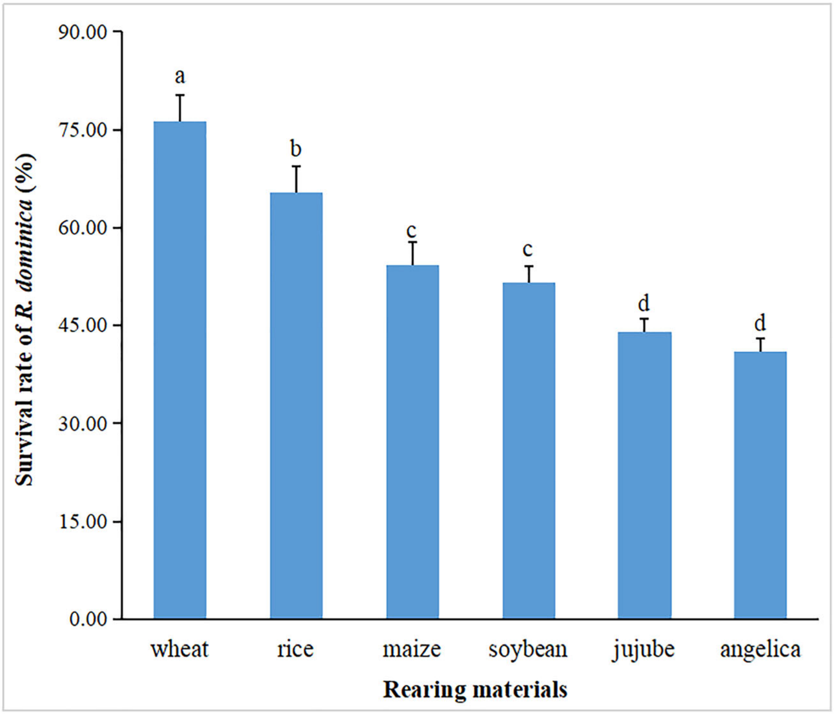

Figure 1 Survival rate (%) of immature stages of *Rhyzopertha dominica* raised on different stored products. Data are means ± SEs. Different letters above bars show significant differences among values (Turkey's HSD test, $P < 0.05$).

51.67% on soybean, 44.00% on jujube, and 41.00% on angelica.

Oviposition

The type of stored product significantly affected the longevity of adult males ($F_{5,12} = 215.47$, $P < 0.01$) and females ($F_{5,12} = 798.94$, $P < 0.01$) of *R. dominica* (Table 2). Fecundity also differed significantly among these stored materials ($F_{5,12} = 451.36$, $P < 0.01$). The materials were ranked, from highest fecundity of *R. dominica* to lowest, as follows: wheat (246.05), rice (150.68), maize (123.44), soybean (106.23), jujube (92.67), and angelica (69.38). Similarly, the highest oviposition rate of *R. dominica* was on wheat (3.25), followed by rice (2.47), maize (2.32), soybean (2.25), jujube (2.13), and finally angelica (1.90) ($F_{5,12} = 154.30$, $P < 0.01$). There were no significant differences in the sex ratios of the offspring ($F_{5,12} = 0.51$, $P = 0.76$) of *R. dominica* among the six stored products.

Life table parameters

Life tables were constructed for the populations of *R. dominica* on the six different stored products (Table 3). The values of R_0 and r_m differed significantly among wheat, rice, maize, soybean, jujube, and angelica, with R_0 values of 68.50, 41.28, 32.32, 27.17, 23.16, and 20.18 ($F_{5,12} = 714.37$, $P < 0.01$), respectively, and r_m values of 0.059, 0.046, 0.042, 0.039, 0.036, and 0.033 ($F_{5,12} = 396.60$, $P < 0.01$), respectively. Like R_0 and r_m, the value of λ for *R. dominica* showed significant differences ($F_{5,12} = 651.20$, $P < 0.01$) among the stored materials. There were also significant differences in T ($F_{5,12} = 211.38$, $P < 0.01$) and DT ($F_{5,12} = 517.37$, $P < 0.01$) of *R. dominica* among the stored materials, but their values showed the opposite trend to those of R_0, r_m, and λ.

Discussion

Our findings revealed the varied responses of *R. dominica* to feeding on different stored products. Considerable differences were detected in the developmental biology, survival, oviposition rate, and population growth of this pest among the six different products. Although *R. dominica* successfully completed its life history on all six stored products, wheat was the most suitable diet for *R. dominica* population development, and angelica was the least suitable diet. This conclusion is supported by the life table parameters of *R. dominica* on these stored materials.

Several studies have indicated that the cultivar of grains can influence the development, survival, and reproductive characteristics of stored product pests (Chougourou *et al.* 2013; Borzoui & Naseri 2016; Naseri & Borzoui 2016; Golizadeh & Abedi 2017). In other studies, the larval and pupal developmental periods of *R. dominica* ranged from 34.1 to 69.1 days on six rice cultivars (Ebadollahi & Borzoui 2019), and the duration of the immature stage of *R. dominica* ranged

Table 2 Fecundity, longevity, and sex ratio of the offspring of *Rhyzopertha dominica* raised on six stored products

Diet	Fecundity (eggs/female)	Oviposition rate (eggs/female/day)	Sex ratio (females/total offspring)	Male longevity	Female longevity
Wheat	246.05 ± 3.54^{a}	3.25 ± 0.06^{a}	0.517 ± 0.009^{a}	98.28 ± 1.03^{a}	90.7 ± 1.18^{a}
Rice	150.68 ± 5.28^{b}	2.47 ± 0.06^{b}	0.510 ± 0.006^{a}	69.37 ± 0.81^{b}	64.51 ± 1.01^{b}
Maize	123.44 ± 1.90^{c}	2.32 ± 0.04^{bc}	0.513 ± 0.003^{a}	64.74 ± 1.18^{b}	56.43 ± 1.79^{c}
Soybean	106.23 ± 1.68^{d}	2.25 ± 0.06^{cd}	0.507 ± 0.007^{a}	56.08 ± 2.88^{c}	49.40 ± 0.46^{d}
Jujube	92.67 ± 0.82^{e}	2.13 ± 0.01^{d}	0.510 ± 0.000^{a}	50.67 ± 0.33^{cd}	45.95 ± 0.66^{e}
Angelica	69.38 ± 0.94^{f}	1.90 ± 0.03^{e}	0.507 ± 0.003^{a}	48.09 ± 0.49^{d}	39.15 ± 0.94^{f}

Data are expressed as mean ± standard error (SE). Different superscript letters in the same column indicate significant differences (one-way analysis of variance followed by Tukey's HSD test, $P < 0.05$).

Table 3 Life table parameters of *Rhyzopertha dominica* raised on different stored products

Diet	R_0	r_m	λ	T	DT
Wheat	68.50 ± 1.09^{a}	0.059 ± 0.001^{a}	1.061 ± 0.001^{a}	71.64 ± 1.15^{e}	11.75 ± 0.12^{f}
Rice	41.28 ± 0.63^{b}	0.046 ± 0.001^{b}	1.047 ± 0.001^{b}	81.01 ± 0.58^{d}	15.10 ± 0.20^{e}
Maize	32.32 ± 0.58^{d}	0.042 ± 0.001^{c}	1.043 ± 0.001^{c}	82.98 ± 0.62^{cd}	16.55 ± 0.20^{d}
Soybean	27.17 ± 0.85^{e}	0.039 ± 0.001^{d}	1.039 ± 0.000^{d}	85.01 ± 0.86^{c}	17.85 ± 0.34^{c}
Jujube	23.16 ± 0.91^{f}	0.036 ± 0.000^{e}	1.037 ± 0.000^{e}	87.78 ± 0.38^{b}	19.37 ± 0.28^{b}
Angelica	20.18 ± 0.84^{f}	0.033 ± 0.000^{f}	1.034 ± 0.000^{f}	91.18 ± 0.95^{a}	21.04 ± 0.30^{a}

Data are expressed as mean ± standard error (SE). Different superscript letters in the same column indicate significant differences (one-way analysis of variance followed by Tukey's HSD test, $P < 0.05$).

from 46.6 to 61.0 days on various barley cultivars (Nemati-Kalkhoran *et al.* 2018). Among six different cereals, *R. dominica* showed the shortest developmental period of the immature stages on wheat (40.42 days), and the longest on maize (65.75 days) (Naseri & Majd-Marani 2022). Similarly, in the present study, the duration of the developmental period of *R. dominica* differed significantly among the six stored products, ranging from 40.20 to 67.04 days. The duration of the immature stages was shortest on wheat, and longest on angelica. These differences may be a result of differences in the physical and biochemical properties, e.g. macronutrient contents, seed hardness, and protein inhibitors, among the different stored products (Majd-Marani *et al.* 2017; Naseri *et al.* 2017).

These factors may also have affected the survival rate and fecundity of *R. dominica* (Nemati-Kalkhoran *et al.* 2018; Ebadollahi & Borzoui 2019; Naseri & Majd-Marani 2022). Another study reported that the survival rate of the immature stages of *R. dominica* was highest (80%) on the rice cultivar Hashemi, and lowest (29%) on the rice cultivar Govhar (Ebadollahi & Borzoui 2019). Similarly, *R. dominica* showed different survival rates at various immature stages among several barley cultivars (Nemati-Kalkhoran *et al.* 2018). The fecundity of *R. dominica* ranged from 72.5 to 287.2 on six rice cultivars (Ebadollahi & Borzoui 2019), and from 217.60 to 348.05 on 12 barley cultivars (Nemati-Kalkhoran *et al.* 2018), and the number of offspring significantly differed among several wheat cultivars (Sinha *et al.* 1988). In the present study, the six stored products were ranked, from highest survival rate and fecundity of *R. dominica* to lowest, as follows: wheat > rice > maize > soybean > jujube > angelica. This ranking is consistent with the results of other studies on different cereal crops (Naseri & Majd-Marani 2022). These results indicate that wheat is a more suitable food source, compared with other cereals and grain products, for the population development of *R. dominica*. Consequently, the focus for control of this pest should be on wheat. The host preference and mechanism of host selection by *R. dominica* should also be further studied (Perišić *et al.* 2018). Other studies have shown that temperature, humidity, and other biological/abiotic factors can affect the development, survival, and oviposition rate of *R. dominica* (Toews *et al.* 2000; Quan *et al.* 2010; Wang *et al.* 2017). On the whole, results of this study are useful information for predicting population growth of *R. dominica* on different stored products; however, further studies should explore the population performance of this pest under a range of storage conditions.

Our results show that the life table parameters of *R. dominica* differed significantly among six stored products. The two most important parameters were r_m and R_0, which describe the growth potential of a population under certain conditions because they represent the sum of the development, survival, and reproduction of the population (Southwood 1978; Carey 1993). In the present study, the r_m ranged from 0.033 to 0.059 and the R_0 ranged from 20.18 to 68.50, similar to the ranges reported in other studies on barley cultivars (r_m, 0.043–0.066; R_0, 55.12–146.79) and cereal grains (r_m, 0.037–0.058; R_0, 28.07–97.39) (Nemati-Kalkhoran *et al.* 2018; Naseri & Majd-Marani 2022). Normally, higher r_m and R_0 values are related to shorter developmental time, lower mortality, and greater fecundity (Razmjou *et al.* 2014; Borzoui *et al.* 2017). Consistently, the r_m and R_0 values of *R. dominica* were higher on wheat than on other products in this study and other studies (Naseri & Majd-Marani 2022). These findings indicate that more offspring are produced, and the damage is more severe, in wheat than in other stored grain products (Chougourou *et al.* 2013). The variations in r_m and R_0 values of *R. dominica* reared on different stored products are likely to have resulted from physicochemical differences among the products or different experimental conditions.

Food quality can significantly affect the population development of phytophagous insects, and digestive enzymes play a crucial role in food intake, consumption, absorption, nutrient use, and growth in insects (Terra 1990; Graça 2001). Enzyme inhibitors in some stored products can significantly affect the amylolytic and proteolytic activity of *R. dominica* (Nemati-Kalkhoran *et al.* 2018; Ebadollahi & Borzoui 2019; Naseri & Majd-Marani 2022). Thus, specific physiochemical characteristics of stored products that affect digestive physiology and their effects on the population performance of *R. dominica* should be studied further. Our results indicate that angelica is not suitable for the population development of *R. dominica* and is, therefore, an unsuitable host. Further studies should investigate the physiochemical characteristics of angelica to determine whether they can be applied in the control or integrated management of this pest.

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Conflict of interest statement

The authors declare that they have no competing interests associated with this work.

References

Bajracharya NS, Opit GP, Talley J *et al*. (2016) Assessment of fitness effects associated with phosphine resistance in *Rhyzopertha dominica* (F.) (Coleoptera: bostrichidae) and *Tribolium castaneum* (Herbst) (Coleoptera: tenebrionidae). *African Entomology* **24**: 39–49.

Borzoui E, Naseri B (2016) Wheat cultivars affecting life history and digestive amylolytic activity of *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae). *Bulletin of Entomological Research* **106**: 464–473.

Borzoui E, Naseri B, Nouri-Ganbalani G (2017) Effects of food quality on biology and physiological traits of *Sitotroga cerealella* (Lepidoptera: Gelechiidae). *Journal of Economic Entomology* **110**: 266–273.

Buonocore E, Monaco DL, Aberlenc HP *et al*. (2017) *Rhyzopertha dominica* (F., 1792) (Coleoptera: Bostrichidae): a stored grain pest on olive trees in Sicily. *EPPO Bulletin* **47**: 263–268.

Carey JR (1993) *Applied Demography for Biologists with Special Emphasis on Insects*. Oxford University Press, Inc., New York.

Chougourou DC, Togola A, Nwilene FE *et al*. (2013) Susceptibility of some rice varieties to the lesser grain borer, *Rhyzopertha dominica* Fab. (Coleoptera: Bostrichidae) in Benin. *Journal of Applied Science* **13**: 173–177.

Cinco-Moroyoqui FJ, Rosas-Burgos EC, Borbosa-Flores J *et al*. (2006) α-Amylase activity of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) reared on several wheat varieties and its inhibition with kernel extracts. *Journal of Economic Entomology* **99**: 2146–2150.

Collins PJ, Falk MG, Nayak MK *et al*. (2017) Monitoring resistance to phosphine in the lesser grain borer, *Rhyzopertha dominica*, in Australia: a national analysis of trends, storage types and geography in relation to resistance detections. *Journal of Stored Products Research* **70**: 25–36.

Dissanayaka DM, Sammani SK, Wijayaratne AMP *et al*. (2020) Effects of aggregation pheromone concentration and distance on the trapping of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) adults. *Journal of Stored Products Research* **88**: 101657.

Ebadollahi A, Borzoui E (2019) Growth performance and digestive enzymes activity of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) feeding on six rice cultivars. *Journal of Stored Products Research* **82**: 48–53.

Edde PA (2012) A review of the biology and control of *Rhyzopertha dominica* (F.) the lesser grain borer. *Journal of Stored Products Research* **48**: 1–18.

Finkelman S, Navarro S, Rindner M *et al*. (2006) Effect of low pressure on the survival of *Trogoderma granarium* Everts, *Lasioderma serricorne* (F.) and *Oryzaephilus surinamensis* (L.) at 30 °C. *Journal of Stored Products Research* **42**: 23–30.

Furlong MJ, Wright DJ, Dosdall LM (2013) Diamondback moth ecology and management: problems, progress, and prospects. *Annual Review of Entomology* **58**: 517–541.

Golizadeh A, Abedi Z (2017) Feeding performance and life table parameters of Khapra Beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) on various barley cultivars. *Bulletin of Entomological Research* **107**: 689–698.

Graça MAS (2001) The role of invertebrates on leaf litter decomposition in streams—a review. *International Review of Hydrobiology* **86**: 383–393.

Kavallieratos G, Athanassiou CG, Nika EP *et al*. (2017) Efficacy of alpha-cypermethrin, chlorfenapyr and pirimiphos-methyl applied on polypropylene bags for the control of *Prostephanus truncatus* (Horn), *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.). *Journal of Stored Products Research* **73**: 54–61.

Lawrence PK, Koundal KR (2002) Plant protease inhibitors in control of phytophagous insects. *Electronic Journal of Biotechnology* **5**: 5–6.

Li C, Li ZZ, Cao Y *et al*. (2009) Partial characterization of stress-induced carboxylesterase from adults of *Stegobium paniceum* and *Lasioderma serricorne* (Coleoptera: Anobiidae) subjected to CO_2-enriched atmosphere. *Journal of Pest Science* **82**: 7–11.

Majd-Marani S, Naseri B, Nouri-Ganbalani G *et al*. (2017) The effect of maize hybrid on biology and life table parameters of the Khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae). *Journal of Economic Entomology* **110**: 1916–1922.

Miliordos DEN, Athanassioua CG, Tsiropoulos NG *et al*. (2017) Persistence and efficacy of indoxacarb against three stored product insect species on wheat and maize. *Journal of Stored Products Research* **73**: 74–86.

Naseri B, Borzoui E (2016) Life cycle and digestive physiology of *Trogoderma granarium* (Coleoptera: Dermestidae) on various wheat cultivars. *Annals of the Entomological Society of America* **109**: 831–838.

Naseri B, Borzoui E, Majd SH *et al*. (2017) Influence of different food commodities on life history, feeding efficiency, and digestive enzymatic activity of *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Journal of Economic Entomology* **110**: 2263–2268.

Naseri B, Majd-Marani S (2022) Different cereal grains affect demographic traits and digestive enzyme activity of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). *Journal of Stored Products Research* **95**: 101898.

Nayak MK, Daglish GJ, Phillips TW *et al*. (2020) Resistance to the fumigant phosphine and its management in insect pests of stored products: a global perspective. *Annual Review of Entomology* **65**: 333–350.

Nemati-Kalkhoran M, Razmjou J, Borzoui E *et al*. (2018) Comparison of life table parameters and digestive physiology of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) fed on various barley cultivars. *Journal of Insect Science* **18**: 1–9.

Pappathi T, Dharani P, Maria Packiam S *et al*. (2021) Repellent and larvicidal activity of medicinal plant extract against *Rhyzopertha dominica* (Coleoptera: Bostrichidae). *Journal of Emerging Technologies and Innovative Research* **8**: 277–284.

Parde VD, Sharma HC, Kachole MS (2010) In vivo inhibition of *Helicoverpa armigera* gut pro–proteinase activation by non–host plant protease inhibitors. *Journal of Insect Physiology* **56**: 1315–1324.

Paudyal S, Opit GP, Osekre EA *et al*. (2017) Field evaluation of the long-lasting treated storage bag, deltamethrin incorporated, (ZeroFly® Storage Bag) as a barrier to insect pest infestation. *Journal of Stored Products Research* **70**: 44–52.

Perišić V, Perišić V, Vukajlović F *et al*. (2018) Feeding preferences and progeny production of *Rhyzopertha dominica* (Fabricius 1792) (Coleoptera: Bostrichidae) in small grains. *Biologica Nyssana* **9**: 55–61.

Quan Y, Deng YX, Lv LS *et al*. (2010) Influence of moisture of maize flour on the development and reproduction of *Rhyzopertha dominica*. *Chinese Bulletin of Entomology* **47**: 498–502.

Rahimi-Alangi V, Bandani AR (2013) Comparison of the effects of three plant protein inhibitors on α-glucosidase of Sunn pest, *Eurygaster integriceps* Putton. *Archives of Phytopathology & Plant Protection* **46**: 318–327.

Razmjou J, Naseri B, Hemati SA (2014) Comparative performance of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on various host plants. *Journal of Pest Science* **87**: 29–37.

Rees DP (2004) *Insects of Stored Products*. CSIRO Publishing, Collingwood, Australia.

Rossi E, Cosimi S, Loni A (2010) Insecticide resistance in Italian populations of *Tribolium* flour beetles. *Bulletin of Insectology* **63**: 251–258.

Rumbos CI, Dutton AC, Athanassiou CG (2016) Insecticidal efficacy of two pirimiphos-methyl formulations for the control of three stored-product beetle species: effect of commodity. *Crop Protection* **80**: 94–100.

Sinha RN, Demianyk CJ, McKenzie RIH (1988) Vulnerability of common wheat cultivars to major stored product beetles. *Canadian Journal of Plant Science* **68**: 337–343.

Sinha RN, Watters FL (1985) *Insect Pests of Flour Mills, Grain Elevators, and Feed Mills and Their Control*. Agriculture Canada, Ottawa.

Southwood TRE (1978) *Ecological Methods with Particular Reference to the Study of Insect Populations*, 2nd edn. Chapman and Hall, London.

Terra WR (1990) Evolution of digestive systems of insects. *Annual Review of Entomology* **35**: 181–200.

Toews MD, Cuperus GW, Phillips TW (2000) Susceptibility of eight U.S. wheat cultivars to infestation by *Rhyzopertha dominica* (Coleoptera: Bostrichidae). *Environmental Entomology* **29**: 250–255.

Wang DX, Zhao HP, Yuan YK *et al*. (2017) Determination and calculation on developmental zero temperature of *Rhyzopertha dominica* (Fabricius). *Journal of Henan University of Technology (Natural Science Edition)* **38**: 51–55 60.

Wang J, Germinara GS, Feng ZY *et al*. (2022) Comparative effects of heat and cold stress on physiological enzymes in *Sitophilus oryzae* and *Lasioderma serricorne*. *Journal of Stored Products Research* **96**: 101949.

Watts VM, Dunkel FV (2003) Postharvest resistance in hard spring and winter wheat varieties of the northern Great Plains to the lesser grain borer (Coleoptera: bostrichidae). *Journal of Economic Entomology* **96**: 220–230.

Wijayaratne LKW, Dissanayaka DMSK, Sammani AMP (2019) Variation in *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) progeny adult emergence in different animal feed stored under ventilated and non-ventilated conditions. *Journal of Stored Products Research* **84**: 101516.

10. Electrophysiological and behavioural responses of *Stegobium paniceum* to volatile compounds from Chinese medicinal plant materials

Yu Cao[1], Onofrio Marco Pistillo[2], Yibin Lou[1], Ilaria D'Isita[2], Filippo Maggi[3], Qiqi Hu[1], Giacinto Salvatore Germinara[2*], Can Li[1*]. *Pest Management Science, 2022, 78(8), 3697-3703.*

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[1] Guizhou Provincial Key Laboratory for Rare Animal and Economic Insect of the Mountainous Region, Department of Biology and Engineering of Environment, Guiyang University, Guiyang, People's Republic of China

[2] Department of Agriculture, Food, Natural Resources and Engineering, University of Foggia, Foggia, Italy

[3] Chemistry Interdisciplinary Project (ChIP), School of Pharmacy, University of Camerino, Camerino, Italy

*Corresponding author

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Electrophysiological and behavioural responses of *Stegobium paniceum* to volatile compounds from Chinese medicinal plant materials

Yu Cao,[a] Onofrio Marco Pistillo,[b] Yibin Lou,[a] Ilaria D'Isita,[b] Filippo Maggi,[c] Qiqi Hu,[a] Giacinto Salvatore Germinara[b*] and Can Li[a*]

Abstract

BACKGROUND: *Stegobium paniceum* (Coleoptera, Anobiidae) is an important pest of stored products causing severe damage to dried Chinese medicinal plant materials (CMPMs). Plant volatiles play an important role in host-searching of insects. The olfactory responses of *S. paniceum* to the most abundant volatile components of some drugstore attractant CMPMs such as *Panax notoginseng*, *Angelica sinensis*, *Gastrodia elata* and *Peucedanum praeruptorum*, namely falcarinol, 3-*n*-butylphthalide, *p*-cresol and β-pinene, respectively, were studied by electroantennography (EAG) and behavioural bioassays in six- and four-arm olfactometers.

RESULTS: EAG recordings showed that male and female antennae are able to perceive the test compounds in a wide range of concentrations and in a dose-dependent manner. Moreover, for each dose of different compounds tested, no significant differences were found between the mean male and female EAG responses. In six-arm olfactometer bioassays, *S. paniceum* exhibited positive responses to falcarinol, 3-*n*-butylphthalide, *p*-cresol and β-pinene at doses of 1, 10, 100, 500 and 1000 µg. The most attractive dose was 500 µg for falcarinol, 100 µg for 3-*n*-butylphthalide, 500 µg for *p*-cresol and 1000 µg for β-pinene. Olfactory preferences of *S. paniceum*, based on comparison of these four compounds at their optimally attractive concentrations in a four-arm olfactometer, were 3-*n*-butylphthalide > *p*-cresol > falcarinol > β-pinene.

CONCLUSION: The results indicated that the four volatiles of CMPMs are perceived by the peripheral olfactory system of *S. paniceum* adults and are able to individually elicit a positive chemotaxis in *S. paniceum* adults confirming the role of chemical cues in host-plant detection and selection of this pest. Further field studies are needed to evaluate the potential of the attractive compounds identified in this study, particularly 3-*n*-butylphthalide, to be applied as a novel monitoring and control tool against this storage-beetle pest.

Keywords: *Stegobium paniceum*; EAG; olfactory response; Chinese medicinal plant material; volatile organic compounds; concentration

1 INTRODUCTION

The drugstore beetle, *Stegobium paniceum* L. (Coleoptera, Anobiidae), is a worldwide pest of stored products. This cosmopolitan insect consumes a wide range of dried plant products, including biological specimens in museum collections.[1–3] *Stegobium. paniceum* adults typically gnaw their way into food storage containers causing direct damage, and then proceed to lay eggs in the stored products. The hatched larvae then infest these stored materials, with the dead beetles and other wastes remaining inside, causing further spoilage and economic loss.[1,2]

Currently, fumigants (e.g. phosphine) are considered to provide the most effective means of protecting and disinfesting stored food and other products.[4,5] However, the repeated and intensive use of various fumigants has resulted in serious problems, including residues with hazard for human health, pest resistance, pest

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[*] Correspondence to: GS Germinara, Department of Agricultural Sciences, Food, Natural Resources and Engineering, University of Foggia, Foggia 71122, Italy, E-mail: giacinto.germinara@unifg.it; or C Li, Guizhou Provincial Key Laboratory for Rare Animal and Economic Insect of the Mountainous Region, Department of Biology and Engineering of Environment, Guiyang University, Guiyang 550009, People's Republic of China. E-mail: lican790108@163.com

[a] Guizhou Provincial Key Laboratory for Rare Animal and Economic Insect of the Mountainous Region, Department of Biology and Engineering of Environment, Guiyang University, Guiyang, People's Republic of China

[b] Department of Agriculture, Food, Natural Resources and Engineering, University of Foggia, Foggia, Italy

[c] Chemistry Interdisciplinary Project (ChIP), School of Pharmacy, University of Camerino, Camerino, Italy

resurgence and lethal effects on nontarget organisms.[6] Therefore, the development of effective alternative methods to control pests of stored products is needed. Integrated pest management (IPM) is considered a more sustainable and environmentally friendly control strategy for combatting these pests.[3,5,7]

Behavioural manipulation based on the responses of insects to special environmental factors is now an important method of pest control. Thus, certain repellents and attractants, whose functional components are derived from specific plants, are applied to manipulate the behaviour of insects to protect stored products from pest infestation.[8–11] In our previous study, *S. paniceum* adults showed significant preferences for volatiles released from several Chinese medicinal plant materials (CMPMs).[12] However, specific volatile components of CMPMs that attracted *S. paniceum* were not identified.

Medicinal plants are widely grown in China and make a substantial contribution to the economy. *S. paniceum* is the main pest species responsible for major losses of stored CMPMs in many provinces of China.[1] Because most of these CMPMs are stored before use in human health protection and disease treatments, it is important to devise methods for the management of *S. paniceum* without risk of contamination of the products. Therefore, there is a keen interest in the development of botanical pesticide alternatives to chemical insecticides for *S. paniceum* control. Indeed, there is a long history of using botanical insecticides to protect stored products from insect pests.[10,13,14] However, volatile compounds or other plant products also could be developed as attractants or repellents for pest management. In our previous study, volatile blends from various CMPMs, including *Panax notoginseng* (Burkill) F.H.Chen (Apiales: Araliaceae), *Angelica sinensis* (Oliv.) Diels (Apiales: Apiaceae), *Gastrodia elata* Blume (Asparagales: Orchidaceae) and *Peucedanum praeruptorum* Dunn (Apiales: Apiaceae), were strongly attractive to *S. paniceum*, with the most abundant components of these four CMPMs being falcarinol, 3-*n*-butylphthalide, *p*-cresol and *β*-pinene, respectively.[12]

In the present study, the olfactory responses of *S. paniceum* to these compounds were investigated by electroactennographic (EAG) tests to assess the sensitivity of male and female *S. paniceum* antennae to the test compounds and by six- and four-arm olfactometer bioassays to evaluate the insects' behavioural response to different concentrations of the same compounds. Such information will help to further elucidate the roles of chemical signals in host selection by *S. paniceum* and provide a basis for further field studies aiming at developing semiochemical-based strategies for the safe and effective control of this beetle pest.

2 METHODS AND MATERIALS

2.1 Insect rearing

Stegobium paniceum have been reared in the laboratory of the Department of Biology and Engineering of Environment, Guiyang University since 2017 and maintained on jujube (*Ziziphus jujuba* Mill.); a laboratory colony from the same population was established at the Department of Agriculture, Food, Natural Resources and Engineering, University of Foggia. The beetles were reared at 28 ± 1 °C, 60 ± 5% relative humidity, and 8 h:16 h, light:dark photoperiod (photophase 09:00 h and 17:00 h), as reported by Li *et al.*[1] Secondary infestation by moisture-sensitive mites was prevented using the method of Steiner *et al.*[15]

2.2 Odour stimuli

Falcarinol was purchased from GlpBio (Montclair, NJ, USA), *p*-cresol and *β*-pinene were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), and 3-*n*-butylphthalide was purchased from Toronto Research Chemicals (North York, Canada). For each compound, mineral oil (Sigma-Aldrich, Milan, Italy) solutions to be used as test stimuli for EAG (0.001, 0.01, 0.1, 1, 10 μg μL^{-1}) and olfactometer (0.1, 1, 10, 50 and 100 μg μL^{-1}) bioassays were prepared. Solutions were stored at −20°C until needed.

2.3 Electroantennography (EAG)

The antennal sensitivity of *S. paniceum* males and females to increasing concentrations of the four test compounds was evaluated by EAG using the technique described in our previous studies.[16,17] The head of a one-week-old specimen was excised using a scalpel and seated between two glass capillary (Micro-glass, Naples, Italy) electrodes filled with Kaissling saline solution[18] and mounted in stainless steel electrode holders (Syntech Laboratories, Hilversum, the Netherlands). The recording electrode (diameter ~ 100 μm) was put in contact with the dorsal surface of the last antennal segment while the neutral electrode was inserted into the base of the head. AgCl-coated silver wires were used to maintain the electrical continuity between the antennal preparation and an AC/DC UN-6 amplifier in DC mode (Syntech Laboratories). The amplifier was connected to a PC equipped with the EAG 2.0 program (Syntech Laboratories).

For each test compound, 10 μL of different mineral oil solutions giving the 0.01, 0.1, 1, 10 and 100 μg doses, was adsorbed onto a filter paper (Whatman No. 1) strip (2 cm^2) inserted in a Pasteur pipette (15 cm long) which was used as an odour cartridge. Using a disposable syringe, vapour stimuli (3 cm^3) were puffed for 1 s (0.35 m s^{-1}) into a charcoal-filtered and humidified air flow (500 mL min^{-1}) passing over the antenna through a stainless-steel delivery tube [1 cm inner diameter (i.d.)] whose outlet was positioned ~1 cm from the antenna. Control (10 μL mineral oil) and standard (5 μL of 10 μg μL^{-1} (*Z*)-3-hexenol mineral oil solution; Sigma-Aldrich) stimuli also were applied at the beginning of the experiment and after each group of four test stimuli. The intervals between stimuli were 1 min. Each dose of the four compounds was tested on five different antennae from different males and females.

The maximum amplitude of negative polarity deflection (−mV) elicited by a stimulus was used to measure the EAG responses.[17] To compensate for solvent and/or mechanosensory artifacts, the absolute amplitude (mV) of the EAG response to each test stimulus was subtracted by the mean EAG response to the two nearest solvent controls.[18] Moreover, to compensate for the decrease in the antennal responsiveness during the experiment, the resulting EAG amplitude was corrected according to the reduction of the EAG response to the standard stimulus.[19] Dose–response curves were calculated based on the corrected EAG values.

2.4 Six-arm olfactometer bioassays

The behavioural responses of adult *S. paniceum* to falcarinol, 3-*n*-butylphthalide, *p*-cresol and *β*-pinene solutions were evaluated in a six-arm olfactometer according to the method reported by Cao *et al.*[20] Each arm was connected to a 25-mL glass vessel via Teflon® tubing, and each glass vessel contained a test or control stimulus (10 μL). Aliquots (10 μL) of each of the five concentrations (0.1, 1, 10, 50 and 100 μg μL^{-1}) of each compound and mineral oil (used as control), adsorbed onto a filter paper disk (1.0 cm diameter), were used as stimuli.

In order to drive the odour source towards the insects, the airflow was set at 200 mL min^{-1}. *Stegobium paniceum* unsexed adults, 2–3 days postemergence and starved for 4 h, were introduced into the olfactometer in groups of 180 individuals, using a brush. After 30 min, *S. paniceum* that entered the arms of the olfactometer were counted and considered as either having made a choice for a particular odour source, or were considered as 'nonresponders'. Bioassays were replicated six times and carried out between 09:00 h and 17:00 h, at room temperature (RT; 25 ± 1 °C). After each replication, the olfactometer was cleaned, dried and the arms were rotated (60°).[12]

2.5 Four-arm olfactometer bioassays

In the six-arm olfactometer bioassays, falcarinol, 3-*n*-butylphthalide, *p*-cresol and *β*-pinene showed the highest attractiveness to *S. paniceum* at concentrations of 50, 10, 50 and 100 μg μL^{-1}, respectively. Therefore, the attractant power of the four compounds at their optimal concentrations were compared in a four-arm olfactometer, using the method of Liu *et al.*[21] The olfactometer consisted of a central glass chamber (15 cm i.d., 6 cm length) with four arms (1.5 cm i.d., 10 cm length), each connected to a glass tube (1.5 cm i.d., 20 cm length). Each arm was connected via Teflon® tubing to a 25-mL glass vessel that contained the test or control stimulus (10 μL), and the airflow was set at 200 mL min^{-1}. Beetles were introduced into the central part of the olfactometer chamber in groups of 120 individuals. 'Responders' and 'nonresponders' were determined using the criteria described for the six-arm olfactometer bioassays. Bioassays were replicated six times and were carried out between 09:00 a.m. and 5:00 p.m, at RT (25 ± 1 °C). After each replication, the olfactometer was cleaned, dried and the arms were rotated (90°). The odour treatments were set as follows:

(1) mineral oil, falcarinol, 3-*n*-butylphthalide and *p*-cresol;
(2) mineral oil, falcarinol, 3-*n*-butylphthalide and *β*-pinene;
(3) mineral oil, falcarinol, *p*-cresol and *β*-pinene;
(4) mineral oil, 3-*n*-butylphthalide, *p*-cresol and *β*-pinene.

2.6 Statistical analysis

In order to verify antennal activation, the corrected mean EAG response of males and females to the last dilution of each test compound was compared to a '0' value using one-sample Student's *t*-test and regarded as 'activated' if significant at $P = 0.05$. Saturation level was taken as the lowest dilution at which the mean response was equal to or less than the previous one.[22] The mean EAG responses of males and females to each stimulus were compared using Student's *t*-test for independent samples at $P = 0.05$. Male and female EAG responses to each test stimulus were not significantly different; therefore, they were pooled and analyzed together. For each of the 0.1, 1, 10 and 100 μg doses of the four compounds the mean EAG responses of adult *S. paniceum* were submitted to ANOVA followed by Tukey's honestly significant difference (HSD) test ($P = 0.05$) for separation of means. Data were log$_{10}$x-transformed to satisfy the assumption of normality (Shapiro–Wilk test) and assessed for homogeneity of variances (Levene's test) before ANOVA.

Because there was no difference in response to odour stimuli between *S. paniceum* males and females,[12] the male and female responses in different behavioural bioassays were pooled and analyzed together. The numbers of insects found in the different arms of the six-arm and four-arm olfactometers were subjected to Friedman two-way ANOVA by ranks and, in the case of significance ($P < 0.05$), the Wilcoxon signed ranks test was used to determine differences among means. All statistical analyses were performed using SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA).

3 RESULTS

3.1 EAG

The EAG responses of *S. paniceum* males and females to increasing doses of falcarinol, 3-*n*-butylphthalide, *β*-pinene and *p*-cresol are reported in Fig. 1. All compounds elicited measurable EAG responses starting from the 0.1-μg dose ($P < 0.05$ in all one-sample Student's *t*-test). In the dose range tested, typical sigmoid-shaped dose–responses were elicited by test compounds in both males and females. The mean EAG response to the highest dose was higher than that to the previous dose for all compounds indicating that no saturation of olfactory receptors occurred at the lowest dose. For each dose of the four compounds no significant differences were found between the mean EAG responses of males and females (falcarinol: $t = 0.447–1.965$, df = 8, $P = 0.086–0.667$; 3-*n*-butylphthalide: $t = 1.095–1.843$; df = 8, $P = 0.103–0.305$; *β*-pinene: $t = 0.485–1.818$; df = 8, $P = 0.107–0.640$; *p*-cresol: $t = 0.615–1.175$; df = 8, $P = 0.274–0.556$).

ANOVA revealed significant differences among the mean pooled male and female EAG responses to the four compounds at the 0.1 ($F = 89.598$, df = 3, $P < 0.001$), 1 ($F = 34.870$, df = 3, $P < 0.001$), 10 ($F = 34.216$, df = 3, $P < 0.001$) and 100 μg ($F = 28.813$, df = 3, $P < 0.001$) doses. At 0.1, 1 and 10 μg, the mean EAG responses elicited by 3-*n*-butylphthalide were significantly higher than those induced by falcarinol, *p*-cresol and *β*-pinene ($P < 0.05$, Tukey's HSD test) (Fig. 2). At 100 μg, the EAG responses to 3-*n*-butylphthalide and falcarinol were statistically similar and significantly higher than those to *p*-cresol and *β*-pinene ($P < 0.05$, Tukey's HSD test).

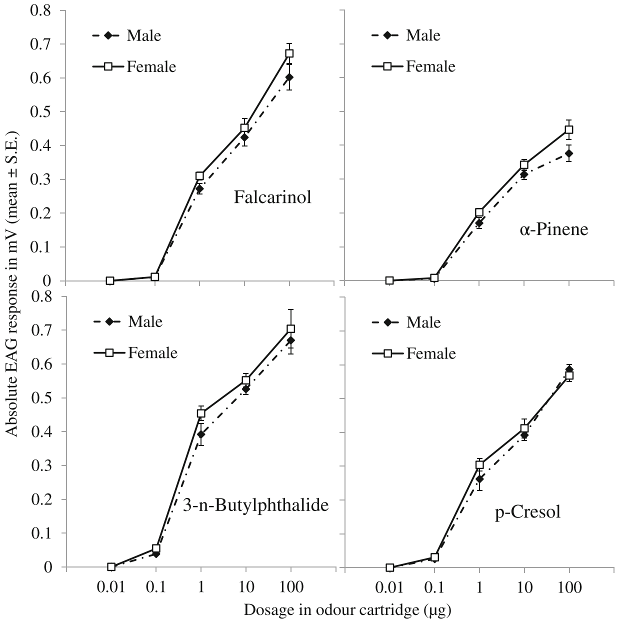

Figure 1. Mean (± SE) EAG dose–response curves of male and female *S. paniceum* antennae to ascending doses of falcarinol, 3-*n*-butylphthalide, *p*-cresol and *β*-pinene. For each dose, mean male and female EAG responses were not significant different (Student's *t*-test for independent samples, $P = 0.05$).

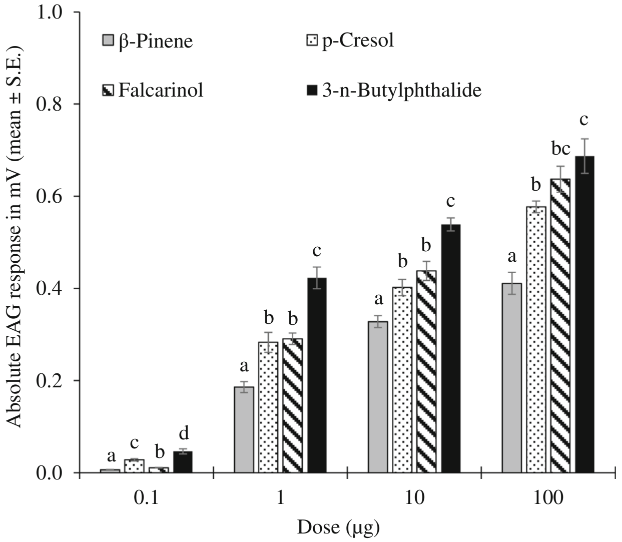

Figure 2. EAG responses of male and female *S. paniceum* antennae to different doses of falcarinol, 3-*n*-butylphthalide, *p*-cresol and *β*-pinene. For each dose, different letters indicate significant differences (Tukey's HSD test, $P < 0.05$).

3.2 Six-arm olfactometer bioassays

In these bioassays, mineral oil controls were significantly less attractive than all doses tested of falcarinol (Friedman test: $\chi^2 = 29.498$, df = 5, $P < 0.001$; Wilcoxon tests: $P = 0.027$–0.028), 3-*n*-butylphthalide (Friedman test: $\chi^2 = 29.238$, df = 5, $P < 0.001$; Wilcoxon tests: $P = 0.026$–0.027), *p*-cresol (Friedman test: $\chi^2 = 29.238$, df = 5, $P < 0.001$; Wilcoxon tests: $P = 0.027$–0.028) and *β*-pinene (Friedman test: $\chi^2 = 29.048$, df = 5, $P < 0.001$; Wilcoxon tests: $P = 0.026$–0.028) (Fig. 3).

There also were significant differences in attractiveness among different doses of each compound. Significantly more insects entered the arms connected to the vessels that contained 500 μg falcarinol (Wilcoxon tests: $P = 0.024$–0.028), 100 μg (Wilcoxon tests: $P = 0.027$) and 500 μg (Wilcoxon tests: $P = 0.024$–0.027) of 3-*n*-butylphthalide, 500 μg (Wilcoxon tests: $P = 0.026$–0.027) and 1000 μg (Wilcoxon tests: $P = 0.027$–0.028) of *p*-cresol, and 500 μg (Wilcoxon tests: $P = 0.024$–0.027) and 1000 μg (Wilcoxon tests: $P = 0.027$) of *β*-pinene relative to the arms with the other doses (Fig. 3).

3.3 Four-arm olfactometer bioassays

Based on the results of six-arm olfactometer bioassays, the most attractive doses of falcarinol, 3-*n*-butylphthalide, *p*-cresol and *β*-pinene were 500, 100, 500 and 1000 μg, respectively. Using these doses, the compounds were compared in multiple-choice tests carried out in four-arm olfactometer bioassays (Fig. 4). In all experiments, mineral oil, the control, was the least attractive stimulus chosen. The beetles significantly preferred 3-*n*-butylphthalide to *p*-cresol and falcarinol ($\chi^2 = 18.000$, df = 3, $P < 0.01$), to falcarinol and *β*-pinene ($\chi^2 = 18.000$, df = 3, $P < 0.01$), and to *p*-cresol and *β*-pinene ($\chi^2 = 18.000$, df = 3, $P < 0.01$). Insects were significantly more attracted to *p*-cresol than to falcarinol and *β*-pinene ($\chi^2 = 18.000$, df = 3, $P < 0.01$) (Fig. 4).

4 DISCUSSION AND CONCLUSIONS

Phytophagous insects rely on semiochemicals to locate suitable food materials, and mating and oviposition sites.[23–25] Qualitatively and quantitatively different blends of volatiles from different plant species allow insects to discriminate and locate their preferred host plants.[25,26] The stored-product pests, *Sitophilus oryzae* (L.),[27] *S. zeamais* Motschulsky,[28,29] *S. granarius* (L.) (Colepotera, Curculionidae),[24] *Oryzaephilus surinamensis* (L.) (Coleoptera, Silvanidae)[30] and *Callosobruchus maculatus* (F.) (Coleoptera, Bruchidae),[31] have been widely reported to be attracted to particular volatiles from some cereals and other stored products.

