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GRAPE PHENOL ACCUMULATION AS RELATED TO CULTURAL PRACTICES INVOLVING CHANGES OF LIGHT MICROCLIMATE

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ABSTRACT

Light is known as an important factor influencing grape quality, especially its phenolic content that is essential for the organoleptic properties and the nutraceutical effect of this fruit.

The present study focuses on some influences of the manipulation of canopy light quantity and quality on grape final attributes.

Three experiments were carried out. The 1st experiment compared four treatments (ND, MD, D50% and DA having respectively 0%, 32%, 50% and 63% defoliation intensities). The phenol concentration of Negroamaro grapes was adequately improved by a high illumination regime induced by the defoliation. The D50% grapes reached the highest sugar accumulation. This treatment, differently from the others, had leaf removal not concentrated in the bunch zone but distributed along the entire canopy, thus likely benefited from a better leaf illumination and higher leaf CO_2 uptake per leaf area unit. The DA grapes, that were the most lighted, were the richest in skin anthocyanins, especially the methylated ones, and produced the wine richest in color, although its anthocyanin content did not differ from that of D50% wine. The color tonality of D50% wine was intermediate between that of DA and ND wine. The color tonality of MD wine was the worst, likely due to the lowest percentage of Delphinidin 3-*O*-glucoside and Malvidin 3-*O*-glucoside. The wine obtained by DA treatment, compared to the other ones, had the highest percentage of Delphinidin 3-*O*-glucoside, that in known to give a blue nuance to the wine colour, improving its visual appreciation.

In the 2nd experiment, Black Magic grape grown under neutral plastic film gave the best results. This behaviour favours the nutraceutical proprieties of the grape production. In photoselective thesis the main effects on grape quality seemed exerted by the PAR reduction, more than by the specific modification of light quality; the canopy trained to overhead "tendone" system filters and attenuates at a great extent the light available at bunch level. Between the photoselective films, the Pink one tended to induce a lower concentration of polyphenols; in the second year this gap increased and became statistically significant, likely due to the rapid opacification of this type of film. The anthocyanin profile showed a lower methylation in the grapes of the Yellow treatment. This is likely correlated to a higher reddish nuance and to a lower blue nuance found in the berry skin colour. On the other hand, the higher methylation level showed by the anthocyanins produced by the grape of the Pink treatment was likely correlated to a high green nuance of its skin colour respect to that of the grape of the yellow treatment, and to a high blue nuance compared to the grapes of both Yellow and Neutral treatments. Delphinidin, the precursor of blue/purple petunidin and malvidin derivatives was higher in the Neutral treatment and similar betweenYellow and Pink thesis. In the present study, the difference in Delphinidin concentration seemed not reflected by the berry skin colour.

The 3rd experiment showed that the photoselective nets did not improve the polyphenol content in grape skins of Italia grapes. The coloured nets, especially the red one, gave grape with less total polyphenols, flavonoids and proanthocyanidins, although the differences of these latter did not rich the level of statistical significance. Light quality modifications by means of hail-nets did not appear to influence chemical composition of grape juices.

Italy is one of the most important world producers of grapes, having an annual production of about 8 million tons (FAOSTAT, 2013). Our country represents the second in the world for the highest grape production after China (10.386.674 million tons) and it is followed by United States, Spain and France (7.054.429, 6.175.872 and 5.847.970 million tons, respectively). Apulia is the first region for grape production in Italy, having the greatest agricultural surface especially devoted to table grapes.

Light is known as an important factor influencing grape quality, especially its phenolic content that is essential for the organoleptic properties and the nutraceutical (by the words "*nutrition*" and "*pharmaceutical*") effects of this fruit.

The aim of this research was to investigate the influence exerted on grape quality by the manipulation of sunlight intercepted by leaves and grapes. Table grape or wine grape cultivars offered different problematic and practical approaches for this study. Hence, the effects of canopy thinning, in particular leaf removal, has been studied on wine grape varieties Negroamaro; the effects of colored plastic cover films and nets on table grape varieties has been tested on cv Black Magic and Italia, respectively. The study is important to increase knowledge about quantitative characteristics of grapes obtained under manipulated light conditions realized by means of different techniques, which are also useful to improve parameters of grape quality, according to its utilization.

The objective of this study was to assess:

- the influence of defoliation on fruit quality in berries of *Vitis vinifera* L. cv *Negroamaro*, especially as concerns the anthocyanic profile;
- fruit quality, in particular, anthocyanin profile of the. cv *Black Magic* and possible changes induced by colored plastic films used for vineyard protection;
- the influence of the coloured hail-net on berry composition in Vitis vinifera L. cv *Italia*.

1. PLANTS AND LIGHT ENVIRONMENT

As described in Smart (1985) the most important components of grapevine canopy microclimate, together with solar radiation, are temperature, wind speed, humidity and evaporation. It is known that canopy temperature is influenced by radiation and wind speed. The absorption of shortwave solar radiation heats, in particular, the outer leaves and berries in condition of low wind speed that is decreased by a dense canopy. Humidity mainly depends on the transpiration of leaves and fruits. Another factor influencing canopy microclimate is evaporation that is mainly related to humidity: the more the evaporation is lower, the more the humidity is high, with a risk of fungal diseases. Evaporation is reduced in the centre of canopy.

Downey and co-workers (2006) demonstrate that, considering a given site, if soil and nutrition are the same, the predominant influence on grape quality was exerted by climatic factors, mainly sunlight and temperature. Grape development was assessed to depend by sunlight, temperature, and humidity. The climate has mainly an effect on vine phenology through the GDD (Growing Degree Days) and water status of the vineyard (Coombe, 1986; Jones and Davies, 2000). The temperature during the berry ripening is a determining factor in the quality of the grapes, especially for winemaking (Jones et al. 2005, Hall and Jones 2009, Keller 2010, Iland et al. 2011). In addition, many studies show the climatic influence on the synthesis of biomolecules and of the activity antioxidant in different tissues of the grape. For

instance, the same cultivars show different behaviour in the polyphenol synthesis when they grow in different climate zones (Downey et al. 2006; Cohen and Kennedy, 2010).

In nature the sun is the main source of energy and the flow rate of radiant energy, seen as electromagnetic wave, is a radiant flux, measured as W. This flux can be absorbed, scattered and transmitted by media, such us plant canopy. Irradiance is a radiometric expression of the radiant flux incident on a surface from all directions, per unit area of surface (unit: W m^{-2}). Absorptance, reflectance and transmittance are the fraction of the incident flux that are absorbed, reflected and transmitted by a medium, respectively. The wavelength of the sunlight radiation is important because influences every property of the radiant flux (McCree, 1972b). When it needs to know the acquisition of energy by plants, it is important to consider the Photosynthetically active radiation (PAR).

Photosynthetically active radiation (PAR) represents the main range of wavelengths absorbed by leaves (Smart, 1973). A study carried out by McCree (1972a), defined the Photosynthetically active radiation. In that work, they were measured the action spectrum, the absorptance and the spectral quantum yield of CO_2 uptake in about twenty species of crop plants in different environmental conditions (species, variety, age of leaf, growth conditions, test conditions such as temperature, CO_2 concentration, flux of monochromatic radiation, flux of supplementary white radiation, orientation of leaf). It was found that in the range of wavelength between 350 to 750 nm all the considered species had the quantum yield curve characterized by two broad maxima, at 440 and 620 nm, with a shoulder at 670 nm, in all environmental conditions. The average height of the blue peak was 70% of that of the red peak. Photosynthetically active radiation (PAR) is the incident power per unit area in the spectral range between 400 and 700 nm, perceived by plants and essential to practice photosynthesis. PAR is usually expressed in W m^{-2} and it can be adequately evaluated by the photosynthetic photon flux density (PPFD) in 400-700 nm wavebands. PPFD is the number of the incident photons in that range of wavelengths, per unit time on a unit surface. It was expressed as $\mu molm^{-2}$ s^{-1} (McCree, 1972b).

The solar radiation is important for plants, allowing them to get energy and for its regulatory function in all physiological processes, influenced in both quantity and quality of light (Shahak et al., 2004b). Considering PAR radiation, the main range of wavelenght for plant physiological processes are Blue (400-500 nm), Green (500-600 nm) and red (600-700 nm) light (Nobel, 1983; Grant, 1997; Combes et al., 2000; Corelli-Grappadelli, 2003).

The radiation available for photosynthesis is a source of energy influenced by foliage disposition, which depends on pruning, training, and other cultural practices. The exterior leaf layer absorbs the main quantity of PAR, transmitting and reflecting only a little percentage of this radiation in the inner crop positions (Smart, 1973).

It is known that light quality under vegetation canopies consists of unfiltered and filtered radiations. The first ones can be direct or diffuse, pass through the gaps in vegetation, arriving unmodified in the inner position of the vegetation canopy (Smith, 1982). Unfiltered component of light is known to exert an important light spectrum modification in fruit trees (Grant, 1997; Baldini et al., 1997). Furthermore, Sinclair and co-authors (1973) showed that the unfiltered radiations, arriving in the inner position of vegetation through the “sunflecks”, are not completely unmodified. The far-red radiations that are not absorbed by the outer leaves exert a slight modification in the light conditions of the sunflecks. Kasperbauer (1971) showed the existence of a shift of Red: Far Red ratio within a canopy, due to the modification of the spectral distribution of light, especially red light. Moreover, it was showed as red light reduction is due to the external leaf absorption.

The amount of filtered solar radiations inside the canopy is the result of absorption, reflection and scattering processes. The ratio between filtered and unfiltered radiation depends

by nature, structure, density and depth of the crop. The absorption of light by photosynthetic pigments is generally highest in the outer crop positions, with a reduction of blue and red ranges of the sunlight spectrum wavelengths and Red: Far red ratio at level of the below canopy (Smith, 1982). The latter is a parameter useful to describe light conditions, important with the aim to evaluate light plant perception of environmental changes (Smith, 1982; Franklin and Whitelam, 2005). Morgan and Smith (1981), in a study on *Chenopodium album*, defined R: FR ratio as an optimal index of vegetation shading because of their capacity to know, for instance, the reduction of red light absorbed by leaves and the increase in far red light inside the canopy with a reduction of R: FR ratio.

Leaves absorb and transmit perceived radiation in different way depending on the perceived wavelengths (Szeicz, 1974). Moss (1952) found that crop, especially leaves, absorb about 92% of radiation in the range between 400 and 500 nm, 71 % between 500 and 600 nm and 84% between 600 to 700 nm. Overall, in the range of 400-700 nm, the 82% of the light perceived is absorbed, while reflection represents about the 10%. Moreover, the absorption of light it is known to decrease beyond 700 nm. Wooley (1971) study light absorption by leaves, showing that violet and ultraviolet radiations are relatively the more absorbed than the others. Leaves are shown to absorb about 80-90% of the PAR, 20% of and IR, transmitting or reflecting the unabsorbed part. Hence, consequently, PAR perceived by leaves was decreased by crop depth (Szeicz, 1974). Awad and co-workers (2001) in a study on apple trees found that light perceived by the inner positions of the canopy was significantly lower than the outer ones. In particular, inside the canopy, it was found in proportion more far red light (700-750 nm) and less light in UV-A (330-750 nm), blue (400-450nm), green (450-530 nm) and red (600-700 nm) spectrum regions.

The majority of the understandings on plant light-response were obtained from research on *Arabidopsis thaliana.* Halliday and co-workers (1994) in a study on *Arabidopsis* shown that plants, in canopy shade condition, react to the relative modifications in red and far -red ratio, increasing internode extension and reducing leaf area.

As reported by Smart and colleagues (1990) technological advances developed in agriculture after World War Two, provoke an excessive enhancement of vineyard vigour. Consequently, it was necessary to study new techniques to avoid shading conditions, by changing the position or the amount of leaves, shoots and fruit in the space. One solution was offered by canopy management through shoot vigour control, shoot trimming, leaf removal in the fruit zone and improved training system, techniques that were shown to reduce vigour when they are applied to vineyard in the right way. Canopy microclimate depends on canopy management that, hence, can be used to improve grape and wine quality, decreasing also the plant disease incidence and making easier the mechanisation (Travis, 1987). Spayd and coll. (2002) showed that the choice of practices in viticulture (trellis systems and leaf, shoot, or partial shoot removal), influencing leaf area, give the possibility to regulate the right exposure of grapes to sunlight.

A uniform microclimate is important in order to avoid canopy shading and grant a good grape exposure. For instance, it is known that grape contents of K, pH and malic acid are increased in shading conditions. On the contrary, sugar, tartaric acid, phenol and anthocyanin concentrations have a decrease (Smart, 1985; Shaulis, 1982; Smart et al., 1988; Kliewer & Smart, 1988).

It is important to avoid vigorous canopy in order to have a good balance between vegetative and fruit growth in which photosynthates are appropriately partitioned between shoot and fruit production (Smart et al., 1990). For instance, leaf photosynthetic efficiency can be improved by partial leaf removal (Hunter and Visser, 1988), remaining a satisfying leaf area necessary to grant a good fruit ripeness (Peterson and Smart, 1975; Koblet, 1987).

A study on *Vitis vinifera* L. cv Sangiovese in different sunlight conditions through the use of shading nets, assessed the effects on the leaves of plants 50% and 20% of shading environment compared to full light conditions. The less the plants received light, the more leaf thickness and leaf mass per area are reduced. This behaviour was accentuated when UV filters were used (Pollastrini *et al.,* 2011).

The plants react in different ways according to light conditions. For instance, under light stress conditions photoprotection is mainly exerted through thermal energy dissipation of absorbed light (Niyogi, 1999). According to Darnell, (1991 and 1996), in blueberry, photosynthetic rate can be reduced by the action of high irradiance and temperature; in these conditions there is a decrease in carbohydrate accumulation and fruit yields. During (1998) showed that the behaviour of grapevine is modified, in condition of increased sunlight radiation, in order to allow an adaptation to light.

On the contrary, in shading conditions a set of responses, depending on the plant species, involves leaf physiology and biochemistry, leaf anatomy and morphology and the whole plant. Therefore, these responses showed as shade tolerance cannot simply be expressed as a minimum of light needed by plant species to be alive, but it is a broader concept (Valladares *et al*., 2008). Irradiance is the most important variable that, driving photosynthesis, influences plants physiological and morphological responses, such for instance the flowering (Thomas *et.al.*, 2006). When light regimen is low, the plants intercept more light in order to survive, thanks to morphological changes used by plants in order to have a light acclimation. For instance, a broader and thicker leaves production and an increased chloroplast volume is a strategy aimed to have an increase in P max increase in irradiance and therefore in the photosynthetic carbon gain (Oguchi et al., 2008; Valladares and Niinemets, 2008).

Morgan and Smith (1979) studied the influence of shade light on plant development, showing that, in several plant species, far-red light effects on stem extension rate and leaf dry weight; supplementary far-red light was added to natural radiation with the aim to simulate natural shade conditions. Hence, it was showed that plant response in that environment is phytochrome controlled. Stem extension rate and leaf dry weight are linearly related to red/far red energy ratio. The same Authors, in 1981, found that stem extension is induced by a low Red: Far red ratio and the intensity of the response was influenced by fluence rate between 400 nm and 700 nm: a lower fluence rate, induced by a low red:far red ratio, leads to a lower response. High red:far red ratio produce a lower ratio between leaf dry weight and stem dry weight, that is instead not influenced by a lower red: far red ratio.

Moreover, photosynthesis is also influenced by the interaction between UV-B radiation and water deficit; both, in combination, influence photosynthesis efficiency, decreasing it for the reduction in the concentration of photosynthetic pigments, and grape berry ripening is delayed (Martìnez-Lüscher *et al*., 2015). As resulted in a study of Kakani and co-workers (2003), the decrease of photosynthesis consequently causes a reduction of the plant biomass.

A high solar UVB increases the accumulation of antioxidant compounds and other photo protective pigments in the leaves (Berli *et al*., 2013). The plants more exposed to elevated UV-B radiation, as a precaution, develop a lower leaf area and a reduced number of leaves (Tevini and Teramura, 1989), change their leaf inclination angle to increase tolerance to UVB irradiance (Grant, 1999) and have inhibited hypocotyls elongation (Ballarè et al., 1995). The increase of phenolic compounds is one of the plant responses, aimed to enhance the protection by radiation between 280 and 340 nm (UV-A and UV-B) radiation (Li *et al*., 1999; Landry *et al*., 1995; Winkel-Shirley, 2001). Hence, phenolic metabolites are useful in stress conditions, due to high temperatures and radiation, because of their antioxidant capacity (Koes *et al*., 1994). As shown by Bieza and Lois, Liakoura and co-workers in 2001, UV radiations enhance flavonoids pathway, acting on gene expression to produce more UV-

absorbing pigments and increasing the resistance to these radiations. However, it must be also considered the seasonal fluctuations in the concentration of UV-absorbing compounds, principally correlated with the stage of plant development (Dixon *et al.*, 1995; Liakoura *et al*., 2001). Leaves response to UV-B irradiation changes between younger and older leaves; young leaves, in fact, produce more UV-B absorbing compound in conditions of increased of UV-B radiations (Majer et al., 2012).

Vines adapted to restricted light conditions seem to develop a limited capacity for photoprotective responses. Sun-adapted plants are less sensitive to increasing PAR (Photosynthetically active radiation) up to a maximum value, after which react with photoinhibition.

Morgan and Smith (1981) showing that stem elongation, together with increased specific leaf area, was a typical response to shade conditions of the plants, suggested that decreased R:FR ratio provokes this response. Smith (1982), in a study about the effects of light on several vegetal species, reported that both quality and quantity of light perceived by plants are important in reaction to shade light conditions. The response confirmed the previous study of Morgan and Smith (1981), showing the involvement of stem extension, the change in leaf size and structure and number of chloroplast. On the other hand, it was showed that plant of the same species have a different reaction to light, similar to shade avoidance when they perceive the same PAR but different R:FR ratio.

Light perception by grapevine is one of the most important aspect in order to have quality both in grape and wine. Hence, it is known that excessive density of the vineyard canopy, due to both shoot growth and leaf area, should be reduced in order to favour light interception by fruits (Koblet, 1984; Smart, 1985; Smart et al., 1985a; 1985b). Shaded and highly vigorous vine are known to give scarcely coloured wines because of their lower anthocyanin contents and proportion of their ionized form (Smart, 1980; Smart et al., 1985b).

A study on *Vitis vinifera* (cv. *Rhine Riesling, Shiraz, Ohanez, Gordo, Sultana*) showed the different responses of vineyard to light and temperature conditions, with a resulting good fruitfulness in conditions of highest temperature and light. The optimal levels were assessed to change among the cultivars. For instance in cv *Rhine Riesling* and *Shiraz*, when maximum temperature was up to 30° C, there was an increase of number and weight of bunch primordial, while at 20° C fruitfulness was lower, in some varieties even barren (Buttrose, 1970).

The light exposure was well-known to influence grape composition. The more the fruit are exposed, the more their content of sugars, anthocyanins, and phenolic compounds. On the other hand, titratable acidity, malate, and pH, decrease when grape is in shading conditions (Kliewer, 1977; Kliewer and Antcliff, 1970; Kliewer and Lider, 1968; Morrison, 1988; Reynolds et al., 1986). Moreover, in grapes it was showed that glucose and fructose have the same tendency of the anthocyanin content; berry sugars in fact stimulate the anthocyanins accumulation (Pirie, 1976 and 1979; Pirie and Mullins, 1980).

The effect of cluster light exposure on fruit growth and composition, during the main stages of grape development, were investigated in a study carried out by Dokoozlian and coll. (1996) on Cabernet Sauvignon and 'Pinot noir' grapevines (*Vitis vinifera* L.). In that study, it was possible to distinguish between the influence direct radiation and temperature because the theses were in condition of similar temperature. The treatments that were unexposed during the first stages of fruit development resulted to have a lower weight and diameters with a delay of berry coloration. It was also assessed that the delay could not be recovered exposing cluster only in the final stage of grape development. The clusters grown without light in one or all grape developmental stages have a lower malate concentration compared to the exposed clusters, with a decrease intensity depending by cultivars. Tartrate concentration and pH of grape juice were not influenced by light conditions. The exposed treatment (control) had

greater concentrations of skin anthocyanins and phenolic compounds compared to light unexposed treatments. However, a higher anthocyanin content was found in grape developed without light only in the third phase of growth, compared to the treatments obscured in the other phases (Dokoozlian, 1996).

Reynolds and co-workers (1986) in a study carried out on cv. *Seyval blanc* grown in four different cluster shading conditions (from full exposure to full shade) showed that the exposed treatments produce grapes containing more SST and less total acidity than shaded fruits. Malate and tartrate trend is the same of total acidity. Wine quality evaluation tends to score higher in exposed treatments although the differences in that study were no statistically significant. Reynolds suggested that leaves near exposed clusters, having a higher photosynthetic rate, exports much more photosynthates to berries, allowing a higher content in° Brix.

In a study of Zibordi and co-workers (2009) it was showed that a reduction of intercepted light by means of shading, during a small period after bloom, may be useful in order to provoke fruit abscission of about 23% at 44 days after full bloom (DAFB) on apple without the use of chemical thinners. As previously seen in apple, the reduction of the number of berries for each cluster has been recently assessed to increase the grape production in a study (2013), carried out on shading *Vitis vinifera* cv Black Magic, grown in greenhouse in different environmental conditions. It has been supposed that the behaviour of increased fruit drop under shading conditions is presumably due to the decrease of net photosynthetic rate. Hence, it has been assessed in shaded treatment that a decreased incident light at bloom gives a good alternative to the chemical thinning method, with the aim to diminish fruit set.

1.1 Light conditions and plants strategy

Light is known to have a direct influence on plant metabolism through photosynthesis. An indirect effect is exerted on both plant grown and development through morphogenetic control that is responsible for many stages of plant development, starting from seed germination, stem extension, leaf expansion, chlorophyll synthesis, flower induction, etc. (Fitter, 2012).

Light regulation involves many factors the most important of which are the photoreceptors response, the physiological stages of plant development, the seasonal and environmental conditions, and the agricultural practices (Shahak et al., 2004a).

1.1.1 Photoreceptors

Light absorbed by plants is useful not only for photosynthetic reactions, but also for influencing plant metabolic response to environmental conditions, by means of photomorphogenesis (Chen *et al.*, 2004). Specific photoreceptors are very sensible to variation in the red (R; 650 – 670 nm), far-red (FR; 720 – 740 nm), and blue (B; 400 – 500 nm) wavelengths of electromagnetic spectrum hence, through photomorphogenesis, they can modulate biological activity of plants (Schettini *et al.*, 2011). Smith (1982) defined acclimation as the plant capacity to modify their physiology adapting to environmental condition modifications. Photoreceptors, absorbing photons in a process called photoreception, perceive light signal and transmit it with a consequent transduction. This process resulted useful in reaction to changed environmental conditions with the aim to have the appropriate metabolism response.

The number of photoreceptors is not the same during plant growth. Hence, the response to the same light signal can be different depending on the plant stage of development. Plants have a series of photoreceptors that monitor light quality, quantity and temporal/spatial patterns of light.
The photoreceptors responsible for the perception of radiant energy by plants and for the monitoring of changes in the ambient light conditions are:

- cryptochrome: it is involved in the UV-B absorption (Rajapakse et. al., 1999) and it has the capacity to mainly detect blue light (Smith, 1982). In conditions of low light intensity, blue light perceived by cry1 and cry2 was shown to exert hypocotyl inhibition and cotyledon opening in *Arabidopsis* seedlings (Lin et al., 1998);
- phytochromes, the most important photoreceptors (Batschauer, 1998), which detect wavelengths of light spectrum between 300 and 800 nm, with maximum absorption between red (600-700 nm) and far red (700-800 nm) region of the spectrum. This pigments are red/far-red reversible consisting in two forms: P_r form adsorbs red light and it is transformed into the P_{fr} form, i.e. active form that controls signal transduction and plant response. P_{fr} form absorbs far-red light and return to P_r form (Rajapakse et al., 1999). Phytochromes were shown significant for their responsibility in the regulation of several important responses in plants, such us, shade avoidance, germination of seeds, growth, flowering and chloroplast development (Casal et al. 2003, Chen et al. 2004, Franklin and Whitelam 2005). It was found that light influences phytochrome localization in plant cells. Hence, the inactive form, i. e. Pr, was localized in the cell cytosol, whereas the active form P_{fr}, is transported in the nucleus (Huq *et al.*, 2003). Phytochromes had the capacity to detect R: FR ratio changes, hence, these photoreceptors represent important sensors of light quality with the aim to establish the photo-equilibrium P_{fr}/P_{total} (Pratt, 1978; Smith, 1982; Franklin and Whitelam, 2005);
- phototropins: they control blue-light responses, chloroplast positioning, leaf expansion and stomatal opening (Kagawa, 2003).

It was assessed that modifications of light environment might involve, in synergy, several photoreceptors. For instance, floral induction was mediated by the antagonistic action of blue/UV-A light receptors cryptocroms and the red/far red light receptor phytochromes. The latter had inhibition function in the regulation of flowering time, while cryptocroms enhanced this process (Mockleret al., 1999). Devlin and co-workers (1998) assessed that flowering was stimulate through the reaction of both phytochromes and cryptocroms to far red and blue light, respectively.

The spectral quality and intensity of light has an important role to ensure development to through the regulation of phytochromes (Fitter, 2012).

Several studies have shown that blue light influences growth and development of higher plants, because blue-light photoreceptors participate in many events of photomorphogenesis (Briggs and Huala 1999, Christie and Briggs, 2001). Blue light enhances photomorphogenesis and it was assessed to be an important source for plant life because influences plant development and physiology (Thomas, 1981). For instance, there is a positive relationship between the dry matter production and the blue region of the light spectrum, which promotes stomatal opening more than other light spectrum wavelengths, with a consequent enhancement of dry matter production (Sharkey and Raschke, 1981). The opening of stomata was shown to be also influenced by red light, with a response depending on the intensity of fluence rate: the only blue light lead stomata opening when the fluence rate was lower, while, the only red light had the same capacity in condition of higher fluence rate. Isolated

epidermial strips under blue light show a stronger stomatal opening than under red light, while there is no influence exerted under green light (Hsiao et al., 1973).

As reported by Ballarè and co-workers (2014), phytochromes, cryptochromes, and phototropins are responsible for shading effects; in particular, phytochromes react in conditions of decreased Red light and consequent R:FR ratios reduction that in *Arabidopsis* it was found to reduce the accumulation of soluble phenolics, anthocyanins.

The competition for light occurs when plants grow too close together, or have excessive vegetation, therefore the shadowing causes a reduction of the perceived radiation, because leaves filter out blue and red-light and transmit green and FR wavelengths. In response to the effect of shading there is a decrease of R:FR ratio perceived by phytochrome, due to the increment of FR radiations (Smith, 1982; Franklin and Whitelam, 2005). When the described situation occurs for a long time, flowering is accelerated (Halliday et al., 1994). Smith and Whitelam (1997) suggested that the latter is a mechanism used by plants to survive until environment conditions improve producing. However the Authors reported also the possibility of reduced germinability that sometimes occurs when R: FR ratio was reduced by a accelerated flowering.

1.2 Influence of light on fruit composition

The amount of light availability influences the shoot/fruit carbohydrate partitioning. As demonstrated in apple tree, full-lighted shoots are capable to export their photo-assimilates into fruit already three weeks after bloom, while shoots receiving about 1/3 of the available sunlight export same amount of photosynthates two weeks later. (Corelli-Grappadelli, 2003). A relevant reduction of light availability at the early stages of fruit development reduces fruit growth (McArtney et al., 2004) as a possible result of a low CO_2 uptake (Zibordi et al., 2009). Nevertheless, under field conditions of warm growing areas, leaves and fruits are often exposed to light excess that, most times, are associated to other environmental stress factors. In facts, as it has been widely studied in grapevine, berry composition depends on sunlight exposure and temperature of fruit. A research was carried out by Bergqvist and co-workers (2001), at berry set, on cluster of red wine grape cv Cabernet Sauvignon and Grenache at different levels of light exposures. The more exposed grapes had a greater concentration of TSS and pH; on the contrary, titratable acidity had a decrease when sunlight exposure was higher (Bergqvist et al., 2001). Moreover, clusters exposed to very high summer irradiance, and related temperature, apart from loss of acidity, increase the potassium content and the pH; in addition, they may be subjected to sunburns and to the decrease of anthocyanin content (Bergqvist et al., 2001; Haselgrove et al., 2000; Spayd et al., 2002). Glucose and fructose showed the same tendency of the anthocyanins, because berry sugars stimulate the anthocyanins accumulation (Pirie, 1976 and 1979; Pirie and Mullins, 1980).

Moreover, in a study on Cabernet Sauvignon, Smart and coll. (1988) showed that excessively shaded grape-berries might show a delayed ripening, herbaceous aroma, a decrease of sugar content, an increase of titratable acidity and malic acid. In that research, as in the study of Morrison (1988), it was assessed a decrease of polyphenols, anthocyanins in particular. On the contrary, in those conditions, pH was not affected by shade environment. Moreover, in the same study, it was assessed the importance of red light supplementations, in shading conditions, in order to have an earlier veraison and an increased concentration of glucose and fructose at harvest (Smart *et al*., 1988). Jeong (2004), Mori (2007) and coll. showed that also TSS content is generally influenced by shading conditions and high temperatures, with the same tendency of the anthocyanin contents. However, in a study on cluster shading carried out by Cortel and colleagues (2006) no differences in soluble solids

(°Brix) were found between shaded and light exposed treatment while there was a little difference in titratable acidity and pH that are higher in the exposed treatment.
As showed by Haselgrove (2000), flavonoid composition in grapes is generally influenced by light radiation perceived hence its concentration is enhanced in the treatments more exposed. Sunlight exposure and temperatures generally stimulate polyphenol accumulation (Bergqvist et al., 2001; Dokoozlian et al., 1996).

The effects of sunlight on berry development and accumulation of flavonoids were also evaluated in a study carried out by Downey and co-workers (2004). The bunches of Shiraz grapes were covered, prior to flowering, by opaque boxes excluding light, realized to minimise changes in temperature and humidity. Berry sugar accumulation and weight were not influenced by shading conditions. During the three year-trial, a slight change in anthocyanin content emerged only in one year. At veraison, the main gene in anthocyanin synthesis had the same expression in normal and shading treatments. In particular, in the shaded fruits, the dioxygenated anthocyanins (that is Cyanidin and Peonidin in glycosidic form), were the most represented in grape skins. Proantocianins content in skins and seeds of the ripe grape was not influenced by shading conditions. At harvest, it was shown a change of flavonol composition that considerably decreased (about 10%) in shading conditions (Downey et al., 2004b).

In a study on cluster shading (*Vitis vinifera* L. cv. Pinot noir) realized using light exclusion boxes total flavonoid concentration in grape-berry skins was found similar between shaded treatment and exposed control, while the relative composition of flavonoids changed between the two treatments. Flavonols and skin proanthocyanidins are decreased in unexposed treatment. On the contrary, anthocyanin composition was not significantly influenced by shading conditions, even if, considering percentage values, anthocyanins were more concentrated in the exposed treatment (+4% of skin flavonoids), compared to the shaded one. Total skin proanthocyanidin concentration in exposed treatment was higher than the other one. At harvest, flavonol concentration in the shaded clusters was about eight times higher than the others (Cortell *et al*., 2006).

