The Impact of Prohexadione-calcium on Grape Vegetative and Reproductive Development and Wine Chemistry

by

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(ABSTRACT)

Prohexadione-calcium (P-ca), as Apogee™, was evaluated in 2000 and 2001 for impact to grape vegetative and reproductive development. In 2000, P-ca (250 mg/L) was applied to Seyval, Cabernet Sauvignon, and Cabernet franc (125, 250, and 375 mg/L). P-ca reduced primary shoot growth for all cultivars and decreased cane pruning weight of Seyval. P-ca (375 mg/L) increased Cabernet franc canopy gaps but increased Cabernet Sauvignon lateral leaf area and leaf layer number. P-ca reduced components of yield for all cultivars. In 2001, P-ca (250 mg/L) was applied singularly at weekly intervals to Cabernet Sauvignon clusters and pre and post-bloom to Cabernet franc and Chardonnay canopies. Application at E-L stages 21 and 23 decreased Cabernet Sauvignon fruit set whereas application at E-L stages 26, 27, and 29 reduced berry weight without impacting fruit set. Berry weight reduction correlated to higher color intensity (420+520 nm), anthocyanins, total phenols and phenol-free glycosyl-glucose (PFGG). Cabernet franc vegetative and reproductive development was generally not affected yet treatment increased absorbance at 280, 420, and 520 nm, color intensity, anthocyanins and total phenols. Pre-bloom applications inhibited Chardonnay vegetative development, and reduced components of yield, and fruit chemistry values: hydroxycinnamates, total phenols, flavonoids, PPFG and absorbance at 280 and 320 nm. Post-bloom applications did not affect Chardonnay vegetative or reproductive development, yet increased PFGG. Treatment did not affect Chardonnay wine chemistry but two post-bloom applications increased Cabernet franc wine anthocyanins and total phenols. Wine aroma and flavor triangle difference tests did not indicate significant treatment differences.

DEDICATION

This thesis is dedicated to my father, Joseph, my mother Diane, and my stepmother Kathy. Three wonderful people whose combined influence kept me out of trouble and focused on my education.

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CHAPTER ONE: LITERATURE REVIEW

INTRODUCTION

Due to the role that gibberellins play in grape vegetative and reproductive development, the inhibition of gibberellin biosynthesis in *Vitis spp*. is a means by which grape producers may modify grape and grapevine development to improve fruit and wine quality. In the following review, the structure and function of gibberellin will be presented as well as the role of gibberellins in grape reproductive and vegetative development. Subsequently, the role of gibberellin-biosynthesis inhibitors will be discussed as they relate to current, and perhaps future, viticultural practice.

REVIEW OF THE LITERATURE

History, structure, and function of gibberellin. The first mention of gibberellin in the literature is its identification by Kurosawa in 1912 as the causal "toxin" of Bakanae or "Foolish Seedling" disease of rice, a disease characterized by elongation of rice seedling stems (Phinney, 1983). It was not until 1938 when this "toxin" was isolated and referred to as gibberellin. Only in the late 1950s was gibberellin actually identified as present in higher plants (Phinney et al., 1957). This period saw a sudden increase of literature regarding every aspect of gibberellin, and not long after research was undertaken to assay its impact on grapevines (Coombe, 1959; Weaver and McCune 1959). Due to this period of intense research, we currently know a great deal about the structure and function of gibberellin.

Gibberellin is a diterpene and therefore the product of the isoprenoid biosynthetic pathway (Coolbaugh, 1983). There are various forms of gibberellin, which differ from each other mainly by the location of hydroxyl groups. As of the last comprehensive review on

gibberellins by Graebe in 1987, 72 different gibberellins were identified, however, not all of these are present in higher plants. The main role of gibberllin in higher plants is cell elongation, possibly due to a suppression of cell wall rigidification thereby extending the period of cell wall extension (Jones, 1985).

Gibberellin biosynthesis: Gibberellins are synthesized in three stages, the biosynthesis of ent-kaurene, the biosynthesis of gibberellin A_{12}-aldehyde (GA_{12}), and the individual biosythenthetic pathways after GA_{12} (Graebe, 1987). The first stage, the biosynthesis of ent-kaurene, can be further divided into three steps. These three steps are the conversion of mavalonate to geranylgeranyl pyrophosphate (GGPP), the conversion of GGPP to copalyl pyrophosphate, and the final conversion of copalyl pyrophosphate to ent-kaurene. The second stage is a six-step conversion of ent-kaurene to GA_{12}. The third and last stage of gibberellin biosynthesis, the conversion of GA_{12} to many different gibberellin-aldehydes, is the most complex. It can generally be thought of as a series of five subsequent oxidations, each of which results in a form of gibberellin. Within these steps are species and organ specific reactions that produce the approximately 72 different gibberellins (Graebe, 1987).

Impact of Gibberellin on Grape Vegetative Development: There is a dearth of information concerning the endogenous levels of gibberellin in grapes. Of this literature, the majority concerns the role of endogenous gibberellins in reproductive rather than vegetative tissues (Perez et al., 2000; Takeno et al., 1983). Nevertheless, it is known that gibberellins play a role in both timing budbreak and the number of breaking buds each season. Prebloom applications of gibberellic acid postponed and reduced budbreak (Considine, 1983).

Of all the phytohormones, gibberellin is the only one shown to promote shoot growth (Considine, 1983). However, most of the viticultural research on gibberellins and vegetative growth focused on gibberellin inhibition rather than gibberellin metabolism itself (Reynolds et al., 1992; Hunter and Procter, 1992; Coombe, 1967). The preponderance of our information on gibberellin metabolism results from research conducted with *Pisum*, *Phaseolus*, and *Spinacia*

species. In one Israeli study (Lavee, 1993) where the movement of hydrogen-3 labeled gibberellic acid (^{3}H-GA_3) applied post-bloom to grape petioles, was traced throughout the vine; the applied GA_3 moved primarily acropetally. Supporting evidence for this acropetal movement is that GA_{20} in peas (*Pisum sativum*) is biosynthesiszed in leaves and translocated to growing regions where it then is converted to GA_1 (Graebe, 1987). Some workers, citing additional evidence from pea plants, propose that GA_1, and potentially other gibberellins, are metabolized more quickly in rapidly elongating cells than in "resting" cells. Of all the gibberellin-aldehydes, GA_1 is thought to be the most important in causing internode elongation. Ingram et al. (1986), working with dwarf mutants of pea, found a proportional relationship between shoot growth and the accumulation of GA_8. These researchers cite the rapid turnover from GA_{20} to GA_1 to GA_8, and its subsequent accumulation, as evidence of the importance of GA_1 for internode elongation. This would explain results showing no difference in GA_3 concentrations in the leaves of vigorously and moderately growing 'Queen of the vineyard' grapevines (Levee, 1993).

Impact of gibberellin on grape berry development: Grape formation is a multi-step process spanning a two-year period. For this reason, the viticultural practices of one year can impact flowering of the following season. During the first season, this process begins with the formation of anlagen by apices of lateral buds followed by the differentiation of anlagen as either tendril or inflorescence primordia. During the subsequent season, flowers form from the inflorescence primordia (Mullins, 1986). There is evidence that gibberellin application during the differentiation of anlagen can cause these uncommitted primordia to form tendril primordia rather than inflorescence primordia, thereby reducing total clusters the following season (Srinivasan and Mullins, 1980a). GA_3 and GA_4 applications prior to anthesis however, increased flower numbers, and the branching and length of inflorescences (Kurshid et al., 1992). This conflicts with other research indicating that cytokinin is the main hormone involved in flower formation (Srinivasan and Mullins, 1980b).

After flower differentiation, anthesis occurs, followed by fruit set. Inflorescences cultured on a simple nutrient medium have produced seeded fruit (Mullins 1967), which indicates that

inflorescences are self sufficient in regards to the hormones required for fruit set. There are low levels of gibberellins in grapes during fruit set compared to the following period of berry growth and enlargement (Perez et al., 2000). However, exogenous GA_3 applications to inflorescences increased fruit set in seeded *Vitis vinifera* L. (Coombe, 1965).

The size of grape berries is additionally impacted by gibberellin. B.G. Coombe at the Waite Agricultural Research Institute in South Australia was at the forefront of this research. Application of GA_3 at anthesis to Black Corinth, a parthenocarpic grape cultivar, at anthesis resulted in a linear increase in berry size as concentration increased (Coombe, 1965). Application of GA_3 on Thompson Seedless, a stenospermocarpic cultivar, similarly increased berry size. The increase in berry size has been attributed to greater cell enlargement and cell division along the longitudinal axis in the pericarp (Coombe, 1965). These results are supported by a study of the histological effect of gibberellic acid on individual grape berries in 1968 by Sachs and Weaver. Berry enlargement was observed for Thompson Seedless and Black Corinth grapes dipped in gibberellic acid 7-10 days after anthesis (Sachs and Weaver, 1968). Berry enlargement in both cultivars was the result of parenchyma cell growth in the pericarp. Greatest growth was observed in the tissue zone surrounding the locule and extending out to, but not including, the outermost tannin-filled cells.

The role of photoassimilates in berry enlargement adds another facet to gibberellin-induced berry growth. It is thought that the majority of dry matter increase in grape berries is due to photosynthesis (Coombe, 1992). In a joint study by Australian and Italian researchers, the rate of increase of glucose and fructose versus berry weight was equal for large and small berries (Coombe et al., 1987). This research demonstrates that berries accumulate photoassimilates in relation to berry size. Therefore, while there is more glucose and fructose in a large berry compared to a small berry, the concentration in each is equal. Gibberellin applications have been shown to increase the amount of photosynthates moving into the flower clusters. In one experiment, inflorescences treated with gibberellic acid had increased uptake of ^{14}C-photoassimilates than untreated inflorescences (Motomura, 1986). However, growing shoots have also been suggested as competing sinks for these compounds, a hypothesis that has spurred

research into the use of gibberellin biosynthesis inhibitors to decrease vegetative growth (Reynolds et al., 1992).

Suppressing grapevine vegetative growth: There are numerous justifications for the reduction of grape vegetative growth, all concerning fruit quality. Ideal dimensions of a grapevine canopy have been proposed by a number of researchers. According to Smart (1985), an ideal canopy has an average leaf layer of one and gaps comprise 40% of the canopy surface area. Dokoozlian (1995) describes high-density and low-density canopies as those with $\leq$1% and $\geq$5% ambient light in the fruit-zone, respectively. Although some researchers (Cavallo et al., 2001) have recently challenged some of the methods for determining "unacceptably" dense canopies, these approximate values are still the industry standard. There is evidence that canopies with values outside of these aforementioned ranges inhibit proper fruit zone light penetration and increase disease incidence and severity (Smart, 1985; Smart and Robinson, 1991; English et al., 1989). Additionally, actively growing shoots may compete with developing fruit for photoassimilates. A great deal of research has been conducted on the manipulation of grapevine canopies to improve fruit quality (Smart, 1985; Smart and Robinson, 1991; Zabadal and Dittmer, 1998; Cavallo, 2001; Staff et al., 1997).

Light is required for good fruit coloration, particularly in the case of red-fruited cultivars. Workers have recently studied the effects of light on phenylalanine ammonia-lyase (PAL), the enzyme that channels phenylalanine away from protein synthesis and towards flavonoid and anthocyanin production (Roubelakis-angelakis and Kliewer, 1986). In this study, grapes, with pedicels immersed in a nutrient solution, were subjected to light and dark condition. Both PAL activity and anthocyanin accumulation in the skin were found to be light-dependant. There was no discernable anthocyanin concentration in the skins of berries subjected to completely dark conditions. Conversely, berries in constant light conditions rapidly accumulated anthocyanins. Results from another study on artificial and natural shading (Bureau, 2000) indicated that the total amount of bound phenols was lower in the berries of shaded Syrah bunches compared to unshaded bunches. In particular, levels of C_{13}-norisoprenoids and glycosides of methyl vanillate,

zingerone, vanillol, guaiacyl ethanol, and methyl syringoate were lower in shaded berries. Modifications in grape canopies that reduced density improved the quality of grapes at harvest. In Virginia, fruit zone leaf thinning increased fruit zone light penetration and leaf thinned vines had higher concentrations of glycosyl-glucose, a measure of glycosides, and select monoterpenes (Zoecklein et al., 1998).

Dense grape canopies also foster disease by reducing the penetration of fungicides, decreasing wind speed, and increasing humidity. Research on *Botrytis cinerea* infections demonstrated an increased incidence and severity of *B. cinerea* was related to a combination of increased humidity, temperature, leaf wetness, and decreased wind speed (English et al., 1989). Fruit zone leaf thinning by these researchers changed these unfavorable microclimate characteristics and reduced disease. Trellising systems designed to promote open canopies have also led to reduced disease (Zabadal and Dittmer, 1998).

An additional reason for vegetative suppression is that growing shoots may compete with developing fruit for photoassimilates (Motomura, 1986). This justification is less established than the prior two. The current practice of tipping and pruning vines, does not completely resolve the issue, as these practices can increase the development of lateral branching if growth conditions are favorable.

The use of growth retardants on grape vegetative structures: From the mid 1960s to present we have seen increased interest in evaluating gibberellin biosynthesis inhibitors for the purpose of retarding vegetative growth and achieving the goals currently accomplished by manual canopy manipulation (Coombe, 1967; Reynolds et al. 1992; Kumar et al., 1998). Quaternary ammonium compounds such as chlormequat chloride (Cylcocel or CCC) and triazole-type compounds such as paclobutrazol have been evaluated for use in suppressing vegetative growth on grapes *Vitis vinifera* (Kumar et al., 1998 Reynolds et al., 1992, Wolf and Miller, 1995; Coombe, B.G., 1967). These compounds suppress shoot growth by reducing internode length and often causing a total reduction in node number (Hunter, 1992; Reynolds et al., 1992). One advantage to paclobutrazol is that it has not been shown to increase lateral shoot

growth (Reynolds et al., 1992). Although these compounds were never registered on grapes in the United States, there remains a desire in commercial grape production for a growth regulator to substitute for labor-intensive canopy management practices.

Distribution of flavor and aroma compounds in the grape berry: Wine flavor and aroma are determined by numerous compounds, some of which are detectable in extremely low concentrations. The location of these compounds within the grape berry varies. In general, glucose and fructose are most concentrated in the flesh of the berry. Inorganic ions, phenolics, potassium, and tartrate are more concentrated in the skin (Coombe and Iland, 1987).

Phenolic and non-phenolic compounds play different roles in wine chemistry. Phenols impart wine with color and structure while non-phenolic compounds, such as monoterpenes and norisoprenoids, are largely responsible for aroma and flavor (Williams et al., 1992). Total phenols comprise flavonoid and non-flavonoid phenols; the former group dominated by hydroxycinnamates, and the latter by anthocyanins. Hydroxycinnamates are the major non-flavonoid phenols in both white and red cultivars (Zoecklein et al., 1999). Flavonoid phenols, particularly anthocyanins, are a greater proportion of total phenols in red cultivars, concentrated in the skins (Fernandez de Simon, 1993). Anthocyanins, which are largely responsible for imparting the color to red wine (Jackson and Lombard, 1993), are found in the vacuoles of cells located a few cell layers below the epidermis (Roubelakis-Angelakis, 1986). Non-flavonoid phenolic compounds such as hydroxycinnamates, (Fernandez de Simon et al., 1992) and non-phenolic monoterpenes such as geraniol and nerol (Park and Noble, 1993) are more concentrated in the skin than in the flesh of the berry. A decrease in berry size would lead to an increase in the surface to volume ratio, theoretically increasing the proportion of these compounds in the must and wine. As an increase in aroma and flavor precursors ostensibly would increase wine quality, this adds credence to a widely held belief that crop yield and wine quality are inversely related (Jackson and Lombard, 1993; Miller et al., 1993).

Recently there has been increased interest in studying glycosidically-bound flavor and aroma compounds. In many grape cultivars, fruit and must have the same aroma and flavor

characteristics as will be found in the wine. This is exemplified by "floral" cultivars such as Muscat, Riesling, and Gewurztraminer that owe their sensory characteristics primarily to monoterpenes. However, other grape cultivars do not exhibit their optimum flavor and aroma profile until many years of aging has occurred. For cultivars of this description, a method is needed to predict the future characteristics of the wine. Williams et al. (1992) found that the sensory characteristics of hydrolyzed glycosidically-bound compounds found in fruit were the same as those in the resulting wine. Research by Park et al. (1993) has shown that for wines produced in warm regions, 90% of monoterpenes may be glycosidically bound. These results indicated that glycosides were suitable flavor and aroma precursors. Researchers in both Australia and the U.S.A. have evaluated glycosyl-glucose as a method of estimating the concentration of glycosides (Williams et al., 1995; Iland et al., 1996). Because not all glycosidically-bound compounds contribute to the sensory attributes of wine, red-free (minus anthocyanins) and phenol-free glycosyl-glucose assays have also been evaluated (Zoecklein et al., 2000). These methods are currently being refined (Whiton and Zoecklein, unpublished data).

The use of growth retardants on grape reproductive structures: With regards to grape reproductive structures, the use of gibberellin biosynthesis inhibitors has been restricted to the modification of fruit set, and berry size. The majority of research has focused on quaternary ammonium compounds and triazole-type growth compounds such as CCC and paclobutrazol respectively. Both compounds inhibit the synthesis of ent-kaurene in the first stage of gibberellin biosynthesis.

The exact mode of action of CCC is not completely understood, despite the fact that it is one of the most widely used growth regulators (Hedden, 1988). CCC increases fruit set in seeded vinifera cultivars when applied before anthesis (Coombe, 1965 and 1967; Considine, 1983). This is interesting, as pre-anthesis GA_3 applications also increased flower number and fruit set (Kurshid et al., 1992). However, some researchers have noted that after high concentration applications, the effects of CCC were not completely reversed by GA_3 applications, indicating that CCC may have multiple modes of action (Hedden, 1988). CCC also

decreased berry size in seeded cultivars (Considine, 1983).

