

Cultural Practice and Environmental Impacts on the Flavonoid Composition of Grapes and Wine: A Review of Recent Research

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Abstract: Flavonoids are a large and diverse group of compounds that, by their presence or absence, contribute greatly to wine quality. While the flavonoid content and composition of a wine reflects the vinification process to some extent, the primary determinant is the composition of the grapes at harvest. Thus, considerable research has been directed toward understanding the nature of flavonoids in grapevines, the factors that influence their biosynthesis, and how this knowledge might be used to manage and manipulate the flavonoid composition of berries at harvest. This review examines the flavonoids as a class of compounds, the role these compounds play in the plant, their contributions to wine quality, and recent research on the impacts of environmental factors and cultural practices on the flavonoid content and composition of grape berries.

Key words: phenolic, polyphenol, flavonoid, anthocyanin, flavanol, flavan-3-ol, tannin, proanthocyanidin

Distribution and Function of Flavonoids

The flavonoids are a group of compounds based on the polyphenolic flavan skeleton, variously substituted with hydroxyl, methyl, galloyl, glucosyl, and acyl moieties. Flavonoids can form complexes with other flavonoids, metal ions, and numerous other molecules (Asen et al. 1972, Brouillard et al. 1989, Strack and Wray 1993, Haslam 1998). Flavonoids can also form oligomers and extensive polymers under a range of biological and chemical conditions (Harborne 1967, Delcour et al. 1983, Haslam 1998).

There are more than 4,000 known flavonoids of diverse physiological function (Stafford 1991, Koes et al. 1994, Shirley 1996). Flavonoids occur in all vascular plants and in most mosses, but they have not been detected in algae or bacteria and are only rarely found in fungi (Markham

1982, Stafford 1991, Weiss et al. 1999). Historically, flavonoids, particularly anthocyanins, have been extensively studied in their role as floral pigments and various color mutants have aided the elucidation of the biosynthetic pathway (Harborne 1958, 1967, Stafford 1990). Studies of these compounds in numerous species, including grapes, have been instrumental to our understanding of the genetic and molecular basis of pigmentation (Bateson 1901, Lawrence 1950, Barritt and Einset 1969, Sparvoli et al. 1994, Holton and Cornish 1995, Boss et al. 1996a,b).

The chromophoric nature of the flavonoid ring structure results in the absorption of light in both the ultraviolet and visible spectra (Markham 1982). Absorbing ultraviolet light protects plants from UV radiation, which has been proposed as the archetypal role of flavonoid compounds (Koes et al. 1994, Shirley 1996, Smith and Markham 1998). Flavonoids that absorb both UV and visible light, including pigments such as aurones, chalcones, and anthocyanins, act as attractants for pollination and seed dispersal (Saito and Harborne 1992, Koes et al. 1994, Shirley 1996). Other physiological roles attributed to flavonoids include facilitating conditional male fertility in pollen (Ylstra et al. 1992, Taylor 1995) and the establishment of symbioses with nitrogen-fixing bacteria in the rhizosphere (Djordjevic et al. 1987, Clarke et al. 1992, Recourt et al. 1992). Flavonoids also act as deterrents to herbivory through the bitter and astringent nature of the flavan-3-ols and condensed tannins (Horowitz 1964, Shaver and Lukefair 1969, Feeny 1976, Elliger et al. 1980, Dreyer et al. 1981, Nef 1988, Wagner 1988, Mole 1989, Harborne and Grayer 1993, Lu and Bennick 1998). In addition, many flavonoids and isoflavonoids have phytoalexin activity with roles in plant defense against fungal and bacterial pathogens (Skipp and Bailey 1977, Smith 1982, Dixon and Lamb 1990, Dakora et al. 1993).

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Flavonoids in Grapes and Wine

Three classes of flavonoids are commonly detected in grapes and wine: anthocyanins, flavonols, and tannins. Anthocyanins are responsible for the color of red wine and grapes (Ribéreau-Gayon and Glories 1986). Flavonols are generally considered to act as UV protectants and free-radical scavengers (Flint et al. 1985, Smith and Markham 1998, Downey et al. 2003b). Although colorless, flavonols are also thought to contribute to wine color as anthocyanin copigments (Asen et al. 1972, Scheffeldt and Hrazdina 1978, Boulton 2001). In winegrapes, tannins include a range of polyphenolic compounds ranging from small oligomeric forms to large proanthocyanidin polymers, also known as condensed tannins. These oligomers and polymers are composed of monomeric subunits analogous to the flavan-3-ol monomers, such as catechin and epicatechin. The role of condensed tannins in grape is uncertain, but their bitterness and astringency is thought to act as a feeding deterrent to herbivorous animals and insects (Feeny 1976, Harborne and Grayer 1993). In wine, tannins are an important component of its gustatory impact, with flavan-3-ols and proanthocyanidins contributing to body and mouthfeel (Glories 1988, Thorngate 1997, Gawel et al. 2000). Tannins also contribute to color stability of wine by forming long-lived polymeric complexes with anthocyanins (Timberlake and Bridle 1976, Gawel 1998, Malien-Aubert et al. 2002, Mateus et al. 2002, Vidal et al. 2002). In red wines there are commonly two types of tannin, condensed and hydrolyzable. Hydrolyzable tannins are derived from wood, such as oak barrels or chips, or can be added to wine in powdered form (Ribéreau-Gayon 1972). Hydrolyzable tannins are not flavonoid compounds and will not be considered here. Of the three classes of flavonoids, condensed tannins are present in the greatest proportion in grapes, followed by anthocyanins, with flavonols present at relatively low levels (Souquet et al. 1996).

Flavonoid Biosynthesis

The biosynthesis of flavonoids is the culmination of two metabolic pathways, the shikimate and the phenylpropanoid. The phenylpropanoid pathway synthesizes flavonoids from carboxylated acetyl-CoA (malonyl-CoA) and the amino acid phenylalanine, which is produced via the shikimate pathway (Dewick and Haslam 1969, Heller and Forkmann 1993). Under normal growth conditions, ~20% of the carbon fixed by plants flows through the shikimate pathway, while ~2% of all carbon fixed by the plant flows from the shikimate pathway into phenylpropanoid metabolism (Markham 1982, Herrmann 1995).

The phenylpropanoid pathway is generally considered to culminate in anthocyanin synthesis. Branches in this pathway produce a range of other compounds, such as hydroxycinnamates, stilbenes, lignin, lignan, aurones, flavones, isoflavonoids, as well as the flavonoids, which include flavonols, tannins, and anthocyanins (Harborne 1967, Gerats and Martin 1992, Haslam 1998). Much of the

biosynthetic pathway was elucidated through characterization of color mutants, and genes encoding many enzymes in the pathway have been cloned (Forkmann 1993, Helariutta et al. 1993, Sparvoli et al. 1994, Charrier et al. 1995, Tanaka et al. 1996, Gong et al. 1997, Tanner et al. 2003, Xie et al. 2003). The study of flavonoid biosynthesis in grapevines has primarily focused on anthocyanin biosynthesis in the skin of the berry (Boss et al. 1996a, Ford et al. 1998, Downey et al. 2004a). Research has also been extended to the synthesis of flavonols in developing Shiraz and Chardonnay berries (Downey et al. 2003b) and tannin biosynthesis in Shiraz grape berries and grapevine leaves (Bogs et al. 2005).

In red grapes, anthocyanin accumulation commences at veraison, the onset of ripening (Somers 1976, Mullins et al. 1992, Boss et al. 1996a). Initially, only glucosides of the dihydroxylated anthocyanins cyanidin and peonidin accumulate, followed by the trihydroxylated anthocyanins based on delphinidin, petunidin, and malvidin (Mazza and Miniati 1993, Boss et al. 1996b, Katalinic and Males 1997, Keller and Hrazdina 1998). The accumulation of anthocyanins in the skin of red grapes coincides with expression of the gene encoding the final step in anthocyanin biosynthesis, UDP-glucose:flavonoid 3-*O*-glucosyl transferase (UFGT) (Boss et al. 1996a). In white grapes, there are no anthocyanins in the skin because the gene encoding UFGT is not expressed (Boss et al. 1996b). Flavonol biosynthesis in the grape also occurs only in the skin of the berry (Downey et al. 2003b). However, unlike anthocyanin biosynthesis, there are two distinct periods of flavonol synthesis in grape berries, the first around flowering and the second beginning one to two weeks after veraison and continuing throughout ripening.

Tannins in the grape berry are found in both the seeds and skin, with trace amounts also detected in the vasculature of the berry (Coombe 1987a). The main period of tannin synthesis in the seeds occurs immediately after fruit set with the maximum level observed around veraison. As with flavonols, the level of tannin in the skin is high at flowering and accumulation continues from fruit set until 1 to 2 weeks after veraison (Kennedy et al. 2001, Downey et al. 2003a, Bogs et al. 2005). The bulk of the tannin in both the seeds and the skin is present as proanthocyanidin polymers. Proanthocyanidin composition differs between seeds and skin with the seeds having shorter polymers comprised of similar amounts of catechin and epicatechin subunits (Downey et al. 2003a). In the skin, proanthocyanidin polymers tend to be much longer and comprised mainly of epicatechin subunits (Cheynier et al. 1997a, Kennedy et al. 2001, Downey et al. 2003a). The level of extractable tannins was observed to decrease in both seeds and skin between veraison and harvest. Physiologically, the decreasing extractability of tannins, particularly from grape skins, represents a decrease in the overall bitterness and astringency of tannins in the grape berry and is likely part of the seed dispersal strategy that includes sugar accumulation and anthocyanin biosynthesis in the berry.