In our previous investigation, *S. paniceum* showed significant olfactory preferences for the volatiles of four CMPMs (i.e. *P. notoginseng*, *A. sinensis*, *G. elata* and *P. praeruptorum*). In the

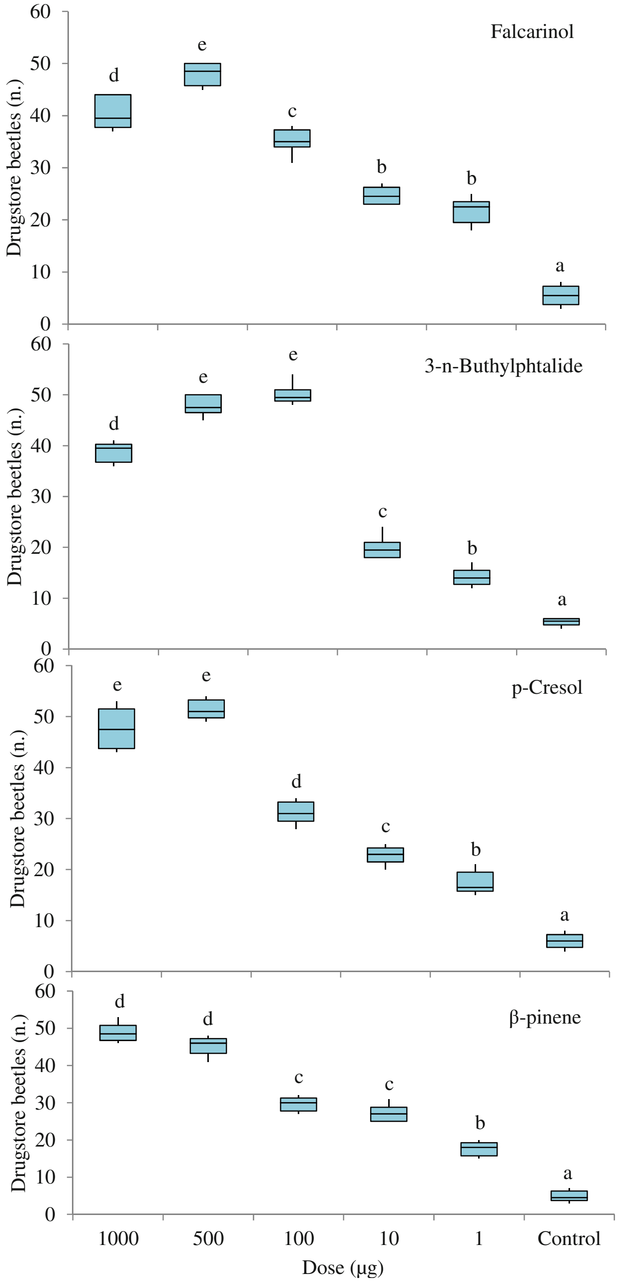

Figure 3. Olfactory responses of *S. paniceum* to different doses of falcarinol, 3-*n*-butylphthalide, *p*-cresol and *β*-pinene in a six-arm olfactometer. The control was mineral oil. Each box plot represents the median and its range of dispersion (lower and upper quartiles and outliers). Above each box plot, different letters indicate significant differences (Wilcoxon test, $P < 0.05$).

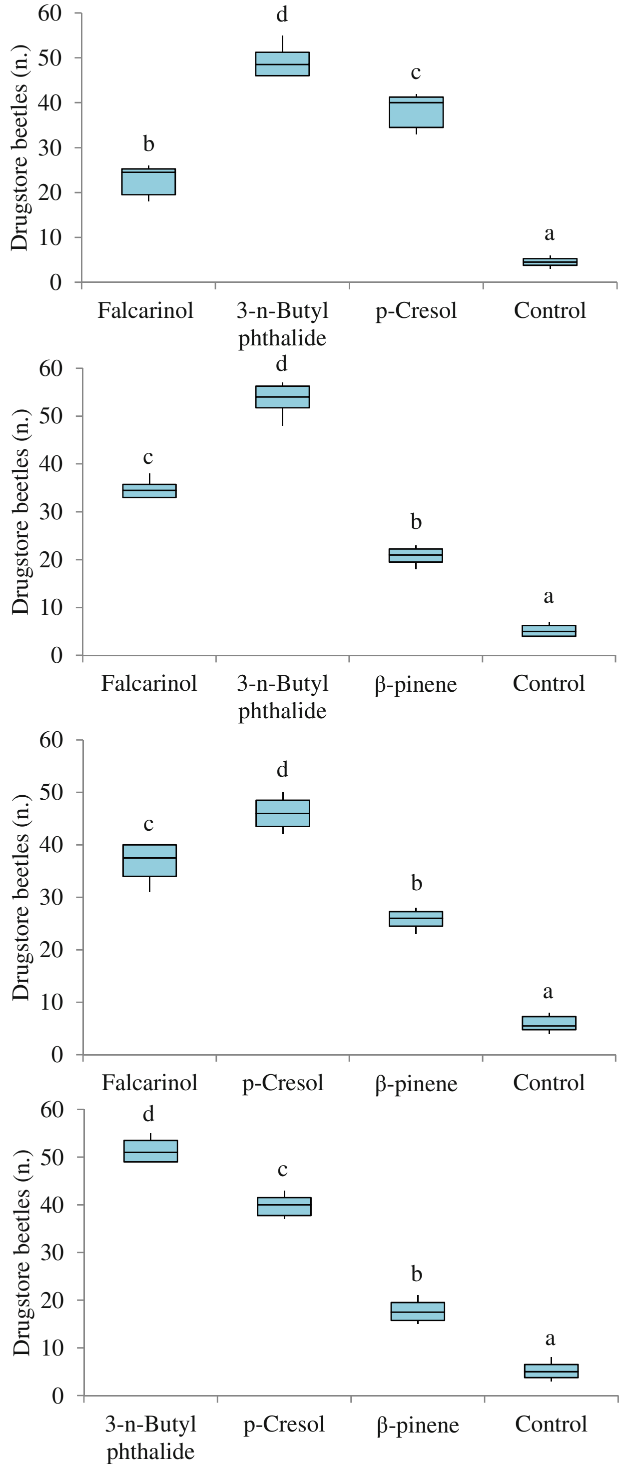

Figure 4. Olfactory responses of *S. paniceum* to falcarinol, 3-*n*-butyl phthalide, *p*-cresol and *β*-pinene (at their most attractive concentrations) in a four-arm olfactometer. The control was mineral oil, and the tested concentrations for falcarinol, 3-*n*-butylphthalide, *p*-cresol and *β*-pinene were 50, 10, 50 and 100 μg μL^{-1}, respectively. Each box plot represents the median and its range of dispersion (lower and upper quartiles and outliers). Above each box plot, different letters indicate significant differences (Wilcoxon test, $P < 0.05$).

present study, falcarinol, 3-*n*-butylphthalide, *p*-cresol and *β*-pinene, as the most abundant volatiles of the aforementioned CMPMs, respectively, were shown to be perceived by the peripheral olfactory systems of *S. paniceum* males and females in a dose-dependent manner.

Once the electrophysiological activity of the four compounds was ascertained, their biological activity was further investigated in six- and four-arm olfactometer bioassays. All compounds were significantly attractive to *S. paniceum* in a range of concentrations (0.1–100 μg μL^{-1}) and when the four compounds were compared at their optimally attractive concentrations, the order of olfactory preferences was 3-*n*-butylphthalide > *p*-cresol > falcarinol > *β*-pinene. Therefore, it seems that, amongst these volatiles, 3-*n*-butylphthalide has the greatest potential for development as a lure for *S. paniceum*. Although it is well-known that the behavioural response elicited by a compound is not directly related to its electrophysiological activity, it is worth noting that, in the dose range tested, 3-*n*-buthylphthalide also was the strongest antennal stimulant of *S. paniceum* adults. This compound has a wide range of pharmacological effects and is widely used for the treatment of ischaemic stroke because of its low toxicity and safety.[32] It also showed neuroprotection by reducing *β*-amyloid-induced toxicity in neuronal cells.[33] Furthermore, it was shown to possess larvicidal and adulticidal activity against *Drosophila melanogaster* (Meig)[34] and antifungal activity against *Candida albicans* (CP Robin) Berkhout.[35] In a previous study, of 33 plant species tested, the *Angelica sinensis* essential oil (EO) showed the best repellent activity against *Aedes aegypti* (L.), and 3-*n*-buthylphthalide was identified as one of the main EO components.[36] However, to the best of our knowledge, there are no reports investigating the behavioural response of any insect species to this compound.

More than 40 years ago, a female-produced sex pheromone of *S. paniceum* was identified,[37,38] and has received commercial interest for pest monitoring in herbarium collections.[39–41] Combined application of host plant volatiles and pheromones might be more effective for monitoring and control of *S. paniceum*, as shown for other pests.[27,42–46]

Interestingly, the order of olfactory preference of *S. paniceum* for the odours of the above mentioned CMPMs previously was shown to be *P. notoginseng* > *A. sinensis* > *G. elata* > *P. praeruptorum*,[12] whereas the preference ranking for their main components at the most attractive dose was 3-*n*-butylphthalide (*A. sinensis*) > *p*-cresol (*G. elata*) > falcarinol (*P. notoginseng*) > *β*-pinene (*P. praeruptorum*). This discordance might result from differences in the levels of the most abundant compounds among the volatiles from the four CMPMs, under natural conditions. In addition, although insects exhibited responses to individual compounds, their behaviour also can be influenced by specific blends of volatiles.[47–49] Further research is needed to determine the behavioural activity of additional individual volatiles of these four CMPMs and their various blends to confirm the functional compounds and/or mixtures, which mediate the behavioural responses involved in the olfactory preferences of *S. paniceum* to different CMPMs. In addition, additive or synergistic attractants might be identified for the management of this pest beetle based on these bioassays.

This study confirmed that semiochemical volatiles participate directly in the interactions between *S. paniceum* and host CMPMs. Two parasitic wasps, *Lariophagus distinguendus* Forster and *Theocolax elegans* Westwood, which are natural enemies of *S. paniceum*, are attracted to volatiles of stored cereal grains which represent the preferred substrates of their hosts.[16,22,50] Therefore volatile compounds identified from CMPMs that could be attractive to both *S. paniceum* and *S. paniceum* parasitoids deserve further research. This information could provide a theoretical framework for establishing a biocontrol system, based on CMPM volatiles, in which parasitoids are used to control *S. paniceum* infesting CMPMs.

Overall, this study demonstrated that semiochemical volatiles of CMPMs are involved in host-plant selection by *S. paniceum*. It also showed how individual compounds previously identified as the main volatile components of different attractive CMPMs are able to stimulate the peripheral olfactory systems and elicit a positive chemotactic response in adult drugstore beetle. The most attractive 3-*n*-butylphthalide found in this study provides a basis for further field-trapping experiments to develop semiochemically-based monitoring tools and direct control options for *S. paniceum*.

AUTHOR CONTRIBUTIONS

YC, GSG and CL conceived and designed the research; YBL, OMP, JW, ID and QQH conducted the experiments; YC and GSG analyzed the data; and YC, FM and GSG wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in [repository name] at [DOI], reference number [reference number].

REFERENCES

1 Li C, Li ZZ, Cao Y, Zheng XW and Zhou B, Partial characterization of stressinduced carboxylesterase from adults of *Stegobium paniceum* and *Lasioderma serricorne* (Coleoptera: Anobiidae) subjected to CO_2-enriched atmosphere. *J Pest Sci* **1**:7–11 (2009).

2 Benelli G, Pacini N, Conti B and Canale A, Following a scented beetle: larval faeces as a key olfactory cue in host location of *Stegobium paniceum* (coleoptera: anobiidae) by *Lariophagus distinguendus* (hymenoptera: Pteromalidae). *Chem* **2**:129–136 (2013).

3 Hironaka M, Kamura T, Osada M, Sasaki R, Shinoda K, Hariyama T *et al.*, Adults of *Lasioderma serricorne* and *stegobium paniceum* (Anobiidae: Coleoptera) are attracted to ultraviolet (uv) over blue light leds. *J Econ Entomol* **110**:1–5 (2017).

4 Zettler JL and Keevr DW, Phosphine resistance in the cigarette beetle (Coleoptera: Anobiidae) associated with tobacco storage in the southeastern United States. *J Econ Entomol* **87**:546–550 (1994).

5 Querner P, Insect pests and integrated pest management in museums, libraries and historic buildings. *Insects* **2**:595–607 (2015).

6 Jovanović Z, Kostić M and Popović Z, Grain protective properties of herbal extracts against the bean weevil *Acanthoscelides obtectus* say. *Ind Crops Prod* **26**:100–104 (2007).

7 Kim S, Park C, Ohh MH, Cho HC and Ahn YJ, Contact and fumigant activity of aromatic plant extracts and essential oils against *Lasioderma serricorne* (Coleoptera: Anobiidae). *J Stored Prod Res* **39**: 11–19 (2003).

8 Kaplan I, Attracting carnivorous arthropods with plant volatiles: the future of biocontrol or playing with fire? *Biol Control* **60**:77–89 (2012).

9 Guo SS, Geng ZF, Zhang WJ, Liang JY, Wang CF, Deng ZW *et al.*, The chemical composition of essential oils from *Cinnamomum camphora* and their insecticidal activity against the stored product pests. *Int J Mol Sci* **17**:1836 (2016).

10 Pavela R and Benelli G, Essential oils as ecofriendly biopesticides? challenges and constraints. *Trends Plant Sci* **21**:1000–1007 (2016).

11 Liang JY, Wang WT, Zheng YF, Zhang D, Wang JL, Guo SS *et al.*, Bioactivities and chemical constituents of essential oil extracted from *Artemisia anethoides* against two stored product insects. *J Oleo Sci* **66**:71–76 (2017).

12 Cao Y, Li S, Benelli G, Germinara GS, Yang J, Yang WJ *et al.*, Olfactory responses of *Stegobium paniceum* to different Chinese medicinal plant materials and component analysis of volatiles. *J Stored Prod Res* **76**:122–128 (2018).

13 Isman MB, Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu Rev Entomol* **51**:45–66 (2006).

14 Dubey NK and Srivastavab KA, Current status of plant products as botanical pesticides in storage pest management. *J Biopest* **1**:182–186 (2008).

15 Steiner S, Steidle JLM and Ruther J, Host-associated kairomones used for habitat orientation in the parasitoid *Lariophagus distinguendus* (hymenoptera: Pteromalidae). *J Stored Prod Res* **43**:587–593 (2007).

16 Germinara GS, De CA and Rotundo G, Electrophysiological and behavioral responses of *Theocolax elegans* (Westwood) (hymenoptera: Pteromalidae) to cereal grain volatiles. *Biomed Res Int* **2016**:1–8 (2016).

17 Germinara GS, Pistillo M, Griffo R, Garonna AP and Di Palma A, Electroantennographic responses of *Aromia bungii* (Faldermann, 1835) (Coleoptera, Cerambycidae) to a range of volatile compounds. *Insects* **10**:274 (2019).

18 Kaissling KE and Thorson J, Insect olfactory sensilla: structural, chemical and electrical aspects of the functional organization, in *Receptors for Neurotransmitters, Hormones, and Pheromones in Insects*, ed. by Satelle DB, Hall LM and Hildebrand JG. Elsevier/North-Holland Biomedical Press, New York, pp. 261–282 (1980).

19 Den Otter CJ, Tchicaya T and Schutte AM, Effects of age, sex and hunger on the antennal olfactory sensitivity of tsetse flies. *Physiol Entomol* **16**:173–182 (1991).

20 Cao Y, Benelli G, Germinara GS, Maggi F, Zhang Y, Luo SL *et al.*, Innate positive chemotaxis to paeonal from highly attractive chinese medicinal herbs in the cigarette beetle, Lasioderma serricorne. *Sci Rep* **9**:6995 (2019).

21 Liu XF, Chen HH, Li JK, Zhang R and Chen L, Volatiles released by Chinese liquorice roots mediate host location behaviour by neonate *Porphyrophora sophorae*, (Hemiptera: Margarodidae). *Pest Manage Sci* **72**:1959–1964 (2016).

22 Germinara GS, Cristofaro AD and Rotundo G, Antennal olfactory responses to individual cereal volatiles in *Theocolax elegans*, (Westwood) (hymenoptera: Pteromalidae). *J Stored Prod Res* **3**:195–200 (2009).

23 Bruce TJA, Wadhams LJ and Woodcock CM, Insect host location: a volatile situation. *Trends Plant Sci* **10**:269–274 (2005).

24 Germinara GS, Cristofaro AD and Rotundo G, Behavioral responses of adult *Sitophilus granarius* to individual cereal volatiles. *J Chem Ecol* **4**:523–529 (2008).

25 Knolhoff LM and Heckel DG, Behavioral assays for studies of host plant choice and adaptation in herbivorous insects. *Annu Rev Entomol* **59**: 263–278 (2014).

26 Beyaert I and Hilker M, Plant odour plumes as mediators of plant–insect interactions. *Biol Rev* **89**:68–81 (2014).

27 Phillips TW, Jiang XL, Burkholder WE, Phillips JK and Tran HQ, Behavioural responses to food volatiles by two species of stored-product Coleoptera, *Sitophilus oryzae* (Curculionidae) and *Tribolium castaneum* (Tenebrionidae). *J Chem Ecol* **19**:723–734 (1993).

28 Tang Q, Wu Y, Liu B and Yu Z, Infochemical-mediated preference behavior of the maize weevil, *Sitophilus zeamais* Motschulsky, when searching for its hosts. *Entomol Fenn* **19**:257–267 (2008).

29 Ukeh DA, Birkett MA, Bruce TJ, Allan EJ, Pickett JA and Mordue AJ, Behavioural responses of the maize weevil, *Sitophilus zeamais*, to

host (storedgrain) and non-host plant volatiles. *Pest Manage Sci* **1**: 44–50 (2010).
30 Trematerra P, Sciaretta A and Tamasi E, Behavioural responses of *Oryzaephilus surinamensis*, *Tribolium castaneum* and *Tribolium confusum* to naturally and artificially damaged durum wheat kernels. *Entomol Exp Appl* **94**:195–200 (2000).
31 Ndomo-Moualeu AF, Ulrichs C and Adler C, Behavioral responses of *callosobruchus maculatus*, to volatile organic compounds found in the headspace of dried green pea seeds. *J Pest Sci* **3**:1–10 (2015).
32 Abdoulaye IA and Guo YJ, A review of recent advances in neuroprotective potential of 3-n-butylphthalide and its derivatives. *Biomed Res Int* **2016**:1–9 (2016).
33 Peng Y, Xing C, Lemere CA, Chen G, Wang L, Feng Y *et al*., L-3-*n*-butylphthalide ameliorates β-amyloid-induced neuronal toxicity in cultured neuronal cells. *Neurosci Lett* **434**:224–229 (2008).
34 Tsukamoto T, Ishikawa Y and Miyazawa M, Larvicidal and adulticidal activity of alkylphthalide derivatives from rhizome of *Cnidium officinale* against *Drosophila melanogaster*. *J Agric Food Chem* **53**:5549–5553 (2005).
35 Gong Y, Liu W, Huang X, Hao L, Li Y and Sun S, Antifungal activity and potential mechanism of N-butylphthalide alone and in combination with fluconazole against Candida albicans. *Front Microbiol* **10**:1461 (2019).
36 Champakaew D, Junkum A, Chaithong U, Jitpakdi A, Riyong D, Sanghong R *et al*., *Angelica sinensis* (Umbelliferae) with proven repellent properties against *Aedes aegypti*, the primary dengue fever vector in *Thailand*. *Parasitol Res* **114**:2187–2198 (2015).
37 Kuwahara Y, Fukami H, Howard R, Ishii S, Matsumura F and Burkholder WE, Chemical studies on the Anobiidae: sex pheromone of the drugstore beetle, *Stegobium paniceum* (L.) (Coleoptera). *Tetrahedron* **34**:1769–1774 (1978).
38 Kodama H, Ono M, Kohno M and Ohnishi A, Stegobiol, a new sex pheromone component of drugstore beetle (*Stegobium paniceum* L.). *J Chem Ecol* **13**:1871–1879 (1987).
39 Child RE and Pinniger DB, Insect trapping in museums and historic houses. *Stud Conserv* **39**:129–131 (1994).
40 Phillips TW, Semiochemicals of stored-product insects: research and applications. *J Stored Prod Res* **33**:17–30 (1997).
41 Phillips TW and Throne JE, Biorational approaches to managing stored-product insects. *Annu Rev Entomol* **55**:375–397 (2010).
42 Landolt PJ and Phillips TW, Host plant influence on sex pheromone behavior of phytophagous insects. *Annu Rev Entomol* **42**:371–391 (1997).
43 Reddy GVP and Guerrero A, Interactions of insect pheromones and plant semiochemicals. *Trends Plant Sci* **9**:253–261 (2004).
44 Pope TW, Campbell C, Hardie J, Pickett JA and Wadhams LJ, Interactions between host-plant volatiles and the sex pheromones of the bird cherry-oat aphid, *Rhopalosiphum padi* and the damson-hop aphid, Phorodon humuli. *J Chem Ecol* **33**:157–165 (2007).
45 Varela N, Avilla J, Anton S and Gemeno C, Synergism of pheromone and host-plant volatile blends in the attraction of *Grapholita molesta* males. *Entomol Exp Appl* **141**:114–122 (2011).
46 Baroffio CA, Sigsgaard L, Ahrenfeldt EJ, Borg-Karlson AK, Bruun SA, Cross JV *et al*., Combining plant volatiles and pheromones to catch two insect pests in the same trap: examples from two berry crops. *Crop Prot* **109**:1–8 (2018).
47 Rojas CJ, Electrophysiological and behavioural responses of the cabbage moth to plant volatiles. *J Chem Ecol* **25**:1867–1883 (1999).
48 Tasin M, Backamanì AC, Bengtsson M, Ioriatti C and Witzgall P, Essential host plant cues in the grapevine moth. *Naturwissenschaften* **93**:141–144 (2006).
49 Najar-Rodriguez AJ, Galizia CG, Stierle J and Dorn S, Behavioral and neurophysiological responses of an insect to changing ratios of constituents in host plant-derived volatile mixtures. *J Exp Biol* **213**:3388–3397 (2010).
50 Querner P and Biebl S, Using parasitoid wasps in integrated Pest management in museums against biscuit beetle (*Stegobium paniceum*) and webbing clothes moth (*Tineola bisselliella*). *J Entomol Acarol Res* **2**:169–175 (2012).

11. Attraction of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) to the semiochemical volatiles of stored rice materials

Yu Cao[1], Qiqi Hu[1], Lujie Huang[1], Christos G. Athanassiou[2], Filippo Maggi[3], Ilaria D'Isita[4], Yangyang Liu[1], Onofrio Marco Pistillo[4], Mingzhi Miao[1], Giacinto Salvatore Germinara[4*], Can Li[1*]. *Journal of Pest Science, 2024, 97(1), 73-85.*
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[1] Guizhou Provincial Key Laboratory for Rare Animal and Economic Insect of the Mountainous Region, Guizhou Provincial Engineering Research Center for Biological Resources Protection and Efficient Utilization of the Mountainous Region, Department of Biology and Engineering of Environment, Guiyang University, Guiyang, People's Republic of China

[2] School of Agricultural Sciences, University of Thessaly, Volos, Greece

[3] School of Pharmacy, Chemistry Interdisciplinary Project (ChIP) research center, Via Madonna delle Carceri 9/B, Camerino, Italy

[4] Department of Agricultural Sciences, Food, Natural Resources and Engineering, University of Foggia, Foggia, Italy

*Corresponding author

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ORIGINAL PAPER

Attraction of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) to the semiochemical volatiles of stored rice materials

Yu Cao[1] · Qiqi Hu[1] · Lujie Huang[1] · Christos G. Athanassiou[2] · Filippo Maggi[3] · Ilaria D'Isita[4] · Yangyang Liu[1] · Onofrio Marco Pistillo[4] · Mingzhi Miao[1] · Giacinto Salvatore Germinara[4] · Can Li[1]

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Abstract

In order to explore the influence of stored cereal volatiles on the behavior of *Sitophilus oryzae,* the olfactory responses of adult rice weevils to the volatiles of different rice cultivars [Red brown rice (RBR), Daohuaxiangmi (DHXM), Baishuigongmi (BSGM), Yashuixinmi (YSXM), and white glutinous rice (WGR)] were studied using electroantennography (EAG) and behavioural bioassays in different types of olfactometers. *S. oryzae* showed significantly different preferences for these rice cultivars, in the order RBR > DHXM = YSXM ≥ BSGM > WGR. Furthermore, 26 components were identified in the volatile profile of RBR. Nonanal (29.37%), hexanal (16.08%), and 1-octen-3-ol (8.83%) were the most abundant compounds. EAG recordings showed that the antennae of *S. oryzae* were able to perceive these three compounds in a dose-dependent manner. The compounds elicited significant EAG responses at various concentrations, with the strongest responses at 100 μg μL^{-1}. *S. oryzae* had a significant positive behavioural response to nonanal, hexanal, and 1-octen-3-ol at various concentrations, with the most attractive being 50, 100, and 100 μg μL^{-1}, respectively. The olfactory preferences of *S. oryzae*, based on a comparison of these compounds at their optimal concentrations, were nonanal > 1-octen-3-ol = hexanal. These results indicated that the volatiles of the preferred rice cultivar (RBR) were perceived by the peripheral olfactory system of *S. oryzae* adults and individually elicited positive chemotaxis. These findings offer new insights into the mechanism of host preferences of stored-grain pests. Nonanal showed the greatest potential for use as a novel monitoring and control tool against this storage-beetle pest.

Keywords *Sitophilus oryzae* · Rice cultivars · Volatiles · GC–MS · EAG responses · Behavioral responses · Pest control

Key message

- *Sitophilus oryzae* damages stored cereals and is attracted to rice grain volatiles.
- Nonanal, 1-octen-3-ol, and hexanal are the main volatiles of red brown rice.
- These three compounds differed in terms of the optimal concentrations that were attractive to *S. oryzae*.
- In bioassays, *S. oryzae* preferred nonanal to 1-octen-3-ol and hexanal
- Nonanal has the greatest potential as a lure to monitor and/or control S. *oryzae*.

Introduction

Grain storage losses can account for up to 50% of the total harvest, resulting in the loss of several billion dollars globally (Tian et al. 2023). Damage and loss of stored products by insect pests is one of the most common challenges in grain storage. Postharvest losses of approximately 9% in developed countries and up to 50% in developing countries have been reported, with considerable economic losses. Serious qualitative degradation that may endanger human health is also of concern (Berhe et al. 2022).

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✉ Giacinto Salvatore Germinara
giacinto.germinara@unifg.it

✉ Can Li
lican790108@163.com

Extended author information available on the last page of the article

The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is among the most widespread and destructive primary pests of stored cereals such as rice, wheat, maize, barley, sorghum, buckwheat, pulses, dried beans, cashew nuts, and products derived from them (Nwaubani et al. 2014; Mehta and Kumar 2020). Control of this pest is difficult because the immature stages develop inside grain kernels, which hinders the accurate detection of infestations and the effectiveness of control measures, resulting in widespread damage to stored cereals (Trematerra et al. 1999; Nwaubani et al. 2014; Mehta et al. 2021).

Sitophilus oryzae shows host preferences for different stored products in terms of its feeding, development, oviposition, and degree of damage (Trematerra et al. 2013; Gvozdenac et al. 2020; Jalaeian et al. 2021; Mehta and Kumar 2021). In addition, the behavior and performance of insects differ depending on the host's physicochemical characteristics, such as the occurrence of toxins, inhibitors, volatiles, macronutrients, and micronutrients, as well as kernel hardness and texture. Infestations of *S. oryzae* have been observed in different types of stored commodities, in terms of both damage and the progeny production capacity (Swamynarayana et al. 2014; Mehta et al. 2021; Mehta and Kumar 2021; Jalaeian et al. 2021). In places where storage facilities are inadequate, the resistance/susceptibility of the stored grain to *S. oryzae* might be influenced by one or a few factors, which together determine the "varietal resistance" (Badii et al. 2013; Mehta and Kumar 2020).

In the past two decades, managing the loss of stored food products to insect damage has relied heavily on the use of synthetic insecticides (Nayak et al. 2020; Brito et al. 2021). This strong reliance on a particular range of chemicals has led to complications such as toxic residues, resistance, pollution, and control failures (Isman 2006; Nayak et al. 2020). These challenges and the growing awareness of environmental issues have prompted researchers to explore suitable alternatives to chemical pesticides. One such alternative is the use of plant products because they are biodegradable, environmentally friendly, and safe for human health (Phillips and Throne 2010; Pavela and Benelli 2016; Hubert et al. 2018). The use of certain plant products as grain protectants has shown a good degree of success against *S. oryzae* (Bala 2015; Bhanderi et al. 2015; Mehta and Kumar 2020; Kundu et al. 2020).

Behavioral manipulation is an important and eco-friendly insect control method to manage pest populations. Previous studies have revealed that rice is the preferred host of *S. oryzae* in terms of oviposition, grain damage, and F1 progeny under free-choice conditions, followed by wheat, barley, and maize (Subedi et al. 2009 and references therein). In this study, the behavioral and electrophysiological responses of *S. oryzae* to the volatiles of different rice cultivars were investigated. We tested the following hypotheses: (1) *S. oryzae* is attracted to odors from rice cultivars; (2) *S. oryzae* shows a preference for certain rice cultivars; and (3) *S. oryzae* can perceive and behaviorally respond to individual volatile compounds. The results will provide new insights into the mechanism of host selection in *S. oryzae*, based on the chemical ecology of interactions between stored products and pests. Furthermore, our results will narrow the gap between theoretical research and the practical application of behavior regulation for pest control. In particular, the study will provide useful information for the development of new attractants for the sustainable control of *S. oryzae* in stored products, through natural resource-based substances.

Methods and materials

Insect rearing

Sitophilus oryzae have been reared in Guizhou Provincial Key Laboratory for Rare Animal and Economic Insect of the Mountainous Region, Guiyang University, China, since 2019. *S. oryzae* are maintained on wheat kernels in 5 L glass jars at 28 ± 1 °C, 60 ± 5% relative humidity, and an 8:16 h light/dark photoperiod, as reported by Li et al. (2009) and Wang et al. (2022b). Secondary infestation by moisture-sensitive mites was prevented using the method of Steiner et al. (2007).

Behavioral responses of *S. oryzae* to rice-borne volatiles

Odor sources

Red brown rice (Guihong No.1), Daohuaxiangmi (Wuyoudao No.4), Baishuigongmi (Zhaoyou 5455), Yashuixinmi (Qianyou 64), and white glutinous rice (Tongnian No.1) (abbreviated as RBR, DHXM, BSGM, YSXM, and WGR, respectively) were purchased from the Guiyang Grain Commodity Market, Guiyang, Guizhou, China. No insecticides had been applied to these rice cultivar materials. Peeled rice grains were used for the behavioral responses and GC–MS bioassays.

Y-tube bioassays

Two types of two-way comparisons were made: 1) rice (25 g) *versus* clean air (CA); 2) pairings of all combinations of the five rice varieties (25 g each). The olfactory responses of *S. oryzae* were evaluated in a Y-tube olfactometer using the method described in our previous studies (Cao et al. 2018, 2019). Unmated *S. oryzae* adults (2–3 days old) were used in the Y-tube bioassays. The air flow was set to

250 mL min^{-1} arm^{-1}. Each insect was observed for 5 min and if the insect had not made a choice within 5 min, it was discarded and reported as ‘no choice’. In total, 50–60 adults were used in each odor test. The olfactometer was cleaned after each tested insect (Carpita et al. 2012). All experiments were conducted between 9:00 AM and 5:00 PM. Choices made by male and female *S. oryzae* were tested separately. If there were no significant differences in the variance between males and females, the data were pooled and the choices were considered independent of sex (Collins et al. 2004, Cao et al. 2018).

Six-arm olfactometer bioassays

The behavioral responses of adult *S. oryzae* to the blends of volatile organic compounds (VOCs) emitted by different rice cultivars were evaluated in a six-arm olfactometer using the methods of Liu et al. (2016) and Cao et al. (2019). In brief, the six-arm olfactometer consisted of a central chamber (12 cm internal diameter) with six arms, each connected to a glass tube that projected outwards at an equal distance; angles between pairs of tubes were all 60°. Each arm was connected with Teflon tubing to a glass vessel, which was used to contain the control or rice materials of each of the five cultivars. The airflow was set at 250 L min^{-1} to drive the odor from the source toward the insects. *Sitophilus oryzae* adults (2–3 days old), starved for 6 h, were introduced in groups (150 individuals per group) into the six-arm olfactometer with a brush. The *S. oryzae* that entered an arm of the olfactometer within 20 min were considered to have chosen that odor source.

Collection and analysis of volatiles

Rice VOCs were collected as described previously (Cao et al. 2018, 2019). The collected VOCs were analyzed by gas chromatography–mass spectrometry (GC–MS) (HP6890/5975C, Agilent Technologies, Santa Clara, CA, USA). An apolar chromatographic column (ZB-5MSI 5% phenyl-95% dimethylpolysiloxane 30 m × 0.25 mm, film thickness 0.25 µm) was used. Temperature was programmed to rise from 40 to 255 °C at 5 °C min^{-1}, and was then maintained for 2 min. The temperatures of the vaporizing chamber, interface, and quadrupole rod were set at 250, 280, and 150 °C, respectively. The chemical identities of the main peaks in the chromatograms were determined by comparing the mass spectra of compounds with those in databases (NIST 2017 and WILEY 275). An additional criterion for peak assignment was consistency between the temperature-programmed retention indices (RIs) obtained and those recorded in the NIST database (2017).

Behavioral responses of *S. oryzae* to rice VOCs

Odor treatment

The VOC mixture from RBR was the most attractive to *S. oryzae*, as assessed by the Y-tube and six-arm olfactometer bioassays. The behavioral responses of *S. oryzae* to nonanal, 1-octen-3-ol, and hexanal, the most abundant VOCs of RBR, were then assessed in subsequent experiments.

Electroantennography

The antennal sensitivity of male and female *S. oryzae* to increasing concentrations of hexanal, 1-octen-3-ol, and nonanal was evaluated by electroantennography (EAG), as described elsewhere (Germinara et al. 2007; Paventi et al. 2021). Briefly, the head of an adult insect was excised using a scalpel and placed between two glass capillary electrodes (Micro-glass, Naples, Italy) filled with saline solution. The recording electrode (diameter ~ 100 µm) was put in contact with the abaxial surface of the antennal club, and the neutral electrode was introduced into the base of the head. AgCl coated silver wires were used to maintain electrical continuity between the antennal preparation and an AC/DC UN-6 amplifier in DC mode. The amplifier was connected to a computer equipped with the EAG 2.0 program (Syntech Laboratories, Hilversum, The Netherlands). The EAG response of *S. oryzae* to each test compound at different doses (0.01, 0.1, 1, 10, 100, and 1000 µg) was measured using the method detailed in Cao et al. (2022).

Y-tube bioassays

The Y-tube olfactometer described above was also used to test the olfactory responses of *S. oryzae* to hexanal, 1-octen-3-ol, and nonanal. Mineral oil was used as the control. The test compound (10 µL of a compound solution at concentrations ranging from 0.1 to 100 µg μL^{-1}) or control (10 µL mineral oil) stimulus was adsorbed onto a filter paper disk (1.0 cm diameter) (Cao et al. 2019), which was suspended in the center of the cross section of the odor chamber by a cotton thread. *Sitophilus oryzae* individuals were allowed to choose between the test compound at a specific dose (1, 10, 100, 500, or 1000 µg) and mineral oil. Bioassays were conducted using the method detailed above using 2- to 3-day-old unmated *S. oryzae* adults. In total, 50–60 adults were tested for each test stimulus.

Six-arm bioassays

The behavioral responses of adult *S. oryzae* to different doses of each of the three compounds (hexanal, 1-octen-3-ol, and nonanal) were also evaluated in a six-arm olfactometer as described above. Each test compound (10 µL of compound solution at concentrations ranging from 0.1 to 100 µg μL^{-1}) or control (10 µL of mineral oil) was placed in an odor chamber that was connected to one of the six olfactometer arms with Teflon tubing (Cao et al. 2019). The odor from each source was driven through the connector tube to the olfactometer compartment to allow adult *S. oryzae* to choose among the odors. Hexanal, 1-octen-3-ol, and nonanal at different doses were tested. Bioassays were replicated six times using 150 individuals per replication.

Four-arm bioassays

In the six-arm olfactometer bioassays, hexanal, 1-octen-3-ol, and nonanal were most attractive to *S. oryzae* at concentrations of 100, 100, and 50 µg μL^{-1}, respectively. Therefore, the attractant power of the three compounds at their optimal concentrations was compared in further four-arm olfactometer bioassays, using the method reported by Liu et al. (2016). The details for the four-arm olfactometer have been described elsewhere (Cao et al. 2022). Hexanal, 1-octen-3-ol, and nonanal at the concentrations mentioned above were used as test stimuli, and mineral oil was used as the control. These four types of odor sources were placed in the odor chambers of the olfactometer system, and the airflow was set at 250 mL min^{-1} to drive the odor toward the insects. *S. oryzae* adults were tested in groups of 120 individuals, and bioassays were replicated six times.

Statistical analyses

The null hypothesis that *S. oryzae* adults showed no preference for either Y-tube arm (a response equal to 50:50) was tested using a chi-square goodness-of-fit test. The numbers of insects found in the different arms of the six-arm and four-arm olfactometer were subjected to Friedman two-way ANOVA by ranks. In the case of significance ($p < 0.05$), Wilcoxon signed ranks test was used for the separation of means. The corrected mean EAG response of males and females to the last dilution of each test compound was compared with the "0" value using one-sample Student's *t*-test and regarded as "activated" if significant at $p < 0.05$. The saturation level was taken as the lowest dilution at which the mean response was equal to or less than the previous one (Germinara et al. 2016). The mean EAG responses of males and females to each stimulus were compared using Student's *t*-test for independent samples at $p = 0.05$. However, because no significant differences were found between males and females, the data were pooled and analyzed together. For each dose of the three compounds, the mean EAG responses of *S. oryzae* adults were submitted to ANOVA followed by Tukey's HSD test ($p = 0.05$) for separation of means. Before ANOVA, data were submitted to Shapiro–Wilk's test to verify the normal distribution of data and to Levene's test to assess the homogeneity of variances. All statistical analyses were performed using SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Behavioral responses of S. oryzae to rice-borne volatiles

Y-tube bioassays

Sitophilus oryzae showed significant responses when offered a choice between the odor of rice materials in one chamber and clean air (CA) in the other (Fig. 1), responding positively to the volatiles of RBR, DHXM, BSGM, YSXM, and WGR (Fig. 1A).

Given a choice between pairs of these five rice cultivars, *S. oryzae* showed positive responses to the odors of RBR paired with DHXM, BSGM, YSXM, or WGR (Fig. 1B). However, *S. oryzae* showed no significant responses when DHXM, BSGM, YSXM, and WGR were paired with each other.

Six-arm bioassays

In the six-arm bioassays, odors emitted by grains of different rice cultivars attracted significantly more insects than did the control air (Friedman test: $\chi^2 = 26.676$, $df = 5$, $p < 0.001$, Wilcoxon tests: $p = 0.026–0.027$) (Fig. 2). Furthermore, RBR attracted significantly more *S. oryzae* adults than the other cultivars (Wilcoxon tests: $p = 0.026–0.028$).

Analysis of RBR volatiles

According to the GC–MS analysis, twenty-six components were identified in the volatiles from RBR (Table 1). The most abundant component was nonanal (29.37%), followed by hexanal (16.08%), and then 1-octen-3-ol (8.83%). No other component accounted for more than 5% of the volatiles of RBR, except for dodecane (5.05%).

EAG analyses

We evaluated the EAG responses of *S. oryzae* males and females to increasing doses of hexanal, 1-octen-3-ol, and nonanal (Fig. 3). Measurable EAG responses were elicited by the three compounds starting from the 0.1 µg dose. In both males and females, typical sigmoid-shaped dose responses were elicited by each compound for the dose range tested. For each compound, the mean EAG response to the

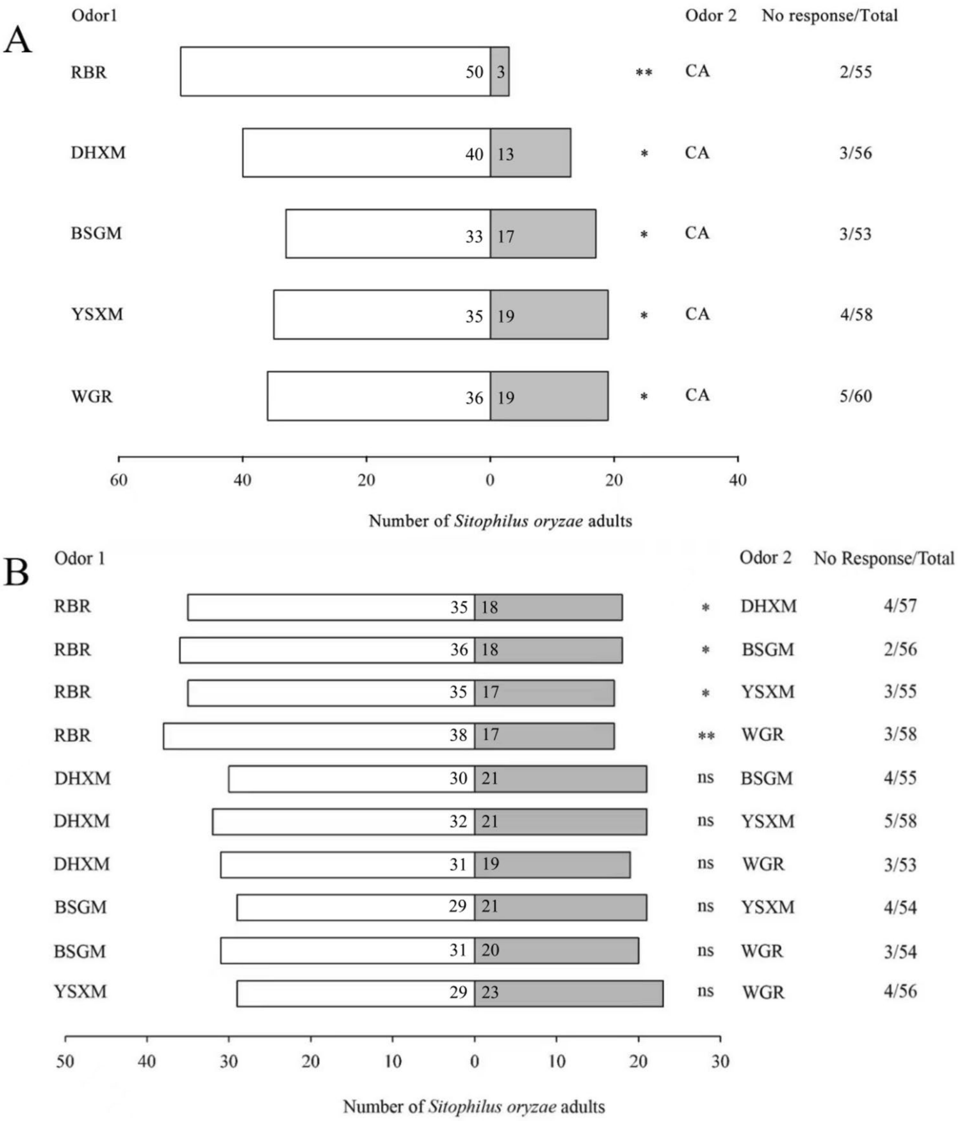


Fig. 1 Behavioral responses of *Sitophilus oryzae* to volatiles of different rice cultivars: Red brown rice (RBR), Daohuaxiangmi (DHXM), Baishuigongmi (BSGM), Yashuixinmi (YSXM), and white glutinous rice (WGR). (**A**) Attraction of rice volatiles. *S. oryzae* showed significant preferences for volatiles from different rice cultivars: RBR ($\chi^2 = 41.68$, $df = 1$, $^{**}p < 0.001$), DHXM ($\chi^2 = 5.45$, $df = 1$, $^{*}p = 0.020$), BSGM ($\chi^2 = 5.12$, $df = 1$, $^{*}p = 0.024$), YSXM ($\chi^2 = 4.74$, $df = 1$, $^{*}p = 0.029$), and WGR ($\chi^2 = 5.26$, $df = 1$, $^{*}p = 0.022$). Control was clean air (CA). (**B**) Strong attraction of RBR volatiles. RBR was more attractive to *S. oryzae* than DHXM ($\chi^2 = 4.45$, $df = 1$, $^{*}p = 0.020$), BSGM ($\chi^2 = 6.00$, $df = 1$, $^{*}p = 0.014$), YSXM ($\chi^2 = 6.23$, $df = 1$, $^{*}p = 0.013$), or WGR ($\chi^2 = 8.02$, $df = 1$, $^{**}p = 0.005$), when these rice cultivars were compared with each other. No significant differences were observed between other pairs of rice cultivars.

highest dose (1000 µg) was higher than that to the previous dose, indicating that the olfactory receptors had not become saturated. For each compound at each dose, no significant differences were observed in the mean EAG responses between males and females (hexanal: $t = 0.489$–1.29, $df = 8$, $p = 0.291$–0.636; 1-octen-3-ol: $t = 0.274$–1.392; $df = 8$, $p = 0.201$–0.791; nonanal: $t = 0.087$–1.899; $df = 8$, $p = 0.094$–0.933).

When the mean EAG results of males and females were pooled, significant differences in the EAG responses of *S. oryzae* were observed among the three compounds at doses of 10 and 100 µg (Fig. 4). At 10 µg, the mean EAG responses elicited by hexanal and 1-octen-3-ol were statistically similar but significantly higher than that induced by nonanal ($F = 8.246$, $df = 3$, $p < 0.01$). At 100 µg, the mean EAG response to hexanal was significantly higher than those to 1-octen-3-ol and nonanal ($F = 16.705$, $df = 3$, $p < 0.001$).

Y-tube bioassays with individual volatile compounds

Next, we evaluated *S. oryzae* sensitivity to nonanal, hexanal, and 1-octen-3-ol in two choice behavior experiments (Fig. 5A–C). The results clearly show that adult *S. oryzae* were attracted to all three compounds at each dose for 1, 10, 100, 500, and 1000 µg, when they were paired with mineral oil as the control.