Paclobutrazol, a triazole-type plant growth retardant, has been researched more extensively than has CCC on grapes (Wolf and Miller, 1995; Reynolds et al., 1992; Kumar et al., 1998). Paclobutrazol, like other triazole-type compounds, inhibits the biosynthetic pathway in an identical manner as CCC (Rademacher et al., 1987). In a study by Reynolds et al. in British Columbia, (1992) paclobutrazol increased the soluble solids concentration in treated grapes. However, this effect was attributed to an altering of the source-sink relationship due to reduced lateral growth, and increased fruit exposure to light rather than a modification of endogenous gibberellins in reproductive tissues. Paclobutrazol has also increased fruit set (Kumar et al., 1998), although these results are not consistent among all cultivars (Hunter, 1992).

The use of prohexadione-calcium as a growth retardant: Prohexadione-calcium, is a cyclohexanetrion (acylcyclohexanedione) 3-oxido-4-propionyl-5-oxo-3-cylohexene-carboxylate. The mode of action of prohexadione-calcium differs from other gibberellin biosynthesis inhibitors currently in use in commercial agriculture. Many of these growth regulators operate, as previously discussed, by interrupting the synthesis of gibberellin early in the biosynthesis pathway. Overwhelmingly these growth inhibitors affect the first stage of gibberellin biosynthesis, the synthesis of ent-kaurene. Among the growth inhibitors that act in such a manner are the quaternary ammonium compounds, substituted pyrimidines, norbornenodiazetine derivatives, and triazole derivatives (Graebe, 1987). Prohexadione-calcium is known to interfere with certain steps beyond the GA_{12}-aldehyde in the gibberellin biosynthesis pathway, a step that directly leads to the bioactive gibberellins, particularly GA_1. The primary target of prohexadione-calcium appears to be 3-ß hydroxylation of GA_{20} to GA_1. The net effect is the reduction of immobile, biologically active GA_1 and an increase in the levels of $GA_{20,}$ which is a mobile, yet inactive form of gibberellin. (Evans et al., 1999; Graebe, 1987).

Current uses of prohexadione-calcium on fruit crops: Prohexadione-calcium has been extensively studied on fruit crops such as apples and peaches for its use of suppressing

vegetative growth. Apogee™, produced by Badische Anilin & Soda-Fabrik (BASF) of Ludwigshafen, Germany, consists of 27.5% prohexadione-calcium (Evans et al., 1999). Studies on apples have indicated that Apogee, when applied in sufficient concentrations and during the first 30 days after bloom, can suppress vegetative growth (Byers and Yoder, 1999). However, the same study indicates that Apogee has little if any impact on apple fruit quality. The only significant improvement in fruit quality, an increase in red color, was attributed to increased light exposure due to reduced vegetative growth. The ability of prohexadione-calcium to act as a vegetative growth suppressant in apples is confirmed by other studies (Owens and Stover, 1998). In studies conducted by Unrath (1999), multiple applications of low dosages were necessary to control vegetative growth throughout the season.

An interesting application of prohexadione-calcium has been in the field of plant pathology. Since prohexadione-calcium suppresses shoot growth, it also suppresses the pathogen *Erwinia amylovora*, which more readily infects rapidly growing shoot tips and induces the disease known as Fire Blight (Yoder et al., 1999).

LITERATURE CITED

Bureau, S. M., R.L. Baumes, and A.J. Raxungles. Effects of vine or bunch shading on the glycosylated flavor precursors in grapes of *Vitis vinifera* L. cv. Syrah. J. Agric. Food Chem. 48:1290-1297 (2000).

Byers, R.E. and Yoder, K.S. Prohexadione-calcium inhibits apple, but not peach, tree growth, but has little influence on apple fruit thinning or quality. HortScience 34:1205-1209 (1999).

Cavallo, P., S. Poni, A. Rotundo. Ecophysiology and vine performance of cv. 'Aglianico' under various training systems. Sci. Hortic. 87:21-32 (2001).

Considine, J.A. Concepts and practice of use of plant growth regulating chemicals in viticulture. In Plant Growth Regulating Chemicals Volume I. L.G. Nickell (Ed.), pp. 89-184. CRC Press, Boca Raton (1983).

Coolbaugh, R.C. Early stages of gibberellin synthesis. *In* The Biochemistry and Physiology of Gibberellins. A. Crozier (Ed.), pp. 53-98. Praeger Publishers, New York (1983).

Coombe, B.G. Fruit set and development in seeded grape varieties as affected by defoliation, topping, girdling, and other treatments. Am. J. Enol. Vitic. 10:85-100 (1959).

Coombe, B.G. Increase in fruit set of Vitis vinifera by treatment with growth retardants. Nature 205:305-306 (1965).

Coombe, B.G. Effects of growth retardants on *Vitis vinifera* L. Vitis 6:278-287 (1967).

Coombe, B.G. Research on development and ripening of the grape berry. Am. J. Enol. Vitic. 43:101-110 (1992).

Coombe, B.G., M. Bovio, and A. Schneider. Solute accumulation by grape pericarp cells. J. Exp. Bot. 38:1789-1798 (1987).

Coombe, B.G. and P.G. Iland. Grape berry development. In Proceedings of the Sixth Australian Wine Industry Technical Conference. Australian Industrial Publishers, Adelaide. pp. 50-54 (1987).

Dokoozlian, N.K. and W.M. Kliewer. The light environment within grapevine canopies. II. Influence of leaf area density on fruit zone light environment and some canopy assessment parameters. Am. J. Enol. Vitic. 46:219-226 (1995).

English, J.T., C.S. Thomas, J.J. Marois, and W.D. Gubler. Microclimates of grapevine canopies associated with leaf removal and control of Botrytis bunch rot. Phytopath. 79:395-401 (1989).

Evans, J.R., R.R. Evans, and C.L. Regusci. Mode of action, metabolism, and uptake of BAS 125W Prohexadione-calcium. HortScience 34:1200-1201 (1999).

Fernandez de Simon, B., T. Hernandez, and I. Estrella. Phenolic composition of white grapes (var. Airen). Changes during ripening. Food Chem. 47:47-52 (1993).

Graebe, J.E. Gibberellin biosynthesis and control. A Review. Annu. Rev. Plant Physiol. 38:419-465 (1987).

Hedden, P. The action of plant growth retardants at the biochemical level. *In* Plant Growth Plant Growth Substances. R.P. Pharis and S.B. Rood (Eds.), Springer-Verlag, Berlin pp. 322-332 (1988).

Hunter, D.M. Paclobutrazol affects growth and fruit composition of potted grapevines. HortScience 27:319-321 (1992).

Iland, P.G., W. Cynkar, I.L. Francis, P.J. Williams, and B.G. Coombe. Optimisation of methods for the determination of total and red-free glycosyl glucose in black grape berries of *Vitis vinifera*. Aust. J. Grape Wine Res. 2:171-178 (1996).

Ingram, T.J., J. B. Reid, J. MacMillan. The quantitative relationship between gibberellin A1 and internode growth in *Pisum sativum* L. Planta 168:414-420 (1986).

Jackson, D.I. and P.B. Lombard. Environmental and management practices affecting grape composition and wine quality-a review. Am. J. Enol. Vitic. 44: 409-430 (1993).

Jones, R.L. The control of plant cell elongation by auxin and gibberellin. *In* Plant Growth Substances. M. Bopp (Ed.), Springer-Verlag, Berlin (1985).

Khurshid, T, D.I. Jackson, and R.N. Rowe. Effect of plant growth regulators on flower development in the grapevine (*Vitis vinifera* L.) cv. Cabernet Sauvignon. N. Z.J. Crop Hortic. Sci. 20:351-356 (1992).

Kumar, A.K, G.S.R. Murti, and S.D. Sikhamany. Effect of Cycocel and Paclobutrazol on morphological attributes, bunch characteristics and endogenous gibberellin levels in 'Arkavati' Grape (*Vitis vinifera* L.) trained on two systems. Gartenbauwissenschaft. 63:63-65 (1998).

Lavee, S. M. Ziv, H. Melamud, and Z. Bernstein. The involvement of gibberellins in controlling bud development of grape vines (*Vitis vinifera* L.). Acta Hortic. 329:177-182 (1993).

Motomura, Y. Effects of gibberellin and daminozide on the distribution of 14C-assimilates in grape shoots. Acta Hortic. 179:421-424 (1986).

Mullins, M. G. Regulation of fruit set in the grapevine. Aust. J. Biol. Sci. 20:1141-1147 (1967).

Mullins, M.G. Hormonal regulation of flowering and fruit set in the grapevine. Acta Hortic. 179:309-315 (1986).

Owens, C.L. and E. Stover. Vegetative frowth and flowering of young apple trees in response to Prohexadione-calcium. HortScience 34:1194-1196 (1999).

Park, S.K. and A.C. Nobel. Monoterpenes and monoterpene glycosides in wine aromas. *In* Beer and Wine Production: Analysis, Characterization, and Technological Advances. B.H. Gump (Ed.), pp. 98-109. Am. Chem. Soc., Washington, D.C (1993).

Perez, F.J., C. Viani, J. Retamales. Bioactive gibberellins in seeded and seedless grapes: identification and changes in content during berry development. Am. J. Enol. Vitic. 51:315-318 (2000).

Phinney, B.O. The history of gibberellins. *In* The Biochemistry and Physiology of Gibberellins. A. Crozier (Ed.), pp.19-52. Praeger Publishers, New York (1983).

Phinney, B.O., C. A. West, M. B. Ritzel, and P. M. Neely. Evidence for gibberellin-like substances from flowering plants. Proc. Nat. Acad. Sci. 43:398-404 (1957).

Rademacher, W., H. Fritsch, J. E. Graebe, H. Sauter, and J. Jung. Tetcyclacis and triazole-type plant growth retardants: Their influence on the biosynthesis of gibberellins and other metabolic processes. Pestic. Sci. 21:241-252 (1987).

Reynolds, A.G., D.A. Wardle, A.C. Cottrell, and A.P. Gaunce. Advancement of 'Riesling' fruit maturity by Paclobutrazol-induced reduction of lateral shoot growth. J. Am. Soc. Hortic. Sci. 117:430-435 (1992).

Roubelakis-Angelakis, K. A. and W. M. Kliewer. Effects of exogenous factors on phenylalanine ammonia-lyase activity and accumulatin of anthocyanins and total phenolics in grape berries. Am. J. Enol.Vitic. 37:275-280 (1986).

Sachs, R.M., and R.J. Weaver. Gibberellin and Auxin-induced berry enlargement in *Vitis vinifera* L. Hortic. Sci. 43:185-95 (1968).

Smart, R.E. Principles of grapevine canopy microclimate manipulation with implications for yield and quality. A Review. Am. J. Enol. Vitic. 36:230-239 (1985).

Smart, R., and M. Robinson. Sunlight into Wine. p.14. Winetitles, Adelaide (1991).

Srinivasan C. and M. G. Mullins. Effects of temperature and growth regulators on formation of anlagen, tendrils, and inflorescences in *Vitis vinifera* L. Ann. Bot. 45:439-446 (1980a).

Srinivasan C. and M. G. Mullins. Flowering in Vitis: Effects of genotype on cytokinin-induced conversion of tendrils into inflorescences. Vitis 19:293-300 (1980b)

Staff, S. L., D. C. Percival, J. A. Sullivan, K. H. Fisher. Fruit zone leaf removal influences vegetative, yield, disease, fruit composition, and wine sensory attributes of *Vitis vinifera* L. 'Optima' and 'Cabernet franc'. Can. J. Plant Sci. 77:149-153 (1997).

Takeno, K., M. Kohioka, R.P. Pharis, K. Rajasekara, M.G. Mullins. Endogenous gibberellin-like substances in somatic embryos of grape (Vitis vinifera x Vitis rupestris) in relation to embryogenesis and the chilling requirement for subsequent development of mature embryos. Plant Physiology. 73:803-808 (1983).

Unrath, C.R. Prohexadione-Ca: A promising chemical for controlling vegetative growth of apples. HortScience 34:1197-1200 (1999).

Weaver, R.J. and S.B. McCune. Effects of giberellin on seedless Vitis vinifera. Hilgardia. 29:247-271 (1959).

Williams, P.J., M.A. Sefton, and I. L. Francis. Glycosidic precursors of varietal grape and wine flavor. *In* Flavor Precursors. R. Teranishi, G.R. Takeoka, and M. Guntert (Eds.), pp. 74-86. Am. Chem. Soc., Washington, D.C. (1992).

Williams, P.J., W. Cynkar, I. L. Francis, J.D. Gray, P.G. Iland, and B.G. Coombe. Quantification of glycosides in grapes, juices, and wines through a determination of glycosyl glucose. J. Agric. Food Chem. 43:121-128 (1995).

Wolf, T.K. and M.K. Miller. Shoot growth rate and density affect bud necrosis of Riesling grape vines. J. Am. Soc. of HortScience. 120:989-996 (1995).

Yoder, K. S., S. S. Miller, and R. E. Byers. Suppression of Fireblight in apple shoots by Prohexadione-calcium following experimental and natural inoculation. HortScience 34:1202-1204 (1999).

Zabadal, T.J. and T.W. Dittmer. Vine management systems affect yield, fruit quality, cluster compactness, and fruit rot of 'Chardonnay' grape. HortScience 33:806-809 (1998).

Zoecklein, B.W., T.K. Wolf, J.E. Marcy, and Y. Jasinski. Effect of fruit zone leaf thinning on total glycosides and selected aglycone concentrations of Riesling. (Vitis vinifera L.) grapes. Am. J. Enol. Vitic. 49:35-42 (1998).

CHAPTER TWO: EFFECT OF PROHEXADIONE-CA ON GRAPE VEGETATIVE DEVELOPMENT

ABSTRACT

Prohexadione-calcium (P-ca) in the form of Apogee™, was applied to whole canopies of Seyval, Cabernet Sauvignon, and Cabernet franc in 2000. In 2000 Seyval and Cabernet Sauvignon treatments consisted of multiple 250 mg/L applications. Cabernet franc was treated thrice with either 125, 250, or 375 mg/L. Primary shoot growth was reduced for all cultivars. P-ca decreased cane pruning weight of Seyval but did not impact that of Cabernet franc or Cabernet Sauvignon. Point quadrat analysis of treatments found increased percent gaps for the 375 mg/L Cabernet franc treatment and increased leaf layer number for the twice-applied 250 mg/L Cabernet Sauvignon treatment. Lateral leaf area increased for Cabernet Sauvignon treatments. Fruit zone photosynthetic photon flux density was not affected for 2000 treatments. In 2001, vegetative experimentation with 250 mg/L P-ca was continued with Cabernet franc, and Chardonnay. Two pre-bloom applications to Chardonnay reduced shoot length, primary node number, and internode length. Shoot length differed for Cabernet franc pre-bloom and post-bloom treatments.

Keywords: prohexadione-calcium, Apogee, growth regulators, gibberellin, grape

INTRODUCTION

An abundance of literature describes how grapevine canopy modification can be used to improve fruit quality (Smart and Robinson, 1991; Zabadal and Dittmer, 1998; Smithyman et al., 1997). Common goals throughout much of this research have been reduction of fruit-zone canopy density and primary shoot growth (Smart, 1985). There is evidence linking dense grapevine canopies and canopy microclimates which favor poor fruit ripening and fungal pathogens such as *Botrytis cinerea* (English et al., 1989; Smart and Robinson, 1991). Excessive canopy density has been cited as a factor in poor fruit ripening due to its reduction of fruit-zone light infiltration (Dokoozlian, 1995 and 1996) while *B. cinerea* infections are increased by a combination of increased humidity, temperature, leaf wetness and reduced wind speeds (English et al., 1989). Current industry canopy management practices, for vigorous vines, focus on trellis systems and procedures such as shoot and leaf thinning to achieve sparse canopies and adequate light infiltration into the fruit zone. (Smart and Robinson, 1991; Zabadal and Dittmer, 1998; Dokoozlian, 1995).

From the early 1960s to present there has been increased interest in evaluating gibberellin biosynthesis inhibitors for the purpose of retarding vegetative growth and achieving the goals currently accomplished by manual canopy manipulation (Coombe, 1967; Reynolds et al., 1992, Kumar et al., 1997). Quaternary ammonium compounds such as chlormequat chloride (Cyclocel™ or CCC) and triazole-type compounds such as paclobutrazol have been evaluated for use in suppressing vegetative growth on grapes *Vitis vinifera* (Kumar et al., 1998; Reynolds et al., 1992; Wolf and Miller, 1995; Coombe, B.G., 1967). Although these compounds were never registered on grapes in the United States, there remains a desire in commercial grape production for a growth regulator to substitute for current costly labor-intensive canopy management practices.

Prohexadione-calcium (P-ca) is a gibberellin biosynthesis inhibitor with a slightly different mode of action from that of quaternary ammonium compounds and triazole-type growth regulators (Evans et al., 1999; Hedden, 1988; Graebe, 1987). P-ca makes up 27.5% of Apogee^{TM}, a product recently introduced by Badische Anilin & Soda-Fabrik (BASF) of Ludwigshafen, Germany (Evans et al., 1999). Studies conducted at the Alson H. Smith Research and Extension Center in Winchester, VA indicated that Apogee suppressed vegetative growth in apples (Malus x domestica Borkh.) when applied in sufficient concentrations during the first 30 days after bloom (Byers and Yoder, 1999). The ability of P-ca to act as a vegetative growth suppressant is confirmed by other studies (Owens and Stover, 1999). In both of these studies, timing of applications and cultivar type have had major impacts on resulting vegetative development. Positive results from this and other work (Unrath, 1999) have led to the E.P.A. registration of Apogee on Apple. This registration is encouraging for grape growers if similar growth suppression can be demonstrated with grapevines (*Vitis spp.*)

Virginia is noted for climatic conditions that favor excessive vegetative growth and fruit-zone canopy density. Field experiments in northwest Virginia in 2000 and 2001 evaluated foliar sprays of P-ca on *Vitis vinifera* and hybrid grape species. Established parameters that define acceptable canopy architecture (Smart and Robinson, 1991) were used to evaluate the impact of Apogee treatments. The objectives of this study were to evaluate prohexadione-calcium for suppression of grapevine vegetative growth to modify grapevine canopy architecture in Virginia. An ancillary goal was the reduction of primary shoot growth to obviate summer pruning necessity.