Hasergrove and coll. (2000) studied the influence exerted by a different grade of shading on polyphenol synthesis and accumulation. The study examined phenolic composition comparing shaded bunches with fully exposed bunches, confirming in the second thesis the significantly highest concentration of anthocyanins. In addition to sun exposure, also temperature is considered important: if it is too high (exceeded 35 °C) there is a limitation in the anthocyanin synthesis. A long period at high temperature (between 32 and 37°C) seemed to inactivated the enzyme systems for anthocyanin synthesis and sugars in the berries (Kliewer, 1977). After veraison, polyphenol accumulation in grapevine tissues was shown to be in relationship with soluble sugar content (Pirie, 1976 and 1979b). Reducing sugars in the cell cytoplasm were found to exert a regulator property on the phenolic biosynthetic pathway (Pirie, 1977). In a study on apple, Faust (1965) showed that the enzyme responsible for enhancing the metabolism of sugars via the pentose phosphate pathway, is also responsible for the anthocyanin increase in the skins. Also Kliewer (1977) and Pirie (1979) found a positive relationship between berry-juice sugars and skin anthocyanin content.

A work carried out by Bergqvist and coworkers (2001), on cluster of red wine grape produced by the cv Cabernet Sauvignon and Grenache showed that the less shading treatments at berry set, provoke an increase in polyphenols, in particular anthocyanin concentrations, with an intensity depending on the grade of shading. An inverse tendency was found in the less exposed treatments. However, also in conditions of excessive sunlight exposure, there was a decrease of the anthocyanin concentrations. Anthocyanin concentration was also assessed to depend on fruit temperature: exaggerate temperatures decrease anthocyanin concentrations in sun-exposed grape berries; shading completely the grape is not

good because some sunlight is very important to have maximum anthocyanin production; shading partially fruit is useful to guarantee the quality of grapes (Spayd et al., 2002). Hence, at similar exposure level, temperature was show to influence phenolic composition (Bergqvist *et al.*, 2001).

A study of Azuma and co-authors (2012) confirmed genetically that the accumulation of anthocyanins was dependent on both low temperature and light. Light and temperature were shown to separately control anthocyanin biosynthesis pathway, acting on the expression of VlMYBA1-3 VlMYBA1-2 and VlMYBA2 genes.

Dokoozlian e Kliewer (1996) carried out a study on Cabernet Sauvignon and Pinot noir grapevines. It was assessed the importance of light, perceived by grapes in the first phase of the fruit development, with the aim to positively regulate the activity of enzymes responsible for the anthocyanin synthesis and to have a maximum pigment production during the following stages of development. In the second phase, the light was in used by grapes to maintain higher the activity of the enzymes (Roubelakis-Angelakis, Kliewer, 1986; Dokoozlian, Kliewer, 1996).

Koyama and co-workers (2008) assessed that anthocyanin concentrations were reduced in grapes shaded during ripening, but the behaviour was not the same for grapes shaded on the early stage of development; in the latter conditions anthocyanins concentrations were not reduced, but changed proportion of tri-hydroxylated anthocyanins. Jeong and co-workers (2006) studied the relationship between anthocyanin composition and expression of the genes F 3'H (Flavonoid 3′-hydroxylase) and F3′5′H (flavonoid 3′,5′-hydroxylase) with the aim to verify their responsibility for specific anthocyanin forms in different stage of berry development. It was shown that F3'H and F3′5′H are involved in the biosynthetic pathway of cyanidin- and delphinidin-based anthocyanins. For instance, berry skins of Cabernet Sauvignon at the early stage of berry development, accumulated a higher level of mRNA of the gene Flavonoid 3′-hydroxylase (F 3'H) than flavonoid 3′, 5′-hydroxylase (F3′5′H). Conversely, at harvest stage it was found a higher level of mRNA of the gene F3′5′H than F 3'H and this results were associated to the major content of delphinidin-based anthocyanins in the considered berry skins. Light exclusion significantly reduced both anthocyanin concentration and composition (Spayd *et al*., 2002; Guan *et al*., 2015), because it changes the expression of the genes responsible for anthocyanin biosynthesis (Guan *et al*., 2015).

In a study of Liang and co-workers (2008) it was relieved that generally in *Vitis Vinifera* grapes Malvidin 3-*O*-glucoside together with its derivatives and Peonidin 3-*O*-glucoside (methylated anthocyanins) were the most concentrated. Guan and co-workers (2015) assessed that light exclusion on the grapes produced by Gamay cv enhances the proportion of methylated anthocyanins in the skins compared to the exposed treatments.

Red and UV radiations are known to enhance anthocyanin synthesis in fruit skins (Bastias et al., 2012). In a study carried out on apples, it was assessed that the most important enzyme involved in the anthocyanin synthesis is UDP-Galactose: flavonoid-3-O-glucosyltransferase (UFGalT) which increases its activity in conditions of greater UV light (Ju et al., 1995 and 1999).

It was assessed that the reaction of conversion from leucoanthocyanidins to anthocyanidins is catalyzed by an enzyme, a dioxygenase which is synthesized under UV receptor influence. The conversion is increased under UV-light augment (Gallop et al, 2001). A crescent exposure to sunlight was showed to increase total phenol concentration also in a study carried out by Price and coll. (1995) in *Pinot noir* grape skins. Moreover, it was assessed on *Vitis vinifera* L. cv Pinot Noir, in condition of both ambient light and filtered UV, that polyphenol content in grape skins is proportional to UV radiations perceived, increasing when exposition is maximum (Cortel et al., 2006).

As shown by Jenkins (2007), UV-B radiations enhance some protection processes, by means of photomorphogenic responses, at level of gene expression, cell physiology and biosynthesis. Radiation influences in superficial plant tissues the synthesis of phenolic compounds, responsible of UV radiation absorption avoiding damages to macromolecules, as DNA.

In general, it was assessed that UV-light positively influences polyphenol compound. A consequence of high UVB radiations, is an increased accumulation of antioxidant compounds and other photo protective pigments in both leaves (Berli *et al*., 2013) and grapes (Berli et al., 2011), as it was also assessed by Keller and Torres-Martinez (2004) in a research on visible and UV light effects. The aim of that studies was to separately investigate the effects of visible and UV light on wine grape composition. Moreover, it was showed that the content of both anthocyanins and hydroxycinnamil tartaric acids is unaffected by UV radiation, while visible radiations are responsible to produce more flavonols in the treatment covered by diacetate films that screen about 98% of all UV-A and UV-B radiations. In that study, results also showed that flavonols protect grape tissue against excessive visible radiation.

Blue light was in particular showed to be the most important sunlight range responsible for anthocyanin accumulation (Chen et al., 2006). Wang and co-workers (2012) assessed that blue, UV-A and UV-B radiations in Turnip Seedlings are responsible for anthocyanin biosynthesis responses, with synergistic effects depending on the considerate organs of the plants. In *Brassica napus* anthocyanin accumulation was shown to depends on the main blue-light photoreceptor, i.e. cryptocrome with direct correlation (Chatterjee et al., 2006).

2. GRAPE COMPOSITION

2.1 Phenolic composition

2.1.1 Total polyphenols

The most important parameter that should be considered with the aim to evaluate grapes, in addition to total acidity, pH and sugar composition, is the phenolic content that is important especially for consumer health (Leighton *et al.*, 1998) and organoleptic characteristic (Kosir *et al.*, 2004; Soleas *et al.*, 2002).

The secondary metabolites of grape varieties are not directly involved in the main physiological functions of plants, although from the quantitative point of view may be affected by climatic and environmental factors (Dixon et al., 2001; Di Stefano, 1996a and 1996b). Polyphenols and grape aromas belong to this class of compounds. Among the polyphenols there are in particular, anthocyanins, acids hydroxycinnamil tartaric acids, flavonols, flavans, playing an essential role in colour and taste of both grapes and wine. These compounds are also important for their antioxidant properties and protection against cardiovascular diseases (Riberau-Gayon et al., 1998).

Hence, the study of the chemical composition of grapes and wine plays an important role for the improvement of fruit quality and the characterization of the product. In wine grape, the study is also important in order to estimate the potential wine grape compounds that can be transferred to the wine (Sun et al., 1999; Costacurta et al., 2001).

2.1.2 Flavonols

The flavonols belong to the class of flavonoids and they can be located in the skins of red and white grapes. The main grape flavonols consist of two benzene rings linked by a heterocyclic oxygenated, which according to the ring substituents can be quercetin, myricetin and kaempferol. They are absent, or present only in traces, in white grapes and absent in white wines (Vuorinen et al., 2000). In grapes, flavonols are detectable mainly in the glycoside forms, in particular quercetin, myricetin and the kaempferol in the forms of glucosides and glucuronides, although there are often other minor flavonols (Di Stefano, 1996b). The content of aglycones in red wines is approximately 100 mg L^{-1}; in white wines, fermented in the absence of the solid parts of the grape, aglycone concentration is between 1-3 mg L^{-1} according to the variety (Ribereau-Gayon *et al.*, 1998). The importance of flavonols as potential anti-mutagenic and anti-cangerogenic and their influence on the color given by copigmentation with anthocyanins has been demonstrated in red wines and fruits (Cheynier *et al.*, 1989; Asen *et al.*, 1972).

2.1.3 Flavanols and proanthocyanidins

The flavan-3-ols and their polymers (proanthocyanidins) are present mainly in the solid parts of the grape, in particular in grape seeds. The (+)-catechin and (-)-epicatechin stereoisomers, are the main flavan-3-ols of grapes because they represent the more stable stereoisomers of the two asymmetric carbons in the oxygenated heterocycle. In grape seeds it

was found (-) epicatechin gallate (gallic acid is esterified carbon C3) even if in small amounts (Pastor del Rio and Kennedy, 2006).

In grapes, dimer, trimer, oligomeric and polymeric proanthocyanidins were found. They are divided into two types: A dimers, in which the two monomers can be linked by a bond C4-C8 or C4-C6 and an ether bond C5-C7 or C2-C2; B dimers in which the two monomers are related by a only bond C4-C8 (B1 to B4) or C4-C6 (B5 to B8). The main dimere procyanidins in grape seeds are B1 and B2. When the polymerization degree exceeds three molecules, procyanidins are considered condensed tannins (De Freitas et al., 2000) and they are able to influence the colour and the taste of the wine. The tannins are compounds formed by monomeric units of a phenolic nature able to bind proteins in a stable way and for this purpose are also used for tanning leather. They differ in hydrolysable tannins, or gallic (gallotannins and ellagiotannins) and condensed tannins (Ribéreau-Gayon et al., 1998).

2.1.4 Anthocyanins

The anthocyanins are the characteristic pigments of red grapes, located primarily in the skins, but also in the leaves, at the end of the vegetation period.

Anthocyanins are important class of polyphenols in both table and wine grapes, because of their property to increase fruit quality and, consequently, wine colour.

Anthocyanins (from the Greek words *anthos* = flower and *kianos* = blue) are the most important pigments of the vascular plants; they are natural water-soluble colorants because of their capacity of easy incorporation in aqueous media (Pazmiño-Durán *et al.,* 2001). They are the most abundant polyphenolic compounds in coloured grapes, being responsible for red, purple and blue pigmentation of the grape berries (Liang *et al*., 2011b).

As shown by Jackson and Lombard (1993) polyphenols in red wine grapes change with environmental and management factors (climate, soil conditions, canopy management species), but it also depends on variety.

The distribution of anthocyanins was shown to be dependent on variety (Ribereau-Gayon, 1982). Malvidin 3-O-glucoside is the main anthocyanin, as it happens in most of grapes (Ribereau-Gayon, 1959; Wulf and Nagel, 1978). Comparing the results of previous studies, Mazza (1995) noted that each cultivar is characterised by a different quantity of anthocyanins, but has the same qualitative pattern. Bakker and Timberlake (1985) analysing anthocyanin profile of 26 grape cultivars show that malvidin based anthocyanins were the main anthocyanins in all variety, having a content between 33 and 60%. They also revealed that the monoglucosidic form of delphinidin, petunidin and cyanidin were the lower in all cultivars.

Differently by *Vitis vinifera, Vitis rotundifolia, V. amurensis* and other species of *Vitis* were shown to have also diglucoside form of anthocyanins (Ribereau-Gayon, 1982).

Anthocyanin content in grape skins is an index of quality. The concentration of these compounds depends on environmental, cultural, physiological, and genetic factors (Winkler *et al.*, 1972). Among environmental factor, temperature (Kliewer, 1970b; Kliewer and Torres, 1972) and solar radiation (Kliewer, 1970b; LeRoux, 1953; Weaver and McCune, 1960) are the most important factors aim to improve grape skin colour.

Solar radiation in a study carried out by Crippen and Morrison (1986), was found to be very important for the grape colour. In that research, sun-exposed grapes resulted to have a higher concentration of anthocyanins compared to shaded ones.

The fundamental unit of the anthocyaninmolecule is formed by two benzene rings connected together by means of an oxygenated heterocycle, unsaturated and equipped with positive charge (flavylium cation). The main anthocyanins present in the grapes are Delphinidin (Dp), Cyanidin (Cy), Petunidin (Pt), Peonidin (Pn) and Malvidin (Mv). These molecules can be in

form of aglycones (anthocyanidins) that is the form less stable than the glycoside one (anthocyanins) (Ribereau-Gayon *et al.*, 1998).

In the grapes of *Vitis vinifera* they were identified anthocyanins in 3-O-monoglucoside form and their acylated derivatives with the para-coumaric acid, acetic acid and, only in the case of Malvidin, caffeic acid. It is known also the presence of diglucoside compounds, in which a second molecule of glucose is bonded to the hydroxyl on the carbon in position 5 of the molecule. However, they were found in grapes produced by several species of *Vitis*, such as *V. riparia* and *V. rupestris*, but no in *Vitis vinifera*. The capacity to form diglucoside compounds was due to a genetic trait transmitted on dominant line, that it is also found in the first generation hybrids resulting from a cross between *V. vinifera* and one of the species mentioned above. Hence, the presence of diglucoside anthocyanins is exclusive only of "hybrids direct producers "(Ribéreau-Gayon et al., 1998; Flamini et al., 2000; 2009).

Anthocyanins, alongside the other phenolic compounds, can be modified by hydroxyl, acyl, glycosyl and methyl groups, which influenced some characterististics of plant tissues. Cyanidin 3-O-glucoside, the stable form of cyanidin, was glycosylated by uridine diphosphoglycosyltransferase enzime (VvGT1 or 3GT), condition that needs to be satisfied before passing from cytosol to vacuole with the aim to accumulate during fruit ripens (Offen *et al.*, 2006). Hydroxyl group at the position 3 of the heterocyclic C-ring, was known to give instability to anthocyanidins in physiological conditions (Barton et al., 1999) hence, as reported by Lucker and coworkers (2010), UDP-glucose: flavonoid 3-O-glucosyltransferase enzyme (3GT) lead glycosilation to promote their stabilization.

Malvidin was the predominant anthocyanin in black grape berries and the reason was explained by Fournier-Level (2011), who shown as the same gene controls the transcription of two important genes of anthocyanins biosynthesis: UFGT and AOMT, responsible for glycosylation on C3 and methylation, respectively. Moreover, it was shown that methylation of Delphinidin 3- glucoside into both Peonidin 3- glucoside and Malvidin 3-O-glucoside, and that of Cyanidin 3-O-glucoside into Peonidin 3- glucoside were all catalyzed by the same enzime (O-methyltransferase) (Hugueney *et al*., 2009). As reported by Ibrahim and co-workers (1998) O-methyltransferases (OMTs) are the enzymes responsible for methylation of anthocyanins and other polyphenols in plants. Their action is aim to transfer a methyl group from S-adenosyl L-methionine onto a hydroxyl group (Noel *et al.*, 2003).

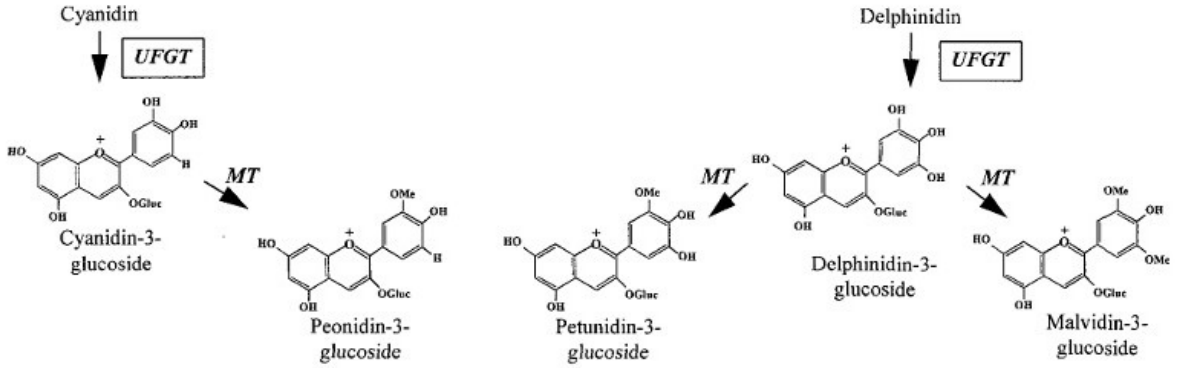


Fig. 2.1 – Anthocyanins methylation (MT: methyltransferase enzyme; UFGT: Flavonol 3-O-glucosyltransferase)
(Boss *et al.*, 1996b)

Fournier-Level (2011) and co-workers reported that, when the phenolic B ring is methylated, anthocyanins increase their stability. It was showed that methylation of free hydroxyl groups increases metabolic stability and improved health effects due to their antioxidant power (Walle, 2009). Methylation of anthocyanins follows glycosylation process because O-methyltransferase enzyme has a higher relative specific activity for glycosylated substrates, in particular towards Delphinidin 3-O-glucoside (Lucker *et al*., 2010); this may be the reason of the lowest content of unmethylated anthocyanins in grape composition and, consequently, the highest content in Malvidin 3-O-glucoside. After their synthesis, anthocyanins are transported from tonoplasts to vacuols with the aim to be accumulated. It has been recently studied a protein named ATP binding cassette (ABC) responsible for transport of non-acylated anthocyanin monoglucosides; in particular, it was shown that the di-methylated malvidin 3-glucoside was transported with efficiency higher than the unmethylated delphinidin 3-O-glucoside, hence the first anthocyanin has a major accumulation in grape skins (Francisco *et al.*, 2013).

As shown by Martin (2013) and co-workers, anthocyanins are significant for their antioxidant capacity and because of their action as phytonutrients which prevent chronic diseases. Hence, it is important to produce grapes with maximum content of anthocyanins. It was also shown that different hydroxylations and glycosylation of anthocyanins influence their antioxidant properties. For instance, antioxidant activity measured by means of ORAC (automated oxygen radical absorbance capacity) assay, as shown by Wang and co-workers (1997), decrease among Cyanidin-3-O-glucosyde, Cyanidin-3-O-rhamnoglucoside (Keracyanin) and Cyanidin: glucose and rhamnoglucose increased antioxidant activity of the same aglycone, i.e. Cyanidin.

A study on Merlot grape carried out in different conditions of fruit exposition. It showed that an increase of light exposure, together with a higher temperature, reduced the ratio between dihydroxylated and trihydroxylated anthocyanins (Tarara *et al.*, 2008). It was shown that anthocyanin accumulation was delayed by light shading conditions (Rojas-Lara *et al.*, 1989)

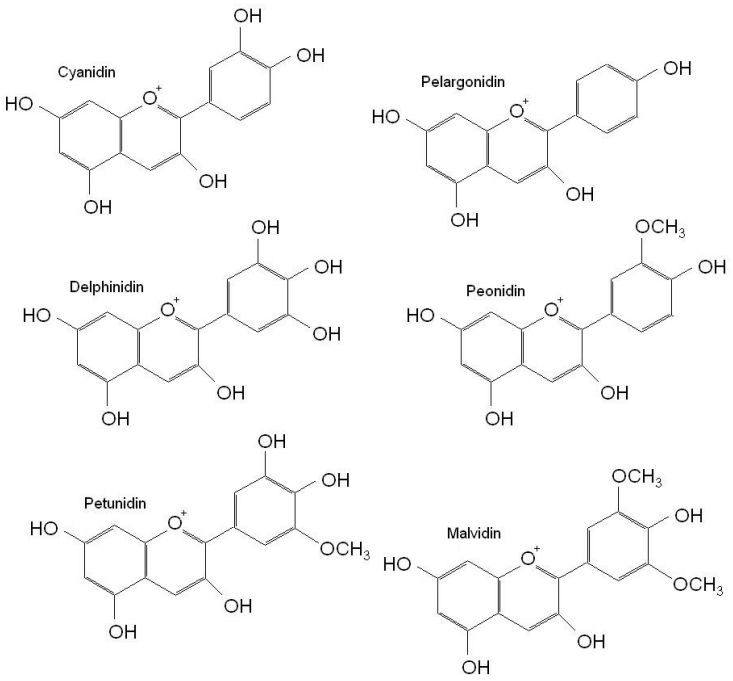


Fig. 2.2 – The main anthocyanins in red and black berry grape skins.

Rice-Evans and co-workers (1996b) showed the positive effects induced by variation in the O-dihydroxy structure in the B ring on the increase of antioxidant activity evaluated with method TEAC. Hence, Cyanidin and Delphinidin, compared to Peonidin and Malvidin, resulted to have the major antioxidant activity (fig. 2.3). In the same study, it was also showed that glycosylation of anthocyanidins in the 3 position of the C ring reduced the antioxidant activity and it can be also decreased by a sugar change; for instance, Cyanidin and Keracyanin (Cyanidin-3-rutinoside) are different only for the sugars in their molecule, i. e. glucose and rutinose.

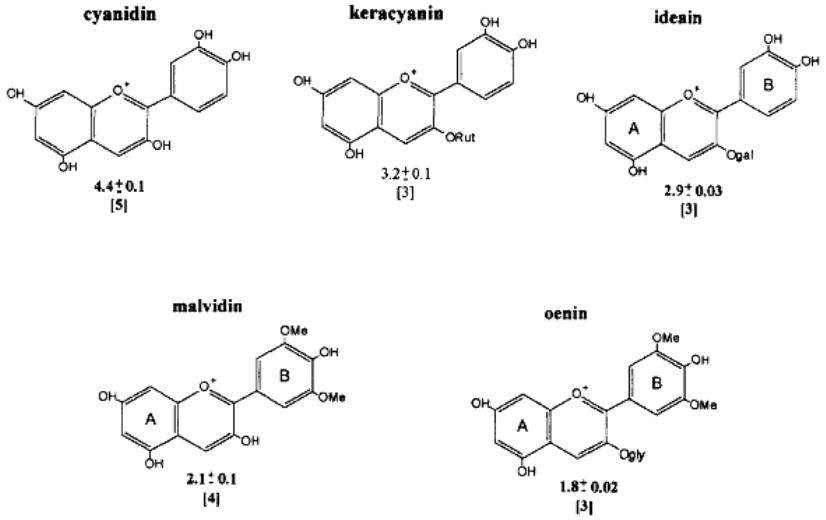


Fig. 2.3 – Antioxidant activity of anthocyanidins and anthocyanins. (Rice_Evans et al.,1996b)

In solution, the anthocyanins assume coloration depending on the composition of the medium (pH, SO_2), by their molecular structure and by copigmentation with other substances. The replacement of the β-ring causes a bathochromic shift of the wavelength of maximum absorption (towards the mauve colour), while the glycosylation and acylation moving the colour in the reverse direction (to orange). The other polyphenols present in solution can copigment changing the colour. Hydroxicinnamiltartaric acids (HCTA), tartaric esters of cinnamic acids, are the major phenolic acids of both white and red grapes. The general structure of these acids, which differ in para-coumaric acid, caffeic acid and ferulic acid, according to the substituents of the benzene ring. In grapes, the HCTA are present both in the skins than in the fleshes and they can be estherified beyond that glycosylated, to a lesser extent (Ribereau-Gayon et al., 1998).

2.1.4.1 Anthocyanins and grape skin colour

It is known that each class of anthocyanins was more or less concentrated depending on genetic, environmental, agronomic factors (cultivar, sunlight exposure, UV irradiation, temperature) and canopy management (Jackson *et al*., 1993; Downey et al., 2006; He et al., 2010). Another factor influencing anthocyanin content was the stability of these compounds, influenced by pH, oxygen, solvents, presence of enzymes, flavonoids, proteins and metallic ions (Kennedy et al., 2001).

Colour hue and difference between pink and deep red skins were shown to depend on the anthocyanin concentration in the skin cell vacuoles (Markham *et al*., 2000), where they

are transferred after their synthesis in the citosol by flavonoid pathway multienzyme complex (Boss *et al.*, 1996b). Moreover, it is well-know that the relative proportion of Cyanidin- and Delphinidin-based anthocyanins was controlled by genes. They were responsible for the red/blue colour of grape berry-skins by vacuolar accumulation (Castellarin *et al*., 2006).

As reported by Wrolstad and co-workers (2005) Pauling in 1939 proposed the responsibility of the resonant structure of the flavylium ion to explain the intensity of anthocyanin colours. Six anthocyanins are the most common in vascular plants, Peonidin, Cyanidin, Malvidin, Petunidin and Delphinidin (Clifford, 2000). The most diffuse in nature, are three non-methylated anthocyanins in glycosidic form, i.e. Cyanidin, Delphinidin they represent about 69% in fruits, 80% in pigmented leaves, and 50% in flowers (Dey and Harborne, 1993).

The differences among anthocyanins depend on: the number of hydroxyl and methoxyl groups of the B-ring; the number and the position of sugars attached to the aglycon; the aliphatic or aromatic acids eventually bonded to the sugar residues (Kong *et al*., 2003).

Heredia and co-workers (1998), in a study on red grapes, found the relationship between anthocyanin content and chromatic characteristics in grape skins. In that study, it was shown that one or more substituents in the B ring of the anthocyanins were very important for the resulting colour of grape skins. Two-substituted anthocyanins (Cyanidin 3-*O*-glucoside and Peonidin 3-*O*-glucoside), tended to orange hue, while three-substituted ones (Delphinidin-3-*O*-glucoside, Petunidin-3-*O*-glucoside, Malvidin-3-*O*-glucoside) had a colour between red and purple. Also methoxylation was shown to influence grape skin colour, shifting it in the wavelengths near purple. Saturation and chroma were found to depend on the number of hydroxyl groups in the B ring (Heredia, 1998). The increase of pH was demonstrated to provoke a reduction in chromaticity (tab. 2.1).

Table 2.1 - CIE 1976 (L*a*b*) colour space (Heredia, 1998)

Anthocyanin	pH	Chromatic coordinates			Chroma	Hue
		L^*	a^*	b^*	C^*_{ab}	h_{ab}
Dp3g	1.5	63.85	68.55	26.97	73.66	21.48
	3.5	88.20	20.55	−1.89	20.63	−5.28
Cy3g	1.5	70.44	60.06	33.90	68.97	29.46
	3.5	88.18	22.69	3.23	22.92	8.14
Pt3g	1.5	63.17	65.71	18.97	68.40	16.13
	3.5	90.18	16.53	−1.16	16.57	−4.03
Pn3g	1.5	73.17	55.63	25.27	61.09	24.45
	3.5	92.14	13.81	1.81	13.93	7.46
Mv3g	1.5	67.85	65.36	11.37	66.34	9.89
	3.5	93.28	11.70	−1.41	11.78	−6.87

Skin colour tend to blue when the most represented anthocyanin belong to those classes in which free hydroxyl group prevails, while with methylation of the hydroxyl groups the prevalent skin colour was red (Jackson, 2008).

Total anthocyanin concentration generally show a negative correlation with the L* and b* colorimetric coordinates of the CIE-LAB system, while the a* coordinate had a positive correlation. In cultivars characterized by dark skins, methylated anthocyanins, such as Malvidin 3-O-glucoside, are the most represented (Liang et al., 2011). In a study carried out on *Vitis vinifera* cultivars (*Flame seedless, Monastrell and Exotic*) by Fernandez-Lopez and co-authors (1998), it was shown the relationship between grape skin colour and anthocyanin content, as previously resulted by a work of Carreno and co-workers (1999). It was found the prevalence of Cyanidin-3-glucoside in the low-pigmented cultivars, while Malvidin-3-

glucoside was shown to be the most concentrated anthocyanin in the highly pigmented cultivars. These results were explained by means of the higher capacity of hydroxylation in 5' position of the ring B, with a consequent highest concentration of methylated anthocyanins in the most pigmented cultivars. Hence, the ratio between unmethylated and methylated anthocyanins may be an index of the level of skin pigmentation. The observation of the studied varieties suggested that the three phase of grape development, from veraison to ripen, had a duration correlated with the anthocyanin content, together with other characteristic typical for each cultivar (Fernandez-Lopez et al., 1998). The proportion of di-substituted (Cyanidin 3-O-glucoside and Peonidin 3-O-glucoside) and tri-substituted anthocyanins (Delpinidin 3-O- glucoside and Malvidin 3-O- glucoside) makes possible the distinction between the cultivars, as demonstrated also by Roggero and co-workers (1986).

Roggero and co-authors (1986) in a study on Syrah grapes found that except for cyanidin, that is the precursor of the other pigments, the anthocyanin composition was set up after veraison and it was almost stable until the grapes ripen. The content of delphinidin was reduced after the end of biosynthesis and Malvidin was increased. As reported by Sichery (2015), Cyanidin and Delphinidin give violet colour to the skins, so that, comparing grape wines such us Negroamaro, Uva di Troia, and Sangiovese, they have blue, violet and violet-black colour, respectively, likely due to the tendencially crescent content of Delphinidin. Anthocyanins have the main responsibility in red wine colour (Ribereau-Gayon, 1982; Mazza et al., 1999). Wine colour is a result of the synergy of anthocyanins, polymeric pigments and tannins (Ribereau-Gayon, 1982). Anthocyanins concentration change with species, variety, season, and a wide range of environmental and management factors such as climate, soil conditions and weather (Ribereau-Gayon, 1982). Anthocyanins are wine colorant pigment extracted from grape skins during crushing, pressing and fermentation and after that, they can interact with colourless phenolics such us (+)-catechin, (-)-epicatechin, quercetin, kaempferol, and phenolic acids with consequent modifications in the wine colour (Liao *et al*.1992). During wine making, a strong crushing allows more anthocyanins to pass in the must, although it is important to avoid an excessive extraction because it can give an astringent wine (Riva, 1980).

Monomeric anthocyanin content was shown to decrease during red wine maturation because of the development of pyranoanthocyanins and polymeric anthocyanins by which wine colour was also influenced (Monages *et al.*, 2009). Anthocyanins contribute to influence wine colour (hue, brightness and saturation) because of their concentration and composition (He *et al*., 2012). It has been well known that berry colour is influenced not only by pigment concentration, but also by their proportion, depending by different factors, such as the level of anthocyanin methylation and hydroxylation. The increase of hydroxyl group number enhances blue intensity, while methylated groups exert the same influence on red colour intensity (Fournier-Level *et al*., 2011).

Fruits and the vegetables are well known to exert protective action against human chronicle diseases thanks to the anthocyanin contents responsible for their antioxidant activity (Record *et al.,* 2001; Heinonen *et al.,* 1998; Rice-Evans *et al*., 1996b). They are natural pigments aim to prevent several diseases and to improve the eyesight (Tsuda, 2012; de Pascual-Teresa *et al*., 2008) and they exert anti-inflammatory and platelet inhibitory effects (Maier *et al*., 2009; Park *et al.*, 2003).

