

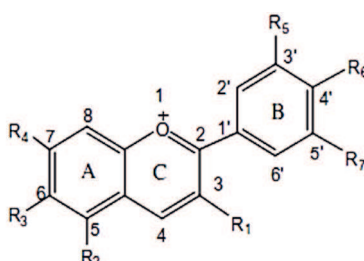
## ENVIRONMENTAL AND VITICULTURAL PRACTICE EFFECTS ON THE PHENOLIC COMPOSITION OF GRAPES: IMPACT ON WINE SENSORY PROPERTIES

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### Phenolic compounds in grapes: localization and biosynthesis

Grape-derived secondary metabolites are the principal sources of wine color, aroma and flavor. Among them, phenolic compounds are classified as non-flavonoid (benzoic and cinamic acids and stilbenes) and flavonoid (anthocyanins, flavonols and tannins).



<b>Anthocyanidin</b>	R1	R2	R3	R4	R5	R6	R7
cyanidin	OH	OH	H	OH	OH	OH	H
delphinidin	OH	OH	H	OH	OH	OH	OH
petunidin	OH	OH	H	OH	OMe	OH	OH
peonidin	OH	OH	H	OH	OMe	OH	H
malvidin	OH	OH	H	OH	OMe	OH	OMe

Figure 1: General structure of grape anthocyanidins

Among the latter, anthocyanidins are pigmented compounds located in the skins of grape berries in red cultivars while tannins derive from both skins and seeds of berries. Anthocyanins are glycosides of anthocyanidins. In red grape *Vitis vinifera* varieties, the most common 3-O-glucoside derivatives of anthocyanidins are delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside, and malvidin-3-O-glucoside, the latter being the most abundant (Figure 1) (Kallithraka et al. 2005). *Vitis labrusca* and *Vitis rotundifolia* grapes contain both anthocyanidin monoglucosides and diglucosides. Although anthocyanin profile is mostly genetically driven, the relative amounts of anthocyanins were also shown to depend to a lesser extent on the degree of grape ripeness (Roggero et al. 1986) and on the growing conditions (Kotseridis et al. 2012)

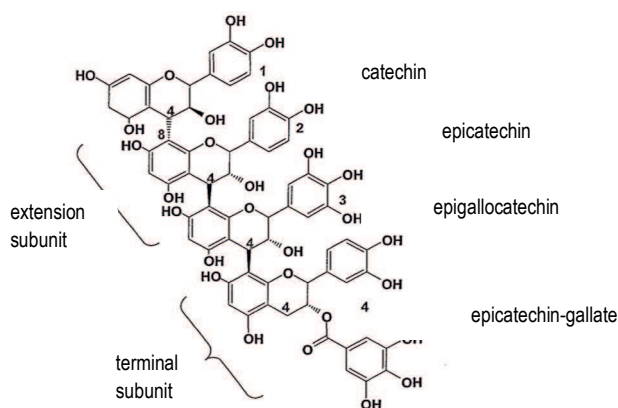


Figure 2: General structure of a proanthocyanidin polymer.

Proanthocyanidins are polymers composed of terminal and extension subunits of flavan-3-ols, mainly (+)-catechin (C), (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG) and (-)-epigallocatechin (EGC) (Figure 2). Grape tannins derived from skins and seeds vary in their relative amount, length, subunit composition and sensory properties. Tannin content of the skins is reported to be lower than that of seeds (Prieur et al. 1994). Seed tannins are also shorter, with a lower mean degree of polymerization (mDP) while skin tannins are generally longer with a higher mDP (Chira et al. 2009). Seed proanthocyanidins are usually composed of C, EC and ECG (Hanlin and Downey 2009) whereas skin proanthocyanidins also contain EGC as extension subunit (Li et al. 2014) and have a lower proportion of ECG (Gil et al. 2012). EC is the major extension subunit in the skins (Monagas et al., 2003) while seeds were found to contain similar amounts of C and EC subunits (Downey et al. 2003).