MATERIALS AND METHODS

Experimentation in 2000 involved Seyval, Cabernet Sauvignon, and Cabernet franc. Treatments consisted of foliar applications of Apogee™. Apogee is comprised of 27.5% Prohexadione-calcium in the form of free flowing dark tan granules. The spray mixture included two adjuvants; Regulaid™, a spreader-activator, and ammonium sulfate $(NH_4)_2SO_4$. Ammonium sulfate has been used to increase the efficacy of P-ca where the spray water contains high concentrations of calcium (Byers, unpublished data). All treatments, except where indicated, included 900 mg/L ammonium sulfate and 1.3 mL/L Regulaid. For each treatment, P-ca was applied when there was no rain predicted for a minimum of 24 hours. Treatments were applied to both sides of the canopy. Solutions applied to the control plots included the two adjuvants in concentrations equal to those in the treatment applications. In 2001, experiments were continued on Cabernet Sauvignon, Cabernet franc, and Chardonnay, and data was recorded for vegetative measurements on the Cabernet franc and Chardonnay experiments.

2000 Seyval. Own-rooted, 10-year-old Seyval vines, located at the Alson H. Smith Jr. AREC in Winchester, VA, were used. Vines were cordon-trained and spur-pruned with a vertical shoot positioned (VSP) canopy. Row x vine spacing was 3.7 x 2.1 m. The experimental design was completely randomized with five replicates of three-vine treatment plots.

Treatments consisted of three foliar applications of a 250 mg/L P-ca solution compared to a control. The initial application was made pre-bloom on 15 May. The second application was 31 May during bloom with the third application post-bloom on 30 June. The amount of active ingredient applied (a.i.) per vine varied depending on the volume of spray water used. The 15 May application was made with 7.57 liters of spray water. The two subsequent applications were each made with 13.25 liters of spray water. The approximate amount of a.i. applied per vine on

15 May, 31 May, and 30 June was 126, 221, and 221 mg respectively. Applications were made with a hand-held pressure sprayer.

Shoot length measurements, point quadrat analysis (PQA), photosynthetic photon flux density (PPFD) measurements, and cane pruning weights were recorded. Six shoots, of similar height and cluster number, were selected per vine in early spring. Shoots were measured on 17 May, 31 May, and 14 June. Measurements were taken from the base of the shoot to the youngest emerging leaf at the apex. Shoot measurements were discontinued after 14 June due to the need for summer pruning. The first two measurements coincided with the first and second P-ca applications.

The procedures used for PQA analysis were similar to those outlined by Smart and Robinson (1991). A total of 21 measurements in the fruit-zone, each 8 centimeters apart, were taken for each three-vine plot. The number of probe contacts with fruit and leaves, as well as canopy gaps, were recorded. Leaf layers, percent exterior fruit and percent gaps were subsequently calculated.

Photosynthetic photon flux density (PPFD) was measured to determine the photosynthetic light penetration in the fruiting zone of the canopy. These measurements, were made with a 1.0–meter line quantum sensor (model LI-191SB, LI-COR Inc. Lincoln, NE) held in the fruit zone. Measurements were made between 1100 and 1600 EDT on clear days in July. Three readings were taken for each vine, one reading with the instrument held so that the sensor panel directly faced skyward (0º), a second reading with the sensor held 45º to the east and a third reading taken 45º to the west; on the North-South oriented rows. These readings were then averaged together to obtain a single observation for the experimental unit. As a point of comparison, ambient light was measured outside the canopy for each plot, with the sensor aimed at the sun for a maximum reading. The averaged fruit zone light readings were divided by the ambient readings to provide a value for the percentage of ambient light in the fruit zone. Cane pruning weights were recorded at dormant pruning.

Cabernet Sauvignon 2000. Nine-year-old Cabernet Sauvignon (FPMS clone #7) vines,

located at the Alson H. Smith Jr. AREC in Winchester, VA, were used. Vines were cordon-trained and spur-pruned on a lyre trellis system. Row x vine spacing was 3.7 x 2.1 m. The experimental design was completely randomized with five replicates of three-vine plots.

Cabernet Sauvignon treatments consisted of an untreated control, a twice-applied treatment (2xP-ca) and a thrice-applied treatment (3xP-ca). The P-ca rate for all applications was 250 mg/L. The 2xP-ca treatment was sprayed twice pre-bloom (15 and 31 May) while 3xP-ca included the two pre-bloom applications plus an additional post-bloom application (30 June). Full bloom occurred 5 June. The first application required 18.93 liters of spray water per five treatment plots while the second and third applications each used 30.28 liters of spray water per treatment. Each vine received amount 315 mg a.i. on the first date and 505 mg on the second and third dates. Applications were made with a hand-held pressure sprayer.

As with the Seyval vines, canopy architecture measurements consisted of shoot length, PQA, PPFD and cane pruning weight measurements. Primary leaves, lateral shoots, and lateral leaves were counted and primary and lateral leaf area was measured.

For shoot length measurements, 24 shoots were selected per treatment plot. These shoots were equally divided between the east and west canopies of the North-South oriented rows. Therefore, a total of 120 shoots per treatment were measured on 16 May, 30 May, and 14 June. Shoot measurements were discontinued after 14 June due to the necessity of hedging the vines. The procedures used for PQA were identical to those made on the Seyval canopy with the exception that since the Cabernet Sauvignon was trained to a lyre system, PQA was performed for both the east and west side of the canopy.

PPFD was measured on 17 August with the same instrumentation as used with the Seyval. Due to the structure of the lyre canopy, six readings were taken within each three-vine plot. These readings were divided between the east and west sides of the canopy so that each vine had an east and west reading. The quantum line sensor was held so that the sensor panel directly faced skyward (0º) for each of these readings.

Leaf area was measured between 23 August and 12 September. To estimate leaf area for each treatment, two shoots of equal length, one from each side of the horizontally divided

canopy, were selected per vine. The three treatments consisting of 15 vines each and a total of 90 shoots were destructively sampled. Leaf area was recorded with an area meter (LI-3000, LI-COR Inc. Lincoln, NE). The number of primary nodes, primary leaves, lateral shoots, lateral leaves, and primary and lateral leaf area were recorded for each shoot. Primary leaf area, and lateral leaf area were determined per shoot. Cane pruning weights were recorded at dormant pruning.

2000 Cabernet franc. Two-year-old Cabernet franc, grown at Indian Springs Vineyard in Woodstock, VA, was used. Vines were cordon-trained and spur-pruned with the canopy trained to a VSP system. Row x vine spacing was 2.7 x 2.1 m. The experimental design was completely randomized with each treatment consisting of 10 single-vine replicates. Treatments were 125, 250 and 375 mg a.i./L. To determine the effect of ammonium sulfate in the spray mixture, two controls were included. One control was treated with a solution of ammonium sulfate and Regulaid and the other control was only treated with Regulaid.

All treatments and controls were sprayed once pre-bloom (18 May) and twice post bloom (1 June, and 30 June). Full bloom occurred 26 May. As applications were made to a continuously increasing canopy, the volume of spray needed to provide complete coverage likewise increased. However, for each application date spray water volume was uniform between treatments. The amount of spary solution useed was 5.70 L 7.57 L and 11.37 L respectively on 18 May, 1 June and 30 June. The amount of a.i. applied per vine for the 125 mg/L treatment, from the first to last application date, was 71 mg, 95 mg, and 142 mg. The amount of a.i. applied per vine for the 250 mg/L treatment, from the first to last application date, was 142 mg, 189 mg, and 284 mg. The amount of a.i. applied per vine for the 375 mg/L treatment, from the first to last application date, was 235 mg, 284 mg, and 426 mg.

Shoot length measurements, PQA, and cane pruning weights were the only canopy characterizations performed on these vines. Shoot length was measured for Cabernet franc on 18 May, 1 June, and 15 June. After 15 June, length measurements were discontinued due to summer pruning necessity. For these measurements, two shoots per vine were selected, for a total of 20

shoots per treatment. The procedures used for PQA were similar to those made on the Seyval canopy, except there were only six probes per vine. Cane pruning weights were recorded at dormant pruning.

2001 Cabernet franc and Chardonnay. Cabernet franc and Chardonnay experiments were identical in every respect except where explicitly noted. The experiments were conducted using spur-pruned cordon-trained Cabernet franc and Chardonnay at Indian Springs vineyard in Woodstock, VA, Shenandoah County. Vines were planted in 1998 with 2.7 x 2.1 m (row x vine) spacing. The canopy was trained in VSP system. The experimental design was completely randomized and experimental units consisted of four-vine plots. Each treatment had five replicates for a total of 20 vines. Applications were made to runoff at a rate of 250 mg/L. Applications were made to both sides of the canopy in a zone defined, at its lowest point, by the fruiting wire and extending vertically 0.5 meters. The first pre-bloom application covered the canopy in entirety due to the short length of the shoots. All treatment solutions contained Regulaid and ammonium sulfate. The experiments had two controls and three treatments. Treatment 2prb consisted of two applications pre-bloom (7 May and 23 May). Treatment 1pstb consisted of a single post-bloom application (19 June), and treatment 2pstb consisted of two post-bloom applications (19 June and 3 July). The E-L growth stage, modified by Coombe (1995), of the vines was recorded at each application date. For Cabernet franc, the two pre-bloom applications were made at E-L stages 15 and 17 and the post-bloom applications were made at E-L stage 29 and 31. For Chardonnay, the two pre-bloom applications were made at E-L stages 15 and 19 and the post-bloom applications were made at E-L stage 30 and 32 .

Because applications were concentrated on the fruit zone, a considerably lower volume of spray was used for these experiments than in 2000. Both pre-bloom applications used 3.79 liters of spray water per treatment and both post-bloom applications used 5.69 liters of spray water per treatment. The amount of a.i. per vine per application date was 47.4 mg for each of the two pre-bloom applications and 71.2 mg for each of the two post-bloom applications.

One of the controls was crop-thinned to an expected crop reduction level due to P-ca. The expected crop reduction was based on mid-season cluster weights and counts for the pre-bloom and post-bloom treatments. Due to the slight difference in average cluster weight between the 2prb, 1pstb and 2pstb treatments, the crop per vine of the thinned control was reduced to a level intermediate to that of the three treatments. The other control was left with its original cluster count.

Shoot lengths were measured on 3 July for 2prb, 1pstb, and the un-thinned control. The shoot lengths of 2pstb were not measured because 2pstb had not yet received its second post bloom application. Measurements were taken to quantify an observed difference in shoot length between Chardonnay control and 1prb vines. For this procedure two shoots were selected per vine, one from each arm of the bilateral cordon. Nodes per shoot were also recorded to calculate internode length.

Data Analysis. In the Seyval experiment a pooled t-test was used to examine differences in PQA, pruning weight, and PPFD between the treatment and control replicates. Seyval shoot growth was analyzed using repeated measures analysis of variance.

As the Cabernet Sauvignon experiment involved two treatments differing by one application, single degree of freedom contrasts were used to compare results from PQA, PPFD, light readings, leaf area, and pruning weight. Shoot growth was analyzed with repeated measures analysis of variance.

The rate-based treatments of the 2000 Cabernet franc experiment were analyzed using single degree of freedom contrasts. PQA values for the treatments were evaluated with single degree of freedom contrasts as well as linear regression analysis. Cabernet franc shoot growth was analyzed using repeated measures analysis. Difference in pruning weights was analyzed with contrasts and linear regression.

2001 Cabernet franc and Chardonnay shoot growth was analyzed using single degree of freedom contrasts. All analyses were performed using the SAS System (v.8e, Cary, NC).

RESULTS

2000 Seyval. Three applications of P-ca at 250 mg/L did not significantly decrease fruit zone canopy density, as measured by PQA, or PPFD readings within the fruit zone (Table 2-1). There was no treatment effect on shoot growth during the first measurement period between 17 and 31 May. However, primary shoot growth rate was significantly decreased by treatment during the second measurement period between 31 May and 14 June (Figure 2-1). After 14 June, growth measurements were stopped due to the need for summer pruning. Therefore, the significant decrease in shoot growth that we observed in this second measurement period was not large enough to obviate the need for shoot topping. The decrease in primary shoot growth was accompanied by a significant decrease in pruning weight for vines treated with the three 250 mg/L applications (Table 2-1). Cropload was not significantly influenced by treatment.

Table 2-1. Seyval canopy characteristics: leaf layer number, percent external fruit, percent canopy gaps, percent photosynthetic photon flux density (PPFD) in the fruit zone, cane pruning weight, and cropload due to three applications of prohexadione-calcium (P-ca) during the 2000 season.

	PQA			Fruit zone PPFD	Pruning data	
Treatment	Leaf layer #	External fruit %	Canopy gaps %	Ambient light % µmol x m^{-2} x s^{-1}	Cane pruning weight (kg)	Cropload (yield/pruning weight)
Control	2.1	41	4	4.3	0.9	11.9
P-ca	2.3	52	1	3.7	0.7	10.0
Significance (*P*-value)						
t-test[a]	0.318	0.328	0.290	0.561	0.012	0.257

[a]Pooled data

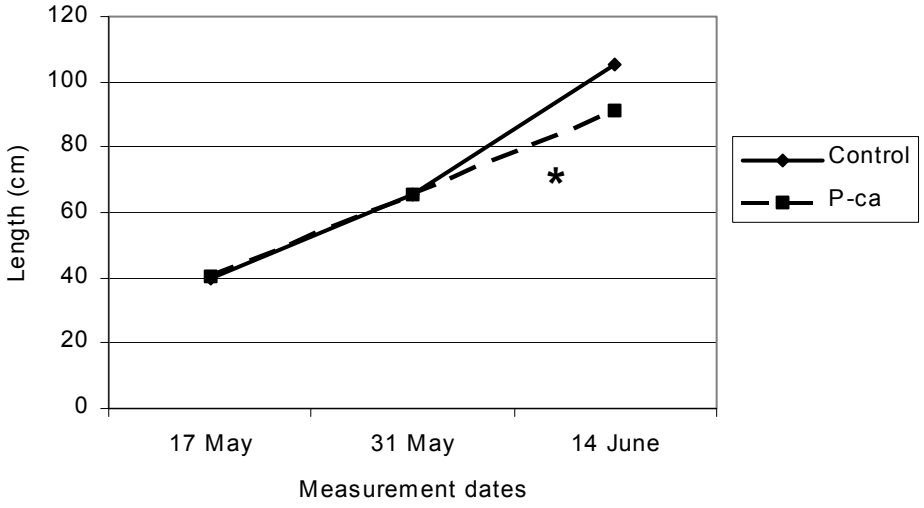


Figure 2-1. Primary shoot length of Seyval affected by 250 mg/L prohexadione-calcium (P-ca) compared to control. Shoot length analyzed by repeated measures analysis of variance. Significant treatment effect depicted by asterisk.

2000 Cabernet Sauvignon. There was no treatment effect on shoot growth during the first measurement period between 16 and 30 May. During the second measurement period, 30 May to 14 June, primary shoot growth decreased due to the two pre-bloom applications (Figure 2-2). Because the third application of 3xP-ca was made two months after the primary shoot measurements stopped, no comparison is available between 2xP-ca and 3xP-ca. The 2xP-ca and 3xP-ca treatments were similar for all canopy architecture measurements. Leaf layer number was greater for 2xP-ca while percent external fruit and percent canopy gaps remained relatively constant (Table 2-2). Treatment did not affect fruit zone PPFD (Table 2-2). Pruning weight was not significantly altered but crop load was decreased by both treatments (Table 2-2). P-ca applications significantly reduced the primary leaf area per shoot but increased lateral leaves per shoot (Table 2-3). A comparison between the control and 2xP-ca indicated an increase in lateral shoots per primary shoot with a *P*-value = 0.062 (data not shown). Average leaf area decreased for both primary and lateral leaves with a significant decrease recorded for the latter (Table 2-3). However, this decrease in average leaf area was offset for the lateral shoots by the significant increase in total lateral leaves per shoot. (Table 2-3).

Table 2-2. Cabernet Sauvignon canopy characteristics: leaf layer number, percent external fruit, percent canopy gaps, percent photosynthetic photon flux density (PPFD) in the fruit zone, cane pruning weight, and cropload due to two (2xP-ca) and three (3xP-ca) 250 mg/L prohexadione-calcium applications during the 2000 season.

	PQA			Fruit zone PPFD	Pruning data	
Treatment	Leaf Layer #	External fruit %	Canopy gaps %	Ambient light % µmol x m^{-2} x s^{-1}	Cane pruning weight (kg)	Cropload (yield/pruning wt.)
Control	1.8	52	3	2.9	4.6	2.0
2x P-ca	2.3	64	3	3.4	4.3	0.7
3x P-ca	2.1	54	2	3.1	4.4	0.9
Significance of Contrast[z] (*P*-value)						
Control vs. mean of treatments	0.010	0.448	0.866	0.444	0.250	0.000
2xP-ca vs. 3xP-ca	0.120	0.325	0.769	0.448	0.750	0.087

[z] Single-degree of freedom contrasts of specified treatments.

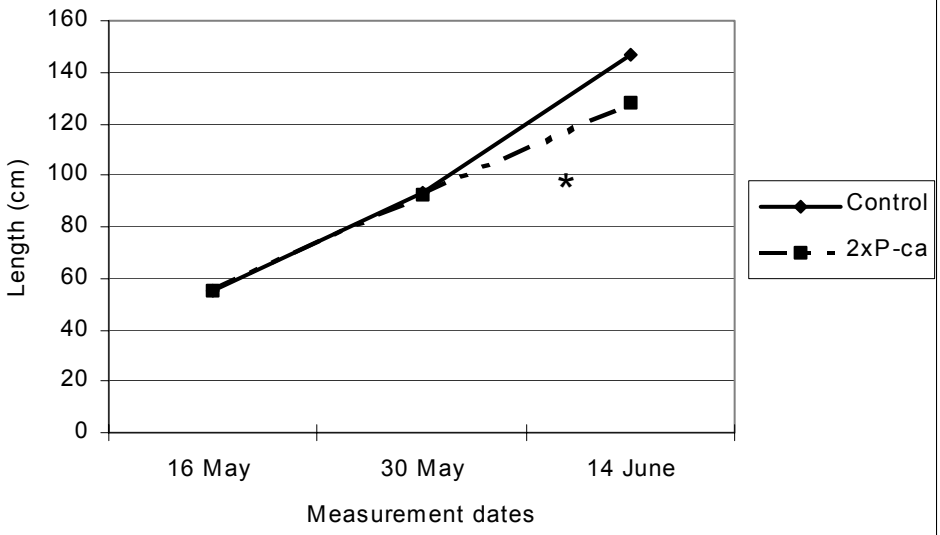


Figure 2-2. Primary shoot length of Cabernet Sauvignon affected by 250 mg/L prohexadione-calcium (P-ca) compared to control. Shoot length analyzed by repeated measures analysis of variance. Significant treatment effect depicted by asterisk.