Six-arm behavioral bioassays with individual compounds

In the six-arm bioassays, all doses of hexanal (Friedman test: $\chi^2 = 29.048$, $df = 5$, $p < 0.001$, Wilcoxon tests: $p = 0.026$–0.027), 1-octen-3-ol (Friedman test: $\chi^2 = 27.995$, $df = 5$, $p < 0.001$, Wilcoxon tests: $p = 0.026$–0.028), and nonanal (Friedman test: $\chi^2 = 29.524$, $df = 5$, $p < 0.001$, Wilcoxon tests: $p = 0.024$–0.027) were significantly

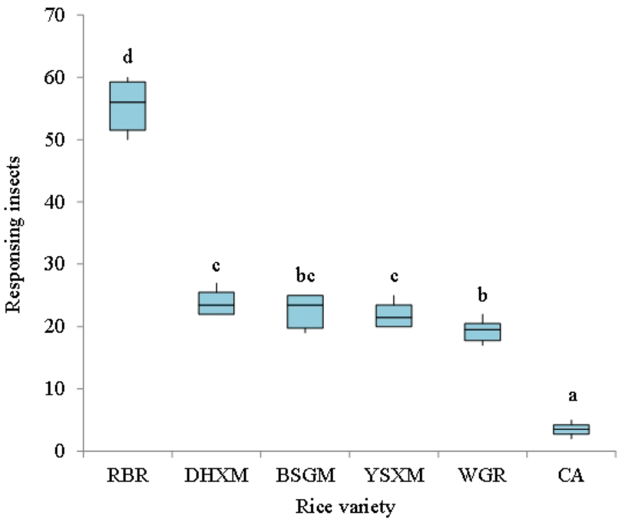


Fig. 2 Olfactory responses of *Sitophilus oryzae* adults to odors of different rice cultivars in a six-arm olfactometer. Control was clean air (CA). Each box plot represents the median and its range of dispersion (lower and upper quartiles and outliers). Different letters above box plots indicate significant differences (Wilcoxon test, $p < 0.05$). Cultivars: Red brown rice (RBR), Daohuaxiangmi (DHXM), Baishuigongmi (BSGM), Yashuixinmi (YSXM), and white glutinous rice (WGR)

more attractive than the mineral oil control (Fig. 6). Furthermore, the most attractive doses of hexanal (Wilcoxon tests: $p = 0.026–0.028$), 1-octen-3-ol (Wilcoxon tests: $p = 0.026–0.027$) and nonanal (Wilcoxon tests: $p = 0.026–0.027$) were 1000, 1000, and 500 μg, respectively.

Four-arm behavioral bioassays with individual compounds

According to the results of the six-arm olfactometer bioassays, hexanal, 1-octen-3-ol, and nonanal were compared at their most attractive doses (1000, 1000, and 500 μg, respectively) in four-arm olfactometer bioassays. All three compounds at their respective doses were significantly more attractive than the mineral oil control (Friedman test: $\chi^2 = 16.932$, $df = 3$, $p < 0.01$, Wilcoxon tests: $p = 0.027$) (Fig. 7). In addition, *S. oryzae* adults significantly preferred nonanal to hexanal or 1-octen-3-ol (Wilcoxon tests: $p = 0.027–0.028$) but showed no significant preference between hexanal and 1-octen-3-ol (Wilcoxon test: $p = 0.414$).

Discussion

Plant volatiles are important cues for many insects when searching for suitable food, oviposition, and mating sites (Visser 1986; Knolhoff and Hecke 2014; Dyer et al. 2018; Wang et al. 2022a), and for non-host substrate avoidance (Angelopoulos et al. 1999). Identification of behaviourally active compounds, either attractants or repellents, can provide the means for monitoring and direct control of insect pests.

Behavioural responses to plant VOCs have been investigated for many stored-cereal insect pests (Trematerra et al. 2000; Germinara et al. 2008; Ukeh et al. 2010; Ndomo-Moualeu et al. 2015), including *S. oryzae*. In fact, adult rice weevils are known to be attracted by the fresh grain volatiles valeraldehyde, maltol, and vanillin (Phillips et al. 1993) but repelled by postharvest waste of cardamom plants (*Elettaria cardamomum* (L.) Maton) (Widiyaningrum et al. 2019) and some individual volatile compounds such as propionic acid, menthone, and *α*-pinene (Germinara et al. 2007; Fouad et al. 2021).

Rice is recognized as a preferred host plant for *S. oryzae* (Subedi et al. 2009). Despite behavioral evidence, little attention has been given to the olfactory response of rice weevils to individual rice VOCs. In this study, therefore, we assessed the sensitivity and behavioral responses of *S. oryzae* adults to the odor stimuli of different rice cultivars, illustrating notable results for the different substrates tested.

In the Y-tube olfactometer bioassays, *S. oryzae* adults were strongly attracted by the VOCs emitted by all rice cultivars. In the six-arm bioassays, *S. oryzae* adults exhibited significantly different preferences among the five rice cultivars. The rice cultivars were ranked, from most to least preferred by *S. oryzae*, as follows: RBR > DHXM ≥ BSGM = YSXM ≥ WGR. This clear ranking preference of *S. oryzae* adults confirmed that plant-borne VOCs provide important cues for the selection of a preferred host by this pest. These results might partially explain why *S. oryzae* showed faster and larger population growth on RBR than on other materials in tests carried out by Wang et al. (2022b).

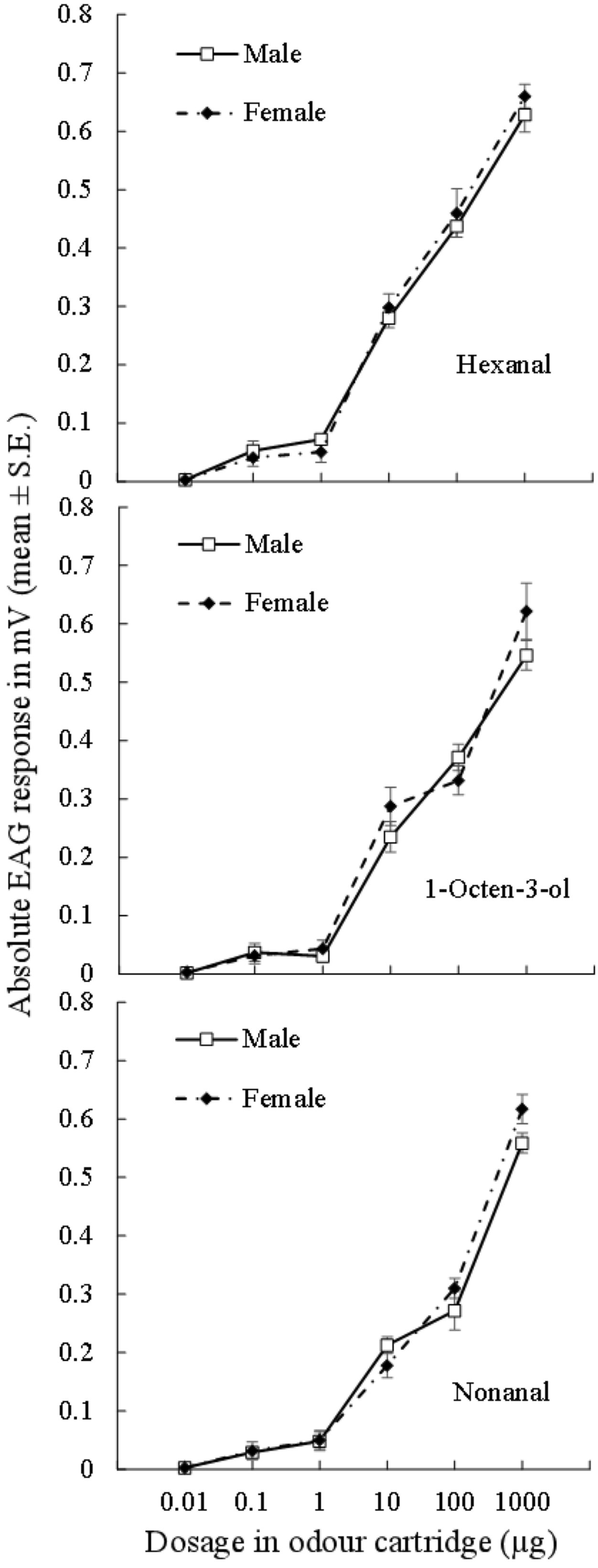
The GC–MS analyses detected 26 compounds in the VOC profile of RBR, among which, nonanal, hexanal, and 1-octen-3-ol were the most abundant components. The results of the EAG analyses showed that these three main compounds were perceived by the peripheral olfactory systems of *S. oryzae* males and females in a wide range of concentrations and in a dose-dependent manner. Once the electrophysiological activity of the three compounds was ascertained, their biological activity was further investigated in Y-tube, six-, and four-arm olfactometer bioassays. In the bioassays, hexanal, 1-octen-3-ol, and nonanal were all attractive to *S. oryzae* at various concentrations, and their most attractive concentrations were 100, 100, and 50 μg μL^{-1}, respectively. When the three compounds were compared at their most attractive concentrations, *S. oryzae* preferred nonanal over the other two, and showed equal preferences for 1-octen-3-ol and hexanal. These findings suggest that nonanal has the greatest potential for development as a kairomonal lure for *S. oryzae*. Nonanal has previously been

Table 1 Components of RBR volatiles

Number	Compound	RI	Molecular Formula	Molecular weight	Relative peak area (%)
1	Ethanol	470	C_2H_6O	46	3.40
2	Pentanal	704	$C_5H_{10}O$	86	1.30
3	1-Pentanol	765	$C_5H_{12}O$	88	1.14
4	2,3-Butanediol	787	$C_4H_{10}O_2$	90	1.54
5	Hexanal	800	$C_6H_{12}O$	100	16.08
6	1-Hexanol	821	$C_6H_{14}O$	102	2.04
7	Heptanal	905	$C_7H_{14}O$	114	0.80
8	Benzaldehyde	960	C_7H_6O	106	0.23
9	1-Octen-3-ol	974	$C_8H_{16}O$	128	8.83
10	6-Methyl-5-hepten-2-one	988	$C_8H_{14}O$	126	1.87
11	2,2,4,6,6-Pentamethyl-heptane	990	$C_{12}H_{26}$	170	2.57
12	2-Pentyl-furan	994	$C_9H_{14}O$	138	1.04
13	Decane	1000	$C_{10}H_{22}$	142	1.71
14	Octanal	1006	$C_8H_{16}O$	128	0.54
15	2-Ethyl-1-hexanol	1031	$C_8H_{18}O$	130	2.40
16	Nonanal	1104	$C_9H_{18}O$	142	29.37
17	Isophorone	1123	$C_9H_{14}O$	138	0.21
18	(*E*)-2-Nonenal	1162	$C_9H_{16}O$	140	1.62
19	Dodecane	1200	$C_{12}H_{26}$	170	5.05
20	Decanal	1206	$C_{10}H_{20}O$	156	0.82
21	6-Methyl-dodecane	1251	$C_{13}H_{28}$	184	1.39
22	4-Methyl-dodecane	1260	C13H28	184	1.03
23	Tridecane	1300	$C_{13}H_{28}$	184	0.66
24	4,6-Dimethyl-dodecane	1322	$C_{14}H_{30}$	198	2.12
25	2,4-Dimethyldodecane	1326	$C_{14}H_{30}$	198	2.63
26	Tetradecane	1400	$C_{14}H_{30}$	198	2.69
27	Unidentified		1.62		
28			1.02		

identified in oat volatiles and, among stored-product insect pests, it was found to be attractive to the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera, Silvanidae) (White et al. 1989). Moreover, nonanal has been reported as an attractant for many other Coleoptera species, including the curculionids *Listronotus maculicollis* Kirby (McGraw et al. 2011) and *Trypophloeus klimeschi* Eggers (Gao et al. 2018). Hexanal is commonly used as an indicator of lipid oxidation in cereals (Piggot et al. 1991), and its production increases over time in stored native oats (Heiniö et al. 2002) and raw oat flour (Molteberg et al. 1996). It is the main VOC released by cereal-based macaroni pasta (Trematerra et al. 2021), which has been reported as an attractant for some important secondary pests of processed cereals, such as *O. surinamensis* and the merchant grain beetle *Oryzaephilus mercator* (Fauvel) (Coleoptera: Silvanidae) (Pierce et al. 1990). This compound was found to exert a strong repellent effect against the adults of the granary weevil, *Sitophilus granarius* (L.) (Germinara et al. 2008) and even to inhibit their olfactory orientation towards wheat grains, a highly attractive food source for this species (Germinara et al. 2015). 1-Octen-3-ol is formed when grain is contaminated by mold (Kaminski et al. 1973) and is an attractant for *O. surinamensis* (White et al. 1989).

All the currently known *S. oryzae* host-plant attractants are listed in the FDA's official database on food additives (EAFUS, Everything Added to Food in the United States). These additives are readily available on the market, at relatively low cost, which would simplify the preparation of a valuable lure for practical applications. In contrast with other stored-product beetle species, where attractants have been successfully developed and thoroughly utilized at the commercial scale, there is still inadequate information on the development of a lure for *S. oryzae*, as well as for other species of this genus (Athanassiou et al. 2006; Trematerra et al. 2015). To this end, future field trapping experiments (Fields and White 2002; Cook et al. 2007) would provide new insights for the development of novel monitoring and control strategies for



◀ **Fig. 3** Electroantennography dose–response curves of *Sitophilus oryzae* males and females to increasing doses of hexanal, 1-octen-3-ol, and nonanal. Mean values are shown. At each dose, mean male and female EAG responses were not significantly different at $p=0.05$ (Student's *t*-test for independent samples, $p=0.05$)

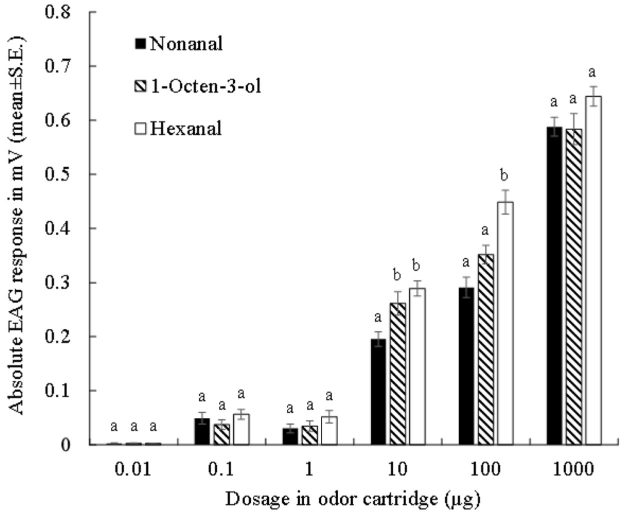


Fig. 4 Electroantennography responses of *Sitophilus oryzae* males and females to different doses of hexanal, 1-octen-3-ol, and nonanal. Mean values are shown. For each dose, different letters indicate significant differences at $p<0.05$ (Tukey's HSD test)

this pest (Wakefield et al. 2005; Phillips and Throne 2010; Guo et al. 2020). Because many studies have highlighted the importance of the ratio and concentrations of plant volatiles for host location by phytophagous insects (Najar-Rodriguez et al. 2010; Webster et al. 2010; Cha et al. 2011), it is important to evaluate different dosages and mixtures of kairomones. From a management perspective, it would also be useful to evaluate kairomone mixtures in combination with (4S,5R)-5-hydroxy-4-methyl-3-heptanone, the aggregation pheromone of *S. oryzae* (Walgenbach et al. 1987). In fact, additive or synergistic interactions between food odors and insect pheromones strongly suggest that more effective traps can be devised to manage this insect pest (Phillips et al. 1993; Wakefield et al. 2005; Athanassiou et al. 2006).

Volatile compounds emitted by the hosts' food can elicit long-range attraction in parasitoids (Vinson 1985; Nordlund et al. 1988; Lewis et al. 1990). Therefore, future studies should also test the attractiveness of VOCs identified from the stored rice materials to the natural enemies of *S. oryzae*, for example, *Anisopteromalus calandrae* (Howard), *Lariophagus distinguendus* (Förster), and *Theocolax elegans* (Westwood)

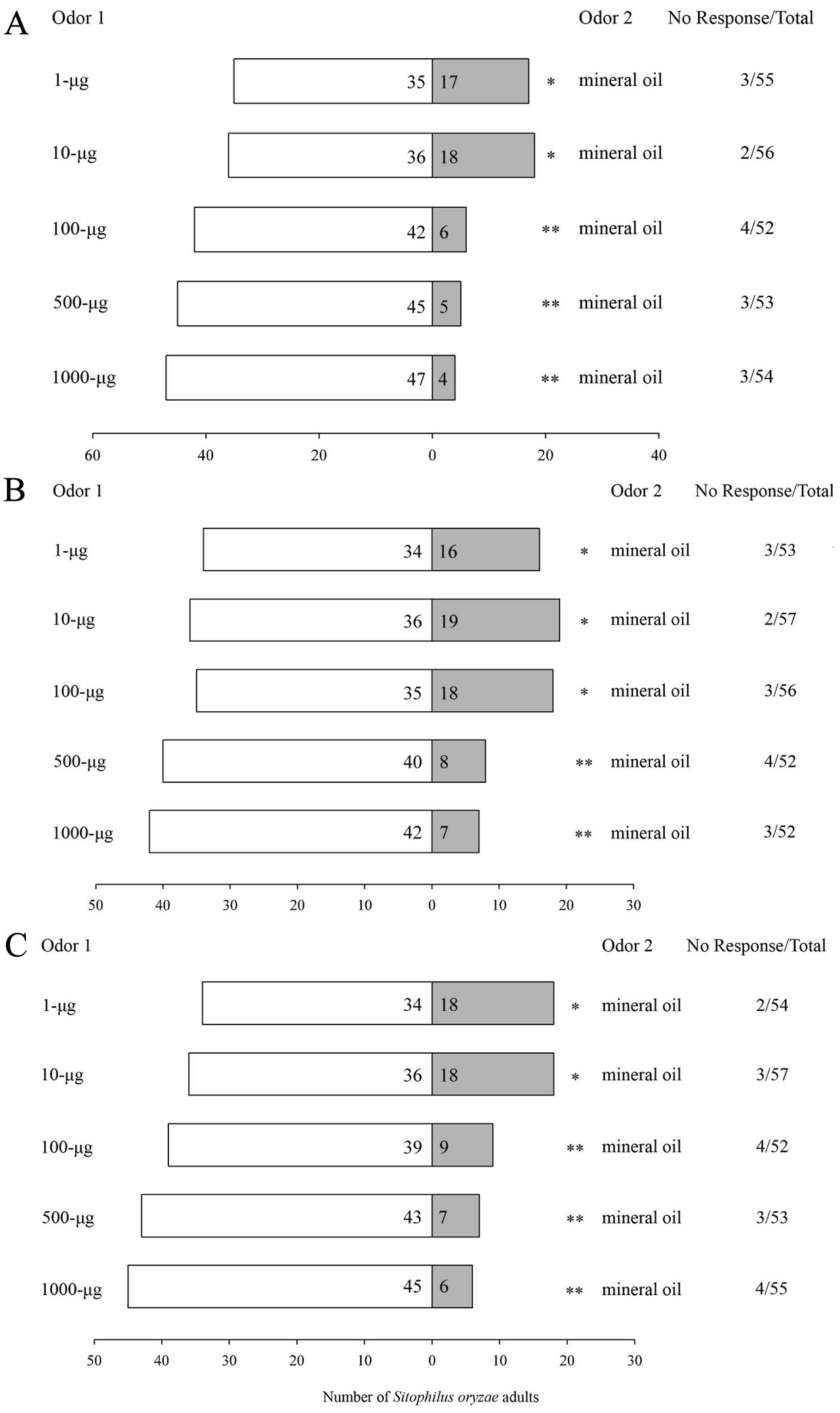


Fig. 5 Behavioral responses of *Sitophilus oryzae* to volatile compounds of red brown rice. (**A**) Attraction to nonanal, which was detected at 1 µg ($\chi^2 = 6.23$, $df = 1$, $*p = 0.013$), 10 µg ($\chi^2 = 6.00$, $df = 1$, $*p = 0.014$), 100 µg ($\chi^2 = 27.00$, $df = 1$, $**p < 0.01$), 500 µg ($\chi^2 = 32.00$, $df = 1$, $**p < 0.01$), and 1000 µg ($\chi^2 = 36.26$, $df = 1$, $**p < 0.01$). (**B**) Attraction to hexanal, which was detected at 1 µg ($\chi^2 = 6.48$, $df = 1$, $*p = 0.011$), 10 µg ($\chi^2 = 5.26$, $df = 1$, $*p = 0.022$), 100 µg ($\chi^2 = 5.45$, $df = 1$, $*p = 0.02$), 500 µg ($\chi^2 = 21.33$, $df = 1$, $**p < 0.01$), and 1000 µg ($\chi^2 = 25.00$, $df = 1$, $**p < 0.01$). (**C**) Attraction to 1-octen-3-ol, which was detected at 1 µg ($\chi^2 = 4.92$, $df = 1$, $*p = 0.027$), 10 µg ($\chi^2 = 6.00$, $df = 1$, $*p = 0.014$), 100 µg ($\chi^2 = 18.75$, $df = 1$, $**p < 0.01$), 500 µg ($\chi^2 = 25.92$, $df = 1$, $**p < 0.01$), and 1000 µg ($\chi^2 = 29.82$, $df = 1$, $**p < 0.01$). Mineral oil was used as the control in all tests.

(Wen et al. 1994; Lucas and Jordi 2002; Germinara et al. 2009). Such information would be useful to develop and refine biocontrol management strategies based on the use of natural enemies to control *S. oryzae* in stored rice. At the same time, rice varieties that are less susceptible than others to infestations of *S. oryzae* should be further investigated under a varietal resistance-based strategy.

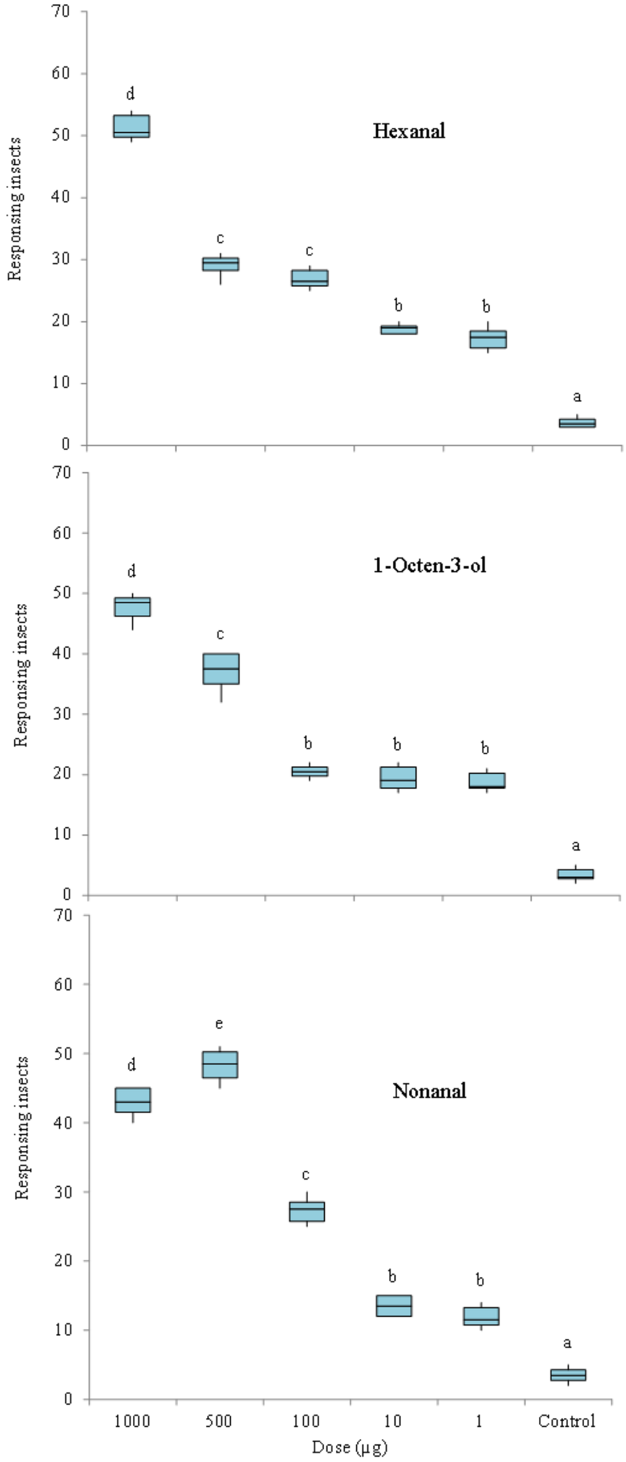


Fig. 6 Olfactory responses of *Sitophilus oryzae* adults to different doses of hexanal, 1-octen-3-ol, and nonanal in a six-arm olfactometer. Control was mineral oil. Each box plot represents the median and its range of dispersion (lower and upper quartiles and outliers). Different letters above box plots indicate significant differences (Wilcoxon test, $p < 0.05$)

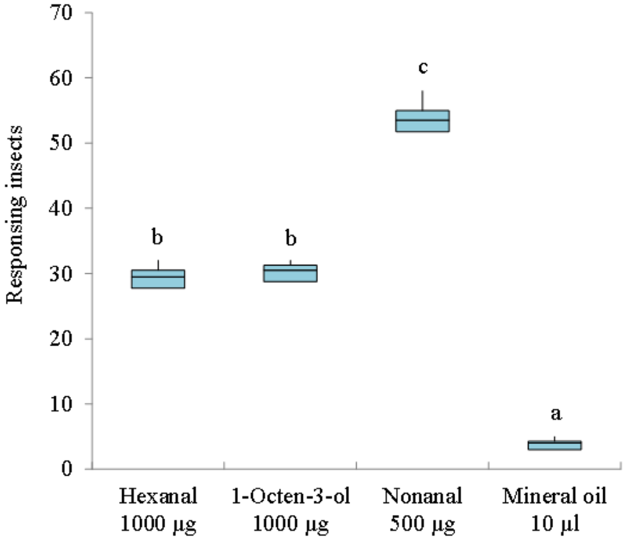


Fig. 7 Olfactory responses of *Sitophilus oryzae* to hexanal, 1-octen-3-ol, and nonanal at their most attractive concentrations in a four-arm olfactometer. Stimuli were 10 µL mineral oil (control) and mineral oil solutions of hexanal, 1-octen-3-ol, and nonanal at concentrations of 100, 100, and 50 µg µL^{-1}, respectively. Each box plot represents the median and its range of dispersion (lower and upper quartiles and outliers). Different letters above box plots indicate significant differences (Wilcoxon test, $p < 0.05$)

Our results confirm the three tested hypotheses: *S. oryzae* was attracted to odors from rice cultivars, showed a clear preference for the odor of RBR, and perceived and responded to three of the main components of the volatiles of RBR. Overall, the results of this study show that semiochemical volatiles of stored rice grains are involved in host-plant selection by *S. oryzae*. Individual compounds among the main volatile components of the preferred rice cultivar (RBR) were able to stimulate the peripheral olfactory systems of adult *S. oryzae* and elicit a positive chemotactic response. The most attractive compound identified in this study, nonanal, alone and in combination with other attractants, has potential application in the development of a kairomonal lure for trapping rice weevil adults at different stages of rice production, storage, and processing.

Author contributions

YC, GSG and CL conceived and designed the research. QQH, LJH, IDI, YYL, MP, and MZM conducted the experiments. YC, FM and GSG analyzed the data. YC, CGA and GSG wrote the manuscript. All of the authors read and approved the manuscript.

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Data availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This research did not involve any human participants and/or animals, only the stored products pest *Sitophilus oryzae*.

Informed consent Not applicable.

References

Angelopoulos N, Birkett MA, Hick AJ, Hooper AM, Pickett JA, Pow EM et al (1999) Exploiting semiochemicals in insect control. Pestic Sci 55:225–235

Athanassiou CG, Kavallieratos NG, Trematerra P (2006) Responses of *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae) to traps baited with pheromones and food volatiles. Eur J Entomol 103:371–378

Badii KB, Asante SK, Adarkwa C (2013) Varietal differences in the susceptibility of new rice for Africa (NERICA) to *Sitophilus oryzae* L. (Coleoptera: Curculionidae). Afr J Agr Res 8(16):1375–1380

Bala S (2015) Ecofriendly management of khapra beetle, *Trogoderma granarium* and rice weevil, *Sitophilus oryzae* through plant products in the stored wheat. J Entomol Res 39:249–252

Berhe M, Subramanyam B, Chichaybelu M, Demissie G, Abay F, Harvey J (2022) Post-harvest insect pests and their management practices for major food and export crops in East Africa: an ethiopian case study. InSects 13:1068

Bhanderi GR, Radadia GG, Patel DR (2015) Ecofriendly management of rice weevil *sitophilus oryzae* (linnaeus) in sorghum. Indian J Entomol 77(3):210–213

Brito VD, Achimón F, Pizzolitto RP, Sánchez AR, Torres EAG, Zygadlo JA, Zunino MP (2021) An alternative to reduce the use of the synthetic insecticide against the maize weevil *Sitophilus zeamais* through the synergistic action of *Pimenta racemosa* and *Citrus sinensis* essential oils with chlorpyrifos. J Pest Sci 94:409–421

Cao Y, Li S, Benelli G, Germinara GS, Yang J, Yang WJ, Li C (2018) Olfactory responses of *Stegobium paniceum* to different Chinese medicinal plant materials and component analysis of volatiles. J Stored Prod Res 76:122–128

Cao Y, Benelli G, Germinara GS, Maggi F, Zhang YJ, Luo SL, Yang H, Li C (2019) Innate positive chemotaxis to paeonal from highly attractive Chinese medicinal herbs in the cigarette beetle. Lasioderma Serricorne Sci Rep 9(1):6995

Cao Y, Pistillo OM, Lou YB, D'isita I, Maggi F, Hu QQ, Germinara GS, Li C (2022) Electrophysiological and behavioural responses of *Stegobium paniceum* to volatile compounds from Chinese medicinal plant materials. Pest Manag Sci 78:3697–3703

Carpita A, Canale A, Raffaelli A, Saba A, Benelli G, Raspi A (2012) (Z)-9-Tri-cosene identified in rectal glands extracts of *Bactrocera oleae* males: first evidence of a male-produced female attractant in olive fruit fly. Naturwissenschaften 99:77–81

Cook SM, Khan ZR, Pickett JA (2007) The use of push-pull strategies in integrated pest management. Ann Rev Entomol 52(1):375–400

Dyer LA, Philbin CS, Ochsenrider KM, Richards LA, Massad TJ, Smilanich AM, Forister ML, Parchman TL, Galland LM, Hurtado PJ, Espeset AE, Glassmire AE, Harrison JG, Mo C, Yoon S, Pardikes NA, Muchoney ND, Jahner JP, Slinn HL, Shelef O, Dodson CD, Kato MJ, Yamaguchi LF, Jeffrey CS (2018) Modern approaches to study plant–insect interactions in chemical ecology. Nat Rev Chem 2:50–64

Fields PG, White N (2002) Alternatives to methyl bromide treatments for stored-product and quarantine insects. Annu Rev Entomol 47(1):331–359

Fouad HA, Tavares WDS, Zanuncio JC (2021) Toxicity and repellent activity of monoterpene enantiomers to rice weevils (*Sitophilus oryzae*). Pest Manag Sci 77(7):3500–3507

Gao G, Dai L, Gao J, Wang J, Chen H (2018) Volatile organic compound analysis of host and non-host poplars for *Trypophloeus klimeschi* (Coleoptera: Curculionidae: Ipinae). Russ J Plant Physiol 65:916–925

Germinara GS, Rotundo G, De Cristofaro A (2007) Repellence and fumigant toxicity of propionic acid against adults of *Sitophilus granarius* (L.) and *S. oryzae* (L.). J Stored Prod Res 43:229–233

Germinara GS, De Cristofaro A, Rotundo G (2008) Behavioral responses of adult *Sitophilus granarius* to individual cereal volatiles. J Chem Ecol 34(4):523–529

Germinara GS, De Cristofaro A, Rotundo G (2009) Antennal olfactory responses to individual cereal volatiles in *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae). J Stored Prod Res 45:195–200

Germinara GS, De Cristofaro A, Rotundo G (2015) Repellents effectively disrupt the olfactory orientation of *Sitophilus granarius* to wheat kernels. J Pest Sci 88(4):675–684

Germinara GS, De Cristofaro A, Rotundo G (2016) Electrophysiological and behavioral responses of *Teocolax elegans* (Westwood) (Hymenoptera: Pteromalidae) to cereal grain volatiles. Biomed Res Int 2016:1–8

Guo XJ, Yu QQ, Chen DF, Wei JL, Yang PC, Yu J, Wang XH, Kang L (2020) 4-Vinylanisole is an aggregation pheromone in locusts. Nature 584:584–588

Gvozdenac S, Tanaskovi S, Vukajlovi FN, Prvulovic D, Viacki V (2020) Host and ovipositional preference of rice weevil (*Sitophilus oryzae*) depending on feeding experience. Appl Ecol Env Res 18(5):6663–6673

Heiniö RL, Lehtinen P, Oksman-Caldentey KM, Poutanen K (2002) Differences between sensory profiles and development of rancidity during long-term storage of native and processed oat. Cereal Chem 79:367–375

Hubert J, Stejskal V, Athanassiou CG, Throne JE (2018) Health hazards associated with arthropod infestation of stored products. Annu Rev Entomol 63:553–573

Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 51:45–66

Jalaeian M, Mohammadzadeh M, Mohammadzadeh M, Borzoui E (2021) Rice cultivars affect fitness-related characteristics and digestive physiology of the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). J Stored Prod Res 93:101821

Kaminski E, Stawicki S, Wasowicz E, Przybylski R (1973) Detection of deterioration of grain by gas chromatography. Ann Technol Agric 22:401–408

Knolhoff LM, Heckel DG (2014) Behavioral assays for studies of host plant choice and adaptation in herbivorous insects. Annu Rev Entomol 59:263–278

Kundu B, Hath TK, Chakraborty D (2020) Evaluation of different wheat germplasms against rice weevil *Sitophilus oryzae* (L.). J Entomol Res 44(2):291

Lewis WJ, Vet LEM, Tumilson JH, Van Lenteren JC, Papaj DR (1990) Variations in parasitoid foraging behaviour. essential element of a sound biological control theory. Environ Entomol 19:1183–1193

Li C, Li ZZ, Cao Y, Zheng XW, Zhou B (2009) Partial characterization of stress-induced carboxylesterase from adults of *Stegobium paniceum* and *Lasioderma serricorne* (Coleoptera: Anobiidae) subjected to CO_2-enriched atmosphere. J Pest Sci 82:7–11

Liu XF, Chen HH, Li JK, Zhang R, Chen L (2016) Volatiles released by Chinese liquorice roots mediate host location behaviour by neonate *Porphyrophora sophorae* (Hemiptera: Margarodidae). Pest Manag Sci 72(10):1959–1964

Lucas É, Jordi R (2002) Biological and mechanical control of *Sitophilus oryzae* (Coleoptera: Curculionidae) in rice. J Stored Prod Res 38(3):293–304

McGraw BA, Rodriguez-Saona C, Holdcraft R, SzendreiZ KAM (2011) Behavioral and electrophysiological responses of *Listronotus maculicollis* (Coleoptera: Curculionidae) to volatiles from intact and mechanically damaged annual bluegrass. Environ Entomol 40:412–419

Mehta V, Kumar S (2020) Influence of different plant powders as grain protectants on *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) in stored wheat. J Food Protect 83(12):2167–2172

Mehta V, Kumar S (2021) Relative susceptibility and influence of different wheat cultivars on biological parameters of *Sitophilus oryzae* L. (Coleoptera: Curculionidae). Int J Trop Insect Sci 41:653–661

Mehta V, Kumar S, Jayaram CS (2021) Damage potential, effect on germination, and development of *Sitophilus oryzae* (Coleoptera: Curculionidae) on wheat grains in Northwestern Himalayas. J Insect Sci 21(3):1–7

Molteberg EL, Magnus EM, Bjørge JM, Nilsson A (1996) Sensory and chemical studies of lipid oxidation in raw and heat-treated oat flours. Cereal Chem 73:579–587

Nayak MK, Daglish GJ, Phillips TW, Ebert PR (2020) Resistance to the fumigant phosphine and its management in insect pests of stored products: a global perspective. Annu Rev Entomol 65:333–350

Ndomo-Moualeu AF, Ulrichs C, Adler C (2015) Behavioral responses of *Callosobruchus maculatus* to volatile organic compounds found in the headspace of dried green pea seeds. J Pest Sci 3:1–10

Nordlund DA, Lewis WJ, Altieri MA (1988) Influences of plant-produced allelochemicals on the host/prey selection behaviour of entomophagous insects. In: Barbosa P, Letourneau D (eds) Novel aspects of insect-plant interactions. Wiley, New York, pp 65–90

Nwaubani SI, Opit GP, Otitodun GO, Adesida MA (2014) Efficacy of two Nigeria- derived diatomaceous earths against *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Rhyzopertha Dominica* (Coleoptera: Bostrichidae) on wheat. J Stored Prod Res 59:9–16

Pavela R, Benelli G (2016) Essential oils as ecofriendly biopesticides? Challenges and constraints. Trends Plant Sci 21:1000–1007

Paventi G, Rotundo G, Pistillo OM, Disita I, Germinara GS (2021) Bioactivity of wild hop extracts against the granary weevil, *Sitophilus granarius* (L.). InSects 12:564

Phillips TW, Throne JE (2010) Biorational approaches to managing stored-product insects. Annu Rev Entomol 55(1):375–397

Phillips TW, Jiang XL, Burkholder WE, Phillips JK, Tran HQ (1993) Behavioural responses to food volatiles by two species of stored-product Coleoptera, *Sitophilus oryzae* (Curculionidae) and *Tribolium castaneum* (Tenebrionidae). J Chem Ecol 19:723–734

Pierce AM, Pierce HD, Oehlschlager AC, Borden JH (1990) Attraction of *Oryzaephilus surinamensis* (L.) and *Oryzaephilus mercator* (Fauvel) (Coleoptera: Cucujidae) to some common volatiles of food. J Chem Ecol 16(2):465–475

Piggot JR, Morrison WR, Clyne J (1991) Changes in lipids and in sensory attributes on storage of rice milled to different degrees. Int J Food Sci Tech 26:615–628

Steiner S, Steidle JLM, Ruther J (2007) Host-associated kairomones used for habitat orientation in the parasitoid *Lariophagus distinguendus* (Hymenoptera: Pteromalidae). J Stored Prod Res 43:587-e593

Subedi S, GC YD, Thapa RB, Rija JP (2009) Rice weevil (*Sitophilus oryzae* L.) host preference of selected stored grains in Chitwan Nepal. J Inst Agr Ani Sci 30:151–158

Swamynarayana KC, Mutthuraja GP, Jagadeesh E (2014) Biology of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) on stored maize grains. Curr Biol 8(1):76–81

Tian XM, Wu FH, Zhou GX, Guo J, Liu XQ, Zhang T (2023) Potential volatile markers of brown rice infested by the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Food Chem X 17:100540

Trematerra P, Fontana F, Mancini M, Sciarretta A (1999) Influence of intact and damaged cereal kernels on the behaviour of rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). J Stored Prod Res 35:265–276

Trematerra P, Sciaretta A, Tamasi E (2000) Behavioural responses of *Oryzaephilus surinamensis*, *Tribolium castaneum* and *Tribolium confusum* to naturally and artificially damaged durum wheat kernels. Entomol Exp Appl 94:195–200

Trematerra P, Lupi C, Athanassiou C (2013) Does natal habitat preference modulate cereal kernel preferences in the rice weevil? Arthropod Plant Interact 7:287–297

Trematerra P, Ianiro R, Athanassiou CG, Kavallieratos NG (2015) Behavioral interactions between *Sitophilus zeamais* and *Tribolium castaneum*: the first colonizer matters. J Pest Sci 88:573–581

Trematerra P, Pistillo MO, Germinara GS, Colacci M (2021) Bioactivity of cereal- and legume-based macaroni pasta volatiles to adult *Sitophilus granarius* (L.). InSects 12:765

Ukeh DA, Birkett MA, Bruce TJA, Allan EJ, Luntz AJM (2010) Behavioural responses of the maize weevil, *Sitophilus zeamais*, to host (stored-grain) and non-host plant volatiles. Pest Manag Sci 1:44–50

Vinson SB (1985) The behavior of parasitoids. In: Kerkut GA, Gilbert LI (eds) Comprehensive insect physiology, biochemistry and pharmacology. Pergamon Press, New York, pp 417–469

Visser JH (1986) Host odor perception in phytophagous insects. Annu Rev Entomol 37:141–172

Wakefield ME, Bryning GP, Chambers J (2005) Progress towards a lure to attract three stored product weevils, *Sitophilus zeamais* Motschulsky, *S. oryzae* (L.) and *S. granarius* (L.) (Coleoptera: Curculionidae). J Stored Prod Res 41:145–161

Walgenbach CA, Phillips JK, Burkholder WE, King GGS, Slessor KN, Mori K (1987) Determination of chirality in 5-hydroxy-4-methyl-3-heptanone, the aggregation pheromone of *Sitophilus oryzae* (L.) and *S. zeamais* (Motschulsky). J Chem Ecol 13:2159–2169

Wang B, Dong WY, Li HM, D'Onofriox C, Bai PH, Chen RP, Yang LL, Wu JA, Wang XQ, Wang B, Ai D, Knoll W, Pelosi P, Wang GR (2022a) Molecular basis of (E)-β-farnesene-mediated aphid location in the predator *Eupeodes corollae*. Curr Biol 32:1–12

Wang J, Germinara GS, Feng ZY, Luo SL, Yang SY, Xu S, Li C, Cao Y (2022b) Comparative effects of heat and cold stress on physiological enzymes in *Sitophilus oryzae* and *Lasioderma serricorne*. J Stored Prod Res 96:101949

Wen BR, Smith L, Brower JH (1994) Competition between *Anisopteromalus calandrae* and *Choetospila elegans* (Hymenoptera: Pteromalidae) at different parasitoid densities on immature maize weevils (Coleoptera: Curculionidae) in corn. Environ Entomol 23(2):367–373

White PR, Chambers J, Walter CM, Wilkins JPG, Mellar JG (1989) Saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Coleptera, Silvanidae) collection, identification, and bioassay of attractive volatiles from beetles and oats. J Chem Ecol 15(3):999–1013

Widiyaningrum P, Candrawati D, Indriyanti DR, Priyono B (2019) Behavioral response of *Sitophilus oryzae* L. to repellent effect from postharvest waste of local cardamom. J Phys: Conf Ser 1321:032028

Authors and Affiliations

Yu Cao[1] · Qiqi Hu[1] · Lujie Huang[1] · Christos G. Athanassiou[2] · Filippo Maggi[3] · Ilaria D'Isita[4] · Yangyang Liu[1] · Onofrio Marco Pistillo[4] · Mingzhi Miao[1] · Giacinto Salvatore Germinara[4] · Can Li[1]

Yu Cao
yucaosuccess@126.com

Qiqi Hu
2228785344@qq.com

Lujie Huang
2324251564@qq.com

Christos G. Athanassiou
athanassiou@uth.gr

Filippo Maggi
filippo.maggi@unicam.it

Ilaria D'Isita
ilaria.disita@unifg.it

Yangyang Liu
523965979@qq.com

Onofrio Marco Pistillo
marco.pistillo@unifg.it

Mingzhi Miao
383517039@qq.com

[1] Guizhou Provincial Key Laboratory for Rare Animal and Economic Insect of the Mountainous Region, Guizhou Provincial Engineering Research Center for Biological Resources Protection and Efficient Utilization of the Mountainous Region, Department of Biology and Engineering of Environment, Guiyang University, Guiyang, People's Republic of China

[2] School of Agricultural Sciences, University of Thessaly, Volos, Greece

[3] School of Pharmacy, Chemistry Interdisciplinary Project (ChIP) research center, Via Madonna delle Carceri 9/B, Camerino, Italy

[4] Department of Agricultural Sciences, Food, Natural Resources and Engineering, University of Foggia, Foggia, Italy

12. Bioactivity of wild hop extracts against the granary weevil, *Sitophilus granarius* (L.)

Gianluca Paventi[1*], Giuseppe Rotundo[2*], Marco Pistillo[3], Ilaria D'Isita[3], Giacinto Salvatore Germinara[3*]. *Insects, 2021, 12(6), 564.*
https://doi.org/10.3390/insects12060564

[1] Department of Medicine and Health Sciences "V. Tiberio", University of Molise, via de Sanctis,
86100 Campobasso, Italy
[2] Department of Agricultural, Environmental and Food Sciences, University of Molise, via de Sanctis, 86100 Campobasso, Italy
[3] Department of Agricultural Sciences, Food, Natural Resources and Engineering, University of Foggia, via Napoli 25, 71100 Foggia, Italy

*Corresponding author

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Article

Bioactivity of Wild Hop Extracts against the Granary Weevil, *Sitophilus granarius* (L.)

Gianluca Paventi [1,*], Giuseppe Rotundo [2,*], Marco Pistillo [3], Ilaria D'Isita [3] and Giacinto Salvatore Germinara [3,*]

[1] Department of Medicine and Health Sciences "V. Tiberio", University of Molise, via de Sanctis, 86100 Campobasso, Italy
[2] Department of Agricultural, Environmental and Food Sciences, University of Molise, via de Sanctis, 86100 Campobasso, Italy
[3] Department of Agricultural Sciences, Food, Natural Resources and Engineering, University of Foggia, via Napoli 25, 71100 Foggia, Italy; marco.pistillo@unifg.it (M.P.); ilaria.disita@unifg.it (I.D.)
* Correspondence: paventi@unimol.it (G.P.); rotundo@unimol.it (G.R.); giacinto.germinara@unifg.it (G.S.G.)