Colour is very important to evaluate food quality (Bridle *et al*., 1997). In grapes, the main anthocyanins are Malvidin, Cyanidin, Delphinidin, Peonidin, Petunidin and Pelargonidin (Liang *et al*., 2008); their content and relative concentrations are very important for skin colour (Carreno and. Martínez, 1995; Corrales *et al*, 2009). However, anthocyanin concentration depends on genetic, environmental and agronomic factors (cultivar, sunlight exposure, UV irradiation, temperature), canopy management (Downey *et al*., 2006; He *et al*.,

2010), but also on their stability that is influenced by pH, oxygen, solvents, the presence of enzymes, flavonoids, proteins and metallic ions (Kennedy *et al.*, 2001).

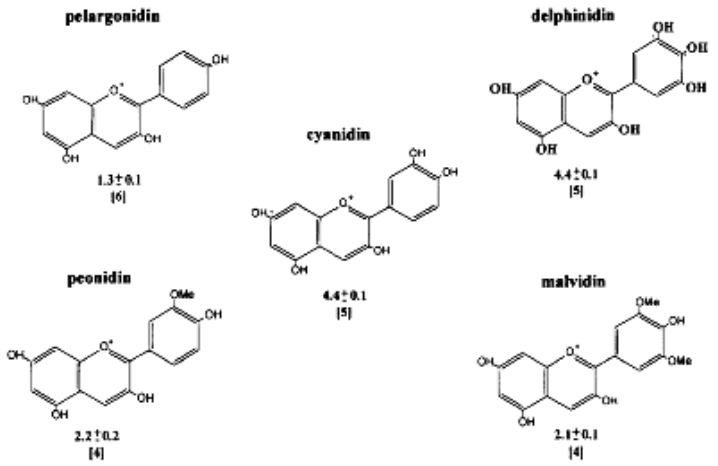


Fig 2.3: The effect of variation in the B ring on the antioxidant activities of the anthocyanins (Rice-Evans et al., 1996b)

The expression of flavonoid 3'5'-hydroxylase (F3'5'H) and flavonoid 3'-hydroxylase (F3'H) genes is important, being responsible for the anthocyanin content in red and black grape skins; when the first gene prevails, resulting colour of the skins may be blue-purple. On the contrary, red colour prevails when F3'5'H genes have a major expression. The colour change between red and blue depending on the synthesized anthocyanins: Delphinidin, is the precursor of blue/purple Petunidin and Malvidin derivatives; Cyanidin, is the precursor of the red peonidin derivatives (Kuhn *et al*., 2014). It is known that anthocyanin colour is due to the number and the position of the hydroxyl groups (Strack *et al*., 1989). Anthocyanidins, the aglycon form of anthocyanins, tend to be bluer and more stable when they have just one free hydroxyl group on the B ring of the molecule (Borkowski *et al*., 2005). Anthocyanin colour changes from yellow-orange to red when C-3 links a hydroxyl group not glycosylated, (Ahamed *et al*., 2004). As shown by Cabrita (2000) and co-authors, the glycosidic form of anthocyanins has a colour depending on pH of solution. In the range of pH between 1 and 3 red is the prevalent colour, which is typical of flavylium forms; when pH is neutral, red decrease and there is a shift to blue colour; at pH above 8.1 monoglycosidic form of peonidin and Malvidin increases the blue colour. In the same range of pH, 3-glycosidic form of Cyanidin, Petunidin, and Delphinidin enhances the red colour. Glycosidic substitution gives more electrophilicity to flavylium cation, hence between aglyconic and glycosidic form of the same anthocyanin there is a loss of colour (Borkowski *et al*., 2005).

The hydroxyl group at C-3, a frequently glycosylated position, is highly significant because it shifts the colour of anthocyanins from the yellow-orange to the red.

The colour of the anthocyanins varies according to the number and position of the hydroxyl groups and they show distinctive Band I peaks in the 450-560 nm region of the sunlight spectrum (due to the B ring hydroxyl cinnamoyl system) and Band II in the 240-280 nm region due to the A ring benzoyl system (Mabry *et al*., 1970; Wollenweber and Dietz, 1981).

It was shown on apples that the most important enzyme involved in the anthocyanin synthesis is UDPGalactose: flavonoid-3-O-glucosyltransferase (UFGalT) which increases its activity in conditions of greater UV light (Ju *et al*., 1995 and 1999). It was known that red plastic nets, used in agriculture, increase light transmission in the range of Red (600 – 700 nm) and Far

Red (700 – 800 nm) visible spectrum of sunlight (Oren-Shamir *et al*., 2001). Schmelzer and coworkers (1998) suggested that the function of flavonoids, such as anthocyanins, was to protect absorbing UV and visible light and to attract pollinator with the aim to disperse seeds.
In conditions of a slight water deficit, anthocyanin contents and TSS are generally influenced by UV-B radiation, and low temperatures (Castellarin *et al.*, 2007; Berli *et al.*, 2010; Mori *et al.*, 2005). On the contrary, shading conditions and high temperatures reduces anthocyanin contents (Jeong *et al.*., 2004; Mori *et al.*, 2007)
Crippen and Morrison (1986) found that, after veraison, anthocyanin concentration was more increased in clusters exposed to light compared to unexposed ones, but this difference was not perceptible at harvest.

2.1.5 Hydroxycinnamil tartaric acids

Hydroxycinnamic compounds are important compounds for their effects on the digestibility of foods and on microbial, taste bitter, their anti-mutagenic and anti-cancer and to their use for therapeutic and pharmacological (Teixeira *et al.*, 2013). It was recently shown that HCTA and phenolic acids are colourless but can assume yellow colour after oxidation. They are responsible for the browning of musts caused by enzymatic oxidation as substrates ideals of polyphenol oxidase (PPO) (Cheynier et al ., 1989). The levels of HCTA in grapes may vary in the shortly before the maturation, though their relative composition is characteristic of the variety (Di Stefano, 1996b). The increase in HCTA occurs in young fruits until a few days after the start of veraison, then decrease rapidly with a small increase at the decrease of sugar accumulation.

2.1.6 Biological properties of polyphenols

Polyph enols are secondary metabolites in plants and foods. Anthocyanins, flavonoids, catechins, procyanidins, phenolic acids and stilbenes are the main phenolic constituents. They are present in grapes, wine and winemaking residues. Generally, these polyphenolic compounds are characterized by the following properties: antioxidants; protective of the cardiovascular system; anticancer; anti-inflammatory; anti-aging; antimicrobial. Polyphenols exert also an influence reducing intestinal glucose absorption and its blood level by inhibition of α – glucosidase (Hanamura *et al*., 2006).

Fruit flavanols are mainly represented by catechin and epicatechin. Gallocatechin, epigallocatechin, and epigallocatechin gallate are largely found in grapes and tea. Catechin and epicatechin are the main flavanols in fruit, whereas gallocatechin, epigallocatechin, and epigallocatechin gallate are found in certain seeds of leguminous plants, in grapes, and more importantly in tea (Arts *et al*., 2000).

As reported by Flint (1985) and co-authors, UV radiations perceived by leaves increase their content in flavonoids, because of their protective action by UV absorption.

Antioxidant properties

The antioxidant activity is one of the most important properties of the phenolic compounds in grapes and wines. Oxidative stress causes the alteration of biological macromolecules, such as lipids, proteins and nucleic acids and it is considered the factor responsible for both the onset of degenerative disorders and alteration processes of food (Shahidi and Naczk, 2003).

The polyphenols, acting as natural antioxidants, are excellent electron donors of hydrogen atom to the free radical molecules that form in the fabrics and substrates for work of initiators, such as UV radiation, oxygen, metals, heat, and enzymes. The phenoxy radical that is formed by the reaction of the phenol with the molecule radical is stabilized by the delocalization of the unpaired electrons on the aromatic ring.

The functional chemical group that determines the antioxidant capacity of the polyphenols is the hydroxyl group and the greater is the number of hydroxyl substituents in polyphenols, the greater is their antioxidant activity. On the contrary, the substitution by methyl groups results in a decreased antioxidant capacity (Xia *et al.*, 2010). The characteristics of the antioxidants polyphenols are also related to their ability to chelate metals and to influence the cell signalling pathways and gene expression (Xia et al., 2010). Polyphenols are present in the various parts in grapes, and many methods (Folin, DPPH, TEAC, ORAC, FRAP) were used to estimate the antioxidant activity of polyphenolic extracts of grape, wine and other by products (Haung *et al.*, 2005; De Beer *et al.*, 2002).

The risk of cardiovascular disease is associated with high cholesterol levels and high protein density (HDL) in plasma, in addition to excessive lipid peroxidation and LDL cholesterol itself. The consumption of polyphenols limits the development of atheromatous lesions (Hayek *et al*., 1997; Miura *et al*., 2001). Antiatherosclerotic effect is due to the reduced low-density lipoproteins (LDL) and their oxidation and decreased tendency to aggregation (Kaplan *et al*., 2001). These results were shown in particular by administrating to rabbits extract of grape seeds rich in proanthocyanidins (Yamakoshi *et al*., 1999).

The development of atherosclerosis, according to the "oxidative theory", is related to macrophages that attack the oxidized LDL and become foam cells, precursors of fibrous plaques. A healthy endothelium produces nitric oxide and prostacyclins that prevent the adhesion of the plates. In conditions of damaged endothelium, underlying collagen fibers are exposed. Hence, the plates will adhere initiating atherogenesis (Cos *et al*., 2003). In vitro studies have shown that tannins, flavonols, benzoic and cinnamic acids, stilbenes and anthocyanins have the ability to inhibit the oxidation of LDL, and that their action is even higher than that exerted by the most common antioxidants, such as vitamin E and D (Yilmaz and Toledo, 2004). The grape polyphenols also work through other mechanisms. They can act as the LDL receptor activation of SREBP-2, which are fundamental in the process of removing LDL from the blood and tissues. Polyphenols can also increase the expression of the gene CYP7A1, which regulates excretion bile acids increasing the levels of cholesterol in the liver and reducing them in the plasma (Chen et al., 2011). Quercetin inhibits the expression of metalloproteinase 1 (MMP 1) promoting the destruction of atherosclerotic plaques. Resveratrol prevents plaque formation by inhibiting the activity of the enzyme named cyclooxygenase 1 (COX-1) with a consequent reduction of thromboxane 2 (a vaso-constrictor and promoter of the aggregation of plaques). The ability to stimulate the channels Ca^{2+} - K^{+} and increase of nitric oxide signal in the endothelium are other mechanisms by which resveratrol exerts its vessel-relaxing activity (Pandey and Rizvi, 2009).

According to Fukumoto and Mazza (2000), using different methods of analysis, on average, anthocyanin antioxidant activity, is enhanced by an increase in hydroxyl groups and a decrease of glycosylation. Three hydroxyl groups on the B ring of flavonoids gave more antioxidant activity to molecule. When one hydroxyl group is lost, there is a slight decrease of antioxidant activity; it was significantly reduced when two hydroxyl groups are lost. By means of ORAC assay application, it has been shown in decreasing order of antioxidant activity: Cyanidin 3-O-glucoside (3.491 ± 0.011), Cyanidin (2.239 ± 0.029), Malvidin (2.009 ± 0.167), Delphinidin (1.809 ± 0.068), Peonidin 3-O-glucoside (1.805 ± 0.014), Malvidin 3-O-glucoside (1.404 ± 0.052).

Anticancer properties

The polyphenols act in the mechanisms of cancer prevention readily interacting with the reactive oxygen species (ROS) and with the free radicals to form relatively stable compounds, hence protecting cells from oxidative stress. They can also stop or reverse the process of carcinogenesis by acting on the signal molecules involved in the process of contributing to the initiation and regulation of proliferation and the immune response (Yu and Ahmedna, 2013). Extracts of grape seed, thanks to the strong antioxidant capacity, have been shown to protect the tissues of the skin from free radicals, inhibiting oxidative stress caused by UV radiation (Bagchi *et al*., 2000). They also possess the property of inhibiting the proliferation of tumour cells in the pancreas, also inducing their apoptosis (Ahmedna and Yu, 2013). Anthocyanins were shown to have in vitro anti-cancer activity towards different types of cancer cells, reducing their proliferation, inflammation and inducing apoptosis. In vivo studies shown that anthocyanins inhibit cancer in the gastrointestinal tract and skin cancer (Wang and Stoner, 2008; de Pascual-Teresa and Sanchez-Ballesta, 2008). Resveratrol, as chemo-preventive agent, was shown to inhibit the initiation, promotion and development of tumors; it was shown that the major organs of interest are the liver and kidneys (Ahmedna and Yu, 2013).

Polyphenols represent important constituents for the positive effects given by their assumption. Vegetables were shown to prevent cardiovascular diseases and cancer (Ames *et al*., 1993) thanks to their antioxidant activity. Polyphenols are antioxidants widely diffuse in plants and they present phenolic hydroxyl groups as common property that is responsible for the antioxidant activity of these compounds (Bors *et al*., 1990; Rice-Evans *et al*., 1997). The antioxidant property is mainly increased by: the 3-hydroxy group on the unsaturated C ring; the 2,3-double bond with the 3-OH group and the 4-one in the C ring; the o-dihydroxy structure in the B ring (Rice-Evans *et al*., 1996a).

Several studies showed that they have anticarcinogenic properties associated to the polyphenol potential to reduce DNA oxidation damages (Bellion *et al*., 2010), protective action in prevention of cardiovascular diseases and cancer (Hollman *et al*., 2011). It was shown that polyphenol administration exerts a protective action inducing a reduction of the number and growth of human tumors (Yang *et al*., 2001).

Anti-aging properties

Due to their antioxidant and anti-radical, polyphenols are useful in preventing damage to the tissues of the central nervous system. Large consume of fruits and vegetables more rich in polyphenols was shown to reduce Alzheimer and others diseases occurring in aging, because of the high content in antioxidants that reduces oxidative stress and inflammations at level of central nervous system and acts improving cognitive processes. It was studied antioxidant healthy effect in the transmission of neural cell signals and was revealed that an important role was exerted by the combinations of antioxidant and anti-inflammatory property of polyphenolic compounds mainly found in fruits and vegetables (Joseph *et al*., 2005; Uttara *et al*., 2009; Singh *et al*, 2004).

Balu et al. (2006) report that the supplement of extract of grape seeds (100 mg per kg of body weight) in rats for 30 days inhibits the accumulation of oxidative damage related to aging in the DNA of neural tissues. Recent studies shown that resveratrol, through inhibition of NADPH oxidase, may exert a neuroprotective role against ischemia and neurodegenerative disorders (Markus and Morris, 2008; Zhang et al., 2010). The epigallocatechin gallate (EGCG) in animal models has been shown to exert a protective role towards the neurotoxin MPTP (N-methyl-4-phenyl-1,2,3,6-tetrahidropiridina), an inducer of Parkinson's disease.

EGCG also protects neurons by activating various pathways of cellular communication involving the MAP kinase critical for cell survival (Pandey and Rizvi, 2009).

Antimicrobial properties

Polyphenolic extracts of plants have demonstrated potential antibacterial activity, antifungal and antiviral properties. These results confirmed the potential application of these compounds as natural food preservatives (Xia et al., 2010). The polyphenols in grapes and wine have shown antimicrobial activity against specific strains of bacteria, such as *Streptococcus mutans, Staphylococcus aureus, Escherichia coli* and *Candida albicans* in which cause the destruction of the bacterial cell wall. Resveratrol has a strong antibacterial and antifungal activity towards several human pathogens and Gram-positive bacteria (Yu and Ahmedna, 2013).

Metabolism and bioavailability of polyphenols

The intake of polyphenols from fruits, drinks, vegetables and legumes is largely influenced by individual habits and food preferences; it is estimated that the daily intake of polyphenols varies between 500 to 1000 mg. Even if the consumption of polyphenols from the diet is high, these compounds are metabolized rapidly and their absorption in the digestive tract is low, variable between 2% and 20%, and minimum quantities of polyphenols were found in plasma. It is known that among the polyphenols, individual anthocyanins are very sensible to oxidation, so after their biosynthesis, they are stabilized by O-Glycosylation and then they were stored in cell vacuoles (Jackson, 2008). However, it has been shown by Miyazawa and co-workers (1999) that anthocyanins found in human plasma are not in aglyconic form; hence, in contrast to flavonoids, in which glycosidic bond is hydrolyzed in the gastrointestinal tract, anthocyanins are assimilated in glycosidic form. The same authors suppose that glycosidic is the form more available in humans.
The concentration of bioactive compounds, and therefore their absorption, can be significantly increased by the intake of dietary supplements and foods that contain polyphenols. This, however, involves an increased risk arising from too high concentrations of polyphenols as antioxidants that can start a process of auto-oxidation and behave as a pro-oxidant, that is, initiate and promote the process of oxidation (Martin and Appel, 2010).

Anthocyanins and health

As shown by Martin and co-workers (2013), anthocyanins are important for their antioxidant capacity and because of their action as phytonutrients, which prevent chronic diseases. Cardiovascular diseases were shown to be reduced the moderate consumption of wine (Renaud and Lorgeril, 1992).

Many studies on wine showed beneficial effects of the polyphenolic compounds, due to their antioxidant activity that is responsible for the decrease of coronary heart disease risks and atherosclerosis (Renaud and Lorgeril, 1992) and contribute to decrease the multiplication of cancer cells and development of cancer (Hou , 2003). Red wine is known for its rich content in anthocyanins and other phenolic compounds (Mazza et al., 1999). A study carried out by Crippen and Morrison (1986), on phenolic compounds in Cabernet Sauvignon, showed the important contribute exerted by anthocyanin compounds, to the antioxidant activity of red wines. Hence, it was found that the amount of anthocyanins is important for understanding of antioxidant potency of red wines. Also in a study of De Beer and co-workers (2003) it was

recognized the importance of anthocyanin concentration in grapes in order to evaluate the antioxidant activity of red and white cultivar wines.

2.2 Acids, sugars and mineral compounds

Grape berry is a source of water (70-80%), sugars (between 150 to 250 g L^{-1}), organic acids (mainly tartaric), minerals (the main are Potassium and Copper) vitamins and pectic substances (INRAN, 2000). All these substances are in relationship each other's and their equilibrium changes during fruit grown and ripen.

The main sugars in grape juice are glucose and fructose, which are both derivates from the leaves thanks to photosynthesis and transported through the phloem Sugar relative content varies during grape ripening. At veraison, glucose and fructose are usually present in equal amounts and their ratio changes among the grape varieties. At harvest, the concentration of fructose exceeds that of glucose, hence their ratio is about 0.9-1.0 (Watson, 2003).

The organic acids are together with sugars the most abundant solids present in grape juice. They are mainly tartaric and malic acids (90% of total acids) and represent the secondary products of the sugar metabolism. In grape juices, malic acid represents the main organic acid after tartaric (Saito *et al.*, 1968); they are produced mainly in the grapes, but also in the grape skins. During the maturation process their content decrease, then it is stable, but at the same time grape sizes increase, hence there is a consequent decrease of titratable acidity.

Generally the reduction in malic acid is greater than tartaric one hence, also at maturity, their fruit contains are different. Tartaric is the main acid in grapes differently to the other fruits. As well known, malic and tartaric acids, are usually present as salts (malate, tartrate) and free acids. Their dominant form depends on variety, season, location, cultural practices, but also on state of maturity. Malic and tartaric acids are mainly in acidic form with a concentration increasing until veraison; those acids decrease at harvest, the period in which salt form is increased (Kliewer *et al.*, 1967).

Grapes contain also a combination of both tartaric and mail acids, as anions, together with potassium or calcium. The salts resulting by this combination together with the acids are important in grape juices because contribute to determine pH (Martin *et al.*, 2014).

The vine takes up minerals from the soil. They represent approximately the 7% of the dried weight of the fruit. It is well-known that environmental factors, such us water availability and temperature, may influence mineral composition of grapevine leaves and berries (Gastol *et al.*, 2014). The main mineral compounds include potassium (50% of the juice cations), sodium, iron, phosphates, sulfate, and chloride. Potassium content is increased during ripening. Its transport into fruit leads to the development of potassium bitartrate, which reduces the fruit acidity and increases the juice pH (Martin *et al.*, 2014).

In a study carried out by Kliewer (1977) it was shown that nitrogen may be responsible by a reduction of fruit ripen and colour. The latter was explained supposing the delayed fruit maturity because of the increased vegetative growth. Potassium (K) is the most abundant macro-element (Etchebarne *et al.*, 2010; Rogiers *et al.*, 2006) and its content in berry juice generally depends on soil water availability (Etchebarne *et al.*, 2010). K is important because it is an enzyme activator (Leigh et al., 1984; Walker et al., 1998) and responsible for osmotic potential regulation (Kendrick et al., 1986). K concentration in grape juice, at harvest, was higher in treatment with shaded microclimate, than the control grown in normal conditions of light. pH was in line with K concentration (Smart et al., 1985b; Rojas-Lara et al, 1989)

Hale and colleagues (1977), knowing the influence of mineral composition on grape titratable acidity, investigated the effect of potassium on malate and tartrate concentration, in

particular at grape ripeness. It was shown that, during berry development, malate concentration is initially similar between fleshes and skins. At first, the decrease of malate concentration in the fleshes depends on the dilution effect of berry growth, and then on malate degradation. On the other hand, tartrate concentration (expressed as percentage of fresh weight) fall at early ripening for dilution effect of berry growth. Tartrate concentration is usually higher in the fleshes compared with the skins, in which, at harvest, is two times less concentrated. Spayd and co-workers (2002) showed as the organic acid decrease was an effect to the increase of temperature.

As reported by Davies (2006) potassium content is in relationship with the acid levels in berries. Moreover, Hale and colleagues (1977) assessed that potassium and malate correlation start during ripening, when there is malic acidic degradation. As reported by Vikery (1973), the more potassium is concentrated the less membrane permeability is increased. It is known that malate is mainly in the cell vacuole. Hence, potassium influencing tonoplast permeability, is indirectly responsible for malate concentration; when there is an increase of potassium concentration, titratable acidity (T.A.) enhancement is due to the highest concentration in malic acid (Hale *et al.* 1977).

During fruit development K^+ increases generally to guarantee high osmotic value (Pierce and Higinbotham, 1970), while Ca^{2+} increases up to 30-40 days after anthesis and then has a decrease, likely due to the end of its action in contribution to the cell division and cell wall building (Possner et al., 1985). Davies and colleagues (2006) investigated the role of potassium in transport and accumulation of sugars during berry development, at veraison, characterized by cell multiplication followed by a stage of cell expansion after veraison. It was analyzed the action of two genes, two potassium transporters (VvKUP1 and VvKUP2) during grape development, comparing the relationship between their expression pattern with potassium accumulation (expressed as micrograms per gram of berry). Finally, it was assessed a similar tendency between them. Hence, consequently, it was suggested the association between potassium accumulation and the increase of berry size.

It was shown in a study on mandarin that there is an inverse trend between K and Ca^{2+} with an increased concentration of K in shaded fruit (Cronje *et al.* 2011). According to a study of Pereira and co-workers (2006) on grape in condition of microclimate modifications, K and Ca^{2+} content are not influenced by modification of light conditions, while between shaded and exposed treatments statistically significant differences are been shown in total N concentration. Studies on grape showed that K was in general much more concentrated than Ca^{2+} (Etchebarne et al., 2010; Sensoy et al., 2015). Mpelasoka and colleagues (2003) suggested that potassium enhances the transport of sugars into the berry, so that SST are increased by this element.

As resulted by a study of Pereira and co-workers (2006) on grape in condition of different light exposure realized by means of leaf removal at different intensity, a reduction of organic acids was enhanced by an increase of radiation perceived. Moreover, Smart and co-workers (1985b) showed that sugar content in the juice of grape berries was likely reduced by shading conditions. The hexoses glucose and fructose represent the large part of berry dry matter as shown by Coombe (1992), so they were considered to evaluate the main solutes in the juices.

In addition, considering also the differences between SST and glucose + fructose, it was shown that those absolute values are usually higher in less mature grapes (Kliewer et al., 1967). Furthermore, it is known (Pereira et al., 2006) that generally the more the berries are exposed, the more they are small, have a lower content of organic acids and nitrogen, the more are concentrated in SST.

3. LIGHT MANIPULATION

Light quantity and quality levels within the canopy were shown as the main factors influencing plant microclimate and yield and fruit composition (Champagnol, 1984; Smart, 1985a; Smart, 1987).

3.1 Manipulation of light quantity

The quantity of light perceived by plants can be efficiently manipulated by means of cultural practices (Corelli-Grappadelli, 2003). As reported by Smart and coll. (1990), canopy management is applied to vineyard with the aim to reduce the amount of leaves or changing their position, the shoot number and the yield. The most important techniques in order to improve canopy management are pruning, leaf removal and an adequate training systems. These interventions are principally aim to reduce shade, as well as decrease and improve the fruit production.

3.1.1 Defoliation

As shown in a study carried out by Hunter (1988) on the effects of *Vitis vinifera* L. cv Cabernet Sauvignon, the partial defoliation influences plant development. A consequence of partial leaf remotion was that the remaining leaves were proportionally photosynthetically more active, likely with the aim to balance the lower number of leaves. In a study carried out by de Palma and coll. (2011) it was akso hypnotized that the enhancement of photosynthesis per leaf area unit as a possible vine response to leaf removal; this enhancement seemed do not have stomatal origin.

Moreover, Hunter (1988) found that defoliated treatments, compared with not-defoliated control, received also more light. Hence, the Author suggested that the major photosynthetic activity consequential to the more radiation passing through the crop might give fruits that are more coloured. It was also supposed that in not-defoliated control the excessive foliage was an inhibition factor of the optimal photosynthetic activity of the leaves.
As reported by Smart (1990), in preveraison, leaf removal localized in the cluster zone enhances fruit exposure in dense canopies. Consequently, it is possible to improve grape quality applying defoliation, having also a decrease of the herbaceous wine characters. Moreover, it would seem that canopy density might be reduced by a removal of up to 66% leaves at veraison stage without deleteriously affecting the yield.

In several studies on *Vitis vinifera* it was showed an increased fruit coloration as a consequence of partial leaf remove (Koblet, 1987 and 1988; Marquis *et al.,* 1989). A study to investigate specific influence exerted by partial defoliation on grape pigments was carried out by Hunter (1991) on Cabernet Sauvignon. It was considered also the influence on relative SST and berry volume and on the resulting wine. It was shown that applying defoliation in four different stages of development (starting from bud break, from berry set, from pea size and from veraison), the more delayed treatment, near veraison, produces a higher value of grape anthocyanin concentration, as assessed in a previous study of Somers (1976). The average content of polyphenols per berry was not influenced by defoliation. Berry volume was positively influenced in the defoliated treatments.

The wine made by grapes produced by defoliated treatment was evaluated to have a major quality compared with the un-defoliated treatment one. Hence, as previously showed

(Smart, 1982; Smart et al., 1985b and 1988), an exaggerate crop vigour in vineyards penalizes wine quality.

3.2 Manipulation of light quantity and quality

A technological alternative to light quantity modification techniques is a specific manipulation of light quality perceived by plant tissues, aimed to modify spectral composition and, consequently, to improve plant growth, yield and quality. Plants composition of sunlight and response depends by photoreceptors, that are important co-workers aim to give specific response to specific light signals perceived by plants (Smith *et al.*, 2000; Bastías *et al.*, 2012).

The light quality has also a relevant importance for fruit crops. Modifications of light composition in the wavelength range of Red (600-700 nm) Far-Red (FR, 700-800 nm), and blue (400-500 nm), phytochrome cryptochrome sensitive respectively, are known to change some morphological and photosynthetical crop responses of the plants. For instance, classical studies on peach trees observed that a low R: FR ratio in the light available for the plant organs stimulates the shoot elongation, which is considered a typical phytochrome-mediated response (Baraldi et al., 1994). Moreover, a higher proportion of blue light respect to the red one reduces the shoot elongation inducing a dwarfing habit, which is considered a cryptochrome -mediated response (Rapparini *et al.*, 1999). As recently proved on apple, changing blue, red and far red light composition may be useful to manipulate the photosynthetic and morphogenetic process regulating the carbohydrate availability for fruit growth (Bastías et al., 2012).

Light quality manipulation can be useful to strategically modify the efficiency of specific processes, which have response influenced by light (Basile et al., 2012). Changes in the light quality and quantity perceived by plants can be realized by means of coloured nets or plastic cover films that are commonly used in agriculture to protect plants against hailstorms, flying pest and damage due to excessive exposure to sunlight (Bastías *et al*., 2012). As reported by Novello and de Palma (2013), studies on protective function of hail-nets on fruit crops by excessive irradiance started in Italy in the 1970's (Romisondo, 1968; Giulivo and Ganzini, 1971).

The crop covering with plastic film modifies the internal microclimatic mainly increasing soil and air temperature (greenhouse effect). These changes give the possibility to mature and harvest fruit out of season. However, a too high temperature limits photosynthetic and reproducing processes: in vineyard, when average temperatures under cover overcome 30 °C, the photosynthetic assimilation rate and the glucidic accumulation are reduced; consequently, a delay of fruit ripening is often observed (Winkler *et al.*, 1972; Kliewer, 1977; Di Lorenzo *et al.* 1989; Novello *et al.*, 2000a; Novello *et al.*, 2008).

An important factor to consider when the plants need be covered with either nets or film, is the shading factor, defined by Italian Standard UNI 10335/94 as the ratio between luxmeter measurements with and without the net sample. An important radiometric property in order to evaluate these coverage materials is the PAR transmittance, because the radiations in the range between 400 and 700 nm are the most important for plant photosynthesis and growth (Castellano *et al.*, 2008).

3.2.1 Use of coverage materials

The evolution of the surfaces in the worldwide plastic cover in agriculture was characterized, as regards the areas covered with plastic products, by a constant development in the period between the end of World War II and the present day. However, it results difficult to provide, especially for periods prior to the twenty-first century, a precise measure on the extent of such areas and trends of expansion. This is mainly due to lack of statistical

data for some countries and, secondly, the lack of detail figures for other nations, sometimes proved too optimistic or, on the contrary, appeared underestimated (Jouet, 2004).
In 1948, polyethylene was used for the first time as a greenhouse film by professor Emmert at the University of Kentucky with the aim to replace the more expensive glass (Anderson and Emmert, 1994; Jensen, 2004; Kasirajan and Ngouajio, 2012). Among the many reasons that led to the birth of the application of direct covers in agriculture, there is the interest of a German manufacturer of low density polyethylene. He launched a technique of semi-forcing, characterized by the only use of plastics. Hence, in 1970, the first perforated polyethylene film was used in German (Jensen and Malter, 1995). However, the diffusion of polyethylene film was in France, around 1975, when the manager of a company producing in woven towel fabric had the idea to spread the same on his garden. From that moment, the agro-textiles, typically made of polypropylene, have experienced a growing success, not only in Europe but also in the USA (A.A.V.V., 1994; Garnaud, 2000; Espi *et al*., 2006).

According to Jouët (2001), the diffusion of direct covers in the world was about 60% from 1991 to 1999, when there were about 86 000 hectares. The use of this technique has been characterized by strong growth in Europe, rather than the other continents. In the Mediterranean region, in the 90's, the application was not widespread and about 80% of the area was concentrated in France with 8 000 hectares, mainly for the production of high quality salads (Castilla and Hernandez, 1995). The greater diffusion was found in the northern European countries, particularly in the UK with approximately 9000 hectares of direct covers (Jouët, 2001). In the early years of this millennium, the technique was further developed globally (in 2002 about 105 000 hectares were estimated in the world) and especially in Europe, where there was an evolution in the amount of 20%, mainly in Germany (+ 30%) and in our country, with the grown surfaces up to 20%. A rapid development has recently characterized some regions of Africa, such as Cameroon, with 8000 hectares that showed a progression of 75% in three years. Similarly, great importance is attributed to this application in Venezuela (Jouët, 2004). The latest estimates indicate CIPA, for 2005, covers an area world to direct amounted to 111 000 hectares.