Table 2-3. Cabernet Sauvignon leaf area measurements: nodes on the primary shoot, lateral shoots per primary shoot, primary leaf area, lateral leaf area, primary leaves per primary shoot, lateral leaves per primary shoot, average primary leaf area, and average lateral leaf area as affected by two (2x P-ca) and three (3x P-ca) applications of prohexadione-calcium during the 2000 season.

Treatment	Primary nodes/ shoot	Lateral shoots/ shoot	Primary leaf area/ shoot (cm^2)	Lateral leaf area/ shoot (cm^2)	Primary leaves/ shoot	Lateral leaves/ shoot	Primary leaf area/ leaf (cm^2)	Lateral leaf area/ leaf (cm^2)
Control	15.7	8.5	1690.9	1888.6	14	44	125	43
2xP-ca	16.1	10.2	1397.4	2357.9	13	72	109	32
3xP-ca	15.0	9.0	1282.9	2117.4	12	62	112	33
Significance of Contrast[z] (*P*-value)								
Control vs. mean of treatments	0.812	0.152	0.003	0.340	0.263	0.044	0.062	0.000
2x P-ca vs. 3x P-ca	0.339	0.218	0.349	0.599	0.322	0.469	0.731	0.762

[z] Single-degree of freedom contrasts of specified treatments.

2000 Cabernet franc. There was no treatment effect on shoot growth during the first measurement period between 18 May and 1 June. There was a significant negative linear concentration effect on Cabernet franc primary shoot length in the second measurement period between 1 June and 15 June (Figure 2-3).

With few exceptions, there was little difference between the three treatment canopies with respect to each other or the control. No significant difference between treatments was observed for fruiting zone leaf layer number (Table 2-4). However, percent canopy gaps was significantly higher for the 375 mg/L treatment compared to the two controls while percent external fruit was significantly lower for this treatment than the control excluding ammonium sulfate (Table 2-4). There was no statistical difference between treatment pruning weights and pruning weight was not affected by the addition of ammonium sulfate (Table 2-4).

Table 2-4. Cabernet franc point quadrat analysis: leaf layer number, percent external fruit, percent canopy gaps, and pruning data: cane pruning weight and cropload, as affected by three rates of prohexadione-calcium (125, 250, and 375 mg/L) and two controls, with (N) and without (noN) ammonium sulfate during the 2000 season.

	PQA			Pruning data	
Treatment	Leaf layer #	External fruit %	Canopy gaps %	Cane pruning weight (kg)	Cropload (yield/pruning weight)
noN	2.5	45	0	1.2	4.5
N	2.7	28	0	1.2	4.0
125 mg/L	2.2	33	0	1.3	1.9
250 mg/L	2.6	40	0	1.3	1.1
375 mg/L	2.6	0	7	1.4	0.4
Significance of Contrast[z] (*P*-value)					
N vs. no N	0.547	0.277	1	0.718	0.154
N vs. mean of treatments	0.987	0.0953	0.233	0.272	0.000
Linear	0.698	0.130	0.011	0.320	0.000
Quadratic	0.251	0.0456	0.052	0.610	0.001

[z] Single-degree of freedom contrasts and trend analysis of specified treatments.

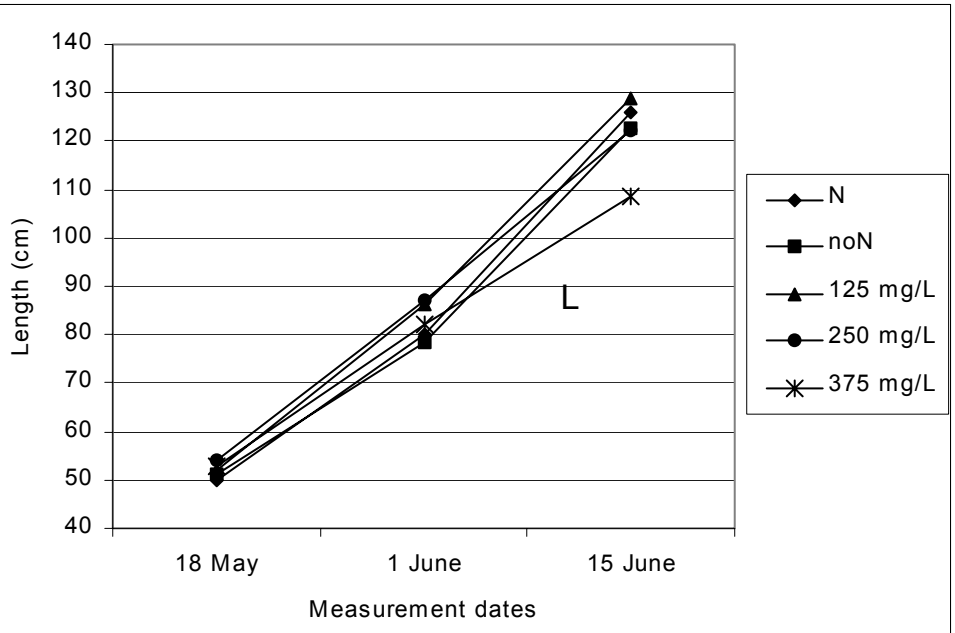


Figure 2-3. Primary shoot length of Cabernet franc affected by three rates of prohexadione-calcium (125, 250, 375 mg/L) compared to two controls with (N) and without (noN) ammonium sulfate . Shoot length analyzed by repeated measures analysis of variance. Significant linear treatment effect depicted by L.

2001 Chardonnay and Cabernet franc. There was no difference in shoot length, internode length or node number between the unthinned control (UT) and pre-bloom (2prb) and post-bloom (1pstb) treatments for Cabernet franc (Table 2-5). . However, Cabernet franc 1pstb had increased shoot length and internode length compared to 2prb. Chardonnay 2prb had reduced shoot length, primary nodes, and internode length compared to UT and reduced shoot length, and primary nodes compared to 1pstb.

Table 2-5. Cabernet franc shoot length measurements as affected by two pre-bloom (2prb) and one post-bloom (1pstb) applications of 250 mg/L prohexadione-calcium during the 2001 season compared to an unthinned control (UT).

Treatment	Shoot length (cm)	Node number	Internode length (cm)
UT	120.9	21.9	5.3
2prb	117.4	22.6	5.0
1pstb	139.7	24.2	5.7
Significance of Contrast[z] (*P*-value)			
2prb vs. UT	0.750	0.560	0.241
1pstb vs. UT	0.096	0.066	0.179
2prb vs. 1pstb	0.047	0.196	0.0127

[z]Single degree of freedom contrasts between specified treatments.

Table 2-6. Chardonnay shoot length measurements as affected by two pre-bloom (2prb) and one post-bloom (1pstb) applications of 250 mg/L prohexadione-calcium during the 2001 season compared to an unthinned control (UT).

Treatment	Shoot length (cm)	Node number	Internode length (cm)
UT	116.6	21.4	5.2
2prb	69.6	14.5	4.5
1pstb	100.5	20.4	4.6
Significance of Contrast[z] (*P*-value)			
2prb vs UT	0.000	0.000	0.028
1pstb vs. UT	0.167	0.481	0.077
2 prb vs. 1 pstb	0.009	0.000	0.658

[z]Single degree of freedom contrasts between specified treatments.

DISCUSSION

P-ca treatments decreased primary shoot growth for Cabernet Sauvignon, Cabernet franc, and Seyval in the period between. The reduction however, did not eliminate the need for summer pruning under the conditions of these experiments. PQA and PPFD measured during the 2000 season did not indicate decreased canopy density resulting from P-ca applications. Two 250 mg/L applications to Cabernet Sauvignon increased fruit zone leaf layer. High leaf layer number was a factor in creating a dense canopy, which creates a particular type of canopy microclimate (Dokoozlian, 1995; Smart, 1985; Smart and Robinson, 1991). Although certain publications place all of our observed leaf layer values within the range of "acceptable" values (Smart, 1985), the optimum value proposed by Smart and Robinson (1991) is an average of one leaf layer number.

Two possible explanations for the increase in fruit-zone canopy leaf layer are increased lateral shoot and leaf area, and decreased internode length. In 2001, decreased primary shoot growth of Chardonnay corresponded to shorter internodes. This is not completely unexpected, as reduced internode length was observed in other studies where gibberellin biosynthesis inhibitors were evaluated on grapes (Coombe, 1967). A decrease in internode length coupled with a suppression of primary shoot growth could itself lead to a more compact and dense canopy. Lateral shoots were only counted for the 2000 Cabernet Sauvignon experiment. Results from this experiment showed an increase of lateral shoots per primary shoot from 8.5 for the control to 10.2 for 2xP-ca. Because the *P*-value for this contrast was 0.062 the results should not be dismissed without further study. The 2xP-ca treatment has the most lateral leaves per primary shoot. However this was balanced by a significant decrease in average lateral leaf area. P-ca is causing greater numbers of smaller lateral leaves. These opposing factors resulted in no significant difference between 2xP-ca and the control in terms of lateral leaf area per primary shoot.

The increase in lateral shoots (P = 0.062) coupled with the increase in lateral leaves suggests that higher rates of P-ca than used in this study may have the potential to increase lateral leaf area of Cabernet Sauvignon and perhaps other cultivars. Unfortunately the failure to measure the lengths of primary and lateral shoots involved in 2000 Cabernet Sauvignon leaf area measurements precludes our calculation of internode lengths for this experiment. However, considering reduction of Chardonnay internode length in 2001 and the effect of gibberellin on elongating grapevine internodes, a general decrease in internode length due to P-ca application is probable. Considering the results from these experiments, the use of P-ca as a canopy management tool has not yet been justified.

Although all rates of P-ca decreased primary shoot growth there was no corresponding decrease in pruning weight for Cabernet franc or Cabernet Sauvignon. There are a few possible explanations why pruning weight was not decreased. The weight of additional lateral shoots could plausibly nullify the reduction in prunings from shorter primary shoots. However, P-ca treatments decreased pruning weight for Seyval treatments. Other work with P-ca on Vidal Blanc also showed a reduction in pruning weight, this at a rate of 500 mg/L (Ferree, unpublished data). Another possible reason for a lack of decreased pruning weight is a reduction in crop per vine. Cabernet franc and Cabernet Sauvignon crop was reduced (data not shown) with the 375 mg/L Cabernet franc treatment exhibiting the most severe reduction. Regarding the cropload data, one can see that there is a negative linear relationship between cropload (crop per vine/pruning weight) and concentration of P-ca. Supporting this hypothesis are various studies, which suggest that crop reduction can significantly stimulate vine size (Miller et al., 1993; Howell, 1999).

The 2001 shoot growth results for Cabernet franc and Chardonnay differed although the cultivars shared most application dates. A likely reason for this disparity is the difference in developmental stage at time of application for these cultivars. By recording the E-L growth stage during 2001 application dates, we observed that Chardonnay and Cabernet franc vines involved in our experiment developed at different rates; Chardonnay was usually one growth stage ahead of Cabernet franc. This could explain why identical applications on the same date

significantly decreased shoot growth, internode length, and node number of Chardonnay but not Cabernet franc.

Cabernet franc 2001 shoot growth results did not duplicate those of the 2000 season, where shoot growth was suppressed. Since shoot lengths were recorded in 2000 after two pre-bloom applications, the pre-bloom treatments for the two seasons had the same frequency of application. Therefore, the difference between 2000 and 2001 Cabernet franc shoot growth results is likely due to a difference in developmental stage at treatment, not frequency of application.

CONCLUSION

Our experiments show that while P-ca does have the potential to significantly reduce primary shoot growth, the growth reduction was insufficient to eliminate the summer pruning normally practiced with VSP training under Virginia conditions. Cultivars of *Vitis vinifera* differed in their response to P-ca in terms of impact to canopy architecture. In 2000, Cabernet Sauvignon responded to treatment with increased lateral leaves and fruit-zone leaf area while Seyval and Cabernet franc did not. P-ca increased pruning weight in Seyval, but not other cultivars that season. Similar cultivar-specific reactions were reported for apples treated with P-ca. Besides the influence of cultivar, changing the frequency and rate of application might cause greater reductions in shoot growth. Additionally, our 2001 season results indicate that the grapevine developmental stage at treatment is very influential in the ability of P-ca to reduce vegetative growth. As cultivars develop at different rates, and as the same cultivar will develop differently from one season to the next, it is imperative for future research to evaluate treatments based on developmental stage rather than date.

LITERATURE CITED

Byers, R.E. and Yoder, K.S. Prohexadione-calcium inhibits apple, but not peach, tree growth, but has little influence on apple fruit thinning or quality. HortScience 34:1205-1209 (1999).

Coombe, B.G. Effects of growth retardants on *Vitis vinifera* L. Vitis 6:278-287 (1967).

Coombe, B.G. Adoption of a system for identifying grapevine growth stages. Aust. J. Grape Wine Res. 1:100-110 (1995).

Dokoozlian, N.K. and W.M. Kliewer. The light environment within grapevine canopies. II. Influence of leaf area density on fruit zone light environment and some canopy assessment parameters. Am. J. Enol. Vitic. 46:219-226 (1995).

Dokoozlian, N.K. and W.M. Kliewer. Influence of light on grape berry growth and composition varies during fruit development. J. Am. Soc. Hortic. Sci. 121:869-874 (1996).

English, J.T., C.S. Thomas, J.J. Marois, and W.D. Gubler. Microclimates of grapevine canopies associated with leaf removal and control of Botrytis bunch rot. Phytopath. 79:395-401 (1989).

Evans, J.R., R.R. Evans, and C.L. Regusci. Mode of action, metabolism, and uptake of BAS 125W Prohexadione-calcium. HortScience 34:1200-1201 (1999).

Graebe, J.E. Gibberellin biosynthesis and control. A Review. Annu. Rev. Plant Physiol. 38:419-465 (1987).

Hedden, P. The action of plant growth retardants at the biochemical level. *In* Plant Growth Plant Growth Substances. R.P. Pharis and S.B. Rood (Eds.), Springer-Verlag, Berlin pp. 322-332 (1988).

Howell, G.S. Sustainable grape productivity and the growth-yield relationship: A Review. Am. J. Enol. Vitic. 52:165-174 (2001).

Kumar, A.K, G.S.R. Murti, and S.D. Sikhamany. Effect of Cycocel and Paclobutrazol on morphological attributes, bunch characteristics and endogenous gibberellin levels in 'Arkavati' Grape (*Vitis vinifera* L.) trained on two systems. Gartenbauwissenschaft. 63:63-65 (1998).

Miller, D.P., G.S. Howell, and R.K. Striegler. Reproductive and vegetative response of mature grapevines subjected to differential cropping stresses. Am. J. Enol. Vitic. 44:435-440 (1993).

Owens, C.L. and E. Stover. Vegetative growth and flowering of young apple trees in response to Prohexadione-calcium. HortScience 34:1194-1196 (1999).

Reynolds, A.G., D.A. Wardle, A.C. Cottrell, and A.P. Gaunce. Advancement of 'Riesling' fruit maturity by Paclobutrazol-induced reduction of lateral shoot growth. J. Am. Soc. Hortic. Sci. 117:430-435 (1992).

Smart, R.E. Principles of grapevine canopy microclimate manipulation with implications for yield and quality. A Review. Am. J. Enol. Vitic. 36:230-239 (1985).

Smart, R., and M. Robinson. Sunlight into Wine. p.14. Winetitles, Adelaide (1991).

Smithyman, R.P., G.S. Howell, and D.P. Miller. Influence of canopy configuration on vegetative development, yield, and fruit composition of Seyval blanc grapevines. Am. J. Enol. Vitic. 48:482-491 (1997).

Unrath, C.R. Prohexadione-Ca: A promising chemical for controlling vegetative growth of apples. HortScience 34:1197-1200 (1999).

Wolf, T.K. and M.K. Miller. Shoot growth rate and density affect bud necrosis of Riesling grape vines. J. Am. Soc. of Hortic. Sci. 120:989-996 (1995).

Zabadal, T.J. and T.W. Dittmer. Vine management systems affect yield, fruit quality, cluster compactness, and fruit rot of 'Chardonnay' grape. HortScience 33:806-809 (1998).

CHAPTER THREE. THE EFFECT OF PROHEXADIONE-CA ON GRAPE REPRODUCTIVE DEVELOPMENT AND FRUIT AND WINE CHEMISTRY

ABSTRACT

In 2000 and 2001, prohexadione-calcium (P-ca) was evaluated for impact to grape reproductive development. In 2000, P-ca (250 mg/L) was applied to Seyval, Cabernet Sauvignon, and Cabernet franc (125, 250, and 375 mg/L). P-ca reduced components of yield for all cultivars. In 2001, P-ca (250 mg/L) was applied singularly at weekly intervals to Cabernet Sauvignon clusters and pre and post-bloom to Cabernet franc and Chardonnay canopies. Cabernet Sauvignon applications at E-L stages 21 and 23, decreased fruit set whereas applications at E-L stages 26, 27, and 29 reduced berry weight with no impact on fruit set. Berry weight reduction correlated to increased color intensity (420+520), anthocyanins, total phenols and phenol-free glycosyl-glucose (PFGG). Cabernet franc vegetative and reproductive development was generally not affected yet treatment increased absorbance at 280, 420, and 520 nm, color intensity, anthocyanins and total phenols. Pre-bloom applications inhibited Chardonnay vegetative development, and reduced components of yield, and fruit chemistry values: hydroxycinnamates, total phenols, flavonoids, PPFG and absorbance at 280 and 320 nm. Post-bloom applications did not affect Chardonnay vegetative or reproductive development, yet increased PFGG. Treatment did not affect Chardonnay wine chemistry but two post-bloom applications increased Cabernet franc wine anthocyanins and total phenols. Wine aroma and flavor triangle difference tests did not indicate significant treatment differences.