Simple Summary: One of the outstanding problems in pest control is the extensive use of synthetic compounds characterized by concerns such as risks to non-target organisms, slow degradation, and development of resistance. For these reasons, the interest in more ecofriendly pesticides, such as botanicals, is progressively increased in the last two decades. In this regard, having recently found that essential oil obtained by wild hop has biological activity against *Sitophilus granarius*, here we checked whether and how three different crude extracts obtained by the same hop ecotype also presented toxicity (contact, ingestion, inhalation) and/or repellent activity against the same insect, which is one of the most damaging pests of stored products. Results reposted here clearly show that, in addition to the essential oil, hop crude extracts (methanol, acetone, and *n*-hexane) preserve interesting activities against pests. Moreover, since they can be easily obtained and produce high yields, hop crude extracts could represent a valid tool for *S. granarius* control.

Abstract: The use of bioinsecticides, rather than synthetic compounds, appears a goal to be pursued in pest control, especially for species such as *Sitophilus granarius* (L.) which attack stored products. Since *Humulus lupulus* (L.) is a remarkable source of bioactive compounds, this study investigated the bioactivity of hop flower extracts against *S. granarius* adults by evaluating toxic (contact, inhalation, and ingestion), repellent, antifeedant, and nutritional effects as well as their anticholinesterase activity and olfactory sensitivity. Hop extracts were obtained by soaking dried and ground hop cones in solvents of decreasing polarity: methanol, acetone, and *n*-hexane. Dried crude extracts were resuspended in each solvent, and used in topical application, ingestion, and fumigation toxicity assays, as well as in contact and short-range repellency tests, in vitro anticholinesterase activity evaluation, and electroantennographic tests. No inhalation toxicity for the extracts was found. On the contrary, all extracts showed adult contact toxicity 24 h after treatment (LD_{50}/LD_{90} 16.17/33.20, 25.77/42.64, and 31.07/49.48 μg/adult for acetone, *n*-hexane, and methanol extracts, respectively); negligible variations for these values at 48 h were found. The anticholinesterase activity shown by all extracts suggested that the inhibition of this enzyme was one of the mechanisms of action. Interestingly, flour disk bioassays revealed a significant ingestion toxicity for the acetone extract and a lower toxicity for the other two extracts. Moreover, all extracts affected insect nutritional parameters, at the highest dose checked. Filter paper and two-choice pitfall bioassays showed repellent activity and a strong reduction of insect orientation to a highly attractive food odor source, with minor differences among extracts, respectively. Finally, the presence of volatile compounds in the different extracts that are perceived by insect antennae was confirmed by electroantennography. All these findings strongly suggest a possible use of hop cone extracts against *S. granarius*, thus further confirming this plant as an interesting species for pest control.

Keywords: botanical insecticides; pest control; plant extracts; *Humulus lupulus*

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1. Introduction

Botanical pesticides represent a valuable alternative to synthetic chemicals since their use significantly reduces the risk to non-target organisms due to their rapid degradation in the environment; moreover, by providing novel and multiple modes of action, the probability of pest resistance development is reduced as well. Thus, the Integrated Pest Management (IPM) approach [1–3] strongly encourages the search for novel active botanicals. Accordingly, the number of papers describing plant extracts active against different pests is progressively increasing, as shown by a simple search using the keyword couple “plant extract” and “pest” (Scopus database, https://www.scopus.com, accessed date 19 February 2021) which returned 20, 185, 679, and 1304 results for the decades 1981–1990, 1991–2000, 2001–2010, and 2011–2020, respectively. In this regard, extracts and/or essential oils from several plants proved to exert insecticidal activity against vegetables and stored product pests and have been proposed for practical application [4–7]. Among the plethora of plant species investigated [8–12], hop, *Humulus lupulus* (L.), is receiving increasing interest for possible re-utilization after commercial use (e.g., beer production) and for its bioactivity against bacteria, yeast, fungi, and insects [13–16]. Besides spent hop, wild hop also showed remarkable activity against pests, such as Colorado potato beetles (*Leptinotarsa decemlineata* Say) (Coleoptera: Chrysomelidae) [17–19], as well as noxious insects and other invasive species [14,20]. Recently, wild hop essential oil (EO) and its main constituents (α-humulene, β-myrcene, and β-caryophyllene) proved to exert interesting properties against the granary weevil, *Sitophilus granarius* (L.) (Coleoptera, Curculionidae): A high contact and a lower inhalation toxicities, as well as a good repellent activity, were found against adult insects [21]. On the other hand, the limited yield of hop EO extraction may limit its use for pest control purposes. Moreover, the insecticidal activities of EOs and crude extracts of the same plant may significantly vary due to the different nature of extracted compounds [22]; in addition, home-made plant crude extracts could offer a low-cost acceptable alternative for farmers [5]. To provide a wider scenario on the hop biological activity in insects, the insecticidal, electrophysiological, and behavioral activities of *n*-hexane, methanol, and acetone crude extracts from wild hops of Central Italy were investigated against the granary weevil adults.

2. Materials and Methods

2.1. Chemicals

Solvents (methanol, n-hexane, and acetone) and all other chemicals were obtained from Sigma–Aldrich (Milan, Italy) and were at the purest grade available.

2.2. Plant Material

Aerial parts of hop, *Humulus lupulus* (L.) Cannabaceae, were collected in September 2019 during the flowering stage in Bojano (Molise region, Italy) at 482 m a.s.l. The area (N 41°47′840″ E 14°49′428″) had an average annual rainfall of 700 mm, and mean annual temperature of 14–15 °C. The soil where hop plants were harvested has neutral pH (7.25), a sandy texture (fine sand 54%, coarse sand 23%), a low organic carbon content (10.7 g/Kg), and a low C/N ratio (5.9), as measured in [23]. It is also a strongly calcareous soil ($CaCO_3$ 37.26%), with a very low content of available phosphorus (P_2O_5 5.14 mg/kg) for plants. Voucher specimen n. 20,348 was deposited in the herbarium of the University of Molise.

2.3. Insect Rearing

Sitophilus granarius were reared for several generations (2 years) at the Department of Agriculture, Environment and Food of the University of Molise. Insects were maintained on wheat grains (*Triticum aestivum* L., cv Bologna) in glass cylindrical containers (Ø 15 × 15 cm) closed by metallic net (1 mm) put in the dark in a climatic chamber set at 25 ± 2 °C and 60 ± 5% r.h. Adult beetles, 2–4 weeks old, were used only once for the experiments.

2.4. Plant Extracts

Hop cones were oven-dried at 36 ± 2 °C for 72 h and ground, and aliquots of powder (50 g) were extracted for 24 h at room temperature using solvents with different polarity: methanol, acetone, or *n*-hexane (250 mL). Then, each crude extract was filtered (Whatman No. 113, Cytiva, Marlborough, MA, USA), dried under vacuum in a rotary evaporator (Laborota 4000, Heidolph, Schwabach, Germany), and stored at −20 °C until further use. The residues obtained were 126.9 ± 20.6, 121.1 ± 3.3, and 96.6 ± 11.6 g/kg dry weight (mean values ± SE) for methanol, acetone, and *n*-hexane extracts, respectively.

2.5. Contact Toxicity

The contact toxicity of hop extracts to granary weevil adults was determined by topical application [8,24]. Plant extract samples were prepared by dissolving the residues of *n*-hexane, methanol, and acetone extracts in *n*-hexane, acetone:methanol (1:1), and acetone, respectively. For each sample, two-fold serial dilutions (150.00–4.69 µg/µL) were prepared and for each dilution an aliquot (0.5 µL) was applied on the pronotum of *S. granarius* adults in thanatosis using a Hamilton's syringe (700 series, MicroliterTM Hamilton Company, Reno, NV, USA). Each dilution was assayed on 40 unsexed adults of *S. granarius* divided in 8 replicates and an equal number of individuals was treated with the respective solvent as a control. For each replicate, insects were confined in a Petri dish within a metal rings (Ø 4.0 × 2.5 cm), covered with metallic net (mesh 1 mm) to prevent insects escape, with 5 wheat kernels, and maintained in the dark under controlled conditions (26 ± 2 °C and 60 ± 5% r.h.). Insect mortality was recorded after 24 and 48 h. The percentage mortalities were transformed to arcsine square-root values for one-way analysis of variance (ANOVA). Treatment means were compared and separated by Tukey's HSD test. The lethal dose 50 (LD_{50}) and 90 (LD_{90}) values, the confidence upper and lower limits, regression equations, and chi-square (χ^2) values were calculated using probit analysis [25]. Statistical analyses were performed with SPSS (Statistical Package for the Social Sciences) v.23 for Windows (SPSS Inc., Chicago, IL, USA).

2.6. Inhalation Toxicity

The inhalation toxicity was assessed by using a fumigation chamber made up of a plastic container (135 mL) in which a perforated septum separated a lower chamber from an upper one. The lower chamber was assigned to contain increasing doses (2.5–150 mg) of each hop extract residue whilst the upper chamber contained adult weevils (n = 20) and intact kernels (n = 20). For each sample three replicates were set up. Dead insects were counted, after incubation in the dark at 26 ± 2 °C and 60 ± 5% r.h. for 24 or 48 h.

2.7. Ingestion Toxicity, Antifeedant, and Nutritional Activity

Effects of hop extracts on the feeding activity and nutrition of granary weevil adults were evaluated by the flour disk bioassay [8,26]. Wheat flour (10 g) was uniformly suspended in distilled water (50 mL) by magnetic stirring. To obtain flour disks, aliquots (200 µL) of suspension were dropped onto a plastic Petri dish and left overnight at 26 ± 2 °C and 60 ± 5% r.h. to dry. Plant extract samples and their dilutions were prepared by dissolving the residues as described in Section 2.5. Disks were treated with sample solutions (5 µL) corresponding to different concentrations (46.87, 93.75, 187.50, 375.00, 750.00 µg/disk) or the solvent alone as a control. Disks were held at room temperature for 2 h for solvent evaporation. In a pre-weighed glass vial (Ø 2.5 × 4.0 cm) 2 flour disks were introduced and the weight measured; later, 10 group-weighed weevil adults were added and each vial was re-weighed and maintained in the dark at 26 ± 2 °C, 60 ± 5% r.h. for 5 days. At the end of the test, for each glass vial, insects were removed, the number of dead insects recorded, and the weight of both the 2 flour disk residues and live insects were separately measured. As a control, glass vials containing treated flour disks but without insects were prepared to determine any decrease in weights due to evaporation of solvent and sample. For each sample concentration, as well as for the control, 5 replicates were set up. The following nutritional indices [4,19,20] for each replicate were calculated:

$$\text{Relative Growth Rate (RGR)} = (\text{A} - \text{B})/\text{B} \times \text{day}^{-1} \quad (1)$$

$$\text{Relative Consumption Rate (RCR)} = \text{D}/\text{B} \times \text{day}^{-1} \quad (2)$$

$$\text{Efficiency Conversion of Ingested food (ECI)} = (\text{RGR}/\text{RCR}) \times 100 \quad (3)$$

$$\text{Feeding Deterrence Index (FDI) (\%)} = [(\text{C} - \text{T})/\text{C}] \times 100 \quad (4)$$

where A = mean weight (mg) of live insects on fifth day; B = initial mean weight (mg) of insects; C = consumption of control disks; D = biomass ingested (mg)/no. of living insects on the fifth day; and T = consumption of treated disks.

Data were submitted to ANOVA followed by Tukey's HSD test for mean comparisons.

2.8. AChE Assay

Anticholinesterase activity of hop extracts was investigated [8,21] by detecting AChE activity photometrically (λ = 412 nm, 25 °C) by means of a Jasco V-570 spectrophotometer (Tokyo, Japan). Briefly, about 0.01 EU of enzyme (EC 3.1.1.7, from *Electrophorus electricus*, Sigma–Aldrich, Milan, Italy) were incubated in phosphate buffer (0.1 M, pH 8.00) plus 5,5′dithiobis(2-nitrobenzoic) acid (DTNB, 0.2 mM) either in the absence or in the presence of different aliquots of hop extracts. For *n*-hexane extract, the addition of Tween-20 (0.4%, *v*/*v*) allowed its re-suspension in phosphate buffer. Reaction was started by the addition of saturating concentration (2.5 mM) of acetylthiocholine iodide and the rate of absorbance change was obtained as tangent to the initial part of the progress curve. IC_{50} values were calculated by means of Grafit 4.0 (Erithacus Software Ltd., East Grinstead, UK). Results were expressed as % of the control (reaction rate measured in the absence of plant extract). Data were submitted to ANOVA followed by Tukey's HSD test for mean comparisons.

2.9. Two-Choice Behavioural Bioassays

The capability of different hop extracts to disrupt granary weevil orientation to odors of wheat grains was evaluated in a two-choice pitfall bioassay [27]. The test arena was a steel container (Ø 32 × 7 cm height) with two diametrically opposed holes (Ø 3 cm) located 3 cm from the side wall. A filter paper disc (Ø 0.7 cm) was suspended at the center of each hole by a cotton wire taped to the lower surface of the arena. Glass flasks (500 mL), assigned to collect the responding insects, were positioned under each hole. The inside necks of the collection flasks were coated with mineral oil to prevent insects from returning to the arena. Thirty unsexed insects, left for at least 4 h without food, were placed under an inverted Petri dish (Ø 3 cm × 1.2 cm high) at the center of the arena and allowed 30 min to acclimatize prior to release. During the assay, the arena was covered with a steel lid to prevent insects from escaping. In each experiment, insects were given a choice between the odors emitted by wheat grains (200 g; 14.5% moisture content) left in a collection flask alone or plus a set dose (10 μL) of an extract solution adsorbed onto the overlying filter paper disc and, as a control, the respective solvent (10 μL) adsorbed onto the opposed paper disc. In each set of experiments, five doses (0.094, 0.188, 0.375, 0.750, 1.500 mg) of each hop extracts were assessed. Tests lasted 3 h and were carried out in the dark at 26 ± 2 °C and 60 ± 5% r.h. Each bioassay was replicated 4 times. In each experiment, a response index (RI) was calculated by using:

$$\text{RI} = [(\text{T} - \text{C})/\text{Tot}] \times 100 \quad (5)$$

where T is the number responding to the treatment, C is the number responding to the control, and Tot is the total number of insects released [28]. For each test stimulus, the significance of the mean RIs was evaluated by comparing the mean number of insects in the treatment and control using a Student's *t*-test for paired comparisons. The mean numbers of insects found in the treatment and in the control and the mean RIs at different doses of hop extracts alone and in the presence of wheat grain odors were subjected to ANOVA and ranked according to Tukey's HSD test.

2.10. Repellence in Filter Paper Disc Bioassay

Repellent activity of hop extracts was further evaluated using the area preference method [24]. A filter paper disc (Whatman No. 1, Ø 8.0 cm, area = 50.2 cm^2) was divided in half. One half was treated with 500 µL of plant extracts solution using a micropipette and the other half was treated with an equal volume of the respective solvent used as control. Both treated and control halves were air-dried for about 10 min to allow complete solvent evaporation, joined with transparent adhesive tape and the full disc fixed on the bottom of a Petri dish (Ø 9.0 cm). Ten weevil unsexed adults were confined to each filter paper disc within a metal O-ring (Ø 8.0 × 4.0 cm) covered with metallic net (mesh 1 mm) to prevent insect escape. The experiment was run in the dark at 26 ± 2 °C and 60 ± 5% r.h. Solutions of hop extracts, prepared as described in Section 2.4, were tested corresponding to the doses of 0.37, 0.75, 1.49, and 2.98 mg/cm^2, respectively. Each bioassay was replicated 4 times. The number of weevils on the treated (Nt) and control (Nc) portion of paper disc was recorded at the following intervals: 10, 30 min, 1, 2, and 24 h, respectively. Percentage repellency (PR) values were calculated as follows:

$$\mathrm{PR} = (\mathrm{Nc} - \mathrm{Nt})/(\mathrm{Nc} + \mathrm{Nt}) \times 100 \tag{6}$$

where positive PR values indicate repellence, whereas negative values indicate attraction. For each dose, PR values at different exposure times were submitted to ANOVA followed by Duncan's HSD test for separation of means.

2.11. Electroantennography (EAG)

The olfactory sensitivity of male and female *S. granarius* antennae to ascending concentrations of the three hop extracts was assessed by EAG using the technique reported in previous studies [29,30]. Briefly, the head of a 2- to 3-week-old insect was excised from the prothorax using a scalpel and mounted between two glass capillary electrodes (Microglass, Naples, Italy) filled with Kaissling saline solution [31]. The recording electrode (diameter~100 µm) was put in contact with the dorsal surface of the antennal club while the neutral electrode was inserted into the base of the head. The electrical continuity between the antennal preparation and an AC/DC UN-6 amplifier in DC mode was maintained using AgCl-coated silver wires. The amplifier was connected to a PC equipped with the EAG 2.0 program (Syntech Laboratories, Hilversum, The Netherlands).

Five two-fold dilution of n-hexane, methanol, and acetone hop cone extracts (100, 50, 25, 12.5, 6.25 µg/µL) in the corresponding solvents (Sigma–Aldrich, Milan, Italy) were prepared. The test stimulus (10 µL of an extract solution) was adsorbed onto a filter paper (Whatman No. 1) strip (2 cm^2) inserted in a Pasteur pipette (15 cm long) after solvent evaporation. The vapor stimuli (3 cm^3) were puffed, using a disposable syringe, for 1 s into a charcoal-filtered and humidified air stream (500 mL/min) flowing over the antenna through a stainless-steel delivery tube (Ø 1.0 cm) with the outlet positioned~1 cm from the antenna. Control (10 µL of a solvent) and standard (10 µL of a 10 µg/µL (Z)-3-hexenol solution) stimuli were also applied at the beginning of the experiment and after each group of 5 test stimuli. The intervals between stimuli were 1 min. Each dose of the three hop extracts was tested on three antennae from different males and females.

EAG responses were measured by the maximum amplitude of negative polarity deflection (-mV) elicited by a stimulus [32]. To compensate for solvent and/or mechanosensory artefacts, the amplitude (mV) of the EAG responses to each test stimulus was subtracted by the mean EAG response of the two nearest solvent controls [33]. To compensate for the decrease in the antennal responsiveness during the experiment, the resulting EAG amplitude was corrected according to the reduction of the EAG response to the standard stimulus [34]. Dose–response curves were calculated based on the corrected EAG values. To verify antennal activation, the corrected mean male and female EAG response to the last dilution of each hop extract was compared to "0" value using one-sample Student's *t*-test and regarded as "activated" if significant at $p = 0.05$. Saturation level was taken as the lowest dilution at which the mean response was equal to or less than the previous one [35]. Mean male and female EAG responses to each stimulus were compared using a Student's *t*-test for independent samples at $p = 0.05$. Since no significant differences were found between the male and female EAG responses to each test stimulus, individual male and female EAG responses were pooled and analyzed together. For each dose tested, the mean EAG responses of adult insects to the three hop extracts were submitted to ANOVA followed by Tukey HSD test ($p = 0.05$). Levene's test was used to assess homogeneity of variances.

3. Results

3.1. Contact and Inhalation Toxicity

Mortalities of *S. granarius* adults obtained 24 and 48 h after topical application of hop extracts are reported in Tables 1–3. For all samples, a dose-dependent increased mortality was found. Twenty-four hours after extract application, mortalities were significantly higher than the control starting from the 9.37 µg/adult dose of *n*-hexane extract (Table 1), and 18.75 µg/adult of both acetone (Table 2) and methanol (Table 3) extracts; 48 h after application, n-hexane and acetone extracts showed a decrease in the lowest active dose which was 4.69 and 9.37 µg/adult, respectively. Contact toxicity, 24 h after topical application returned LD_{50} values of 16.17, 25.77, and 31.07 µg/adult and LD_{90} values of 33.20, 42.64, and 49.48 µg/adult for acetone, *n*-hexane, and methanol extracts, respectively; these values slightly decreased 48 h after application (Tables 1–3).

Table 1. Contact toxicity of different concentrations of *n*-hexane extract against *S. granarius* adults 24 and 48 h after topical application. For each exposure time, mean mortality values followed by same letter are not significantly different at $p \leq 0.05$ (Tukey HSD test).

Dose (µg/Adult)	Exposure Time (h)	% Mortality (Mean ± S.E.)	Regression Equation	χ^2	LD_{50} (95% CL, µg/Adult)	LD_{90} (95% CL, µg/Adult)
75.00	24 h	100.00 ± 0.00 a	$y = 3.33x - 4.24$	8.77	25.77 (20.34–34.50)	42.64 (34.05–61.18)
37.50		77.50 ± 4.50 b				
18.75		32.50 ± 7.50 c				
9.37		22.50 ± 5.90 c				
4.69		2.50 ± 2.50 d				
2.34		0.00 ± 0.00 d				
0.00		0.00 ± 0.00 d				
F		95.88				
d.f.		6				
p		<0.001				
75.00	48 h	100.00 ± 0.00 a	$y = 0.087x - 2.027$	10.60	22.94 (17.79–31.08)	38.69 (30.67–56.18)
37.50		85.00 ± 3.27 a				
18.75		37.50 ± 4.53 b				
9.37		27.50 ± 6.50 b				
4.69		5.00 ± 3.27 c				
2.34		0.00 ± 0.00 d				
0.00		0.00 ± 0.00 d				
F		128.68				
d.f.		6				
p		<0.001				

Table 2. Contact toxicity of different concentrations of acetone extract against *S. granarius* adults 24 and 48 h after topical application. For each exposure time, mean mortality values followed by same letter are not significantly different at $p \leq 0.05$ (Tukey HSD test).

Dose (μg/Adult)	Exposure Time (h)	% Mortality (Mean ± S.E.)	Regression Equation	χ^2	LD$_{50}$ (95% CL, μg/Adult)	LD$_{90}$ (95% CL, μg/Adult)
75.00	24 h	100.00 ± 0.00 a	$y = 4.10x - 4.38$	16.37	16.17 (9.65–28.85)	33.20 (20.96–157.85)
37.50		97.50 ± 2.50 a				
18.75		57.50 ± 4.53 b				
9.37		7.50 ± 3.66 c				
4.69		5.00 ± 3.27 c				
2.34		0.00 ± 0.00 c				
0.00		0.00 ± 0.00 c				
F		290.48				
d.f.		6				
p		<0.001				
75.00	48 h	100.00 ± 0.00 a	$y = 3.84x - 4.51$	6.21	14.91 (12.82–17.41)	32.14 (26.29–42.77)
37.50		97.50 ± 2.50 a				
18.75		60.00 ± 5.34 b				
9.37		15.00 ± 3.27 c				
4.69		7.50 ± 3.66 cd				
2.34		0.00 ± 0.00 d				
0.00		0.00 ± 0.00 d				
F		241.28				
d.f.		6				
p		<0.001				

Table 3. Contact toxicity of different concentrations of methanol extract against *S. granarius* adults 24 and 48 h after topical application. For each exposure time, mean mortality values followed by same letter are not significantly different at $p \leq 0.05$ (Tukey HSD test).

Dose (μg/Adult)	Exposure Time (h)	% Mortality (Mean ± S.E.)	Regression Equation	χ^2	LD$_{50}$ (95% CL, μg/Adult)	LD$_{90}$ (95% CL, μg/Adult)
75.00	24 h	100.00 ± 0.00 a	$y = 0.07x - 2.163$	2.25	31.07 (27.33–36.03)	49.48 (43.19–59.09)
37.50		67.50 ± 5.26 b				
18.75		17.50 ± 4.53 c				
9.37		7.50 ± 3.66 cd				
4.69		5.00 ± 3.27 cd				
2.34		2.50 ± 2.50 d				
0.00		0.00 ± 0.00 d				
F		137.14				
d.f.		6				
p		<0.001				
75.00	48 h	100.00 ± 0.00 a	$y = 0.06x - 1.89$	0.64	28.66 (25.01–33.52)	48.08 (41.71–57.81)
37.50		72.50 ± 3.66 b				
18.75		22.50 ± 5.90 c				
9.37		12.50 ± 3.66 cd				
4.69		5.50 ± 3.27 d				
2.34		5.50 ± 3.27 d				
0.00		2.50 ± 2.50 d				
F		118.39				
d.f.		6				
p		<0.001				

Negligible mortality in inhalation toxicity assays was found for all the extracts in the dose range tested (0.018–1.111 g/L hop extract residue, data not shown).

3.2. Ingestion Toxicity, Antifeedant and Nutritional Activity

Ingestion toxicity and nutritional effects of hop extracts towards adult granary weevils are presented in Tables 4–6. Methanol (Table 4) and *n*-hexane extracts (Table 6) induced low insect mortality values only at the highest dose checked; a more toxic effect was shown by the acetone extract which caused about 60% insect mortality at the highest dose (Table 5). The highest dose of each extract significantly decreased the relative growth rate (RGR, F = 4.73–10.43; df = 5; $p < 0.001$–0.004), the efficiency conversion of ingested food (ECI, F = 4.58–11.14; df = 5; $p < 0.001$–0.005), and the relative consumption rate (RCR, F = 3.78–8.73; df = 5; $p < 0.001$–0.012), except for RCR in the case of acetone extract treatment. Doses of all extracts elicited positive FDI values; a significant dose-dependent increase in this value (FDI, F = 0.93–11.58; df = 4; $p < 0.001$–0.467) was found for methanol and *n*-hexane extracts, but not for the acetone one.

Table 4. Nutritional indices, mortality, and food deterrence of *S. granarius* adults of different concentrations of methanol extract. Means in the same column with the same letter are not significantly different at the 0.05 level determined by the Tukey's HSD test.

Concentration (µg/Disk)	Mortality (%)	RGR [1]	RCR	ECI	FDI (%)
750.00	16.00 a	−0.011 ± 0.008 a	0.066 ± 0.047 a	−31.915 ± 11.695 a	74.000 ± 20.199 a
375.00	4.00 b	−0.020 ± 0.006 ab	0.188 ± 0.009 b	−10.363 ± 2.465 b	27.951 ± 7.782 b
187.50	0.00 b	−0.0130 ± 0.007 b	0.199 ± 0.007 b	−6.304 ± 2.620 b	21.222 ± 7.185 b
93.75	0.00 b	−0.003 ± 0.003 c	0.230 ± 0.015 b	−1.134 ± 1.173 b	12.621 ± 4.576 b
46.87	0.00 b	−0.001 ± 0.002 c	0.224 ± 0.018 b	−0.416 ± 0.780 b	15.780 ± 4.405 b
Control	0.00 b	0.012 ± 0.003 c	0.278 ± 0.039 b	6.973 ± 0.513 b	

[1] RGR, relative growth rate; RCR, relative consumption rate; ECI, efficiency conversion of ingested food; FDI, feeding deterrent index.

Table 5. Nutritional indices, mortality, and food deterrence of *S. granarius* adults of different concentrations of acetone extract. Means in the same column with the same letter are not significantly different at the 0.05 level determined by the Tukey's HSD test.

Concentration (µg/Disk)	Mortality (%)	RGR [1]	RCR	ECI	FDI (%)
750.00	62.00 a	−0.091 ± 0.020 a	0.122 ± 0.024 ab	−86.710 ± 20.378 a	41.033 ± 13.712 a
375.00	34.00 ab	−0.035 ± 0.009 ab	0.110 ± 0.020 a	−46.266 ± 21.573 abc	55.427 ± 9.801 a
187.50	40.00 ab	−0.060 ± 0.015 a	0.129 ± 0.043 ab	−75.607 ± 26.856 ab	44.223 ± 21.273 a
93.75	16.00 bc	−0.048 ± 0.016 ab	0.123 ± 0.036 ab	−65.118 ± 24.932 abc	48.037 ± 16.664 a
46.87	4.00 c	0.008 ± 0.007 bc	0.207 ± 0.016 ab	3.228 ± 3.222 bc	18.489 ± 5.192 a
Control	0.00 c	0.023 ± 0.077 c	0.242 ± 0.020 b	9.028 ± 2.166 c	

[1] RGR, relative growth rate; RCR, relative consumption rate; ECI, efficiency conversion of ingested food; FDI, feeding deterrent index.

Table 6. Nutritional indices, mortality, and food deterrence of *S. granarius* adults of different concentrations of *n*-hexane extract. Means in the same column with the same letter are not significantly different at the 0.05 level determined by the Tukey's HSD test.

Concentration (µg/Disk)	Mortality (%)	RGR [1]	RCR	ECI	FDI (%)
750.00	38.00 a	−0.015 ± 0.002 a	0.132 ± 0.009 a	−11.596 ± 1.410 a	40.463 ± 6.509 a
375.00	10.00 b	−0.008 ± 0.004 a	0.161 ± 0.003 ab	−5.248 ± 2.352 ab	21.816 ± 2.537 ab
187.50	6.00 b	−0.003 ± 0.011 ab	0.186 ± 0.016 bc	−0.529 ± 6.876 ab	4.827 ± 5.844 b
93.75	0.00 b	0.006 ± 0.002 ab	0.189 ± 0.009 bc	3.337 ± 1.064 b	4.616 ± 3.320 b
46.87	0.00 b	0.005 ± 0.003 ab	0.190 ± 0.007 bc	2.756 ± 1.426 b	7.376 ± 3.119 b
Control	0.00 b	0.016 ± 0.002 b	0.204 ± 0.009 c	7.735 ± 0.854 b	

[1] RGR, relative growth rate; RCR, relative consumption rate; ECI, efficiency conversion of ingested food; FDI, feeding deterrent index.

3.3. Anticholinesterase Activity

The effect of the different extracts on the AChE was investigated (Figure 1). For each hop extract, a dose-dependent inhibitory activity was found (*n*-hexane extract: F = 51.21, df = 7, $p < 0.001$; methanol extract: F = 34.41, df = 7, $p < 0.001$; acetone extract: F = 25.60, df = 7, $p < 0.001$). No significant differences among the samples were found, except for the dose of 0.50 μg/mL. Since in these experiments Tween-20 (0.4%, v/v) was used to resuspend *n*-hexane extract in phosphate buffer, the control was made so that the Tween-20 (up to 1%, v/v) did not affect enzyme activity. IC_{50} was calculated for each extract returning the following values: 0.331 ± 0.025, 0.440 ± 0.108, and 0.505 ± 0.041 μg/mL for *n*-hexane, methanol, and acetone extract, respectively.

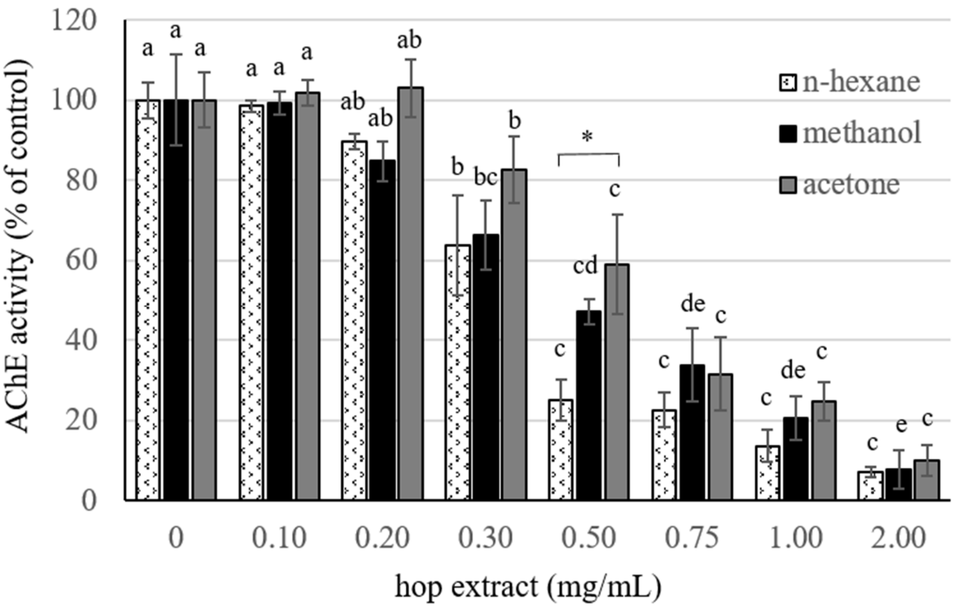

Figure 1. The anticholinesterase (AChE) activity of hop extracts. Mean values (±SE) of AChE activity obtained either in the absence or in the presence of different doses of hop *n*-hexane, methanol, and acetone extracts. Values ($n = 3$) were calculated as % of the control (enzyme activity measured in the absence of hop extracts). Among each series (hop extract), different letters indicate a significant difference ($p < 0.05$, Tukey's HSD test); among each dose, * indicate a significant difference ($p < 0.05$, Tukey's HSD test).

3.4. Two-Choice Behavioural Bioassays

Results of two-choice behavioral bioassays evaluating the possible disruptive effects of different hop extracts on granary weevil orientation towards wheat grain odors are reported in Figure 2. Wheat grain odors elicited a highly positive and significant RI, indicating insect attraction. In the dose range tested, the RI to wheat grains was significantly decreased by the presence of methanol (F = 57.11, df = 5, $p < 0.001$), acetone (F = 41.19, df = 5, $p < 0.001$), and *n*-hexane extract (F = 40.46, df = 5, $p < 0.001$) with methanol and *n*-hexane extracts showing a dose-dependent effect. For these extracts, the highest dose (1.50 mg) resulted in negative and significant RIs, indicating actual repellency (Figure 2), with methanol extract eliciting the highest repellent effect (−17.50).

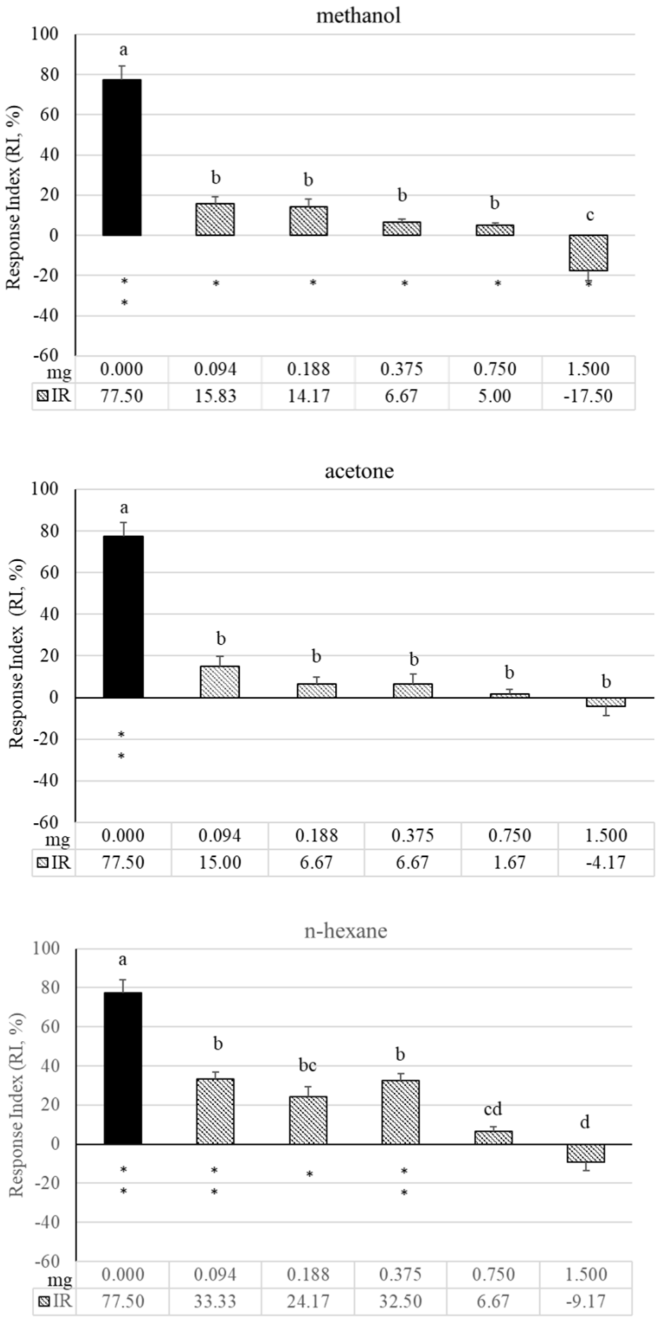

Figure 2. Response index (RI) of *S. granarius* adults to odors of wheat grains (200 g) alone (black bars) or in the presence of ascending doses of hop extracts in two-choice bioassays. For each set of experiments, values with the same letter are not significantly different ($p < 0.05$, Tukey's HSD test); asterisks indicate significant differences between the number of insects in the treatment and the control (* $p < 0.05$, ** $p < 0.01$; Student's *t*-test).

3.5. Repellence in Filter Paper Disc Bioassay

Contact repellency of different hop extracts was further evaluated in filter paper disc bioassays (Figure 3). In the dose range tested, all the extracts repelled insects with the methanol one being the most active at the lowest dose tested (F = 5.61, df = 2, $p < 0.05$). Repellent activity was found to significantly decrease as a function of time of application, particularly for the acetone and methanol extract (methanol extract: F = 1.47–4.37, df = 4, $p < 0.016$–0.260; acetone extract: F = 1.77–17.55, df = 4, $p < 0.001$–0.188) (Figure 3). The two higher doses of the *n*-hexane extract exhibited a remarkable contact repellency over time.

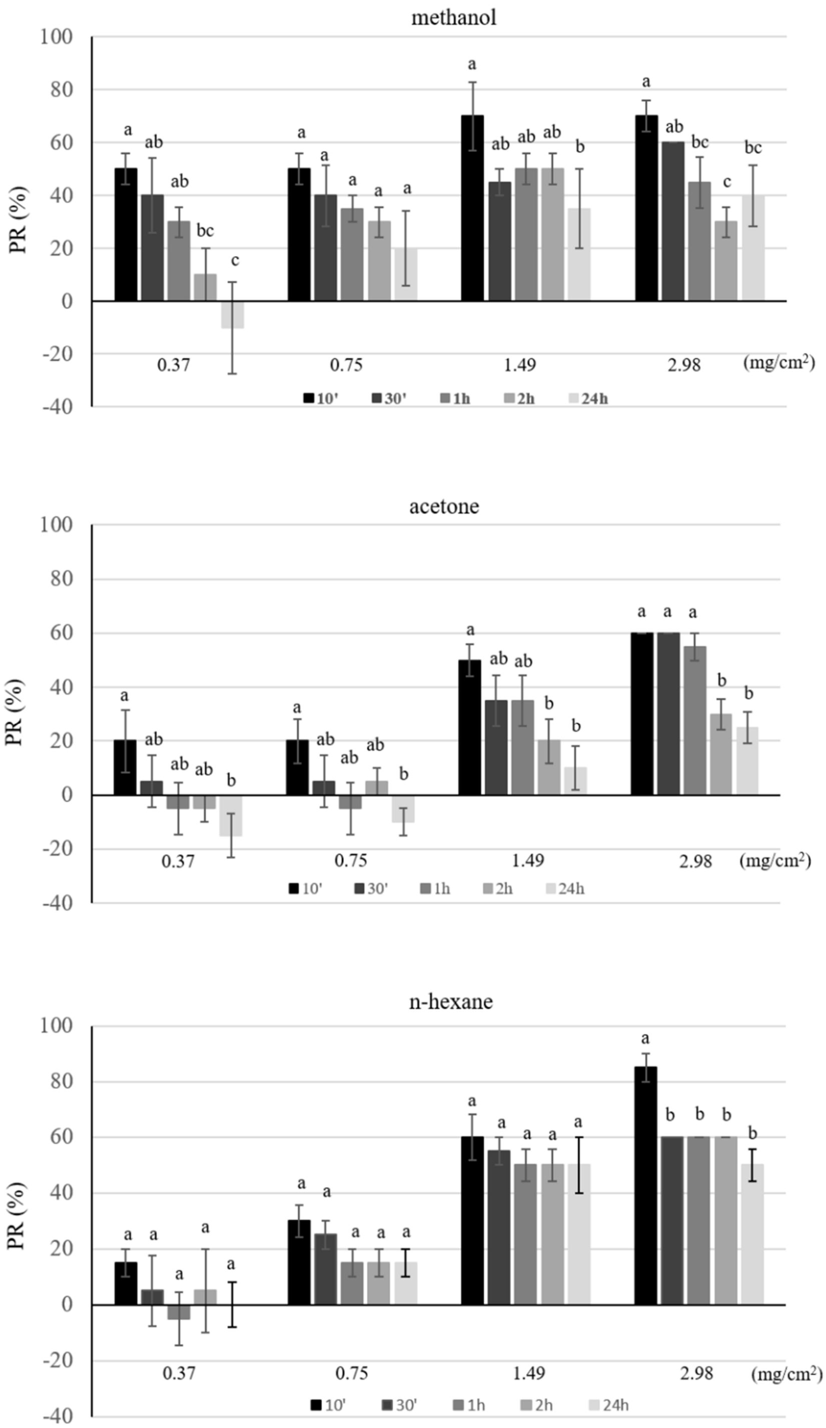

Figure 3. Percent repellency (PR) of different concentrations of hop extracts against *S. granarius* adults in filter paper disc bioassays. PR mean value (±S.E.), calculated as reported in the Methods section, obtained in four different experiments, were reported as a function of both dose and time after exposure. For each dose, different letters indicate significant differences among time ($p < 0.05$, Duncan MRT's test).

3.6. EAG

The antennal sensitivity of granary weevil adults to increasing doses of *n*-hexane, methanol, and acetone hop cone extracts is presented in Figure 4. In the dose range tested, all extracts elicited measurable ($p < 0.05$ in all one-sample Student's *t*-test) and dose-dependent EAG responses in both sexes without significant differences between males and females (*n*-hexane extract: $t = 0.402$–1.687, df = 4, $p = 0.096$–0.708; methanol extract: $t = 0.340$–1.478; df = 4, $p = 0.214$–0.751; acetone extract: $t = 0.276$–2.325; df = 4, $p = 0.081$–0.797). The amplitude of the mean EAG response to *n*-hexane and acetone decreased from the 0.5 to 1 mg doses, indicating saturation of receptors at the lowest one.

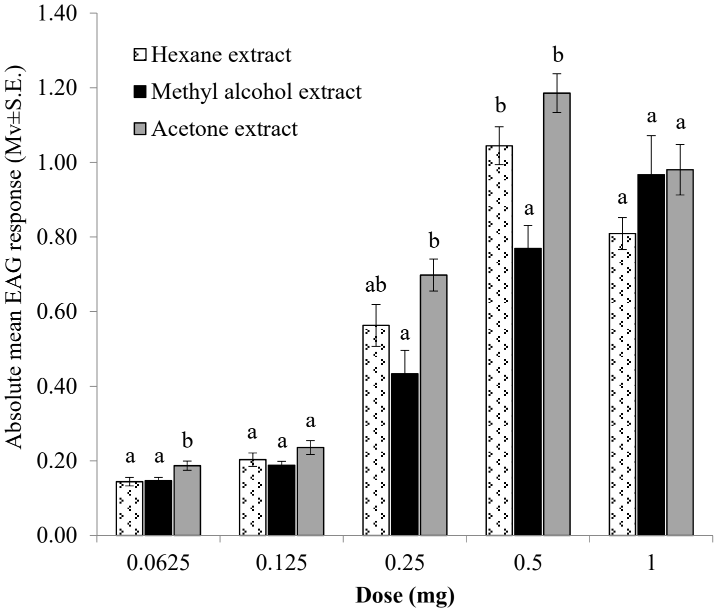

Figure 4. EAG responses (mean values ± SE) of adult *S. granarius* antennae ($n = 6$) to ascending doses of different hop cone extracts. For each dose, different letters indicate significant differences at ($p < 0.05$; Tukey's HSD test).

At the lowest dose tested, the mean EAG response to acetone extract was significantly higher than those to *n*-hexane and methanol extracts (F = 4.91, df = 2, $p < 0.023$). The mean EAG responses elicited by the same extract were significantly higher than those of methanol extract at the 0.25 (F = 5.85, df = 2, $p = 0.013$) and 0.5 mg (F = 14.83, df = 2, $p < 0.001$) doses, but not statistically different than those recorded at the same doses of the *n*-hexane extract.

4. Discussion

As part of the ongoing research on the biological activity of hop plant, results reported in this study shows that *n*-hexane, methanol, and acetone hop extracts exert different bioactivities against the pest *S. granarius*. Topical application of all three extracts resulted in a high contact mortality, reaching the 100% value at the highest dose tested (75.00 µg/adult). Among the extracts, the *n*-hexane was found to be the most active showing significant mortality starting from 9.37 µg/adult. The high contact toxicity of hop extracts was supported by the respective LD_{50} values which were comparable among them and lower than the values reported for similar extracts of other plants, such as *Scrophularia canina* L. [36] and *Dittrichia viscosa* (L.) [8], against the same insect species. However, LD_{50} value of acetone extract, which was found to be the most toxic, was about 4-fold higher than that of the pyrethrin extract (DL_{50} 4.29 µg/adult) against the congener *S. zeamais* Motschulsky [37]. Moreover, lower insecticidal activity for hexane and acetone root extracts of *Decalepis hamiltonii* against *S. oryzae* (L.) was found [38]. Interestingly, acetone and especially methanol extracts obtained from wild hop used in this study appeared to be more toxic than those obtained from a different hop ecotype against *S. granarius* [39], thus confirming the importance of ecotypes in determining hop properties [40–42]. A possible mechanism by which hop extracts exert contact toxicity may rely on the anticholinesterase action. In fact, all of them showed a dose-dependent inhibition of this enzyme, with limited differences in IC_{50} values among the extracts. In this regard, the absence of major differences in both LD_{50} and enzyme IC_{50} among the several extracts strongly support the hypothesis of anti-AChE mediated toxic activity. Notice that anticholinesterase activity was already found in water and ethanolic extracts of several hop ecotypes [43], but not in the EO of the same ecotype used in this study [21].