In Italy, the first applications of cover films, in the late '50, were finalized to have early ripening of table grapevine (Zito, 1957) and peach trees (Bargioni, 1957; Mercuri, 1957). In those years it was also studied the possibility to use plastic films with the aim to delay ripening (Lalatta, 1967). In recent times, as reported by Novello and de Palma (2008), the application of plastic films is increased because it is useful to meet the growing demand of extra-seasonal production.

According to Foury (2003), the direct covers are the easiest crop protection. Their peculiarity consists in placing the plastic film or non-woven tissue directly above the crop, so that the plants themselves are to hold the cover. They are characterized by the low cost and simplicity of use. The main effects due to the use of direct covers can be summarized as:

- they increase the temperature of air and soil below them, creating a microclimate most suitable to the development of vegetable crops, especially in the period immediately subsequent to the planting of young seedlings (Gimenez et al., 2002; Wells and Loy, 1985)
- they favour the growth of the plants and lead to an advance of the production (Wells and Loy, 1985)
- they constitute a physical barrier against the majority of harmful insects, thereby reducing the incidence of infection by viruses, as they prevent that the carriers reach the aerial part of the plants (Pentangelo et al., 1999).
- allow to directly carry out the irrigation, thanks to the porosity of the woven towel or the use of perforated plastic film. In addition to the single direct cover it is possible to realize a double coverage that although guaranteeing thermal conditions more

favorable to plants, may run into problems related to an excessive reduction of light transmission, with negative repercussions on growth and crop production (Benoit and Ceustermans, 1990)

Cover material used in agriculture are characterized by the type of chosen polymer (LDPE, EVA, LLDPE, etc.), the number of layers, the additive in the polymer composition (stabilizing compounds or pigments), thickness of the cover and manufacture (Espi *et al.*, 2006).
The main optical properties of the coverage materials used in agriculture are total light transmittance and haze (Waaijenberg et al., 2004). Haze is realized by means of mineral or white pigments (Espi *et al.*, 2006). The choice of their best characteristics depends on the geographic area in which the coverage is realized. For instance, in the Mediterranean area is preferred hazing materials in order to prevent plant burnings.

3.2.1.1 Plastic films

Polypropylene (PP) is generally one of the most used materials for the production of these films (Castellano, 2008). Low density polyethylene (LDPE) is used with the same aim, because of its good transparency to solar radiations and its convenience compared to PVC and EVA. The LDPE is poor in both anti-droplet and screen properties (Takakura, 1989; Novello, 2000). Drop-like behaviour is one of the disvantage of plastic films because of their hydrophobic nature (Gbiorczyk et al., 2002). The combination of two coverage materials is often used in order to have cover films with the best properties. For instance, the combinations of low and high density polyethylene, i.e. the co-extruses LDPE+EVA and HDPE+LDPE are used in a study carried out by Novello (2000) on *Vitis vinifera* cv. Matilde. The cover materials were compared and results showed their different transmittance to solar radiation (380-1110 nm), 88 and 80%, respectively. Moreover, the same co-extrused films (LDPE+EVA and HDPE+LDPE), have approximately the same transmittance in the range of wavelengths between 7500 and 12500 nm (long IR): 35 and 34%, respectively. Long infrared radiations (IR) are emitted by soil and other solid bodies and they influence temperature under covers; it is important to use a film with no excessive transmittance in IR_{long} region in order to avoid the lost of thermal energy that reduces the air tenìmperature especially during the nights with low temperature. The major transmittance to sunlight, together with IR restrained under LDPE+EVA cover film, enhanced, in *Vitis vinifera* cv. Matilde, budbreack date and berry ripening date because it increase the air temperature under cover (Novello, 2000).

Transparent plastic films are useful in order to delay the dispersions and hold a part of the energy accumulated thanks to radiation that pass through them. This aspect resulted useful, for instance, to enhance vine budbreak. The other stages of development can be also influenced by the use cover films, especially the early ripen in the grapes. For instance, it is possible to have an early ripen grapes. Grape quality can be improved by the protective cultivation (Novello and de Palma, 2008).
It is know also the existence of termic films, opaque to infrared and transparent to PAR radiations, useful in order to advance the vegetative development and with the aim to obtain an early harvest. The termic capacity of these films can be due to the use of mineral additive (synthetic or natural silicates, sulphates, etc.) or the combination or coextrusion of copolymers of EVA or EBA. It also exist film be modified in the NIR range using reflecting materials, with the aim to avoid the excessive heating effect under cover. These films contain pigment absorbing in the PAR and NIR regions of the spectrum, reflecting platelets, interference materials. Overall, they are made in order to selectively exclude the radiations responsible for the increase of temperature but it is not necessary for plant photosynthesis (Espì et al., 2006).

Plastic films filter the sunlight modifying the quantity and the quality of the radiation input into the system culture. In addition, plant microclimate is influenced by plastic film application with several possible effects on physiology and quality of production. For instance, modification of quanti-, qualitative of production and timing of harvest, can be found, with a dependence by variety, plastic film properties and type of coverage (de Palma *et al*, 1999; Novello *et al*., 2000a; Tarricone, 2003; Novello *et al*., 2004). The techniques of semi-forcing by means of film coverage can be used in order to delay or to anticipate the harvest (de Palma *et al.*, 2002)

The films have spectroradiometric properties that can be regulated by adding a quantity of polymeric compounds in function of the desiderate transmissivity (Castellano, 2008). It is possible to influence microclimatic change, covering vineyard by means of plastic films, to advance or delay ripeness, at the end or in the first part of annual cycle, respectively. Film protection structures are close in case of "early-covering", partially closed, with the lateral part of vineyard uncovered, in "late covering". Both the types of covering were shown to modify microclimatic conditions, but they need the correct management especially concerning temperature, with the aim to have a good quality product. (Novello and de Palma, 2008).

The main characteristic of both coloured nets and plastic films in agriculture is that the radiation, passing through the coloured covering material, is modified in a specific region of the spectrum. A coverage material appears to have a specific colour because it transmits mainly radiations correspondent to that that specific colour and it will absorb its complementary colours. Hence, it could be possible to choose the colour of cover material according to radiation useful to enhance a specific plant developmental process (Castellano, 2008).

Schettini and co-authors (2011) worked to investigate the effects of spectral modification of solar radiation when photoluminescent and photoselective films are used on peach and cherry trees. In particular, the photoselective films were shown to enhance shoot growth in both peach and cherry trees because of the R:FR ratio decrease, with a reduction from 1.14 in open field to 0.93. The same consistent growth of shoots together with a rapid production of biomassa was obtained in a previous study on *Triticum durum,* cv Cappelli, carried out by De Salvador and co-workers (2008). It was showed that the quality of solar radiation can be altered by means of covering films, influencing photomorphogenesis, with the aim to improve cultivation; this is possible, but it was suggested the importance to consider that the results can change depending on the total amount of radiation, species, wavelength range involved and temperature. Photoselective plastic covers films were developed during 1990s. They contain pigment to selectively absorb or reflect specific wavelengths, aimed, in particular, to reduce temperatures under the cover film and to avoid damage to plants; in this way it is also possible to reduce energy and cooling requirements when the crop coverage is total (Verlodt et al., 1999).

3.2.1.2 Anti-hail nets

The anti-hail nets are known to protect fruit and ornamental crop by hail, wind, snow and strong rainfall. Photoselective netting, early used in agriculture primarily for protective function, can be also employed in order to screen out particular spectral bands of light, with the aim to positively influence physiological processes dependent by light (Shahak et al., 2004b). It is possible to choose among three main kinds of nets: Italian mono wire woven; English double woven thread, resistant to hail storms; Raschel, consisting of a chain knitting that give a particular resistance to wind High density polyethylene (HDPE) was generally used for nets. The nets can be made by Polypropylene (PP) that is also the favourite material

for making plastic cover films (Castellano, 2008). In addition, biodegradable materials can be used in agriculture but they are rarely employed because of their high costs and lower resistance to physical and mechanical damages due to climatic conditions, in particular solar radiation (Scarascia et al., 2004).

In 2004 were developed coloured nets in order to modify light quality and quantity intercepted by plants growing underneath. These nets generally include pigment absorbing UV, blue, green, yellow, red, far-red or infrared wavelengths with a different relative rate (Rajapakse and Shahak, 2007).

Net colour depends on the used mix of chromatic additives in High density polyethylene. Light quality is unmodified when passes through the net holes, while it changes if arrives to the threads. Modifications depend on the additives contained in the plastic and on the knitting design (Shahak *et al*, 2004a and b). Sometimes transparent or semitransparent threads are used in order to reduce excessive radiation that can damage plants (Castellano, 2008). Light passing through the nets can arrive to the plants filtered, unmodified or diffuse (Shahak *et al*, 2004b).

Transparent nets are commonly used on table grape vines in Southern Italy, Apulia region in particular, with the aim to protect shoots, inflorescences and grapes from hail, but also to protect vine by direct visible radiations (Novello and de Palma, 2013).

Colored nets are filters of solar radiation, exerting function of physical protection and changing spectral light composition. The first applications of nets were in ornamental plants. Oren-Shamir (2001) and Ovadia (2009) with co-workers, studied the development of ornamental crop grown under photoselective net. The work highlighted the increasing of vegetative vigour under red and yellow net, dwarfing under the blue one and selective effects on leaf size and flowering time. In horticulture, it was shown that coloured nets decrease photosynthetically active radiation (PAR) and Red:Far-red ratio. It was also found the potential of coloured shade nets in order to manipulate light quality and increase the quantity of diffuse light (Stamps et al., 2009), known as responsible for the increment in radiation use efficiency and yields (Gunter *et al.*, 2008). Gu et co-workers (2002) showed that diffuse radiation is more efficiently used by plants.

On fruit crops, initial efforts aim to control quantity of light by means of coloured plastic film and nets were carried out with an important difference: the shading factor used in the case of fruit crops is lower than the factor used for ornamental crops which usually arrives to 50%. This choice is due to the greater number of physiological stages during fruit trees development and a higher shading factor can reduce the productivity (Shahak *et al.*, 2004b)

Shade nets generally scatter mainly UV radiation (Shahak *et al*., 2004; Wong, 1994), because cover films and nets are product together with additive substances, aimed to protect them by UV-radiation damages (Wong, 1994). Scattered light is important for plants because has the capacity to penetrate into dense canopy. It has a R/FR ratio lower than natural light.

It is well-known the importance of red, far-red and blue spectra perceived by plants. For instance, studies on peach trees showed the dependence of phytochrome response by R:FR ratio, and the mediation of cryptochome depending on the relative amount of blue and red light. The proportion of blue and red light was assessed as important because influences the photosynthesis rate (Bastías *et al*., 2012).

Nets are useful because of their protective function from excess of irradiance, reducing net photosynthetic rate of the leaves, as reported by Shao (2014), in a research on shade nets. Retamales and co-workers (2006) showed that net shading could be also useful for reducing temperatures and prevent photosynthesis inhibition.

In a study carried out by Shahak and co-workers (2004b), it was recognized the importance of the use of coloured nets in agriculture because of their protective function by excessive solar radiation, environmental hazards and flying pests. The research confirmed that

covers reduce the total radiation and the PAR available for the crop. Moreover, it was assessed that this decrease can be seen most markedly using plastic film rather than hail nets. It was also showed that the attenuation of radiation perceived by plants changes during the day (Rana et. al., 2004).

As showed by a study carried out by Rana and coworkers (2004), covering technique influences microclimate, acting on leaf water potential Ψ_b and stomatal conductance (C_s). In the same research, it was assessed how stomatal conductance was influenced by coverage technique: after irrigation, at first has maximum value in uncovered thesis, medium under net and minimum under plastic film. Seven days after irrigation, Cs acquires opposite trend, so it is maximum under plastic film. Under nets and films, compared to uncovered treatment, vineyards have lower air temperatures and higher air humidity near cluster (Rana et. al., 2004).

Using photoselective films, it is important to know that a high concentration of dye inside them provokes a reduction of red and far-red light transmission, but it is also decreased the transmission of photosynthetic light. Therefore, it is important to consider this factor, to avoid fruit quality decrease due to photosynthetic light reduction (Rajapakse *et al.*, 1999). The amount of photosynthetic rate decrease obtainable under nets depends on the percentage of shading and the differences can be perceived after ten days of treatment. For instance, Shao and coll. (2014) comparing 5%, 20%, 30% and 50% light-shading treatments assessed that the 50% thesis have maximum reduction of net photosynthetic rate. This difference increases after 40 days of treatment. The highest net photosynthetic rate was observed in plants under 30% of shading. Plants grown in 30% shading conditions, show more developed chloroplast in compare to excessively irradiated treatments and optimized light absorption thanks to increased chlorophyll content per unit leaf area (Wittmann *et al*., 2001; Shao *et al*., 2014).

Studies on apples show that the main effects on fruit growth are due to the reduction in light availability. Photosynthetically active radiation, B:R and R:FR the light relations can influence plant grown so, manipulation of light transmission is important for plants. Several studies demonstrate the possibility of using photo-selective shade nets to manipulate light spectrum perceived by apple trees (Bastías *et al*., 2012). For instance, it was known that red cover, increases light transmission in the range of Red (600 – 700 nm) and Far Red (700 – 800 nm) (Oren-Shamir *et al.*, 2001)

Bastías *et coll*. in 2012 show that photo-selective nets affect apple fruit growth, modifying photosynthetic (leaf photosynthesis) and morphogenetic (leaf area, shoot growth) process on Carbonium assimilation rate and carbohydrate availability for fruit growth. Results depend on net color. Blue net for example, when compared to red net, increased the midday leaf stomatal conductance in apple trees, incrementing Photosystem II efficiency. Red and blue nets on apple orchard reduce PAR by 27%. Blue net can indirectly enhance photosynthesis , Red:Far-red ratio, total leaf area and fruit weight (Bastias *et al.*, 2012).

Lobos and coll. (2012) studied in *Vaccinium corymbosum* L. the influence of red, white and black nets with several shading intensity (25%, 50% and 75%), compared to full sun (control). In that study, several differences among treatments were found. Firstly, the red net strongly decrease the visible spectrum (400–700 nm) and increase the infrared wavelengths. Secondly, under white nets UV radiation is decreased, with a consequent little reduction of visible spectrum, while the amount of infrared radiation is higher than control; there are no effects on light environment when black nets are used. As reported in recent studies by Oren-Shamir et al. (2001), Shahak et al. (2004a) and Al-Helal and Abdel-Ghany (2010), white nets, comparing to the other ones, have the highest proportion of diffuse light.

Study on apple trees under 30% shading ColorNets (Red, Blue, Grey and Pearl) and 15% shading White and Red net, shown for all experimental nets, an increase of fruit set, a larger fruit size; the latter characteristic, enhanced under red nets, is probably favoured by a

lower potential due to a reduced water stress under nets. Also red fruit color is more visible in fruit grown under all colored nets (Shahak *et al*., 2004). A study on blueberry (Vaccinium corymbosum L.), showed that white, gray and red nets, with maximum shading factor of 50%, enhance yields because improve fruit set; flower induction was not influenced by treatments (Retamales *et al.*, 2008).

Moreover, a study on coloured nets carried out by Shahak (2004) on peach orchard shown that size and red coloration of fruit are enhanced by the use of all employed nets. In addition, the peaches growing up under red and yellow nets are firmer, sweeter and larger. Applied nets do not influence acidity. Fruit set is favoured by the influence of red net, while fruit size is enhanced by grey, red, yellow and pearl nets and reduced by blue net. In the same study it was not demonstrated a reduction in productivity caused by the use of nets, but beneficial effects also on trees, such as health and absence of moulds, that are present on the control (Shahak *et al*., 2004).

The synergic effect of colour and porosity of the net was shown to influence Far red light composition. The latter is enhanced by the increase of the net brightness together with the decrease of porosity. Far red light was shown higher in beige, orange and white treatments than in green and black ones. As suggested by Shahak (2004b) comparing net knitting with the same design, spectrally modified light depends on chromatic additives contained in the plastic meshes. Studies by Oren-Shamir et al. (2001), Shahak et al. (2004a) and Al-Helal and Abdel-Ghany (2010) showed that white nets, comparing to the other ones, have the highest proportion of diffuse light; as reported by Stamps (2009), diffuse light is important because increases radiation use efficiency, influencing plant development and growth. Moreover, in a study on light modification by means of nets, it was shown by Lobos and co-workers (2012) that Red net enhances light transmission in Red and Far Red region of the spectrum. As reported also in some studies by Oren-Shamir (2001), Shahak (2004b), Abdel-Ghany and Al-Helal (2010) and coll., Neutral nets, compared to the other ones, have the highest proportion of diffuse light.

Abdel and coll. (2010) assessed that the diffused beam transmittance (t_{bd}) is very poorly influenced by net colour, porosity and construction, although the latter affects slightly more control compared to the others. Total direct beam transmittance (t_b) is positively influenced, in particular by porosity, but also by colour and brightness. For instance, the diffused beam represents about the 2% and the 20% of the total beam under black and blue nets

3.3 Influence of light manipulation on fruit composition

As shown by Gonzalez and co-workers (2015) manipulation of vine light environment can improve grape quality increasing its nutraceutical power; changing light quality perceived by photoreceptors, i. e. phytochromes and cryptochromes, light manipulation could have positive effects on photomorphogenesis, so that crop and quality of production can be improved.

On the other hand, the coloured plastic film influence the yield in both quantitative and qualitative way because they modify the relative absorption in specific range of wavelengths. For instance, in some experiments, yellow and purple films improved productivity and precocity, while gray and red nets are known to increase the skin colour. Red and pearl-coloured nets are shown to enhance fruit size (Tesi, 1999; Shahak *et al.*, 2004b).

It was assessed the potential of coloured shade nets with the aim to manipulate light quality and increase the quantity of diffuse light, known as responsible of the increment in radiation use efficiency and yields (Stamps *et al.*, 2009). Gu et co-workers (2002) showed that diffuse radiation is more efficiently used by plants and it causes less easily saturation. Shade

nets generally scatter mainly UV radiation, because cover films and nets are product adding additive substances aimed to protect them by UV-radiation damages (Wong, 1994). In a recent study carried out on *Vitis vinifera*, it was found that an addition of red light at cluster level. At harvest, results showed that the sugar content of the fruits, titratable acidity or berry size are unaffected by red light add-on (González *et al.*, 2015).

3.3.1 Light manipulation and fruit phenolic content

Downey and co-workers (2006) showed that viticulture practices (canopy management, irrigation, yield regulation, and timing of harvest) could influence the content of flavonols, anthocyanins, and proanthocyanidins in grapes. Light manipulation was also showed to influence grape phenolic content.

A clear understanding of the influences exerted by light manipulation in vineyard and grape quality is far from being elucidated. It is important to consider that light transmitted by cover films, passing throughout canopy was modified by leaves absorption before arrive to cluster. As shown on apples, crop foliage absorbs the main amount of radiations reducing the differences perceived above the crops (Awad *et al*., 2001). Hence, considering a vineyard, the light perceived in the inner position of the crop, where grape clusters grow, was significantly lower compared to that in the outer positions of the canopy.

Moreover, it was assessed that, considering a given site, in the same conditions of soil and nutrition, sunlight and temperature exert the predominant influence on grape quality. The effect of cluster light exposure on growth and composition, during the main stages of grape development, were investigated in a study carried out by Dokoozlian and coll. (1996) on Cabernet Sauvignon and Pinot noir grapevines (*Vitis vinifera* L.). light exposed treatments show greater concentrations of skin phenolic compounds, anthocyanins in particular, compared to unexposed treatments. Each cultivar needs a minimum intensity of light under which, in the skins of grapes, there is no production of anthocyanins (Kliewer, 1977). Light exposition can also positively influence flavonol concentration in fruit and leaves. The more the tissues receive sunlight, the more flavonols were present inside them (Downey *et al.*, 2004).

Furthermore, the effects of light manipulation change according to the grape developmental stage in which modifying intervention occurs. It is known that berry grown can be divided in two phases: from bloom to pre-veraison; from veraison to fruit ripening (Robinson and Davies, 2000; Coombe and McCarthy, 2000). During the first phase, berries grow through division and expansion of cells, flavonols and proanthocyanidins are produced in the skins. Only a few anthocyanins are synthesized. At veraison proanthocyanidin synthesis continue until 1 to 2 weeks after veraison, anthocyanins and sugar are increased and titratable acids is reduced (Bogs *et al.*, 2005). Hence, it is important to decide the stage of grown in which manipulation should start, because flavonols, flavan-3-ol monomers, and proanthocyanidins are biosynthesized during the first phase of berry growth, whereas anthocyanins are biosynthesized during fruit ripening.

According to Cheng and co-worker (2015), when photo-selective plastic films are used to modify light quality perceived by *Vitis vinifera* (cv. Yatomi Rosa) there are effects on soluble solid content (°Brix), which is higher in grape grown under blue films than in the berry grown under red, orange, green and white ones. Grapes harvested under blue and white films are more richly coloured than the others. At harvest, anthocyanin composition is similar between grapes grown under blue and white films. Anthocyanins in red, orange and green film treatments are similar and less concentrated than the other ones. In the study is shown that blue film increases the activity of some genes in grape-berry, PAL and CHI, which encode enzymes involved in the anthocyanin biosynthesis.

It was shown in grapes that the activity of phenylalanine ammonia-lyase, the main enzyme in the secondary metabolism, is improved by light exposure of fruit in the first stages of growth in order to grant the maximum accumulation of anthocyanins in the next growth stage (Roubelakis-Angelakis and Kliewer, 1986). Hence, as supposed by Takeda (1988) light is important during the initial berry growth, presumably enhancing the concentration or activity of the enzymes responsible for anthocyanin biosynthetic. During the third stage of growth, accumulation beginning light is important to preserve the maximum activity of these enzymes during ripening.

In a study on *Vitis vinifera* cv Red Globe, it was assessed that white net with about 10-15% of shading factor was responsible for a decrease in berry-skin colour and in weight of both berries and cluster, caused by the lower production of photo-assimilate (Pugliese, 2009).

polyphenol concentrations are significantly lower when UV radiations are filtered, (Pollastrini *et al.*, 2011). The phenols are known to absorb UV-B light, hence, reduce its penetration trough essential tissues (Li et al. 1993, Burger and Edwards 1996, Bieza and Lois 2001), but also act as antioxidants (Markham et al. 1998, Gould et al. 2002, Steyn et al. 2002). Polyphenol synthesis enhancement may be due to the protective function of these antioxidant substances by stress conditions. Studies of light shading influence on the phenolic composition in grape berries show that phenolic content has about a double concentration when there is no shading (Spayd *et al.*, 2002; Downey *et al.*, 2004b; Cortell and Kennedy, 2006).

Several studies concerning anthocyanins showed their protective function on the photosynthetic tissues against photoinhibition, under light stress conditions. In those conditions, anthocyanins are known to exert their protective function by filtering visible light (Smillie and Hetherington, 1999).

During the synthesis of anthocyanins in apple skins the range of visible spectrum between 640 nm and 670 nm (red light) was shown to be effective, with maximum adsorption occurring near 600 nm and similar to red cabbage, turnip and mustard seedlings (Siegelman, 1957). Lange and coll. (1971) confirm that a major relative preponderance of the longer wavelength radiations, especially red ones, over the others are responsible for enhanced synthesis production of anthocyanin and flavonoid. Also in a study carried out by Zhou and Singh (2004) on cranberries (*Vaccinium macrocarpon* Ait) it was demonstrated that Red light is more effective in increasing the total anthocyanin content (41.5%), than far-red light (34.7%) compared with a dark control sample. Red and UV radiations are well known to enhance anthocyanin synthesis in fruit skins (Arakawa, 1991; Bakhshi and Arakawa, 2006; Bastias *et al.*., 2012). In a study carried out on *Vitis vinifera*, it was found that an addition of red light at cluster level stimulate polyphenolic synthesis, anthocyanins in particular. In the same study, at harvest, it was assessed that red light supplementation, directed on the clusters, was responsible for the enhanced synthesis and accumulation of phenolic compounds, especially anthocyanins, in grape berry skins. The response was mediated by specific berry photoreceptor that is phytochrome (González *et al.*, 2015). Hence, an increase of red light in proportion to the other wavelength seems to be useful to improve fruit quality.

Moreover, anthocyanins have the capacity to absorb a part of light incident on chloroplasts, changing it in quantity and quality (Krol *et al.*, 1995; Ntefidou and Manetas, 1996). The red anthocyanins present in vegetative tissues preferentially absorb green and ultraviolet (UV) light, a lower quantity of blue light and a little red light (McClure, 1975). Absorbance of blue-green light by anthocyanins reduces light available to chlorophyll (Pietrini & Massacci, 1998; Smillie & Hetherington, 1999) in proportion to the anthocyanin concentration (Neill & Gould, 1999). This presents a mechanism to modulate light absorption in accordance with environmental and developmental requirements (Pietrini and Massacci, 1998). In a study carried out by Cheng and co-workers (2015), photo-selective plastic film

bags were used to modify light quality perceived by clusters of *Vitis vinifera* (cv. Yatomi Rosa). According to results, at harvest, clusters grown covered with blue and white bags, had grapes more richly coloured compared to the other treatments (Red, Orange, and Green). Hence, there were effects on anthocyanin concentrations: Neutral film had a major content compared with the Red treatment. In a study on apples (Malus pumila Mill.) white light and UV-B light singularly and in synergy where shown as factors responsible to stimulate anthocyanin production (Arakawa et al., 1985).

4. EXPERIMENT I:

DEFOLIATION INFLUENCE ON ANTHOCYANIN COMPOSITION OF NEGROAMARO WINE GRAPE

4.1 Materials and Methods

a) Preliminary assessments

Negroamaro

Fig. 4.1 – Negroamaro grape.

Negroamaro is an autochthonous wine grape cultivar of Southern Italy, which is becoming very important for the Italian wine market (Toci et al., 2012). This cultivar grows almost exclusively in Apulia region. Wine produced by Negroamaro grapes is characterised by a very deep colour, with a very rustic character, appreciated by the consumers for its perfume that, together with an earthy bitterness, give a pleasant organoleptic complexity (Capone et al., 2013). Negroamaro was classified as a cultivar with mid-early period of budding, early both in flowering and in veraison time. Also physiological maturity of the berry has been defined as early.

This variety is characterized by clusters of average size and length, medium compactness. Berry grapes are spheroid, with small and blue-black colour skins and seeded and coloured fleshes. The grapes have neutral taste (Italian Vitis database, 2015)

It was found that Negroamaro and other Apulia cultivars (Uva di Troia and Primitivo) according to its anthocyanin content, are characterized by a high percentage of Delphinidine-3-glucoside and Cyanidin -3-glucoside but no acylated forms. (Lovino *et al.*, 2006).

In this experiment, different light microclimates at cluster-zone were induced by applying four “defoliation” treatments.

The field part of the experiment was carried out, in Southern Italy (Apulia region), at a vineyard located at a private farm (Conti Zecca, Leverano, Lecce province, 40º17'0"N, 18º5'0"E). Negroamaro variety was grafted onto 140 Ru rootstock, at 1.0 x 2.5 m apart, trained to Vertical Shoot Positioned, and pruned to 5 two-bud spurs per vine. Shoots were topped after overcoming the last trellis wire. The vineyard was managed according to the normal farm cultural practices. Soil is deep and rainfalls are abundant during winter, thus the vineyard, although provided with drip irrigation system, normally does not receive irrigation water supplies. Since June of 2009, the farm experimented different types of leaf removal, included the mechanical one.

In this trial, four defoliation treatments were applied when vines were in the phenological stage of “berry pea size” (early June): DA=cluster-zone farm defoliation, that is, hand removal of all leaves and laterals from the base of the shoot until the first node above the last cluster; MD= cluster-zone mechanical defoliation low intensity, obtained by an appropriate machine regulation; D50%=defoliation of almost half of the vine canopy,that is, hand removal of the main leaf and the lateral shoot, at alternate nodes, from the base to the top of the canopy; ND= non-defoliation, that is, no leaf removal (Fig. 4.2).

Fig. 4.2 – Defoliation treatments: ND (above left)**, MD** (above right), **D50%** (below left)**, DA** (below, right)**.**

The removed leaf area was estimated using the weight-to-area ratio (250 leaf dishes per treatment, of known area, were cut and immediately weighted). Measurements were taken on 5 single-vine replicates per treatment.

The Red:Far red ratio (R:Fr) of the light interceptable at the cordon level was measured at the phenological stage of “majority of berries touching”, in late June, when summer climatic conditions were still mild, and at the stage of “veraison”, in late July, when climate became hot and dry. Readings were taken when sun was over the vine row (25 readings per

treatment, SKR 660/730 sensor, Skye Instruments). It is a considered a reliable indicator of the light microclimate at the cluster-zone. At veraison, the midday temperature of illuminated bunch portions was also measured, using an infra-red thermometer (TRI-88 Lafayette Electronic Supply Inc.) (25 readings per treatment), and the percentage of bunches with sunburn damages was visually assessed.

At the farm harvest (late September), the yield components were evaluated on 10 vines per treatment. On five 100-berry sample per treatment, total soluble solids, pH and titratable acidity (expressed as tartaric acid) were assessed. About 100 kg of grapes per treatment were wine-processed according to a protocol already described (Suriano and Tarricone, 2006). At wine raking (end of October), concentrations of total polyphenols and flavonoids (both expressed as (+)catechin), total anthocyanins (expressed as malvidin monoglucoside) and proanthocyanidins (expressed as cyanidin chloride) were analyzed according to Di Stefano and coll. (1989). Moreover, the wine alcohol by volume, the color intensity (sum of spectrophotometric absorbance at 420, 520 and 620 nm; spectrophotometer Shimadzu UV-1700) and tonality (420/520 nm absorbance ratio) were also assessed (according to the EC 2676/90 regulation). Wine analyses were performed in triplicate.

Data were statistically processed by means of the ANOVA analysis; for the significant sources of variation, the average values were separated by means of the Duncan test (ASSISTAT 7.7 software procedures).

b) Anthocyanin profile

Reagents used for the study are: Acetonitrile anhydrous of HPLC-grade (99.8%), Perchloric acid solution, Tartaric acid, Methanol and Waters for HPLC were obtained by Sigma-Aldrich; Ethanol used to extract the polyphenols was obtained by Carlo Erba Reagents;

Anthocyanin profile was obtained by means of Revilla and coll. HPLC method (2000) with some differences. Individual anthocyanins were identified by using HPLC Agilent 1200 instrument on a column Zorbax SB C18 (100 mm X 4.6 mm, 1.8 µm; Agilent, Santa Clara, CA), protected by a guard column, and eluted at a flow rate of 0.5 mL/min. DAD detector (model G1315C) was used to measure the UV absorption of the effluent.

Tab. 4.1 – Linear gradient used for the separation of phenolic compounds

Time (min)	Solvent A(%)	Solvent B (%)
0	95	5
4.8	90	10
16.8	80	20
21.6	70	30
31.2	60	40
40.8	55	45
48	0	100
58	0	100

Grape extracts, after passing through a nylon membrane filters (0,45 µm – diameter of 25 mm) in order to remove solid particles from it, were injected in the chromatographic column.

The mobile phase consisted of a linear gradient of water – acetonitrile (50:50) as solvent B and water – acetonitrile (95:5) as solvent A at a flow-rate of 0.5 ml/min (Tab. 4.1). Both solvents were adjusted to pH 1.8 with perchloric acid.