Keywords: Prohexadione-calcium, Apogee, Growth Regulator, Gibberellin, PGR

INTRODUCTION

Research on the ability of gibberellins to modify grape berry (*Vitis vinifera*) development had its origins in experiments at the University of California, Davis beginning in the late 1950s (Coombe, 1959; Weaver and McCune, 1959). The results of those and subsequent experiments (Coombe, B.G. 1965; Sachs and Weaver, 1968) showed that exogenous gibberellins can significantly alter fruit set and modify other canopy characteristics depending on timing and rate of applications. One of the uses of gibberellin resulting from these experiments is its application on seedless table grape cultivars of *V. vinifera* to increase fruit set and berry enlargement (Coombe, 1965; Khurshid et al., 1992). Exogenous gibberellin-induced berry enlargement in seedless cultivars increases parenchyma cell growth in the pericarp. The greatest response is observed in the tissue surrounding the locule and extending out to, but not including, the outermost tannin-filled cells of the berry (Sachs and Weaver, 1968).

In the period between that early work and present, a great deal has been learned about the physiology of berry development and the organization of flavor and aroma compounds within the berry (Coombe and McCarthy, 2000). Glucose and fructose are located in highest concentrations in the flesh of the berry whereas inorganic ions, phenolics, potassium, and tartrate are more concentrated in the skin (Coombe, 1988).

Phenolic and non-phenolic compounds play different roles in wine chemistry. Phenols impart wine with color and structure while non-phenolic compounds, such as monoterpenes and norisoprenoids, are largely responsible for aroma and flavor (Williams et al., 1992). Total phenols comprise flavonoid and nonflavonoid phenols; the former group dominated by hydroxycinnamates, and the latter by anthocyanins. Hydroxycinnamates are the major non-flavonoid phenols in both white and red cultivars (Zoecklein et al., 1999). Flavonoid phenols, particularly anthocyanins, are found in greater amounts in red cultivars, particularly in the skins (Fernandez de Simon, 1993). Anthocyanins, which are largely responsible for imparting the

color to red wine (Jackson and Lombard, 1993), are found in the vacuoles of cells located a few cell layers below the epidermis (Roubelakis-Angelakis, 1986). Ample evidence indicates that nonflavonoid phenolic compounds such as hydroxycinnamates, (Fernandez de Simon et al., 1992) and non-phenolic monoterpenes such as geraniol and nerol (Park and Noble, 1993) are more concentrated in the skin than in the flesh of the berry. A decrease in berry size would lead to an increase in the surface to volume ratio, theoretically increasing the proportion of these compounds in the must and wine. As an increase in aroma and flavor precursors ostensibly would increase wine quality, this adds credence to a widely held belief that crop yield and wine quality are inversely related (Jackson and Lombard, 1993; Miller et al., 1993).

Many aroma and flavor compounds are glycosidically-bound. Williams et al. (1992) found that the sensory characteristics of hydrolyzed glycosidically-bound compounds found in fruit were the same as those in the resulting wine. These results indicated that glycosides are aroma and flavor precursors. Researchers in both Australia and the U.S.A. have evaluated glycosyl-glucose, red-free glycosyl-glucose, and phenol-free glycosyl-glucose as a method of estimating the concentration of glycosides (Williams et al., 1995; Iland et al., 1996). To better determine the concentration of aroma and flavor precursors, methods to determine non-phenolic glycosides have been developed (Zoecklein et al., 2000). These methods are currently being refined (Whiton and Zoecklein, unpublished data).

There is also interest in means to modify grape cluster architecture in order to loosen the cluster and reduce the incidence of Botrytis bunch rot disease. Recent work on the impact of grape cluster architecture and susceptibility of berries to Botrytis bunch rot has quantified bunch "tightness" and found significant correlations between tightness and susceptibility of clusters to *B. cinerea* infections (Vail and Marois, 1991). Many grape-producing regions endure high humidity, which further favor *B. cinerea* infection (Smart and Robinson, 1992). The cumulative effect of these factors results in higher *B. cinerea* incidence and severity among compact-cluster cultivars. Small berries may decrease cluster compactness.

For these and other reasons, there has been a shift from high yield, large berry wine grape production to low-yield quality production. Growers may desire reduced fruit set, berry weight,

and crop load for reasons varying from a reduction in labor (cluster thinning) to reduced fungal pathogens to improvements in fruit and wine quality. As the majority of flavor and aroma compounds are located in the skin of grapes, a decrease in berry weight could increase the proportion of these compounds in must and wine. Some growers achieve smaller berries and lower yields by deficit irrigation (Coombe, B.G. and McCarthy, 2000; Esteban et al., 1999; and Reynolds and Naylor, 1994). While this is a relatively common practice, its success hinges on the grape growing region having minimal annual rainfall. A characteristic not shared but all American viticultural areas.

Prohexadione-calcium (P-ca) is a gibberellin biosynthesis inhibitor, which makes up 27.5% of Apogee™, a product recently introduced by Badische Anilin & Soda-Fabrik (BASF) of Ludwigshafen, Germany (Evans et al., 1999). P-ca is currently registered on apples for the reduction of vegetative growth (Byers and Yoder, 1999). During the 2000 and 2001 growing seasons we evaluated the vegetative impact of P-ca on various cultivars of *V. vinifera* and one interspecific hybrid. While the original intent of the experimentation was vegetative growth suppression, we noted a significant effect on berry size and fruitset. Procedures were modified in 2001 to evaluate the impact of P-ca on reproductive development with the goal of decreasing fruit set and berry size.

MATERIALS AND METHODS

Research on the effects of P-ca on the fruit chemistry began in 2000 with experimentation on Seyval, Cabernet Sauvignon, and Cabernet franc. Treatments consisted of foliar applications of P-ca. All treatments, except where indicated, included 18.9 mL/L of Regulaid^{TM}, a spreader-activator, and 900 mg/L of ammonium sulfate. Ammonium sulfate has been used to increase the efficacy of P-ca where the spray water contains high concentrations of calcium (Byers, unpublished data). For each application Apogee was applied when there was no rain predicted for a minimum of 24 hours. If rain occurred within 12 hours after application, reapplications were made. Treatments were applied to both sides of the canopy. Solutions applied to the control panels included the two spray adjuvants in concentrations equal to those in the treatment applications. In 2001 experimentation was conducted with Cabernet Sauvignon, Cabernet franc, and Chardonnay. Except where indicated, treatment applications were made in a manner similar to those of the 2000 season.

Cabernet Sauvignon 2000. Nine-year-old Cabernet Sauvignon (FPMS clone #7) vines, located at the Alson H. Smith Jr. AREC in Winchester, VA, were used. Vines were cordon-trained and spur-pruned on a lyre trellis system. Row x vine spacing was 3.7 x 2.1 m. The experimental design was completely randomized with five replicates of three-vine plots.

Cabernet Sauvignon treatments consisted of an untreated control, a twice-applied treatment (2xP-ca) and a thrice-applied treatment (3xP-ca). The P-ca rate for all applications was 250 mg/L. The 2xP-ca treatment was sprayed twice pre-bloom (15 and 31 May) while 3xP-ca included the two pre-bloom applications plus an additional post-bloom application (30 June). Full bloom occurred 5 June. The first application required 18.93 liters of spray water per five treatment plots while the second and third applications each used 30.28 liters of spray water per treatment. Each vine received amount 315 mg a.i. on the first date and 505 mg on the second and

third dates. Applications were made with a hand-held pressure sprayer.

Cabernet Sauvignon was harvested on 20 October. At harvest, clusters per vine, total fruit weight, and average berry weight were recorded. On this same date, prior to harvest, berry samples were collected from each of the five treatment replicates. Berry samples consisted of fifty berries from each treatment replicate, randomly selected.

2000 Cabernet franc. Two-year-old Cabernet franc, grown at Indian Springs Vineyard in Woodstock, VA, was used. Vines were cordon-trained and spur-pruned with the canopy trained to a VSP system. Row x vine spacing was 2.7 x 2.1 m. The experimental design was completely randomized with each treatment consisting of 10 single-vine replicates. Treatments were 125, 250 and 375 mg a.i./L. To determine the effect of ammonium sulfate in the spray mixture, two controls were included. One control was treated with a solution of ammonium sulfate and Regulaid and the other control was only treated with Regulaid.

All treatments and controls were sprayed once pre-bloom (18 May) and twice post bloom (1 June, and 30 June). Full bloom occurred 26 May. As applications were made to a continuously increasing canopy, the volume of spray needed to provide complete coverage likewise increased. However, for each application date spray water volume was uniform between treatments. The amount of spray solution used was 5.70 L 7.57 L and 11.37 L respectively on 18 May, 1 June and 30 June. The amount of a.i. applied per vine for the 125 mg/L treatment, from the first to last application date, was 71 mg, 95 mg, and 142 mg. The amount of a.i. applied per vine for the 250 mg/L treatment, from the first to last application date, was 142 mg, 189 mg, and 284 mg. The amount of a.i. applied per vine for the 375 mg/L treatment, from the first to last application date, was 235 mg, 284 mg, and 426 mg.

Cabernet franc was harvested on 11 October. At harvest, clusters per vine, and total fruit weight, were recorded. Before harvest, 15-berry samples were randomly collected from each vine. Each of the 10-vine treatments was divided into three groups of vines: the first two groups consisted of three vines and the remaining consisted of four vines. The 15-berry samples for the vines in each of the three groups were pooled to provide each treatment with two 40-berry

samples and one 60-berry sample. Average berry weight was calculated from these replicates. Berry samples were taken in this manner to avoid removing a great deal of fruit per vine at harvest.

2000 Seyval. Own-rooted, 10-year-old Seyval vines, located at the Alson H. Smith Jr. AREC in Winchester, VA, were used. Vines were cordon-trained and spur-pruned with a vertical shoot positioned (VSP) canopy. Row x vine spacing was 3.7 x 2.1 m. The experimental design was completely randomized with five replicates of three-vine treatment plots.

Treatments consisted of three foliar applications of a 250 mg/L P-ca solution compared to a control. The initial application was made pre-bloom on 15 May. The second application was 31 May during bloom with the third application post-bloom on 30 June. The amount of active ingredient (a.i.) applied per vine varied depending on the volume of spray water used. The 15 May application was made with 7.57 liters of spray water. The two subsequent applications were each made with 13.25 liters of spray water. The approximate amount of a.i. applied per vine on 15 May, 31 May, and 30 June was 126, 221, and 221 mg respectively. Applications were made with a hand-held pressure sprayer.

Seyval was harvested on 6 September. On this same date, before harvest, 50-berry samples were randomly collected from each of the five treatment replicates. At harvest, clusters per vine, total fruit weight and average berry weight were recorded. After clusters were harvested, they were visually inspected. Clusters with rot were recorded and rot severity was calculated in the following manner:

Rot Severity = # rotten clusters/[# clusters total x % of rotten berries in each affected cluster]x100

Seyval fruit chemistry data was also recorded from the harvest berry sample. Soluble solids were measured using a temperature compensated refractometer (model 10430, Reichert Scientific Instruments) pH was measured using a Fisher Accumet pH meter (model 815MP) titratable acidity was measured with procedures by Zoecklein et al. (1999).

Evaluation of second year treatment effects from 2000 season treatments. In the 2001 season, clusters per shoot were counted for the vines treated the prior year to determine if

there were any second-year reproductive effects from applications made during 2000. Although the assessment was restricted to cluster counts, observable differences in canopy structure between treated and control panels were also noted. Average clusters/shoot values were compared between treatments and controls using a t-test for Seyval, and single degree of freedom contrasts for Cabernet Sauvignon and Cabernet franc. All data analysis was performed using the SAS system (v8e. Cary, NC).

Cabernet Sauvignon single cluster experiment 2001. This experiment was conducted using cordon-trained, spur-pruned nine-year-old Cabernet Sauvignon vines grown at the Alson H. Smith Jr. AREC in Winchester, VA. The vines were trained on a lyre-trellis system with a row x vine spacing of 3.7 x 2.1 m with three vines per plot.

The purpose of this experiment was to determine the effect of single applications of P-ca on clusters of Cabernet Sauvignon at different phenological stages. This thirteen-week experiment consisted of thirteen treatments and an untreated control each comprised of single-cluster experimental units. Treatments were represented by the following notation: T_0, $T_1 \ldots T_{13}$. The subscripts indicate the week during which a single application of P-ca was applied to each treatment, with T_0 representing the control. Clusters used in the experiment were the basal clusters of two-cluster shoots. Each treatment included a minimum of ten single-cluster replicates. Clusters were located on ten vines, chosen for their uniformity. Each of these vines constituted a block on which all treatments, including T_0 the untreated control, were represented at least once in order to minimize the potential influence of an aberrant vine on experimental results. Treatments were randomly located within the vine so that no cluster had the same placement on each vine. Treatments T_0, T_2, T_5, and T_8 were chosen for mid-season destructive sampling in order to chart the development of treated clusters. Consequently, these treatments consisted of an additional twenty clusters to provide an adequate sample size for these mid-season sampling dates. The treatment solution consisted of a 250 mg/L P-ca, including concentrations of Regulaid and ammonium sulfate identical to those in previous experiments. Applications were made with a hand-held spray bottle. Each cluster was sprayed approximately

four times from different angles to completely coat the cluster with solution. The amount of solution applied in four sprays with a hand held sprayer was 7.6 mL. With this application volume, the amount of prohexadione-calcium applied per cluster was 1.91 µg.

As the intention was to observe the effects of a direct cluster application of P-ca, care was taken to avoid any accidental foliar application. Plastic bags were used to isolate the desired cluster during treatment applications to avoid spraying other clusters or leaves. The E-L growth chart, as modified by Coombe (1995), was used to classify the phenological stage at each date of application. The first treatment T_1 was sprayed one month after bud break at E-L stage 18 (Table 3-1).

Table 3-1. Description of Cabernet Sauvignon single cluster treatments 2001: application date, and E-L growth stage at each application date.

Treatment	Application date	E-L growth stage
T_0*	Control	N/A
T_1	5/24/01	18
T_2*	5/31/01	21
T_3	6/6/01	23 (Full Bloom)
T_4	6/14/01	26
T_5*	6/21/01	27
T_6	6/28/01	29
T_7	7/6/01	32
T_8*	7/13/01	33
T_9	7/20/02	33
T_{10}	7/27/01	33
T_{11}	8/2/01	33
T_{12}	8/9/01	34
T_{13}	8/16/01	37

Asterisk indicates those treatments sampled at mid-season sampling dates.

The four mid-season sampling dates were 21 June 13 July, 2 August and 14 September. On each of these dates five clusters were collected from T_0, T_2, T_5, and T_8. Berries were removed from the cluster, counted, weighed and average berry weight was calculated for each cluster. Final harvest was conducted for all treatments on 12 October 2001. On this date, each treatment, consisting of ten clusters, was harvested. Each of the ten clusters for each treatment was assigned a replicate number. Total berries were counted and average berry weight calculated for each cluster replicate. After the calculation of berry weight, the berries for each cluster were frozen at -37 ºC. On 24 January 2002 sample processing began. Samples were thawed to room temperature and processed based on replicate number rather than treatment. This was done to avoid any bias that sampling procedures or timing of processing might have on fruit chemistry results. For each cluster replicate, 80 berries were randomly selected for fruit chemistry samples. When clusters had fewer than 80 berries, samples consisted of the total number of berries in the cluster. After thawing to room temperature, samples were machine blended, separated from skins with a filter bag (Stomacher Labsystem model 400) and centrifuged at 17,000 rpms at 10°C for 30 minutes. Immediately following processing, soluble solids, titratable acidity, and pH were calculated. Soluble solids was measured using a temperature compensated refractometer (model 10430, Reichert Scientific Instruments, Buffalo, NY) pH was measured using a Fisher Accumet pH meter model 815MP titratable acidity was calculated according to Zoecklein et al. (1999)**.** Samples were then refrozen at –37 ºC. After all samples were processed, frozen must was thawed on a per replicate basis for further analyses.

After samples were thawed to room temperature and inverted, a 10 mL sample was pipetted into a test tube. This sample was subsequently used in both spectral color estimation and a phenol-free glycosyl-glucose assay. Spectral Color Estimation was done with a Spectronic Genesys model-5 spectrophotometer according to procedures outlined by Zoecklein et al. (1999). In this procedure, juice absorbance values were measured at 280, 420, and 520 nm wavelengths. Color intensity, anthocyanins, ionized anthocyanins, and total phenols were estimated from absorbance values.

Procedures for the phenol-free glycosyl-glucose assay came from Zoecklein et al (2000)

modified by Whiton and Zoecklein (unpublished data). This assay is a modification of previous methods to determine glycosides in juice and wine samples by measuring glucose (Williams et al., 1995; Iland et al., 1996). Must samples were raised to pH 13 to exclude phenolic glycosides. Samples were then run through a solid-phase extraction and eluted with ETOH. Elutions were hydrolyzed for one hour, neutralized and glucose determined by enzymatic assay. The spectrophotometer used in the enzymatic assay was Labsystems Multiskan MCC/340 from Fisher Scientific.

The experimental design was completely randomized. The Cabernet Sauvignon fruit chemistry and harvest data were analyzed using proc GLM and means separated by Duncans Multiple Range Test. Correlations were made using Pearson's correlation coefficient. All data analysis was performed using the SAS System (v.8e, Cary, NC).

Cabernet franc 2001. Three year old Cabernet franc, located at Indian Springs Vineyard in Woodstock, VA, was used. Vines were cordon-trained and spur-pruned with the canopy trained to a VSP system. Row x vine spacing was 2.7 x 2.1 m. The experimental design was completely randomized with five replicates of four-vine plots. P-ca was applied to the canopy fruit zone at a rate of 250 mg/L. Applications were made to both sides of the canopy in a zone defined, at its lowest point, by the fruiting wire and extending vertically 0.5 meters. Applications were made to the point of runoff so that foliage was consistently coated. All treatment solutions contained Regulaid and ammonium sulfate. The experiment comprised two controls and three treatments. Treatments consisted of a two pre-bloom application treatment (2prb), (7 May and 23 May), a one post-bloom application treatment (1pstb) (19 June), and a two post-bloom application treatment (2pstb) (19 June and 3 July). Bloom occurred 6 June. The E-L growth stage, modified by Coombe, of the vines was recorded at each application date. The E-L stages for Cabernet franc, in order of first to last, were 15, 17, 29, and 31; with 15 representing the developmental stage during the first pre-bloom application. As treatments were concentrated on the fruit-zone, a considerably lower volume of spray water was used for these experiments than the 2000 season evaluations. Both pre-bloom applications used 3.79 liters of spray water per

treatment and both post-bloom applications used 5.69 liters of spray water per treatment. The amount of a.i. per vine per application date was 47.4 mg for each of the two pre-bloom applications and 71.2 mg for each of the two post-bloom applications.