Flavonoid compounds are synthesized via the phenyl-propanoid biosynthetic pathway which is modulated by both the biotic and abiotic factors (Cassasa et al. 2015). Biosynthesis of tannins occurs after anthesis, reaching a maximum at veraison (Ollé et al. 2011). Downey et al. (2003) observed the highest concentration of flavan-3-ols in Shiraz skins before veraison followed by a continuous decrease until complete ripeness while in other studies, skin tannins were found to change little from veraison to harvest (Harbertson et al. 2002). Concerning seed tannins, most studies report a general decline during ripening associated with seed coat browning (Kennedy et al. 2000). The degree of polymerization of skin tannins was generally reported to increase with ripening especially in the polymeric fraction (Kyrleou et al. 2015b). On the contrary, seed proanthocyanidins mDP was either found to remain approximately constant (Pastor del Rio and Kennedy 2006) or it followed a decreasing trend during ripening (Bordiga et al. 2011). Accumulation of anthocyanins commences at veraison and presents a maximum around harvest period (Castellarin et al. 2007) but some authors have observed a decline just before harvest or during over-ripening (Bucchetti et al. 2011). Kyrleou et al. (2016b) found that maximum anthocyanin accumulation of Syrah berries, grown under semiarid conditions, occurred 18-24 days after veraison.

### Sensory properties of phenolic compounds

The types and amounts of various anthocyanins in grape skins determine the color and quality of the produced wines as they undergo co-pigmentation with other compounds to produce more stable pigments (Bolton 2001) but they are tasteless or indistinctly flavored (Vidal et al. 2004). On the other hand, proanthocyanidins are responsible for the bitter and astringent sensation of red wines. The intensity of astringency is positively related with proanthocyanidin concentration but it is mostly determined by polymer size, the longer molecules being more astringent than the smaller ones (Vidal et al. 2003). However, astringency of proanthocyanidins could also decrease at high mDP since

large molecules become either less soluble or too bulky to bind with proteins (Sun et al. 2013). Moreover, the extraction of tannins becomes more difficult as their polymerization degree increases, thus, larger tannins may be less easily released from skins during the maceration phase (Quijada-Morin et al. 2015). Astringency also increases with the degree of galloylation (presence of ECG subunits) while it decreases in the presence of prodelfinidins (proanthocyanidins with subunits constituted of EGC) (Vidal et al. 2003). Bitterness is more associated with lower molecular weight compounds such as flavan-3-ols monomers and oligomers (Peleg et al. 1999). Seed and skin tannins were found to be equally astringent when tasted at the same concentration in a wine or buffer medium in some studies (Brossaud et al. 2001) while other works reported a higher astringency sensation of seed extracts compared to those of the skins (Kyrалеou et al., 2016a). However, skin tannins commonly have a higher contribution to the polymer composition of the wine (Monagas et al. 2003) due to the increasing extractability of skin cell walls with the progress of ripening (Nunan et al. 1998) as compared to the lignified seed coat (Cadot et al. 2006). Finally, the bitter and astringent perception of tannins is also affected by the formation of complexes with soluble polysaccharides and proteins present in the grape must (Gil et al. 2012).

### Vineyard factors affecting phenolic composition of grapes

Grape and wine phenolic content has been related to many agronomical factors such as soil and climate (Koundouras et al. 2006), rootstocks (Koundouras et al. 2009), irrigation (Kyrалеou et al. 2016b), training systems (Kyrалеou et al. 2015a) and summer pruning techniques (Kotseridis et al. 2012).

Environmental factors (topographical, agro-pedological, climatic) have been acknowledged to influence grape and wine phenolic composition, mainly by controlling vine vigour as a result of soil rooting depth, fertility and water storage (Koundouras et al. 1999). Therefore, phenolic composition of grapes usually shows a well structured and time-stable spacial pattern within the vineyard, mostly related to soil physical properties. Bramley (2003) demonstrated how variation in soil depth controlled by variation in topography affected vine growth in a Coonawarra vineyard. In a work conducted in a cv. Agiorgitiko vineyard in Central Greece situated on a steep slope (Figure 3, unpublished data), berries with the highest phenolic content were located in the upper part of the vineyard with the more shallow soil and the lowest water reserves, associated with low vine vigour (estimated as winter pruning weight).