For winemakers, a key issue is how the relative contributions of individual flavonoid components affect wine quality. For viticulturists, the challenge is to discover strategies that enrich winegrapes in the particular flavonoids considered most desirable for different styles of wine.

Factors Affecting Flavonoid Biosynthesis

There are many factors reputed to affect flavonoid biosynthesis in plants, including light, temperature, altitude, soil type, water, nutritional status, microbial interactions, pathogenesis, wounding, defoliation, plant growth regulators, and various developmental processes. While many of these factors have been investigated in grapevines, with the primary focus on grape color, much of the published research has been conducted on model plant species, such as *Arabidopsis*, or in other crops.

Nutrient availability has a fundamental influence on plant growth (Russell 1961, Marschner 1995, Keller et al. 1998) and has been shown to effect the flavonoid composition of plant tissues. Of the three nutrients commonly applied as fertilizer—nitrogen, potassium, and phosphate—only nitrogen and potassium have thus far attracted viticultural research. Both low and excessively high levels of nitrogen fertilizer have been shown to decrease color in grape berries (Kliewer 1977, Keller and Hrazdina 1998, Delgado 2004), while high potassium has been reported to decrease color in grapes (Morris et al. 1983, Jackson and Lombard 1993). The most likely mechanism for decreasing phenolic content at high nutrient levels is excessive vigor.

Vine vigor has also been reported to impact upon the tannin content and composition of grape skins in Pinot noir. In the berry skin, proanthocyanidins were higher in low-vigor vines, with an increase in the proportion of epigallocatechin subunits in proanthocyanidin polymers and an increase in the average size of polymers observed with decreasing vine vigor (Cortell et al. 2005). It is uncertain whether this change is due to the difference in vine vigor or is an indirect effect of changes in canopy architecture resulting in differential bunch exposure effects.

Physical characteristics can also affect flavonoid accumulation (Jackson and Lombard 1993, McDonald et al. 1998). Such characters as the parent material and the age of the soil that largely determine the micronutrient pool, structure, and texture of soil have a significant effect on plant growth (Russell 1961, Northcote 1992, Marschner 1995). However, the major consequence of soil type is the capacity of the soil to hold water while remaining sufficiently well-drained to avoid waterlogging (Russell 1961, Northcote 1992). Irrigation can alleviate water-stress-related reductions in plant growth and development, although some reports suggest water deficit increases tannin and anthocyanin content in grapes (Nadal and Arola 1995, Dry et al. 1998). In grape cell cultures, anthocyanin biosynthesis is extremely sensitive to osmotic stress (Do and Cormier 1991, Suzuki 1995). Osmotic stress results in

increased anthocyanin accumulation, which suggests that deficit irrigation could be a powerful tool for managing anthocyanins in the vineyard. However, some research suggests that while excessive water application decreased tannin content (Kennedy et al. 2000), water deficit had little or no effect on tannin or anthocyanin accumulation in the grape berry (Kennedy et al. 2000, 2002, Stoll 2000). Rather, the primary effect of water deficit was to decrease berry size and thus change the ratio of skin weight to total berry weight and therefore anthocyanin and tannin concentration in the berry. Closer investigation of this phenomenon suggested that changes in anthocyanin and tannin concentration did in fact occur with deficit irrigation aside from any effect related to berry size (Roby et al. 2004). However, the authors considered that changes in the structure and development of the skin were responsible rather than any direct effect on flavonoid biosynthesis (Roby and Matthews 2004, Roby et al. 2004).

The difficulty with interpreting deficit irrigation treatments is that water availability impacts on a wide range of plant processes apart from flavonoid biosynthesis. For example, stomatal closure in response to water deficit reduces photosynthesis, thereby reducing all metabolite accumulation and resulting in decreased root and shoot growth (Jones 1992). In extreme cases this may lead to senescence of some tissues and alter source-sink relationships within the plant (Coombe 1989).

Many such responses are regulated by plant growth regulators such as abscisic acid, ethylene, cytokinins, gibberellins, and auxins, and the influence of these compounds has been specifically examined with respect to their influence on flavonoid biosynthesis. In grapevines, abscisic acid has been shown to increase anthocyanin accumulation in grape berries of the cultivars Olympia, Kyo-ho, and Cabernet Sauvignon (Matsushima et al. 1989, Hiratsuka et al. 2001, Ban et al. 2003, Dan and Lee 2004, Jeong et al. 2004). The application of gibberellins (GA_3) to grapes has generally been reported to decrease anthocyanin levels in the fruit (Dan and Lee 2004). Gibberellic acid is usually applied to table grapes to increase berry size and the decrease in anthocyanin levels under these circumstances is likely to occur through an effective dilution of anthocyanin concentration in individual berries. A similar effect has been reported with the growth regulator forchlorfenuron (N-(2-chloro-4-pyridyl)-N'-phenylurea; CPPU) (Dan and Lee 2004).

Auxins and cytokinins are also plant growth regulators that were extensively used to manage plant production. While these were shown to increase anthocyanin biosynthesis in plants generally (Deikman and Hammer 1995, Nakamura et al. 1980, Ozeki and Komamine 1981), the application of the auxin naphthaleneacetic acid was shown to inhibit the anthocyanin accumulation in Cabernet Sauvignon grape berries (Jeong et al. 2004). Application of the synthetic auxin benzothiazole-2-oxyacetic acid to Shiraz vines delayed the onset of ripening, including anthocyanin accumulation in the grape skin (Davies et al.

1997), a result of a delay in the expression of genes encoding critical steps in anthocyanin biosynthesis such as chalcone synthase and UFGT. A similar result was reported in the Kyoho cultivar with auxin 2,4-dichlorophenoxyacetic acid inhibiting anthocyanin accumulation and expression of the flavonoid pathway genes phenylalanine ammonia-lyase, chalcone synthase, chalcone isomerase, dihydroflavonol reductase, and UFGT, while the same genes were upregulated and anthocyanin content increased with the application of abscisic acid (Ban et al. 2003).

Ethylene has also been identified as a plant growth regulator that has a particular effect on fruit ripening (Burg and Burg 1965). Application of ethylene had no effect on anthocyanin accumulation in *Arabidopsis* (Deikman and Hammer 1995); however, in grape berries there seems to be a requirement for low levels of endogenous ethylene for anthocyanin biosynthesis (Chervin et al. 2004). Exogenous application of ethylene or ethylene-releasing compounds has also been shown to enhance grape skin color (Roubelakis-Angelakis and Kliewer 1986, El-Kereamy et al. 2003).

Wounding and pathogenesis have also been observed to impact upon phenylpropanoid biosynthesis in grapevines. While this has been extensively investigated in relation to the phytoalexins resveratrol and viniferin (Langcake and Pryce 1976, 1977a,b, Langcake and McCarthy 1979, Pool et al. 1981, Creasy and Coffee 1988, Dercks and Creasy 1989, Jeandet et al. 1995), to date this work has not been extended to flavonoids.

The effect of altitude on grape berry development and composition has also been examined. In the Spanish red *Vitis vinifera* cultivars Touriga Nacional and Touriga Francesca, anthocyanin content was observed to increase with increasing altitude from 150 to >250 m above sea level (Mateus et al. 2002). In contrast, flavan-3-ol monomer, dimer, and total proanthocyanidin content in the skin of both cultivars decreased with increasing altitude, while seed monomers and proanthocyanidins decreased in Touriga Nacional but increased in Touriga Francesca (Mateus et al. 2001). However, it is unlikely that these results are strictly effects of altitude but rather the effects of different climatic conditions at each site, with the higher latitude sites cooler than the lower sites.

Many variables impact flavonoid composition in grapevines and many of these factors are closely interrelated and difficult to isolate experimentally. Added to this is the genetic variation between plant species and varieties. In Vitaceae alone there are ~10,000 known cultivars (Mullins et al. 1992), resulting in an enormous diversity of flavonoid content and composition. Despite the variability conferred by any or all of these parameters, the greatest influences on the flavonoid content of any cultivar are site and season (Bakker et al. 1986, Gonzalez-San Jose et al. 1990, Revilla et al. 1997, McDonald et al. 1998, de Freitas and Glories 1999, Guidoni et al. 2002). For a given site in an irrigated vineyard, it is assumed that characteristics such as soil will remain invariant, nutrition will be ad-

equate, and viticultural practices will not vary greatly from year to year. Thus, the primary seasonal difference will be climatic, predominantly sunlight and temperature.

Influence of Light and Temperature on Grape Composition

Investigations into the effects of light on flavonoid biosynthesis in grapes have taken a range of approaches, most involving the application of physical shade treatments, including plastic sheeting (Kliewer et al. 1967), shade cloth (Smart et al. 1988), bags (Weaver and McCune 1960, Kliewer and Antcliff 1970), and foil-clad cages (Haselgrove 1997). Others have tried binding canes under the canopy (Archer and Strauss 1989), sampling different parts of the canopy where the light regimen was perceived to be different (Price et al. 1995, Haselgrove et al. 2000, Bergqvist et al. 2001), and defoliation (Kliewer and Antcliff 1970, Hunter et al. 1995). In addition, treatments have been applied at different developmental stages from fruit set to veraison. These approaches resulted in a range of different exposure levels of fruit and, in some cases, different levels of foliage exposure with concomitant impacts on photosynthesis.

Where shading of the foliage has occurred, the flavonoid content of the grapes was observed to decrease significantly (Buttrose et al. 1971, Kliewer 1977, Crippen and Morrison 1986, Smart et al. 1988). However, there was also a general decrease in carbon fixation in these grapevines, which resulted in lower levels of other metabolites such as sugars and organic acids (Kliewer et al. 1967, Kliewer 1977, Smart et al. 1988, Rojas-Lara and Morrison 1989).