One issue in 2000 was the lack of crop standardization between the control and treated vines. To avoid that situation in 2001 we had two sets of control vines. On 18 May, shoots and clusters on all vines were counted and thinned to 16 shoots/meter of vine. In late July, one of the controls was cluster thinned to an anticipated P-ca induced crop level. The expected crop reduction was based on mid-season cluster weights and counts for all treatment plots. Due to the slight difference in average cluster weight between 1prb, 1pstb, and 2pstb, the crop per vine of the thinned control was reduced to a level intermediate to that of the three treatments. The other control was left with its original cluster count.

Cabernet franc was harvested on 10 October. At harvest, clusters per vine, and total fruit weight were recorded. On this same date, prior to harvest, berry samples were collected from each of the five treatment replicates. Fifty-berry samples were randomly collected from each treatment plot. These berry samples were processed and analyzed in a manner identical to that of the Cabernet Sauvignon experiment.

Wine was made from all of the Cabernet franc treatments. Pre-fermentation chemical analysis including titratable acidity, pH, soluble solids, and formol (data not shown) was performed on each of the two replicates. Clusters from each treatment were de-stemmed and berries divided into two 11.4-liter fermentation lots in Nalgene^{TM} fermentors to avoid loss of data from possible contamination during fermentation. The containers were placed in a 7 ºC cooler for a cold soak. After the wine cold-soaked for 48 hours it was warmed to room temperature for yeast addition. The following compounds were added to each fermentation replicate: 3.79 g/L of yeast, 158 µl/L Scott ColorX Enzyme (Scott Laboratories), 1 g/L tartaric acid, 30 mg/L of SO_2, and 5.69 g/L of FermaidK yeast nutrient. Twenty-four hours after the addition of yeast, the caps were punched down on all replicates. The caps were punched three times per day until the wines fermented to dryness (2.0 g/L residual sugar) at a fermentation temperature of 65 ºC. The wines were then pressed and racked into full glass containers. After

neither replicate was found subject to contamination, replicates were pooled for subsequent analyses. Titratable acidity, pH, ethanol, absorbance at 280, 420, and 520 nm and phenol-free glycosides were measured post fermentation. Ethanol was measured using an Ebuliometer and pH was measured with a Fisher Accumet pH meter (model 815MP). Color intensity, anthocyanins, ionized anthocyanins, and total phenols were estimated from absorbance values. The wine phenol-free glycosyl-glucose assay was performed in a similar manner to that of the must, with an additional dilution to compensate for the opacity of wine samples.

Chardonnay 2001. Three-year-old Chardonnay vines, grown at Indian Springs vineyard in Woodstock, VA, were used. Vines were cordon-trained and spur-pruned with the canopy trained to a VSP system. Row x vine spacing was 2.7 x 2.1. The experimental design was completely randomized with five replicates of four-vine plots. At the time of the experiment vines did not yet completely fill up the canopy. Experimentation with Chardonnay was an exact replicate of the Cabernet franc experiment. Treatments, applications rates and dates, and measurements were equal with the exception that the first application date was 8 May rather than 7 May for Cabernet franc. Bloom occurred on 2 June. The E-L growth stage, modified by Coombe, of the vines at each application date was recorded. Chardonnay was slightly more developmentally advanced than was Cabernet franc, which is indicated by the E-L stages for each application date. The E-L stages for Chardonnay, in order of first to last, were 15, 19, 30, and 32; with 15 representing the developmental stage during the first pre-bloom application. Due to the susceptibility of this cultivar to Botrytis bunch rots we additionally evaluated rot severity at harvest.

Chardonnay was harvested on 30 September. After clusters were harvested, they were visually inspected. Clusters with rot were recorded and rot severity was calculated in the following manner:

Rot Severity = # rotten clusters/[# clusters total x % of rotten berries in each affected cluster] x 100

Berry samples were collected in a manner consistent with that of the Cabernet franc experiment. Components of yield and fruit chemistry calculations were made in a similar manner to that of Cabernet franc with one notable exception. Spectrophotometric measurements were made at 280

and 320 nm wavelengths for spectral colors analysis.

The wine treatments for Chardonnay were similar to those of the Cabernet franc with the exception that the Chardonnay 2prb treatment was excluded from wine making due to poor fruit set, which severely reduced crop yield. Chardonnay grapes were pressed the day after harvest. Pre-fermentation chemical analysis including titratable acidity, pH, Brix, and formol was performed on each of the two replicates data not shown). Must was allowed a to settle for 24 hours at 7 ˚Celsius and then racked into two 11.4-liter fermentation vessels. The following compounds were added to each fermentation replicate: 3.79 g/L of yeast VL-1 yeast from Scott Laboratories), 20 µl/L Pec5L enzyme Scott Laboratories), 3.79 g/L of FermaidK yeast nutrient, and 20 mg/L SO_2. After it was determined that neither replicate was subject to contamination, replicates were pooled for subsequent analyses. Wines fermented to dryness (2.0 g/L residual sugar) and were racked into full glass containers. Titratable acidity, pH, and ethanol, absorbance at 280 and 320 nm, and phenol-free glycosides were measured post-fermentation. Ethanol was measured using an Ebuliometer and pH was measured using a Fisher Accumet pH meter model 815MP. Total phenols, hydroxycinnamates, and total flavonoids, were estimated from absorbance values. The wine phenol-free glycosyl-glucose assay was performed in a similar manner to that of the must, with an additional dilution to compensate for the opacity of wine samples.

Triangle difference test. A triangle difference test was performed on Cabernet franc and Chardonnay treatments. Triangle test procedure was based on that of Larmond (1982). The panel used was a consumer panel with some limited sensory training. At each flight, panelists were presented with three coded glasses of wine. Panelists were told that two of these wines were the same, and one was different. They were instructed to smell the wines and indicate the different wine. After this was completed, they were presented with three different wines upon which they conducted a sensory analysis based on taste and again instructed to indicate the different wine. Treatment wines were always compared to untreated controls. As the unthinned control had berry weights closest to those of the treatments, this control was chosen as the

comparison wine. Each session was designed for four flights of eight panelists per flight. However, in actuality, the number of observations varied slightly depending on the number of panelists who participated in each flight.

Session one compared Chardonnay UT to 1pstb with 28 observations. Session two compared Chardonnay UT to the 2pstb with 32 observations. Session three compared Cabernet franc UT to the 2prb with 30 observations. Session four compared Cabernet franc UT to 1pstb with 29 observations. Session five compared Cabernet franc UT to 2pstb with 29 observations.

Data analysis. Cabernet franc and Chardonnay fruit chemistry results were analyzed with single degree of freedom contrasts using the SAS System (v.8e, Cary, NC). Because there were not wine treatment replicates, statistical analysis was not performed on wine chemistry data. Mean values for pre-fermentation fruit chemistry analysis were calculated from the five treatment replicates, analyzed with ANOVA procedures and means separated with Duncan's Multiple Range Test. Triangle test results were analyzed with a rapid analysis table prepared by Roessler et al.(1948).

RESULTS

Cabernet Sauvignon 2000. Average cluster weight, berries per cluster, berry weight and crop per vine were all decreased by applications of 250 mg/L P-ca (Table 3-2). The 3xP-ca treatment had higher berries per cluster and crop per vine than 2xP-ca.

In the 2001 evaluation of second-year treatment effects we observed a significant reduction in clusters per shoot due to the twice-applied P-ca treatment. No significant differences were noted between the twice and thrice applied treatments.

Table 3-2. Cabernet Sauvignon components of yield due to two (2xP-ca) and three (2xP-ca) 250 mg/L prohexadione-calcium applications during the 2000 season.

Treatment	Clusters per vine	Cluster wt. (g)	Berries per cluster	Berry wt. (g)	Crop per vine (kg)
Control	72.7	127.03	92	1.38	9.24
2xP-ca	75.3	36.11	46	0.83	2.75
3xP-ca	76.1	48.41	58	0.81	3.71
Significance of Contrast[z] (*P*-value)					
Control vs. mean of treatments	0.243	0.000	0.000	0.000	0.000
2xP-ca vs. 3xP-ca	0.781	0.063	0.046	0.523	0.024

[z]Single-degree of freedom contrasts between specified treatments.

Table 3-3. Cabernet Sauvignon clusters per shoot in 2001 from vines treated twice (2xP-ca) and thrice (3xP-ca) with 250 mg/L prohexadione-calcium during the 2000 season.

Treatment	Clusters/shoot
Control	1.5
2xP-ca	1.4
3xP-ca	1.4
Significance of Contrast[z] (*P*-value)	
Control vs. mean of treatments	0.003
2xP-ca vs. 3xP-ca	0.389

[z]Single-degree of freedom contrasts between specified treatments.

Cabernet franc 2000. There were few significant differences noted between the controls that excluded or included ammonium sulfate. The inclusion of ammonium sulfate did appear to slightly improve the efficacy of P-ca as seen in a decrease in average berry weight (Table 3-4). However, as the differences between the two controls were slight, treatments were compared to the control including ammonium sulfate. A significant reduction in clusters per vine, cluster weight, berries per cluster, berry weight and crop per vine was determined using single degree of freedom contrasts between the three treatments and the control (Table 3-4). A negative linear relationship was observed between concentration of application and clusters per vine, berries per cluster and berry weight. A quadratic relationship was observed for cluster weight and crop per vine (Table 3-4).

For the 2001 evaluation of second-year treatment effects, we found a negative linear relationship between clusters per shoot and increasing concentration of the 2000 application (Table 3-5).

Table 3-4. Cabernet franc components of yield, as affected by three rates of prohexadione-calcium (125, 250, and 375 mg/L) and two controls, with (N) and without (noN) ammonium sulfate during the 2000 season.

Treatment	Clusters per vine	Cluster wt. (g)	Berries per cluster	Berry wt. (g)	Crop per vine (kg)
noN	53.5	100.84	58	1.74	5.4
N	46.8	105.41	69	1.55	4.9
125 mg/L	34.6	68.02	48	1.44	2.4
250 mg/L	31.0	42.47	38	1.11	1.4
375 mg/L	19.1	25.43	26	0.95	0.5
Significance of Contrast[z] (*P*-value)					
N vs. no N	0.054	0.530	0.083	0.001	0.173
N vs. mean of treatments	0.000	0.000	0.000	0.000	0.000
Linear	0.000	0.000	0.000	0.000	0.000
Quadratic	0.950	0.052	0.295	0.544	0.001

[z] Single-degree of freedom contrasts and trend analysis of specified treatments.

Table 3-5. Cabernet franc clusters per shoot in 2001from vines affected by three rates of prohexadione-calcium (125, 250, and 375 mg/L) and two controls, with (N) and without (noN) ammonium sulfate during the 2000 season

Treatment	Clusters/shoot
noN	1.6
N	1.6
125 mg/L	1.3
250 mg/L	1.3
375 mg/L	1.1
Significance of Contrast[z] (*P*-value)	
N vs. no N	0.711
N vs. mean of treatments	0.000
Linear	0.000
Quadratic	0.103

[z] Single-degree freedom contrasts and trend analysis

Seyval 2000. Cluster weight, berries per cluster, berry weight and crop per vine were all decreased by P-ca applications (Table 3-6). The rot severity of P-ca treatments (3.5%) was similar to that of controls (5.6%). Seyval was the only cultivar for which fruit chemistry measurements were made in 2000. We did not note any significant changes in soluble solids or pH due to treatment (Table 3-7). Visual inspection of the berries after verasion revealed slightly looser clusters, perhaps a result of decreased fruit set, and reddening of berries compared to controls. These observations were not quantified.

Unlike Cabernet Sauvignon and Cabernet franc, the 2001 analysis of Seyval treated in 2000 did not show a treatment effect. Seyval clusters per shoot of P-ca treated vines (1.4) did not differ significantly from that of the control (1.5) (data not shown in table).

Table 3-6. Seyval components of yield affected by three applications of prohexadione-calcium (P-ca) during the 2000 season.

Treatment	Clusters per vine	Cluster wt. (g)	Berries per cluster	Berry wt. (g)	Crop per vine (kg)
Control	48.8	198.02	79	2.53	9.8
P-ca	45.5	131.84	63	2.10	6.0
Significance (*P*-value)					
t-test[a]	0.455	0.004	0.047	0.000	0.020

[a]Pooled data

Table 3-7. Fruit chemistry of Seyval: soluble solids (ºBrix) and pH affected by three applications of prohexadione-calcium (P-ca) during the 2000 season.

Treatment	ºBrix	pH
Control	19.5	3.28
P-ca	18.6	3.26
Significance (*P*-value)		
t-test[a]	0.1771	0.5511

[a]Pooled data

Cabernet Sauvignon Single-Cluster Experiment 2001. At the first of four midseason sampling dates, we noted a decrease in berries per cluster and berry weight for T_2 compared to the control (T_0) (Table 3-8). By the second measurement date we noted a significant decrease in berry weight for T_5. No observable differences were noted between the control and T_8 throughout the four measurement dates (Table 3-8). This data concurred with berry weight data collected at harvest.

P-ca applications before and during bloom (T_1, T_2, T_3) severely and significantly reduced fruit set (Figure 3-1). Clusters from T_2 and T_3 applications were so severely affected it was impossible to gather any fruit chemistry results from them. However, once all caps were off and berries began to set, P-ca applications had no more impact on fruit set. This is best exemplified by T_4, sprayed at E-L growth stage 26, at the start of berry set. T_4 exhibited no reduction in fruit set and actually had a higher number of berries per cluster at harvest than any other treatment including the control (Table 3-9). T_4 responded to treatment with a 51% reduction in berry weight. A reduction of berry weight was evident for applications through T_6 although less intense and statistically insignificant after T_4. These immediate post-bloom applications had no impact on soluble solids (ºBrix), pH, or titratable acidity (Table 3-10). However, these post-bloom treatments had elevated absorbances of 280, 420, and 520nm wavelengths. Additionally, low berry weight treatments had elevated concentrations of phenols, anthocyanins, and ionized anthocyanins as well as color intensity (420 + 520) (Table 3-11). Phenol-free glycoside concentration was additionally increased for these low berry weight treatments with T_4 having the highest value (Table 3-12). Correlations were done to further explain the relationship between berry weight and fruit chemistry. Negative correlations between berry weight and fruit chemistry measurements were significant across all treatments, but were most pronounced for these low berry weight treatments as is evident in comparing the Pearson's correlation coefficients in tables 3-13 and 3-14.

Treatments were ineffective in reducing berry weight after week six. Accompanying this return to "normal" berry weight was a return to absorbance values and phenol-free glycosyl-glucose measurements statistically equal to the untreated control.

Table 3-8. Average berry weight four mid-season sampling dates for Cabernet Sauvignon treated with prohexadione-calcium at three distinct dates compared to an untreated control (T_0) in 2001.

Treatment	21 June	13 July	2 August	14 September
T_0	0.06 a	0.61 a	0.77 a	1.44 a
T_2	0.02 b	0.23 b	0.48 b	0.87 b
T_5	0.04 a	0.46 b	0.58 b	0.99 b
T_8	0.05 a	0.60 a	0.77 a	1.41 a

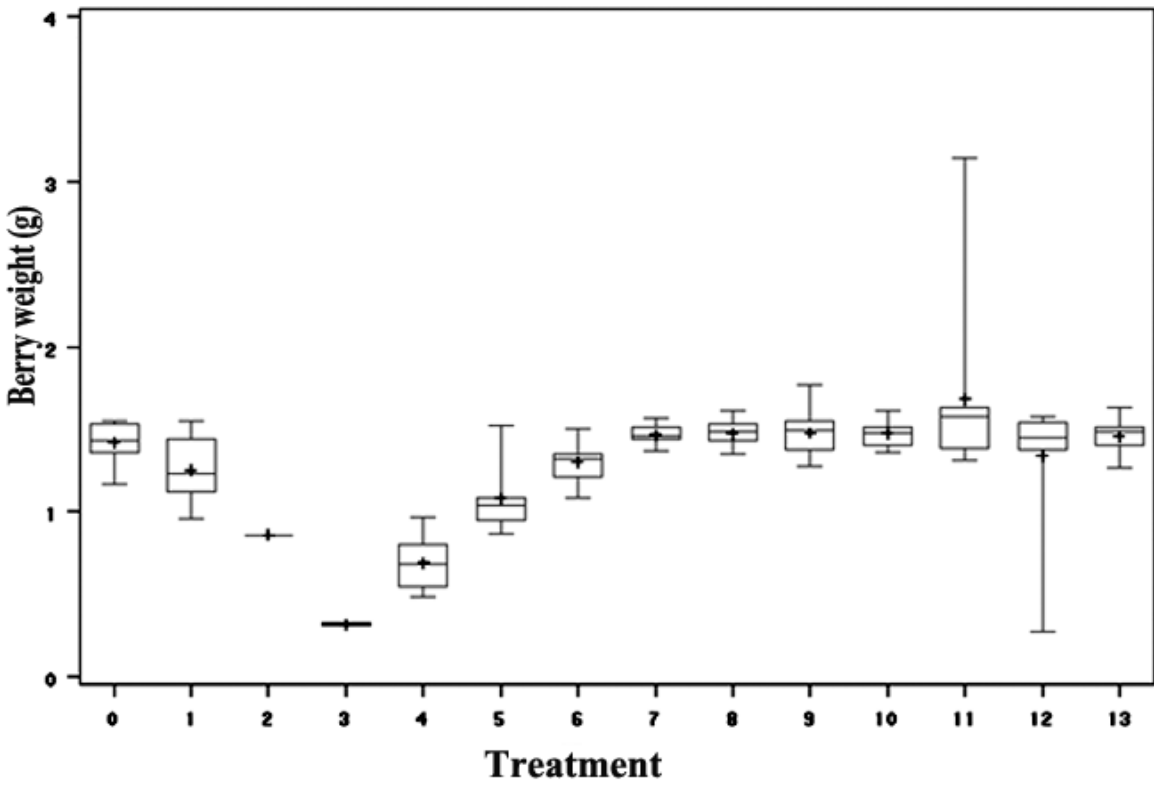
Significance determined by ANOVA procedures. Means separated by Duncans multiple range test

Table 3-9. Berries per cluster and berry weight of Cabernet Sauvignon treated with single cluster applications of 250 mg/L prohexadione-calcium in 2001.