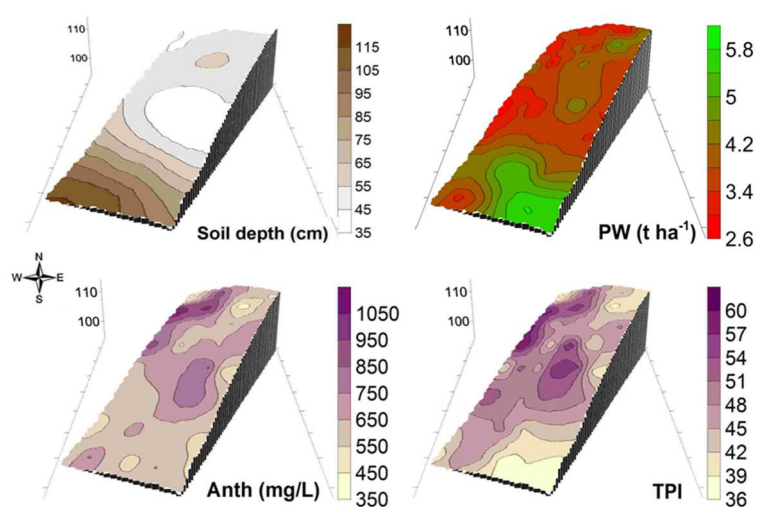


Figure 3: Spatial variability of the phenolic composition of 'Agiorgitiko' grapes as affected by topography (2010, Central Greece); PW, winter pruning weight; Anth, Total Anthocyanins in the juice; TPI, Total Grape Phenolics Index (OD280).

However, it is generally accepted that the direct manipulation of water availability through irrigation is the single most important management factor in determining berry and wine phenolic composition. More notably, many studies have reported a positive effect of a moderate water restriction imposed by deficit irrigation on the accumulation of anthocyanins in grapes in a variety of cultivars. The positive effects of water deficits on grape anthocyanin content are mostly attributed to suppressed vegetative growth, associated with improved canopy microclimate and better carbohydrate partitioning to the ripening berries (Romero et al. 2010) or to increased berry skin-to-pulp ratio (Koundouras et al. 2009). However, according to Roby et al. (2004), increases in skin polyphenol levels under limited irrigation can occur independently of any effect related to berry size modification. Recent studies have shown that the expression of genes of the flavonoid pathway is triggered earlier in grapevines submitted to water deficit thus stimulating anthocyanin biosynthesis (Castellarin et al. 2007). Anthocyanin accumulation pattern should also be considered when studying the effects of irrigation, since anthocyanin content is reported to undergo a decrease prior to harvest, especially under warm climate. In a recent study conducted in Greece (Kyraleou et al. 2016b), Syrah skin anthocyanins were higher in berries of water stressed vines 2 to 3 weeks after veraison, but no differences were observed compared to irrigated vines by harvest time.

In contrast to anthocyanins, reports on the effects of water availability on berry tannins are fewer. As regards skin tannins, most studies show an increasing trend of skin proanthocyanidins with water restriction (Roby et al. 2004, Casassa et al. 2015). Less uniform results are published concerning seed polyphenols, some studies showing increased concentration of seed flavanols with water deficiency (Chacón et al. 2009, Casassa et al. 2015) while other authors found higher levels of polyphenols in the seeds of irrigated vines (Kennedy et al. 2000, Koundouras et al. 2009). In the case of an irrigation trial under the semiarid conditions of Greece (Kyraleou et al. 2016a), irrigation was found to increase contents of total tannins and flavan-3-ol monomers and dimers in Syrah seeds, which might explain the stronger astringency of seed extracts of irrigated vines as perceived by sensory analysis (Figure 4). Regarding the effects of water conditions on the structural properties of skin and seed proanthocyanidins, such as polymerization, galloylation, and subunit composition, the amount of research is limited. In a recent study (Kyraleou et al. 2015b) water deficit increased skin tannin polymerization throughout ripening leading to increased levels of polymeric tannins at harvest as opposed to grapes of irrigated vines; in the same study, an opposite trend was observed for seed tannins, decreasing polymerization with water limitation (data not shown).

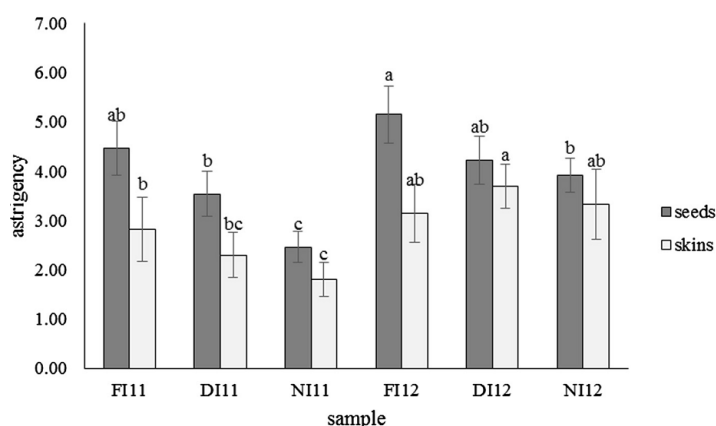


Figure 4: Mean astringency intensity of seed and skin extracts of Full Irrigated (FI), Deficit irrigated (DI) and Non Irrigated (NI) Syrah vines in 2011 (FI11, DI11, NI11) and 2012 (FI12, DI12, NI12). Grapes were collected at commercial harvest. Different letters within each year's samplings indicate statistical significant difference ( $p < 0.05$ ). Skin samples were treated separately from seed samples (from Kyraleou et al., 2016a).