At 520 nm, it was possible to carry out qualitative analysis thanks to comparison of the retention times (min) and maximum absorption peak among the resulting chromatograms and the spectra of the different pure standards. Column temperature was set to 25 °.

4.2 Results and Discussion

a) Preliminary assessments

Assuming that D50% treatment removed about 50% of the vine foliage, Negroamaro total leaf area per vine at the stage of "berry pea-size" was estimated about 2 m^2. Since at that stage shoots were still young, most of the leaf area was concentrated in the proximal third of the canopy, that is, at the cluster zone. Assuming that all vines had same total leaf area when the three treatments were applied, DA removed about 63% of the vine leaf area, resulting the most severe treatment, while MD removed about 32%, that is, almost one half of the leaf area removed by DA. Hence, D50% resulted in a quite intermediate defoliation intensity (Tab 4.2). In late June, the R:Fr of sunlight filtering at the cordon level was 0.42 in DA and almost half in MD: the "shading difference" between these treatments was coherent with their "defoliation difference". On the other hand, the R:Fr at the cordon of MD and D50% canopies was almost the same: in the latter treatment, the leaf removal along all the foliage modified the relationship between defoliation intensity and canopy shading respect to the treatments were leaf removal was concentrated at the cluster-zone. In non defoliated vines, the shading at the cordon level was higher by 80% respect to DA, and by 65% respect to MD.

In late July, at veraison, the R:Fr at the cordon level of defoliated vines decreased because of the shoot growth and their related shade; this decrement was relevant in DA vines, where it reached about -30%. At veraison, R:Fr at cordon level of DA vines was still about 50% higher than that found in the other defoliated vines.

Tab. 4.2 – Estimated percentage of removed leaf area, R:FR of sunlight filtering at the cordon level, temperature of illuminated bunch portions, percentage of sunburned bunches in Negroamaro vines, after defoliation treatments.

Parameter	ND	MD	D50%	DA
Defoliation (%)	-	32.3 ± 1.40 c	51.03 ± 0.69 b	63.4 ± 4.6 a
Cluster-zone R:Fr (late June)	0.08 ± 0.03 c	0.23 ± 0.02 b	0.24 ± 0.04 b	0.42 ± 0.04 a
Cluster-zone R:Fr (late July)	0.12 ± 0.02 b	0.19 ± 0.03 ab	0.21 ± 0.04 ab	0.30 ± 0.01 a
Temperature of sun exposed bunch portions (°C) (late July)	32.9 ± 0. 5 c	36.20 ± 1.51 ab	35.00 ± 1.42 b	37.90 ± 1.45 a
Sunburned bunches (%) (late July)	0.00 ± 0.00 b	15.00 ± 5.10 a	15.00 ± 5.00 a	18.00 ± 4.18 a

Within row, different letters indicate significant difference at $p \leq 0.05$.

At this time, the pattern of differences in temperature of illuminated bunch portions did not follow properly that of the cordon R:Fr, since the temperature of MD bunches did not differ from that of D50% ones. The air temperature in late July was very high, with maximum temperatures of 40 °C for some consecutive days; this factor likely produced a thermal inertia in grape tissues. On the other hand, the bunch temperature was 2.9 °C higher in DA than in D50%, pointing out the strong effect induced by the total leaf removal around clusters on the berry thermal regime. Nonetheless, the percentage of bunches with sunburns, although slightly higher in DA vines, did not differ among defoliated vines. The temperature of small illuminated bunch portions of ND vines was "only" 32.9 °C: this treatment was the only one that did not show sunburn damages on grape.

At farm harvest, it was seen that, among defoliated vines, the grape yield increased with the leaf surface that had been left at the time of defoliation, that is, at the "berry pea size" stage (Tab. 4.2); nonetheless, grape yield of non defoliated vines did not differ from that of MD vines. Compared to DA vines, which produced each about 2.6 kg, the yield increased by 16% in D50% and by 31-35% in ND and MD vines. This response seemed related either to the number of bunches that achieved the ripeness or to the berry weight. Compared to the about 2.4 g of DA berry mass, D50% berry was 13% lighter, while ND and MD had berry was 22% and 33% heavier. It is like to suppose that both the leaf surface (by "source-sink" relationship) and the microclimate at the cluster level (by berry water loss due to transpiration) affected final the berry weight.

Tab. 4.3 – Yield components at farm harvest in Negroamaro vines, after defoliation treatments.

Parameter	ND	MD	D50%	DA
Grape per vine (kg)	3.38 ± 0.25 a	3.49 ± 0.48 a	3.00 ± 0.34 ab	2.58 ± 0.23 b
Bunches per vine (n)	10.60 ± 0.86 ab	12.80 ± 1.80 a	11.80 ± 1.07 ab	10.40 ± 1.07 b
Bunch weight (g)	341.74 ± 38.84 a	273.24 ± 32.12 ab	250.45 ± 17.59 b	263.61 ± 27.64 b
Berry weight (g)	2.89 ± 0.20 a	3.17 ± 0.18 a	2.14 ± 0.07 c	2.37 ± 0.07 b

Within row, different letters indicate significant difference at $p \leq 0.05$.

Berry juice reached about 22 °Brix in D50%, but only 19 °Brix in MD (-12 %) which did not achieve an optimal ripeness (Tab 4.4); grapes of the other treatments had intermediate TSS level. The best result obtained in with D50% treatment was likely related to an improving of canopy illumination, and thus of potential leaf CO_2 uptake per leaf area unit, allowed by the regular leaf thinning along the canopy.

The titratable acidity into the Negroamaro berry juice was penalized by the high summer temperature, especially in DA and D50% treatments, which clusters were less leaf-protected than in ND and MD; temperature are well known to increase the cell respiration and thus the organic acid respiration, reducing the titratable acidity. The pH differed significantly only between MD, that had the lowest value (3.36) and all the other treatments (that ranged around 3.4); the lowest MD pH is coherent with the highest acidity of berry juice of this treatment.

Tab. 4.4 – Technological parameters, at farm harvest, of Negroamaro grapes after defoliation treatments.

Parameter	ND	MD	D50%	DA
Total Soluble Solids (°Brix)	20.44 ± 0.24 ab	19.32 ± 0.41 b	22.00 ± 0.35 a	20.64 ± 0.39 ab
Titratable Acidity (g/L)	6.69 ± 0.12 a	6.75 ± 0.13 a	6.47 ± 0.28 ab	6.01 ± 0.16 b
pH	3.42 ± 0.02 a	3.36 ± 0.02 b	3.49 ± 0.02 a	3.43 ± 0.02 a

Within row, different letters indicate significant difference at $p \leq 0.05$.

The wine alcohol increased according to the berry juice TSS concentration. It was 12.93% in D50%, 12.33% in DA, 11.52% in ND and 11.36% in MD; all the differences were statistically significant. The wines obtained from DA and D50% vines were significantly richer in all the classes of polyphenols than those obtained from MD and ND vines (Tab. 4.5). The MD treatment was not able to improve the phenolic performance of Negroamaro respect to non defoliation treatment. Even worse, MD penalized anthocyanins richness respect to ND, probably due also to the combined effect of "not-too-high" light intensity and "not-to-low" temperature that MD induced at the cluster-zone. The importance of these two micro-environmental factors on anthocyanin and total phenol accumulation has been deeply investigated (Dokoozlian and Kliewer, 1996; Haselgrove *at al*., 2000; Bergqvist *et al*., 2001; Spayd *et al*., 2002).

Tab. 4.5 – Indices of total phenol content and colour of wines obtained by Negroamaro grapes after defoliation treatments

Parameter	ND	MD	D50%	DA
Total polyphenols (mg L^{-1})	915.66 ± 4.06 c	905.00 ± 44.80 c	1182.00 ± 20.82 b	1488.66 ± 11.41 a
Total flavonoids (mg L^{-1})	802.61 ± 12.72 b	759.67 ± 2.91 b	930.00 ± 8.89 a	975.671 ± 28.43 a
Total anthocyanins (mg L^{-1})	144.33 ± 2.19 b	121.33 ± 1.86 c	207.67± 4.33 a	213.00 ± 3.06 a
Total proanthocyanidins (mg L^{-1})	564.66 ± 8.57 c	564.33 ± 27.91 c	783.66 ± 31.06 b	949.00 ± 10.97 a
Color intensity (A420+A520+A620)	4.86 ± 0.35 c	5.19 ± 0.12 c	7.31 ± 0.28 b	8.57 ± 0.41 a
Color tonality (A 420/520)	0.78 ± 0.02 ab	0.83 ± 0.01 a	0.72 ± 0.03 bc	0.67 ± 0.01 c

Within row, different letters indicate significant difference at $p \leq 0.05$.

The DA treatment, respect to the D50% one, improved significantly the wine total polyphenol content (+26%) and the proanthocyanidin content (+21%). The anthocyanin richness was almost the same. These results seem to indicate that the phenol store of Negroamaro grape, if adequately improved by a high illumination regime, is quite resistant to the oxidation induced by high temperatures. Although DA and D50% wines had very close anthocyanin content, the former, that derived from more lighted grapes, had a higher color intensity and an almost better color tonality than the latter. Moreover, the color tonality of D50% wine was intermediate between that of DA and ND wine, while the color tonality of

MD wine was the worst. The investigation of grape anthocyanin profile could give information helpful to explain some of these occurrences.

b) Anthocyanin profile

The following anthocyanins detected in the grape skins of MD, D50% and DA Negroamaro treatments, in crescent order of quantity, are: Cyanidin, Malvidin, Keracyanin (Cyanidin-3-*O*-*β*-rutinoside), Cyanidin 3-*O*-glucoside, Peonidin 3-*O*-glucoside, Delphinidin-3-*O*-glucoside and Malvidin-3-*O*-glucoside. That crescent trend in concentration was followed by MD, D50% and DA treatment, while it changes in ND, in which Keracyanin is more concentrated than Cyanidin 3-*O*-glucoside.

Anthocyanin accumulation was not reduced by defoliation, probably because it was not severe, hence temperature was not excessively increased by defoliation causing anthocyanin decrease shown by Mori and co-workers (2007).

The concentration of total identified anthocyanins was highest in the grape skins of farmer defoliation (DA) treatment. It resulted that Negroamaro wine obtained from "farm defoliated" vines (DA) was the richest in total polyphenols and total anthocyanins (+14 % compared to D50% thesis, 100 % compared to MD). The average values of total anthocyanin identified follow the same trend of the first part of the study.

Malvidin 3-*O*-glucoside was the most concentrated anthocyanin, as it happens in most of grapes (Ribereau-Gayon, 1959). In Negroamaro it ranged between 62-69% of total anthocyanin content detected in the extract prepared in ethanolic solutions and 74-78% in those extracted in tartaric solution (pH=3.2) prepared in order to simulate polyphenol extraction during wine-making process.

It seems (Tab. 4.7) that leaf-removal was likely responsible for the enhancement of total anthocyanin concentration, especially Malvidin 3-*O*-glucoside, the major concentrated grape skin anthocyanin. Moreover, on percentage, it was more representative in not defoliation condition compared to the other treatments. This difference can be seen either in total or in tartaric skin extracts. In tartaric extract of polyphenols Malvidin 3-*O*-glucoside was reduced by defoliation of about 3%, 4% and 7% in MD, D50% and DA, respectively. In total skin extracts, it was decreased of about 5%, 7% and 4% in MD, D50% and DA, respectively. In total extracts of polyphenols, the anthocyanin percentages were lower than those of the tartaric extracts, likely because less anthocyanins were identified in the latter extracts, in which there was no Keracyanin.

Tab. 4.6 – Average retention times (min) of identified anthocyanins in Negroamaro grapes –ethanolic and tartaric solutions

Tr (min)	*Ethanolic solutions*				*Tartaric solutions*			
Anthocyanins	ND	MD	D50%	DA	ND	MD	D50%	DA
Dp 3-O-gluc	17.30	17.55	17.34	17.26	17.53	17.51	17.47	17.59
Ker	20.12	20.44	20.17	20.06	-	-	-	-
Cn 3-O- gluc	20.93	21.22	20.98	20.90	21.16	21.14	21.10	21.22
Pn 3-O- gluc	24.56	24.68	24.45	24.47	25.40	25.38	25.36	25.44
Mv 3-O-gluc	25.89	26.13	25.90	25.86	26.02	26.01	25.98	26.04
Cn	30.69	31.09	30.72	30.67	30.82	30.81	30.79	30.86
Mv	39.40	39.45	39.44	39.37	39.60	39.57	39.55	39.65

Both not-glycosylated and mono-glycosylated anthocyanins were identified. No acylated forms were found in both ethanolic and tartaric extracts of polyphenols. Glycosylated anthocyanins identified in this study can be classified in Cyanidin-derivatives (Cyanidin 3-*O*-glucoside, Keracyanin, Peonidin 3-*O*-glucoside) and Malvidin-derivatives (Delphinidin-3-*O*-glucoside and Malvidin-3-*O*-glucoside).

The 3-monoglucoside form represented about 99% of the total identified pigments in our study, both in ethanolic (fig. 4.7) and tartaric extracts (fig. 4.8). Hence, grape skins were very poor in of aglyconic form of Cyanidin and Malvidin. Individual anthocyanins were in fact very sensible to oxidation, so after their biosynthesis they were stabilized by means of *O*-Glycosylation and then stored in cell vacuoles (Jackson, 2008). Anthocyanins, alongside the other phenolic compounds, can be modified by hydroxyl, acyl, glycosyl and methyl groups, which influenced some characterististics of plant tissues. Cyanidin 3-O-glucoside, the stable form of cyanidin, was glycosylated by uridine diphosphoglycosyltransferase enzime (VvGT1 or 3GT), condition that needs to be satisfied before passing from cytosol to vacuole with the aim to accumulate during fruit ripens (Offen et al., 2006). Hydroxyl group at the position 3 of the heterocyclic C-ring, was know to give instability to anthocyanidins in physiological conditions (Barton et al., 1999) therefore, as reported by Lucker and coworkers (2010), UDP-glucose:flavonoid 3-O-glucosyltransferase enzyme (3GT) lead glycosilation to promote their stabilization.

Tab. 4.7 - Anthocyanin content in berry skins of Negroamaro grapes

Anthocyanins	ND		MD		D50%		DA	
	mg kg^{-1} skins	%*	mg kg^{-1} skins	%*	mg kg^{-1} skins	%*	mg kg^{-1} skins	%*
Dp 3-O-gluc.	256.7 ± 22.1 d	9.8	378.3 ± 47.4 c	11.4	612.7 ± 62.3 b	11.9	754.6 ± 39.5 a	13.8
Ker	157.6 ± 14.8 b	6.0	165.9 ± 15.6 b	5.0	303.1 ± 31.7 a	5.9	274.1 ±14.9 a	5.0
Cn 3-O- gluc.	139.7 ± 9.9 c	5.3	240.9 ± 26.3 b	7.3	409.4 ± 38.3 a	7.9	357.8 ± 38.8 a	6.6
Pn 3-O- gluc.	232.2 ± 23.2 c	8.9	368.8 ± 37.3 b	11.2	590.9 ± 52.3 a	11.4	452.7 ± 45.7 b	8.3
Mv 3-O-gluc.	1807.4 ± 118.3 b	69.0	2122.6 ± 274.5 b	64.2	3193.9 ± 334.1 a	61.8	3563.0 ± 342.5 a	65.3
Cn	6.6 ± 0.4 d	0.3	8.1 ± 0.9 c	0.2	11.8 ± 0.3 b	0.2	13.8 ± 0.4 a	0.3
Mv	18.4 ± 2 b	0.7	21.5 ± 2.2 b	0.6	42.4 ± 3.9 a	0.8	38.7 ± 3.1 a	0.7
Total	2618.6	100	3305.9	100	5164.05	100	5454.6	100

* percentage on content of identified anthocyanins
different letters indicate significant difference at $p \leq 0.05$

Concentrations (milligrams per kilogram of skins) and relative percentage of anthocyanin identified in skin extracts obtained in ethanolic solutions, under different vine defoliation conditions. (ND= not-defoliated control; DA = cluster zone farm hand defoliation; MD = cluster zone low intensity mechanical defoliation; D50%= hand canopy defoliation, from the canopy base to the top. at alternate nodes. Dp:Delphinidin; Ker: Keracyanin; Cn: Cyanidin; Pn: Peonidin; Mv: Malvidin)

In our study, anthocyanins with some hydroxyl groups methylated, Peonidin 3-*O*-glucoside, Malvidin and Malvidin 3-*O*-glucoside were contained from 74 to 79%, with a maximum percentage value in ND, intermediate in MD (76%) and minimum in D50% and

DA. Considering tartaric extracts, methylated forms prevail in ND and MD (85%) and it was slightly less concentrated in D50% (-3%) and minimum in DA (-3% compared to D50% treatment).

Fournier-Level (2011) and co-workers reported that, when phenolic B ring is methylated, anthocyanins increase their stability. Glycosylation on C3 and methylation are regulated by the same gene. Methylation of anthocyanins follows glycosylation process because O-methyltransferase enzyme has a higher relative specific activity for glycosylated substrates (Lucker *et al*., 2010); this may be the reason of the lowest content of unmethylated anthocyanins in grape composition and, consequently, the highest content in Malvidin 3-O-glucoside.

The reason of the predominance of Malvidin in black grape berries were explained by Fournier-Level (2011), who shown as the same gene controls the transcription of two important genes of anthocyanins biosynthesis: UFGT and AOMT, responsible for glycosylation on C3 and methylation, respectively. Moreover, it was shown that methylation of Delphinidin 3- glucoside into both Peonidin 3- glucoside and Malvidin 3-O-glucoside, and the methylation of Cyanidin 3-O-glucoside into Peonidin 3- glucoside were all catalyzed by the same enzime (O-methyltransferase) (Hugueney *et al*., 2009). As reported by Ibrahim and co-workers (1998) O-methyltransferases (OMTs) are the enzymes responsible for methylation of anthocyanins and other polyphenols in plants.

Tab. 4.8 - Anthocyanin content in berry skins of Negroamaro grapes

Anthocyanins	ND		MD		D50%		DA	
	mg kg^{-1} skins	%*	mg kg^{-1} skins	%*	mg kg^{-1} skins	%*	mg kg^{-1} skins	%*
Dp 3-O- gluc.	266.7 ± 46.61 bc	11.1	135.3 ± 11.0 c	7.5	385.9 ± 19.0 b	11.3	580.6 ± 117.1 a	13.7
Cn 3-O- gluc.	83.0 ± 4.1 c	3.5	126.5 ± 8.0 bc	7.0	200.7 ± 22.9 ab	5.9	267.5 ± 58.2 a	6.3
Pn 3-O- gluc.	136.7 ± 6.04 c	5.7	172.3 ± 9.7 bc	9.5	259.8 ± 32.3 ab	7.6	332.3 ± 64.5 a	7.8
Mv 3-O-gluc.	1883.7 ± 179.5 bc	78.4	1357.4 ± 73.3 c	75.0	2528.2 ± 116.8 ab	74.1	3017.4 ± 487.0 a	71.1
Cn	17.2 ± 2.7 b	0.7	9.5 ± 0.9 b	0.5	17.1 ± 1.8 b	0.5	22.8 ± 3.7 a	0.5
Mv	13.8 ± 1.7 bc	0.6	9.5 ± 0.7 c	0.5	18.3 ± 3.8 ab	0.5	21.5 ± 3.1 a	0.5
Total	2402.1	100	1810.4	100	3410.0	100	4242.2	100

* percentage on content of identified anthocyanins
different letters indicate significant difference at $p \leq 0.05$

Concentrations *(milligrams per kilograms of skins)* and relative *percentage of anthocyanin identified in skin extracts obtained in tartaric solutions, under different vine defoliation conditions. (ND= not-defoliated control; DA = cluster zone farm hand defoliation; MD = cluster zone low intensity mechanical defoliation; D50%= hand canopy defoliation, from the canopy base to the top. at alternate nodes. Dp:Delphinidin; Ker: Keracyanin; Cn: Cyanidin; Pn: Peonidin; Mv: Malvidin)*

Moreover, it was shown that the di-methylated malvidin 3-glucoside during fruit ripen was transported from cell cytosol to vacuole in order to be accumulated, with efficiency higher than the unmethylated delphinidin 3-O-glucoside, therefore the first has a major

anthocyanin accumulation in grape skins (Francisco *et al.*, 2013). However, it was showed that methylation of free hydroxyl groups increases metabolic stability and improved health effects due to their antioxidant effect (Walle, 2009).

Results are in line with a study of Lovino and co-workers (2006), in which Negroamaro was compared to other Apulia cultivars (Uva di Troia and Primitivo) and classified, according to its anthocyanin content, as a variety containing, in particular, a higher percentage of Delphinidine-3-glucoside and Cyanidin -3-glucoside but no acylated forms.

It was shown that different hydroxylations and glycosylation of anthocyanins influence their antioxidant properties. Antioxidant activity measured by means of ORAC (automated oxygen radical absorbance capacity) assay, as shown by Wang and co-workers (1997), decrease among Cyanidin-3-*O*-glucosyde, Cyanidin-3-*O*-rhamnoglucoside (Keracyanin) and Cyanidin: glucose and rhamnoglucose increased antioxidant activity of the same aglycone, i.e. Cyanidin. Hence, in ND treatment there is a slight prevalence of Cyanidin-3-*O*-rhamnoglucoside (+1%) compared to Cyanidin-3-*O*-glucosyde so a slight lower antioxidant activity compared to the other treatments.

Fukumoto and Mazza (2000), using different methods of analysis found that anthocyanin antioxidant activity, on average, is enhanced by the increase of hydroxyl groups and the decrease of glycosylation. Three hydroxyl groups on the B ring of flavonoids gave more antioxidant activity to molecule. When one hydroxyl group is lost, there is a slight decrease of antioxidant activity; it was significantly reduced when two hydroxyl groups are lost.

By means of ORAC assay the following mean value has been resulted in decreasing order of antioxidant activity: Cyanidin 3-O-glucoside (3.491 ± 0.011), Cyanidin (2.239 ± 0.029), Malvidin (2.009 ± 0.167), Delphinidin (1.809 ± 0.068), Peonidin 3-O-glucoside (1.805 ± 0.014), Malvidin 3-O-glucoside (1.404 ± 0.052). Therefore, in our study, D50% and DA treatments, having a higher content of Cyanidin 3-O-glucoside and Peonidin 3-O-glucoside, in addition to a maximum content of Malvidin 3-O-glucoside (fig. 2), presumably had more antioxidant activity compared to the other treatments. The major antioxidant activity can be seen as a caracteristic improving grape quality because may increase its nutraceutical property.

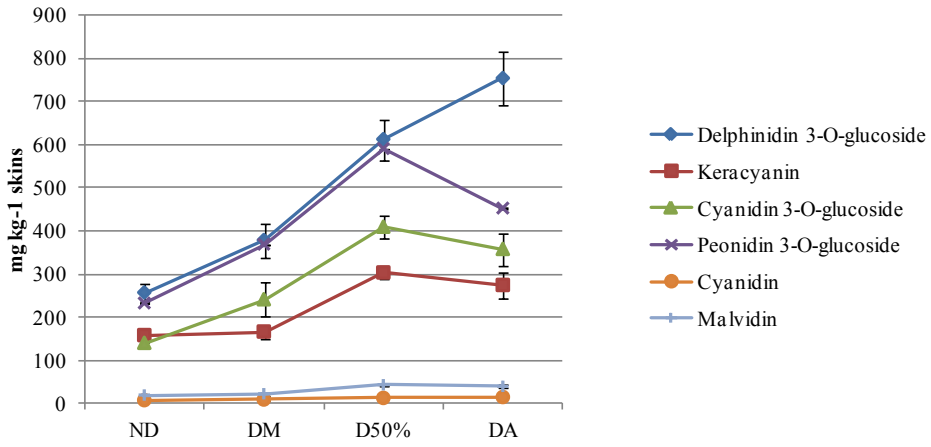


Fig. 2 – Concentration of all identified anthocyanins, except Malvidin 3-O-glucoside, expressed in milligrams per kilogram of skins, from the experimental control (ND**) to the more defoliated treatment (**DA**) (***vertical lines represent standard errors from the means***).**

According to average concentrations, Keracyanin, Cyanidin C-3-*O*-glucosyde, Peonidin 3-*O*-glucoside and Malvidin had approximately the same tendency among the treatments (fig. 4.3). They had maximum concentration in D50% thesis, intermediate in DA and DM and minimum in ND. The only exception was Keracyanin content in ND, because of its higher concentration compared to Cyanidin 3-*O*-glucoside. Considering that Keracyanin, Cyanidin-3-*O*-glucosyde, Peonidin 3-*O*-glucoside are Cyanidin-based derivatives, it is possible to suppose that the defoliation likely influenced, in particular, the concentration of these compounds, increasing their concentration, mainly in D50%. The latter is the treatment with intermediate foliage remotion (about 48%), not limited to cluster zone, but distributed on the vine, from the canopy base to the top. The found differences may be due to the more diffuse defoliation.

The lower content of cyanidin-based derivative anthocyanins in DA compared with D50% may be likely due to the increase of light perceived by cluster when defoliation was more intensive. When leaves remotion was maximum and limited in the cluster zone as in DA treatment, Cyanidin-derivated had a concentration between D50% and DM treatment. Among the three defoliated treatments, the minimum average content of the Cyanidin-based derivative anthocyanins were found in condition of less intensive defoliation DM (about 32%). However, it is well-know that the relative proportion of cyanidin- and delphinidin-based anthocyanins was controlled by genes. (Castellarin *et al*., 2006) that was likely influenced by different levels of defoliation. Cyanidin concentration among treatments had approximately the same trend of its derivated with DA content slightly more concentrated than D50%.

Malvidin was the less concentrated anthocyanin in all treatments; in crescent order was in treatment ND, DM, D50%, and DA, the same tendency of its glycosilate form.

It is interesting that Delphinidin-3-*O*-glucoside was much more concentrated in DA treatment than in the other ones (fig. 4.3). It is possible to note the same behaviour of its methylated form, Malvidin 3-*O*-glucoside, that is a Delphinidin-based derivative, as Delphinidin-3-*O*-glucoside. Delphinidin-3-*O*-glucoside had concentration similar to Peonidin 3-*O*-glucoside, in ND, DM and D50%, except for DA, respect to which Delphinidin-3-*O*-glucoside resulted about 1.7 times more concentrated. In cv Negroamaro, Cyanidin 3-*O*-glucoside is more present in all the defoliated theses, than in ND control.

Hence, in DA, the highest defoliated treatment (about 63%), Delphinidin- based derivatives were more concentrated in compare with the other treatments, while cyanidin-derivates were tendencially less concentrated than D50%, the treatment with intermediate defoliation (about 48%). In D50% treatment, cyanidin- based derivatives forms were the most concentrated among thesis. The similar concentration between Delphinidin-3-*O*-glucoside and Peonidin 3-*O*-glucoside in all treatments except for DA (fig. 4.3), may depend on light conditions, that influencing the regulation of anthocyanin synthesis, was likely responsible for the enhancement of the more stable Delphinidin 3-*O*-glucoside, intermediate of Malvidin 3-*O*-glucoside sinthesis. The methylation of both Delphinidin 3- glucoside and Cyanidin 3-O-glucoside into Peonidin 3- glucoside and Malvidin 3-O-glucoside, and into Peonidin 3-glucoside, respectively, were all catalyzed by the same enzyme (O-methyltransferase) (Hugueney *et al*., 2009), hence it is possible to suppose an effect of light in the regulation of the activity of this enzyme.

Malvidin and Delphinidin concentration in their mono-glucosidic forms, seemed to be enhanced by farm hand defoliation near clusters (DA) that was also the treatment with maximum defoliation (63%), in which much more light has been perceived by grapes. The highest exposure, together with a higher temperature were shown to reduce the ratio between dihydroxylated and trihydroxylated anthocyanins in a study on Merlot grape carried out in different conditions of fruit exposition (Tarara *et al*., 2008). In our study, dihydroxylated and

trihydroxylated anthocyanins were Cyanidin and Delfinidin, respectively. The treatment more exposed to light is DA, in which Delphinidin 3-*O*-glucoside was the most concentrated, while Cyanidin 3-*O*-glucoside was decreased by the highest light exposition due to defoliation (fig. 4.9). Hence, our results are in line with Tarara and co-authors (2008).

In slightly shading conditions, as in D50% in which the main leaves and the lateral shoots were removed, at alternate nodes, from the canopy base to the top (D50%), results showed a content of cyanidin- based derivatives higher than MD, that is the treatment with the lowest intensity of defoliation (about 63%).

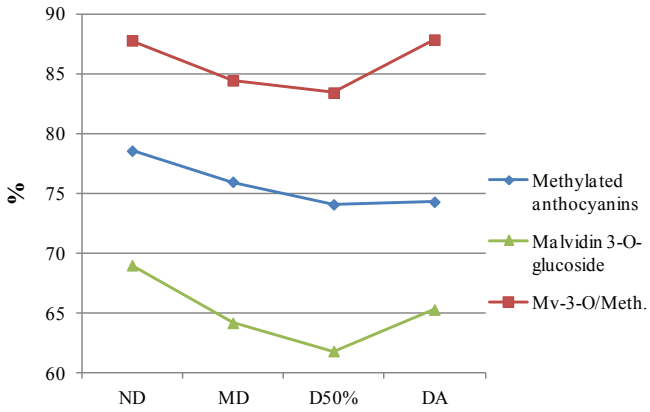


Fig. 4.4 – Comparison among the percentages of Malvidin 3-O - glucoside (Mv 3-O**), methylated anthocyanins and rate between Mv 3-O and methylated anthocyanins in skin extracts of Negramaro, from experimental control (**ND**) to the more defoliated treatment (**DA**).**

The percentage values of total methylated-anthocyanins were compared with the most representative of them, i. e. Malvidin 3-*O*-glucoside. It was possible to see (fig. 4.4) that in D50% and DA treatment methylated anthocyanins had similar percentage concentration although the lower content of Malvidin 3-*O*-glucoside in D50%; this fact may be explained considering that the other methylated anthocyanins, Peonidin 3-*O*-glucoside with Malvidin, are more represented in D50% than in DA.

The slight difference between relative anthocyanin concentrations may influence wine colour. As shown by Fournier-Level and coll. (2011) berry colour is influenced not only by pigment concentration, but also by their proportion, depending by different factors, such us the level of anthocyanin methylation and hydroxylation. The increase of hydroxyl group number enhances blue intensity, while methylated groups exert the same influence on wine red colour intensity (tab. 4.5). The wine made by grapes from the most defoliated treatment (farm defoliation) was evaluated as richer in colour intensity. The reason may be the most concentrated anthocyanins.

Overall, the total anthocyanin concentrations such us average contents of methylated anthocyanin, are significantly highest in the treatments of leaf removal named as DA and D50% (table 4.8), that are the thesis with more leaves-remotion (about 63% and 48% respectively), compared with MD, in which only about 32% of leaves were removed. It seems that anthocyanin accumulation was likely reduced by shading conditions (Rojas-Lara *et al*, 1989), suggesting that this is presumably, in our experiment, the reason for the less anthocyanin content, at harvest, in the grapes of the more shaded treatments.

Anthocyanins contribute to influence wine colour (hue, brightness and saturation) because of their concentration and composition (He *et al*., 2012). It has been well-known that berry

colour is influenced not only by pigment concentration, but also by their proportion, depending by different factors, such us the level of anthocyanin methylation and hydroxylation. The increase of hydroxyl group number enhances blue intensity, while methylated groups exert the same influence on red color intensity, that is important especially because of its influence on wine colour (Fournier-Level *et al.*, 2011).