Treatment	Berries/cluster		Berry weight	
T_0	127.5	bc	1.42	ab
T_1	44.1	d	1.25	bc
T_2	0.2	e	0.09	e
T_3	4.5	e	0.06	f
T_4	166.2	a	0.69	e
T_5	132.0	a-c	1.09	cd
T_6	133.4	a-c	1.30	bc
T_7	136.4	ab	1.47	ab
T_8	108.1	bc	1.48	ab
T_9	128.6	bc	1.48	ab
T_{10}	131.7	a-c	1.48	ab
T_{11}	98.30	c	1.69	a
T_{12}	108.8	bc	1.34	bc
T_{13}	98.0	c	1.46	ab

Significance determined by ANOVA procedures. Means separated by Duncans multiple range test

Figure 3-1. Cabernet Sauvignon harvest berry weight.



Berry weight affected by single applications of 250 mg/L prohexadione-calcium at weekly intervals in 2001. Week of treatment is on the x axis with berry weight in grams on the y axis. The tails of the box plot go out to the extreme values for each sample. The box is defined by the first and third data quartiles with the cross bar indicating the median. The + symbol indicates the mean.

Table 3-10. Must soluble solids (°Brix), pH, titratable acidity (TA), absorbance at 280, 420, and 520 nm, estimation of anthocyanins (Ant), ionized anthocyanins (Ion ant), total phenols, color intensity (INT) and phenol-free glycosyl-glucose (PFGG) for Cabernet Sauvignon single cluster applications of 250 mg/L prohexadione-calcium 2001.

Treatment	°Brix	pH	TA (g/L)	280 nm	420 nm	520 nm	Ant (mg/L)	Ion ant (mg/L)	Total phenols (au)	INT (420+520)	PFGG (μM)
T_0	21.1 a-d	3.87 a	5.4 a-d	8.8 bc	0.7 b-d	1.0 b-d	95 bc	16.5 c	4.8 bc	16.5 c	289.2 bc
T_1	21.5 ab	3.73 b	5.4 b-d	10.2 b	0.8 b	1.2 b	103 a-c	20.5 ab	6.2 b	20.5 ab	318.3 bc
T_2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
T_3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
T_4	21.0 a-d	3.72 b	6.1 a	14.3 a	1.3 a	1.9 a	139 a	22.8 a	10.3 a	22.8 a	542.6 a
T_5	21.6 a	3.82 ab	5.6 a-d	10.2 b	0.9 b	1.2 bc	109 ab	17.8 bc	6.2 b	17.8 bc	333.4 b
T_6	20.6 c-e	3.78 ab	5.8 ab	8.5 bc	0.7 b-d	0.1 b-d	82 bc	17.8 bc	4.5 bc	17.8 bc	347.1 b
T_7	20.5 c-e	3.79 ab	5.5 a-d	7.6 bc	0.6 b-d	0.9 b-d	83 bc	18.0 bc	3.6 bc	18.0 bc	219.3 bc
T_8	20.2 e-f	3.77 ab	5.6 a-c	7.5 bc	0.5 d	0.8 b-d	80 bc	16.4 c	3.5 bc	16.4 c	242.1 bc
T_9	20.0 ef	3.81 ab	5.5 a-d	7.6 bc	0.5 d	0.7 cd	83 bc	15.0 c	3.6 bc	15.0 c	334.2 b
T_{10}	20.9 a-d	3.79 ab	5.3 b-d	7.1 bc	0.5 d	0.7 cd	72 bc	14.4 c	3.1 bc	14.4 c	299.1 bc
T_{11}	19.6 f	3.80 ab	5.1 cd	7.2 bc	0.5 d	0.7 d	69 c	16.5 c	3.2 bc	16.5 c	284.9 bc
T_{12}	20.4 c-e	3.86 a	5.1 cd	6.7 c	0.5 d	0.6 d	64 c	15.8 c	2.7 c	15.8 c	179.4 c
T_{13}	20.7 b-e	3.85 a	4.9 d	7.3 bc	0.5 d	0.6 d	70 bc	16.2 c	3.3 bc	16.2 c	292.5 bc

Significance determined by ANOVA procedures. Means separated by Duncans multiple range test

Table 3-11. Correlations of berry weight and absorbance at 280, 420, and 520 nm, color intensity (420 + 520nm), anthocyanins (Ant), ionized anthocyanins (Ion ant), and total phenols across all Cabernet Sauvignon treatments 2001.

	Berry weight (g)	
Spectrophotometric Measurements	Pearson's correlation coefficient	*P*-value
280 nm	-0.55432	0.000
420 nm	-0.57586	0.000
520 nm	-0.54762	0.000
Intensity	-0.56045	0.000
Ant (mg/L)	-0.42195	0.000
Ion ant (mg/L)	-0.42499	0.000
Total phenols (au)	-0.55432	0.000

Table 3-12. Correlations of berry weight and absorbance at 280, 420, and 520 nm, color intensity (420 + 520nm), anthocyanins (Ant), ionized anthocyanins (Ion ant), and total phenols for Cabernet Sauvignon treatment four (T_4).

	Berry Weight (g)	
Spectrophotometric Measurements	Pearson's correlation coefficient	*P*-value
280 nm	-0.76821	0.010
420 nm	-0.68656	0.028
520 nm	-0.66542	0.036
Intensity	-0.67464	0.032
Ant (mg/L)	-0.58315	0.077
Ion ant (mg/L)	-0.72857	0.017
Total phenols (au)	-0.76821	0.009

Cabernet franc 2001. Although the thinned had significantly fewer clusters than the unthinned treatment (UT) this did not impact cluster weight or crop per vine. Due to the lack of significant differences between the controls, treatments were compared to UT. Cabernet franc treatments did not exhibit the reduction in crop observed in 2000 season experiments (Table 3-13). Only the 2pstb treatment differed significantly from either of the controls in terms of components of yield measurements. Two post-bloom applications reduced crop per vine for this treatment without any significant reduction in cluster weight or berries per cluster. Although not significant on their own, collectively the low berry weight and low berries per cluster could have significantly reduced crop per vine. Clusters per vine were also reduced, which could have cause a crop per vine reduction.

All treatments increased pH. Soluble solids and titratable acidity (TA) were generally not affected with the exception of a decrease in TA by two post-bloom applications (Table 3-14). Two pre-bloom applications increased absorbance at 280, 420 and 520 nm (P=0.065), anthocyanins (P=0.057), total phenols (P=0.059), and color intensity. The 1pstb treatment had significantly higher absorbance values for 280, 420, and 520 nm and higher values for phenols, anthocyanins, and color intensity. Fruit chemistry for 2pstb was not affected by treatment. There were no significant differences for phenol-free glycosides for any treatment (Table 3-14).

Wine chemistry results were similar among treatments and controls for ethanol content, titratable acidity, and pH. Most treatments had elevated absorbance at 280, 420, and 520 nm, anthocyanins, total phenols, and color intensity compared to UT. Of the treatments, 2pstb had the greatest increase in values, particularly with regards to anthocyanins (Table 3-15).

Despite fruit and wine chemistry differences between the treatments, results from the triangle difference test showed no significance at an alpha level of 0.05. The consumer panel could not discern the different wine in significant numbers for any of the comparisons (Table 3-16).

Table 3-13. Components of yield for Cabernet franc affected by two pre-bloom (2prb), one post-bloom (1pstb), and two post-bloom applications of 250 mg/L prohexadione-calcium (P-ca) during the 2001 season compared to an unthinned (UT) and a thinned (T) control.

Treatment	Clusters/ vine	Cluster wt (g)	Berries/ cluster	Berry wt (g)	Crop/vine (kg)
UT	54.5	77.35	52.1	1.49	4.24
T	39.4	93.27	62.2	1.50	3.72
2prb	52.8	69.44	49.5	1.40	3.67
1pstb	53.3	69.81	51.0	1.38	3.72
2pstb	47.1	61.68	44.6	1.39	2.94
Significance of Contrast[z] (*P*-value)					
UT vs. T	0.000	0.078	0.075	0.864	0.344
UT vs. 2prb	0.568	0.367	0.635	0.178	0.303
UT vs. 1pstb	0.695	0.390	0.843	0.103	0.342
UT vs. 2pstb	0.023	0.082	0.184	0.128	0.025

[z]Single degree of freedom contrasts of specified treatments.

Table 3-14. Cabernet franc must chemistry: soluble solids (ºBrix), pH, titratable acidity (TA), absorbance at 280, 420, and 520 nm, anthocyanins (Ant), ionized anthocyanins (Ion ant), total phenols, color intensity (INT) and phenol-free glycosyl-glucose (PFGG) affected by two pre-bloom (2prb), one post-bloom (1pstb), and two post-bloom (2pstb) applications of 250 mg/L prohexadione-calcium during the 2001 season compared to an unthinned (UT) and a thinned (T) control.

Treatment	ºBrix	pH	TA (g/L)	280 nm	420 nm	520 nm	Ant (mg/L)	Ion ant (mg/L)	Total phenols (au)	INT (420+520)	PFGG (µM)
UT	22.3	3.44	5.35	21.9	2.5	3.1	108	11.9	17.9	5.5	298
T	22.6	3.51	5.50	21.5	2.4	2.9	107	4.3	17.5	5.2	577
2prb	23.0	3.49	5.38	25.6	3.0	4.0	153	4.1	21.6	6.9	289
1pstb	22.5	3.50	5.10	26.4	3.1	4.6	179	4.1	22.4	7.6	340
2pstb	22.6	3.51	4.86	22.8	2.6	3.1	113	4.0	18.8	5.6	233
Significance of Contrast[z] (*P*-value)											
UT vs. T	0.212	0.035	0.425	0.836	0.559	0.668	0.976	0.134	0.836	0.634	0.190
UT vs. 2prb	0.368	0.046	0.867	0.059	0.009	0.065	0.057	0.123	0.059	0.038	0.967
UT vs. 1pstb	0.469	0.023	0.195	0.025	0.002	0.004	0.005	0.121	0.025	0.003	0.847
UT vs. 2pstb	0.337	0.007	0.018	0.636	0.589	0.993	0.830	0.118	0.636	0.884	0.764

[z]Single degree of freedom contrasts of specified treatments

Table 3-15. Cabernet franc wine chemistry: alcohol (ETOH), titratable acidity (TA), pH, absorbance at 280, 420, and 520 nm, anthocyanins (Ant), ionized anthocyanins (Ion ant), total phenols, color intensity (INT) and phenol-free glycosyl-glucose (PFGG) affected by two pre-bloom (2prb), one post-bloom (1pstb), and two post-bloom (2pstb) applications of 250 mg/L prohexadione-calcium during the 2001 season compared to an unthinned (UT) and a thinned (T) control.

Treatment	ETOH	TA (g/L)	pH	280 nm	420 nm	520 nm	Ant (mg/L)	Ion ant (mg/L)	Total phenols (au)	INT (420+520)	PFGG (µM)
UT	11.30	7.48	3.40	32.6	2.2	4.0	377	20.7	28.6	6.3	104
T	11.95	7.63	3.47	35.3	2.5	4.5	390	22.5	31.3	7.1	110
2 prb	11.70	7.50	3.43	34.3	2.4	4.5	384	22.8	30.3	6.9	101
1pstb	11.80	7.44	3.43	37.7	3.0	5.4	424	24.7	33.7	8.4	102
2pstb	12.00	7.68	3.44	44.8	4.5	8.9	473	37.6	40.8	13.4	113

Table 3-16 Cabernet franc triangle test of wine aroma and flavor comparing an unthinned control (UT) with either two prebloom applications (2prb), one post-bloom application (1pstb) or two post-bloom applications (2pstb) of 250 mg/L prohexadione-calcium in 2001.

Comparison	Observations	Correct Responses	*P*-value
UT vs. 2prb Aroma	30	9	>0.40
UT vs. 2prb Flavor	30	13	0.20
UT vs. 1pstb Flavor	29	11	>0.40
UT vs. 1pstb Taste	29	9	>0.40
UT vs. 2pstb Aroma	29	12	0.30
UT vs. 2pstb Flavor	29	12	0.30

Chardonnay 2001. The thinned control (T) differed slightly from the unthinned (UT) control due to the few clusters removed from the latter during mid-season thinning. This light thinning did not significantly reduce clusters per vine. However, thinning did slightly reduce crop per vine (Table 3-17). Due to the lack of significant differences between the controls, treatments were compared to UT. The 2prb treatment reduced clusters per vine, cluster weight, berries per cluster, berry weight and crop per vine (Table 3-17). The 1pstb treatment reduced cluster weight, berries per cluster, berry weight and crop per vine however, the 2pstb only reduced crop per vine. On average the controls had higher values for all fruit chemistry measurements with the exception of PFGG, which was higher for 2pstb. The 2prb treatment had lower soluble solids, pH, absorbance of 280 and 320nm, total phenols, hydroxycinnamates, total flavonoids and PFGG but greater TA (Table 3-18). One post bloom application reduced absorbance at 320 nm, and hydroxycinnamates. Two post-bloom applications lowered absorbance at 280 and 320 nm and values for all spectrophotometrically estimated compounds yet increased PFGG.

Spectrophotometric color estimation and the PPFG assay showed few differences between treatments. The single post-bloom application did have a higher concentration of PPFG, but this was the only notable exception. Spectrophotometric estimates for phenols, hydroxycinnamates, and flavonoids are based on light absorbance at 280 and 320 nm respectively. A typical value of non-phenolic and non-hydroxcinnamate compounds is subtracted from these absorbances, which may produce negative values (Table 3-19). These spectrophotometric estimates should be used on a comparative basis among treatments and not as precise values (Table 3-19).

Results from the triangle difference test showed no significance at an alpha level of 0.05. Our consumer panel could not discern the different wine in significant numbers for any of the comparisons (Table 3-20).

Table 3-17. Components of yield for Chardonnay affected by two pre-bloom (2prb), one post-bloom (1pstb), and two post-bloom applications of 250 mg/L prohexadione-calcium (P-ca) during the 2001 season compared to an unthinned (UT) and a thinned (T) control 2001.

Treatment	Clusters/ vine	Cluster wt (g)	Berries/ cluster	Berry wt (g)	Crop/vine (kg)
UT	27.9	138.23	72.6	1.90	3.89
T	25.6	114.98	60.7	1.90	2.85
2prb	9.9	49.31	36.3	1.39	0.50
1pstb	25.0	99.43	57.5	1.73	2.28
2pstb	24.1	125.58	68.2	1.85	3.01
Significance of Contrast[z] (*P*-value)					
UT vs. T	0.504	0.065	0.080	0.996	0.003
UT vs. 2prb	0.000	0.000	0.000	0.000	0.000
UT vs. 1pstb	0.440	0.004	0.030	0.036	0.000
UT vs. 2pstb	0.317	0.300	0.500	0.525	0.009

[z]Single degree of freedom contrasts of specified treatments.

Table 3-18. Chardonnay must soluble solids (ºBrix), pH, titratable acidity (TA), , absorbance at 280, 320nm, total phenols, hydroxycinnamates (absorption units au and caffeic acid equivalents CAE), flavonoids, and phenol-free glycosyl-glucose (PFGG) affected by one post-bloom (1pstb) and two post-bloom applications of 250 mg/L prohexadione-calcium (P-ca) during the 2001 season compared to an unthinned (UT) and a thinned (T) control.

Treatment	ºBrix	PH	TA (g/L)	280 nm	320 nm	Total phenols (au)	CinOH (au)	CinOH (CAE)	Flavonoids (au)	PFGG (µM)
UT	20.4	3.50	6.27	13.0	8.2	9.0	6.8	75.4	4.5	718
T	20.7	3.52	5.84	11.9	7.5	7.9	6.1	68.0	3.8	382
2prb	18.8	3.41	7.51	9.0	4.6	5.0	3.2	35.4	2.9	334
1pstb	20.2	3.55	5.61	11.9	6.4	7.9	5.0	55.1	4.6	1023
2pstb	19.9	3.54	5.28	9.0	4.4	5.0	3.0	33.7	3.0	1225
Significance of Contrast[z] (*P*-value)										
UT vs. T	0.589	0.884	0.467	0.370	0.387	0.370	0.387	0.387	0.401	0.030
UT vs. 2prb	0.003	0.008	0.047	0.004	0.000	0.004	0.000	0.000	0.050	0.014
UT vs. 1pstb	0.647	0.594	0.270	0.354	0.026	0.354	0.026	0.026	0.940	0.080
UT vs. 2pstb	0.303	0.846	0.105	0.004	0.000	0.004	0.000	0.000	0.073	0.002

[z]Single degree of freedom contrasts of specified treatments

Table 3-19. Chardonnay wine alcohol (ETOH), titratable acidity (TA), pH, absorbance at 280, 320nm, total phenols, hydroxycinnamates (absorption units and caffeic acid equivalents CAE) flavonoids, and phenol-free glycosyl-glucose (PFGG) affected by one post-bloom (1pstb) and two post-bloom applications of 250 mg/L prohexadione-calcium (P-ca) during the 2001 season compared to an unthinned (UT) and a thinned (T) control.

Treatment	ETOH	TA (g/L)	pH	280 nm	320 nm	Total phenols (au)	CinOH (au)	CinOH (CAE)	Flavonoids (au)	PFGG (µM)
UT	11.70	9.05	3.31	0.7	0.5	-0.7	-0.9	-9.7	-2.8	30
T	12.48	8.95	3.30	0.6	0.5	-0.8	-0.9	-9.8	-2.8	30
1pstb	12.45	8.65	3.31	0.6	0.5	-0.8	-0.9	-10.3	-2.8	46
2pstb	12.05	8.55	3.30	0.6	0.5	-0.8	-0.9	-10.5	-2.8	28

Phenols, hydroxycinnamate and flavanoids values are estimations and can only be compared between treatments.