The application of shading treatments has also been shown to substantially alter temperature and humidity within grapevine canopies (Kliewer et al. 1967, Rojas-Lara and Morrison 1989, Haselgrove et al. 2000). Increased humidity in the canopy has a 2-fold effect. First, by lowering vapor pressure deficit, transpiration and photosynthesis are decreased, reducing growth and subsequently flavonoid accumulation. Second, high humidity increases the risk of pathogenesis through fungal or bacterial infection (Emmett et al. 1992), which may cause a general wound response inducing flavonoid accumulation (Mehdy and Lamb 1987, Vogt et al. 1994).

Increased temperature in the plant, either through direct heating by incident radiation or increased air temperature, will increase the rate of metabolic processes in the plant with an associated increase in development and metabolite accumulation (Hawker 1982, Jones 1992, Ebadi et al. 1995, Dokoozlian and Kliewer 1996). However, at high temperatures many metabolic processes stop or are significantly reduced (Jones 1992). In grapevines it is thought that this temperature is ~30°C (Coombe 1987b).

There is much historical data on the effects of sun exposure on grape composition, including phenolic and flavonoid composition of the berry. Reports on the effects of shading on grape color have fascinated growers and re-

searchers. One study on table grapes showed that when grown in the dark, Tokay berries remained green (Weaver and McCune 1960). While this study shaded ~40 cultivars, most of which colored normally, the Tokay result made a vivid impression on the viticultural industry, leading to numerous subsequent investigations.

These subsequent investigations showed that low light also reduced color in Emperor table grapes and in the winegrape Pinot noir (Kliewer 1970, 1977). Similar results were later reported in Shiraz (Smart et al. 1985) and Cabernet Sauvignon (Morrison and Noble 1990, Hunter et al. 1991, Dokoozlian and Kliewer 1996). Together these results created a strong impression that light was necessary for color formation in grapes, an impression reinforced by observations from other plant species, such as apple, where light is an absolute requirement for anthocyanin biosynthesis (Siegelman and Hendricks 1958, Chalmers and Faragher 1977, Lancaster 1992, Dong et al. 1998). Generally, the consensus developed that low light reduced anthocyanins and other flavonoids, while increasing light increased the flavonoid content of grapes (Wicks and Kliewer 1983, Dokoozlian and Kliewer 1996).

As investigations into the effects of exposure on grape color continued, a growing body of contradictory data began to appear. Some studies failed to observe any change in total anthocyanins with shading, while others reported that high light resulted in decreased anthocyanin levels (Hunter et al. 1995, Bergqvist et al. 2001, Spayd et al. 2002). In other cases, there was no change in the total, but shifts in anthocyanin composition were observed (Price et al. 1995, Haselgrove et al. 2000, Spayd et al. 2002, Downey et al. 2004a). Many possible explanations have been suggested for the range of results, including differences in cultivar, site, and season as well as sampling and analytical techniques. In addition, it has often been difficult to separate the effects of light and temperature.

Separating the Effects of Light and Temperature on Anthocyanin Biosynthesis

The first serious attempts at separating the effects of light and temperature on flavonoid biosynthesis in grapevines used potted vines of Pinot noir and Cabernet Sauvignon in a phytotron (Kliewer and Torres 1972, Dokoozlian and Kliewer 1996). The authors present a convincing case for increased anthocyanin biosynthesis in exposed compared to shaded fruit, which clearly demonstrates that anthocyanin biosynthesis in grapevines can be manipulated by bunch exposure. Nevertheless, it can be reasonably argued that a phytotron is a highly artificial environment and that these results may in part be an artifact of both the phytotron and potted vines. In the field, Bergqvist et al. (2001) closely monitored the light and temperature environment of Cabernet Sauvignon and Grenache grape berries as well as anthocyanin accumulation in the fruit. These authors observed that anthocyanin accumulation increased with increasing light up to 100 mmol/

m²/s on the north (shaded) side of the canopy. However, on the south (sunny) side of the canopy, when exposure exceeded 100 mmol/m²/sec anthocyanin accumulation began to decrease in both Cabernet Sauvignon and Grenache berries (Bergqvist et al. 2001).

Spayd et al. (2002) examined north-south oriented rows of Merlot. The east side of rows received only morning sun, while the west side of rows was exposed to afternoon sun. Because of this, temperature of fruit on the west side of rows was substantially higher than fruit on the east side. Fruit from the east side of rows had higher levels of anthocyanins than fruit from the west side, exposed to hot afternoon sun, consistent with the observations of Bergqvist et al. (2001). To separate the effects of light and temperature, Spayd et al. (2002) artificially cooled more exposed fruit and heated less exposed fruit. Cooling the highly exposed fruit on the west side of the row increased the level of anthocyanins in that fruit, while heating the less exposed fruit resulted in a reduction in anthocyanin levels, demonstrating that accumulation of anthocyanin is more a function of temperature than of light.

Using an alternative approach, Downey et al. (2004a) developed a lightproof box that excluded light from bunches of Shiraz grapes without modifying other microclimate parameters such as temperature and humidity. In two out of three seasons, there was no difference in total anthocyanins between the shaded and normally exposed fruit. However, in one season the level of anthocyanins in the shaded fruit was significantly lower throughout berry development and at harvest than in the normally exposed fruit. The authors concluded that anthocyanin biosynthesis in Shiraz was not, on the whole, influenced by light or dark in Shiraz and that observations for the second season were temperature related, as it was extremely hot.

Generally, the extant literature suggests that temperature has a greater influence on anthocyanin biosynthesis in grapes than light. There is a strong indication that above 100 mmol/m²/s incident solar irradiation, the temperature load on the berry has a negative impact on anthocyanin biosynthesis. At precisely what berry temperature that occurs remains uncertain, although it has been observed in Cabernet Sauvignon grape berries that anthocyanins were greater in the fruit when the day temperature was a constant 20°C rather than 30°C (Buttrose et al. 1971). Complicating this is the effect of diurnal differences in temperature, where lower night temperature (15°C) resulted in greater anthocyanin accumulation than a constant temperature of 30°C (Mori et al. 2005). When the day temperature was between 30 and 35°C, anthocyanin synthesis in Pinot noir was unaffected, while it was virtually inhibited in Cardinal grape berries at similar temperatures (Kliewer and Torres 1972). Elsewhere, Ferrini et al. (1995) suggest that in the grape cultivar Trebbiano, the critical leaf temperature for photosynthesis occurs between 27.5 and 35°C. Thus, as Coombe (1987b) suggested for primary metabolism of the berry, the critical metabolic temperature may very well be ~30°C.

Both Bergqvist et al. (2001) and Spayd et al. (2002) observed that the temperature of fully exposed fruit was $>10^{\circ}\text{C}$ than shaded fruit and that the fully exposed fruit was also significantly warmer than the ambient air temperature. That is consistent with the conclusions Smart and Sinclair (1976) had drawn from excised grape berries and suggests that grape berry temperature, particularly in warm-climate viticulture (Winkler regions IV and V; Winkler et al. 1974), frequently reaches a level that would inhibit anthocyanin synthesis.

While anthocyanin biosynthesis might be reduced by high temperature, in forage legumes high temperature stress increases tannin accumulation in leaves (Lees et al. 1994). A salient observation from research on forage legumes might wisely be noted: at the optimum temperature for growth, tannin levels were significantly reduced (Carter et al. 1999), implying that all secondary metabolite biosynthesis might be reduced under optimum conditions for biomass accumulation. That is analogous with the high-vigor, low-quality observations of Cortell et al. (2005), but also suggests that some degree of stress stimulates the production of secondary metabolites in plants. Indeed, this acts as a pertinent reminder that much of the physiological role of secondary metabolites is a defensive response to environmental stresses (Koes et al. 1994, Sparvoli et al. 1994, Dixon and Paiva 1995).

Nevertheless, the observation that higher temperatures result in decreased anthocyanin levels energized growers and researchers alike to examine mechanisms to manage vineyard temperature. This research has primarily focused on the use of evaporative cooling in vineyards, which was found an effective means of reducing temperature with concomitant increases in grape color in the Tokay, Cardinal, and Carignane cultivars (Gilbert et al. 1970, Kliever and Schultz 1973). To date this research has not been extended to winegrapes; although generally the application of excessive water is likely to result in increased vigor, and possibly increased berry size, which may have undesirable impacts in winegrape quality. Furthermore, increased water use has implications for disease pressure (Gilbert et al. 1970) and may be impractical where the cost or availability of water is a limitation.

Changes in Anthocyanin Composition with Bunch Exposure

Apart from the effect of various light and temperature regimes on total anthocyanins, anthocyanin composition has also been closely examined. Anthocyanins in grape (*Vitis vinifera* L.) are based on cyanidin, peonidin, delphinidin, petunidin, and malvidin. Each of these is glucosylated at the 3 position of ring C. The glucoside can be further substituted with acetyl and coumaroyl moieties, giving rise to some 15 different anthocyanins commonly found in grape berries (Mazza 1995). Recent research has shown shifts in anthocyanin composition, with shaded fruit having a higher proportion of trihydroxylated anthocyanins: those based on delphinidin, petunidin, and

malvidin (Spayd et al. 2002, Downey et al. 2004a). This shift had not previously been reported, although shifts in composition based on glucoside substitution have been reported with a proportional shift from nonacylated glucosides to coumaroylglucosides with shading (Gao and Cahoon 1994, Price et al. 1995, Haselgrove et al. 2000).