Hop extracts showed different ingestion toxicity. In particular, the acetone extract was the most active, reaching about 60% mortality at the highest dose, whereas the methanol was the less active, causing only 16% mortality at the same dose. These findings further suggest the occurrence of different active compounds in the extracts. Notice that no ingestion toxicity was reported for extracts of other plants, such as *S. canina* [36] and *D. viscosa* [8], against the same insect pest as well as for the plant-based commercial product Margosan® (0.25% azadirachtin) [26].

In addition to ingestion toxicity, the highest doses of hop extracts affected nutritional indices with methanol extract showing the highest deterrence (about 75%) and the acetone extract providing the lowest ECI. The low conversion indices of ingested food may explain the higher mortality caused by ingestion observed for the acetone extract. Insect nutritional parameters obtained in the control (untreated disks flour) were in fairly good agreement with those calculated in previous studies [8,24,44]. Notice that the antifeedant activity of hop extracts is not unique since similar properties were reported for extracts of several plant species, particularly for methanolic extracts [45,46].

In distinction with what reported for similar plant extracts [38,47,48], hop extracts did not show any inhalation toxicity. A moderate inhalation toxicity (LC_{50} 136.37 mg/L; LC_{90} 201.48 mg/L) was found for the EO obtained from the same hop ecotype [21]. These differences between solvent extracts and EO could be due to the occurrence of different bioactive compounds or to the loss of the volatile compounds exerting inhalation toxicity during crude extracts preparation. In this regard, resins, essential oil, and polyphenols are the main components of hop cones [49]. Hop resin is usually classified as soft and hard: the former contains α- and β-acids (humulones and lupulones, respectively) and is characterized by a good solubility in *n*-hexane [50]; the latter, probably deriving from the oxidation of the soft resin, is completely insoluble in hexane, but soluble in methanol and diethyl ether [50]. Thus, we can speculate that hard resin was restricted to the methanol extract, whereas the n-hexane fraction was enriched in α- and β-acids. In addition to resin, further active components of hop cones are polyphenols, which include flavonols, flavan-3-ols, phenolic carboxylic acids, and others phenolic compounds as prenylflavonoids (xanthohumol, isoxanthohumol, desmethylxanthohumol, and 6- and 8-prenylnaringenin) [51]. For some of these, such as xanthohumol [39] and catechin [52], insecticidal activity was reported. However, identification of possible active components in these extracts strictly requires their chemical characterization since the relative abundance of compounds in hop cones is highly affected by both intrinsic and extrinsic factors, such as the variety and the agronomic-environmental conditions, respectively [53–56].

The observed repellency of hop extracts was investigated by using two different experimental approaches in order to assess both contact and short-range effects. All hop extracts exerted contact repellency towards granary weevil with the methanol extract being active even at low doses. Interestingly, the *n*-hexane extract maintained a good contact repellency over time with respect to other extracts, suggesting a possible long-lasting activity. The disruptive effects of hop extracts on adult granary weevil orientation were also exerted at a distance and even in the presence of a highly attractive food source, as shown by the arena behavioral bioassays with wheat grains. In these experiments, the attractiveness of wheat grains was significantly reduced by the presence of all the extracts, with the methanol one exerting actual repellency at the highest dose. In agreement with these results, electrophysiological tests confirmed the presence in all the extracts of volatile compounds able to stimulate the peripheral olfactory system of granary weevil males and females in a dose-dependent manner. Repellent extracts may be used to control hidden infestation before fresh grain is introduced [57] or they may be incorporated into packaging materials to prevent insects from entering packaged cereal [58].

All these findings strongly suggest good potential of hop crude extracts for the development of sustainable control strategy of this pest.

5. Conclusions

In this study, we showed that methanol, acetone, and *n*-hexane extracts of hop exerted a significant bioactivity against *S. granarius* adults. All the extracts showed contact and short-range repellent effects and, more importantly, reduced the attractiveness of stored food. In particular, a good contact and a moderate ingestion toxicity was found for the acetone extract. Although differences in bioactivity among the different extracts were found, all of them provided interesting results and are worthy of further investigation in order to identify the bioactive compounds.

Author Contributions: G.R., G.S.G., and G.P. conceived and designed the experiments, analyzed the data, and wrote the paper; G.R. performed the experiments reported in Tables 1–3 and Figures 2 and 3; G.P. carried out the experiments reported in Figure 1; G.R. and G.P. carried out experiments reported in Tables 4–6; G.S.G., I.D., and M.P. performed experiments of Figure 4. All authors have read and agreed to the published version of the manuscript.

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References

1. Nerio, L.S.; Olivero-Verbel, J.; Stashenko, E. Repellent activity of essential oils: A review. *Bioresour. Technol.* **2010**, *101*, 372–378. [CrossRef]
2. Pavela, R.; Benelli, G. Essential oils as ecofriendly biopesticides? Challenges and constraints. *Trends Plant Sci.* **2016**, *21*, 1000–1007. [CrossRef]
3. Zoubiri, S.; Baaliouamer, A. Potentiality of plants as source of insecticide principles. *J. Saudi Chem. Soc.* **2014**, *18*, 925–938. [CrossRef]
4. Karkanis, A.C.; Athanassiou, C.G. Natural insecticides from native plants of the Mediterranean basin and their activity for the control of major insect pests in vegetable crops: Shifting from the past to the future. *J. Pest Sci.* **2021**, *94*, 187–202. [CrossRef]
5. Pavela, R. History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects—A review. *Plant Prot. Sci.* **2016**, *52*, 229–241. [CrossRef]
6. Isman, M.B. Commercial development of plant essential oils and their constituents as active ingredients in bioinsecticides. *Phytochem. Rev.* **2020**, *19*, 235–241. [CrossRef]
7. Jairoce, C.F.; Teixeira, C.M.; Nunes, C.F.P.; Nunes, A.M.; Pereira, C.M.P.; Garcia, F.R.M. Insecticide activity of clove essential oil on bean weevil and maize weevil. *Rev. Bras. Eng. Agric. Ambient.* **2016**, *20*, 72–77. [CrossRef]
8. Rotundo, G.; Paventi, G.; Barberio, A.; De Cristofaro, A.; Notardonato, I.; Russo, M.V.; Germinara, G.S. Biological activity of hexane extract fractions of *Dittrichia viscosa* (L.) Greuter against *Sitophilus granarius* (L.) (Coleoptera, Curculionidae) and identification of active compounds. *Sci. Rep.* **2019**, *9*, 6429. [CrossRef] [PubMed]
9. Islam, T.; Iqbal, J.; Abdullah, K.; Khan, E.A. Evaluation of some plant extracts against maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae) under laboratory conditions. *Pak. J. Agric. Sci.* **2017**, *54*, 737–741. [CrossRef]
10. Mpumi, N.; Machunda, R.L.; Mtei, K.M.; Ndakidemi, P.A. Insecticidal efficacy of *Syzygium aromaticum*, *Tephrosia vogelii* and *Croton dichogamus* extracts against *Plutella xylostella* and *Trichoplusiani* on *Brassica oleracea* crop in Northern Tanzania. *AIMS Agric. Food* **2020**, *6*, 185–202. [CrossRef]

11. Couto, I.F.S.; Souza, S.A.; Valente, F.I.; da Silva, R.M.; de Paula Quintão Scalon, S.; Pereira, F.F.; da Silva, S.V.; de Carvalho, E.M.; Mussury, R.M. Changes in the biological characteristics of *Plutella xylostella* using ethanolic plant extracts. *Gesunde Pflanz.* **2020**, *72*, 383–391. [CrossRef]
12. Rohimatun, Y.S.; Winasa, I.W. Dadang efficacy of selected piperaceae, asteraceae, and zingiberaceae plant extracts against *Helopeltis antonii* sign. *J. Int. Soc. Southeast Asian Agric. Sci.* **2020**, *26*, 145–157.
13. Jirovetz, L.; Bail, S.; Buchbauer, G.; Denkova, Z.; Slavchev, A.; Stoyanova, A.; Schmidt, E.; Geissler, M. Antimicrobial testings, gas chromatographic analysis and olfactory evaluation of an essential oil of hop cones (*Humulus lupulus* L.) from Bavaria and some of its main compounds. *Sci. Pharm.* **2006**, *74*, 189–201. [CrossRef]
14. Bocquet, L.; Rivière, C.; Dermont, C.; Samaillie, J.; Hilbert, J.L.; Halama, P.; Siah, A.; Sahpaz, S. Antifungal activity of hop extracts and compounds against the wheat pathogen *Zymoseptoria tritici*. *Ind. Crop. Prod.* **2018**, *122*, 290–297. [CrossRef]
15. Bedini, S.; Flamini, G.; Girardi, J.; Cosci, F.; Conti, B. Not just for beer: Evaluation of spent hops (*Humulus lupulus* L.) as a source of eco-friendly repellents for insect pests of stored foods. *J. Pest Sci.* **2015**, *88*, 583–592. [CrossRef]
16. Jackowski, J.; Hurej, M.; Rój, E.; Popłoński, J.; Kosny, L.; Huszcza, E. Antifeedant activity of xanthohumol and supercritical carbon dioxide extract of spent hops against stored product pests. *Bull. Entomol. Res.* **2015**, *105*, 456–461. [CrossRef] [PubMed]
17. Gökçe, A.; Isaacs, R.; Whalon, M.E.M.E. Dose-Response relationships for the antifeedant effects of *Humulus lupulus* extracts against larvae and adults of the Colorado potato beetle. *Pest Manag. Sci.* **2012**, *68*, 476–481. [CrossRef] [PubMed]
18. Gökçe, A.; Whalon, M.E.; Çam, H.; Yanar, Y.; Demirtaş, I.; Gören, N. Plant extract contact toxicities to various developmental stages of Colorado potato beetles (Coleoptera: Chrysomelidae). *Ann. Appl. Biol.* **2006**, *149*, 197–202. [CrossRef]
19. Gökçe, A.; Whalon, M.E.; Çam, H.; Yanar, Y.; Demirtaş, I.; Goren, N. Contact and residual toxicities of 30 plant extracts to Colorado potato beetle larvae. *Arch. Phytopathol. Plant. Prot.* **2007**, *40*, 441–450. [CrossRef]
20. Bedini, S.; Flamini, G.; Cosci, F.; Ascrizzi, R.; Benelli, G.; Conti, B. *Cannabis sativa* and *Humulus lupulus* essential oils as novel control tools against the invasive mosquito *Aedes albopictus* and fresh water snail *Physella acuta*. *Ind. Crop. Prod.* **2016**, *85*, 318–323. [CrossRef]
21. Paventi, G.; de Acutis, L.; De Cristofaro, A.; Pistillo, M.; Germinara, G.S.; Rotundo, G. Biological activity of *Humulus lupulus* (L.) essential oil and its main components against *Sitophilus granarius* (L.). *Biomolecules* **2020**, *10*, 1108. [CrossRef]
22. Benelli, G.; Pavela, R.; Maggi, F.; Nkuimi Wandjou, J.G.; Yvette Fofie, N.G.B.; Koné-Bamba, D.; Sagratini, G.; Vittori, S.; Caprioli, G. Insecticidal activity of the essential oil and polar extracts from *Ocimum gratissimum* grown in Ivory Coast: Efficacy on insect pests and vectors and impact on non-target species. *Ind. Crop. Prod.* **2019**, *132*, 377–385. [CrossRef]
23. Di Martino, C.; Palumbo, G.; Vitullo, D.; Di Santo, P.; Fuggi, A. Regulation of mycorrhiza development in durum wheat by P fertilization: Effect on plant nitrogen metabolism. *J. Plant Nutr. Soil Sci.* **2018**, *181*, 429–440. [CrossRef]
24. Germinara, G.S.; Di Stefano, M.G.; De Acutis, L.; Pati, S.; Delfine, S.; De Cristofaro, A.; Rotundo, G. Bioactivities of *Lavandula angustifolia* essential oil against the stored grain pest *Sitophilus granarius*. *Bull. Insectol.* **2017**, *70*, 129–138.
25. Finney, D.J. *Probit Analysis*, 3rd ed.; Cambridge University Press: London, UK, 1971.
26. Xie, Y.; Bodnaryk, R.; Fields, P. A rapid and simple flour-disk bioassay for testing substances active against stored-product insects. *Can. Entomol.* **1996**, *128*, 865–875. [CrossRef]
27. Germinara, G.S.; De Cristofaro, A.; Rotundo, G. Behavioral responses of adult *Sitophilus granarius* to individual cereal volatiles. *J. Chem. Ecol.* **2008**, *34*, 523–529. [CrossRef]
28. Phillips, T.W.; Jiang, X.-L.; Burkholder, W.E.; Phillips, J.K.; Tran, H.Q. Behavioral responses to food volatiles by two species of stored-product coleoptera, *Sitophilus oryzae* (curculionidae) and *Tribolium castaneum* (tenebrionidae). *J. Chem. Ecol.* **1993**, *19*, 723–734. [CrossRef] [PubMed]
29. Germinara, G.S.; Rotundo, G.; De Cristofaro, A. Repellence and fumigant toxicity of propionic acid against adults of *Sitophilus granarius* (L.) and *S. oryzae* (L.). *J. Stored Prod. Res.* **2007**, *43*, 229–233. [CrossRef]
30. Germinara, G.S.; Beleggia, R.; Fragasso, M.; Pistillo, M.O.; De Vita, P. Kernel volatiles of some pigmented wheats do not elicit a preferential orientation in *Sitophilus granarius* adults. *J. Pest Sci.* **2019**, *92*, 653–664. [CrossRef]
31. Kaissling, K.E.; Thorson, J. Insect olfactory sensilla: Structural, chemical and electrical aspects of the functional organization. In *Receptors for Neurotransmitters, Hormones, and Pheromones in Insects*; Satelle, D.B., Hall, L.M., Hildebrand, J.G., Eds.; Elsevier/North-Holland Biomedical Press: New York, NY, USA, 1980; pp. 261–282.
32. Germinara, G.S.; Pistillo, M.; Griffo, R.; Garonna, A.P.; Di Palma, A. Electroantennographic responses of *Aromia bungii* (Faldermann, 1835) (Coleoptera, Cerambycidae) to a range of volatile compounds. *Insects* **2019**, *10*, 274. [CrossRef] [PubMed]
33. Raguso, R.A.; Light, D.M. Electroantennogram responses of male *Sphinx perelegans* hawkmoths to floral and "green-leaf volatiles". *Entomol. Exp. Appl.* **1998**, *86*, 287–293. [CrossRef]
34. Otter, C.J.D.; Tchicaya, T.; Schutte, A.M. Effects of age, sex and hunger on the antennal olfactory sensitivity of tsetse flies. *Physiol. Entomol.* **1991**, *16*, 173–182. [CrossRef]
35. Germinara, G.S.; De Cristofaro, A.; Rotundo, G. Electrophysiological and behavioral responses of *Theocolax elegans* (Westwood) (Hymenoptera: Pteromalidae) to cereal grain volatiles. *Biomed. Res. Int.* **2016**, *2016*, 1–8. [CrossRef]
36. Rotundo, G.; Paventi, G.; Germinara, G.S. Attività insetticida di estratti di *Scrophularia canina* L. verso adulti di *Sitophilus granarius* (L.) (Coleoptera, Curculionidae). *Tec. Molit.* **2014**, *45*, 90–96.
37. Chu, S.S.; Liu, Q.R.; Liu, Z.L. Insecticidal activity and chemical composition of the essential oil of *Artemisia vestita* from China against *Sitophilus zeamais*. *Biochem. Syst. Ecol.* **2010**, *38*, 489–492. [CrossRef]

38. Rajashekar, Y.; Gunasekaran, N.; Shivanandappa, T. Insecticidal activity of the root extract of *Decalepis hamiltonii* against stored-product insect pests and its application in grain protection. *J. Food Sci. Technol.* **2010**, *47*, 310–314. [CrossRef]
39. Aydin, T.; Bayrak, N.; Baran, E.; Cakir, A. Insecticidal effects of extracts of *Humulus lupulus* (hops) L. cones and its principal component, xanthohumol. *Bull. Entomol. Res.* **2017**, *107*, 543–549. [CrossRef]
40. Maliar, T.; Nemeček, P.; Ürgeová, E.; Maliarová, M.; Nesvadba, V.; Krofta, K.; Vulganová, K.; Krošlák, E.; Kraic, J. Secondary metabolites, antioxidant and anti-proteinase activities of methanolic extracts from cones of hop (*Humulus lupulus* L.) cultivars. *Chem. Pap.* **2017**, *71*, 41–48. [CrossRef]
41. Ghiselli, L.; Tallarico, R.; Romagnoli, S.; De Acutis, L.; Benedettelli, S. Antioxidant and mineral element characterization in spontaneous hop (*Humulus lupulus* L.) in central Italy. *Agrochimica* **2015**, *59*, 319–334. [CrossRef]
42. Ocvirk, M.; Nečemer, M.; Košir, I.J. The determination of the geographic origins of hops (*Humulus lupulus* L.) by multi-elemental fingerprinting. *Food Chem.* **2019**, *277*, 32–37. [CrossRef] [PubMed]
43. Kobus-Cisowska, J.; Szymanowska-Powałowska, D.; Szczepaniak, O.; Kmiecik, D.; Przeor, M.; Gramza-Michałowska, A.; Cielecka-Piontek, J.; Smuga-Kogut, M.; Szulc, P. Composition and in vitro effects of cultivars of *Humulus lupulus* L. hops on cholinesterase activity and microbial growth. *Nutrients* **2019**, *11*, 1377. [CrossRef]
44. Guedes, N.M.P.; Guedes, R.N.C.; Silva, L.B.; Cordeiro, E.M.G. Deltamethrin-Induced feeding plasticity in pyrethroid-susceptible and -resistant strains of the maize weevil, *Sitophilus zeamais*. *J. Appl. Entomol.* **2009**, *133*, 524–532. [CrossRef]
45. Nawrot, J.; Harmatha, J. Phytochemical feeding deterrents for stored product insect pests. *Phytochem. Rev.* **2012**, *11*, 543–566. [CrossRef]
46. Boussaada, O.; Ben Halima Kamel, M.; Ammar, S.; Haouas, D.; Mighri, Z.; Helal, A.N. Insecticidal activity of some Asteraceae plant extracts against *Tribolium confusum*. *Bull. Insectol.* **2008**, *61*, 283–289.
47. Abdelkhalek, A.; Salem, M.Z.M.; Kordy, A.M.; Salem, A.Z.M.; Behiry, S.I. Antiviral, antifungal, and insecticidal activities of Eucalyptus bark extract: HPLC analysis of polyphenolic compounds. *Microb. Pathog.* **2020**, *147*, 104383. [CrossRef]
48. Yunshou, L.; Huaying, Z.; Luxiang, W.; Zhu, N.; Wanyi, L.; Xiaoyan, N.; Shaozong, T.; Yizhang, Y. Insecticidal activity of extracts from *Eupatorium adenophorum* against four stored grain insects. *Kunchong Zhishi* **2001**, *38*, 214–216.
49. Astray, G.; Gullón, P.; Gullón, B.; Munekata, P.E.S.; Lorenzo, J.M. *Humulus lupulus* L. as a natural source of functional biomolecules. *Appl. Sci.* **2020**, *10*, 5074. [CrossRef]
50. Taniguchi, Y.; Taniguchi, H.; Yamada, M.; Matsukura, Y.; Koizumi, H.; Furihata, K.; Shindo, K. Analysis of the components of hard resin in hops (*Humulus lupulus* L.) and structural elucidation of their transformation products formed during the brewing process. *J. Agric. Food Chem.* **2014**, *62*, 11602–11612. [CrossRef]
51. Česlová, L.; Holčapek, M.; Fidler, M.; Drštičková, J.; Lísa, M. Characterization of prenylflavonoids and hop bitter acids in various classes of Czech beers and hop extracts using high-performance liquid chromatography-mass spectrometry. *J. Chromatogr. A* **2009**, *1216*, 7249–7257. [CrossRef]
52. Adfa, M.; Hattori, Y.; Yoshimura, T.; Komura, K.; Koketsu, M. Antifeedant and termiticidal activities of 6-alkoxycoumarins and related analogs against *Coptotermes formosanus* Shiraki. *J. Chem. Ecol.* **2011**, *37*, 598–606. [CrossRef]
53. Aberl, A.; Coelhan, M. Determination of volatile compounds in different hop varieties by headspace-Trap GC/MS—In comparison with conventional hop essential oil analysis. *J. Agric. Food Chem.* **2012**, *60*, 2785–2792. [CrossRef]
54. Katsiotis, S.T.; Langezaal, C.R.; Scheffer, J.J.C.; Verpoorte, R. Comparative study of the essential oils from hops of various *Humulus lupulus* L. cultivars. *Flavour Fragr. J.* **1989**, *4*, 187–191. [CrossRef]
55. Eyres, G.; Dufour, J.-P. Hop essential oil: Analysis, chemical composition and odor characteristics. In *Beer in Health and Disease Prevention*; Elsevier: Amsterdam, The Netherlands, 2009; pp. 239–254.
56. Donner, P.; Pokorný, J.; Ježek, J.; Krofta, K.; Patzak, J.; Pulkrábek, J. Influence of weather conditions, irrigation and plant age on yield and α-acids content of Czech hop (*Humulus lupulus* L.) cultivars. *Plant Soil Environ.* **2020**, *66*, 41–46. [CrossRef]
57. Germinara, G.S.; De Cristofaro, A.; Rotundo, G. Bioactivity of short-chain aliphatic ketones against adults of the granary weevil, *Sitophilus granarius* (L.). *Pest Manag. Sci.* **2012**, *68*, 371–377. [CrossRef] [PubMed]
58. Germinara, G.S.; Conte, A.; Lecce, L.; Di Palma, A.; Del Nobile, M.A. Propionic acid in bio-based packaging to prevent *Sitophilus granarius* (L.) (Coleoptera, Dryophthoridae) infestation in cereal products. *Innov. Food Sci. Emerg. Technol.* **2010**, *11*, 498–502. [CrossRef]

13. Insecticidal activity of a diatomaceous earth and a natural Cuban zeolite against adults of the bean weevil, *Acanthoscelides obtectus*

Ilaria D’Isita*, Onofrio Marco Pistillo, Federica Lo Muzio, Ammara Asghar, Marco Coduti, Michele Acquaviva, Antonella Marta Di Palma, Giacinto Salvatore Germinara. *Accepted for publication in Acta Horticulturae*

Department of Agricultural Sciences, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Via Napoli 25, 71122 Foggia, Italy

*Corresponding author

Insecticidal activity of a diatomaceous earth and a natural Cuban zeolite against adults of the bean weevil, *Acanthoscelides obtectus*

I. D'Isita, O.M. Pistillo, F. Lo Muzio, A. Asghar, M. Coduti, M. Acquaviva, A.M. Di Palma, G.S. Germinara

Department of Agricultural Sciences, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Via Napoli 25, 71122 Foggia, Italy

Abstract

The bean weevil, *Acanthoscelides obtectus* (Say) (Coleoptera, Bruchidae), is one of the most serious pests of stored legumes worldwide. There is a global interest to replace chemical insecticides due to their effects on environment, human health, and pest resistance. Many inert dusts, manly including diatomaceous earth (DE), proven to be effective in controlling stored-product insect pests, while the insecticidal activity of zeolites is still little investigated. The present study aimed at evaluating the insecticidal activity of a DE (Insectosec, Newpharm) and a natural Cuban zeolite (Zeokill, Bioagrotech) against *A. obtectus* applied on seeds of different legumes. Chickpea or lentil samples (80 g) put in cylindrical plastic containers (Ø 6 x 8 cm) were treated with increasing doses (0, 0.5, 1.0, 2.0, 4.0, 8.0 kg/ton) of DE or zeolite, infested with 20 newly emerged *A. obtectus* adults, and maintained at 25±2°C, 60% r.h., and L12:D12 photoperiod. The number of dead specimens was recorded at 3, 7, 10, 14, 21 days-exposure and the mean percentage of insect mortality calculated. For both legumes, the DE and zeolite treatments induced adult mortality increasing with the dose and exposure time. On chickpea, the total insect mortality was achieved at the lowest dose tested (0.5 kg/ton) after 3 and 7 days-exposure to DE and zeolite, respectively. Similarly, on lentil the total insect mortality was recorded at 0.5 kg/ton and 1 kg/ton doses after 7 day-exposure to DE and zeolite, respectively. Overall, both inert dusts were effective in controlling *A. obtectus* at lower doses tested and in short exposure-times, with the DE showing a faster insecticidal activity. Notably, the insecticidal activity of inert dusts can vary with the substrate treated, probably due to a different adherence of the particles to the surface of the seeds.

Keywords: stored-product insect pests, inert dusts, mortality bioassays, physical control means, IPM.

Introduction

Legumes represent a category of foods essential for the diet of the populations in developed and developing countries (Rawal and Navarro, 2019). Rich source of protein, calcium, iron, phosphorus, and other minerals, legumes are considered more eco-friendly compared to other food, such as meat or dairy products (Semba *et al.*, 2021). Furthermore, growing legumes represent an advantage in crop rotation to enhance soil fertility and crop production reducing the use of nitrogen fertilizers (Foyer *et al.*, 2016). During long periods of storage, insect pests can cause substantial economic loss to stored commodities, including dried legume seeds (Stejskal *et al.*, 2014; Mengistu, 2022). Among these, the bean weevil, *Acanthoscelides obtectus* (Say), is a widespread primary pest of domesticated legumes, including the common bean as its primary host plant, and other species such as chickpea, lentil, broad bean, and common pea (Savković *et al.*, 2012; Naroz *et al.*, 2019).

Acanthoscelides obtectus can reproduce without diapause (Labeyrie, 1990), thus it is highly competitive when infesting stored products (Soares *et al.*, 2015). Furthermore, *A. obtectus* has a short life cycle and high reproductive potential in warm climates, producing several generations per year under favourable conditions often recorded in storage facilities (Soares *et al.*, 2015). Infestation of crop by *A. obtectus* can start in field where females lay eggs inside pods. The larvae bore and develop into seeds causing hidden grain infestation. Then, adults emerge from the seeds through an emergence hole. Damage legume seeds show a low weight due to the trophic activity of the larvae, poor germination capacity and are unsuitable for human and animal consumption (Johnson, 1989). Pest management in stored dried legumes commonly involves treatments with chemical insecticides, in particular fumigants (Paul *et al.* 2009). However, due to the legislation limits on the use of chemical insecticides in particular in developed countries (Handford *et al.*, 2015), the fast developing of resistant strains of target insects (Lorini *et al.*, 2007; Song *et al.*, 2011; Boyer *et al.*, 2012; Kocak *et al.*, 2015; Afful *et al.*, 2017; Nayak *et al.*, 2020; Sakka *et al.*, 2022), and environmental and human health risks due to pesticides residues (Tudi *et al.*, 2021; Tuet *et al.*, 2021), low-impact control tools towards storage pests are required (Field and White, 2002; Germinara *et al.*, 2007; Rajendran and Sriranjini, 2008; Chaudhari *et al.*, 2021; Urrutia *et al.*, 2021). In recent years, the research interest for inert dust powders as sustainable control means of stored-product insect pests is increasing (Golob, 1997; Eroglu, 2014; De Smedt *et al.*, 2015; Zeni *et al.*, 2021). In particular, the insecticidal mode of action of inert powders could be mainly linked to their capability of damaging insect cuticle causing abrasion and/or adsorption of the epicuticular waxy layer resulting in rapid moisture evaporation and death of insects by desiccation (Alexander *et al.*, 1944). The insecticidal activity of some inert dusts has been reported against different storage pest species (Athanassiou *et al*., 2005; Kljajic *et al*.,2010, 2011; Andrić *et al*., 2012; Daniel *et al*., 2013; Rumbos *et al*., 2016; Lü *et al*., 2017; Eroglu *et al*., 2019). To the best of our knowledge, the research on inert dusts as sustainable tools to control *A. obtectus* was mainly focused on diatomaceous earths (DEs) (Dal Bello, 2006; Romano *et al*., 2006; Willie *et al*., 2019), whilst less information is available about the insecticidal effect of zeolite formulation on *A. obtectus* (Floros *et al*., 2018). With the aim to expand the knowledge on the insecticidal activity of a natural Cuban zeolite against the been weevil, in the present study laboratory bioassays were carried out to evaluate the mortality rate of *A. obtectus* adults maintained on chickpea and lentil seeds treated with the Cuban zeolite or a diatomaceous earth.

Materials and Methods

Insects

Insect colonies of *A. obtectus* were obtained from infested storage bean collected in storage facilities of the Apulia region and maintained in the dark at 25±2°C, 60±5% relative humidity (r.h.) in cylindrical glass containers (Ø 15 × 15 cm) covered with a fine mesh net (0.5 mm). Newly emerged adults of *A. obtectus* were used for the experiments.

Laboratory bioassays

To evaluate the insecticidal activity of a DE (Insectosec, Newpharm, Padova, Italy) and a natural Cuban zeolite (Clinoptilolite-Heulandite 67.5%, Mordenite 32.5%) (Zeokill, Bioagrotech, Dogana, San Marino Republic) applied on chickpea (*cv.* Pasha) and lentil (*cv.* Eston) against adults of *A. obtectus* proper laboratory bioassays were carried out. Samples (80 g) of chickpea or lentil were put in cylindrical plastic containers (Ø 6 x 8 cm) covered with screw caps provided with a central hole (2 cm) screened by a metallic net (mesh size 1 mm) to allow air exchange. Chickpea and lentil seeds were treated with increasing doses (0, 0.5, 1.0, 2.0, 4.0, 8.0 kg/ton) of DE or zeolite and shaken by hand for 5 min to ensure an uniform treatment. For each food substrate, inert powder and dose tested, 3 replicates were set up. Additional sets of untreated grains were used as controls. After treatment, each replicate was infested with 20 newly emerged *A. obtectus* adults and maintained at 25±2°C, 60±5% r.h., and L12:D12 photoperiod. After 3, 7, 10, 14, 21 days-exposure, the total number of dead specimens in each replicate was recorded and used to calculate the mean adult mortality rate.

Statistical analysis

Data were submitted to Shapiro-Wilk's test to verify the normal distribution of data and to Levene's test to assess the homogeneity of variances. Then, data were analysed using analysis of variance (ANOVA) followed by Tukey's HSD test for mean comparisons. Statistical analyses were performed with SPSS (Statistical Package for the Social Sciences) v.18 for Windows (SPSS Inc., Chicago, IL).

Results

Laboratory bioassays

The mean mortality rates of *A. obtectus* adults after 3, 7, 10, 14, and 21 day-exposure to chickpea or lentil seeds treated with different doses of DE and Cuban zeolite are reported in Tables 1, 2, 3, and 4. For chickpea treated with DE, the mean mortality rate of *A. obtectus* adults after 3 day-exposure to all doses tested were significantly higher compared to control and reached the 100% adult mortality at the lowest dose of 0.5 kg/ton (Table 1). Similar results were observed for chickpea treated with Cuban zeolite, but the 100% adult mortality at the lowest dose of 0.5 kg/ton was recorded after 7 day-exposure (Table 2).

In the case of lentil treated with DE, the mortality rate of *A. obtectus* adults after 3 day-exposure to all doses tested were significantly higher compared to control; moreover, the total insect mortality at the lowest dose of 0.5 kg/ton was achieved after 7 day-exposure (Table 3).

For lentil treated with Cuban zeolite, after 3 days of exposure, the mean mortality rate of *A. obtectus* adults was significantly higher than control starting from to 2 kg/ton dose (Table 4). After 7 days, the insect mortality rate was significantly higher than control starting from the 1 kg/ton at which the total insect mortality was achieved. After 10 day-exposure to lentil treated with Cuban zeolite, the mortality rate of *A. obtectus* adults was significantly higher than control starting from the lowest dose tested of 0.5 kg/ton (Table 4).

Discussion

The research and development of low-impact tools to control stored-product insect pests is urgently needed and inert dusts, including DE and zeolites, due to their physical proprieties, could represent valuable alternatives to synthetic insecticides in Integrated Pest Management (IPM) strategies (Nikpay *et al*., 2006; Rumbos *et al*., 2016). In the present study, the adult mortality rates of *A. obtectus* adults maintained on dried chickpea and lentil seeds treated with different doses of a DE and a natural Cuban zeolite after 3, 7, 10, 14, 21 days of exposure was recorded.

The total mortality of *A. obtectus* maintained on both legumes treated with both DE and a natural Cuban zeolite was reached within short times, with some slight differences among inert dusts and food substrates. The DE treatment achieved the 100% of insect mortality rate at the lowest dose tested (0.5 kg/ton) after 3 and 7 days of exposure on chickpea and lentil, respectively. Considering the Cuban zeolite, on chickpea the insect total mortality at the lowest dose tested was induced after 7 day-exposure, whereas on lentil the total insect mortality was reached at the 1 kg/ton dose after 7 day-exposure. As personal observation, after 21 days from the start of the experiment chickpea and lentil seeds of the control reported visible damages and emergence hole of newly adults; whilst no damage was observed on both legume seed of treated thesis. Our results regarding the effectiveness of DE against *A. obtectus* are in accordance with previous studies (Dal Bello *et al*., 2006; Romano *et al*., 2006; Willie *et al*., 2019). Willie *et al*. (2019) tested a DE against *A. obtectus* and *S. zeamais* maintained on treated common bean and maize, respectively. The control of *A. obtectus* was greater than that of *S. zeamais* probably due to different cuticle characteristics of insects (Fields and Korunić, 2000), but also to different grain types considering that DEs have differences in grain adhesion depending on plant species (Korunić, 1997; Willie at al., 2019). Similar results were obtained by Dal Bello *et al*. (2006) which tested a DE originating from Argentina alone and in combination with fungal formulations of *Beauveria bassiana* (Balsamo) against *A. obtectus* and *S. oryzae* adults. The insect mortality caused by DE alone was specie-dependent and the 100% of mortality was induced only in the case of *A. obtectus* adults, probably due to its lower complexity of the epicuticular hydrocarbons compared to *S. oryzae* (Baker *et al*., 1984; Crespo *et al*., 2002; Dal Bello *et al*., 2006). In addition, the insecticidal efficacy of the DEs might be enhanced by using low-risk methods, including entomopathogenic fungi (Athanassiou and Steenberg, 2007; Ashraf *et al*., 2017; Luz *et al*., 2012; Athanassiou *et al*., 2016). Indeed, the combination of DE and the fungus *B. bassiana* or *Trichoderma harzianum* Rifai was more effective of DE alone against *A. obtectus* (Dal Bello *et al*., 2006; Gad *et al*., 2020), probably due to the DE particles capability to improve spore access into the insect cuticle (Dal Bello *et al*., 2006).

Zeolites represent a broad range of microporous, crystalline aluminosilicates of natural or synthetic origin with high variability in composition (De Smedt *et al*., 2015). Thus, the insecticidal activity of each type of zeolite has to be carefully investigated before to use them to control harmful stored-product insect pests (Germinara *et al*., 2019). To the best of our knowledge, lesser information is available in scientific literature on the use of natural Cuban zeolite to control *A. obtectus* compared to DE. However, our results are in accordance with Floros *et al*. (2018), which tested a natural zeolite on dry beans infested with *A. obtectus* and found a high insecticidal activity after few days of exposure.

Our study confirms that the little studied Cuban zeolite (Germinara *et al*., 2019) is very interesting for practical application in the control of *A. obtectus*. Indeed, Cuban zeolite show a similar effectiveness compared to the more well-known DEs, even if its slightly slower speed of action. However, from a practical point of view, this difference is negligible considering the long storage times of legumes which allow the zeolite to fully exercise its insecticidal activity.

Conclusion

In conclusion, the slight differences in days to reach the total insect mortality of DE compared to Cuban zeolite could be connected to their different physical properties, such as dust particle size, shape, and also to the particles tendency to adhere to grain surface (Korunić, 1997; at al., 2005; Rumbos *et al*., 2016; Willie *et al*., 2019). However, tested formulations at usually doses registered for inert dust direct application on grain showed a high insecticidal activity against adults of the bean weevil. Considering the long period of storage both dusts could be effective in the control of *A. obtectus* adults. However, further large-scale studies are required to define the possible application of DEs and zeolites in the control of *A. obtectus* in storage facilities.

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References

Afful, E., Elliott, B., Nayak, M. K., & Phillips, T. W. (2018). Phosphine resistance in North American field populations of the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae). J. Econ. Entomol. *111(1)*, 463-469.

Alexander, P., Kitchener, J. A., & Briscoe, H. V. A. (1944). Inert dust insecticides: Part I. Mechanism of action. Annals of Applied Biology *31(2*), 143-149.

Andrić, G. G., Marković, M. M., Adamović, M., Daković, A., Golić, M. P., & Kljajić, P. J. (2012). Insecticidal potential of natural zeolite and diatomaceous earth formulations against rice weevil (Coleoptera: Curculionidae) and red flour beetle (Coleoptera: Tenebrionidae). J. Econ. Entomol. *105(2)*, 670-678.

Ashraf, M., Farooq, M., Shakeel, M., Din, N., Hussain, S., Saeed, N., ... & Rajput, N. A. (2017). Influence of entomopathogenic fungus, Metarhizium anisopliae, alone and in combination with diatomaceous earth and thiamethoxam on mortality, progeny production, mycosis, and sporulation of the stored grain insect pests. ESPR *24*, 28165-28174.

Athanassiou, C. G., & Steenberg, T. (2007). Insecticidal effect of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreaes) in combination with three diatomaceous earth formulations against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Biological *40*(3), 411-416.

Athanassiou, C. G., Rumbos, C. I., Sakka, M. K., Vayias, B. J., Stephou, V. K., & Nakas, C. T. (2016). Insecticidal effect of the combined application of spinosad, Beauveria bassiana and diatomaceous earth for the control of Tribolium confusum. Biocontrol Sci. Techn. *26*(6), 809-819.

Athanassiou, C. G., Vayias, B. J., Dimizas, C. B., Kavallieratos, N. G., Papagregoriou, A. S., & Buchelos, C. T. (2005). Insecticidal efficacy of diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) on stored wheat: influence of dose rate, temperature and exposure interval. J. Stored Prod. Res. *41(1)*, 47-55.

Boyer, S., Zhang, H., & Lempérière, G. (2012). A review of control methods and resistance mechanisms in stored-product insects. Bull. Entomol. Res. *102(2)*, 213-229.

Chaudhari, A.K., Singh, V.K., Kedia, A., Das, S., and Dubey, N.K. (2021). Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: prospects and retrospects. ESPR *28*, 18918-18940.

Dal Bello, G., Padín, S., Juárez, P., Pedrini, N., & De Giusto, M. (2006). Biocontrol of Acanthoscelides obtectus and Sitophilus oryzae with diatomaceous earth and Beauveria bassiana on stored grains. Biocontrol Sci. Techn. *16*(2), 215-220.

Daniel, C., Dierauer, H., & Clerc, M. (2013). The potential of silicate rock dust to control pollen beetles (Meligethes spp.). IOBC wprs Bulletin *96*, 47-55.

De Smedt, C., Someus, E., & Spanoghe, P. (2015). Potential and actual uses of zeolites in crop protection. Pest Manag. Sci. *71(10)*, 1355-1367.

Eroglu, N. (2014). A review: Insecticidal potential of Zeolite (Clinoptilolite), toxicity ratings and general properties of Turkish Zeolites. In 11th IWCSPP 755-767.

Eroglu, N., Sakka, M. K., Emekci, M., & Athanassiou, C. G. (2019). Effects of zeolite formulations on the mortality and progeny production of *Sitophilus oryzae* and *Oryzaephilus surinamensis* at different temperature and relative humidity levels. J. Stored Prod. Res. *81*, 40-45.

Fields, P.G., and White, N. D. (2002). Alternatives to methyl bromide treatments for stored-product and quarantine insects. Annu. Rev. Entomol. *47(1)*, 331-359.

Floros, G. D., Kokkari, A. I., Kouloussis, N. A., Kantiranis, N. A., Damos, P., Filippidis, A. A., & Koveos, D. S. (2018). Evaluation of the natural zeolite lethal effects on adults of the bean weevil under different temperatures and relative humidity regimes. J. Econ. Entomol. *111*(1), 482-490.

Foyer, C. H., Lam, H. M., Nguyen, H. T., Siddique, K. H., Varshney, R. K., Colmer, T. D., ... & Considine, M. J. (2016). Neglecting legumes has compromised human health and sustainable food production. Nat. plants *2*(8), 1-10.

Gad, H. A., Al-Anany, M. S., Sameer, W. M., & Al-Anany, F. S. (2020). Control of *Acanthoscelides obtectus* with *Trichoderma harzianum* applied alone or in combination with diatomaceous earth on a stored common bean. Plant. Prot. Sci. *56(2)*, 107–115.

Germinara, G.S., Rotundo, G., and De Cristofaro, A. (2007). Repellence and fumigant toxicity of propionic acid against adults of *Sitophilus granarius* (L.) and *S. oryzae* (L.). J. Stored Prod. Res. *43(3)*, 229-233.

Germinara, Giacinto S., Roberto Albanese, and Onofrio M. Pistillo (2019). Evaluation of a Cuban zeolite against three stored-product pests. Tecnica Molitoria *70 (1)*, 38-46.

Golob, P. (1997). Current status and future perspectives for inert dusts for control of stored product insects. J. Stored Prod. Res. *33(1)*, 69-79.

Handford, C. E., Elliott, C. T., & Campbell, K. (2015). A review of the global pesticide legislation and the scale of challenge in reaching the global harmonization of food safety standards. Integrat. Environ. Assess. Manag. *11(4)*, 525-536.

Johnson, C. D. (1989). Adaptive radiation of Acanthoscelides in seeds: examples of legume-bruchid interactions, Monogr. Syst. Bot. Missouri Bot. 747-779.

Kljajić, P., Andrić, G., Adamović, M., & Golić, M. P. (2010). Laboratory evaluation of insecticidal effectiveness of a natural zeolite formulation against *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst) in treated wheat. Julius-Kühn-Archiv, *(425)*, 863.

Kljajić, P., Andrić, G., Adamović, M., & Pražić-Golić, M. (2011). Possibilities of application of natural zeolites in stored wheat grain protection against pest insects. Journal on Processing and Energy in Agriculture *15(1)*, 12-16.

Kocak, E., Schlipalius, D., Kaur, R., Tuck, A., Ebert, P., Collins, P., & Yilmaz, A. (2015). Determining phosphine resistance in rust red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) populations from Turkey. Turk. J. Entomol, *39*, 129-136.

Labeyrie, V. (1990). The bean beetle (Acanthoscelides obtectus) and its host, the French bean (Phaseolus vulgaris): a two-way colonization story. In Biological invasions in Europe and the Mediterranean basin. (Dordrecht: Springer Netherlands), pp. 229-243.

Lorini, I., Collins, P. J., Daglish, G. J., Nayak, M. K., & Pavic, H. (2007). Detection and characterisation of strong resistance to phosphine in Brazilian *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Pest Manag. Sci. *63(4)*, 358-364.

Lü, J., Sehgal, B., & Subramanyam, B. (2017). Insecticidal potential of a synthetic zeolite against the cowpea weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). J. Stored Prod. Res. *72*, 28-34.

Luz, C., Rodrigues, J., & Rocha, L. F. (2012). Diatomaceous earth and oil enhance effectiveness of *Metarhizium anisopliae* against *Triatoma infestans*. Acta trop. *122(1)*, 29-35.

Mengistu, H. K. (2022). Abiotic and biotic stress factors affecting storage of legumes in tropics. In Legumes Research-Volume 1. (IntechOpen).

Naroz, M. H., Ahmed, S. S., Abdel-Aziz, S. Y., & Abdel-Shafy, S. (2019). First record of *Acanthoscelides obtectus* (say) (Coleoptera: Chrysomelidae: Bruchinae) in Egypt: development and host preference on five species of legume seeds. Coleopt. Bull. *73(3)*, 727-734.

Nayak, M. K., Daglish, G. J., Phillips, T. W., & Ebert, P. R. (2020). Resistance to the fumigant phosphine and its management in insect pests of stored products: a global perspective. Annu. Rev. Entomol. *65*, 333-350.

Paul U. V., Lossini J. S., Edwards P. J., Hilbeck A. (2009) Effectiveness of products from four locally grown plants for the management of *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman) (both Coleoptera: Bruchidae) in stored beans under laboratory and farm conditions in Northern Tanzania. J. Stored Prod. Res. *45*, 97-107.

Rajendran, S., and Sriranjini, V. (2008). Plant products as fumigants for stored-product insect control. J. Stored Prod. Res. *44(2)*, 126-135.

Rawal, V. & Navarro, D. K. (2019). The Global Economy of Pulses. Rome, FAO.

Romano, C. M., Móras, A., Oliveira, M. D., Pereira, J. M., Gularte, M. A., & Elias, M. C. (2006). Control of *Acanthoscelides obtectus* in black beans with diatomaceus earth. Alternative Methods to Chemical Control 877-882.

Rumbos, C. I., Sakka, M., Berillis, P., & Athanassiou, C. G. (2016). Insecticidal potential of zeolite formulations against three stored-grain insects, particle size effect, adherence to kernels and influence on test weight of grains. J. Stored Prod. Res. *68*, 93-101.

Sakka, M. K., Jagadeesan, R., Nayak, M. K., & Athanassiou, C. G. (2022). Insecticidal effect of heat treatment in commercial flour and rice mills for the control of phosphine-resistant insect pests. J. Stored Prod. Res. *99*, 102023.

Savković, U., Vučković, I., & Stojković, B. (2012). The growth on different stored legume species affects the profiles of cuticular hydrocarbon (CHC) in Acanthoscelides obtectus (Say). J. Stored Prod. Res. *50*, 66-72.