Moreover, it may be that by MD treatment was produced the wine with the worst colour tonality because of its lower percentage content of Delphinidin 3-*O*-glucoside and Malvidin 3-*O*-glucoside. DA treatment contained not only less Delphinidin 3-*O*-glucoside than D50% and DA, as ND treatment, but also the lowest percentage of Malvidin 3-*O*-glucoside.

In addition, the wine obtained by DA treatment, compared to the other ones, had the highest Delphinidin 3-*O*-glucoside percentage content that give blue nuance to the wine, influencing positively the wine intensity.

5. EXPERIMENT II:

INFLUENCE OF LIGHT MICROCLIMATE ON ANTHOCYANIN PROFILE OF A BLACK-BERRY TABLE GRAPE VARIETY

5.1 Materials And Methods

a) Preliminary assessments

Fig. 5.1 – Black Magic grape.

Black Magic variety (Codreanca or Kodrianka) is a hybrid of *Moldova* x *Marsaliskii* origined from *Vitis labrusca,* constituted in the institute of Viticulture in Chisinau in Moldavia in 1849. This cultivar has a good productivity, early ripen and it is characterized by sensitive to colatura and to millerandage, especially in cold and rainy springs and fertile land. It has medium-high resistance to downy mildew, powdery mildew and *Botrytis cinerea Pers*. Black Magic variety produces a table grape with cluster medium-large, conical-pyramidal, weighing approximately 400-500 g. The savoury berries are blue-black, oblong, of medium size and they weigh about 6.5 g. Grapes are poorly resistant to transport, because of the easy detachment of the berries from the rachis (Clematisy, 2015; ASSOFRUIT, 2015; Vitifera, 2015).

Productivity is about 12-13 t ha^{-1} (Sauron, 2015). Cultivated areas are about: 100 ha in Italy, 300 ha in Moldova, 50 ha in Ukraine, 50 ha in Russia, 20 ha in Serbia, 80 ha in North Africa, 30 ha in ex-Yugoslavia and 20 ha in Moldova (Pizzuto, 2013). The cultural costs are medium-high, requiring the thinning of the grapes and the forcing with plastic sheeting to anticipate maturation and harvesting. It is a cultivar very sensitive to low temperatures during the flowering stage. It is very fertile, so the pruning should be average, 8 buds per fruit cane are usually sufficient to have a good production. The grape flesh has a neutral flavor and good

resistance to crushing. Shelf life is estimate about 13 days (ASSOFRUIT, 2015; Clematisy, 2015; Vitifera, 2015).
In Apulia region, carpometric increases of about 25-35% can be obtained by producing clusters of about 500-600 g. The harvest occurs between late July and the second week of August. In "early semi-forcing" the grapes arrives to 15-17 ° Brix between the second and third decade of July. The variety is characterized by is high vigour and the canopy density. The actual fertility is about one cluster per bud (de Palma *et al*., 2012).

In this experiment, the light microclimate was manipulated using photoselective plastic covers.

The field part of the experiment was carried out, in Southern Italy (Apulia region), at a vineyard was located at a private farm (Bari province, Lat. N 41° 8'; Long E 16° 24'). Cv. Black Magic was grafted onto 34 E.M. rootstock, at 2.3 x 2.3 m apart, trained to overhead *tendone* trellis, Puglia type, and pruned to four 10-bud canes. Crop load was regulated at one cluster per shoot. Vineyard received about 2000 m^3 ha^{-1} of irrigation water.

Since the beginning of March 2009, the vineyard was protected with a "closed type" polyethylene plastic cover. The top of the cover was realized with three different types of films: Neutral, Yellow and Pink-red coloured. This trial was conducted in two consecutive years. Each type of cover represented a treatment; the treatment consisted of three replicates, each of one formed by a group of three adjacent rows; the replicates were alternated (Fig. 5.2). After the stage of berry pea-size, the lateral plastic sheets were wound up in order to improve air circulation and reduce air overheating, according to the best practices.

Fig. 5.2–Vineyard covered with photoselective and neutral plastic films.

In mid June and July of the first year, the light filtering through the plastic film was measured, over the canopy, by means of a solar bar, in the 400-700 nm wavelength range (AccuPAR PAR/LAI LP-80, DecagonDev.); the air temperature and relative humidity were measured at several canopy height. These measurements pointed out that the light filtering through the covers decreased from Neutral (74%) to colored films (Yellow 66%, Pink 63% of that available outside); the air temperature (about 34 °C) and humidity (38%), did not change among treatments.

Black Magic grapes were "farm-harvested" in the last week of July. Ten bunches per replicate were sampled (from the central row) in order to assess the grape quality parameters. Bunch weight, berry number per bunch and berry weight were recoded. Fifty berries per replication were sampled to analyze, after crushing, juice total soluble solids (TSS, digital

refractometer Atago VM-7) and titratable acidity (TA, determined using standard EEC methods and expressed as tartaric acid). Before berry crushing, the skin chromatic coordinates were measured by means of a colorimeter (CR 400, Minolta Co.;CIE L*a*b* system). Moreover, on 20 berry samples per replicate, the main skin phenol content was assessed, that is, total polyphenols, total flavonoids (both expressed as (+)catechin), proantocyanidins (as cyanidine chloride), and total anthocyanins (as malvidin monoglucoside). The phenolic analyses were performed according to the method described by Di Stefano and Cravero (1991).

The reagents used were Ethanol to polyphenols extraction, obtained by Carlo Erba Reagents and Sodium hydroxide 1 N by J.T.Baker.
Data were statistically processed by means of the ANOVA analysis; for the significant source of variation, the average values were separated by means of the Duncan test (ASSISTAT 7.7 software procedure).

b) Anthocyanin profile

The reagents used in this part of the study were: Acetonitrile anhydrous of HPLC-grade (99.8%), Perchloric acid solution, Tartaric acid, Methanol and Waters for HPLC obtained by Sigma-Aldrich; Ethanol used to extract polyphenols was obtained by Carlo Erba Reagents. Anthocyanin profile was obtained by means of Revilla and coll. HPLC method (2000) with some differences. Individual anthocyanins were identified by using HPLC Agilent 1200 instrument on a column Zorbax SB C18 (100 mm X 4.6 mm, 1.8 µm; Agilent, Santa Clara, CA), protected by a guard column, and eluted at a flow rate of 0.5 mL/min. DAD detector (model G1315C) was used to measure the UV absorption of the effluent.

Tab. 1 - Linear gradient used for the separation of phenolic compounds

Time (min)	Solvent A(%)	Solvent B (%)
0	95	5
4.8	90	10
16.8	80	20
21.6	70	30
31.2	60	40
40.8	55	45
48	0	100
58	0	100

Grape extracts, after passing through a nylon membrane filters (0,45 µm – diameter of 25 mm) with the aim to remove solid particles from it, were injected in the chromatographic column. The mobile phase consisted of a linear gradient (Tab. 1) of water – acetonitrile (50:50) as solvent B and water – acetonitrile (95:5) as solvent A (Tab. 5.1) at a flow-rate of 0.5 ml/min. Both solvents were adjusted to pH 1.8 with perchloric acid.

At 520 nm, it was possible to carry out qualitative analysis thanks to comparison of the retention times (min) and maximum absorption peak among the resulting chromatograms and the spectra of the different pure standards. Column temperature was set to 25 °C.

5.2 Results and discussion

a) Preliminary assessments

The carpological characteristics of Black Magic grape were not significantly affected by the treatment (Tab. 5.2).

In the first year the cluster weight ranged about from 630 g (Neutral film) to 660 g (Yellow film), and berry weight ranged from 6.20 g (Yellow film) to 6.55 g (Neutral film); a compensation between berry number per bunch and berry weight occurred, hence, the bunch mass showed only very small and not significant differences. Consequently, very small differences of productivity *vs*. Neutral film were expected (+2% with Pink film, +4% with Yellow film), since the number of buds per vine and the number of bunches per shoot were regulated and uniformed at the time of the winter pruning and of the summer pruning. By the way, the carpometric values were very close to those typical of this varieties, that is characterized by a medium sized berry (~ 6 g) and a medium cluster weight (~ 600 g).

In the second year, the berry number per bunch and, at a much lesser extent, the berry weight were lower than in the first year (from -25% to -31% for berry number and from 6% to 8% for berry weight; the highest percentages corresponded to the Yellow film). As a consequence, also the bunch weight decreased (from -25% for grapes under Yellow cover to -17% for grapes under Pink cover) respect to the first year. Differences between treatments were quite small and not statistically significant, but the grape under the Pink film showed a slight tendency for a higher berry number per bunch (+8%) and, thus, for a higher bunch weight (+8%). An analogous slight difference of productivity could be estimated between treatments.

Tab. 5.2 – Carpological traits of Black Magic table grape produced under different plastic cover.

Parameter	*1st year*			*2nd year*	
	Neutral film	Yellow film	Pink film	Yellow film	Pink film
Cluster weight (g)	628.30 a ± 70.62	655.6 a ±38.18	641.50 a ±83.42	493.15 a ±37.38	532.31 a ± 42.65
Berry/bunch (n)	93.30 a ± 9.3	105.30 a ± 8.49	103.50 a ± 14.52	72.20 a ± 4.96	78.30 a ± 9.70
Berry weight (g)	6.55 a ± 0.17	6.20 a ± 0.19	6.21 a ± 0.23	5.71 a ±0.33	5.81 a ± 0.39

Within row, different letters indicate significant difference at $p \leq 0.05$

At the date of harvest, the Neutral cover gave, in the first year, the highest berry sugar concentration (16.43 °Brix), likely due to its highest light transparency, and thus, to its potential capability to allow a higher rate of leaf photosynthesis. The lowest TSS was obtained with the Yellow film (14.63 °Brix), to which corresponded a slightly heavier bunch. This TSS difference was statistically significant (Tab. 5.3). The grape produced under the Pink film reached an intermediate of sugar level, but had the lowest acidity (3.95 g L^{-1}); consequently, it resulted in the highest sugar/acid ratio (40), that is an important index of maturity for table grapes. At the same date, the TSS/TA ratio was about 35 and 30 with and

Neutral and Yellow films, respectively. According to the OIV resolution VITI 1/2008, the black-berry table grapes are considered ripen with TSS ≥16 °Brix; when TSS is <16 °Brix and >12.5 °Brix, table grapes are considered ripen if the TSS/TA ratio is higher than 20. Hence, in the present experiment, all the grapes were ripen, and thus the decreasing TSS/TA order may be considered as an order of decreasing advance in berry ripening among treatments.

Tab. 5.3 – Technological parameters of Black Magic table grape produced under different plastic covers.

Parameter	*1st year*			*2nd year*	
	Neutral film	Yellow film	Pink film	Yellow film	Pink film
Total Soluble Solids (°Brix)	16.43 a ±0.47	14.63 b ± 0.31	15.56 ab ± 0.23	17.72 a ±0.51	15.36 b ± 0.42
Titratable Acidity (g/L)	4.36 a ± 0.10	4.83 a ± 0.11	3.95 b ± 0.10	5.57 a ±0.14	5.70 a ± 0.23
TSS/TA	35.79 b ± 1.67	30.36 c ± 0.93	39.60 a ± 0.51	31.96 a ±1.41	27.20 b ± 1.62

Within row, different letters indicate significant difference at $p \leq 0.05$.

In the second year, grapes under the Yellow cover reached a markedly higher TSS concentration (+21%) respect to that found in first year: this increment was very similar to the decrement observed for cluster weight. At the opposite, the grape under Pink film had almost same TSS concentration of the first year, hence, it did not benefit of the productivity decrement. By comparing the two treatments in the 2nd year, the Pink one had a significantly lower TSS concentration and TSS/TA ratio (-13% and -15%, respectively). In this year it was noticed that the Pink film appeared as visually deteriorated it terms of transparency (more opaque) and of mechanical resistance (it was broken in some points). This fact, which probably depended on the additive incorporated in the plastic material, likely reduced the transmittance of the Pink cover to the solar radiation; this factor, in turn, could exerted a negative effect on the leaf functionality and on the final accumulation of berry sugar.

Black Magic berry skin colour, in the first year changed significantly among treatments: the brightness (L*) and the red component (a*) of the skin color (that is opposite to the green component) increased progressively moving from the Neutral to the Pink and, finally, to the Yellow cover (Tab. 5.4).

In the second year, the values of a* were slightly lower, and those of b* were a little higher (less negative) respect to the first year, irrespectively of the treatment. The brightness (L*) varied between the treatments, but the difference was very small.

At a visual level, it was not possible to appreciate these small differences of berry skin color.

The skin phenol content, which was quite high in Black Magic table grape, in the first year decreased passing from the Neutral cover, to the Yellow and to the Pink one (Tab. 5.5), although the differences in total polyphenol and in proanthocyanidin and anthocyanin content did not reach any statistical significance (due to a wide range of variation among replicates).

Tab. 5.4 – Colorimetric coordinates of Black Magic table grape produced under different plastic covers.

Coordinate	*1st year*			*2nd year*	
	Neutral film	Yellow film	Pink film	Yellow film	Pink film
L*	23.57 b ± 0.35	26.87 a ± 0.36	24.88ab ± 0.31	26.47 a ±1.44	26.87 a ± 0.78
a*	1.84 b ± 0.08	4.72 a ± 0.19	3.02ab ± 0.14	1.07 a ±0.62	2.40 a ± 0.78
b*	-1.09 a ± 0.69	-0.89a ± 0.14	-1.26 a ± 0.08	-0.67 a ± 0.29	-0.54 a ± 0.3

Within row, different letters indicate significant difference at $p \leq 0.05$.

In the second year, the overall skin phenol content of grapes produced with Yellow cover was much higher than in the first year (from +69% for total polyphenols to +109% for total anthocyanins), while that of grapes produced with Pink cover increased at a lower extent (from +18% for total proanthocyanidins to +36% for total anthocyanins). Both treatments seemed to benefit, in terms of skin phenol accumulation and concentration, from the lower productivity of this year, but at a markedly different extent. For all the classes of skin phenols, grapes under the Yellow cover reached a significantly higher amount.

Tab. 5.5– Indices of total phenol content in berry skin of Black Magic table grape produced under different plastic covers.

Phenol compounds (mg kg^{-1} of grapes)	*1st year*			*2nd year*	
	Neutral film	Yellow film	Pink film	Yellow film	Pink film
Total Polyphenols	842.91 a ± 27.72	776.92 a ± 25.36	656.15a ± 45.5	1287.78 a ± 64.53	802.07 b ± 45.50
Total Flavonoids	1039.02 a ±50.34	898.73ab ± 32.30	794.69 b ± 60.05	1760.27 a ± 165.90	1002.30 b ± 60.05
Total Anthocyanins	319.61 a ± 19.84	228.69b ± 6.50	202.54 b ± 23.72	478.94 a ±59.94	276.86 b ± 37.59
Total Proanthocyanidins	863.23 a ± 24.55	832.61 a ± 16.27	752.99 a ± 50.13	1493.76 a ± 69.83	885.01 b ± 59.141

Within row, different letters indicate significant difference at $p \leq 0.05$.

b) Anthocyanin profile

Five main anthocyanins were detected in Black Magic berry skins: Malvidin-3-*O*-glucoside, Peonidin-3-*O*-glucoside, Delphinidin-3-*O*-glucoside, Keracyanin and Delphinidin (Tab. 1 and 2). They were present as monoglycoside derivatives of the non-methylated (Delphinidin-3-*O*-glucoside, Keracyanin and Delphinidin) and methylated anthocyanidins (Malvidin-3-*O*-glucoside, Peonidin-3-*O*-glucoside); no acetylated anthocyanins were found.

Malvidin 3-*O*-glucoside was the main anthocyanin, as it happens in most of grapes (Ribereau-Gayon, 1959). In Black Magic it represented about 68-71% of total anthocyanin content detected in the grape produced in the first year and 62-72% in those produced in the second year. Nevertheless, in terms of absolute content, the concentration in berry skins varied considerably between the two years, that is, 1462 mg kg^{-1} of skin in the I year and 2658 mg kg^{-1} of skin in the II year. At the opposite, Delphinidin 3-*O*-glucoside was the less represented anthocyanin: it ranged between about 1 and 3% of total anthocyanin content in both years.

Comparing the three treatments, the skins of the grapes grown under neutral and yellow plastic films contained the following anthocyanins, listed for decreasing content: Malvidin 3-*O*-glucoside, Delphinidin, Keracyanin (Cyanidin-3-*O*-*β*-rutinoside), Peonidin 3-*O*-glucoside and Delphinidin 3-*O*-glucoside. Thus, the cyanidin-based derivated anthocyanins Peonidin 3-*O*-glucoside and Keracyanin were less concentrated than the delphinidin-based derivated ones such as Malvidin 3-*O*-glucoside and Delphinidin 3-*O*-glucoside.

Generally speaking, total anthocyanin concentration was maximum in grapes grown under Neutral plastic film. The plastic films used in the present study were also adopted in a previous research carried out by de Palma and co-authors (2012), in which it resulted that the photosynthetic photon flux passing throughout the cover and intercepted over the canopy of vine grown under Neutral, Yellow and Pink films, was about 74%, 66% and 63% of that available in open air, respectively. Hence, we can assume that grapes under Neutral film were more lighted than the others. As shown by Jeong and co-workers (2004), anthocyanin accumulation in grape skins decreases with shading, which influences the expression of anthocyanin biosynthetic genes, thus, it is likely that the higher transparency of the Neutral film to the solar radiation had a positive effect on anthocyanin accumulation. It was shown on apples that the most important enzyme involved in the anthocyanin synthesis is UDPGalactose: flavonoid-3-*O*-glucosyltransferase (UFGalT) which increases its activity in conditions of greater UV light (Ju *et al.*, 1995 and 1999). The Neutral, Yellow and Pink films plastic films used in the present trial had UV transmissivity of 57%, 24% and 26%, thus it could not explain the difference of anthocyanin content observed between grapes of yellow and pink treatments.

Tab. 5.6 – Average retention times (min) of identified anthocyanins in Black Magic table grape – 1st and 2nd year

Tr (min)	*1st year*			*2nd year*	
Anthocyanins	Neutral film	Yellow film	Pink film	Yellow film	Pink film
Delphinidin 3-O-glucoside	19.51	19.51	19.84	17.57	17.57
Keracyanin	24.27	24.27	24.55	22.79	22.80
Delphinidin	25.79	25.77	26.03	24.93	24.92
Peonidin 3-O-glucoside	26.26	26.27	26.55	25.39	25.48
Malvidin 3-O-glucoside	27.08	27.11	27.42	25.98	26.00

Tab. 5.7 - Anthocyanin content in berry skins of Black Magic table grape – 1st year

Anthocyanins	Neutral Film		Yellow Film		Pink Film	
	mg kg^{-1} skins	%*	mg kg^{-1} skins	%*	mg kg^{-1} skins	%*
Delphinidin 3-O-glucoside	71.4 ± 4.2 a	2.26	59.7 ± 7.2 a	2.77	20 ± 2.9 b	0.97
Keracyanin	249.25 ± 10.3 a	7.91	195.6±14.9 ab	9.07	156.52 ± 15.8 b	7.60
Delphinidin	377.5 ± 24.1 a	11.98	250.6 ± 10.1 b	11.62	246.75 ± 33.3 b	11.98
Peonidin 3-O-glucoside	238.7 ± 8.4 a	7.57	174.2 ± 12.1 b	8.08	173.54 ± 14.9 b	8.43
Malvidin 3-O-glucoside	2215.5 ± 104.7 a	70.28	1475.4 ± 47.8 b	68.45	1462.2 ± 166.5 b	71.01
Total	3152.4	100	2155.5	100	2059.1	100

* percentage on content of identified anthocyanins
In columns, different letters indicate significant difference at $p \leq 0.05$

A comparison between the two photoselective films showed that, in the first year study (Tab. 5.7 and 5.8), differences in anthocyanin compositions were no statistically significant for four out of five anthocyanins: the only statistically significant difference was in Delphinidin 3-*O*-glucoside. On the second year, all the identified anthocyanins were significantly more concentrated under yellow films. Considering the values as a percentage of total anthocyanins identified, Malvidin 3-*O*-glucoside, was more concentrated in the Pink treatment. Between Yellow and Pink treatment, 3-monoglucosides and aglycon form there was only a slightly difference of 1%, so may be an equilibrium between the forms. The highest total anthocyanin concentrations were found under Neutral plastic film, likely due to its high transparency; the results showed that it also occur for each anthocyanin. As concerns the coloured (photoselective) plastic films, Keracyanin and Delphinidin 3-*O*-glucoside were more concentrated in the skin of grapes grown under yellow film than under the pink one.
The results of the first year showed that, with a 95% confidence interval, White films produced grapes with a significantly higher anthocyanin concentration compared to the photoselective films; for four out of five anthocyanins there were no statistically significant differences between treatments “Yellow” and “Pink”.

Tab. 5.8 - Anthocyanin content in berry skin of Black Magic table grape – 2nd year

Anthocyanins	Yellow Film		Pink Film	
	mg kg^{-1} skins	%*	mg kg^{-1} skins	%*
Delphinidin 3-O-glucoside	118.4 ± 8.8 a	2.74	36.1 ± 2.4 b	1.59
Keracyanin	538.3 ± 30.7 a	12.45	238.9 ± 20.7 b	10.52
Delphinidin	472.0 ± 53.8 a	10.92	249.9 ± 21.1 b	11.00
Peonidin 3-O-glucoside	535.8 ± 52.6 a	12.39	106.9 ± 6.1 b	4.71
Malvidin 3-O-glucoside	2658.5 ± 237. 7 a	61.50	1639.7 ± 119.7 b	72.18
Total	4323.0	100	2271.6	100

* percentage on content of identified anthocyanins
In columns, different letters indicate significant difference at $p \leq 0.05$

As shown by Awad and co-workers (2001) in a study on apple trees, light perceived by the inner positions of the canopy was significantly lower compared to the outer ones. In particular it was relived more far red light (700-750 nm) inside the canopy and less UV-A (330-750), blue (400-450), green (450-530 nm) and red light (600-700 nm). Wooley (1971)

showed the capacity of leaves to absorb mainly violet and ultraviolet radiations. It is known that violet colour is derivated by blue and red ones; therefore we can suppose that the inner position of the crop perceived less blue and red radiations. Kasperbauer (1971) showed that within a canopy there was a shift or Red:Far Red ratio, because there was a modification of the spectral distribution of light, especially red light, that was reduced by the external leaf absorption. Hence, also in our conditions, the effects of coloured film were presumable attenuated by leaf shading effect. As reported by Ballarè and co-workers (2014), the main photoreceptors of the light spectrum are phytochromes, cryptochromes and phototropins; in particular, phytochromes react in conditions of decreased Red light compared to the other ranges of the light spectrum with a consequent R:FR ratios reduction that it was assessed to reduce the accumulation of soluble phenolics, especially anthocyanins.

In a recent study of Gonzalez and co-authors (2015) it was shown that red light supplementation, directed on the clusters, was responsible for the enhanced synthesis and accumulation, in grape berry skins, of phenolic compounds, anthocyanins, in particular; the response was mediated by specific berry photoreceptors that is phytochromes. In that study sugar content, acidity and berry size were not influenced. Considering the same study, it is possible to suppose that in our conditions, the slight quality modification of light under Pink film likely did not arrive to cluster level or it was very slight because of the crop shading effect over these fruits. Therefore, the latter consideration may explain the higher concentration of anthocyanins in Yellow treatment compared to the Pink one, although, the first film it is known to absorb red and green light, according the colour theory of Young-Helmholtz.

Red and UV radiations are well known to enhance anthocyanin synthesis in skins of apples, (Arakawa, 1991; Bakhshi and Arakawa, 2006; Bastias *et al.*, 2012). In a recent research carried out on *Vitis vinifera* cv Malbec (wine grape variety) trained with vertical shoot positioning, it was found that the application of a red light source directly to the cluster stimulated, under field conditions, the synthesis of the polyphenols and, in particular, that of anthocyanin compounds (González *et al.*, 2015).

It is known that red nets favour light transmission in the Red (600–700 nm) and Far Red (700 – 800 nm) wavelength ranges, while the yellow nets favour the transmission of the light wavelengths higher than 500 nm, included those associated to the yellow colour (570-600 nm) and penalize the transmission of the red and blue light wavelengths respect to the red cover (Oren-Shamir *et al.*, 2001, Olsen *et al*., 2002; Shahak e Gussakovsky, 2004; Schettini, 2011). A similar behaviour may be supposed for the pink-reddish film and the yellow film utilized in the present study. However, especially in the second year of trial, Black Magic grape skins of yellow treatment had a markedly higher anthocyanin content compared to those of the red one: it is confirmed and emphasized that, in our conditions, an other factor such as the total light availability exerted a major role on berry skin anthocyanin biosynthesis and accumulation.

In general, aglyconic form of Delphinidin prevails on Delphinidin 3-*O*-glucoside in all treatments, probably because glucosidic form was methylated to give Malvidin 3-*O*-glucoside. Malvidin concentrations in our study were in accordance with Boss and co-workers (1996b) who showed, in a work on cv. Shiraz, that Malvidin-based anthocyanin derivatives increased over the ripening period, whereas the percentage of the other compounds decreased slightly during this time. It is possible suppose that in our experiment Delphinidin was converted in Delphinidin 3-*O*-glucoside, its intermediate, but its concentration likely dropped because it was soon converted in Malvidin 3-*O*-glucoside, defined by Wulf and co-workers (1978) as the major anthocyanin in *Vitis vinifera*. Cyanidin was not identified, likely because it was less stable than Peonidin (Roggero *et al*., 1986), so it was presumably converted in

Peonidin 3-*O*-glucoside by means of methylation of the intermediate Cyanidin 3-*O*-glucoside (Boss *et al.,* 1996b).

It is known that each class of anthocyanins was more or less concentrated depending on genetic, environmental, agronomic factors (e.g. cultivar, sunlight exposure, UV irradiation, temperature) and canopy management (Jackson *et al*., 1993; Downey et al., 2006; He et al., 2010). Another factor influencing anthocyanin detection was the stability of these compounds that is influenced by pH, oxygen, solvents, presence of enzymes, flavonoids, proteins and metallic ions (Kennedy *et al*., 2001).

The 3-monoglucoside form represented about 80% of the total identified pigments in the first year and 77-78% of pigments identified in the second year; under yellow treatments, glycosidic form was slightly less concentrated (-1%) than under the pink one. Individual anthocyanins are very sensitive to oxidation; after their biosynthesis, they are stabilized by *O*-Glycosylation and then stored in cell vacuoles (Jackson, 2008). However it has been shown by Miyazawa and co-workers (1999) that anthocyanins relieved in human plasma are not in aglyconic form; hence, in contrast to flavonoids, which glycosidic bond is hydrolyzed in the gastrointestinal tract, anthocyanins are assimilated in glycosidic form. The same authors suppose that glycosidic is the form more available in human body. Hence, the results of our experiment, showing a percentage of the 3-monoglucosidic form up to 80% in Black Magic, indicate a possible healthy effect of this grape.

In our study delphinidin-derivative were less concentrated than delphinidin-based derivative anthocyanins, as in other table grapes varieties (Liang *et al.*, 2008). Jeong and co-workers (2006) studied the relationship between anthocyanin composition and expression of the genes F 3'H (Flavonoid 3′-hydroxylase) and F3′5′H (flavonoid 3′,5′-hydroxylase with the aim to verify their responsibility for specific anthocyanin forms in different stage of berry development. It was shown that F 3'H and F3′5′H are involved in the biosynthetic pathway of cyanidin- and delphinidin-based anthocyanins; for instance berry skins of Cabernet Sauvignon at the early stage of berry development, accumulated a higher level of mRNA of the gene Flavonoid 3′-hydroxylase (F 3'H) than flavonoid 3′,5′-hydroxylase (F3′5′H). Conversely, at harvest it was been relieved a higher level of mRNA of the gene F3′5′H than F 3'H and this results were associated to the major content of delphinidin-based anthocyanins in the considered berry skins.

In a study of Liang and co-workers (2008) it was assessed that generally, in *Vitis Vinifera* grapes, Malvidin 3-*O*-glucoside together with its derivatives and Peonidin 3-*O*-glucoside (methylated anthocyanins) were the most concentrated. This habit was confirmed in our study as concens to Malvidin 3-*O*-glucoside; on the other hand, delphinidin was tendentially more concentrated than Peonidin 3-*O*-glucoside in all treatment, except for grapes grown under Yellow film, particularly in the second year of our study.

When free hydroxyl group prevail among anthocyanins, skin colour tend to blue, while with methylation of the hydroxyl groups prevalent skin colour was red (Jackson, 2008). Total anthocyanin concentration generally show a negative correlation with the L* and b* colorimetric coordinates of the CIE-LAB system, while the a* coordinate had a positive correlation. In cultivars characterized by dark skins, methylated anthocyanins, such as Malvidin 3-O-glucoside, are the most represented (Liang et al., 2011). In our study, anthocyanin methylation increased under yellow plastic film. The berry skin colour of the Black Magic grape analysed in the present study was previously assessed in the first part of this experiment. The value of the a* coordinate increased from the neutral (1.8) to the Pink (3.0) and to the yellow (4.7) treatment, indicating a tendency to enhance the red component of the skin colour. The value of the b* coordinate was very similar among the Yellow (-0.9), the Neutral (-1.1) and the Pink (-1.3) treatment, indicating a poor tendency to change the blue component of the skin colour.

In the first year, the lower methylation in yellow treatment is likely correlated to a reddish colour of the skins (higher *a value) and to a less blue colour (lower *b value). On the other hand, the higher methylation in pink treatment is likely correlated to a value of a* lower than yellow one and a bluest colour (more negative *b) in compare with the other thesis.

Moreover, Cyanidin is considered the precursor of the red Cianidin based derivatives (Kuhn *et al*., 2014; Castellarin *et al*., 2006), i.e. Keracyanin and Peonidin 3-*O*-glucoside, that in our results were more concentrated in Yellow and Pink treatments compared to the Neutral one. Hence, this result confirm the tendency observed basing on colour measurement. Delphinidin, the precursor of blue/purple petunidin and malvidin derivatives was higher in the Neutral treatment and similar between Yellow and Pink thesis. The proportion of the cyanidin- and delphinidin-based anthocyanins is responsible for the colour variation among red, purple and blue berry grape and the mechanism is genetically regulated by flavonoid 3'- and 3',5'-hydroxylases (F3'H and F3'5'H) genes (Castellarin *et al*., 2006). As reported by Sichery (2015), Cyanidin and Delphinidin give violet color to skin, so that, comparing grapewine such us Negroamaro, Uva di Troia, and Sangiovese, they have color blue, violet and violet – black, respectively, likely due to tendencially crescent content of Delphinidin. In the present study, the difference in Delphinidin concentration seemed not reflected by the skin colour.

Methylated anthocyanins, Peonidin 3-*O*-glucoside and Malvidin 3-*O*-glucoside, represented the main part of anthocyanin in all treatments. In the first year of trial, their concentrations in grapes compared to total anthocyanin individuated were about 78%,76%, and 79% under Neutral, Yellow and Pink film, respectively; in the second year their concentrations, under Yellow and Pink treatments, were 74% and 77%, respectively.

Therefore, in the first and in the second year of trial, methylated anthocyanins were slightly more concentrated under Pink film (+3%) compared to the Yellow one.

The average values of methylated anthocyanin content under Neutral, Yellow, Pink and film in the first year (2454.21, 1649.63, 1635.76 mg kg^{-1} of skins) and in second year of study (3194.32 and 1746.73 mg kg^{-1} of skins) were considered. The results showed that methylated anthocyanin in the first year had the maximum concentrations in Neutral treatment while the others were similar each other's; in the second year, Yellow treatment gave more methylated anthocyanins.