Furthermore, temperature is also implicated in altering the anthocyanin composition of grape berries. Downey et al. (2004a) reported a proportional increase in coumaroyl glucosides in a season of high temperature, while Spayd et al. (2002) reported that cooling the fruit decreased coumaroyl derivatives and heating caused a substantial increase. Such shifts in composition with temperature suggest that warm-climate fruit would tend to have a higher proportion of malvidin, petunidin, and delphinidin coumaroyl derivatives, while cool-climate, shaded fruit might have more peonidin and cyanidin nonacylated glucosides and acetylglucosides. What has not yet been firmly established is the relative contribution of each of these components to final wine color. However, research on extractability of anthocyanins from the fruit into the wine suggests that the nonacylated glucosides and acetylglucosides are more readily extracted from the fruit than the coumaroylglucosides (Leone et al. 1984, Roggero et al. 1984). Such an observation may account for some of the reduction in grape berry color observed in very hot seasons. Whether this decrease occurs through degradation of anthocyanins or reduced anthocyanin biosynthesis is not known.

Light and Temperature Effects on Flavonols and Tannins

While the first exposure experiments were primarily concerned with the impact of light on anthocyanin accumulation in the grape, other flavonoid components such as flavonols and tannins have also attracted research interest. Flavonol accumulation in plant tissues has previously been studied in a number of species, with exposure to UV shown to increase flavonol glucosides in vegetative and reproductive tissues (Hrazdina and Parsons 1982, Ryan et al. 1998, Vogt et al. 1999, Reay and Lancaster 2001). This effect has also been reported in winegrapes, with exposed fruit higher in flavonol glucosides, while shaded fruit had lower flavonol content (Price et al. 1995, Haselgrove et al. 2000, Spayd et al. 2002). More recently, Downey et al. (2004a) reported that the level of flavonols in both leaves and fruit of the grapevine were almost negligible when those tissues had not been exposed to light. Subsequent exposure of those tissues to sunlight resulted in a rapid increase in flavonol accumulation and in expression of the gene encoding flavonol synthase (Downey et al. 2004b).

In contrast, tannin accumulation in Shiraz grape berries appears to be largely unaffected by bunch exposure (Downey et al. 2004a). In the seed there was no observable effect of bunch exposure on either the proanthocyanidin content or composition. In the skin of the grape berry at harvest, there was also no appreciable difference in tannin content. However, the study examined proantho-

cyanidin content and composition throughout berry development in both shaded and exposed fruit, revealing significant differences in both content and composition throughout the intermediate stages of berry development, with shaded fruit reaching a much lower maximum in proanthocyanidin content than exposed fruit. The peak in proanthocyanidin accumulation in winegrapes occurred around time of veraison and then decreased toward harvest in what is generally considered to be a decrease in tannin extractability rather than degradation or turnover (Czochanska et al 1979, Amrani-Joutei et al. 1994, Escribano-Bailon et al. 1995, Cheynier et al. 1997b, Saint-Cricq de Gaulejac et al. 1997, de Freitas and Glories 1999, Kennedy et al. 2001, Downey et al 2003a). This decrease in tannin extractability was observed in both shaded and exposed fruit; however, the decrease was greater in exposed fruit such that the levels were virtually the same in shaded and exposed fruit at harvest. The effects of shading on tannin accumulation in grape berries have only been examined in Shiraz, although this is an active area of research in the Australian wine industry.

Managing Wine Flavonoid Composition in the Vineyard

While a plethora of studies on the flavonoid composition of wines have been published, few have attempted to link wine flavonoid composition to that of the grapes at harvest and fewer still have examined the connection between environment or viticultural practice and grape and wine flavonoid composition. Of these, Price et al. (1995) examined the effect of bunch shading on the anthocyanin and flavonol content of the fruit and subsequent wines of the cultivar Pinot noir. Total phenolics were higher in wines made from exposed compared to shaded wines. Both flavonols and anthocyanins were significantly lower in the wines made from the shaded fruit, although the level of anthocyanins was not significantly different in the skin of the grape. Both pigmented polymers and proanthocyanidins were lower in the wines made from shaded fruit, while the level of catechin monomers was significantly higher. Elsewhere, decreasing vine water status in Cabernet Sauvignon has been observed to increase concentration of flavonols, anthocyanins, and skin-derived proanthocyanidins in the wine (Kennedy et al. 2002).

The effects of grape maturity and yield on flavonoid composition of wine have also been researched. In cultivars Tinto Fino and Cabernet Sauvignon, the level of catechin monomers in the wines decreased with increasing grape maturity (Pérez-Magarino and Gonzalez-San Jose 2004). While catechin decreased with maturity, other flavan-3-ol components such as epicatechin and proanthocyanidin dimers and trimers increased in concentration in more mature fruit. Some sensory attributes of Cabernet Sauvignon wines were examined at different yields. Bitterness and astringency, associated with flavan-3-ol and proanthocyanidin content, were rated higher in wines from low-yielding compared with high-yielding vines (Chapman et al. 2004).

These few examples suggest that the content and composition of different flavonoid classes, such as flavonols, anthocyanins, and proanthocyanidins, can be manipulated by viticultural practices such as canopy management, irrigation, yield regulation, and timing of harvest. Of the viticultural approaches to managing and manipulating grape flavonoid content and composition for wine quality, canopy management for bunch exposure has attracted considerable interest and has been widely implemented as a management tool in many countries. As a result, a substantial effort of current research is focused on developing the potential to manage flavonoid composition of the fruit through bunch exposure and understanding the impacts of those manipulations of wine quality.

Conclusions

While much of the extant data on the impact of environment and cultural practice on grape and wine quality is derived from isolated studies—several conducted on a single season's data and limited to comparatively few cultivars—some valuable conclusions emerge. Of the many factors that influence flavonoid content and composition of a grape cultivar, site and season are the most dramatic. Assuming that most other inputs are relatively constant, these influences can be crudely summarized as light and temperature. Long recognized, these have proven difficult to manage, although row orientation, trellising, and other canopy modifications are demonstrated mechanisms for manipulating both parameters to an extent. Furthermore, strategic use of irrigation and cover crops in other horticultural systems suggests that such approaches may be effective in influencing temperature in viticultural systems.

Existing studies also indicate a lack of uniformity of response across grape cultivars. While the inherent plasticity of the plant may account for some of this variation, it may also be an artifact of the different research methodologies used. Nevertheless, there is a clear indication that the response of grapevines to various management practices is cultivar dependent. Thus, applying the same management practice to a multitude of different grape cultivars is unlikely to result in optimum flavonoid content or composition in some or all of those cultivars, and strategies will need to be tailored to suit individual cultivars. This is clearly the antithesis of applying a single, universal practice and is likely impractical, both from a research and a viticultural management perspective. The solution lies somewhere in between, and what is currently beholden to researchers is to determine which cultivars show similar responses such that management strategies can be developed for groups of cultivars.

While developing management strategies for optimizing grapevine flavonoid composition is a major research objective, equally important is the development of standardized tools for measuring flavonoids in fruit and wine that the industry can readily adopt. While many practical analyses have been usefully described (Iland et al. 2000), a standardized measure of tannins has yet to be adopted.

One possible method is that of Harbertson and colleagues (Harbertson et al. 2002, 2003, Downey and Adams 2005). The value of such a method would be greatly enhanced by a database of tannin content across a wide range of cultivars and regions, which, combined with grape color data, may prove a useful tool for predicting final wine color.