Semba, R. D., Ramsing, R., Rahman, N., Kraemer, K., & Bloem, M. W. (2021). Legumes as a sustainable source of protein in human diets. Glob. Food Secur. *28*, 100520.

Soares, M. A., Quintela, E. D., Mascarin, G. M., & Arthurs, S. P. (2015). Effect of temperature on the development and feeding behavior of *Acanthoscelides obtectus* (Chrysomelidae: Bruchinae) on dry bean (Phaseolus vulgaris L.). J. Stored Prod. Res. *61*, 90-96.

Song, X., Wang, P., & Zhang, H., 2011. Phosphine resistance in *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) from different geographical populations in China. AJB *10(72)*, 16367-16373.

Stejskal, V., Aulicky, R., & Kucerova, Z. (2014). Pest control strategies and damage potential of seed-infesting pests in the Czech stores-a review. Plant Prot. Sci. *50*(4).

Tudi, M., Daniel Ruan, H., Wang, L., Lyu, J., Sadler, R., Connell, D., ... & Phung, D. T. (2021). Agriculture development, pesticide application and its impact on the environment. IJRPH *18(3)*, 1112.

Tuet, W. Y., Pierce, S. A., Racine, M. C., Stone, S., Pueblo, E., Dukes, A., ... & Wong, B. (2021). Cardiopulmonary effects of phosphine poisoning: A preliminary evaluation of milrinone. Toxicol. Appl. Pharmacol. *427*, 115652.

Urrutia, R.I., Yeguerman, C., Jesser, E., Gutierrez, V.S., Volpe, M.A., and González, J.O.W. (2021). Sunflower seed hulls waste as a novel source of insecticidal product: Pyrolysis bio-oil bioactivity on insect pests of stored grains and products. J. Clean. Prod. *287*, 125000.

Willie, C. L., Wille, P. E., da Rosa, J. M., Boff, M. I. C., & Franco, C. R. (2019). Efficacy of recovered diatomaceous earth from brewery to control *Sitophilus zeamais* and *Acanthoscelides obtectus*. J. Stored Prod. Res *83*, 254-260.

Zeni, V., Baliota, G. V., Benelli, G., Canale, A., & Athanassiou, C. G. (2021). Diatomaceous earth for arthropod pest control: Back to the future. Mol. *26(24)*, 7487.

Table 1. Mortality percentage of *A. obtectus* adults maintained on chickpea after 3, 7, 10, 14, 21 days-exposure to different doses of diatomaceous earths.

Dose	**Mortality (%) (Mean ± S.E.)**				
(Kg/ton)	**3 days**	**7 days**	**10 days**	**14 days**	**21 days**
0	25.5 ± 10.3 a	66.7 ± 13.6 a	85.0 ± 5.0 a	98.3 ± 1.7 a	100 ± 0 a
0.5	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a	100 ± 0 a
1	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a	100 ± 0 a
2	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a	100 ± 0 a
4	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a	100 ± 0 a
8	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a	100 ± 0 a
F	52040.0	5970.0	9.0	1.0	-
df	5	5	5	5	5
P	0.000	0.005	0.001	0.458	-

In the same column, values with different letters are significantly different at $P < 0.05$.

Table 2. Mortality percentage of *A. obtectus* adults maintained on chickpea after 3, 7, 10, 14, 21 days-exposure to different doses of a natural Cuban zeolite.

Dose	**Mortality (%) (Mean ± S.E.)**				
(Kg/ton)	**3 days**	**7 days**	**10 days**	**14 days**	**21 days**
0	22.1 ± 6.6 a	58.0 ± 6.3 a	84.4 ± 6.6 a	96.8 ± 1.6 a	100 ± 0 a
0.5	92.1 ± 3.2 b	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a
1	98.3 ± 1.7 b	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a
2	98.4 ± 1.6 b	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a
4	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a
8	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a
F	98.426	44.349	5.650	3.974	-
df	5	5	5	5	5
P	0.000	0.000	0.007	0.023	-

In the same column, values with different letters are significantly different at $P < 0.05$.

Table 3. Mortality percentage of *A. obtectus* adults maintained on lentil after 3, 7, 10, 14, 21 days-exposure to different doses of diatomaceous earths.

Dose	**Mortality (%) (Mean ± S.E.)**				
(Kg/ton)	**3 days**	**7 days**	**10 days**	**14 days**	**21 days**
0	11.7 ± 6.0 a	26.7 ± 14.5 a	59.1 ± 5.5 a	72.1 ± 7.2 a	100 ± 0 a
0.5	48.3 ± 9.3 b	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a
1	98.3 ± 1.7 c	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a
2	100 ± 0 c	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a
4	100 ± 0 c	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a
8	100 ± 0 c	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a
F	68.449	25.474	55.449	14863.0	-
df	5	5	5	5	5
P	0.000	0.000	0.000	0.000	-

In the same column, values with different letters are significantly different at $P < 0.05$.

Table 4. Mortality percentage of *A. obtectus* adults maintained on lentil after 3, 7, 10, 14, 21 days-exposure to different doses of a natural Cuban zeolite.

Dose	**Mortality (%) (Mean ± S.E.)**				
(Kg/ton)	**3 days**	**7 days**	**10 days**	**14 days**	**21 days**
0	20.0 ± 7.6 a	45.0 ± 5.8 a	58.3 ± 1.7 a	83.3 ± 6.0 a	100 ± 0 a
0.5	36.2 ± 12.2 ab	66.9 ± 23.6 ab	91.7 ± 8.3 b	98.3 ± 1.7 b	100 ± 0 a
1	63.3 ± 20.5 abc	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a
2	78.3 ± 1.7 bc	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a
4	100 ± 0 c	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a
8	100 ± 0 c	100 ± 0 b	100 ± 0 b	100 ± 0 b	100 ± 0 a
F	10.456	5.745	23.077	6.929	-
df	5	5	5	5	5
P	0.000	0.006	0.000	0.003	-

In the same column, values with different letters are significantly different at $P < 0.05$.

14. General discussion and conclusion

Postharvest wheat losses due to pest attacks are about 10-15% of the global annual production (Rajendran, 2002; Neethirajan *et al.*, 2007) and can reach 50% in developing countries (Fornal *et al.*, 2007). Stored grains are damaged by different insect pests, including *S. granarius* and *R. dominica* which are major primary pests of stored cereals worldwide (Trematerra and Throne, 2012; Majeed *et al.*, 2015; Lemic *et al.*, 2020).

Host plant selection by phytophagous insects is characterize by the phases of host finding and host acceptance (Thorsteinson, 1958). In the host finding, VOCs play a pivotal biological role as first chemicals detected by insects useful to identify host plants for feeding, mating and oviposition and also to avoid unsuitable habitats and hosts (Dickens, 1984; Visser, 1986; Agelopoulos *et al.* 1999; Reddy and Guerrero, 2004; Bernays and Chapman, 2007; Piesik *et al.*, 2020). Whereas, the host acceptance mainly depends on behavioural responses of insects to non-volatile chemical and physical features of the plants (Bernays and Chapman 2007). In particular, in the case of storage pests kernel physicochemical properties influence the demographic performance of insect, causing different susceptibility levels of grains (Throne *et al.*, 2000).

Control of stored-product insect pests for many years was mostly based on the use of synthetic insecticides, in particular phosphine and methyl bromide (Taylor, 1994; Hagstrum and Phillips, 2017). However, the repeated use of chemicals has resulted in the “3 R” problems of Resurgence, Resistance, and Residual effects with negative consequences on human health, environment and no target organism (Parkash *et al.*, 2023). Thus, low-impact control tools towards storage pests are urgently needed (Field and White, 2002; Germinara *et al.*, 2007; Rajendran and Sriranjini, 2008; Chaudhari *et al.*, 2021; Urrutia *et al.*, 2021). Due to their intrinsic characteristics, resistant wheat varieties, semiochemical-based control means, EOs, and inert dusts are considered suitable low-impact alternatives to synthetic insecticides useful in IPM strategies to control storage pests (Cox, 2004 and references therein; De Cristofaro *et al.*, 2010; Phillips and Throne, 2010; Abd El-Aziz, 2011; Germinara *et al.*, 2007, 2011, 2012, 2015; Cao *et al.*, 2023; Throne *et al.*, 2000; Golob, 1997; Eroglu, 2014; De Smedt *et* *al*., 2015; Zeni *et al*., 2021; Trdan *et al*., 2008; Pavela, 2016; Ntalli *et al*., 2021; Chaudhari *et al*., 2021).

Nowadays, the need for a wholesome, traditional, and sustainable food supply resulted in a renewed interest in ancient, old and landraces wheats providing a source of income for farmers, particularly in agriculturally marginal areas (Arzani, 2011). Indeed, their low nitrogen requirements and the high adaptability to adverse soil and climatic conditions (Perrino, 1993; D'Antuono, 1994; D'Antuono and Bravi, 1996) make ancient cereals suitable to low-input agricultural systems (Mefleh *et al*., 2019). Furthermore, the increasing consumer's demand for ancient wheat products contributing to agricultural diversification (Padulosi *et al*., 1996) and safeguard this precious biodiversity, potentially useful in breeding programs (Mefleh *et al*., 2019).

Given the lack of knowledge on the interaction among storage pests and ancient wheats (Trematerra and Gentile, 2002; Almaši and Poslončec, 2014 and reference therein; Gałęcki *et al*., 2019), the PhD thesis mainly focused on the evaluation of attractiveness and susceptibility of landraces, ancient and old wheat species to the primary pests *S. granarius* and *R. dominica* . In particular, the susceptibility level of three old wheat genotypes (Senatore Cappelli, old Saragolla, Dauno III) compared to four modern wheat varieties (Mec, Ofanto, Svevo, Faridur) (Chapter 5), and of two ancient hulled wheat species (the emmer Padre Pio and the spelt Benedetto) (Chapter 7), was investigated to identify genotypes resistant or less-susceptible to both species potentially useful in breeding programs. These studies paved also the way to identify the kernel physicochemical proprieties involved in resistance mechanisms of grains to primary pests. Furthermore, in order to identify source of bioactive compounds useful to develop semiochemical-based control tools, the VOCs profiles and olfactory responses of the same genotypes to *S. granarius* and *R. dominica* were also evaluated (Chapter 6 and 7).

Given the high rusticity of old genotypes varieties, a certain resistance to insect pests compared to modern ones could be hypothesized. Instead, even some significant differences, both old and modern wheat varieties tested were susceptible to *S. granarius* and *R. dominica* attacks.

According with previous studies, kernel hardness emerged as first barrier for the oviposition of *S. granarius* and the penetration of *R. dominica* larvae inside wheat kernels (McGaughey *et al*.,1990; Nawrot *et al*., 2006; Suleiman *et al*., 2015; Antunes *et al*., 2016; Kalsa *et al*., 2019; Željko *et al*., 2020, Amos *et al*.,1986; Toews *et al*., 2000; Arthur *et al*., 2020). However, other kernel features, such as protein content, have probably influenced the susceptibility level of tested genotypes to *S. granarius* and *R. dominica* attacks. Regarding the susceptibility degree of hulled wheat species, glumes and glumelles represented a physical barrier against *S. granarius,* but not against *R. dominica*, in accordance with previous studies where spikelets were natural protection only against some pest species (Kordan *et al*., 2007; Almaši *et al*., 2010; Bodroža-Solarov *et al*., 2010). Avoiding the glumes protectant effect, also in this case the kernel hardness and protein content represented the main factors involved in resistance mechanisms to both species. In general terms, both studies on susceptibility level of old and ancient wheat varieties indicating that wheat susceptibility to stored grain pests probably is the result of different physicochemical features and their interactions. Furthermore, to the best of our knowledge, these are the first studies for the old and ancient wheat varieties investigated. Thus, considering the increasing interest of consumers and farmers in these grains, both studies offer new knowledge also useful for a more rational postharvest management of these cereal resources.

The study on the VOCs profiles and olfactory responses of old and modern genotypes to *S. granarius* and *R. dominica* showed that *S. granarius* adults were significantly attracted to odours of wheat varieties tested, with a significant preference of females for Mec, Ofanto, Faridur, and Old Saragolla. Whereas, *R. dominica* adults were significant attracted only by Mec, Faridur, and Old Saragolla. The olfactory preferences recorded among varieties for both species could be associated with differences in types and concentrations of volatile components and may be affected by synergistic effects of several compounds (Bruce *et al*., 2005; Najar-Rodriguez *et al*., 2010; Bruce and Pickett, 2011; Cha *et al*., 2011). In particular, Faridur, the most attractive variety for both species, was rich in alcohols, alkanes, and terpenes; whilst Svevo, the less attractive one for *S. granarius* females, was rich in aldehydes. These results are in accordance with previous studies on the behavioural responses of granary weevil adults to different cereal VOCs. Indeed, some alcohols acted as attractant (Piesik and Wenda-Piesik, 2015) or repellent (Germinara *et al*., 2008), or showed a dual activity based on their concentrations (Germinara *et al*., 2008). Instead, some aliphatic aldehydes disrupt the granary weevil orientation towards attractive commercial wheat varieties (Germinara *et al*., 2015), inhibit its orientation (Germinara *et al*., 2019), and act as strong repellent (Germinara *et al*., 2008). Thus, the host finding by S. *granarius* is a complex process based on the balance of positive and negative odour stimuli (Germinara *et al*., 2008).

In the study on the behavioural responses of hulled emmer and spelt, *S. granarius* were effectively attracted by both ancient wheat species, with a significant preference of both sexes for the emmer Padre Pio. By contrast, odours from emmer and spelt varieties did not elicit a significant attraction in *R. dominica* adults. In this case, the VOCs profile of emmer and spelt resulted quite similar. Thus, probably the olfactory preferences of *S. granarius* for emmer Padre Pio could be linked to differences in VOCs emissions among the two substrates potential caused by differences in kernels and glumes characteristics. In the case of *R. dominica*, olfactory cues are important to discriminate different kinds of food substrates (Edde and Philips, 2006; Cao *et al*., 2024 b), however little is known about the role of chemical classes or specific VOCs on the orientation towards food substrates. In conclusion, both studies are the first attempt to evaluate attractiveness of modern, old and ancient wheat varieties towards *S. granarius* and *R. dominica* and paves the way for further electrophysiological and behavioural studies aiming at the evaluation of the biological activity of VOCs detected. In fact, defining the biological activity of these VOCs could offer new insights into the mechanism of host finding by both pests. From a practical perspective, this information would be useful to identify bioactive compounds valuable in the development of effective attractants for semiochemical-based control means to enhance the performance of aggregation pheromone-based lures (Chambers, 1990; Dowdy *et al*., 1993; Dissanayaka *et al*., 2020; Morrison *et al*., 2023) or strong repellents deterring host finding by pests (Germinara *et al*., 2008; 2012). Furthermore, clarifying the possible role of VOCs in semiochemical interactions between host plant and postharvest insect pests, is crucial for new breeding programs aiming to develop new varieties producing high levels of repellents that could modify the insect behaviour and increase the storability of wheat grains.

The techniques acquired during the study of the susceptibility and olfactory responses of *S. granarius* and *R. dominica* to ancient and old wheats, were used also to deepening knowledge on storage insects and host plant interactions and to evaluate the efficacy of different low-impact control tools. In this context, fruitful collaborations with national and international research centres were established.

In Chapter 8 and 9 of the thesis, respectively, the host acceptance of a pigmented wheat genotype by *S. granarius* adults and of different stored products by *R. dominica* adults was evaluated.

Pigmented wheat genotypes rich in anthocyanins are very interesting due to the high nutritional value conferred to the final products (Ficco *et al.* 2014; Ficco *et al.* 2016; Ficco *et al.* 2020). Phenolic compounds could modulate growth, development and fitness of insect pests (Kordan *et al.*, 2019). Our results strongly suggested that the anthocyanins accumulated in the pericarp of kernels determined a less susceptibility compared to yellow kernel varieties against granary weevil probably due to antifeedant, deterrent and toxic effects. These findings offer new insight on the role of anthocyanins in host acceptance by *S. granarius* and pave the way for further studies aimed to transfer genes or genetic regions associated with resistance to storage pests in modern breeding programs. Regarding the host acceptance and utilisation by *R. dominica*, our findings revealed a different response of this pest to different stored products. Indeed, although *R. dominica* successfully completed its life history on all stored products tested, wheat was the most suitable diet for *R. dominica* population development, and angelica was the least suitable one. These differences are probably linked with physical and biochemical properties of the different stored products (Majd-Marani *et al.* 2017; Naseri *et al.* 2017) that may influence the host acceptance by *R. dominica*. Thus, the physicochemical properties of angelica should be further explored for potential application in the control *R. dominica*. Furthermore, results of this study are useful information for predicting population growth of *R. dominica* on different stored products, taking into account that the focus for control of this pest should be on wheat.

To provide a basis for developing semiochemical-based strategies, the olfactory responses of *S. paniceum* (Chapter 10) and *S. oryzae* (Chapter 11) adults to VOCs respectively from Chinese medicinal plants and different rice cultivars were evaluated. Both studies offer new insight on host finding by *S. paniceum* and *S. oryzae*, confirming that VOCs participate directly in the interactions between storage insects and host plants. Moreover, 3-n-butylphthalide and nonanal, respectively the most attractive compounds for *S. paniceum* and for *S. oryzae*, seems to have a greatest potential for developing kairomonal lure for these pests.

In the last chapters of the thesis, the insecticidal activity of different wild hop extracts (Chapter 12) against *S. granarius* adults, and of a Cuban zeolite and a diatomaceous earth against *A. obtectus* adults (Chapter 13) was evaluated.

In Chapter 12, emerged that, in addition to the essential oil (Paventi *et al*., 2020), also methanol, acetone, and n-hexane extracts of hop exerted a significant bioactivity against *S. granarius* adults. All the extracts showed contact and short-range repellent effects and, more importantly, reduced the attractiveness of stored food. All these findings strongly suggest a possible use of hop extracts against *S. granarius*, confirming that this plant species is interesting for pest control.

Regarding the use of inert dusts, the Cuban zeolite and the diatomaceous earth showed a high insecticidal activity against adults of *A. obtectus*, even if Cuban zeolite has a slightly slower speed of action. These differences in efficacy could be caused by different susceptibility level to inert dusts in storage pests (Rumbos *et al*., 2016), and also to dust physical properties, such as particle size, shape, and the particles tendency to adhere to grain surface (Kavallieratos *at al*., 2005; Rumbos *et al*., 2016). In this context, further studies are needed to carefully investigate the insecticidal activity of each type of inert dust before to use it to control harmful stored-product insect pests and to define their possible application in storage facilities.

Overall, the findings of the present PhD thesis can provide a significant contribution to the growing scientific knowledge on feeding behaviour of primary pests *S. granarius* and *R. dominica* on ancient and old wheat species, little investigated before. Furthermore, the role of some kernel physicochemical proprieties involved in resistance mechanisms of grains to primary pests was clarified. The acquired knowledge on food finding and food acceptance by storage insect species could represent a solid basis for further studies aimed at identifying and develop resistant genotypes and/or semiochemical-based control tools. As well as, the thesis provides new information on potential low-impact control tools against stored-product insect pests useful in the framework of current IPM approaches. Moreover, the techniques learned during the PhD period are applicable to various basic and applied research contexts paving the way for future studies on insects, in particular stored-product pests.

15. References

Abdel-Aal, E. S. M., & Hucl, P., 2003. Composition and stability of anthocyanins in blue-grained wheat. Journal of agricultural and Food Chemistry, 51(8), 2174-2180.

Abdel-Aal, E. S. M., Young, J. C., & Rabalski, I., 2006. Anthocyanin composition in black, blue, pink, purple, and red cereal grains. Journal of Agricultural and Food Chemistry, 54(13), 4696-4704.

Adler, C., Athanassiou, C., Carvalho, M. O., Emekci, M., Gvozdenac, S., Hamel, D., ... & Trematerra, P., 2022. Changes in the distribution and pest risk of stored product insects in Europe due to global warming: Need for pan-European pest monitoring and improved food-safety. Journal of Stored Products Research, 97, 101977.

Afful, E., Elliott, B., Nayak, M. K., & Phillips, T. W., 2018. Phosphine resistance in North American field populations of the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Journal of Economic Entomology, 111(1), 463-469.

Agelopoulos N., Birkett M. A., Hick A. J., Hooper A. M., Pickett J. A., Pow E. M., Woodcock C. M., 1999. Exploiting semiochemicals in insect control. Journal of Pesticide Science, 55(3), 225-235.

Allison JD, Borden JH & Seybold SJ, 2004. A review of the chemical ecology of the Cerambycidae (Coleoptera). Chemoecology 14,123-150.

Almaši, R., & Poslončec, D. I., 2014. Reproduction of confused flour beetle *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae) on common and spelt wheat and their products. Pesticides and Phytomedicine, 29(3), 197-204.

Almasi, R., Bodroza-Solarov, M., & Posloncec, D., 2010. Development of rice weevils (*Sitophilus oryzae* L.) and lesser grain borers (*Rhizopertha dominica* F.) on kernels and spikes of spelt wheat. Savremena Poljoprivreda, 59(1-2), 92-98.

Amoah, B. A., Mahroof, R. M., Gerken, A. R., & Campbell, J. F., 2019. Effect of delayed mating on longevity and reproductive performance of *Lasioderma serricorne* (Coleoptera: Anobiidae). Journal of Economic Entomology, 112(1), 475-484.

Amos, T.G., Semple, R.L., and Williams, P., 1986. Multiplication of some stored grain insects on varieties of wheat. General and Applied Entomology: The Journal of the Entomological Society of New South Wales, 18, 48-52.

Andrić, G. G., Marković, M. M., Adamović, M., Daković, A., Golić, M. P., & Kljajić, P. J., 2012. Insecticidal potential of natural zeolite and diatomaceous earth formulations against rice weevil (Coleoptera: Curculionidae) and red flour beetle (Coleoptera: Tenebrionidae). Journal of Economic Entomology, 105(2), 670-678.

Antunes, C., Mendes, R., Lima, A., Barros, G., Fields, P., Da Costa, L.B., ... & Carvalho, M.O., 2016. Resistance of rice varieties to the stored-product insect, *Sitophilus zeamais* (Coleoptera: Curculionidae). Journal of Economic Entomology, 109(1), 445-453.

Arthur, F. H., & Puterka, G. J., 2002. Evaluation of kaolinite-based particle films to control *Tribolium* species (Coleoptera: Tenebrionidae). Journal of Stored Products Research, 38(4), 341-348.

Arthur, F.H., Bean, S.R., Smolensky, D., Cox, S., Lin, H.H., Peiris, K.H.S., and Peterson, J., 2020. Development of *Rhyzopertha dominica* (Coleoptera: Bostrychidae) on sorghum: Quality characteristics and varietal susceptibility. Journal of Stored Products Research, 87, 101569.

Arzani, A., 2011. Emmer (*Triticum turgidum* ssp. dicoccum) flour and bread. In Flour and breads and their fortification in health and disease prevention, ed. by Preedy VR, Watson RR and Patel VB. (2011). Academic Press, London, 542.

Astuti, L.P., Mudjiono, G., Rasminah, S.C., and Rahardjo, B.T., 2013. Susceptibility of milled rice varieties to the lesser grain borer (*Rhyzopertha dominica*, F). Journal of Agricultural Science, 5(2), 145.

Athanassiou C.G., Kavallieratos N.G., Sciarretta A., Trematerra P., 2016 - Mating disruption of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) in a storage facility: spatio-temporal distribution changed after long-term application. Journal of Stored Product Research, 67, 1-12.

Athanassiou, C. G., Vayias, B. J., Dimizas, C. B., Kavallieratos, N. G., Papagregoriou, A. S., & Buchelos, C. T., 2005. Insecticidal efficacy of diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) on stored wheat: influence of dose rate, temperature and exposure interval. Journal of Stored Products Research, 41(1), 47-55.

Athanassiou, C. G., Vayias, B. J., Dimizas, C. B., Kavallieratos, N. G., Papagregoriou, A. S., & Buchelos, C. T., 2005. Insecticidal efficacy of diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) on stored wheat: influence of dose rate, temperature and exposure interval. Journal of Stored Products Research, 41(1), 47-55.

Athanassiou, C.G., Kavallieratos, N.G., Evergetis, E., Katsoula, A., Haroutounian, S.A., 2013. Insecticidal efficacy of silica gel with *Juniperus oxycedrus* ssp. *oxycedrus* (Pinales: Cupressaceae) essential oil against *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae). Journal of Economic Entomology, 106, 1902-1910.

Bacaltchuk, B., Beckel, H., Deckers, D., Sundfeld, E., Santos, J.P., Biagi, J.D., Celaro, J.C., Faroni, L.R.D., Bortolini, L. de O.F., Sartori, M.R., Elias, M.C., Guedes R.N.C., Fonseca, R.G. da, Scussel V.M., 2006. Proceedings of the 9th international working conference on stored-product protection, ABRAPOS, Passo Fundo, R.S., Brazil. 15-18 October 2006, 400-407.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M., 2008. Biological effects of essential oils– a review. Food and Chemical Toxicology, 46(2), 446-475.

Banks, J. and Fields, P., 1995 Physical methods for insect control in stored grain ecosystems. In Stored Grain Ecosystems, Marcel Dekker, New York, 353-409.

Barnes, J.H., Groove, A.J., 1916. The insects attacking stored wheat in the Punjab and the methods of combating them, including a chapter on the chemistry of respiration. Memo of the Department of Agriculture. India (Chemical Series) 4, 166-172.

Bashir T., 2002. Reproduction of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) on different host-grains. Pakistan Journal of Biological Sciences, 5, 91-93.

Beck S.D., 1965. Resistance of plant to insect. Annual Review of Entomology, 10, 207-232.

Bell, C.H., 2014. Food safety assurance systems: Infestation Management in Food Production Premises. Encyclopedia of Food Safety, 4, 189-200.

Benelli, G., & Pavela, R., 2018 a. Repellence of essential oils and selected compounds against ticks-A systematic review. Acta Tropica, 179, 47-54.

Benelli, G., & Pavela, R., 2018 b. Beyond mosquitoes - Essential oil toxicity and repellency against bloodsucking insects. Industrial Crops and Products, 117, 382-392.

Bernays E. A., Chapman R. F., 2007. Host-plant selection by phytophagous insects. Springer, Science & Business Media, New York. 2, 14-54.

Binyameen, M., Ali, Q., Roy, A., & Schlyter, F., 2021. Plant volatiles and their role in insect olfaction. Plant-pest interactions: from molecular mechanisms to chemical ecology. Chemical Ecology, 127-156.

Birch, L.C., 1945. The Influence of Temperature, Humidity and Density on the Oviposition of the small Strain of *Calandra oryzae* L. and *Rhizopertha dominica* Fab.(Coleoptera). Australian Journal of Experimental Biology & Medical Science, 23(3), 197-203.

Bodroža-Solarov, M., Almaši, R., Poslončec, D., Filipčev, B., & Šimurina, O., 2010. Protective effect of hulls Triticum aestivum ssp. spelta against insect infestation during storage. 2nd Workshop Feed-to-Food FP7 REGPOT-3. XIV International Symposium feed technology, Proceedings. Novi Sad, Serbia, 19-21.

Bohinc, T., Horvat, A., Andrić, G., Pražić Golić, M., Kljajić, P., Trdan, S., 2020. Natural versus synthetic zeolites for controlling the maize weevil (*Sitophilus zeamais*)–like Messi versus Ronaldo?. Journal of Stored Product Research, 88, 101639.

Bordoni, A., Danesi, F., Di Nunzio, M., Taccari, A., & Valli, V., 2017. Ancient wheat and health: a legend or the reality? A review on KAMUT khorasan wheat. International Journal of Food Sciences and Nutrition, 68(3), 278-286.

Bougherra-Nehaoua, H. H., Bedini, S., Cosci, F., Flamini, G., Belhamel, K., & Conti, B., 2015. Enhancing the insecticidal efficacy of inert dusts against stored food insect pest by the combined action with essential oils. Integrated Protection of Stored Products. IOBC-WPRS Bullettin, 111, 31-38.

Boukid, F., Folloni, S., Sforza, S., Vittadini, E., & Prandi, B., 2018. Current trends in ancient grains-based foodstuffs: insights into nutritional aspects and technological applications. Comprehensive Reviews in Food Science and Food Safety, 17(1), 123-136.

Boyer, S., Zhang, H., & Lempérière, G., 2012. A review of control methods and resistance mechanisms in stored-product insects. Bulletin of Entomological Research, 102(2), 213-229.

Breese, M.H., 1960. The infestibility of stored paddy by *Sitophilus sasakii* (Tak.) and *Rhyzopertha dominica* (F.). Bulletin of Entomological Research, 51(3), 599-630.

Bruce, T.J., & Pickett, J.A., 2011. Perception of plant volatile blends by herbivorous insects–finding the right mix. Phytochemistry, 72(13), 1605-1611.

Bruce, T.J., Wadhams, L.J., & Woodcock, C.M., 2005. Insect host location: a volatile situation. Trends in Plant Science, 10(6), 269-274.

Bruneton, J., 1999. Pharmacognosy, phytochemistry, medicinal plants, laboisier tec & Doc. Paris, France.

Buchelos C.T., Levinson A.R., 1993. Efficacy of multisurface traps and Lasiotraps with and without pheromone addition, for monitoring and mass-trapping of *Lasioderma serricorne* F. (Col., Anobiidae) in insecticide-free tobacco stores. Journal of Applied Entomology, 116: 404-448.

Buerli, M., 2006. Farro in Italy: A Desk-study by Markus Buerli. Global Facilitation Unit for Underutilized Species (GFU), Rome, 20.

Burkholder, W.E., 1982. Reproductive biology and communication among grain storage and warehouse beetles. Journal of the Georgia Entomological Society, 17, 1-10.

Burkholder, W.E., 1990. Practical use of pheromones and other attractants for stored-product insects. Behavior-Modifying Chemicals for Insect Management: Applications of Pheromones and Other Attractants. Marcel Dekker, New York, USA, 497-516.

Burks C.S., McLaughlin J.R., Miller J.R., Brandl D.G., 2011 - Mating disruption for control of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) in dried beans. Journal of Stored Products Research, 47(3): 216-221.

Campbell, A., Sinha, R.N., 1976. Damage of wheat by feeding of some stored product beetles. Journal of Economic Entomology, 69, 11-13.

Campbell, J.F., 2002. Influence of seed size on exploitation by the rice weevil, *Sitophilus oryzae*. Journal of Insect Behavior, 15, 429-445.

Campolo, O., Cherif, A., Ricupero, M., Siscaro, G., Grissa-Lebdi, K., Russo, A., ... & Palmeri, V., 2017. Citrus peel essential oil nanoformulations to control the tomato borer, *Tuta absoluta*: chemical properties and biological activity. Scientific reports, 7(1), 13036.

Campolo, O., Giunti, G., Russo, A., Palmeri, V., & Zappalà, L., 2018. Essential oils in stored product insect pest control. Journal of Food Quality, 1, 6906105.

Campolo, O., Malacrinò, A., Zappalà, L., Laudani, F., Chiera, E., Serra, D., Russo, M. & Palmeri, V., 2014 a: Fumigant bioactivity of five citrus essential oils against *Tribolium confusum*. Phytoparasitica, 42, 233.

Campolo, O., Romeo, F. V., Malacrinò, A., Laudani, F., Carpinteri, G., Fabroni, S., ... & Palmeri, V., 2014 b. Effects of inert dusts applied alone and in combination with sweet orange essential oil against *Rhyzopertha dominica* (Coleoptera: Bostrichidae) and wheat microbial population. Industrial Crops and Products, 61, 361-369.

Campos M., Phillips T. W., 2010 - Contact toxicity of insecticides for attract-and-kill applications against adult *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). Pest Management Science, 66(7): 752-761.

Campos M., Phillips T.W., 2014. Attract-and-kill and other pheromone-based methods to suppress populations of the Indianmeal moth (Lepidoptera: Pyralidae). Journal of Economic Entomology, 107(1), 473-480.

Cao, Y., Hu, Q., Huang, L., Athanassiou, C. G., Maggi, F., D'Isita, I., ... & Li, C., 2024 a. Attraction of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) to the semiochemical volatiles of stored rice materials. Journal of Pest Science, 97(1), 73-85.

Cao, Y., Jian, L., Athanassiou, C. G., Yang, Y., Hu, Q., Zhang, X., ... & Maggi, F., 2024 b. Behavioral responses of *Rhyzopertha dominica* (F.) to volatiles of different stored grains. Journal of Stored Products Research, 105, 102235.

Careddu, M. L., Giunta, F., & Motzo, R., 2023. Lessons from the Varietal Evolution of Durum Wheat in Italy. Agronomy, 14(1), 87.

Carvalho, M.O., Santos, F., Mexia, A., Torres, L., 2006. Use of pheromone traps to assess *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae) infestation in a cigarette factory on the Cape Verde islands. African Entomology, 14(2), 307-315.

Carvalho, M.O., Mexia, A., 2002. The use of pheromone traps for mass- trapping of *Lasioderma serricorne* in a cigarette factory in Portugal. Proceedings of the 8th International Working Conference on Stored-Product Protection, York, 222-229.

Cha, D.H., Linn Jr, C.E., Teal, P.E., Zhang, A., Roelofs, W.L., & Loeb, G.M., 2011. Eavesdropping on plant volatiles by a specialist moth: significance of ratio and concentration. PLoS One, 6(2), e17033.

Chaieb, I., Zarrad, K., Sellam, R., Tayeb, W., Ben Hammouda, A., Laarif, A., & Bouhachem, S., 2018. Chemical composition and aphicidal potential of Citrus aurantium peel essential oils. Entomologia Generalis, 37(1).

Chambers, J., 1990. Overview on stored-product insect pheromones and food attractants. Journal of the Kansas Entomological Society, 490-499.

Chambers, J., Van Wyk, C.B., White, P.R., Gerrard, C.M., & Mori, K., 1996. Grain weevil, *Sitophilus granarius* (L.): antennal and behavioral responses to male-produced volatiles. Journal of Chemical Ecology, 22, 1639-1654.

Chapman, R.F., 2003. Contact chemoreception in feeding by phytophagous insects. Annual Review of Entomology, 48(1), 455-484.

Chaudhari, A.K., Singh, V.K., Kedia, A., Das, S., & Dubey, N.K., 2021. Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: prospects and retrospects. Environmental Science and Pollution Research, 28, 18918-18940.

Chittenden, F.H., 1911. The lesser grain borer and the larger grain borer. Bulletin of United State Bureau of Entomology, 96, 29-47.

Chûjô, M., 1958. Coleoptera: Bostrychidae. Insects of Micronesia 16, 85-104.

Collins, F.W., 1986. Oat phenolics: structure, occurrence, and function, 227-295. In: Oats: Chemistry and Technology. F. W. Webster, ed. Am. Assoc. Cereal Chemistry, St Paul, MN.

Cook, S.M., Khan, Z.R., & Pickett, J.A., 2007. The use of push-pull strategies in integrated pest management. Annual Review of Entomology, 52(1), 375-400.

Cox, P.D., & Collins, L.E., 2002. Factors affecting the behaviour of beetle pests in stored grain, with particular reference to the development of lures. Journal of Stored Products Research, 38(2), 95-115.

Cox, P.D., 2004. Potential for using semiochemicals to protect stored products from insect infestation. Journal of Stored Products Research, 40(1), 1-25.

D'Antuono, L.F. and Bravi, R., 1996. The hulled wheat industry: present developments and impact on genetic resources conservation. In: Padulosi, S., 1996. Hulled Wheats: Proceedings of the First International Workshop on Hulled Wheats, 21-22 July 1995, Castelvecchio Pascoli, Tuscany, Italy (Vol. 4). Bioversity International.

D'Antuono, L.F., 1994. Obsolete wheats in Italy: an overview on cultivation, use and perspectives for their conservation. In Report of the IPGRI Workshop on Conservation and Use of Underutilized Mediterranean Species. Rome, Italy: IPGRI, 41-48.

D'Isita, I., Di Palma, A. M., De Vita, P., & Germinara, G. S., 2023. Acceptance and utilization efficiency of a purple durum wheat genotype by *Sitophilus granarius* (L.). Scientific Reports, 13(1), 14246.

D'Isita, I., Pistillo, O. M., Di Palma, A. M., De Vita, P., & Germinara, G. S., 2024. Susceptibility of old and modern wheat genotypes to *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.). Journal of Stored Products Research, 106, 102265.

Daniel, C., Dierauer, H., & Clerc, M., 2013. The potential of silicate rock dust to control pollen beetles (*Meligethes* spp.). IOBC/WPRS Bulletin, 96, 47-55.

Darwin, C., 1883. The Variation of Animals and Plants Under Domestication. Second Edition. New York: D. Appleton and Co.

Davenport, C.B., 1945. The dietaries of primitive peoples. American Anthropologist, 47(1), 60-82.

De Cristofaro, A., Anfora, G., Germinara, G. S., Ioriatti, C., Mazzoni, V., & Rotundo, G., 2010. Cell responding to pheromone components and plant volatiles could affect semiochemical based control strategies of insect pests in agricultural ecosystems. IOBC/WPRS Bulletin, 54, 410.

De Smedt, C., Someus, E., & Spanoghe, P., 2015. Potential and actual uses of zeolites in crop protection. Pest Management Science, 71(10), 1355-1367.

Deguine, J.P., Aubertot, J.N., Flor, R.J., Lescourret, F., Wyckhuys, K.A., & Ratnadass, A., 2021. Integrated pest management: good intentions, hard realities. A review. Agronomy for Sustainable Development, 41(3), 38.

Devi, S.R., Thomas, A., Rebijith, K.B., & Ramamurthy, V.V., 2017. Biology, morphology and molecular characterization of *Sitophilus oryzae* and *S. zeamais* (Coleoptera: Curculionidae). Journal of Stored Products Research, 73, 135-141.

Di Francesco, A., Cunsolo, V., Saletti, R., Svensson, B., Muccilli, V., De Vita, P., & Foti, S., 2021. Quantitative label-free comparison of the metabolic protein fraction in old and modern italian wheat genotypes by a shotgun approach. Molecules, 26(9), 2596.

Di Palermo, D., Giunti, G., Laudani, F., Palmeri, V., & Campolo, O., 2021. Essential oil-based nano-biopesticides: Formulation and bioactivity against the confused flour beetle *Tribolium confusum*. Sustainability, 13(17), 9746.

Dib, T.A., Monneveux, P., & Araus, J.L., 1992. Adaptation à la sécheresse et notion d'idéotype chez le blé dur. II. Caractères physiologiques d'adaptation. Agronomie, 12(5), 381-393.

Dicke, M., & Sabelis, M.W., 1988. Infochemical terminology: based on cost-benefit analysis rather than origin of compounds?. Functional ecology, 131-139.

Dickens J.C., 1984. Olfaction in the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae): Electroantennogram studies. Journal of Chemical Ecology, 10(12), 1759-1785.

Dinu, M., Whittaker, A., Pagliai, G., Benedettelli, S., & Sofi, F., 2018. Ancient wheat species and human health: Biochemical and clinical implications. The Journal of Nutritional Biochemistry, 52, 1-9.

Dissanayaka, D.M.S.K., Sammani, A.M.P., Wijayaratne, L.K.W., Rajapakse, R.H.S., Hettiarachchi, S., & Morrison III, W.R., 2020. Effects of aggregation pheromone concentration and distance on the trapping of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) adults. Journal of Stored Products Research, 88, 101657.

Do, T.K.T., Hadji-Minaglou, F., Antoniotti, S., & Fernandez, X., 2015. Authenticity of essential oils. TrAC Trends in Analytical Chemistry, 66, 146-157.

Doane, R.W., 1919. Weevils in Australian wheat in California. Journal Economic Entomology 12, 308-312.

Dobie, P., 1974. The laboratory assessment of the inherent susceptibility of maize varieties to postharvest infestation by *Sitophilus zeamais* Motsch. (Coleoptera, Curculionidae). Journal of Stored Products Research, 10(3-4), 183-197.

Domina, G., & Marzio, Z., 2018. Biodiversity in Italy. In Global Biodiversity. Apple Academic Press, 203-253.

Dowdy, A.K., Howard, R.W., Seitz, L.M., & McGaughey, W.H., 1993. Response of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) to its aggregation pheromone and wheat volatiles. Environmental Entomology, 22(5), 965-970.

Drewnowski, A., & Gomez-Carneros, C., 2000. Bitter taste, phytonutrients, and the consumer: a review. The American journal of clinical nutrition, 72(6), 1424-1435.

Dubcovsky, J., & Dvorak, J., 2007. Genome plasticity a key factor in the success of polyploid wheat under domestication. Science, 316(5833), 1862-1866.

Edde, P.A., & Phillips, T.W., 2006. Potential host affinities for the lesser grain borer, *Rhyzopertha dominica*: behavioral responses to host odors and pheromones and reproductive ability on non-grain hosts. Entomologia Experimentalis et Applicata, 119(3), 255-263.

Edde, P.A., 2012. A review of the biology and control of *Rhyzopertha dominica* (F.) the lesser grain borer. Journal of Stored Products Research, 48, 1-18.

Edde, P.A., Phillips, T.W., & Toews, M.D., 2005. Responses of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) to its aggregation pheromones as influenced by trap design, trap height, and habitat. Environmental Entomology, 34(6), 1549-1557.

Eroglu, N., 2014. A review: Insecticidal potential of Zeolite (Clinoptilolite), toxicity ratings and general properties of Turkish Zeolites. In 11th International Working Conference on Stored Product Protection, 755-767.

Eroglu, N., Sakka, M.K., Emekci, M., & Athanassiou, C.G., 2019. Effects of zeolite formulations on the mortality and progeny production of *Sitophilus oryzae* and *Oryzaephilus surinamensis* at different temperature and relative humidity levels. Journal of Stored Products Research, 81, 40-45.

Escribano-Bailón, M.T., Santos-Buelga, C., & Rivas-Gonzalo, J.C., 2004. Anthocyanins in cereals. Journal of Chromatography A, 1054(1-2), 129-141.

EUROPEAN FOOD SAFETYAUTHORITY, 2013 a. Panel on Food Contact Materials, Enyzmes, Flavourings and Processing Aids. EFSA Journal, 11, 3155.

EUROPEAN FOOD SAFETYAUTHORITY, 2013 b. Panel of Additives and Products or Substances used in Animal Feed. EFSA Journal, 11, 3039.

Evans, L.T., 1993, Crop Evolution, Adaptation and Yield. Cambridge University Press, Cambridge.

Fabres, A., de Campos Macedo da Silva, J., Fernandes, K.V., Xavier-Filho, J., Rezende, G. L., & Oliveira, A.E.A., 2014. Comparative performance of the red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae) on different plant diets. Journal of Pest Science, 87, 495-506.

Fabroni, S., Ruberto, G., & Rapisarda, P., 2012. Essential oil profiles of new Citrus hybrids, a tool for genetic citrus improvement. Journal of Essential Oil Research, 24(2), 159-169.

Fadamiro H.Y., Baker T.C., 2002. Pheromone puffs suppress mating by *Plodia interpunctella* and *Sitotroga cerealella* in an infested corn store. Entomologia Experimentalis et Applicata, 102, 239-251.

Fanelli, V., Dellino, M., Taranto, F., De Giovanni, C., Sabetta, W., De Vita, P., & Montemurro, C., 2023. Varietal identification in pasta through an SSR-based approach: a case study. Journal of the Science of Food and Agriculture, 103(11), 5521-5528.

FAO, 2014. Food and agriculture organization of the United Nations. Statistics Division, Crops data.

FAO. 2023. World Food and Agriculture. Statistical Yearbook 2023. Rome.

Faustini, D.L., Giese, W.L., Phillips, J.K., & Burkholder, W.E., 1982. Aggregation pheromone of the male granary weevil, *Sitophilus granarius* (L.). Journal of Chemical Ecology, 8, 679-687.

Ficco, D.B.M., Borrelli, G.M., Miedico, O., Giovanniello, V., Tarallo, M., Pompa, C., ... & Chiaravalle, A.E., 2020. Effects of grain debranning on bioactive compounds, antioxidant capacity and essential and toxic trace elements in purple durum wheats. LWT, 118, 108734.

Ficco, D.B.M., De Simone, V., De Leonardis, A.M., Giovanniello, V., Del Nobile, M.A., Padalino, L., ... & De Vita, P., 2016. Use of purple durum wheat to produce naturally functional fresh and dry pasta. Food Chemistry, 205, 187-195.

Ficco, D.B., De Simone, V., Colecchia, S.A., Pecorella, I., Platani, C., Nigro, F., ... & De Vita, P., 2014 b. Genetic variability in anthocyanin composition and nutritional properties of blue, purple, and red bread (*Triticum aestivum* L.) and durum (*Triticum turgidum* L. ssp. *turgidum* convar. durum) wheats. Journal of Agricultural and Food Chemistry, 62(34), 8686-8695.

Ficco, D.B., Mastrangelo, A.M., Trono, D., Borrelli, G.M., De Vita, P., Fares, C., ... & Papa, R., 2014 a. The colours of durum wheat: a review. Crop and Pasture Science, 65(1), 1-15.

Fields, P., & Korunic, Z., 2000. The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. Journal of Stored Products Research, 36(1), 1-13.

Fields, P.G., and White, N.D., 2002. Alternatives to methyl bromide treatments for stored-product and quarantine insects. Annual Review of Entomology, 47(1), 331-359.

Finch, S., 1986. Assessing host-plant finding by insects. Insect-plant interactions, 23-63.

Fisher, W.S., 1950. A revision of the North American species of beetles belonging to the family Bostrichidae (No. 698). US Government Printing Office.