Another consideration can be done about tendency of methylated forms: it was likely due to UV light modification under plastic films. The cover films used in this experiment were the same reported by de Palma and co-workers (2012). It was shown by means of spectrophotometer that Yellow plastic film, compared to Neutral and Pink ones, had a lower transmission of radiation in the violet wavelength range, while Neutral cover film was showed to transmit in the UV range, more than the photo-selective films. Hence, there were two main differences between coloured films: the Yellow film transmitted in the region of violet less than the Red one and more in the range of about 500 and 650 nm. Our results may wrongly suggest that the more direct UV radiation arrive under cover film the more methylated anthocyanins increase, because it was shown by Chalker-Scott (1999) and co-workers that generally this behaviour was likely due to the protective action of anthocyanins against solar exposure and ultraviolet radiation. As shown by Jenkins (2007), UV-B radiation enhanced some protection processes, by means of the photomorphogenic responses, at level of gene expression, cell physiology and biosynthesis. Radiation influences, for instance, the synthesis in superficial plant tissues of phenolic compounds, responsible of UV radiation absorption and to avoid damages to macromolecules, as DNA.

Koyama and co-workers (2008) showed that anthocyanin concentrations were reduced in grapes shaded during ripening, but it was not the same for grape shaded on the early stage

of development; in the latter conditions anthocyanins concentrations were not reduced, but changed proportion of trihydroxylated anthocyanins, as it happened in our experiment..

These results were in agreement with Dokoozlian e Kliewer (1996) that in a study on Cabernet Sauvignon' and 'Pinot noir' grapevines showed the importance of light, in the first phase of the grape development, with the aim to produce anthocyanin pigments. In the second phase of development it was showed that grape thanks to light can regulate the activity of the enzymes responsible for the anthocyanin synthesis (Roubelakis-Angelakis, Kliewer, 1986; Dokoozlian, Kliewer, 1996). Light exclusion significantly reduced both anthocyanin concentration and composition (Spayd *et al*., 2002; Guan *et al*., 2015), because it changes the expression of the genes responsible for anthocyanin biosynthesis (Guan *et al*., 2015). It may explain the minimum anthocyanin content of Pink film which resulted to have minimum transparency to PAR.

As shown by Martin (2013) and co-workers, anthocyanins are significant for their antioxidant capacity and because of their action as phytonutrients which prevent chronic diseases. Hence, it is important the choice of treatment that give maximum content of anthocyanins, such us in our study, the Yellow film within coloured treatment. The lower transmission of PAR radiation, may be responsible for the less content of anthocyanins in grapes grown under Yellow and Pink film.

According to Fukumoto and Mazza (2000), using different methods of analysis, anthocyanin antioxidant activity, on average is enhanced by an increase in hydroxyl groups and a decrease in glycosylation. By means of ORAC assay application, it has been shown in decreasing order of antioxidant activity: Delphinidin (1.809 ± 0.068), Peonidin 3-O-glucoside (1.805 ± 0.014), Malvidin 3-O-glucoside (1.404 ± 0.052). Hence, in the first year results, considering ORAC assay, under Neutral film the highest antioxidant activity should result and it should be similar between Yellow and Pink treatments. In the second year of study, it should result higher in grapes grown under Yellow film because each anthocyanin is significantly more concentrated under this film.

The differences in anthocyanin concentrations between Yellow and Red films may be due to violet wavelengths range or to the lower absorption in the range of about 400 and 650 nm (blue-green and a few of red region of the spectrum). Blue light was shown to be necessary for anthocyanin accumulation (Chen *et al*., 2006). Chatterjee and coll. (2006) showed as anthocyanin accumulation depends on the main blue-light photoreceptor, i.e. cryptocrome with direct correlation. Therefore, we can suggest that also in our study the lower concentrations of anthocyanins in the grapes grown under Pink film could be slightly correlated to the lower light perceived by grape in the range between about 400 and 500 nm, although in the inner position of the canopy this difference should be very poor.

In a study carried out by Cheng and co-workers (2015), photo-selective plastic film bags were used to modify light quality perceived by clusters of *Vitis vinifera* (cv. Yatomi Rosa). According to results, at harvest, clusters grown covered with blue-and white bags, had grapes more richly coloured compared to the other treatments (Red, Orange, Green); hence, there were effects on anthocyanin concentration: Neutral film had a major content compared with the Red treatment. In a study on apples (*Malus pumila* Mill.) white light and UV-B light singularly and in synergy where shown as factors responsible to stimulate anthocyanin production (Arakawa et al., 1985). Therefore, in our study, considering photo-selective treatments, it seems important the reduction of transparency as factor influencing anthocyanin concentration.

In cv Black Magic, the results showed that it is difficult to change the anthocyanins profile by using photoselective plastic films: the position in the sequence is the same for the most and the less concentrated anthocyanins, but it seems possible to modify their intermediate positions.

6. EXPERIMENT III: INFLUENCE OF COLOURED ANTI-HAIL NETS ON QUALITY OF ITALIA GRAPE

6.1 Materials and Methods

a) Preliminary assessments

Fig. 6.1 – Italia grape.

Italia variety produces a blank table grape obtained by Prof. Alberto Pirovano crossing *Bicane X Muscat Hamburg*. It is the most cultivated and famous in the world. It is a vigorous variety and produces a very good grape with slight aromas of muscat.

Italia cultivar has an average bud break and late ripeness. It is characterised by a high fertility, and production, a good vigour. It is sensitive to downy mildew and to botrytis (Richter, 2015).

The cluster of this variety is big, winged, conic-pyramidal and compact. Its weight, on average, is about 800 g. The berries are ellipsoid, very big, characterised by thick skins amber-yellow coloured. Juicy and firm flesh taste is slightly muscat. According to Colapietra (2004) this is a suitable variety to delayed harvest, because it is resistant and preserve good organoleptic characteristics on the vineyard. It has also aptitude to stores.

The field part of the experiment was carried out in 2014 at a private vineyard located in Trinitapoli (Barletta-Andria-Trani, Southern Italy; 41,3582° N, 16,0882° E). Since 1999, the vineyard has been planted in a deep soil characterized by a sandy-loam texture. Italia variety was grafted onto 1103 P rootstock. The vineyard had a square planting pattern, with 2.40 m as density of plantation. The vines are trained in “tendone” system, and pruned to three 10-buds canes.

Water supply started from the first week of June and it was reduced near the harvest period. It was used a drip system together with the mineral nutrition during fertigation.

The vineyard was covered by yellow, red and white anti-hail nets characterized by a 3x5 mm mesh size. The measured net height above the ground was about 2.85 m. Yellow, red and white treatments were considered, each consisting of three rows by 13 strains covered with one of three types of nets. The central rows were covered by yellow and red nets, while on the

other rows were applied white nets. The coverage of the row was made with sloping gable, exposed respectively to the southwest and northeast.

Fig. 6.2 – Vineyard covered with coloured and white hail-nets.

In mid July, Photosynthetically active radiation (PAR) within plant canopy was measured by means of the AccuPAR sensor (model LP-80). Temperatures and relative humidity were detected using a HD 8501 H, Delta Ohm hygrometer. The measurements were carried out in a clear day, between 10.30 a.m. and 12.30 a.m., in the open air at 2 m above the ground.

On the roof of the canopy, three leaves well-exposed to light on the main shoot site were used to measure rates of exchange-gaseous content, ie stomatal conductance (CD), net photosynthesis (Pn) and transpiration (TR) per unit leaf area (IRGA LC ProPlus, ADC). Moreover, a pressure chamber, Soilmoisture Equipment was used in order to estimate Ψ_{stem}, the vine water status (Scholander et al., 1965). Six leaves for each treatment were closed in plastic and aluminium bags for two hours before the readings.

In September, Italia grapes were harvested.

Chemical and physical parameters of the grape juice

Ten bunches per replicate were sampled in order to assess the grape quality parameters. Bunch weight, berry number per bunch and berry weight were recorded. Fifty berries per replication were sampled to analyze, after crushing, juice total soluble solids (TSS) and titratable acidity (TA). Before berry crushing, the skin chromatic coordinates were measured by means of a colorimeter (CR 400, Minolta Co.;CIE L*a*b* system). Moreover, on 20 berry samples per replicate, the main skin phenol content was assessed.

About 100 berries were manually pressed and the must was centrifuged, with a speed of 4500 RPM for 10 minutes at 20°C with the aim to remove the insoluble interfering fractions. The must obtained was analysed for pH, acidic and sugar content determinations (3 replicates).

Sugar content, determined as total soluble solids (TSS) was measured by means of a digital refractometer (ATAGO WM7 Refractometer). placing some must drops on the instrument and reading the value expressed as °Brix.

A 10 ml volume of must and 40 ml of distillated water and NaOH solution (0,1 N) were used in order to measure the titratable acidity (TA), using standard EEC methods, by means of

a automatic titrator (Titrex – Universal Potentiometric Titrator), expressing the results as tartaric acid, well known like the main acid in the grapes (grams per litre).

$$T.A. = \frac{a \times N \times 75}{V}$$

a = mL of NaOH
N = NaOH concentration
75 = equivalent weight of tartaric acid
V = volume of analyzed must (10mL).

The pH was determined by potentiometric measurement using a Crison Basic 20 pH-meter. All assays were performed at 20°C and provided values are the average of three replicates.

Acidic (L-Ascorbic acid, L- Malic acid, D-Lactic Acid, L-Lactic acid, Pyruvic acid, Citric acid), mineral (α -amino Nitrogen, Ammonia Nitrogen, Readily Assimilable Nitrogen (RAN), Calcium, Potassium, Copper) and sugar composition (Glucose, Glucose + Fructose, Glycerol) were assessed by using BIOGAMMA MIURA ONE Chemistry Analyzer. Each determination needs a specific reagent kit, consisting of two reagents, produced and bought by BIOGAMMA S.r.l.

Indexes of polyphenols

Chloridric ethanol solution used for the polyphenol extraction was obtained by mixing ethanol:water:hydrochloric acid with a volume ratio of 70:30:1. A 300 g L^{-1} $FeSO_4$ $7H_2O$, stock solution was prepared by dissolving the salt in concentrated hydrochloric acid. A 10 % (m/v) sodium carbonate (Na_2 CO_3) solution was prepared by adding distilled water to salt. 4 g of vanillin were dissolved in 100 ml of methanol to obtain a 4% (m/v) vanillin in methanol. Methanol, Folin-Ciocalteu (FC) reagent and Hydrochloric acid were purchased from Sigma Aldrich, Ethanol from Carlo Erba Reagents, Sodium hydroxide 1 N from J.T.Baker.
Grape sampling was carried out according to di Stefano and Cravero (1991) method. Ten berries for each replicate of Black Magic and Italia grapes and twenty berries of Negroamaro grapes were randomly used to prepare the extracts of polyphenols.
An analytical spectrophotometric method (di Stefano *et*. al. 1991), was used to quantify total polyphenols in the skin grape extracts, analyzing the following indexes of polyphenols:

- total polyphenols
- total flavonoids
- proanthocyanidins
- flavans in vanillin

The berry weights were measured. The skins were removed from fleshes and they were dried with paper, to prepare ethanolic extracts of polyphenols. After the weight measure, the skins were stored in 25 mL of 70:30:1 ethanol:water:hydrochloric acid (v/v) stored in the dark at room temperature, for 24 hours, so that polyphenols could be extracted. Next, the extractive solution was filtered using a strainer and stored at −20 °C. Grape seeds extracts were prepared in the same way, but because seeds needed more than 24 hours to have complete polyphenols extraction, so they were stored in solution for 72 hours.

Total flavonoid content

Each skin extract was diluted by chloridric ethanol solution; while distillated water was used for seed extract dilution. Considering extract colour, it was possible to estimate how many dilutions between 10 and 100 were needed to determine flavonoid content. Then, absorption of the solution was measured in the range between 230 and 400 nm, using chloridric ethanol solution and distillated water as blank, for extracts of skins and seeds, respectively.
Total flavonoids concentration was calculated by applying this formula:

$$\text{Total flavonoids (mg L}^{-1}) = 82{,}4 \cdot E'_{280} \cdot d,$$

in which E'_{280} results from graphic method described by Di Stefano and co-workers (1989), *d* are the dilutions of ethanolic extract used for the analysis.

Total polyphenols

0,1 ml of the sample extract of polyphenols were added to 5 ml of distillated water in a 20 ml volumetric flask; 1ml of Folin-Ciocalteu reagent was added and 4 ml of 10% (w/v) sodium carbonate solution. The volumetric flask was finally filled up to 25 ml with distilled water. The blank solution was prepared in the same way, using all the reagents except the extract, (which contains the compounds of interest that absorbs the light), replaced by the same volume of distillated water. After 90 minutes, using blank solution spectrophotometer was calibrated at 750 nm and then sample absorbance was read at 750 nm in a 1-cm pathlength cuvette.
Total polyphenol concentration was expressed as milligrams of (+) Catechin equivalents per liter of extract, calculated by applying the following formula:

$$\text{Total polyphenols (mg L}^{-1}\text{of (+)Catechin)} = 186{,}5 \cdot E_{750} / V,$$

in which V = 0,1 ml, the aliquot of ethanolic extract used for the analysis, E_{750} was the absorption of sample solution measured at 750 nm ,
All spectrophotometric measurements were made in a Shimadzu UV-1700 spectrophotometer using a 1.0 cm optical path length glass cell.

Proanthocyanidins

200µl of extract were pipetted into a 50ml obscured volumetric flask; then ethanol (12,3 ml) and 12,5 ml of $FeSO_4$ $7H_2O$ were added to the solution, putting the flask into a beaker of ice water. The flask was placed on hot-water bath at 90°C for 50 minutes, so Bate-Smith reaction could start. At the end, after refrigeration aim to stop the reaction, absorption was measured between 380 and 700 nm. By means of the spectrophotomether, using a 1cm-cuvette.
Cyanidin was used as a standard and the total proanthocyanidins were expressed as milligrams of cyanidin equivalents per litre of extract, calculated by applying the following formula:

$$\text{Proanthocyanidins (mg L}^{-1}\text{of cyanidin)} = 1162{,}5\ \Delta E/V ,$$

in which V= 0,2 ml, the aliquot of extract used for the analysis, ΔE was a difference obtained by graphical method (di Stefano *et al*., 1989)

Flavans in vanillin

Extracts were diluted in methanol (10 dil.); to 0.5 ml of this solution 3 ml of vanillin in methanol (4% m/v) were added and 1,5 ml of concentrated hydrochloric acid were put in a dark glass tube, which was immersed in an ice-water bath. A blank for each sample was prepared using the same reagents, but replacing vanillin solution with methanol. After 15 min at room temperature, the absorbance of the solution was measured at 500 nm in a 1-cm cuvette, using for each sample the specific blank solution.

Catechin was used as standard and the total flavans in vanillin were expressed as milligrams of (+)Catechin equivalents per liter of extract, calculated by applying the following formula:

Flavans (mg $L^{-1}$of (+)Catechin) = 290,8 · ΔE · d,

in which d = 10, the dilutions of ethanolic extract used for the analysis, ΔE was the difference between absorptions of blank and sample, measured on the spectrophotometer after vanillin reaction.

All grape phenolic indexes were converted by milligrams of equivalents per litre of extract, to milligrams per kilogram of grapes, by multiplication with the factor 25/P, in which P was the ten-berry weight and 25 was the total volume of extract prepared by the skins of 10 grape berries.

Hydroxycinnamil tartaric acids

After the complete remotion of the skins used in order to prepare polyphenolic extracts of skin and seeds, idrossicinnamic acid phenols grapes were analyzed. Before the analysis, the fleshes were pressed with Sodium metabisulfite $Na_2S_2O_5$ (100 μg), then they were centrifuged at 4500 rpm for 10 minutes at 20 °C, to remove insoluble fractions and finally 1 ml of supernatant was diluted 10 times with sulphuric acid 0,1 N to avoid tartaric precipitation. Spectrophotometer was used to measure the solution absorbance at 325 nm (A_{320}), using sulphuric acid 0,1 N as blank solution with and an optical path length of 1cm.
Idrossicinnamic acid were expressed as milligrams of Caffeic acid (3,4-dihydroxycinnamic) equivalents per litre of extract, calculated by applying the following formula:

Flavans (mg $L^{-1}$of Caffeic acid) = A_{325} · (10/0,9) · d,

in which d = 10 were the dilutions, 0.9 absorbance values of caffeic acid at 325 nm, having a concentration of 10 mg L^{-1} .

Antioxidant activity

The antioxidant activity of both grape flashes and skins was determined according to the method of Re and co-workers (1999) from the ability of antioxidant substances in the samples to react with ABTS•+ [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] chromophore

(TEAC assay). The extracts were prepared with methanol, and the sample was homogenized using an Allegra X-22R centrifuge, setting the speed of 4500 RPM for 10 minutes at 20°C. After maceration for 24h at a temperature of 3-4ºC, the samples were filtered and stored at -20 °C.
Two solutions are needed to prepare chromophore: ABTS solution 7 mM prepared by dissolving 19.2 mg of ABTS salt in 5 mL bidistillated water and storing it under refrigeration; a solution 140 mM of potassium persulfate prepared by adding 378 mg of potassium salt in a volumetric flask, which is then filled to 10 ml with bidistilled water. 88 µl of this potassium persulfate solution was added to ABTS and than the solution was stored in the dark at room temperature, for more than 6 hours, so that the absorbance becomes maximal and stable to have a complete oxidation of ABTS.

At the moment of analysis, solution were diluted in ethanol 1:88 to give, when it was compared to ethanol, an absorbance of 0.7±0.02 at 734 nm; if the value of absorbance was not in range, it was adjusted by adding ethanol or ABTS solution, to decrease or increase the value, respectively.

To quantify the antioxidant activity, 200µL of diluted sample extract (50 dil.) were added to 2 mL of the $ABTS^{\bullet+}$ working solution. The absorbance of the sample at 734 nm was read by spectrophotometer, and then it was read again after 15 minutes. A standard curve was previously prepared with Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid), used as antioxidant standard. Trolox (2.5 mM) was prepared in ethanol for the quantification of the antioxidant activity. Trolox equivalent antioxidant capacity (TEAC) were given in millimols of Trolox equivalents per kilograms of homogenized consisting of grape-fleshes and skins.

Statistical analysis

The determinations were conducted in triplicate and results were expressed as mean ± standard error. Statistical analyses were done by one-way ANOVA followed by DUNCAN's test with $P < 0.05$ as a limit of significance, using the pc software ASSISTAT v. 7.7 beta.

6.2 Results and Discussion

At harvest, no significant difference were found between treatments of Italy on regard to the bunch weight that ranged between about 540 g in vines grown under yellow nets and 585 g in those under red net (+ 9%). The number of berries per bunch was fairly constant (56-58), while the weight of the individual berry is increased significantly from 9.2 g, in the bunches products with yellow net, to 10.5 g in those products with red net (+ 14%); the white treatment had an average value. Carpometric values are similar to those found in other vineyards of Italy having a similar age and conducted without interventions forcing (Novello et al., 1999). The pattern of differences between treatments appeared similar to the net photosynthesis rate measured in mid-July. The color coordinates of the skins was not affected by the color of the network.

As shown in fig. 6.4, berry resistance to detachment from rachis (berry removal force) was highest in the grape grown under Red net (R grape), statistically different from grapes grown under White Net (W grapes) but no from Yellow ones (Y grapes). This tendency might be a possible indication of delayed maturation in grapes grown under red net. Nevertheless, also berry diameters were higher in grape grown under red and white net, without statistical difference with berries produced under white net.

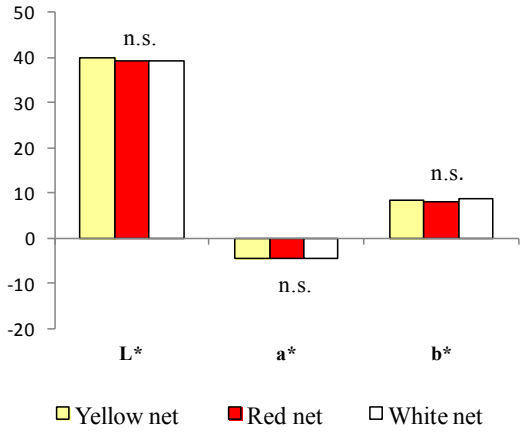


Fig 6.3 - Colorimetric indices of grape skins grown under Yellow, Red and White nets.
different letters indicate significant difference at $p \leq 0.05$

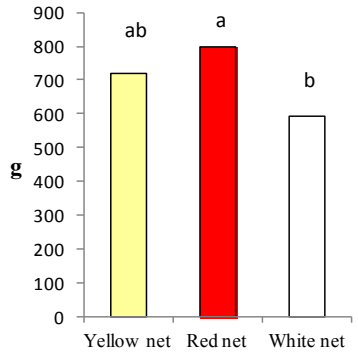


Fig. 6.4 - Grape-berry resistance to detachment of Italia grapes grown under Yellow, Red and White
different letters indicate significant difference at $p \leq 0.05$

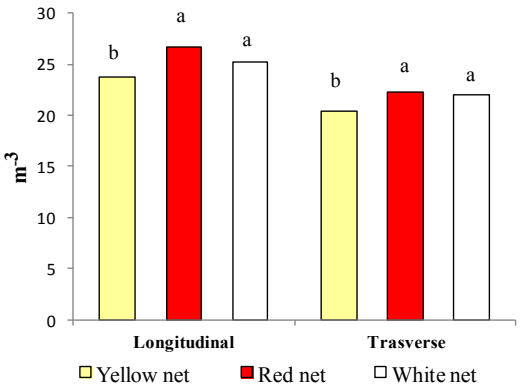


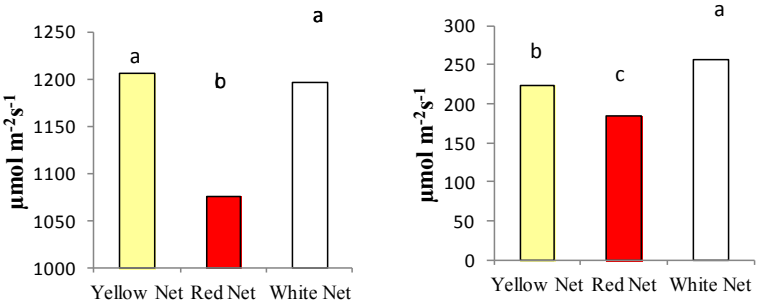
Fig. 6.5 - Berry diameters of Italia grapes grown under Yellow, Red and White nets.
different letters indicate significant difference at $p \leq 0.05$

The micro-environmental conditions found in open field were similar between mid-July and late August. They were characterized by a high intensity of the solar photosynthetically active radiation (on average, the maximum flow intercepted was 2165 ± 70 m^{-2} s^{-1}, the mean

flow available on horizontal plan was 1599 ± 128 m^{-2} s^{-1}) temperature values (30 ± 0.2 ° C) and relative humidity in open field (41 ± 0.3%), moderate for the environment culture.

The main results obtained by field relives, aim to known micro-environmental conditions, are graphically represented in the following graphics in which it is possible to compare average values of R:Fr ratio under nets (fig. 6.7) and PAR (Photosynthetically active radiation) between net and crop, both direct (fig. 6.6 a) and diffuse (fig. 6.6 b). Total PAR under cover was statistically different between the Red nets and the others, which have similar average values. Yellow, Red and White net average values of PAR were respectively 1205 µmol $m^{-2}s^{-1}$, 1075.33 µmol $m^{-2}s^{-1}$ and 1196.50 µmol $m^{-2}s^{-1}$. Diffuse PAR followed the same trend of total PAR, but the differences were statistically significant also between White and Yellow nets. Similar tendency is showed by graphical representation of Red: Far Red ratio (fig. 6.7).

On average, about 90%, 87% and 82% of the radiative flux available outside the canopy was transmitted by white, yellow and red nets, respectively. Hence, comparing the intensity of the PAR reduction, between white and red net there was a difference of about 8%, while between neutral and yellow ones it is only 3%. The yellow net transmitted more than the red one (+5%). It is well known that the darker colour nets are more opaque to sunlight, increasing the shadowing effect on the vineyard. Our results are in line with the study carried out by Schettini (2011) in which yellow and red net with the same shading factor showed a difference of 5%.



(a) (b)

Fig. 6.6 - Total (a) and diffuse PAR (b) under coloured nets

different letters indicate significant difference at $p \leq 0.05$

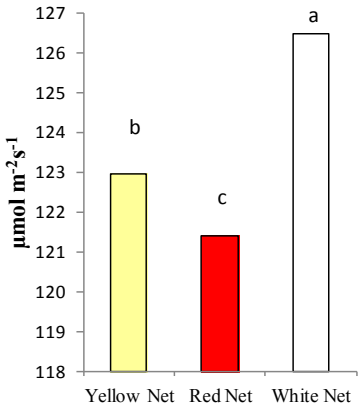


Fig. 6.7 - Red:Far Red ratio under coloured nets

different letters indicate significant difference at $p \leq 0.05$

It was shown by Lobos and co-workers (2012) that Red net enhances light transmission in Red and Far Red region of the light spectrum. As reported also in some studies by Oren-Shamir et al. (2001), Shahak *et al.* (2004b) and Abdel-Ghany and Al-Helal (2010), neutral nets, compared to the other ones, have the highest proportion of diffuse light.

It was recently discovered the potential of coloured shade nets with the aim to manipulate light quality and increase the quantity of diffuse light, known as responsible of the increment in radiation use efficiency and yields (Stamps *et al.*, 2009). Gu et co-workers (2002) showed that diffuse radiation is more efficiently used by plants and it causes less easily saturation. Shade nets generally scatter mainly UV radiation, because they are produced with additive substances aimed to protect them by UV-radiation damages (Wong, 1994).

Under a red net it has also been relived by Bastias and co-workers, a decrease in blue:red light proportion compared to Neutral net, a significant reduction in Net photosynthesis (P_N) and transpiration rate. In the same experiment, the net photosynthetic rate was found higher under a Neutral net than at the external, athough the net reduced the PAR level: this result was explained by admitting that the external conditions were stressing and that the net reduced the environmental stress experienced by leaves.

As shown by Gonzalez and co-workers (2015) the manipulation of light environment can improve fruit quality increasing nutraceutical power of fruit; changing light quality perceived by photoreceptors, i. e. phytochromes and cryptochromes, could have positive effects on photomorphogenesis, so that crop and quality of production could be improved.

Along the rows, microclimate seemed to be warmer that the external one under yellow (+1.1 ° C) red (+1.3%) and white nets (+2.0 ° C) and more humid with an increment of 0.9%, 3.4% and 3.7% RH under white, red yellow net, respectively. A previous observations carried out under the same type of white net, in the absence of hair (Novello and Palma de 2015) can explain the anti-hail nets tendency to increase the air temperature in the hot hours of the day. This increase, according the Authors, due to the lower natural ventilation under nets,while the increased umidity under net can be due to leaf mass transpiration.

The water potential of stem in mid-July are considered indicative of a mild level of water deficit (Van Leeuwen et al., 2009). Water status of the vine covered with yellow and red nets appeared significantly higher (+24-25%) than the vines covered with white net, probably due to the limited values air vapour pressure deficit of the canopy.

Under white nets, receiving maximum irradiance, there were the fewer units of leaf gas exchange than those in red treatment leaves (+55% transpiration) and a higher units compared with the yellow ones (-15% of stomatal conductance). The pattern of differences emerged between treatments in relation to the rates of net photosynthesis and stomatal conductance appeared similar to that observed in a study conducted on the white grape variety grown in Victoria semiforzatura early film-neutral, yellow and pink (de Palma *et al.*, 2012).

It is known that yellow nets, compared to red ones, penalize the transmission of wavelengths of red and blue ranges of the visible spectrum. On the contrary, the yellow nets are known to enhance the transmission of solar radiation of wavelength greater than 500 nm, including those in the range of the yellow colour (570-600 nm). The red nets favour especially the transmission of radiation of wavelength greater than 600 nm, including those of the range of red colour (600-700 nm) (Olsen et al., 2002; Shahak and Gussakovsky, 2004a and b; Schettini, 2011).

During the physiological processes, the various classes of pigments photoreceptors, including chlorophylls depending on the ratio of the red and blue wavelength radiation, influence the foliar functionality. The blue light stimulates, in particular, the stomatal opening, the chlorophyll photon absorption and efficiency of photosystem II; the red light performs actions similar but with lower effects, depending by species (Evans, 1987; Olsen et al., 2002; Aasamaa and Aphalo, 2016). In addition, it is known that leaves developed under

pure red light show physiological disorders that affect on photosystem II, in conditions of low irradiance (100 μmol m^{-2} s^{-1}); in these leaves, the rates of stomatal conductance and net photosynthesis is independent by the level of irradiance (Hogewoning *et al.*, 2010).

The low light transmissivity of the yellow nets in the wavelength range of red and even more than the blue (complementary color to the yellow and hence likely to be more absorbed) may be responsible for the limited gaseous exchanges in mid July. The relative enrichment of radiation in the same ranges under red net may be related to the tendency to intensify the rate of gas exchange. In a study carried out by Shahak and Gussakovsky (2004) it was found that in shading nets yellow (with shading of 49%) and red (with shading of 55%) coloured, the ratio between blue and red light is 0.53 and 0.63, respectively.

Italia grapes grown under the yellow and the red nets, compared to the yellow ones showed a mild tendency of the red net to increase rates of photosynthesis (+ 8%) and transpiration (+ 14%) than. The net carbon fixation capacity, in particular, was significantly greater in the leaves protected by white net than those in the red (+ 24%) and yellow ones (+ 33%). The vines under white net water have been reported significantly lower by 60-80% than that of the other two treatments, reaching relatively high levels of water deficit in response to the crunch he irrigation on the pre -collection. More observations are needed to test of the observed behaviours and study more deeply its causes.

In the following graphics, there are the results of phenolic analysis on skins (fig. 6.8) and seeds (fig. 6.10) extracts of the grape sampled at the end of August.

The concentrations of the main classes of phenolic compounds in grape of Red (R), Yellow (Y) and White (W) thesis are expressed in milligrams of polyphenols per kilogram of grapes. Considering the average values for each class of phenolic compounds in skin extracts, it is possible to see that: grapes grown under Yellow net (Y grapes) had a higher polyphenol concentration than the other thesis. In grapes grown under red net (R grapes) there was a minimum polyphenol content; grapes under White net (W grapes) had polyphenol concentration greater than R grapes. These differences among thesis, were not statistically significant for each class of polyphenols. Results of hydroxycinnamil tartaric (ICT) acid analysis in grape fleshes showed the same tendency of skins.