Literature Cited

- Amrani-Joutei, K., Y. Glories, and M. Mercier. 1994. Localization of tannins in grape berry skins. *Vitis* 33:133-138.
- Archer, E., and H.C. Strauss. 1989. Effect of shading on the performance of *Vitis vinifera* L. cv. Cabernet Sauvignon. *S. Afr. J. Enol. Vitic.* 10:74-77.
- Asen, R., R.N. Stewart, and K.H. Norris. 1972. Co-pigmentation of anthocyanins in plant tissues and its effect on colour. *Phytochemistry* 11:1139-1144.
- Bakker, J., P. Bridle, C.F. Timberlake, and G.M. Arnold. 1986. The colours, pigment and phenol contents of young port wines: Effects of cultivar, season and site. *Vitis* 25:40-52.
- Ban, T., M. Ishimaru, S. Kobayashi, S. Shiozaki, N. Goto-Yamamoto, and S. Horiuchi. 2003. Absciscic acid and 2,4-dichlorophenoxyacetic acid affect the expression of anthocyanin biosynthetic pathway genes in 'Kyoho' grape berries. *J. Hortic. Sci. Biotech.* 78:586-589.
- Barritt, B.H., and J. Einset. 1969. The inheritance of three major fruit colours in grapes. *J. Am. Soc. Hortic. Sci.* 94:87-89.
- Bateson, W. 1901. Experiments in plant hybridisation. *J. Royal Hortic. Soc.* 26:1-32.
- Bergqvist, J., N. Dokoozlian, and N. Ebisuda. 2001. Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the central San Joaquin Valley of California. *Am. J. Enol. Vitic.* 52:1-7.
- Bogs, J., M.O. Downey, J.S. Harvey, A.R. Ashton, G.J. Tanner, and S.P. Robinson. 2005. Proanthocyanidin synthesis and expression of genes encoding leucoanthocyanidin reductase and anthocyanidin reductase in developing grape berries and grapevines leaves. *Plant Physiol.* 139:652-663.
- Boss, P.K., C. Davies, and S.P. Robinson. 1996a. Expression of anthocyanin biosynthesis pathway genes in red and white grapes. *Plant Mol. Biol.* 32:565-569.
- Boss, P.K., C. Davies, and S.P. Robinson. 1996b. Analysis of the expression of anthocyanin pathway genes in developing *Vitis vinifera* L. cv Shiraz grape berries and the implications for pathway regulation. *Plant Physiol.* 111:1059-1066.
- Boulton, R. 2001. The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *Am. J. Enol. Vitic.* 52:67-87.
- Brouillard, R., G. Mazza, Z. Saad, A.M. Albrecht-Gary, and A. Cheminat. 1989. The copigmentation reaction of anthocyanins: A microprobe for the structural study of aqueous solutions. *J. Am. Chem. Soc.* 111:2604-2610.
- Burg, S.P., and E.A. Burg. 1965. Ethylene action and the ripening of fruits. *Science* 148:1190-1196.
- Buttrose, M.S., C.R. Hale, and W.M. Kliever. 1971. Effect of temperature on the composition of 'Cabernet Sauvignon' berries. *Am. J. Enol. Vitic.* 22:71-75.
- Carter, E.B., M.K. Theodorou, and P. Morris. 1999. Responses of *Lotus corniculatus* to environmental change. 2. Effect of elevated CO₂ temperature and drought on tissue digestion in relation to condensed tannin and carbohydrate accumulation. *J. Sci. Food Agric.* 79:1431-1440.
- Chalmers, D.J., and J.D. Faragher. 1977. Regulation of anthocyanin biosynthesis in apple skin. I. Comparison of the effects of cyclohexamide, ultraviolet light, wounding and maturity. *Aust. J. Plant Physiol.* 4:111-121.
- Chapman, D.M., M.A. Matthews, and J.X. Guinard. 2004. Sensory attributes of Cabernet Sauvignon wines made from vines with different crop yields. *Am. J. Enol. Vitic.* 55:325-334.
- Charrier, B., C. Coronado, A. Kondorosi, and P. Ratet. 1995. Molecular characterization and expression of alfalfa (*Medicago sativa* L.) flavanone-3-hydroxylase and dihydroflavonol-4-reductase encoding genes. *Plant Mol. Biol.* 29:773-86.
- Chervin, C., A. El-Kereamy, J.P. Roustau, A. Latché, J. Lamon and M. Bouzayen. 2004. Ethylene seems required for the berry development and ripening in grape, a non-climacteric fruit. *Plant Sci.* 167:1301-1305.
- Cheyrier, V., H. Fulcrand, P. Sarni, and M. Moutounet. 1997a. Reactivity of phenolic compounds in wine: Diversity of mechanisms and resulting products. *In* *In vino analytica Scientia*, pp. 143-154. Bordeaux, France.
- Cheyrier, V., C. Prieur, S. Guyot, J. Rigaud, and M. Moutounet. 1997b. The structures of tannins in grapes and wines and their interactions with proteins. *In* *Wine: Nutritional and Therapeutic Benefits*. T.R. Watkins (Ed.), pp. 81-93. ACS Symp. Ser. 661. American Chemical Society, Washington, DC.
- Clarke, H.R., J.A. Leigh, and C.J. Douglas. 1992. Molecular signals in the interactions between plants and microbes. *Cell* 71:191-199.
- Coombe, B.G. 1987a. Distribution of solutes within the developing grape berry in relation to its morphology. *Am. J. Enol. Vitic.* 38:120-127.
- Coombe, B.G. 1987b. Influence of temperature on composition and quality of grapes. *Acta Hortic.* 206:23-35.
- Coombe, B.G. 1989. The grape berry as a sink. *Acta Hortic.* 239:149-158.
- Cortell, J.M., M. Halbeib, A.V. Gallagher, T.L. Righetti, and J.A. Kennedy. 2005. Influence of vine vigor on grape (*Vitis vinifera* L. cv. Pinot noir) and wine proanthocyanidins. *J. Agric. Food Chem.* 53:5789-5797.
- Creasy, L.L., and M. Coffee. 1988. Phytoalexin production potential of grape berries. *J. Am. Soc. Hortic. Sci.* 113:230-234.
- Crippen, D.D.J., and J.C. Morrison. 1986. The effects of sun exposure on the phenolic content of Cabernet Sauvignon berries during development. *Am. J. Enol. Vitic.* 37:243-247.
- Czochanska, Z., L.Y. Foo, and L.J. Porter. 1979. Compositional changes in lower molecular weight flavans during grape maturation. *Phytochemistry* 18:1819-1822.
- Dakora, F.D., C.M. Joseph, and D.A. Phillips. 1993. Alfalfa (*Medicago sativa* L.) root exudates contain isoflavonoids in the presence of *Rhizobium meliloti*. *Plant Physiol.* 101:819-824.
- Dan, D.H., and C.H. Lee. 2004. The effects of GA₃, CPPU and ABA applications on the quality of Kyoho (*Vitis vinifera* L. x *Labrusca* L.) Grape. *Acta Hortic.* (ISHS) 653:193-197.
- Davies, C., P.K. Boss, and S.P. Robinson. 1997. Treatment of grape berries, a nonclimacteric fruit with a synthetic auxin, retards ripening and alters the expression of developmentally regulated genes. *Plant Physiol.* 115:1155-1161.
- de Freitas, V.A.P., and Y. Glories. 1999. Concentration and compositional changes of procyanidins in grape seeds and skin of white *Vitis vinifera* varieties. *J. Sci. Food Agric.* 79:1601-1606.

- Deikman, J., and P.E. Hammer. 1995. Induction of anthocyanin accumulation by cytokinins in *Arabidopsis thaliana*. *Plant Physiol.* 108: 47-57.
- Delcour, J.A., D. Ferreira, and D.G. Roux. 1983. Synthesis of condensed tannins. Part 9. The condensation sequence of leucocyanidin with (+)-catechin and with the resultant procyanidins. *J. Chem. Soc., Perkin Trans. 1*:1711-1717.
- Delgado, R. 2004. Changes in phenolic composition of grape berries during ripening in relation to vineyard nitrogen and potassium fertilisation rates. *J. Sci. Food Agric.* 84:623-630.
- Dercks, W., and L.L. Creasy. 1989. The significance of stilbene phytoalexins in the *Plasmopara viticola*-grapevine interaction. *Physiol. Mol. Plant Pathol.* 34:189-202.
- Dewick, P.M., and E. Haslam. 1969. Phenol biosynthesis in higher plants: Gallic acid. *Biochem. J.* 113:537-542.
- Dixon, R.A., and C.J. Lamb. 1990. Molecular communications in interactions between plants and microbial pathogens. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 41:339-367.
- Dixon, R.A., and N.L. Paiva. 1995. Stress-induced phenylpropanoid metabolism. *Plant Cell* 7:1085-1097.
- Djordjevic, M.A., J.W. Redmond, M. Batley, and B.G. Rolfe. 1987. Clovers secrete specific phenolic compounds which either stimulate or repress *nod* gene expression in *Rhizobium trifolii*. *EMBO J.* 6:1173-1179.
- Do, C.B., and F. Cormier. 1991. Accumulation of peonidin 3-glucoside enhanced by osmotic stress in grape (*Vitis vinifera* L.) cell suspension. *Plant Cell Tissue Organ Cult.* 24:49-54.
- Dokoozlian, N.K., and W.M. Kliever. 1996. Influence of light on grape berry growth and composition varies during fruit development. *J. Am. Soc. Hortic. Sci.* 121:869-874.
- Dong, Y.H., L. Beuning, K. Davies, D. Mitra, B. Morris, and A. Kootstra. 1998. Expression of pigmentation genes and photo-regulation of anthocyanin biosynthesis in developing Royal Gala apple flowers. *Aust. J. Plant Physiol.* 25:245-252.
- Downey, M.O., and D.O. Adams. 2005. Practical use of a total tannin assay for red winegrapes. *Aust. Vitic. May-June*:68-71.
- Downey, M.O., J.S. Harvey, and S.P. Robinson. 2003a. Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. *Aust. J. Grape Wine Res.* 9:15-27.
- Downey, M.O., J.S. Harvey, and S.P. Robinson. 2003b. Synthesis of flavonols and expression of flavonol synthase genes in developing grape berries of Shiraz and Chardonnay (*Vitis vinifera* L.). *Aust. J. Grape Wine Res.* 9:110-121.
- Downey, M.O., J.S. Harvey, and S.P. Robinson. 2004a. The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Aust. J. Grape Wine Res.* 10:55-73.
- Downey, M.O., J.S. Harvey, and S.P. Robinson. 2004b. Flavonol accumulation and expression of a gene encoding flavonol synthase demonstrates light sensitivity of flavonol biosynthesis in grapevines. *In XXII International Conference on Polyphenols.* A. Hoikkala and O. Soidinsalo (Eds.), pp. 59-60. University of Helsinki.
- Dreyer, D.L., J.C. Reese, and K.C. Jones. 1981. Aphid feeding deterrents in sorghum. *J. Chem. Ecol.* 7:273-284.
- Dry, P.R., B.R. Loveys, P.G. Iland, D.G. Botting, M.G. McCarthy, and M. Stoll. 1998. Vine manipulation to meet fruit specifications. *In Proceedings of the 10th Australian Wine Industry Technical Conference.* R.J. Blair et al. (Eds.), pp. 208-214. AWITC, Adelaide.
- Ebadi, A., P. May, M. Sedgley, and B.G. Coombe. 1995. Fruit set on small Chardonnay and Shiraz vines grown under varying temperature regimes between budburst and flowering. *Aust. J. Grape Wine Res.* 1:1-10.
- El-Kereamy, A., et al. 2003. Exogenous ethylene stimulates the long-term expression of genes related to anthocyanin biosynthesis in grape berries. *Physiol. Plant.* 119:178-182.
- Elliger, C.A., B.C. Chan, and A.C. Waiss Jr. 1980. Flavonoids as larval growth inhibitors. Structural features governing toxicity. *Naturwissenschaften* 67:358-360.
- Emmett, R.W., A.R. Harris, R.H. Taylor, and J.K. McGeachan. 1992. Grape diseases and vineyard protection. *In Viticulture.* Vol. 2. Practices. B.G. Coombe and P.R. Dry (Eds.), pp. 232-278. Winetitles, Adelaide.
- Escribano-Bailon, M.T., M.T. Guerra, J.C. Rivas-Gonzalo, and C. Santos-Buelga. 1995. Proanthocyanidins in skins from different grape varieties. *Z. Lebensm.-Unters.-Forsch.* 200:221-224.
- Feeny, P. 1976. Plant apparency and chemical defence. *In Biochemical Interaction between Plants and Insects.* J.W. Wallace and R.L. Mansell (Eds.), pp. 1-40. Plenum Press, New York.
- Ferrini, F., G.B. Mattii, and F.P. Nicese. 1995. Effect of temperature on key physiological responses of grapevine leaf. *Am. J. Enol. Vitic.* 46:375-379.
- Flint, S.D., P.W. Jordan, and M.M. Caldwell. 1985. Plant protective response to enhanced UV-B radiation under field conditions: Leaf optical properties and photosynthesis. *Photochem. Photobiol.* 41:95-99.
- Ford, C.M., P.K. Boss, and P.B. Hoj. 1998. Cloning and characterization of *Vitis vinifera* UDP-glucose:flavonoid 3-O-glucosyltransferase, a homologue of the enzyme encoded by the maize Bronze-1 locus that may primarily serve to glucosylate anthocyanidins in vivo. *J. Biol. Chem.* 273:9224-9233.
- Forkmann, G. 1993. Genetics of flavonoids. *In The Flavonoids: Advances in Research since 1986.* J.B. Harborne (Ed.), pp. 537-564. Chapman & Hall, London.
- Gao, Y., and G. A. Cahoon. 1994. Cluster shading effects on fruit quality, skin color, and anthocyanin content and composition in Reliance (*Vitis* hybrid). *Vitis* 33:205-209.
- Gawel, R. 1998. Red wine astringency: A review. *Aust. J. Grape Wine Res.* 4:74-95.
- Gawel, R., A. Oberholster, and I.L. Francis. 2000. A 'Mouth-feel Wheel': Terminology for communicating the mouth-feel characteristics of red wine. *Aust. J. Grape Wine Res.* 6:203-207.
- Gerats, A.G.M., and C. Martin. 1992. Flavonoid synthesis in *Petunia hybrida*; genetics and molecular biology of flower colour. *In Phenolic Metabolism in Plants.* H.A. Stafford and R.K. Ibrahim (Eds.), pp. 165-199. Plenum Press, New York.
- Gilbert, D.E., J.L. Meyer, J.J. Kissler, P.D. la Vine, and C.V. Carlson. 1970. Evaporation cooling of vineyards. *Calif. Agric.* 24:12-14.
- Glories, Y. 1988. Anthocyanins and tannins from wine: Organoleptic properties. *In Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular, and Medicinal Properties.* V. Cody et al. (Eds.), pp. 123-134. Alan R. Liss, New York.
- Gong, Z., M. Yamazaki, M. Sugiyama, Y. Tanaka, and K. Saito. 1997. Cloning and molecular analysis of structural genes involved in anthocyanin biosynthesis and expressed in a forma-specific manner in *Perilla frutescens*. *Plant Mol. Biol.* 35:915-927.
- Gonzalez-San Jose, M.L., G. Santa-Maria, and C. Diez. 1990. Anthocyanins as parameters for differentiating wines by grape variety, wine-growing region, and wine-making methods. *J. Food Compost. Anal.* 3:54-66.
- Guidoni, S., P. Allara, and A. Schubert. 2002. Effect of cluster thinning on berry skin anthocyanin composition of *Vitis vinifera* cv. Nebbiolo. *Am. J. Enol. Vitic.* 53:224-226.