Fleurat-Lessard F., Pimaud M.F., Cangardel H., 1986. Effects de doses elevees de Zeta sur *Plodia interpunctella* Huebner (Lépidoptère: Pyralidae) dans le stocks de pruneaux d'angen. In: Les Pheromones Sexuelles des Lépidoptére. Centre de recherches INRA de Bordeaux, 163-169.

Fornal, J., Jeliński, T., Sadowska, J., Grundas, S., Nawrot, J., Niewiada, A., ... & Błaszczak, W., 2007. Detection of granary weevil *Sitophilus granarius* (L.) eggs and internal stages in wheat grain using soft X-ray and image analysis. Journal of Stored Products Research, 43(2), 142-148.

Fuller, D.Q., & Colledge, S., 2008. Recent lessons from Near Eastern archaeobotany: wild cereal use, pre-domestication cultivation and tracing multiple origins and dispersals. Praghara, 18, 105-134.

Gałęcki, R., Bakuła, T., Wojtacki, M., and Żuk-Gołaszewska, K., 2019. Susceptibility of ancient wheat species to storage pests *Sitophilus granarius* and *Tribolium confusum*. Journal of Stored Products Research, 83, 117-122.

Gani, A., Wani, S.M., Masoodi, F.A., & Hameed, G., 2012. Whole-grain cereal bioactive compounds and their health benefits: A review. Journal of Food Processing Technology, 3(3), 146-56.

Gepts, P. 2004. Crop Domestication as a Long-term selection experiment. Plant Breeding Reviews, 24, 1-44

Gerken, A.R., & Campbell, J.F., 2018. Life history changes in *Trogoderma variabile* and *T. inclusum* due to mating delay with implications for mating disruption as a management tactic. Ecology and Evolution, 8(5), 2428-2439.

Germinara G.S., De Cristofaro A., Rotundo G., 2012. Bioactivity of short-chain aliphatic ketones against adults of the granary weevil, *Sitophilus granarius* (L.). Pest Management Science, 68, 371-377.

Germinara G.S., De Cristofaro A., Rotundo G., 2015. Repellents effectively disrupt the olfactory orientation of *Sitophilus granarius* to wheat kernels. Journal of Pest Science, 88 (4), 675-684.

Germinara, G.S., Beleggia, R., Fragasso, M., Pistillo, M.O., & De Vita, P., 2019. Kernel volatiles of some pigmented wheats do not elicit a preferential orientation in *Sitophilus granarius* adults. Journal of Pest Science, 92(2), 653-664.

Germinara, G.S., De Cristofaro, A., & Rotundo, G., 2008. Behavioral responses of adult *Sitophilus granarius* to individual cereal volatiles. Journal of Chemical Ecology, 34, 523-529.

Germinara, G.S., Rotundo, G., De Cristofaro, A., & Giacometti, R., 2002. Risposte elettroantennografiche di *Sitophilus granarius* (L.) e *S. zeamais* Motschulsky a sostanze volatili dei cereali. Tecnica Molitoria, 53, 27-34.

Germinara, G.S., Rotundo, G., and De Cristofaro, A., 2007. Repellence and fumigant toxicity of propionic acid against adults of *Sitophilus granarius* (L.) and *S. oryzae* (L.). Journal of Stored Products Research, 43(3), 229-233.

Germinara, G.S., Albanese, R., Pistillo, O.M., 2019 b. Evaluation of a Cuban zeolite against three stored-product pests. Tecnica Molitoria 70 (1), 38-46.

Giunta, F., Motzo, R., & Pruneddu, G., 2007. Trends since 1900 in the yield potential of Italian-bred durum wheat cultivars. European Journal of Agronomy, 27(1), 12-24.

Giunti, G., Campolo, O., Laudani, F., Palmeri, V., Spinozzi, E., Bonacucina, G., ... & Benelli, G., 2023. Essential oil-based nanoinsecticides: ecological costs and commercial potential. In Development and Commercialization of Biopesticides. Academic Press, 375-402.

Giunti, G., Campolo, O., Laudani, F., Zappalà, L., & Palmeri, V., 2021. Bioactivity of essential oil-based nano-biopesticides toward *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Industrial Crops and Products, 162, 113257.

Giunti, G., Campolo, O., Russo, A., Palmeri, V., & Zappalà, L., 2018. Chemical properties and efficacy of Sweet orange essential oil nanoemulsion applied as cold aerosol against two stored product beetles. Julius-Kühn-Archiv, 463.

Giunti, G., Palermo, D., Laudani, F., Algeri, G.M., Campolo, O., & Palmeri, V., 2019. Repellence and acute toxicity of a nano-emulsion of sweet orange essential oil toward two major stored grain insect pests. Industrial Crops and Products, 142*,* 111869.

Golden, G., Quinn, E., Shaaya, E., Kostyukovsky, M., & Poverenov, E., 2018. Coarse and nano emulsions for effective delivery of the natural pest control agent pulegone for stored grain protection. Pest Management Science, 74(4), 820-827.

Golob, P., 1997. Current status and future perspectives for inert dusts for control of stored product insects. Journal of Stored Products Research, 33(1), 69-79.

Gutiérrez, M.M., Stefanazzi, N., Werdin-González, J.O., Benzi, V.S., & Ferrero, A.A., 2009. Actividad fumigante de aceites esenciales de *Schinus molle* (Anacardiaceae) y *Tagetes terniflora* (Asteraceae) sobre adultos de Pediculus humanus capitis (Insecta; Anoplura; Pediculidae). BLACPMA, 8(3), 176-179.

Hagstrum, D.W., and Phillips, T.W., 2017. Evolution of stored-product entomology: protecting the world food supply. Annual Review of Entomology, 62, 379-397.

Handford, C.E., Elliott, C.T., & Campbell, K., 2015. A review of the global pesticide legislation and the scale of challenge in reaching the global harmonization of food safety standards. Integrated environmental assessment and management, 11(4), 525-536.

Hansson, B.S., 2002. A bug's smell–research into insect olfaction. Trends in Neurosciences, 25(5), 270-274.

Harlan, J.R., De Wet, J.M.J. and Price, E.G., 1973. Comparative evolution of cereals. Evolution, 27, 311-325.

Haryadi, Y., Syarief, R., Hubeis, M., Herawati, I., 1994. Effect of zeolite on the development of *Sitophilus zeamais* Motsch, In: Highley E., Wright, E.J., Banks, H.J., Champ, B.R. (Eds), Stored Products Protection. Proceedings of the Sixth International Working Conference on Stored-product Protection, 633-634.

Hashem, A.S., Awadalla, S.S., Zayed, G.M., Maggi, F., & Benelli, G., 2018. *Pimpinella anisum* essential oil nanoemulsions against *Tribolium castaneum* - insecticidal activity and mode of action. Environmental Science and Pollution Research, 25, 18802-18812.

Hassemer, M.J., Borges, M., Withall, D.M., Pickett, J.A., Laumann, R.A., Birkett, M.A., & Blassioli-Moraes, M.C., 2019. Development of pull and push–pull systems for management of lesser mealworm, *Alphitobius diaperinus*, in poultry houses using alarm and aggregation pheromones. Pest Management Science, 75(4), 1107-1114.

Hatt, S., Xu, Q., Francis, F., & Osawa, N., 2019. Aromatic plants of East Asia to enhance natural enemies towards biological control of insect pests. A review. Entomologia Generalis, 38(4).

Hawkes, J.G., 1983. The Diversity of Crop Plants. Cambridge, Mass.: Harvard University Press.

Hillman, G. and Davis, M.S. 1990 a. Measured Domestication Rates in Wild Wheats and Barley Under Primitive Cultivation. Journal of World Prehistory, 4, 157-222.

Hillman, G.C. and Davis, M.S. 1990 b. Domestication Rates in Wild Wheats and Barley Under Primitive Cultivation. Biological Journal of the Linnaean Society, 39, 39-78.

Hodges R.J., Benton F.P., Hall D.R., Dos Santos Serodio R., 1984. Control of *Ephestia cautella* (Walker) (Lepidoptera: Phycitidae) by synthetic sex pheromone in the laboratory and store. Journal of Stored Products Research, 20, 191-197.

Horber, E., 1983. Principles, problems, progress and potential in host resistance to stored-grain insects. In Proc. 3rd Intl. Conf. Stored-Prod. Entomol., Kansas State University, Manhattan, Kansas, 391-417

Hosseinian, F.S., Li, W., & Beta, T., 2008. Measurement of anthocyanins and other phytochemicals in purple wheat. Food Chemistry, 109(4), 916-924.

Howe, R.W., 1950. The development of *Rhizopertha dominica* (F.) (Col., Bostrichidae) under constant conditions. The Entomologist's Monthly Magazine, 6, 1-5.

Hüsnü Can Başer, K., & Buchbauer, G., 2015. Handbook of essential oils: science, technology, and applications. In Handbook of Essential Oils: Science, Technology, and Applications, CRC Press, Boca Raton, FL, USA.

Ibrahim, M.A., Kainulainen, P., Aflatuni, A., Tiilikkala, K., & Holopainen, J. K., 2001. Insecticidal, repellent, antimicrobial activity and phytotoxicity of essential oils: with special reference to limonene and its suitability for control of insect pests.

Igrejas, G., & Branlard, G., 2020. The importance of wheat. Wheat quality for improving processing and human health, 1-7.

Imura, O., 1990. Life histories of stored-product insects. In Bruchids and Legumes: Economics, Ecology and Coevolution: Proceedings of the Second International Symposium on Bruchids and Legumes (ISBL-2) held at Okayama (Japan), September 6–9, 1989. Springer Netherlands, 257-269.

Islam, M.S., Hasan, M.M., Lei, C., Mucha-Pelzer, T., Mewis, I. & Ulrichs, C., 2010: Direct and admixture toxicity of diatomaceous earth and monoterpenoids against the storage pests *Callosobruchus maculatus* (F.) and *Sitophilus oryzae* (L.). Journal of Pest Science, 83(2), 105-112.

Isman, M.B., 2000. Plant essential oils for pest and disease management. Crop Protection, 19(8-10), 603-608.

Isman, M.B., Miresmailli, S., & Machial, C., 2011. Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. Phytochemistry reviews, 10, 197-204.

Ivie, M.A., 2002 a. Family 69. Bostrichidae. In: Arnett Jr., R.H., Thomas, M.C., Skelley, P.E., Frank, J.H. (Eds.), American Beetles. Polyphaga: Scarabaeoidae through Curculionoidea, vol. 2. CRC Press, Boca Raton, 233-244.

Ivie, M.A., 2002 b. Keys to families of beetles in America north of Mexico. In: Arnett Jr., R.H., Thomas, M.C., Skelley, P.E., Frank, J.H. (Eds.), American Beetles. Polyphaga: Scarabaeoidae through Curculionoidea, vol. 2. CRC Press, Boca Raton, 816-835.

Jaenike, J., 1990. Host specialization in phytophagous insects. Annual Review of Ecology and Systematics, 243-273.

Jankowska, M., Rogalska, J., Wyszkowska, J., & Stankiewicz, M., 2017. Molecular targets for components of essential oils in the insect nervous system-a review. Molecules, 23(1), 34.

Jia, F., Toews, M.D., Campbell, J.F., & Ramaswamy, S.B., 2008. Survival and reproduction of lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) on flora associated with native habitats in Kansas. Journal of Stored Products Research, 44(4), 366-372.

Jyoti, J.L., Shelton, A.M., & Earle, E.D., 2001. Identifying sources and mechanisms of resistance in crucifers for control of cabbage maggot (Diptera: Anthomyiidae). Journal of Economic Entomology, 94(4), 942-949.

Kah, M., Beulke, S., Tiede, K., & Hofmann, T., 2013. Nanopesticides: state of knowledge, environmental fate, and exposure modeling. Critical Reviews in Environmental Science and Technology, 43(16), 1823-1867.

Kalsa, K.K., Subramanyam, B., Demissie, G., Mahroof, R., Worku, A., Gabbiye, N., ... and Abay, F., 2019. Susceptibility of Ethiopian wheat varieties to granary weevil and rice weevil infestation at optimal and sub-optimal temperatures. Journal of Stored Products Research, 83, 267-274.

Kanda, D., Kaur, S., & Koul, O., 2017. A comparative study of monoterpenoids and phenylpropanoids from essential oils against stored grain insects: acute toxins or feeding deterrents. Journal of Pest Science, 90(2), 531-545.

Karabak, S., & Kan, M., 2021. Total economic value of wheat landraces. Wheat landraces, 121-146.

Karabörklü, S., & Ayvaz, A., 2023. A comprehensive review of effective essential oil components in stored-product pest management. Journal of Plant Diseases and Protection, 130(3), 449-481.

Kavallieratos, N.G., Athanassiou, C.G., Pashalidou, F.G., Andris, N.S., & Tomanović, Ž., 2005. Influence of grain type on the insecticidal efficacy of two diatomaceous earth formulations against *Rhyzopertha dominica* (F) (Coleoptera: Bostrychidae). Pest Management Science: formerly Pesticide Science, 61(7), 660-666.

Kavallieratos, N.G., Nika, E.P., Skourti, A., Boukouvala, M.C., Ntalaka, C. ., Maggi, F., ... & Bonacucina, G., 2022 a. Carlina acaulis essential oil nanoemulsion as a new grain protectant against different developmental stages of three stored-product beetles. Pest Management Science, 78(6), 2434-2442.

Kavallieratos, N.G., Nika, E.P., Skourti, A., Perinelli, D.R., Spinozzi, E., Bonacucina, G., ... & Maggi, F., 2022 b. Apiaceae essential oil nanoemulsions as effective wheat protectants against five arthropod pests. Industrial Crops and Products, 186, 115001.

Khare, B.P., 1994. Pests of stored grain and their management. Kalyani Publishers, New Delhi, 304.

Khokhar, D.S., and Gupta, D.S., 1974. Relative resistance of some varieties of wheat to *Sitophilus oryzae* (L.) and *Rhizopertha dominica* (F.) at different temperatures. Bullettin of Grain Technology, 12(3), 117-123.

Khorramshahi, A., & Burkholder, W.E., 1981. Behavior of the lesser grain borer *Rhyzopertha dominica* (Coleoptera: Bostrichidae) Male-produced aggregation pheromone attracts both sexes. Journal of Chemical Ecology, 7, 33-38.

Khush, G.S., 2001. Green revolution: the way forward. Nature Reviews Genetics, 2(10), 815-822.

Kljajić, P., 2008. Control of harmful insects in storage grains. Protection of Stored Plant Products from Harmful Organisms (Kljajić P., ed.) Institute of Pesticides and Environmental Protection, Belgrade, Serbia, 67-100.

Kljajić, P., Andrić, G., Adamović, M., & Golić, M. P., 2010 a. Laboratory evaluation of insecticidal effectiveness of a natural zeolite formulation against *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst) in treated wheat. Julius-Kühn-Archiv, (425), 863.

Kljajić, P., Andrić, G., Adamović, M., & Pražić-Golić, M., 2011. Possibilities of application of natural zeolites in stored wheat grain protection against pest insects. Journal on processing and Energy in Agriculture, 15(1), 12-16.

Kljajić, P., Andrić, G., Adamović, M., Bodroža-Solarov, M., Marković, M., & Perić, I., 2010 b. Laboratory assessment of insecticidal effectiveness of natural zeolite and diatomaceous earth formulations against three stored-product beetle pests. Journal of Stored Products Research, 46(1), 1-6.

Knievel, D.C., Abdel-Aal, E.S., Rabalski, I., Nakamura, T., & Hucl, P., 2009. Grain color development and the inheritance of high anthocyanin blue aleurone and purple pericarp in spring wheat (*Triticum aestivum* L.). Journal of Cereal Science, 50(1), 113-120.

Kocak, E., Schlipalius, D., Kaur, R., Tuck, A., Ebert, P., Collins, P., & Yilmaz, A., 2015. Determining phosphine resistance in rust red flour beetle, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) populations from Turkey. Turkish Journal of Entomology, 39, 129-136.

Kordan, B., Laszczak-Dawid, A., Nietupski, M., & Zuk-Golaszewska, K., 2007. Wplyw formy przechowywania pszenicy orkisz [*Triticum spelta* L.] na rozwoj wolka zbozowego [*Sitophilus granarius* L.]. Progress in Plant Protection, 47(1), 263-266.

Kordan, B., Skrajda-Brdak, M., Tańska, M., Konopka, I., Cabaj, R., & Załuski, D., 2019. Phenolic and lipophilic compounds of wheat grain as factors affecting susceptibility to infestation by granary weevil (*Sitophilus granarius* L.). Journal of Applied Botany and Food Quality, 92, 64-72.

Korunic, Z., 1998. Rapid assessment of the insecticidal value of diatomaceous earths without conducting bioassays. Journal of Stored Products Research, 33(3), 219-229.

Kućerová, Z., & Stejskal, V., 1994. Susceptibility of wheat cultivar to postharvest losses caused by *Sitophilus granarias* (L.) (Coleoptera: Curculionidae)/Attraktivität von Weizensorten für *Sitophilus granarius* (Coleoptera: Curculionidae) und die dadurch verursachten Nachernteverluste. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection, 641-648.

Lampiri, E., Agrafioti, P., Vagelas, I., & Athanassiou, C. G., 2022. Insecticidal effect of an enhanced attapulgite for the control of four stored-product beetle species. Agronomy, 12(7), 1495.

Law J. H. and Regnier F. E., 1971. Pheromones. Annual Review of Biochemistry, 40, 533-548.

Le Gouis, J., Oury, F.X., & Charmet, G., 2020. How changes in climate and agricultural practices influenced wheat production in Western Europe. Journal of Cereal Science, 93, 102960.

Lemic, D., Mikac, K. M., Genda, M., Jukić, Ž., & Pajač Živković, I. 2020. Durum wheat cultivars express different level of resistance to granary weevil, *Sitophilus Granarius* (Coleoptera; Curculionidae) infestation. Insects, 11(6), 343.

Levinson, H.Z., Kanaujia, K.R., 1981. Phagostimulatory responses of male and female *Sitophilus granarius* to newly harvested and stored wheat grains. Naturwissenschaften, 68, 44.

Levinson, A., & Levinson, H., 1999. Inhibition of sexual attraction and mating by pheromone enantiomers in male *Lasioderma serricorne*. Naturwissenschaften, 86, 138-140.

Levinson, H., & Levinson, A., 1994. Origin of grain storage and insect species consuming desiccated food. Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz, 67, 47-60.

Liang, J.Y., Wang, W.T., Zheng, Y. F., Zhang, D., Wang, J.L., Guo, S.S., 2017. Bioactivities and chemical constituents of essential oil extracted from *Artemisia anethoides* against two stored product insects. Journal of Oleo Science, 66(1), 71-76.

Likhayo, P.W., Hodges, R.J., 2000. Field monitoring *Sitophilus zeamais* and *Sitophilus oryzae* using refuge and flight traps baited with synthetic pheromone and cracked wheat. Journal of Stored Products Research, 36: 341-353.

Longstaff, B.C., 1981. Biology of the grain pest species of the genus *Sitophilus* (Coleoptera: Curculionidae): a critical review. Protection Ecology, 3 (2), 83-130.

Lorini, I., Collins, P. J., Daglish, G. J., Nayak, M. K., & Pavic, H., 2007. Detection and characterisation of strong resistance to phosphine in Brazilian *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Pest Management Science: formerly Pesticide Science, 63(4), 358-364.

Lü, J., Sehgal, B., & Subramanyam, B., 2017. Insecticidal potential of a synthetic zeolite against the cowpea weevil, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). Journal of Stored Products Research, 72, 28-34.

Maceljski, M., & Korunić, Z., 1972. Contribution to the knowledge of the mechanism of acting of inert dusts against insects. Plant Protection, 22, 117-118.

Mafra-Neto A., Baker T.C., 1996. Timed metered sprays of pheromone disrupt mating of *Cadra cautella* (Lepidoptera: Pyralidae). Journal of Agricultural Entomology, 13, 149-168.

Maga, J.A., 1978. Cereal volatiles, a review. Journal of Agricultural and Food Chemistry, 26(1), 175-178.

Magan, N., Hope, R., Cairns, V., & Aldred, D., 2003. Post-harvest fungal ecology: impact of fungal growth and mycotoxin accumulation in stored grain. Epidemiology of Mycotoxin Producing Fungi: Under the aegis of COST Action 835 'Agriculturally Important Toxigenic Fungi 1998–2003', 723-730.

Mahroof, R.M., & Phillips, T.W., 2007. Stable isotopes as markers to investigate host use by *Rhyzopertha dominica*. Entomologia Experimentalis et Applicata, 125(2), 205-213.

Mahroof, R.M., & Phillips, T.W., 2014. Mating disruption of *Lasioderma serricorne* (Coleoptera: Anobiidae) in stored product habitats using the synthetic pheromone serricornin. Journal of Applied Entomology, 138(5), 378-386.

Mahroof, R.M., Edde, P.A., Robertson, B., Puckette, T., Phillips, T.W., 2010. Dispersal of *Rhyzopertha dominica* F. in different habitats. Environmental Entomology 39, 30-938.

Maille, J., Gerken, A., Adrianos, S., Arthur, F., Campbell, J., Oppert, B., 2020. Exploiting chemosensory genomics for improved monitoring and control of stored product pests. Insects In press.

Majd-Marani, S., Naseri, B., Nouri-Ganbalani, G., & Borzoui, E., 2017. The effect of maize hybrid on biology and life table parameters of the *Trogoderma granarium* (Coleoptera: Dermestidae). Journal of Economic Entomology, 110(4), 1916-1922.

Majeed, M. Z., Mehmood, T., Javed, M., Sellami, F., Riaz, M. A., & Afzal, M., 2015. Biology and management of stored products' insect pest *Rhyzopertha dominica* (Fab.) (Coleoptera: Bostrichidae). International Journal of Biosciences, 7(5), 78-93.

Malacrinò, A., Campolo, O., Laudani, F., & Palmeri, V., 2016. Fumigant and repellent activity of limonene enantiomers against *Tribolium confusum* du Val. Neotropical Entomology, 45(5), 597-603.

Mathew, G., 1987. Insect borers of commercially important stored timber in the state of Kerala, India. Journal of Stored Products Research, 23(4), 185-190.

Matsuoka, Y., 2011. Evolution of polyploid Triticum wheats under cultivation: the role of domestication, natural hybridization and allopolyploid speciation in their diversification. Plant and Cell Physiology, 52(5), 750-764.

McCain, F.S., Eden, W.G., and Singh, D.N., 1964. A Technique for Selecting for Rice Weevil Resistance in Corn in the Laboratory 1. Crop Science, 4(1), 109-110.

McCallum, J.A., & Walker, J.R.L., 1990. Proanthocyanidins in wheat bran. Cereal Chemistry, 67(3), 282-285.

McGaughey, W.H., Speirs, R.D., and Martin, C.R., 1990. Susceptibility of classes of wheat grown in the United States to stored-grain insects. Journal of Economic Entomology, 83(3), 1122-1127.

Mebarkia, A., Rahbé, Y., Guechi, A., Bouras, A., & Makhlouf, M., 2010. Susceptibility of twelve soft wheat varieties (*Triticum aestivum*) to *Sitophilus granarius* (L.)(Coleoptera: Curculionidae). Agriculture and Biology Journal of North America 1, 571-578.

Mefleh, M., Conte, P., Fadda, C., Giunta, F., Piga, A., Hassoun, G., & Motzo, R., 2019. From ancient to old and modern durum wheat varieties: Interaction among cultivar traits, management, and technological quality. Journal of the Science of Food and Agriculture, 99(5), 2059-2067.

Miller, J.R., & Strickler, K.L., 1984. Finding and accepting host plants. In Chemical ecology of insects. Boston, MA: Springer US, 127-157.

Mohandass, S., Arthur, F. H., Zhu, K.Y., & Throne, J.E., 2007. Biology and management of *Plodia interpunctella* (Lepidoptera: Pyralidae) in stored products. Journal of Stored Products Research, 43(3), 302-311.

Moretti, M.D., Sanna-Passino, G., Demontis, S., & Bazzoni, E., 2002. Essential oil formulations useful as a new tool for insect pest control. AAPs PharmSciTech, 3, 64-74.

Morrison III, W.R., Agrafioti, P., Domingue, M.J., Scheff, D.S., Lampiri, E., Gourgouta, M., ... & Athanassiou, C.G., 2023. Comparison of different traps and attractants in 3 food processing facilities in Greece on the capture of stored product insects. Journal of Economic Entomology, 116(4), 1432-1446.

Morrison III, W.R., Arthur, F.H., Wilson, L.T., Yang, Y., Wang, J., & Athanassiou, C.G., 2020. Aeration to manage insects in wheat stored in the Balkan peninsula: Computer simulations using historical weather data. Agronomy, 10(12), 1927.

Morrison III, W.R., Scully, E.D., & Campbell, J.F., 2021. Towards developing areawide semiochemical-mediated, behaviorally-based integrated pest management programs for stored product insects. Pest Management Science, 77(6), 2667-2682.

Mostafalou, S., & Abdollahi, M., 2013. Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. Toxicology and Applied Pharmacology, 268(2), 157-177.

Motzo, R., Fois, S., & Giunta, F., 2007. Protein content and gluten quality of durum wheat (*Triticum turgidum* subsp. durum) as affected by sowing date. Journal of the Science of Food and Agriculture, 87(8), 1480-1488.

Najar-Rodriguez, A.J., Galizia, C.G., Stierle, J., & Dorn, S., 2010. Behavioral and neurophysiological responses of an insect to changing ratios of constituents in host plant-derived volatile mixtures. Journal of Experimental Biology, 213(19), 3388-3397.

Nansen, C., Phillips, T.W., 2002. Attracticide for control of Indianmeal Moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). In: Proceedings of the 8th International Working Conference on Stored-Product Protection, New York, 306-310.

Nansen, C., Phillips, T.W., 2004. Attractancy and toxicity of an attracticide for the Indianmeal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae). Journal of Economic Entomology, 97. 703-710.

Naseri, B., & Majd-Marani, S., 2022. Different cereal grains affect demographic traits and digestive enzyme activity of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). Journal of Stored Products Research, 95, 101898.

Naseri, B., Borzoui, E., Majd, S., & Mozaffar Mansouri, S., 2017. Influence of different food commodities on life history, feeding efficiency, and digestive enzymatic activity of *Tribolium castaneum* (Coleoptera: Tenebrionidae). Journal of Economic Entomology, 110(5), 2263-2268.

Nawrot, J., Warchalewski, J.R., Piasecka-Kwiatkowska, D., Niewiada, A., Gawlak, M., Grundas, S.T., Fornal, J., 2006. The effect of some biochemical and technological properties of wheat grain on granary weevil (*Sitophilus granarius* L.) (Coleoptera: Curculionidae) development. In Proceedings of the 9th International Working Conference on Stored Product Protection 15, 400-407.

Nawrot, J., Gawlak, M., Szafranek, J., Szafranek, B., Synak, E., Warchalewski, J.R., ... and Fornal, J., 2010. The effect of wheat grain composition, cuticular lipids and kernel surface microstructure on feeding, egg-laying, and the development of the granary weevil, *Sitophilus granarius* (L.). Journal of Stored Products Research, 46(2), 133-141.

Nayak, M. K., Daglish, G. J., Phillips, T. W., & Ebert, P. R., 2020. Resistance to the fumigant phosphine and its management in insect pests of stored products: a global perspective. Annual Review of Entomology, 65, 333-350.

Ndomo-Moualeu, A. F., Ulrichs, C., & Adler, C., 2016. Behavioral responses of Callosobruchus maculatus to volatile organic compounds found in the headspace of dried green pea seeds. Journal of Pest Science, 89, 107-116.

Neethirajan, S., Karunakaran, C., Jayas, D. S., and White, N.D.G., 2007. Detection techniques for stored-product insects in grain. Food Control, 18(2), 157-162.

Niewiada, A., Nawrot, J., Szafranek, J., Szafranek, B., Synak, E., Jeleń, H., & Wąsowicz, E., 2005. Some factors affecting egg-laying of the granary weevil (*Sitophilus granarius* L.). Journal of Stored Products Research, 41(5), 544-555.

Nordlund D. A. and Lewis W. J., 1976. Terminology of chemical releasing stimuli in intraspecific and interspecific interactions . Journal of Chemical Ecology, 2, 211-220.

Ntalli, N., Skourti, A., Nika, E. P., Boukouvala, M.C., & Kavallieratos, N.G., 2021. Five natural compounds of botanical origin as wheat protectants against adults and larvae of *Tenebrio molitor* L. and *Trogoderma granarium* Everts. Environmental Science and Pollution Research, 28, 42763-42775.

Nuovo, P., 2013. Evoluzione delle varietà di grano, della tecnica molitoria e panificatoria. Industrie Grafiche Pacini Editore.

Osman, S.E.I., Swidan, M.H., Kheirallah, D.A., & Nour, F.E., 2016. Histological effects of essential oils, their monoterpenoids and insect growth regulators on midgut, integument of larvae and ovaries of Khapra beetle, *Trogoderma granarium* everts. Journal of Biological Sciences, 16 (3), 93-101

Padulosi, S., 1996. Hulled Wheats: Proceedings of the First International Workshop on Hulled Wheats, 21-22 July 1995, Castelvecchio Pascoli, Tuscany, Italy (Vol. 4). Bioversity International.

Park, S.H., Arthur, F.H., Bean, S.R., & Schober, T.J., 2008. Impact of differing population levels of *Rhyzopertha dominica* (F.) on milling and physicochemical properties of sorghum kernel and flour. Journal of Stored Products Research, 44(4), 322-327.

Parkash, J., Rajat, A.K., & Jeevan, B.G., 2023. Integrated Pest Management: A Comprehensive Overview. Advances in Entomology, 3.

Pavela, R., 2015. Essential oils for the development of eco-friendly mosquito larvicides: a review. Industrial Crops and Products, 76, 174-187.

Pavela, R., 2016. History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects-a review. Plant Protection Science, 52(4), 229-241.

Pavela, R., & Benelli, G., 2016. Essential oils as ecofriendly biopesticides? Challenges and constraints. Trends in Plant Science, 21(12), 1000-1007.

Paventi, G., de Acutis, L., De Cristofaro, A., Pistillo, M., Germinara, G. S., & Rotundo, G., 2020. Biological activity of *Humulus lupulus* (L.) essential oil and its main components against *Sitophilus granarius* (L.). Biomolecules, 10(8), 1108.

Paventi, G., Rotundo, G., Pistillo, M., D'Isita, I., & Germinara, G.S., 2021. Bioactivity of wild hop extracts against the granary weevil, *Sitophilus granarius* (L.). Insects, 12(6), 564.

Pavoni, L., Pavela, R., Cespi, M., Bonacucina, G., Maggi, F., Zeni, V., ... & Benelli, G., 2019. Green micro-and nanoemulsions for managing parasites, vectors and pests. Nanomaterials, 9(9), 1285.

Pease, G., and Storm, C.G., 2010. Efficacy of pheromone-based control system, Exosex^{TM} SPTab, against moth pests in European food processing facili- ties. Proc. 10th Int. Working Conf. Stored-Product Prot. Estoril, 183-189.

Peña-Bautista, R.J., Hernandez-Espinosa, N., Jones, J.M., Guzmán, C., & Braun, H.J., 2017. Wheat-based foods: their global and regional importance in the food supply, nutrition, and health.

Perrino, P., 1993. The farro: an ancient crop to renew. Agricoltura, 21, 9-15.

Pezzutti, R., 1979. Effects of dry air on some beetles infesting stored cereals. In Domenichini, G.(Convener): 2nd symposium on pest control in food processing plant and the protection of foodstuffs.: 2 deg simposio sulla difesa antiparassitaria nelle industrie alimentari e la protezione degli alimenti, 135-144.

Phillips T.W., Throne J.E., 2010. Biorational approaches to managing stored-product insects. Annual Review of Entomology, 55, 375-397.

Phillips T.W., Jiang X.L., Burkholder W.E., Phillips J.K., Tran, H.Q., 1993. Behavioural responses to food volatiles by two species of stored-product Coleoptera, *Sitophilus oryzae* and *Tribolium castaneum*. Journal of Chemical Ecology, 19, 723-734.

Phillips, J.K., Chong, J.M., Andersen, J.F., & Burkholder, W.E., 1989. Determination of the enantiomeric composition of (R*, S*)-1-ethylpropyl 2-methyl-3-hydroxypentanoate, the male-produced aggregation pheromone of *Sitophilus granarius*. Entomologia Experimentalis et Applicata, 51(2), 149-153.

Phillips, T.W., 1997. Semiochemicals of stored-product insects: research and applications. Journal of Stored Products Research, 33(1), 17-30.

Pierce, L.H., 1994. Using pheromones for the location and suppression of phycitid and cigarette beetles in Hawaii – a five-year summary. Proceedings 6th International Working Conference on Stored-Product Protection, Canberra, 439-433.

Piergiovanni, A.R., 2013. Evaluation of genetic variation and grain quality of old bread wheat varieties introduced in north-western Italian environments. Genetic Resources and Crop Evolution, 60(1), 325-333.

Piesik, D., & Wenda-Piesik, A., 2015. *Sitophilus granarius* responses to blends of five groups of cereal kernels and one group of plant volatiles. Journal of Stored Products Research, 62, 36-39.

Piesik, D., Bocianowski, J., Sendel, S., Krawczyk, K., & Kotwica, K., 2020. Beetle orientation responses of *Gastrophysa viridula* and *Gastrophysa polygoni* (Coleoptera: Chrysomelidae) to a blend of synthetic volatile organic compounds. Environmental Entomology, 49(5), 1071-1076.

Plarre, R. & Vanderwel, D.C., 1999. Stored-product Beetles. In: Hardie RJ & Minks AK (Eds.) 1999: Pheromones of Non-Lepidopteran Insects Associated with Agricultural Plants. CAB International, 149-198.

Plarre, R., 2013 b. More than a pest management tool-45 years of practical experience with insect pheromones in stored-product and material protection. Journal of Plant Diseases and Protection, 120, 145-152.

Plarre, R., 2013 a. An attempt to reconstruct the natural and cultural history of the granary weevil, *Sitophilus granarius* (Coleoptera: Curculionidae). European Journal of Entomology, 107(1), 1-11.

Potter, C., 1935. The biology and distribution of *Rhyzopertha dominica* (FAB.). Transactions of the Royal Entomological Society of London, 83, 449-482.

Prevett, P.F., Benton, F.P., Hall, D.R., Hodges, R.J., Dos Santos Serodio R., 1989. Suppression of mating in *Ephestia cautella* (Walker) (Lepidoptera: Phycitidae). Journal of Stored Products Research, 25, 147-154.

Rajendran, S., 2002. Postharvest pest losses. In: Pimentel, D. (Ed.), Encyclopedia of Pest Management. Marcel Dekker, Inc., New York, 654-656.

Rajendran, S., 2020. Insect pest management in stored products. Outlooks on Pest Management, 31(1), 24-35.

Rajendran, S., and Sriranjini, V., 2008. Plant products as fumigants for stored-product insect control. Journal of Stored Products Research, 44(2), 126-135.

Reddy, G.V.P., & Guerrero, A., 2004. Interactions of insect pheromones and plant semiochemicals. Trends Plant Science, 9, 253-261.

Regnault-Roger, C., Vincent, C., & Arnason, J.T., 2012. Essential oils in insect control: low-risk products in a high-stakes world. Annual Review of Entomology, 57(1), 405-424.

Régnière, J., Delisle, J., Dupont, A., & Trudel, R., 2019. The impact of moth migration on apparent fecundity overwhelms mating disruption as a method to manage spruce budworm populations. Forests, 10(9), 775.

Ren, Y., Wang, T., Wang, C., D'Isita, I., Hu, Q., Germinara, G.S., & Cao, Y., 2023. Population development of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) on different stored products. Entomological Research, 53(10), 359-366.

Rotundo G., Paventi G., Barberio A., De Cristofaro A., Notardonato I., Russo M.V., Germinara G.S., 2019. Biological activity of *Dittrichia viscosa* (L.) Greuter extracts against adult *Sitophilus granarius* (L.) (Coleoptera, Curculionidae) and identification of active compounds. Scientific Reports, 9(1): 6429.

Rotundo, G., & Germinara, G.S., 2015. I semiochimici in agricoltura. Georgofili: quaderni: II, 2015, 25-41.

Rotundo, G., Germinara, G.S., & De Cristofaro, A., 2000. Immuno-osmophoretic technique for detecting *Sitophilus granarius* (L.) infestations in wheat. Journal of Stored Products Research, 36(2), 153-160.

Rumbos, C.I., Sakka, M., Berillis, P., & Athanassiou, C.G., 2016. Insecticidal potential of zeolite formulations against three stored-grain insects, particle size effect, adherence to kernels and influence on test weight of grains. Journal of Stored Products Research, 68, 93-101.

Ryne, C., Ekeberg, M., Jonzén, N., Oehlschlager, C., Löftedt C., Anderbrant O., 2006. Reduction in an almond moth *Ephestia cautella* (Lepidoptera: Pyralidae) population by means of mating disruption. Pest Management Science, 62, 912-918.

Ryne, C., Svensson, G.P., Anderbrant, O., Löftedt, C., 2007. Evaluation of long-term mating disruption of *Ephestia kuehniella* and *Plodia interpunctella* (Lepidoptera: Pyralidae) in indoor storage facilities by pheromone traps and monitoring of relative aerial concentrations of pheromone. Journal of Economic Entomology, 100, 1017-1025.

Ryne, C., Svensson, G.P., Löfstedt, C., 2001. Mating disruption of *Plodia interpunctella* in small-scale plots: effects of pheromone blend, emission rates and population density. Journal of Chemical Ecology, 27, 2109-2124.

Saad, A.S., Tayeb, E.H.M., El-Shazli, M.M., and Baheeg, S.A., 2018. Susceptibility of certain Egyptian and imported wheat cultivars to infestation by *Sitophilus oryzae* and *Rhyzopertha dominica*. Archives of Phytopathology and Plant Protection, 51(1-2), 14-29.

Sadras, V.O., Hayman, P.T., Rodriguez, D., Monjardino, M., Bielich, M., Unkovich, M., ... & Wang, E., 2016. Interactions between water and nitrogen in Australian cropping systems: physiological, agronomic, economic, breeding and modelling perspectives. Crop and Pasture Science, 67(10), 1019-1053.

Sakka, M.K., Jagadeesan, R., Nayak, M.K., & Athanassiou, C.G., 2022. Insecticidal effect of heat treatment in commercial flour and rice mills for the control of phosphine-resistant insect pests. Journal of Stored Products Research, 99, 102023.

Salamini, F., Özkan, H., Brandolini, A., Schäfer-Pregl, R., & Martin, W., 2002. Genetics and geography of wild cereal domestication in the near east. Nature Reviews Genetics, 3(6), 429-441.

Sanchez-Mariñez, R.I., Cortez-Rocha, M.O., Ortega-Dorame, F., Morales-Valdes, M., & Silveira, M.I., 1997. End-use quality of flour from *Rhyzopertha dominica* infested wheat. Cereal chemistry, 74(4), 481-483.

Sarwar, M.H., Sarwar, M.F., Sarwar, M., Qadri, N.A., & Moghal, S., 2013. The importance of cereals (Poaceae: Gramineae) nutrition in human health: A review. Journal of Cereals and Oilseeds, 4(3), 32-35.

Savoldelli S., Süss L., 2010. Integrated control of *Ephestia cautella* (Walker) in a confectionary factory. In: Carvalho M.O., Fields P.G., Adler C.S., Arthur F.H., Athanassiou C.G., Campbell J.F., Fleurat-Lessard F., Flinn P.W., Hodges R.J., Isikber A.A., Navarro S., Noyes R.T., Riudavets J., Sinha K.K., Thorpe G.R., Timlick B.H., Trematerra P., White N.D.G. (Eds.), Proceedings of the 10th International Working Conference on Stored-Product Protection 27 June - 2 July, 2010 Estoril. Julius Kühn Institut, Berlin, 991-992.

Scarascia Mugnozza, G.T., 2005. The contribution of Italian wheat geneticists: from Nazareno Strampelli to Francesco D'Amato. Proc. Int. Congr. In the wake of the double helix: from the green devolution to the gene revolution, Bologna, Italy, 52-75.

Schäpers, A., Petrén, H., Wheat, C.W., Wiklund, C., & Friberg, M., 2017. Female fecundity variation affects reproducibility of experiments on host plant preference and acceptance in a phytophagous insect. Proceedings of the Royal Society B: Biological Sciences, 284(1849), 20162643.

Schwardt, H.H., 1933. Life history of the lesser grain borer. Journal of the Kansas Entomological Society 2, 61-66.

Schwartz, B.E., & Burkholder, W.E., 1991. Development of the granary weevil (Coleoptera: Curculionidae) on barley, corn, oats, rice, and wheat. Journal of Economic Entomology, 84(3), 1047-1052.

Seitz, L. M., & Ram, M. S., 2004. Metabolites of lesser grain borer in grains. Journal of Agricultural and Food Chemistry, 52(4), 898-908.

Shani A., Clearwater J., 2001. Evasion of mating disruption in *Ephestia cautella* (Walker) by increased pheromone production relative to that of undisrupted populations. Journal of Stored Products Research, 37, 237-252.

Shankar, U., & Abrol, D.P., 2012. Integrated pest management in stored grains. In Integrated pest management: principles and practice. Wallingford UK, CABI, 386-407.

Shewry, P.R., 2009. Wheat. Journal of Experimental Botany, 60(6), 1537-1553.

Shipp, J., & Abdel-Aal, E.S.M., 2010. Food applications and physiological effects of anthocyanins as functional food ingredients. The Open Food Science Journal, 4(1), 7-22.

Sieminska, E., Ryne, C., Löfstedt, C., Anderbrant, O., 2009. Long-term pheromone mediated mating disruption of the Mediterranean flour moth, *Ephestia kuehniella*, in a flour mill. Entomologia Experimentalis et Applicata, 131, 294-299.

Simmonds, N.W., 1979. Principles of crop improvement. Longman, London, 408.

Smith, S.E., Barker, S.J., & Zhu, Y.G., 2006. Fast moves in arbuscular mycorrhizal symbiotic signalling. Trends in Plant Science, 11(8), 369-371.

Song, X., Wang, P., & Zhang, H., 2011. Phosphine resistance in *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae) from different geographical populations in China. African Journal of Biotechnology, 10(72), 16367-16373.

Sower, L.L., Witmer, G.P., 1977. Population growth and mating success of Indianmeal moths and Almond moths in the presence of synthetic sex pheromone. Environmental Entomology, 6, 17-20.

Stadler, E., 1992. Behavioural responses of insects to plant secondary compounds, in Herbivores: Their Interactions with Secondary Plant Metabolites. Vol. II. Ecological and Evolutionary Processes, ed. by Rosenthal GA and Berenbaum MR. Academic Press, London, UK, 45-88.

Stadler, T., Buteler, M., Weaver, D.K., Sofie S., 2012. Comparative toxicity of nanostructured alumina and a commercial inert dust for *Sitophilus oryzae* (L.) and Rhyzopertha dominica (F.) at varying ambient humidity levels. Journal of Stored Product Research, 48, 81-90.

Stagnari, F., Codianni, P., and Pisante, 2008. Agronomic and kernel quality of ancient wheats grown in central and southern Italy. Cereal Research Communications, 36(2), 313-326.

Stefanazzi, N., Gutierrez, M.M., Stadler, T., Bonini, N.A., Ferrero, A.A., 2006. Biological activity of essential oil of *Tagetes terniflora* Kunth (Astereaceae) against *Tribolium castaneum* Herbst (Insecta, Coleoptera, Tenebrionidae). Boletin de Sanidad Vegetal Plagas, 32(3), 439- 447.

Stoner, K.A., & Shelton, A.M., 1988. Role of nonpreference in the resistance of cabbage varieties to the onion thrips (Thysanoptera: Thripidae). Journal of Economic Entomology, 81(4), 1062-1067.

Subramanyam, B., & Roesli, R., 2000. Inert dusts. Alternatives to pesticides in stored-product IPM, 321-380.

Subramanyam, B., Swanson, C.L., Madamanchi, N., & Norwood, S., 1994. Effectiveness of Insecto, a new diatomaceous earth formulation in suppressing several stored grain insect species. In Proceedings of the 6th International Working Conference on Stored Product Protection, 17, 23.

Suleiman, R., Rosentrater, K.A., and Bern, C.J., 2015. Evaluation of maize weevils *Sitophilus zeamais* Motschulsky infestation on seven varieties of maize. Journal of Stored Products Research, 64, 97-102.

Süss, L., Locatelli, D.P., and Marrone, R., 1996. Possibilities and limits of mass trapping and mating disruption techniques in the control of *Ephestia kuehniella* (Zell.) (Lepidoptera Phycitidae). Bollettino di Zoologia Agraria e di Bachicoltura, 28, 77-89.

Süss, L., Locatelli, D.P., Marrone, R., 1999. Mating suppression of the Mediterranean flour moth (*Ephestia kuehniella* Zeller) (Lepidoptera Pyralidae) in a food industry. Bollettino di Zoologia agraria e di Bachicoltura, 31, 59-66.

Svoboda, K. P., & Greenaway, R. I., 2003. Investigation of volatile oil glands of *Satureja hortensis* L. (summer savory) and phytochemical comparison of different varieties. International Journal of Aromatherapy, 13(4), 196-202.

Tadesse, M., 2020 a. Post-harvest loss of stored grain, its causes and reduction strategies. Food Science and Quality Management, 96, 26-35.

Tadesse, M., Dibaba, K., Bayissa, W., Hunde, D., Mendesil, E., Kassie, M., ... & Tefera, T., 2020 b. Assessment of quantitative and qualitative losses of stored grains due to insect infestation in Ethiopia. Journal of Stored Products Research, 89, 101689.