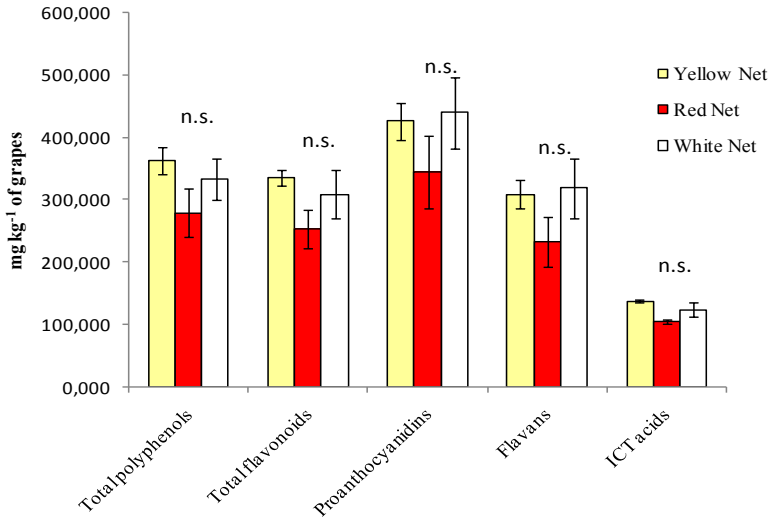


Fig. 6.8 - Polyphenolic composition of the first sampling: skins of Italia grapes grown under Yellow, Red and White nets.
different letters indicate significant difference at $p \leq 0.05$

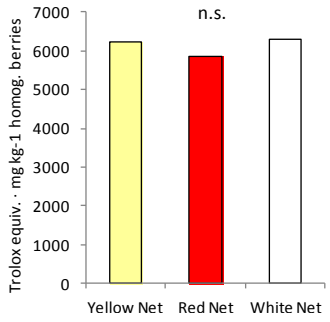


Fig. 6.9 - Antioxidant activity of berry fleshes and skins of grapes (cv. Italia) grown under colored nets (first sampling)
different letters indicate significant difference at $p \leq 0.05$

Antioxidant activity analysis on the same sampling gave results in line with the content of polyphenols, without statistically significant differences (fig. 6.9).

By the seed extract analysis (fig. 6.10) appeared that seeds followed the same trend of skins and hence, both in the skins that seeds, in these first grape sampling there was no statistically significant difference between Y, R and W grape phenolic concentrations.

Figg. 6.11 and fig. 6.13 respectively represents the results of phenolic analysis on skins and seeds of the grapes sampled at harvest, in early September 2014,. On average, R grapes compared to the others had the lowest concentration of each class of polyphenols, with the exception of the hydroxycinnamil tartaric (ICT) acids, that is not statistically different from Y grapes; this last thesis shows an intermediate polyphenol content, instead W grapes tend to have the highest concentration of these substances. W skins have more total polyphenols than R grapes; this difference is statistically significant, but it is not the same for the other classes of phenolic compounds, although the trend of average values of concentration was the same of total polyphenols.

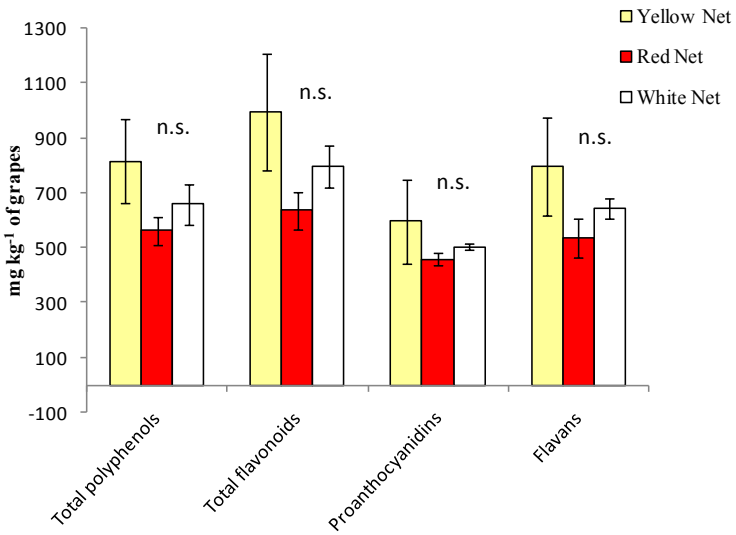


Fig. 6.10 - Polyphenolic composition of the first sampling: seeds of Y, R, W grapes (cv Italia) grown under Yellow, Red and White nets, respectively.
different letters indicate significant difference at $p \leq 0.05$

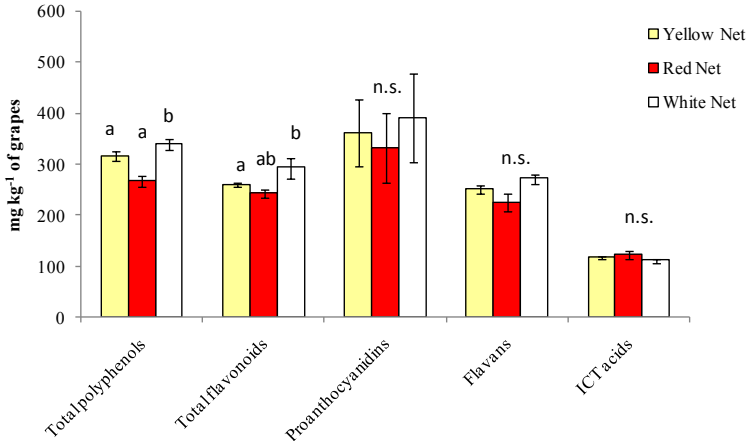


Fig. 6.11 - Polyphenolic composition of the second sampling: skins of Y, R, W grapes (cv Italia) grown under Yellow, Red and White nets, respectively.
different letters indicate significant difference at $p \leq 0.05$

W grapes had a higher concentration of polyphenols than Y ones and the difference was statistically significant in total polyphenol and flavonoid contents and not significant for proanthocyanidins, flavans and ICT acids. As showed by Haselgrove (2000), flavonoid composition is generally influenced by the solar radiation perceived, hence their concentration was enhanced in the more exposed treatments. In our study, the White treatment was more exposed to the PAR; in particular, in our conditions diffuse PAR tendency under nets seems to have more incidence on flavonoid concentration. These differences are not perceived early during the ripening process, but at harvest. the same trend of total polyphenols emerged by the antioxidant activity results (fig. 6.12) but without statistically significant differences.

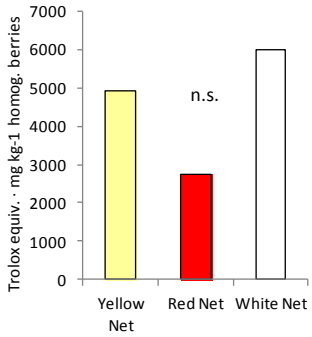


Fig. 6.12 - Antioxidant activity of berry fleshes and skins of grapes (cv. Italia) grown under coloured nets (second sampling).
different letters indicate significant difference at $p \leq 0.05$

The results of the seed extract analyses (fig. 6.13) showed that there were no statistically significant differences in any of the considered classes of polyphenols among the three treatments. According to average values, polyphenols were more concentrated in seeds of Y grapes, and R and W grapes had similar concentrations of proanthocyanidins, total polyphenols and flavonoids; the only difference between R and W grapes is visible in flavan mean values.

Hence, after about 10 days, that is the period between the two sampling, it was possible to see a trend change between W and Y grapes and an increment in polyphenol concentration in W grape skins. It seems that polyphenols production, at the beginning of fruit ripening, was slightly delayed when white nets are used; at the end of ripening there was an inversion of trend, interesting mainly the skins.

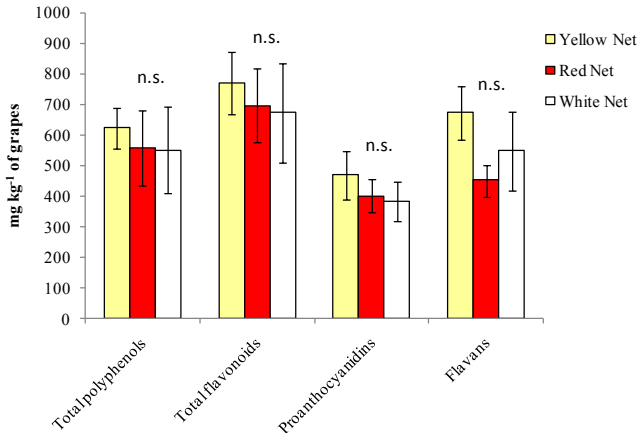


Fig. 6.13 - Polyphenolic composition of the second sampling: seeds of Y, R, W grapes (cv Italia) grown under Yellow, Red and White nets, respectively
different letters indicate significant difference at $p \leq 0.05$

At harvest, grape skin polyphenol composition showed differences, that before were not perceived, between coloured and white nets, the skin of the grapes grown under white nets were more concentrated in total polyphenols. This behaviour may be due to the major radiation filtered by red and yellow nets, that may have exerted on fruits a protective action by sunlight exposure and temperatures that could generally stimulate polyphenol accumulation (Bergqvist *et al*., 2001; Dokoozlian *et al.*, 1996). As suggested by Shahak (2004b) in fact, comparing several nets with the same knitting design, the spectrally modified light depends on chromatic additives contained in the plastic meshes. R grapes were slightly less concentrated in polyphenols than Y grapes, in accordance with the trend of both direct and diffuse PAR. Studies by Oren-Shamir et al. (2001), Shahak et al. (2004a) and Al-Helal and Abdel-Ghany (2010) showed that white nets, comparing to the other ones, have the highest proportion of diffuse light; as reported by Stamps (2009), diffuse light is important because increases radiation use efficiency, influencing plant development and growth. Therefore, more diffuse light may likely explain the highest concentration of polyphenols in W grapes. Comparing white net with the others, the intensity of the PAR reduction was measured to be 8% and 3% in red and yellow nets, respectively. Yellow net, transmits more light (+5%) than the red one. The trend is similar to polyphenol composition and it appears also clear the results of the antioxidant activity analysis on grapes. Results of statistical analysis showed that the differences among treatments were less perceived in total flavonoids and flavans and they were no significant for proanthocyanidins.

However, after ten days from the first sampling, there was an increment of the difference between Y and W grape polyphenol content so, at harvest, under white net grape skins developed more polyphenols than Y grapes. Polyphenol accumulation in the seeds followed about the same trend. It is well known that environmental factors, such us water availability and temperature, may influence mineral composition of grapevine leaves and berries (Gastol *et al*., 2014). In this experiment, the fruit juices were analyzed in order to compare macro- (K, Ca) and microelements (Cu, Fe). Among all treatments showed α -amino Nitrogen, ammonia Nitrogen and Readily Assimilable Nitrogen (RAN) ranged from 30 to 101 mg L^{-1}, from 46 to 92 mg L^{-1} and from 78 to 189 mg L^{-1}, respectively. Calcium, potassium and copper were in the ranges between 9 and 45 mg L^{-1} , 646 and 1689 mg L^{-1}, 1.75 to 2.79 mg L^{-1}, respectively. There were also traces quantities of ionic iron compared to the other elements. All the differences among treatments were no statistically significant. R grapes mean values in comparison with the others showed a tendency for a higher concentration of

Nitrogen, Calcium and Potassium. Copper had a very similar concentration among the considered thesis.

Tab 6.1 - Mineral composition of grape juices

Net colour	α -amino Nitrogen (mg L^{-1})	Ammonia Nitrogen (mg L^{-1})	Readily Assimilable Nitrogen (RAN) (mg L^{-1})	Calcium (mg L^{-1})	Potassium (mg L^{-1})	Copper (mg L^{-1})
Yellow	64.33 ± 20.22 a	68 ± 11.85 a	132.67 ± 32.05 a	19.67 ± 7.69 a	940.33 ± 105.77 a	2.27 ± 0.26 A
Red	78.33 ± 7.69 a	83 ± 6.24 a	161.33 ± 13.57 a	29.67 ± 6.69 a	1335.33 ± 177.35 a	2.04 ± 0.21 A
White	65 ± 18 a	60.67 ± 10.49 a	125.33 ± 27.95 a	25 ± 10.58 a	977.33 ± 188.84 a	2.31 ± 0.24 A

Different letters indicate significant difference at $p \leq 0.05$

The difference was particularly high in the case of Potassium; however, it did not reach any statistical level, due to the great variability found within the replicates. On the other hand, very low differences among replicates were found in the case of copper. At the moment, we are not able to individuate the reason of the large variations. Potassium was the most abundant macro-element in agreement with other researches (Etchebarne *et al.,* 2010; Rogiers *et al*., 2006) and its content in berry juice generally depends on soil water availability (Etchebarne *et al.,* 2010). K is important because it is an enzyme activator (Leigh *et al*., 1984; Walker *et al*., 1998) and responsible for osmotic potential regulation (Kendrick *et al*., 1986). K concentration in grape juice, at harvest, was higher in treatment with shaded microclimate, than the control grown in normal conditions of light. pH was in line with K concentration (Smart *et al*., 1985; Rojas-Lara *et al*, 1989); in our conditions under red net (the treatment with less PAR transmission) K content was the highest and pH among treatments had about the same trend of K concentration.
During fruit development K^+ generally increases to guarantee high osmotic value (Pierce and Higinbotham, 1970), while Ca^{2+}increases up to 30-40 days after anthesis and then has a decrease, likely due to the end of its action in contribution to the cell division and cell wall building (Possner *et al*., 1985). Davies and colleagues (2006) investigated the role of potassium in transport and accumulation of sugars during berry development, at veraison, a developmental stage characterized by cell multiplication, and after veraison with cell expansion. It was analyzed the action of two genes, two potassium transporters (VvKUP1 and VvKUP2), showing that, comparing their expression pattern during grape development with potassium accumulation (expressed as micrograms per gram of berry) there was a similar tendency, so they suggested the association between these potassium accumulation and the increase of berry size. In agreement with that study, in our results, Red treatment shows effectively the highest average values of berry diameters and potassium concentrations, while Yellow treatment, which corresponded to the smallest berry size, had the lower potassium concentration value.

On average, in our experiment, K and Ca^{2+} followed the same trend, having maximum concentrations under Red net and decreasing under Yellow ones. It was shown that in mandarin there is an inverse trend between K and $Ca^{2+.}$ with an increased concentration of K in shaded fruit (Cronje *et al*., 2011). Hence, in our study the slight major content of K in Red treatment was likely due to the lower PAR radiation perceived by this thesis. However, according to a study of Pereira and co-workers (2006) on grape in condition of microclimate

modifications, in the cluster zone K and Ca^{2+} content are not influenced by modification of light conditions, while between shaded and exposed treatments statistically significant differences are been shown in total N concentration. In contrast to those results, in our study N content is not statistically influenced by different light condition, although average values of α -amino Nitrogen, Ammonia Nitrogen and RAN contents are tendencially more pronounced in the juice of grape grown under Red net.
As in our experiment, other studies shown that in grape, K was generally much more concentrated than Ca^{2+} (Etchebarne *et al.*, 2010; Sensoy *et al.*, 2015). Mpelasoka and colleagues (2003) suggested that potassium enhances the transport of sugars into the berry, hence TSS are increased by this element; our results were tendencially in line with that hypothesis.

Tab. 6.2. - Results of the chemical parameter in grape juices of Italia– 1st sampling

Net colour	pH	Titratable acidity (g L^{-1})	SST (°Brix)
Yellow	3.19 ± 0.04 a	6.93 ± 0.031 a	17.90 ± 1.02 a
Red	3.28 ± 0.03 a	6.92 ± 0.41 a	18.30 ± 0.35 a
White	3.20 ± 0.03 a	6.68 ± 0.21 a	16.73 ± 0.55 a

Different letters indicate significant difference at $p \leq 0.05$

The parameters of total soluble solids (SST), titratable acidity and pH, carbohydrates and organic acids in grape juices are shown in Tables 6.2, 6.3 and 6.4, respectively. Sugar compositions analyzed in grape juices show that there are no statistically significant differences among the treatments. Titratable acidity (TA), expressed as grams per litre of tartaric acid that as shown by Kliewer and colleagues is the main responsible for the TA, (1967) and it decreased gradually throughout the maturation period (tab. 6.2 and 6.3), almost in all treatments inversely to sugar content. As shown by Saito and Kasai (1968), tartaric acid was synthesized until the early stage of grape ripening and soon converted to its salt form, tartrate. Kliewer and colleagues (1967) assessed the inverse correlation between free and salt form of malates and tartrate, the first being the decreasing form at ripeness.

On average, results of the first sampling showed a crescent pH in Yellow, White and Red treatments; titratable acidity was maximum in the juice of Yellow and Red treatment and minimum in the White one. As resulted by a study of Pereira and co-workers (2006) on grape in condition of different light exposure, a reduction of organic acids was enhanced by an increase of radiation perceived by grapes. This behaviour is in accord with our results, in which the more exposed treatment, White, had the lowest average value of titratable acidity, although the differences between treatments are not statistically significant and as showed by SST content, in this sampling grapes of W treatment are not enough ripe. SST concentrations under White, Yellow and Red treatments are in crescent order.

In the second sampling mean values show a higher pH, having crescent order from White and Yellow, to Red treatments (Tab. 6.3). Titratable acidity, from the first to the second sampling, was less concentrated in all theses: it is maximum in the juice of White treatment, intermediate in the Yellow and minimum in the Red one. SST concentrations are in crescent order in Red, White and Yellow treatments.
The concentration of total soluble solids in the juice of the date of collection was high, at about 18-19 °Brix (tab. 6.3). The ratio of grape maturity (SST / AT) was always equal to 32-33.

Tab. 6.3. - Results of the chemical parameter in grape juices of Italia– 2nd sampling

Net colour	pH	Titratable acidity (g L^{-1})	SST (°Brix)
Yellow	3.44 ± 0,03 a	5.70 ± 0.23 a	19.03 ± 0.33 a
Red	3.48 ± 0.04 a	5.47 ± 0.08 a	18.20 ± 0.66 a
White	3.43 ± 0.05 a	5.89 ± 0.42 a	18.33 ± 1.12 a

Different letters indicate significant difference at $p \leq 0.05$

In particular, Red treatment in this second sampling, was not the most concentrated in SST. In this situation, it is important to consider berry size, with the aim to understand fruit development during ripen. Comparing the berry sizes in all treatments and for both sampling, in fact, it is possible to see an inverse tendency between SST and longitudinal and transversal berry-diameters, so that i.e. under red net there was the highest berry size and the lowest SST concentration. However, although differences among treatments were no statistically significant, it is possible to see that, at harvest, tendency of sugar composition was slightly influenced by radiation perceived by vineyards. Under Red net, PAR was effectively the lowest and SST are minimum hence, sugar content in the juice of grape berries was likely reduced by shading conditions, as showed Smart and co-workers (1985),.
No statistically significant influence of net-colour berry composition was individuated (pH, Total acidity; °Brix and L-malic content), although grapes produced under yellow net showed a tendency for a higher SST concentration likely related to the tendency for a smaller berry size.

The hexoses glucose and fructose represent the large part of berry dry matter as shown by Coombe (1992), so they were considered to evaluate the main solutes in the juices. The ranges of sugar concentrations found in juices were as follows: glucose from 66.32 to 77.09 g L^{-1}; glucose + fructose from 338.80 to 426 g L^{-1}; glycerol from 0.091 to 0.115 g L^{-1}.
Glucose was slightly less concentrated than results of a study on the grape juices of Sabir and co-workers (2010), which found about 86.4 g L^{-1} on cv Italia, but in that study acidity was slightly lower. Average values in Tab. 6.3 and 6.4 show that: glucose and glucose+ fructose were more concentrated in the juice of R and Y grapes, respectively; glycerol seemed to be unaffected by the use of coloured hail-nets. TSS index, such as Glucose + fructose concentration, is higher in grape Y, probably for the smaller berry size.

Tab. 6.4 - Sugar composition of grape juices

Net colour	**Glucose** (g L^{-1})	**Glucose + Fructose** (g L^{-1})	**Glycerol** (g L^{-1})
Yellow	71.14 ± 0.73 a	402.72 ± 11.97 a	0.10 ± 11.97 a
Red	73.77 ± 1.73 a	388.35 ± 17.41 a	0.10 ± 0.005 a
White	72.76 ± 3.22 a	371.93 ± 17.97 a	0.10 ± 0.004 a

Different letters indicate significant difference at $p \leq 0.05$

In grape juices, malic acid, the main organic acid after tartaric (Saito *et al.*, 1968), ranges from 2.70 to 3.74 g L^{-1} so, in line with other study (Coombe, 1992). This acid was more concentrated than the others in tab. 6.5. Citric acid ranged from 0.166 to 0.302 g L^{-1}; D-

lactic and L-lactic acids ranged from 0,022 to 0,033 g L^{-1} and from 0,038 to 0,057 g L^{-1}, respectively; piruvic acid ranged from 0.010 to 0.024 g L^{-1}, L-ascorbic acid ranged from 35.36 to 52.09 mg L^{-1}.

Tab. 6.5 -Acidic composition of grape juices

Net color	**L- malic acid** (g L^{-1})	**citric acid** (g L^{-1})	**D-lactic acid** (g L^{-1})	**L-lactic acid** (g L^{-1})	**pyruvic acid** (g L^{-1})	**L-ascorbic acid** (mg L^{-1})
Yellow	3.35 ± 0.25 a	0.21 ± 0.03 a	0.03 ± 0.001 a	0.05 ± 0.001 a	0.02 ± 0.001 a	45.05 ± 3.60 a
Red	3.06 ± 0.11 a	0.27 ± 0.02 a	0.02 ± 0.002 a	0.05 ± 0.002 a	0.02 ± 0.001 a	45.00 ± 4.17 a
White	3.10 ± 0.24 a	0.21 ± 0.02 a	0.02 ± 0.008 a	0.05 ± 0.005 a	0.02 ± 0.004 a	37.40 ± 1.22 a

Different letters indicate significant difference at $p \leq 0.05$

Results of the acidic composition analysis in tab. 6.5, showed that the concentrations of L-malic and D-lactic acids were slightly higher when grapes grow under Y nets, while R and W average values are very similar to each other. Citric acid was a little more concentrated in R grapes, compared to the other ones. L-lactic and pyruvic acids seemed to be unaffected by the colour of net. L-ascorbic acid average values were similar between Y and R juice grapes and lower in W grapes. L-malic acid is more concentrated in Y grapes compared to R and W grapes, which had respectively minimum and maximum mean values of total acidity. Therefore, in R grape there was the lower concentration of total acidity and L-malic acid. Malic and tartaric acids were the main acids in the grapes. As well known, they are usually present as salts (malate, tartrate) and free acids. Their dominant form depends on variety, season, location, cultural practices, but also on the stage of maturity. Malic and tartaric acids are mainly in acidic form with a concentration increasing until veraison; those acids decrease at harvest, the period in which salt form is increased (Kliewer *et al*., 1967). Hence, in our conditions, R grapes seemed to be more ripened, because of their lower concentrations of L-malic acid and higher concentration of glucose. However, considering also the differences between SST and glucose + fructose (fig. 6.14), it was shown that they are usually higher in the less mature grapes (Kliewer *et al*., 1967). Hence, it is possible to suppose that in White treatment likely there were the more ripen grapes.

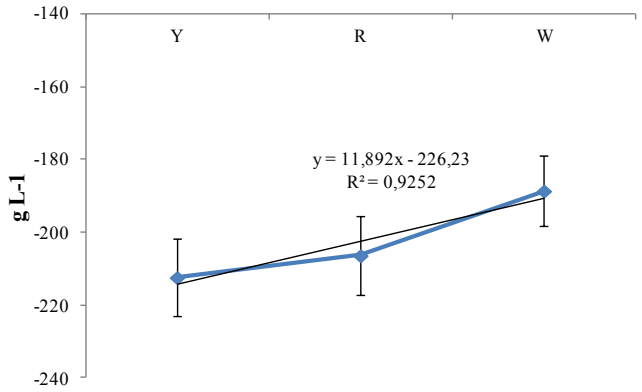
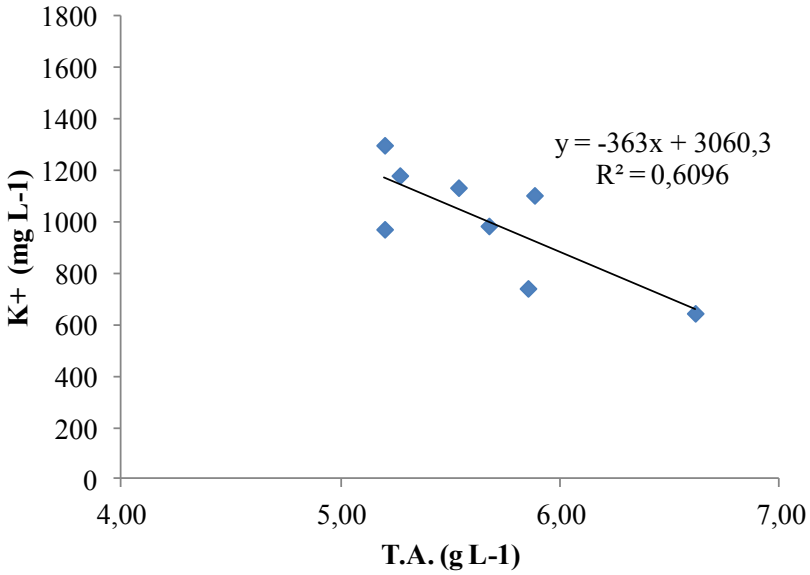
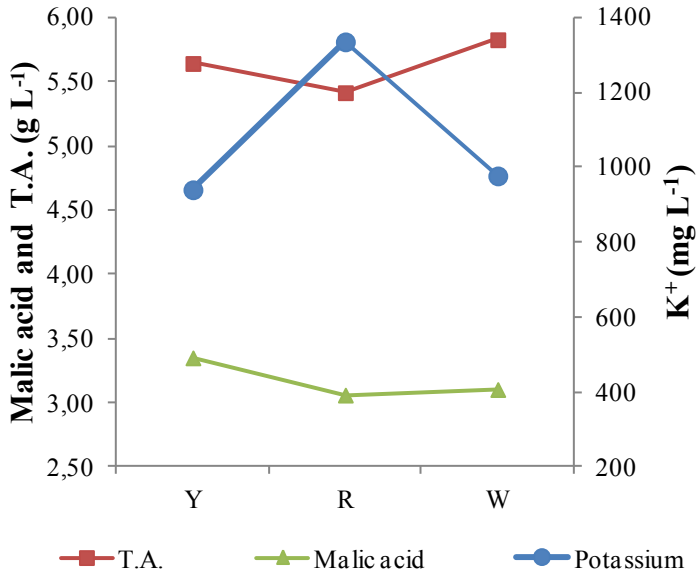


Fig. 6.14 - Differences between SST (in g L^{-1}) and glucose + fructose, in relationship to Yellow(Y), Red (R) and White (W) nets.

It is known (Pereira *et al*., 2006) that generally the more the berries are exposed, the more they are small, have a lower content of organic acids and nitrogen, the more are concentrated in SST. In our study, results showed that less light transmitted to the red treatment. Under this net they were produced the biggest berries, with the highest Nitrogen content and a minimum concentration of SST. Acidic content results are in contrast with the study of Pereira (2006) according which it should be the highest. This contrast, in addition to dilution effect due to the highest berry size, may be also explained considering the study of Spayd and co-workers (2002) showing as the organic acid decrease was an effect to the increase of temperature; results of our experiment showed that temperature was not influenced by the use of net.



(a)



(b)

Fig. 6.15 - Correlation between titratable acidity (g $L^{-1}$of tartaric acid) and potassium concentration (g L^{-1}) in grape juices (a) and tendency of this parameters and malic acid among treatments (b).

It is possible to see in fig. 6.15 that the concentration of potassium seemed to be in relationship with the acid levels in berries, decreasing together with tartaric acid, as reported by Davies (2006). In our study both titratable acidity and malic acid seemed to have been reduced with the same incidence in Yellow and Red treatments. Between Red and White treatments it is possible to note a slight difference of malic acid compared with the increase of the titratable acidity that likely suggest a lower composition in malic acid compared to titratable acidity. In fig. 6.16, in fact, it is visible that the percentage value of malic acid related to the titratable acidity, showed that between Red and White treatment, and between Yellow and Red ones, there were differences of about +3% for both comparisons, although they were no statistically significant. Malic acid on percentage was statistically more preponderant (+6%) in Yellow treatment compared with White one.

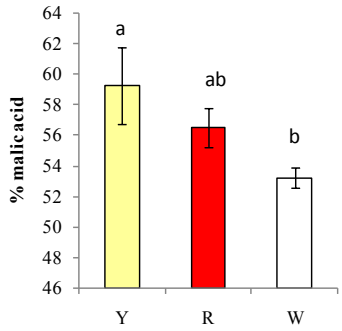


Fig 6.16 - Percentage of malic acid compared to titratable acidity)
Different letters indicate significant difference at $p \leq 0.05$

Hale and colleagues (1977), knowing the influence of mineral composition on grape titratable acidity, investigated the effect of potassium on malate and tartrate concentration, in particular at ripeness. It was shown that, at harvest, the decrease of malate concentration in grapes initially depends on the dilution effect due to berry growth and then on malate degradation.

In the same study Hale shown that potassium and malate correlation start during ripening, when there is malic acidic degradation and, as reported by Vikery (1973), the more potassium is concentrated the less membrane permeability is increased. Malate is mainly in the vacuole, so Hale and colleagues suggested that potassium, influencing tonoplast permeability, is indirectly responsible of malate concentration; when the potassium is more concentrated, titratable acidity (T.A.) increase is due to the highest concentration in malic acid. In our conditions Red treatment compared with the other ones, had a higher concentration in potassium and a minimum concentration of malic acid and the lower titratable acidity. This was in contrast with the study of Hale and colleagues (1977), although the differences among the treatments were no statistically significant in our conditions; Yellow treatment, compared to the other ones had minimum concentration of potassium per volume of juice, slighty more content in malic acid and intermediate mean value of A.T. However, by introducing a correction factor aim to take in consideration berry size (as diameter ratio) in relationship with malic acid and T.A., titratable acidity was still minimum in Red treatment. On the contrary, in the same thesis, malic acid had an intermediate concentration between Yellow and White treatments; by using the correction factor, it is possible to note that Yellow and White treatments had similar K and A.T. The latter parameters were less and more concentrated than Red treatment, respectively. Then, it is

possible to suppose that in our conditions, the slight differences in potassium concentrations seemed not influence T.A. and malic acid.

Summarizing, no significant influence of net-colour on berry juice composition was individuated (pH, Total acidity; °Brix and L-malic content), although Y grapes showed a tendency for a higher SST concentration likely related to the tendency for a smaller berry size. Under Red net, grape juices had tendencially the lower titratable acidity, malic acid content and higher content of Ca^{2+}, K, N so, as resulted by the study of Pereira and co-workers (2006), light condition influences both acidity content and mineral content, but in our experiment the differences are not statistically significant. Sugar content was not maximum in Red treatments as it should be, because, there is generally an inverse tendency between sugar and acidic composition. However, it was possible to note that glucose concentration is tendencially higher in red treatment compared to the other ones. In Italia variety, coloured nets seemed to have some influences on berry growth and the berry resistance to detachment from the rachis that are relevant aspects for table grapes.

Grape composition was analyzed in *Italia* cultivar with the aim to investigate the grapes variability in conditions of different colour net covering on vineyard. The results showed that, at harvest, phenolic composition of Italia cv was slightly affected by the use of coloured nets. Grapes grown under White nets seemed to have more total polyphenols in the skins, probably due to less filtered PAR under White nets, compared to the coloured ones. Acidic, sugar and mineral compositions were only slightly affected by the colour of the net.

The lower PAR intensity under Red net was likely responsible for the lowest phenolic composition of grapes under that net; also the decrease in net photosynthesis (P_N) and may be responsible since reduces the availability of carbon scheleton. The lower contents in polyphenols and flavonoids under Yellow net compared to the White one were likely due to PAR decrease.

However, the use of netting on vineyards is useful because of its protective function against environmental hazards, but it is important to adopt the right shading factor and colour of net, depending on the cultivar in question and climatic conditions, in order not to excessively reduce the polyphenol content and therefore the nutraceutical power of produced grapes.

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