- Harbertson, J.F., J.A. Kennedy, and D.O. Adams. 2002. Tannin in skins and seeds of Cabernet Sauvignon, Syrah, and Pinot noir during ripening. *Am. J. Enol. Vitic.* 53:54-59.
- Harbertson, J.F., E.A. Picciotto, and D.O. Adams. 2003. Measurements of polymeric pigments in grape berry extracts and wines using a protein precipitation assay combined with bisulfite bleaching. *Am. J. Enol. Vitic.* 54:301-306.
- Harborne, J.B. 1958. Chromatographic identification of anthocyanin pigments. *Chromatogr. Rev.* 1:209-224.
- Harborne, J.B. 1967. *Comparative Biochemistry of the Flavonoid*. Academic Press, London.
- Harborne, J.B., and R.J. Grayer. 1993. Flavonoids and insects. In *The Flavonoids: Advances in Research since 1986*. J.B. Harborne (Ed.), pp. 589-618. Chapman & Hall, London.
- Haselgrove, L. 1997. The effect of manipulated bunch exposure on the accumulation of quercetin glycosides and anthocyanins in *Vitis vinifera* L. cv. Shiraz. Masters thesis, University of Adelaide.
- Haselgrove, L., D. Botting, R. van Heeswijck, P.B. Høj, P.R. Dry, C. Ford, and P.G. Iland. 2000. Canopy microclimate and berry composition: The effect of bunch exposure on the phenolic composition of *Vitis vinifera* L. cv. Shiraz grape berries. *Aust. J. Grape Wine Res.* 6:141-149.
- Haslam, E. 1998. *Practical Polyphenolics: From Structure to Molecular Recognition and Physiological Action*. Cambridge University Press, Cambridge, UK.
- Hawker, J.S. 1982. Effect of temperature on lipid, starch and enzymes of starch metabolism in grape, tomato and broad bean leaves. *Phytochemistry* 21:33-36.
- Helariutta, Y., P. Elomaa, M. Kotilainen, P. Seppanen, and T.H. Teeri. 1993. Cloning of cDNA coding for dihydroflavonol-4-reductase (DFR) and characterization of DFR expression in the corollas of *Gerbera hybrida* var. Regina (Compositae). *Plant Mol. Biol.* 22:183-193.
- Heller, W., and G. Forkmann. 1993. Biosynthesis of flavonoids. In *The Flavonoids: Advances in Research since 1986*. J.B. Harborne (Ed.), pp. 499-535. Chapman & Hall, London.
- Herrmann, K.M. 1995. The shikimate pathway: Early steps in the biosynthesis of aromatic compounds. *Plant Cell* 7:907-919.
- Hiratsuka, S., H. Onodera, Y. Kawai, T. Kubo, H. Itoh, and R. Wada. 2001. ABA and sugar effects on anthocyanin formation in grape berry cultured in vitro. *Sci. Hortic.* 90:121-130.
- Holton, T.A., and E.C. Cornish. 1995. Genetics and biochemistry of anthocyanin biosynthesis. *Plant Cell* 7:1071-1083.
- Horowitz, R.M. 1964. Relations between taste and structure of some phenolic glycosides. In *Biochemistry of Phenolic Compounds*. J.B. Harborne (Ed.), pp. 545-571. Academic Press, London.
- Hrazdina, G., and G.F. Parsons. 1982. Induction of flavonoid synthesizing enzymes by light in etiolated pea (*Pisum sativum* cv. Mid-freezer) seedlings. *Plant Physiol.* 70:506-510.
- Hunter, J.J., O.T. de Villiers, and J.E. Watts. 1991. The effect of partial defoliation on quality characteristics of *Vitis vinifera* L. cv. Cabernet Sauvignon grapes. II. Skin color, skin sugar, and wine quality. *Am. J. Enol. Vitic.* 42:13-17.
- Hunter, J.J., H.P. Ruffner, C.G. Volschenk, and D.J. le Roux. 1995. Partial defoliation of *Vitis vinifera* L. cv. Cabernet Sauvignon/99 Richter: Effect on root growth, canopy efficiency, grape composition, and wine quality. *Am. J. Enol. Vitic.* 46:306-314.
- Iland, P.G., A. Ewart, J. Sitters, A. Markides, and N. Bruer. 2000. Techniques for chemical analysis and quality monitoring during winemaking. Patrick Iland, Adelaide.
- Jackson, D.I., and P.B. Lombard. 1993. Environmental and management practices affecting grape composition and wine quality – A review. *Am. J. Enol. Vitic.* 44:409-430.
- Jeandet, P., R. Bessis, B.F. Maume, P. Meunier, D. Peyron, and P. Trollat. 1995. Effect of enological practices on the resveratrol isomer content of wine. *J. Agric. Food Chem.* 43:316-319.
- Jeong, S.T., N. Goto-Yamamoto, S. Kobayashi, and M. Esaka. 2004. Effects of plant hormones and shading on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape berry skins. *Plant Sci.* 167:247-252.
- Jones, H.G. 1992. *Plants and Microclimate. A Quantitative Approach to Environmental Plant Physiology*. Cambridge University Press, Cambridge, UK.
- Katalinic, V., and P. Males. 1997. Compositional changes in grape polyphenols throughout maturation. *J. Wine Res.* 8:169-177.
- Keller, M., and G. Hrazdina. 1998. Interaction of nitrogen availability during bloom and light intensity during veraison. II. Effects on anthocyanins and phenolic development during grape ripening. *Am. J. Enol. Vitic.* 49:341-349.
- Keller, M., K.J. Arnink, and G. Hrazdina. 1998. Interaction of nitrogen availability during bloom and light intensity during veraison. I. Effects on grapevine growth, fruit development and ripening. *Am. J. Enol. Vitic.* 49:333-340.
- Kennedy, J.A., Y. Hayasaka, S. Vidal, E.J. Waters, and G.P. Jones. 2001. Composition of grape skin proanthocyanidins at different stages of berry development. *J. Agric. Food Chem.* 49:5348-5355.
- Kennedy, J.A., M.A. Matthews, and A.L. Waterhouse. 2000. Changes in grape seed polyphenols during ripening. *Phytochemistry* 55:77-85.
- Kennedy, J.A., M.A. Matthews, and A.L. Waterhouse. 2002. The effect of maturity and vine water status on grape skin flavonoids. *Am. J. Enol. Vitic.* 53:268-274.
- Kliewer, W.M. 1970. Effect of day temperature and light intensity on coloration of *Vitis vinifera* L. grapes. *J. Am. Soc. Hortic. Sci.* 95:693-697.
- Kliewer, W.M. 1977. Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes. *Am. J. Enol. Vitic.* 28:96-103.
- Kliewer, W.M., and A.J. Antcliff. 1970. Influence of defoliation, leaf darkening and cluster shading on the growth and composition of Sultana grapes. *Am. J. Enol. Vitic.* 21:26-36.
- Kliewer, W.M., and H.B. Schultz. 1973. Effect of sprinkler cooling of grapevines on fruit growth and composition. *Am. J. Enol. Vitic.* 24:17-26.
- Kliewer, W.M., and R.E. Torres. 1972. Effect of controlled day and night temperatures on grape coloration. *Am. J. Enol. Vitic.* 26:71-77.
- Kliewer, W.M., L.A. Lider, and H.B. Schultz. 1967. Influence of artificial shading of vineyards on the concentration of sugar and organic acids in grapes. *Am. J. Enol. Vitic.* 18:78-86.
- Koes, R.E., F. Quattrocchio, and J.N.M. Mol. 1994. The flavonoid biosynthetic pathway in plants: Function and evolution. *BioEssays* 16:123-132.
- Lancaster, J.E. 1992. Regulation of skin color in apples. *CRC Crit. Rev. Plant Sci.* 10:487-502.
- Langcake, P., and W.V. McCarthy. 1979. The relationship of resveratrol production to infection of grapevine leaves by *Botrytis cinerea*. *Vitis* 18:244-253.
- Langcake, P. and R.J. Pryce. 1976. The production of resveratrol by *Vitis vinifera* and other members of the Vitaceae as a response to infection or injury. *Physiol. Plant Pathol.* 9:77-86.