Among the many seasonal practices that affect the phenolic composition of grapes, cluster exposure by selective leaf removal is accepted as a powerful technique to manipulate flavonoid content of grapes and wines. High light incidence on grapes is generally considered to promote greater anthocyanin accumulation in the skins (Jeong et al. 2004). According to Matus et al. (2009), the specific anthocyanin biosynthetic gene encoding UDP glucose: flavonoid-3-O-glucosyltransferase (UFGT) was particularly enhanced under increased exposure to solar radiation in Cabernet Sauvignon grapes. However, fruit-zone defoliation effects on grape composition are not always consistent depending on the timing (Tardaguila et al. 2010) and the severity of application (Kotseridis et al. 2012). Of particular importance in defoliation trials is the interaction between light intensity and temperature since the concomitant increase in exposed berry temperature, especially under warm climate conditions, may cause lower pigmentation in red grapes (Spayd et al. 2002). Mori et al. (2007) observed a significant reduction of anthocyanin content of Cabernet Sauvignon grapes at 35°C as compared to 25°C.

Contrary to skin anthocyanins, fewer data exist regarding the effect of cluster exposure to light on skin and seed proanthocyanidins. Sunlight exposure increased the accumulation of skin proanthocyanidins in Shiraz (Downey et al. 2004) and Pinot noir (Cortell et al. 2006) and decreased seed tannins (Cortell et al. 2006). Moreover, berry shading was reported to reduce the transcription of the specific proanthocyanidin biosynthesis genes in the skins during berry development while no significant effect was observed in the seeds (Fujita et al. 2007). A significant influence of vine vigor on total flavan-3-ol monomers in seeds has also been reported (Koundouras et al. 2009) with higher levels in high vigor vines possibly because more dense canopies increase shading in the fruit zone. In a post bloom defoliation trial conducted in Greece (Kotseridis et al. 2012), defoliation increased skin anthocyanins in Merlot and Cabernet Sauvignon but significantly reduced seed flavan-3-ols mainly as a result of the reduction in catechin and epicatechin amount. These effects were also largely independent of any variation in berry mass.

## Conclusion

While considerable research has been conducted on the impact of environmental and cultural practice on grape anthocyanins, additional knowledge is required in order to elucidate the dependence of polymeric flavonoids, as well as of their structural properties, on site and viticultural factors. Irrigation and microclimate manipulation are highlighted as the most important tools influencing grape phenolic potential but results are often contradicting depending on variety and experimental conditions. Moreover, the influence of other vineyard factors such as rootstocks, row orientation or training systems remain relatively less studied. The building of a comprehensive database per phenolic compound and cultural practice across a wide range of cultivars would greatly assist grape growers in adjusting vineyard management to winery specifications.

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### **Abstract**

*Grape phenolic compounds are located in the internal layers of grape skins and seeds. They are synthesized via the phenyl-propanoid biosynthetic pathway which is modulated by both biotic and abiotic factors. Considerable research has been conducted to clarify the evolution pattern of grape phenolic compounds and the role of environmental and viticultural factors that can manipulate their levels at harvest. The accumulation of phenolic compounds in grapes may be influenced by grape variety, environmental conditions and viticultural practices. More notably, the influence of irrigation on the accumulation of anthocyanins in grapes has been treated by several authors reporting an overall positive impact of mild water deficit, attributed to changes in berry skin-to-pulp ratio, modifications in grape microclimate or differences in the partitioning of assimilates among vine organs. Moreover, light environment of the grapes, as affected directly by leaf removal, is reported to modify skin anthocyanin content, profile and extractability. However, under hot climate conditions, increased temperatures of exposed berries may hasten phenolic ripening and decouple skin and seed sensory traits. Concerning berry tannins, reports on the effects of environmental and viticultural conditions are fewer and inconsistent. Moreover, there is limited information available concerning the effects of environmental and viticultural conditions on the structural characteristics of grape proanthocyanidins, such as polymerization, galloylation and subunit composition, which define wine sensory properties.*

**Keywords:** *grapevine, anthocyanins, tannins, flavan-3-ols, astringency, bitterness, polymerization, irrigation, microclimate.*