Taylor, R.D., & Koo, W.W., 2015. 2015 Outlook of the US and World Wheat Industries, 2015-2024.

Taylor, R.W.D., 1994. Methyl bromide-Is there any future for this noteworthy fumigant?. Journal of Stored Products Research, 30(4), 253-260.

Thompson, J.D., Chalchat, J.C., Michet, A., Linhart, Y. B., & Ehlers, B., 2003. Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of Thymus vulgaris chemotypes. Journal of Chemical Ecology, 29, 859-880.

Thompson, V., 1966. Biology of the lesser grain borer, *Rhyzopertha dominica* (F.). Bullettin of Grain Technology, 4, 163-168.

Thorsteinson, A.J., 1958. The chemotactic influence of plant constituents on feeding by phytophagous insects. Entomologia Experimentalis et Applicata, 1(1), 23-27.

Throne, J.E., Baker, J.E., Messina, F.J., Karl, J.K., Howard, J.A., 2000. Varietal resistance. In: Subramanyam B., Hagstrum DW (eds) Alternatives to pesticides in stored-product IPM. Kluwer Academic, Massachusetts, 165-192.

Toews, M.D., Cuperus, G.W., and Phillips, T.W., 2000. Susceptibility of eight US wheat cultivars to infestation by *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Environmental Entomology, 29(2), 250-255.

Trdan, S., Žnidarčič, D., Vidrih, M., & Kač, M., 2008. Three natural substances for use against *Altemaria cichorii* on selected varieties of endive: antifungal agents, plant strengtheners, or foliar fertilizers?/Drel gegenüber *Alternaria cichorii* an ausgewählten Endiviensorten wirkende natürliche Substanzen: antimykotische Agenzien, Pflanzenstärkungsmittel oder Blattdünger? Journal of Plant Diseases and Protection, 63-68.

Trematerra, P., and Gentile, P., 2010. Five years of mass trapping of *Ephestia kuehniella* Zeller: a component of IPM in a flour mill. Journal of Applied Entomology, 134, 149-156.

Trematerra, P., 1994. Control of *Ephestia kuehniella* Zeller by sex pheromones in the flour mills. Anzeiger fur Schadlingskunde Pflanzenschutz Umweltschutz, 67, 74-77.

Trematerra, P., 1990. Population dynamic of *Ephestia kuehniella* Zeller in a flour mill: three years of mass-trapping. Proc. 5 Int. Working Conf. Stored- Product Protection, Bordeaux: 1435-1443.

Trematerra, P., 1995. The use of attracticide method to control *Ephestia kuehniella* Zeller in flour mills. Anz Schadlingsk Pflanzenschutz Umweltschutz, 68, 69-73.

Trematerra, P., Athanassiou, C., Stejskal, V., Sciarretta, A., Kavallieratos, N., Palyvos, N., 2011. Large scale mating disruption of *Ephestia* spp. and *Plodia interpunctella* in Czech Republic, Greece and Italy. Journal of Applied Entomology, 135, 749-762.

Trematerra, P., Athanassiou, C.G., Sciarretta, A., Kavallieratos, N.G., Buchelos, C.T., 2013. Efficacy of the auto-confusion system for mating disruption of *Ephestia kuehniella* (Zeller) and *Plodia interpunctella* (Hübner). Journal of Stored Products Research, 55, 90-98.

Trematerra, P., Capizzi, A., 1991. Attracticide method in the control of *Ephestia kuehniella* Zeller: studies on effectiveness. Journal of Applied Entomology*,* 111, 451-456.

Trematerra, P., Oliviero, A., Savoldelli, S., Schoeller, M., 2017. Controlling infestion of a chocolate factory by *Plodia interpunctella* (Hübner) by combining mating disruption and the parasitoid *Habrobracon hebetor* (Say). Insect Science, 24 (3), 503-510.

Trematerra, P., Savoldelli, S., 2013. The use of water traps and presence of spermatophores to evaluate mating disruption in the almond moth, *Ephestia cautella*, during exposure to synthetic sex pheromone. Journal of Pest Science, 86 (2), 227-233.

Trematerra, P., Spina, G., 2013. Mating-disruption trials for control of Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), in traditional flour mills. Journal of Food Protection, 76(3), 456-461.

Trematerra, P. 2012. Advances in the use of pheromones for stored-product protection. Journal of Pest Science, 85, 285-299.

Trematerra, P., & Gentile, P., 2002. Stored insect pests in traditional cultivated hulled wheat crop areas of Central-Southern Italy with emphasis on *Sitotroga cerealella* (Olivier). IOBC/WPRS BULLETIN, 25(3), 27-32.

Trematerra, P., & Throne, J., 2012. Insect and mite pests of durum wheat. Durum wheat, chemistry and technology, Second Edition. AACC International Inc., St. Paul, MN, USA, 73-83.

Trematerra, P., Sciarretta, A., & Tamasi, E., 2000. Behavioural responses to volatiles from durum wheat kernels and host selection in *Oryzaephilus surinamensis* (Linnaeus), *Tribolium castaneum* (Herbst) and *Tribolium confusum* J. du Val. Entomologia Experimentalis et Applicata, 94, 195-200.

Tremblay, E., & Rotundo, G., 1980. I feromoni. En "Prospettive di controllo biologico degli insetti in agricoltura. CNRAQ, 1, 81-121.

Tudi, M., Daniel Ruan, H., Wang, L., Lyu, J., Sadler, R., Connell, D., ... & Phung, D.T., 2021. Agriculture development, pesticide application and its impact on the environment. International journal of environmental research and public health, 18(3), 1112.

Tuet, W.Y., Pierce, S.A., Racine, M.C., Stone, S., Pueblo, E., Dukes, A., ... & Wong, B., 2021. Cardiopulmonary effects of phosphine poisoning: A preliminary evaluation of milrinone. Toxicology and Applied Pharmacology, 427, 115652.

Ukeh, D.A., Birkett, M.A., Bruce, T.J., Allan, E.J., Pickett, J.A., & Mordue, A.J., 2010. Behavioural responses of the maize weevil, Sitophilus zeamais, to host (stored-grain) and non-host plant volatiles. Pest Management Science: formerly Pesticide Science, 66(1), 44-50.

Urrutia, R.I., Yeguerman, C., Jesser, E., Gutierrez, V.S., Volpe, M.A., and González, J.O.W., 2021. Sunflower seed hulls waste as a novel source of insecticidal product: Pyrolysis bio-oil bioactivity on insect pests of stored grains and products. Journal of Cleaner Production, 287, 125000.

Vayias, B.J., & Stephou, V.K., 2009. Factors affecting the insecticidal efficacy of an enhanced diatomaceous earth formulation against three stored-product insect species. Journal of Stored Products Research, 45(4), 226-231.

Vayias, B.J., Athanassiou, C.G., 2004. Factors affecting efficacy of the diatomaceous earth formulation SilicoSec against adults and larvae of the confused beetle *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae). Crop Protection, 23, 565-573.

Vick, K.W., Coffelt, J.A., Sullivan, M.A., 1978. Disruption of pheromone communication in the Angoumois grain moth with synthetic female sex pheromone. Environmental Entomology, 7, 528-531.

Visser J.H., 1986. Host odor perception in phytophagous insects. Annual Review of Entomology, 31(1), 121-144.

Walters, D., 2011. Plant defense: warding off attack by pathogens, herbivores and parasitic plants. John Wiley & Sons.

Webster, B., Bruce, T., Pickett, J., & Hardie, J., 2010. Volatiles functioning as host cues in a blend become nonhost cues when presented alone to the black bean aphid. Animal Behaviour, 79(2), 451-457.

Weiss, E., & Zohary, D., 2011. The Neolithic Southwest Asian founder crops: their biology and archaeobotany. Current Anthropology, 52(S4), S237-S254.

Werdin-Gonzáles, J.O., Murray, A.P., & Ferrero, A.A., 2008. Bioactividad de aceites esenciales de Schinus molle var. areira (Anacardiaceae) en ninfas II de *Nezara viridula* (Hemiptera: Pentatomidae). Boletín de Sanidad Vegetal Plagas, 34, 367-376.

Werdin-González, J.O., Gutiérrez, M.M., Ferrero, A.A., & Band, B.F., 2014. Essential oils nanoformulations for stored-product pest control–Characterization and biological properties. Chemosphere, 100, 130-138.

Wertheim, B.E., Baalen van, J.A., Dicke, M., & Vet, L.E.M, 2005. Pheromone-mediated aggregation in nonsocial arthropods: An Evolutionary Ecological Perspective. Annual Review of Entomology, 50, 321-46.

Williams, H.J., Silverstein, R.M., Burkholder, W.E., & Khorramshahi, A., 1981. Dominicalure 1 and 2: components of aggregation pheromone from male lesser grain borer *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). Journal of Chemical Ecology, 7, 759-780.

Winterbottom, D.C., 1922. Weevil in Wheat and Storage of Grain in Bags. A Record of Australian Experience during the War Period (1915 to 1919). Government Printer, North Terrace, Adelaide, Australian.

Xynias, I.N., Mylonas, I., Korpetis, E.G., Ninou, E., Tsaballa, A., Avdikos, I.D., & Mavromatis, A.G., 2020. Durum wheat breeding in the Mediterranean region: Current status and future prospects. Agronomy, 10(3), 432.

Yang, F.L., Zhu, F., & Lei, C.L., 2010. Garlic essential oil and its major component as fumigants for controlling *Tribolium castaneum* (Herbst) in chambers filled with stored grain. Journal of Pest Science, 83, 311-317.

Zebec, Z., Wilkes, J., Jervis, A.J., Scrutton, N.S., Takano, E., & Breitling, R., 2016. Towards synthesis of monoterpenes and derivatives using synthetic biology. Current Opinion in Chemical Biology, 34, 37-43.

Željko J., Matković A., Liška A., i Jukić K., 2020. Resistance of different wheat cultivars to granary weevil (*Sitophilus granarius* L.). Glasnik Zaštite Bilja, 43(5), 34-41.

Zeni, V., Baliota, G. V., Benelli, G., Canale, A., & Athanassiou, C. G., 2021. Diatomaceous earth for arthropod pest control: Back to the future. Molecules, 26(24), 7487.

Zeven, A. C., 1991. Wheats with purple and blue grains: a review. Euphytica, 56, 243-258.

Zhanda, J., Mvumi, B.M., Machekano H., 2020.. Potential of three enhanced diatomaceous earths against *Sitophilus zeamais* Motschulsky and *Prostephanus truncantus* (Horn) on stored maize grain. Journal of Stored Product Research, 87, 101608.

Zhou, M., Robards, K., Glennie-Holmes, M., & Helliwell, S., 1999. Analysis of volatile compounds and their contribution to flavor in cereals. Journal of Agricultural and Food Chemistry, 47(10), 3941-3953.

Ziaee, M., Moharramipour, S., & Francikowski, J., 2014. The synergistic effects of *Carum copticum* essential oil on diatomaceous earth against *Sitophilus granarius* and *Tribolium confusum*. Journal of Asia-Pacific Entomology, 17(4), 817-822.

Zohary, D. 1969. The progenitors of wheat and barley in relation to domestication and agriculture dispersal in the Old World, 47-66 in Ucko, P. J. and Dimbleby, G. W., The domestication and exploitation of plants and animals. London: Duckworth.

Zohary, D., and Hopf, M., 2000. Domestication of Plants in the Old World. Third. Oxford: Oxford University Press.

Zuzarte, M., & Salgueiro, L., 2015. Essential oils chemistry. Bioactive Essential Oils and Cancer, 19-61.

16. Annexes: Scientific Curriculum

16.1 Education, Research and Professional experiences

1. 2011: Classical High School Graduation, Liceo Classico "Aldo Moro" di Manfredonia (Foggia, Italy).
2. 2015: BSc in Agricultural Science and Technology (*magna cum laude*) L25 - University of Bari "Aldo Moro". Thesis "Protezione integrata dell'asparago e applicazione dei disciplinari GLOBAL GAP".
3. 2017: MSc in Plant Medicine (*magna cum laude*) LM69 - University of Bari "Aldo Moro". Experimental thesis "Efficacy of *Bacillus* spp. against grey mould on strawberry, population dynamics and expression of genes coding for antimicrobial lipopeptides".
4. 2018: Qualification as Agronomist and Forestry Doctor.
5. 11.06.2018 - 10.12.2018: Research fellowship - University of Foggia, DAFNE Department, Laboratory of General and Applied Entomology. Research activities "Conduzione di biosaggi comportamentali per l'identificazione di semiochimici da impiegare per il monitoraggio di *Megaplatypus mutatus* e *Aromia bungii* in Campania". Research project: "Controllo integrato di *Megaplatypus mutatus* (Chapuis) (Coleoptera, Curculionidae, Platypodinae): impiego del feromone sessuale per la messa a punto di strategie ecocompatibili di controllo" granted by the Campania Region D.R. n. 502 del 22.12.2011.
6. 11.12.2018 - 10.06.2019: Research fellowship - University of Foggia, DAFNE Department, Laboratory of General and Applied Entomology. Research activities "Conduzione di biosaggi comportamentali per l'identificazione di semiochimici da impiegare per il monitoraggio di *Megaplatypus mutatus* e *Aromia bungii* in Campania". Research project: "Controllo integrato di *Megaplatypus mutatus* (Chapuis) (Coleoptera, Curculionidae, Platypodinae): impiego del feromone sessuale per la messa a punto di strategie ecocompatibili di controllo" granted by the Campania Region D.R. n. 502 del 22.12.2011.

7. 08.08.2019 - 07.11.2019: Research fellowship - University of Foggia, DAFNE Department, Laboratory of General and Applied Entomology. Research activities “Conduzione di saggi comportamentali per la valutazione dell’ attività biologica di composti organici volatili (VOCs) da impegnare per il monitoraggio di *Megaplatypus mutatus* e *Aromia bungii* in Campania”. Research project: “Controllo integrato di *Megaplatypus mutatus* (Chapuis) (Coleoptera, Curculionidae, Platypodinae): impiego del feromone sessuale per la messa a punto di strategie ecocompatibili di controllo” granted by the Campania Region D.R. n. 502 del 22.12.2011.
8. 11.01.2020 - 10.07.2020: Research fellowship - University of Foggia, DAFNE Department, Laboratory of General and Applied Entomology. Research activities “Allestimento e valutazione di nuovi diffusori e formulati di composti volatili vegetali ad azione attrattiva o repellenti verso gli adulti della Mosca delle olive”. Research project: “DIfesa da organismi nocivi in OLivicoltura tradizionale e intensiva (DI.OL.)” granted by the Italian Ministry of Agricultural Food and Forestry Policies (MASAF, ex MIPAAF) (grant prot. no. 23774).
9. 11.07.2020 - 17.11.2020: Research fellowship - University of Foggia, DAFNE Department, Laboratory of General and Applied Entomology. Research activities “Allestimento e valutazione di nuovi diffusori e formulati di composti volatili vegetali ad azione attrattiva o repellenti verso gli adulti della Mosca delle olive”. Research project: “Difesa da organismi nocivi in olivicoltura tradizionale e intensiva (DI.OL.)” granted by Italian Ministry of Agricultural Food and Forestry Policies (MASAF, ex MIPAAF) (grant prot. no. 23774).
10. 2020-24: PhD Course Management of innovation in the agricultural and food system of the mediterranean region (Cycle XXXVI) University of Foggia, DAFNE Department granted by the Italian Ministry of Education, University and Research within the ESF - ERDF National Operational Programme on “Research and Innovation 2014-2020” (code number DOT13YISJ8).

11. 13-17.03.2023: Participation to the XXVII course of Mass Spectrometry by Division of Mass Spectrometry of Società Chimica Italiana. Certosa di Pontignano, Siena.
12. 2023 (1 month): Training and research period at the CREA Research Centre for Cereal and Industrial Crops of Foggia.
13. 2023 (6 months): Training and research period at the Santacroce Giovanni S.p.a.
14. 2023 (1 month): Training and research period abroad at Department of Agricolture, Crop Productions and Rural Environment, University of Thessaly (Volos, Greece).
15. 2024: Subject expert at the University of Foggia, DAFNE Department in the Disciplinary Scientific Sector AGR/11 - General and Applied Entomology.
16. 2024: Research fellowship - University of Foggia, DAFNE Department, Laboratory of General and Applied Entomology. Research activities: "Studio delle interazioni semiochimiche *Philaenus spumarius*-olivo e identificazione di sostanze volatili ad azione attrattiva o repellente". Research project: "Sviluppo di strategie di controllo sostenibili di *Philaenus spumarius* ed interferenza con la trasmissione di *Xylella fastidiosa* (SOS)" granted by the Italian Ministry of Agricultural Food and Forestry Policies (MASAF).

16.2 Memberships and IDs

1. Società Entomologica Italiana (SEI)
2. Associazione Italiana per la Protezione delle Piante (AIPP)
3. SCOPUS ID: 57192428983
   https://www.scopus.com/authid/detail.uri?authorId=58560013000
4. Web of Science ID: ERD-9353-2022
   https://www.webofscience.com/wos/author/record/20599748
5. ORCID ID: 0000-0001-6001-3610
   https://orcid.org/0000-0001-6001-3610
6. RG page: https://www.researchgate.net/profile/Ilaria-Disita

16.3 Scientific contributions

16.3.1 Published articles indexed in SCOPUS/ISI

1. Paventi G., Rotundo G., Pistillo M., **D'Isita I.**, Germinara G.S., 2021. Insects, 12(6), 564. https://doi.org/10.3390/insects12060564
2. Pistillo O. M., **D'Isita I.**, Germinara G.S., 2021. Olfactory Responses of the Spotted Asparagus Beetle, *Crioceris duodecimpuctata* (L.) to Host Plant Volatile. Journal of Chemical Ecology, 48(1), 41-50. https://doi.org/10.1007/s10886-021-01323-5
3. Cao Y., Pistillo O. M., Lou Y., **D'Isita I.**, Maggi F., Hu Q., Germinara G.S., 2022. Electrophysiological and behavioural responses of *Stegobium paniceum* to volatile compounds from Chinese medicinal plant materials. Pest Management Science, 78(8), 3697-3703. https://doi.org/10.1002/ps.7012
4. Cao Y., Hu Q., Huang L., Athanassiou C. G., Maggi F., **D'Isita I.**, Liu, Y., Pistillo, O. M., Miao, M., Germinara, G. S., Li, C., 2023. Attraction of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) to the semiochemical volatiles of stored rice materials. Journal of Pest Science, 1-13. https://doi.org/10.1007/s10340-023-01616-6
5. Ren, Y., Wang, T., Wang, C., **D'Isita**, I., Hu, Q., Germinara, G. S., & Cao, Y., 2023. Population development of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) on different stored products. Entomological Research, 1-8. https://doi.org/10.1111/1748-5967.12670
6. Avosani, S., Nieri, R., Mazzoni, V., Anfora, G., Hamouche, Z., Zippari, C., Vitale, M. L., Verrastro, V., Tarasco, E., **D'Isita** I., Germinara, G. S., Döring, T. F., Belusic, G., Fereres, A., Thompson, V., Cornara, D., 2023. Intruding into a conversation: how behavioral manipulation could support management of *Xylella fastidiosa* and its insect vectors. Journal of Pest Science, 1-17. https://doi.org/10.1007/s10340-023-01631-7
7. **D'Isita**, I., Di Palma, A. M., De Vita, P., & Germinara, G. S., 2023. Acceptance and utilization efficiency of a purple durum wheat genotype by *Sitophilus granarius* (L.). Scientific Reports, 13(1), 14246. https://doi.org/10.1038/s41598-023-41384-y

8. Toffolatti, S. L., Davillerd, Y., **D'Isita**, I., Facchinelli, C., Germinara, G. S., Ippolito, A., Khamis, Y., Kowalska, J., Maddalena, G., Marchand, P., Marcianò, D., Mihály, K., Mincuzzi, A., Mori, N., Piancatelli, S., Sándor., E, Romanazzi, G. (2023). Are Basic Substances a Key to Sustainable Pest and Disease Management in Agriculture? An Open Field Perspective. Plants, 12(17), 3152. https://doi.org/10.3390/plants12173152
9. Geppert, C., da Cruz, M., Alma, A., Andretta, L., Anfora, G., Battaglia, D., Burgio, G., Caccavo, V., Chiesa, S. G., Cinquatti, F., Cocco, A., Costi, E., **D'Isita**, I., Duso, C., Garonna, A. P., Germinara, G. S., Lo Bue, P., Lucchi, A., Maistrello, L., Mannu, R., Marchesini, E., Masetti, A., Mazzon, L., Mori, N., Ortis, G., Peri, E., Pescara, G., Prazaru, S.C., Ragone, G., Rigamonti, I. E., Rosi, M. C., Rotundo, G., Sacchetti, P., Savoldelli, S., Suma, P., Tamburini, G., Tropea Garzia, G., Marini, L., 2024. Climate and landscape composition explain agronomic practices, pesticide use and grape yield in vineyards across Italy. Agricultural Systems, 215, 103853. https://doi.org/10.1016/j.agsy.2024.103853
10. **D'Isita**, I., Pistillo, O. M., Di Palma, A. M., De Vita, P., & Germinara, G. S., 2023. Susceptibility of old and modern wheat genotypes to *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.). Journal of Stored Products Research, 106, 102265. https://doi.org/10.1016/j.jspr.2024.102265
11. Germinara, G. S., Pistillo, O. M., **D'Isita**, I., Di Palma, A. M., Rotundo, G., Guidotti, M., Psaro., R., Caselli., A., Econdi, S., Gargani, E., Cutino, I., Benvenuti, C., & Roversi, P. F., 2024. Inhibitory activity of some short-chain aliphatic aldehydes on pheromone and ammonium carbonate-mediated attraction in olive fruit fly, *Bactrocera oleae*. Pest Management Science. https://doi.org/10.1002/ps.8264
12. Pistillo, O. M., **D'Isita, I.**, Di Palma, A., & Germinara, G. S., 2024. Identification of the Sex Pheromone of the Asparagus Moth, *Parahypopta caestrum* (Lepidoptera, Cossidae). Journal of Chemical Ecology, 1-9. https://doi.org/10.1007/s10886-024-01504-y
13. El-Khoury, Y., Bari, G., Grujić, N., Poliseno, M., Germinara, G. S., Pistillo, M., **D'Isita, I.**, & Tarasco, E., 2024. Can the return to the natural conditions of

in vivo parasitization increase the infectivity performance of EPNs? Redia, 107, 81-83. http://dx.doi.org/10.1926/REDIA-107.24.12

16.3.2 Published articles not indexed in SCOPUS/ISI

1. Germinara, G.S., Pistillo, M., **D'Isita I.**, Rotundo, G., 2021. I Semiochimici nel controllo sostenibile degli insetti. La Chimica e L'Industria, Anno V, N. 2, 14-18.
2. Germinara, G.S., Pistillo, M., **D'Isita, I.**, De Cristofaro, A., Rotundo, G., 2022. I semiochimici degli insetti e le loro applicazioni. Terra e Vita, N. 19-2022, 43-45.
3. Germinara, G.S., **D'Isita, I.**, Pistillo, M., Di Cataldo, M., Rotundo, G., 2023. Interferire nelle comunicazioni degli insetti dannosi. Terra e Vita, N. 8-2023, 32-34.
4. Germinara, G.S., Guario, A., Lasorella, V., Acquaviva, M., Antonino, N., Grande, O., Iadarola, G., **D'Isita,** I., Pistillo, M., Ladurner, E., Iodice, A., 2024. Tignola dell'olivo: efficacia ottima con la confusione sessuale. L'nformatore Agrario. In press.

16.3.3 In press, under review and submitted articles

1. **D'Isita, I.**, Pistillo, O.M., Lo Muzio, F., Asghar, A., Coduti, M., Acquaviva, M., Di Palma, A.M., Germinara, G.S., 2024. Insecticidal activity of a diatomaceous earth and a natural Cuban zeolite against adults of the bean weevil, *Acanthoscelides obtectus* (Say). Acta Horticulturae. In press.
2. Pistillo, O.M., **D'Isita, I.**, Lo Muzio, F., Acquaviva, M., Iadarola, G., Coduti, M., Di Palma, A.M., Germinara, G.S., 2024. Distribution and flight activity of the asparagus moth, *Parahypopta caestrum*, in Southern Italy as monitored by pheromone traps. Acta Horticulturae. In press.
3. **D'Isita, I.**, Pistillo, O.M., Lo Muzio, F., Pati, S., Di Palma, A.M, De Vita, P., Germinara, G.S., 2024. Behavioural responses of *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) to odours of old and modern wheat genotypes. Accepted for publication in Journal of Stored Products Research.

4. **D’Isita, I.**, Pistillo, O.M., Pati, S., Di Palma, A.M, De Vita, P., Germinara, G.S., 2024. Susceptibility of ancient wheats to *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) and their allelochemical interactions. Submitted for publication to Grain & Oil Science and Technology.
5. **D’Isita, I.**, Sakka, M.K., Germinara, G.S., Athanassiou, G.C., 2024. Insecticidal effect of an enhanced attapulgite for the control of *Sitophilus granarius* (L.). Submitted for publication to Journal of Stored Products Research.
6. **D’Isita, I.**, Sakka, M.K., Germinara, G.S., Athanassiou, G.C., 2024. Insecticidal effect of fungicide-coated maize kernels on adults of the larger grain borer, *Prostephanus truncatus* (Horn). Submitted for publication to Crop protection.

16.3.4 Conference proceedings

1. Pistillo M., **D’Isita I.,** Germinara G. S. 2021. Risposta elettroantennografica e comportamentale di *Crioceris duodecimpunctata* a composti volatili dell’asparago. Atti XXVI CNIE (Congresso Nazionale Italiano di Entomologia), Torino 7-11giugno: 65
2. Germinara G. S., **D’Isita I.**, Pistillo M., Pecchioni N., De Vita P. 2021. Efficienza di utilizzazione alimentare di frumenti a cariosside pigmentata e gialla in adulti di *Sitophilus granarius* mediante indici nutrizionali. Atti XXVI CNIE (Congresso Nazionale Italiano di Entomologia), Torino, modalità online 7-11 giugno: 214.
3. Germinara G. S., **D’Isita I.**, Beleggia R., Pistillo M., Fragasso M., De Vita P., 2021. Risposte olfattive di adulti di *Sitophilus granarius* verso cariossidi di varietà commerciali gialle e di genotipi pigmentati di frumento. Atti XXVI CNIE (Congresso Nazionale Italiano di Entomologia), Torino, modalità online 7-11 giugno: 221.
4. Pistillo O.M., **D’Isita I.**, Lo Muzio F., Di Palma A.M., Germinara G.S., 2023. Identificazione del feromone sessuale del cosside dell’asparago, *Parahypopta caestrum* (Lepidoptera, Cossidae): indagine chimica, elettrofisiologica e di

attrattività in campo. Atti XXVII CNIE (Congresso Nazionale Italiano di Entomologia), Palermo, 12-16 giugno: 52.

5. Figlioli L., Pica F., Griffo R., **D'Isita I.**, Nugnes F., Germinara G.S., Bernardo U., 2023. Uso di differenti attrattivi per migliorare le catture di femmine di *Bactrocera dorsalis*. Atti XXVII CNIE (Congresso Nazionale Italiano di Entomologia), Palermo, 12-16 giugno: 163.
6. Lo Muzio F., **D'Isita I.**, Pistillo O.M., Rotundo G., Germinara G.S., Di Palma A.M., De Cristofaro A., 2023. Tossicità da contatto di estratti di buccia di *Punica granatum* (L.) verso adulti di *Sitophilus granarius* (L.). Atti XXVII CNIE (Congresso Nazionale Italiano di Entomologia), Palermo, 12-16 giugno: 172.
7. **D'Isita I.**, Pistillo O.M., Lo Muzio F., Di Palma A.M., De Vita P., Germinara G.S., 2023. Suscettibilità e attrattività di varietà di grano antiche e moderne verso il punteruolo del grano, *Sitophilus granarius* (L.) (Coleoptera, Curculionidae). Atti XXVII CNIE (Congresso Nazionale Italiano di Entomologia), Palermo, 12-16 giugno: 307.
8. Rotundo R., Paventi G., **D'Isita I.**, Lo Muzio F., Pistillo O.M., Marconi E., De Cristofaro A., Germinara G.S., 2023. Tossicità per inalazione di farine da semi di *Eriobotrya japonica* Lindl. verso adulti di *Sitophilus granarius* (L.) (Coleoptera, Curculionidae). Atti XXVII CNIE (Congresso Nazionale Italiano di Entomologia), Palermo, 12-16 giugno: 312.
9. Convertini S., Puglisi R., Carmignano M., Lamanna M., **D'Isita I.**, Pistillo O.M., Germinara G.S., 2023. Valutazione dell'efficacia di alcuni insetticidi biologici su melograno per il controllo di *Aphis punicae* Passerini. Atti XXVII CNIE (Congresso Nazionale Italiano di Entomologia), Palermo, 12-16 giugno: 401.
10. El-Khoury, Y., Bari, G., Grujić, N., Poliseno, M., Germinara, G. S., Pistillo, M., **D'Isita, I.**, & Tarasco, E., 2023. Can the return to the natural conditions of in vivo parasitization increase the infectivity performance of EPNs? Microbial and Nematode Control of Invertebrate Pests IOBC-WPRS Bulletin 162, 88-92.
11. Germinara, G.S., Guario, A., Iadarola, G., Lasorella, V., Acquaviva, M., Antonino, N., Grande, O., **D'Isita, I.**, Pistillo, M., Ladurner, E., Iodice, A.,

2024. Applicazione della confusione sessuale per il controllo della tignola dell'olivo (*Prays Oleae*): prime esperienze in Italia. Atti Giornate Fitopatologiche, 2024.

12. Pistillo, M., Lasorella, V., Coduti, M., Antonino, N., Grande, O., D'Isita, I., Prencipe, N., Raichini, F., Membola, T., D'Angelo, M., Sitti, A., Corsi, L., Sperandio, G., Ruschioni, S., Riolo, P., D'Ascenzo, D., Guario, A., Germinara, G.S., 2024. Monitoraggio di *Cryptoblabes gnidiella* in vigneti delle regioni adriatiche centro-meridionali. Atti Giornate Fitopatologiche, 2024.

16.4 Current editorial tasks

16.4.1 Peer reviewer

1. Bulletin of Insectology (Department of Agricultural and Food Sciences, Alma Mater Studiorum, University of Bologna).
2. Redia Journal of Zoology (CREA-DC, Research Centre for Plant Protection and Certification).
3. Archives of Phytopathology And Plant Protection (Taylor & Francis).
4. Journal of Pest Science (Springer).
5. Insects (MDPI).

16.5 Attended congresses, workshops, and meetings

1. *Torino, 7 - 11 June 2021*

Poster "Efficienza di utilizzazione alimentare di frumenti a cariosside pigmentata e gialla in adulti di *Sitophilus granarius* mediante indici nutrizionali". Congresso Nazionale Italiano di Entomologia - XXVII CNIE.

2. *Giovinazzo, 11 - 15 October 2021*

Oral presentation "Susceptibility of ancient and modern wheat varieties to the lesser grain borer, *Rhyzopertha dominica* (F.)". 3rd Joint Meeting of Agriculture - oriented PhD Programs.

3. *Firenze, 17 - 19 November 2021*

Oral presentation “Susceptibility of ancient and modern wheat varieties to the lesser grain borer, *Rhyzopertha dominica* (F.)”. XII Annual Meeting: European PhD Network Insect Science.

4. *Foggia, 16 - 21 May 2022*

Oral presentation “Suscettibilità agli attacchi di insetti di varietà locali di frumento”. Settimana della biodiversità pugliese, University of Foggia, DAFNE Department.

5. *Ceské Budejovice, 19 - 22 June 2022*Oral presentation “Can the return to the natural conditions of in vivo parasitization increase the infectivity performance of EPNs?”. Proceedings of the 18th Meeting “Microbial Control Agents in the Age of Global Change”.

6. *Udine, 3 - 7 October 2022* Oral presentation “Susceptibility of different *Triticum* spp. genotypes to *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) and their semiochemical interactions”. 4rd Joint Meeting of Agriculture - oriented PhD Programs.

7. *Vancouver, 13 - 16 November 2022*

Oral presentation “Electroantennogram responses of the asparagus moth, *Parahypoposta caestrum* (L.) to host plant volatiles”. Joint Annual Meeting ESA (Entomological Society of America) “Entomology as inspiration: Insect throught art, science, and culture”.

8. *Palermo, 12 - 16 June 2023*

Oral presentation “Suscettibilità e attrattività di varietà di grano antiche e moderne verso il punteruolo del grano, *Sitophilus granarius* (L.) (Coleoptera, Curculionidae)”. Congresso Nazionale Italiano di Entomologia (XXVII CNIE).

9. *Foggia, 10 October 2023*

Oral presentation “Valutazione dell’efficacia di polveri inerti per il controllo sostenibile di bruchidi infestanti i legumi in postraccolta”. Training day PSR of Apulia Region 2014/2020 - Submeasure 16.2 - Project: “Innovazione e potenziamento della produttività, sostenibilità e redditività della filiera dei legumi tipici pugliesi (PSR_LEG)”.

10. Firenze, 8 - 10 November 2023

Oral presentation “Susceptibility of different *Triticum* spp. genotypes to *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.) and olfactory responses of adult beetles to kernel VOCs”. XIV Annual Meeting: European PhD Network Insect Science.

11. Venosa, 24 November 2023

Oral presentation “La tignoletta della vite: biologia e mezzi di controllo”. Training day “Moderne tecniche di difesa integrata e nuovi strumenti digitali per la protezione del vigneto” by Syngenta and Cantina di Venosa.

12. Foggia, 12 Dicember 2023

Oral presentation “Normativa sull’uso dei prodotti fitosanitari e scenari futuri”. Training day “Ricerca e Sviluppo di Metodi di Produzione Sostenibile” by Fimagri O.P. and the research group of General and Applied Entomology of DAFNE Department, University of Foggia.

13. Foggia, 17 January 2024

Oral presentation “Controllo degli insetti dannosi del mandorlo: innovazione e sostenibilità” Training day PSR of Apulia Region 2014/2020 - Submeasure 16.2 - Project: “Arboricoltura del terzo millennio per il rilancio della capitanata (ATMIRCap)”.

14. Foggia, 15 April 2024

Oral presentation “Monitoring system of *Lymantria dispar* (L.) in Italy”. Joint Meeting “Monitoring Systems in Forest Pests” Life project “eGymer”.

15. Bucharest, 15 May 2024

Oral presentation “Insecticidal activity of a diatomaceous earth and a natural Cuban zeolite against adults of the bean weevil, *Acanthoscelides obtectus* (Say)”. The

European Horticultural Congress – EHC (former Symposium on Horticulture in Europe – SHE).

16. Bucharest, 15 May 2024

Oral presentation “Distribution and flight activity of the asparagus moth, *Parahypopta caestrum*, in Southern Italy as monitored by pheromone traps”. The European Horticultural Congress – EHC (former Symposium on Horticulture in Europe – SHE).

17. Bologna, 12 – 15 May 2024

Poster “Monitoraggio di *Cryptoblabes gnidiella* in vigneti delle regioni adriatiche centro-meridionali”. Giornate Fitopatologiche 2024.

18. Bologna, 12 – 15 May 2024

Poster “Applicazione della confusione sessuale per il controllo della tignola dell’olivo (*Prays oleae*): prime esperienze in Italia”. Giornate Fitopatologiche 2024.

19. Foggia, 23 May 2024

Oral presentation “Recenti acquisizioni e nuove sfide nel controllo sostenibile degli insetti negli arboreti moderni”. Final congress PSR of Apulia Region 2014/2020 - Submeasure 16.2 - Project: “Arboricoltura del terzo millennio per il rilancio della Capitanata (ATMIRCap)”.

20. Foggia, 18 June 2024

Oral presentation “*Parahypopta caestrum*: recenti acquisizioni su biologia, monitoraggio e mezzi biotecnici di controllo”. Final congress PSR of Apulia Region 2014/2020 - Submeasure 16.2 - Project: “Innovazioni e soluzioni sostenibili per l’asparago pugliese (AS_PARA)”.

21. Foggia, 18 June 2024

Oral presentation “Impiego di polveri inerti per il controllo di insetti dannosi ai cereali in postraccolta” Final congress PSR of Apulia Region 2014/2020 - Submeasure 16.2 -

Project: “Ottimizzazione delle pratiche di semina su sodo in frumento duro per migliorare la sostenibilità della cerealicoltura pugliese (SODOSOST)”.

16.5.1 Chairperson activity

1. Chairperson during the 3rd Joint Meeting of Agriculture - oriented PhD Programs. Giovinazzo, 11 - 15 October 2021.
2. Chairperson during the 4rd Joint Meeting of Agriculture - oriented PhD Programs. Udine, 3 - 7 October 2022.
3. XIV Annual Meeting: European PhD Network Insect Science. Firenze, 8 – 10 November 2023.

16.6 Participation to research projects

Due to the limitations of my PhD scholarship, I could not be directly involved in research project. However, I provided free of charge technical and scientific support in the following research projects supported by the Apulian Programme for Rural Development (PSR) 2014/2020 - Submeasure 16.2:

1. Innovazioni e soluzioni sostenibili per l’asparago pugliese - AS_PARA (CUP: B79J20000120009; DDS: 94250025221).
2. Gestione Forestale sostenibile nelle aziende agrosilvopastorali del Gargano - FORGARGANO (CUP: B77H20001830009; DDS: 94250036798).
3. Gestione Olivicola attraverso l’uso di Innovazione e Controllo - OLIVE MATRIX (CUP: B97H20000980009; DDS: 94250026542)
4. Innovazione e potenziamento della produttività, sostenibilità e redditività della filiera dei legumi tipici pugliesi - PSR_LEG (CUP: B77H20001840009; DDS: 94250033001).
5. Ottimizzazione delle pratiche di semina su sodo in frumento duro per migliorare la sostenibilità della cerealicoltura pugliese - SODOSOST (CUP: B79J20000090009; DDS: 94250033811).
6. Arboricoltura del terzo millennio per il rilancio della capitanata - ATMIRCap (CUP: B79J20000110009; DDS: 94250042523).
7. Innovazioni per miglioramento della produttività sostenibile delle aziende biologiche impegnate nel settore delle colture erbacee ed industriali pugliesi – SOFT (CUP: B79J2000008000; DDS: 94250035584).

8. Valorizzazione della mela Limoncella e melicoltura sostenibile nelle aree rurali dei Monti Dauni - VALMELA (CUP: B79J20000150009; DDS: 94250037358).
9. Competitività e sostenibilità della coltura del melograno in Puglia - CO.S.MEL (CUP: B79J2000016000; DDS: 94250036004).
10. Produzione e valorizzazione dell’arachide da frutto in Puglia - PEANUTPUGLIA (CUP: B77H20001660009; DDS: 94250038034).
11. Puglia Vitivinicola dell'Internet of Things - PuVI.o.T (CUP: B39J20000110009; DDS: 94250035345)

16.7 Attended courses, seminars, and lectures

Seminars

1. Research management and founding sources: Research Project Development: Drafting by Prof. Antonio Lo Polito, University of Foggia.
2. Research management and founding sources: Research Project Development: Evaluation by Prof. Maurizio Prosperi, University of Foggia.
3. Do Group Size, Longevity and Production Decisions Affect Geographical Indications’ Prices and Channel Margins? By Dr. Francesco Bimbo, University of Foggia.
4. COVID-19 Impacts on Food Security and Nutrition by Dr. Tarek Ben-Hassen, Qatar University.
5. How probiotics face enemies: they get by a little help by Prof. Giuseppe Spano, University of Foggia.
6. Introduction to Machine Learning by Dr. Andrea Nigri, University of Foggia.
7. RNA interference: a new tool for plant improvement and protection by Dr. Salvatore Arpaia, C.R. ENEA Trisaia.
8. Employing Mediterranean Agrobiodiversity Resources and Agronomic Biofortification Techniques for the Development of High-value Functional Vegetable Products by Prof. Francesco di Gioia, Pennsylvania State University.

9. Agricultural & Environmental Applications of Carbon-rich Materials Obtained from Waste Biomass Using Different Technological Processes by Prof. Elisabetta Loffredo, University of Bari.
10. The dissemination History of the Italian and the other Eurasian Grapevine Populations. Does the Gene Flow Fit with the Migration Routes around the Mediterranean Basin? By Prof. Francesco Sunseri, University of Reggio Calabria.
11. Biologic Assets and Equipments: the case of agro-products in the Beira Interior of Portugal by Prof. Rute Abreu, Polytechnic Institute of Guarda.
12. Plant-soil interactions to sustain agro-environmental challenges in a changing world by Prof. Laura Ercoli, Scuola Superiore Sant'Anna di Pisa.
13. Possibilities of work in the soft fruit industry for PhD scientists by Dr. Daniela Segantini, Molari Berries & Breeding of Cesena.
14. The third mission in academia by Prof. Alessandro Muscio, University of Foggia.
15. Market research: qualitative e quantitative methods by Prof. Biagia De Devitiis e Rosaria Viscecchia, University of Foggia.
16. Social Innovation and entrepreneurship by Prof. Antonio Stasi, University of Foggia.
17. Databases structure, data management and analytics: theory and applications by Dr. Francesco Bimbo, University of Foggia.
18. Insights to write a successful H2020 proposal: the case of the CERERE project by Dr. Giuseppe Nocella, University of Foggia.
19. Digital Agriculture and Sustainability of Agricultural Systems by Prof. Bruno Basso, University of Foggia.
20. Valorization of by-products from the food industry and production of ingredients with added values by Prof. Vesela and Ivanova Chalova-Zhekova, University of Bulgaria.
21. Bioactive organic molecules - natural compounds and the methodologies for the synthesis of important heterocyclic compounds by Dr. Selbi Keskin, University of Turkey.

22. Food Contaminants and Carcinogenicity and Polycyclic aromatic hydrocarbon (PAH): Sources, Exposure and Health Effects by Prof. Sibel Kaçmaz, University of Turkey.
23. OMICS, with a focus on proteomics to understand muscle to meat conversion and discovery of protein biomarkers by Dr. Mohammed Gagaoua, French National Institute for Agriculture, Food, and Environment (INRAE).

Courses

1. Valorization of research results and intellectual property - Writing a Scientific Paper by Prof. Agostino Sevi, University of Foggia.
2. Valorization of research results and intellectual property - Research & Innovation in Biotechnology: the Future in the Tradition by Prof. Antonio Bevilacqua, University of Foggia.
3. Valorization of research results and intellectual property - Research trends in Animal Science by Prof. Antonella Santillo, University of Foggia.
4. Information Technology and Management of Experimental Data by Prof. Fabio Santeramo and Prof. Maria Luisa Amodio, University of Foggia.
5. Chimica Generale e Analitica by Prof. Maurizio Quinto, University of Foggia.

International Lectures

1. Valorization of brewery spent grains as a good example of the food industry side-streams useability by Dr. Małgorzata Korzeniowska, University of Poland.
2. Extraction of bioactive compounds from fruits and vegetables by-products by Dr. Marina Cano Lamadrid, University of Spain.
3. Application of food by-products as potential functional ingredients in new food products by Dr. Lorena Martinez Zamora, University of Spain.
4. Determining afforestation areas by using social, economic and ecological scales by Dr. Seda Erkan Bugday, University of Turkey.
5. Use of Unmanned Aerial Vehicles in Forestry Activities by Ender Bugday, University of Turkey.

6. Upcoming changes in the packaging industry - proposal on packaging and packaging waste regulation (PPWR) by Dr. Karolina Wiszumirska, University of Poland.
7. The possibilities of using intelligent packaging in reducing food waste by Dr. Mariusz Tichoniuk, University of Poland.

16.8 Teaching activities

16.8.1 Academic teaching activities

1. *Foggia, 14 October 2023*

Master di II Livello Addetto Al Controllo Ufficiale Degli Alimenti e delle Bevande e Esperto di Sicurezza Alimentare (ACUAB) - A.A. 2022/2023. DAFNE Department, University of Foggia (4 hours).

2. *Foggia, 7 February 2024*

PCTO (Percorsi per le Competenze Trasversali e l'Orientamento) - Agricoltura sostenibile. DAFNE Department, University of Foggia (3 hours).

3. *Foggia*, *March - June 2024*

Course of Zoology and Agricultural Entomology - BSc in Agricultural Science and Technology. DAFNE Department, University of Foggia (4 CFU).

4. *Foggia*, *11 May 2024*

Master di II Livello Controllo ufficiale degli alimenti e sicurezza alimentare (CUASA) - A.A. 2023/2024. DAFNE Department, University of Foggia (4 hours).

5. *Foggia*, *May - June 2024*

Course of Zoology – BSc in Biology Science. DMCS Department, University of Foggia (2 CFU).

16.8.1 Others teaching activities

1. *Foggia, 25 - 28 March 2022*

Corso di Abilitazione all'acquisto ed utilizzo dei prodotti fitosanitari by Istituto Ricerche Studi Educazione Formazione – IRSEF (6 hours).

2. *Foggia, 18 - 25 May 2022*

Corso di Abilitazione all'acquisto ed utilizzo dei prodotti fitosanitari by Istituto Ricerche Studi Educazione Formazione – IRSEF (10 hours).

3. *Foggia, 27 - 28 April 2023*

Corso di Abilitazione all’acquisto ed utilizzo dei prodotti fitosanitari by Istituto Ricerche Studi Educazione Formazione – IRSEF (8 hours).

4. *Foggia, 11 - 18 October 2023*

Corso di Abilitazione all’acquisto ed utilizzo dei prodotti fitosanitari by Istituto Ricerche Studi Educazione Formazione – IRSEF (10 hours).

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