- Langcake, P., and R.J. Pryce. 1977a. A new class of phytoalexins from grapevines. *Experientia* 33:151-152.
- Langcake, P., and R.J. Pryce. 1977b. The production of resveratrol and the viniferins by grapevines in response to ultraviolet irradiation. *Phytochemistry* 16:1193-1196.
- Lawrence, W.J.C. 1950. 1. Genetic control of biochemical synthesis as exemplified by plant genetics – Flower colour. *Biochemical Society Symp. no. 4. Biochem. Asp. Genetics* 4:3-9.
- Lees, G.L., C.F. Hinks, and N.H. Suttill. 1994. Effect of high temperature on condensed tannin accumulation in leaf tissues of Big Trefoil (*Lotus uliginosus* Schkur). *J. Sci. Food Agric.* 65:415-421.
- Leone, A.M., E. la Notte, and G. Gambacorta. 1984. Gli antociani nelle fasi di macerazione e di elaborazione del vino. L'influenza della tecnica diffusiva sulla loro estrazione. *Vignevini* 4:17-31.
- Lu, Y., and A. Bennick. 1998. Interaction of tannin with human salivary proline-rich proteins. *Arch. Oral Biol.* 43:717-28.
- Malien-Aubert, C., O. Dangles, and M. J. Amiot. 2002. Influence of procyanidins on the colour stability of oenin solutions. *J. Agric. Food Chem.* 50:3299-3305.
- Markham, K.R. 1982. *Techniques of Flavonoid Identification*. Academic Press, London.
- Marschner, H. 1995. *Mineral Nutrition of Higher Plants*. Academic Press, London.
- Mateus, N., J.M. Machado, and V. de Freitas. 2002. Developmental changes of anthocyanins in *Vitis vinifera* grapes grown in the Douro Valley and concentration in respective wines. *J. Sci. Food Agric.* 82:1689-1695.
- Mateus, N., S. Marques, A.C. Goncalves, J.M. Machado and V. de Freitas. 2001. Proanthocyanidin composition of red *Vitis vinifera* varieties from the Douro Valley during ripening: Influence of cultivation altitude. *Am. J. Enol. Vitic.* 52:115-121.
- Matsushima, J., S. Hiratsuka, N. Taniguchi, R. Wada, and N. Suzuki. 1989. Anthocyanin accumulation and sugar content in the skin of grape cultivar 'Olympia' treated with ABA. *J. Jap. Soc. Hortic. Sci.* 58:551-555.
- Mazza, G. 1995. Anthocyanins in grapes and grape products. *CRC Crit. Rev. Food Sci. Nutr.* 35:341-371.
- Mazza, G., and E. Miniati. 1993. Grapes. In *Anthocyanins in Fruits, Vegetables and Grains*. G. Mazza and E. Miniati (Eds.), pp. 149-195. CRC Press, Boca Raton, FL.
- McDonald, M.S., M. Hughes, J. Burns, M.E.J. Lean, D. Matthews, and A. Crozier. 1998. Survey of the free and conjugated myricetin and quercetin content of red wines of different geographical origins. *J. Agric. Food Chem.* 46:368- 375.
- Mehdy, M.C., and C.J. Lamb. 1987. Chalcone isomerase cDNA cloning and mRNA induction by fungal elicitor, wounding and infection. *EMBO J.* 6:1527-1533.
- Mole, S. 1989. Polyphenolics and the nutritional ecology of herbivores. In *Toxicants of Plant Origin*. Vol. IV. Phenolics. P.R. Cheeke (Ed.), pp. 191-223. CRC Press, Boca Raton, FL.
- Mori, K., S. Sugaya, and H. Gemma. 2005. Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature condition. *Sci. Hortic.* 105:319-330.
- Morris, J.R., C.A. Sims, and D.L. Cawthon. 1983. Effects of excessive potassium levels on pH, acidity and color of fresh and stored grape juice. *Am. J. Enol. Vitic.* 34:35-39.
- Morrison, J.C., and A.C. Noble. 1990. The effects of leaf and cluster shading on the composition of Cabernet Sauvignon grapes and on fruit and wine sensory properties. *Am. J. Enol. Vitic.* 41:193-200.
- Mullins, M.G., A. Bouquet, and L.E. Williams. 1992. *Biology of the Grapevine*. Cambridge University Press, Cambridge, UK.
- Nadal, M., and L. Arola. 1995. Effects of limited irrigation on the composition of must and wine of Cabernet Sauvignon under semi-arid conditions. *Vitis* 34:151-154.
- Nakamura, N., H. Nakamae and L. Maekawa. 1980. Effects of light and kinetin on anthocyanin accumulation in the petals of *Rosa hybrida*, Hort. Cv. Ehigasa. *Z. Pflanzenphysiol.* 98:263-270.
- Nef, L. 1988. Interactions between the leaf miner, *Phyllocnistis suffusella*, and poplars. In *Mechanisms of Woody Plant Defenses Against Insects*. Search for Pattern. W.J. Mattson et al. (Eds.), pp. 239-251. Springer-Verlag, New York.
- Northcote, K.H. 1992. Soils and Australian viticulture. In *Viticulture*. Vol. 1. Resources. B.G. Coombe and P.R. Dry (Eds.), pp. 61-90. Winetitles, Adelaide.
- Ozeki, Y., and A. Komamine. 1981. Induction of anthocyanin synthesis in relation to embryogenesis in a carrot suspension culture: Correlation of metabolic differentiation with morphological differentiation. *Physiol. Plant.* 53:570-577.
- Perez-Magarino, S., and M.L. Gonzalez-San Jose. 2004. Evolution of flavanols, anthocyanins, and their derivatives during the aging of red wines elaborated from grapes harvested at different stages of ripening. *J. Agric. Food Chem.* 52:1118-1189.
- Pool, R.M., L.L. Creasy, and A.S. Frackelton. 1981. Resveratrol and the viniferins, their application to screening for disease resistance in grape breeding programs. *Vitis* 20:136-145.
- Price, S.F., P.J. Breen, M. Valladao, and B.T. Watson. 1995. Cluster sun exposure and quercetin in Pinot noir grapes and wine. *Am. J. Enol. Vitic.* 46:187-194.
- Reay, P.F., and J.E. Lancaster. 2001. Accumulation of anthocyanins and quercetin glycosides in 'Gala' and 'Royal Gala' apple fruit skin with UV-B-visible irradiation: Modifying effects of fruit maturity, fruit side, and temperature. *Sci. Hortic.* 90:57-68.
- Recourt, K., A.J. van Tunen, L.A. Mur, A.A.N. van Brussel, B.J.J. Lugtenberg, and J.W. Kijne. 1992. Activation of flavonoid biosynthesis in roots of *Vicia sativa* subsp. *nigra* plants by inoculation with *Rhizobium leguminosarum* biovar *viciae*. *Plant Mol. Biol.* 19:411-420.
- Revilla, E., E. Alonso, and V. Kovac. 1997. The content of catechins and procyanidins in grapes and wines as affected by agroecological factors and technological practices. In *Wine: Nutritional and Therapeutic Benefits*. T.R. Watkins (Ed.), pp. 69-80. American Chemical Society, Washington, DC.
- Ribéreau-Gayon, P. 1972. The tannins. In *Plant Phenolics*. P.H. Heywood (Ed.), pp. 169-248. Oliver & Boyd, Edinburgh.
- Ribéreau-Gayon, P., and Y. Glories. 1986. Phenolics in grapes and wines. In *Proceedings of the 6th Australian Wine Industry Technical Conference*. T. Lee (Ed.), pp. 247-256. Australian Industrial Publishers, Adelaide.
- Roby, G., J.F. Harbertson, D.O. Adams, and M.A. Matthews. 2004. Berry size and vine water deficits as factors in winegrape composition: Anthocyanins and tannins. *Aust. J. Grape Wine Res.* 10:100-107.
- Roby, G., and M.A. Matthews. 2004. Relative proportions of seed, skin and flesh in ripe berries from Cabernet Sauvignon grapevines grown in a vineyard either well irrigated or under water deficit. *Aust. J. Grape Wine Res.* 10:74-82.
- Rojas-Lara, B.A., and J.C. Morrison. 1989. Differential effects of shading fruit or foliage on the development and composition of grape berries. *Vitis* 28:199-208.

- Roggero, J.P., B. Ragonnet, and S. Coen. 1984. Analyse fine des anthocyanes des vins et des pellicules de raisin par la technique HPLC. *Vigne Vins* 327:38-42.
- Russell, E.W. 1961. *Soil Conditions and Plant Growth*. Longmans, Green & Co., London.
- Roubelakis-Angelakis, K., and W.M. Kliever. 1986. Effects of exogenous factors on phenylalanine ammonia-lyase activity and accumulation of anthocyanins and total phenolics in grape berries. *Am. J. Enol. Vitic.* 37:275-280.
- Ryan, K.G., K.R. Markham, S.J. Bloor, J.M. Bradley, K.A. Mitchell, and B.R. Jordan. 1998. UVB radiation induced increase in quercetin:kaempferol ratio in wild-type and transgenic lines of *Petunia*. *Photochem. Photobiol.* 68:323-330.
- Saint-Cricq de Gaulejac, N., M. Augustin, N. Vivas, and Y. Glories. 1997. A biochemical approach to the evolution of procyanidins in grape seeds during the ripening of red grapes (*Vitis vinifera* L. cv. Merlot Noir). *J. Wine Res.* 8:159-167.
- Saito, N., and J.B. Harborne. 1992. Correlations between anthocyanin type, pollinator and flower colour in the Labiatae. *Phytochemistry* 31:3009-3015.
- Scheffeldt, P., and G. Hrazdina. 1978. Copigmentation of anthocyanins under physiological conditions. *J. Food Sci.* 43:517-520.
- Shaver, T.N., and M.J. Lukefair. 1969. Effect of flavonoid pigments and gossypol on growth and development of bollworm, tobacco budworm, and pink bollworm. *J. Econ. Entomol.* 62:643-646.
- Shirley, B.W. 1996. Flavonoid biosynthesis: 'New' functions for an 'old' pathway. *Trends Plant Sci.* 1:377-382.
- Siegelman, H.W., and S.B. Hendricks. 1958. Photocontrol of anthocyanin synthesis in apple skin. *Plant Physiol.* 33:185-190.
- Skipp, R.A., and J.A. Bailey. 1977. The fungitoxicity of isoflavonoid phytoalexins measured using different types of bioassay. *Physiol. Plant Pathol.* 11:101-112.
- Smart, R.E., J.B. Robinson, G.R. Due, and C.J. Brien. 1985. Canopy microclimate modification for the cultivar Shiraz. II. Effects on must and wine composition. *Vitis* 24:119-128.
- Smart, R., and T.R. Sinclair. 1976. Solar heating of grape berries and other spherical fruits. *Agric. Meteorol.* 17:241-259.
- Smart, R.E., S.M. Smith, and R.V. Winchester. 1988. Light quality and quantity effects on fruit ripening for Cabernet Sauvignon. *Am. J. Enol. Vitic.* 39:250-258.
- Smith, D.A. 1982. Toxicity of phytoalexins. In *Phytoalexins*. J.A. Bailey and J.W. Mansfield (Eds.), pp. 218-252. Blackie & Son, Glasgow.
- Smith, G.J., and K.R. Markham. 1998. Tautomerism of flavonol glucosides: Relevance to plant UV protection and flower colour. *J. Photochem. Photobiol. A: Chem.* 118:99-105.
- Somers, T.C. 1976. Pigment development during ripening of the grape. *Vitis* 14:269-277.
- Souquet, J.M., V. Cheynier, P. Sarni-Manchado, and M. Moutounet. 1996. Les composés phénoliques du raisin. *J. Int. Sci. Vigne Vin* 99-107.
- Sparvoli, F., C. Martin, A. Scienza, G. Gavazzi, and C. Tonelli. 1994. Cloning and molecular analysis of structural genes involved in flavonoid and stilbene biosynthesis in grape (*Vitis vinifera* L.). *Plant Mol. Biol.* 24:743-755.
- Spayd, S.E., J.M. Tarara, D.L. Mee, and J.C. Ferguson. 2002. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Vitic.* 53:171-181.
- Stafford, H.A. 1990. *Flavonoid Metabolism*. CRC Press, Boca Raton, FL.
- Stafford, H.A. 1991. Flavonoid evolution: An enzymic approach. *Plant Physiol.* 96:680-685.
- Stoll, M. 2000. Effects of partial rootzone drying on grapevine physiology and fruit quality. Ph.D. thesis. Department of Horticulture, Viticulture and Oenology, University of Adelaide.
- Strack, D., and V. Wray. 1993. The anthocyanins. In *The Flavonoids: Advances in Research since 1986*. J.B. Harborne (Ed.), pp. 1-22. Chapman & Hall, London.
- Suzuki, M. 1995. Enhancement of anthocyanin accumulation by high osmotic stress and low pH in grape cells (*Vitis* hybrids). *J. Plant Physiol.* 147:152-155.
- Tanaka, Y., K. Yonekura, M. Fukuchi-Mizutani, Y. Fukui, H. Fujiwara, T. Ashikari, and T. Kusumi. 1996. Molecular and biochemical characterization of three anthocyanin synthetic enzymes from *Gen-tiana triflora*. *Plant Cell Physiol.* 37:711-716.
- Tanner, G.J., K.T. Francki, S. Abrahams, J.M. Watson, P.J. Larkin, and A.R. Ashton. 2003. Proanthocyanidin biosynthesis in plants. Purification of legume leucoanthocyanidin reductase and molecular cloning of its own cDNA. *J. Biol. Chem.* 278:31647-31656.
- Taylor, L.P. 1995. Flavonols: Effects on fertility and fecundity. *Crop Sci.* 35:1521-1526.
- Timberlake, C.F., and P. Bridle. 1976. The effect of processing and other factors on the colour characteristics of some red wines. *Vitis* 15:37-49.
- Thorngate, J.H. 1997. The physiology of human sensory response to wine: A review. *Am. J. Enol. Vitic.* 48:271-279.
- Vidal, S., D. Carlade, J.M. Souquet, H. Fulcrand, and V. Cheynier. 2002. Changes in proanthocyanidin chain length in winelike model solutions. *J. Agric. Food Chem.* 50:2261-2266.
- Vogt, T., M. Ibdah, J. Schmidt, V. Wray, M. Nimtz, and D. Strack. 1999. Light-induced betacyanin and flavonol accumulation in the bladder cells of *Mesembryanthemum crystallinum*. *Phytochemistry* 52:583-592.
- Vogt, T., P. Pollak, N. Taryln, and L.P. Taylor. 1994. Pollination- or wound-induced kaempferol accumulation in petunia stigmas enhances seed production. *Plant Cell* 6:11-23.
- Wagner, M.R. 1988. Induced defenses in Ponderosa pine against defoliating insects. In *Mechanisms of Woody Plant Defenses Against Insects*. W.J. Mattson et al. (Eds.), pp. 141-155. Springer-Verlag, New York.
- Weaver, R.J., and S.B. McCune. 1960. Influence of light on color development in *Vitis vinifera* grapes. *Am. J. Enol. Vitic.* 11:179-184.
- Weiss, M., J. Schmidt, D. Neumann, V. Wray, R. Christ, and D. Strack. 1999. Phenylpropanoids in mycorrhizas of the Pinaceae. *Planta* 208:491-502.
- Wicks, A.S., and W.M. Kliever. 1983. Further investigations into the relationship between anthocyanins, phenolics and soluble carbohydrates in grape berry skins. *Am. J. Enol. Vitic.* 34:114-116.
- Winkler, A.J., J.A. Cook, W.M. Kliever, and L.A. Lider. 1974. *General Viticulture*. University of California Press, Berkeley.
- Xie, D.Y., S.B. Sharma, N.L. Paiva, D. Ferreira, and R.A. Dixon. 2003. Role of anthocyanidin reductase, encoded by *BANYULS* in plant flavonoid biosynthesis. *Science* 299:396-399.
- Ylstra, B., A. Touraev, R.M.B. Moreno, E. Stoger, A.J. van Tunen, O. Vicente, J.N.M. Mol, and E. Heberle-Bors. 1992. Flavonols stimulate development, germination and tube growth of tobacco pollen. *Plant Physiol.* 100:902-907.