Table 3-20. Chardonnay triangle test of wine aroma and flavor comparing an unthinned control (UT) with either one (1pstb) or two (2pstb) post-bloom applications of 250 mg/L prohexadione-calcium.

Comparison	Observations	Correct Responses	*P*-value
UT vs. 1pstb Flavor	28	10	>0.40
UT vs. 1pstb Taste	28	7	>0.40
UT vs. 2pstb Aroma	32	13	0.30
UT vs. 2pstb Flavor	30	14	0.10

DISCUSSION

In 2000, experiments with P-ca. In this experiment pre and post-bloom P-ca (250 mg/L) applications reduced Cabernet Sauvignon berries per cluster and berry weight. Kurshid et al. (1992) found that pre-bloom applications of GA_3 increased flower number in Cabernet Sauvignon. Therefore, a P-ca application during this same period may have reduced the total flower number, causing the reduction in berries per cluster. An alternate hypothesis is that P-ca reduced fruit set. An experiment, counting the inflorescence number following pre-bloom applications of P-ca, could determine the cause of the berry reduction. The two and three applications of P-ca did not differ for the majority of measurements. The second application, made on 30 June, may have been too late to influence reproductive development. Results of 2000 were confirmed by the results of the single-cluster experiment conducted in 2001.

Results from the 2000 Cabernet franc experiment show that yield components declined linearly with increasing concentration of P-ca. The 375 mg/L treatment caused a particularly severe crop reduction. In 2001 250 mg/L rate did not reduce yield components as in 2000. A possible reason for this discrepency is that the 2000 experiments were subject to three applications of P-ca, compared to two in 2001. However, considering the lack of difference between two and three applications to Cabernet Sauvignon in 2000, it is likely that the third application to Cabernet franc had little effect. The most plausible reason for the inconsistency is that the timing of the pre-bloom applications differed slightly between the two years. These results highlight the importance of correct timing of applications on achieving reduced berry weight and fruit set.

Applications of 250 mg/L P-ca reduced crop 60%, 70% and 30% on Cabernet Sauvignon, Cabernet franc, and Seyval respectively. This suggests that Seyval, an interspecific hybrid, was less responsive to P-ca than were the *Vitis vinifera* cultivars. However, each of these cultivars

have different rates of development and as E-L growth stages were not recorded for the application dates, it is difficult to determine if cultivar type or timing of application caused these variable results. An additional interest in experimenting with Seyval was to decrease cluster compactness. Seyval is a cultivar with tight clusters. Although Seyval fruit set was reduced and clusters appeared less compact, P-ca did not decrease disease incidence or severity. However, these vines were treated in August 2000 with Vanguard^{TM}, a botryticide. Therefore, the lack of significant results may be due to too low an incidence of disease that season. Neither Seyval soluble solids nor pH were affected by treatment.

Second-year effects were determined for 2000 season treatments of Cabernet Sauvignon and Cabernet franc. Current year's buds are formed the previous year with the formation and differentiation of anlagen, so it is reasonable to suspect that 2000 season treatments would effect cluster number in 2001. The clusters of the 2001 season resulted from anlagen which differentiated the season prior into either tendril or inflorescence primordia (Mullin, 1986). What is striking, however, is that gibberellin applications during the differentiation of anlagen cause them to form tendril primordia rather than inflorescence primordia (Srinivasan and Mullins, 1980). This suggests that the application of a gibberellin biosynthesis inhibitor to the differentiating anlagen in 2000 should have increased the cluster number in 2001.

The 2001 single-cluster Cabernet Sauvignon experiment successfully quantified the impact of single P-ca applications to clusters at distinct phenological stages of grape berry development. The most significant effects of the P-ca applications were reduced fruit set for applications made during bloom and reduced berry weight for post-bloom applications.

Although fruit set was reduced by pre-bloom applications in 2000, the severity of fruit set reduction that we observed was unexpected. The literature describing the effect of gibberellin biosynthesis inhibitors on fruit set is conflicting, with some researchers noting decreases in fruit set (Reynolds et al., 1992), some seeing no impact (Hunter, 1992), and others citing increases in fruit set (Coombe, 1965). Moreover, P-ca applications on apple, while suppressing vegetative growth, had no effect on fruit other than a slight increase in red coloration attributed to increased light interception (Byers and Yoder, 1999). However, a reduction in fruit set was not entirely

unexpected considering 2000 experimental results and the volume of literature on the increase in berry number of seedless cultivars of *Vitis vinifera* due to gibberellic acid applications (Coombe, 1965; Khurshid et al., 1992; Weaver and McCune, 1959). Increased berry number has long been attributed to increased fruit set. Recent research with GA_3 on Cabernet Sauvignon indicates that pre-bloom applications may also increase the inflorescence number prior to fruit set, thereby increasing the potential number of berries (Khurshid et al., 1992).

Another outcome of the Cabernet Sauvignon treatments was reduced berry weight. A good deal of research has linked exogenous gibberellic acid applications to increased berry weight (Weaver and McCune, 1959; Sachs and Weaver, 1968). Only recently has the change in concentration of bioactive gibberellins during the course of berry development been studied. New literature in this area describes a sharp increase in GA_3 levels 21 and 65 days after anthesis in seeded cultivars of *Vitis vinifera* (Perez, 2000). These post-bloom increases in GA_3 coincide with the P-ca applications for T_4 through T_6 and T_{12}. We previously reported that T_4 through T_6 had lower berry weights than the control and other treatments. T_{12} likewise exhibited a slight although not significant (alpha = .05) depression in berry weight compared to treatments immediately preceding and following this second increase in GA_3, T_{11} and T_{12} respectively. This would suggest that P-ca inhibits the synthesis of endogenous gibberellin in the grape berry and furthermore, that the lag time between application and inhibition is relatively short. Additionally, these results highlight the importance of endogenous gibberellin levels during these post-bloom periods to berry growth and final harvest berry weight.

Another interesting result from the Cabernet Sauvignon experiment is that elevated levels of phenol-free glycosides corresponded to low berry weight and increased absorbance at 280, 420, 520 nm wavelengths. These results agree with a number of studies which cite the skin as having a greater concentration of glycosidically-bound aroma and flavor compounds than the interior of the berry (Park and Noble, 1993; Fernandez de Simon et al., 1993). Canopy modification to increase light exposure and glycoside composition of fruit have increased total glycosides 17% to 29% in fruit from leaf-thinned canopies (Zoecklein et al., 1998). Bureau et al. (2000) found that light exposure could increase the concentration of C_{13}-norisoprenoids

approximately 40%. In contrast, Cabernet Sauvignon treatment four had phenol-free glycoside concentrations 88% higher than the control. These results suggest that increasing the surface to volume ratio of grape may potentially increase glycosidically-bound compounds, many of which are aroma and flavor precursors. Additionally, it should be emphasized that these values resulted from analyzing grape berry flesh. Analysis of grape skin extractions may have provided even higher results.

As previously discussed, Cabernet franc did not respond to treatment in a similar manner observed in 2000. Timing of applications is critical in the effectiveness of P-ca. Since we did not record the E-L growth stages at 2000 application dates, results from the two years are essentially incomparable. Additionally, as treatments were restricted to the fruit zone of the canopy, a great deal less active ingredient was applied per vine in 2001 compared to 2000. However, timing may seem a more credible explanation for the disparity between 2000 and 2001 results as BASF cites P-ca as being acropetally translocated (Evans et al., 1999). As long as an equal concentration of spray material was applied to the fruit zone over the two seasons, irrespective of the rest of the canopy, reproductive results should have corresponded, given that vines were at similar developmental stages.

Unlike the Cabernet Sauvignon single-cluster experiment, P-ca did not reduce Cabernet franc berry weight. Fruit chemistry results were unrelated to berry weight. Cabernet franc must spectrophotometric measurements were affected by one post-bloom application more strongly than by two post-bloom applications however wine values were more affected by two post-bloom applications. The lack of correspondence between berry size and Cabernet franc fruit chemistry measurements suggests the existence of a treatment effect independent of berry weight. It equally possible that slight variations in fermentation conditions affected wine chemistry of treatments. The use of treatment replicates in future research might compensate for wine making effects on chemistry results.

Chardonnay exhibited severely reduced crop per vine (0.5 kg/vine) due to two pre-bloom applications. The overwhelming contributor to this reduced crop yield was a reduction in fruit set. Although treatment reduced fruit set to an undesirable level, an impact on fruit set is

positive, as lower fruit set might decrease cluster compactness. The compact clusters of Chardonnay have been linked to *Botrytis cinerea* infections (Zabadal and Dittmer, 1998). These experiments did not find a significant treatment-induced decrease in incidence or severity of Botrytis bunch rot. Though visual observations noted looser clusters on treatment vines, Chardonnay cluster compactness was not quantified. It is possible that while P-ca applications lowered berry weight and reduced fruit set, they also inhibited the growth of the rachis, causing a smaller, yet equally compact cluster. Because rachis measurements are laborious undertakings, quantifications of cluster compactness such as were made by Vail and Marois (1991) might be beneficial in future Chardonnay studies.

Interestingly, the E-L growth stages of Chardonnay during the two pre-bloom applications were 15 and 19. These stages are not very different from E-L stages 18 and 21, at which application of P-ca severely decreased the fruit set of Cabernet Sauvignon clusters. The twice-applied post-bloom application also significantly reduced crop per vine, a reduction caused by the cumulative impact of slightly lower clusters per vine, cluster weight, and berry weight. However, this crop reduction from post-bloom applications was not nearly the magnitude of that caused by pre-bloom applications.

It is difficult to decipher the fruit and wine chemistry results from the 2001 Chardonnay experiment. Unlike the Cabernet Sauvignon single-cluster experiment where low berry weight corresponded to higher absorbance and PPFG values, Chardonnay fruit and wine chemistry was negatively impacted by reduced berry weight. Furthermore, spectrophotometric estimations and PFGG concentration of Chardonnay did not correlate to each other. Norisoprenoids, the major aroma and flavor compounds in Chardonnay (Willaims et al., 1992) and phenolic compounds such as hydroxycinnamates and flavonoids, are concentrated in the skin. However, Chardonnay PFGG readings did not correlate to the spectrophotometric estimations of hydroxycinnamates and flavonoids.

Despite differences in fruit and wine chemistry between Chardonnay and Cabernet franc treatments, our consumer panel had difficulty discerning the wines from each other. Although this does not indicate that P-ca will not be a future asset to the commercial wine industry,

certainly our goals of impacted wine chemistry were not met. One certainly can argue that if a general consumer panel cannot differentiate wines, there is no difference.

CONCLUSION

This research indicates that timing, rate of application, and cultivar all significantly influence the effect that P-ca will have on grape berry development. 2000 and 2001 experiments show that P-ca has the ability to reduce fruit set and berry weight on a number on Cabernet Sauvignon, Cabernet franc, Seyval, and Chardonnay. Reductions in berry number are attributed to reduction in fruit set by pre-bloom applications while reduced berry weight is ascribed to post-bloom applications. Often the effectiveness of application coincides with increases of endogenous gibberellins, a fact that emphasizes the important role of gibberellins in grape fruit set and berry development. If timing and rate of P-ca can be determined for different cultivars, it may be a useful commercial tool to reduce fruit set and berry weight. However, for timing and rate to be effectively determined, a single-cluster experiment, such as was conducted with Cabernet Sauvignon, should be performed for cultivars of interest with varying concentrations at different developmental stages.

Fruit chemistry measurements of 2001 Cabernet Sauvignon found that reduced berry weight corresponded with higher phenol-free glycosyl-glucose concentrations and increased absorbance at 280, 420, 520 nm wavelengths. These results were not duplicated by 2001 applications to Cabernet franc and Chardonnay fruit zones. Excepting a decrease in yield components due to pre-bloom applications of Chardonnay, results from the 2001 Cabernet franc and Chardonnay experiment were negligible to those of the Cabernet franc single-cluster experiment. Because Cabernet franc fruit and wine chemistry results were unrelated to berry weight, the possibility of a treatment-effect unrelated to berry weight should not be discounted.

Although 2001 Cabernet franc and Chardonnay wine results were inconclusive, P-ca has been shown to increase the concentration of PPFG in berries of Cabernet Sauvignon. Most aroma and flavor compounds are non-phenolic, a large proportion of which are glycosidically-

bound in must and young wine. Admittedly, not all glycosidically-bound compounds are aroma and flavor precursors. However, an increase in must PFGG indicates an increase in aroma and flavor precursos, which would ostensibly improve wine sensory characteristics.

LITERATURE CITED

Bureau, S. M., R.L. Baumes, and A.J. Raxungles. Effects of vine or bunch shading on the glycosylated flavor precursors in grapes of *Vitis vinifera* L. cv. Syrah. J. Agric. Food Chem. 48:1290-1297 (2000).

Byers, R.E. and Yoder, K.S. Prohexadione-calcium inhibits apple, but not peach, tree growth, but has little influence on apple fruit thinning or quality. HortScience 34(7):1205-1209 (1999).

Coombe, B.G. Fruit set and development in seeded grape varieties as affected by defoliation, topping, girdling, and other treatments. Am. J. Enol. Vitic. 10:85-100 (1959).

Coombe, B.G. Increase in fruit set of Vitis vinifera by treatment with growth retardants. Nature. 205:305-306 (1965).

Coombe, B.G. Research on Berry Composition and Ripening. In Proceedings of the Seventh Australian Wine Industry Technical Conference. pp. 150-152. (1988).

Coombe, B.G. Research on development and ripening of the grape berry. Am. J. Enol. Vitic. 43:101-110 (1992).

Coombe, B.G. Adoption of a system for identifying grapevine growth stages. Aust. J. Grape Wine Res. 1:100-110 (1995).

Coombe, B.G., M. Bovio, and A. Schneider. Solute accumulation by grape pericarp cells. J. Exp. Bot. 38:1789-1798 (1987).

Coombe, B.G. and P.G. Iland. Grape berry development. In Proceedings of the Sixth Australian Wine Industry Technical Conference., Adelaide. pp. 50-54 (1987)

Coombe, B.G. and M.G. McCarthy. Dynamics of grape berry growth and physiology of ripening. Aust J. Grape Wine Res. 6:131-135 (2000).

Esteban, M.A., M.J. Villanueva, J.R. Lissarrque. Effect of irrigation on changes in berry composition of tempranillo during maturation. Am. J. Enol. Vitic. 50(4):418-434 (1999).

Evans, J.R., R.R. Evans, and C.L. Regusci. Mode of action, metabolism, and uptake of BAS 125W Prohexadione-calcium. HortScience. 34:1200-1201 (1999).

Fernandez de Simon, B., T. Hernandez, and I. Estrella. Phenolic composition of white grapes (var. Airen). Changes during ripening. Food Chem. 47(1):47-52 (1993).

Hunter, D.M. Paclobutrazol affects growth and fruit composition of potted grapevines. HortScience 27(4)319-321 (1992).

Iland, P.G., W. Cynkar, I.L. Francis, P.J. Williams, and B.G. Coombe. Optimisation of methods for the determination of total and red-free glycosyl glucose in black grape berries of Vitis vinifera. Aust J. Grape Wine Res. 2:171-178 (1996).

Jackson, D.I. and P.B. Lombard. Environmental and management practices affecting grape composition and wine quality-a review. Am. J. Enol. Vitic. 44:409-430 (1993).

Larmond, E. Laboratory methods for sensory evaluation of food: publication 1637. Canada Department of Agriculture. (1982).

Miller, D. P., G. S. Howell, and R. K. Striegler. Reproductive and vegetative response of mature grapevines subjected to differential cropping stresses. Am J. Enol. Vitic. 44:435-440 (1993).

Motomura, Y. Effects of gibberellin and daminozide on the distribution of 14C-assimilates in grape shoots. Acta Hortic. 179:421-424 (1986).

Mullin, M.G. Hormonal regulation of flowering and fruit set in the grapevine. Acta Hortic. 179:309-315 (1986).

Jackson, D.I. and P.B. Lombard. Environmental and management practices affecting grape composition and wine quality-a review. Am. J. of Enol. Vitic. 44: 409-430 (1993).

Khurshid, T, D.I. Jackson, and R.N. Rowe. Effect of plant growth regulators on flower development in the grapevine (*Vitis vinifera* L.) cv. Cabernet Sauvignon. N. Z. J. Crop Hortic. Sci. 20:351-356 (1992).

Park, S.K. and A.C. Nobel. Monoterpenes and monoterpene glycosides in wine aromas. *In* Beer and Wine Production: Analysis Characterization, and Technological Advances. B.H. Gump (Ed.), pp. 98-109. Am. Chem. Soc., Washington, D.C (1993)

Perez, F.J., C. Viani, J. Retamales. Bioactive gibberellins in seeded and seedless grapes: identification and changes in content during berry development. Am. J. Enol. Vitic. 51(4):315-318 (2000).

Reynolds, A.G. and A.P Naylor. Pinot Noir and Riesling grapevines respond to water stress duration and water holding capacity. HortScience 29(12):1505-1510 (1994).

Reynolds, A.G., D.A. Wardle, A.C. Cottrell, and A.P. Gaunce. Advancement of 'Riesling' fruit maturity by Paclobutrazol-induced reduction of lateral shoot growth. J. Am. Soc. Hortic. Sci. 117(3):430-435 (1992).

Roessler, E.B., J. Warren, and J.F. Guymon. Significance in triangular taste tests. Food Res. 13:503-505 (1948).

Roubelakis-Angelakis, K. A. and W. M. Kliewer. Effects of exogenous factors on phenylalanine ammonia-lyase activity and accumulatin of anthocyanins and total phenolics in grape berries. Am. J. Enol.Vitic. 37(4):275-280 (1986).

Sachs, R.M., and R.J. Weaver. Gibberellin and Auxin-induced berry enlargement in *Vitis vinifera* L. Hortic. Sci. 43:185-95 (1968).

Srinivasan C. and M. G. Mullins. Effects of temperature and growth regulators on formation of anlagen, tendrils, and inflorescences in Vitis vinifera L. Ann. Bot. 45:439-446 (1980a).

Vail, M.E. and J.J. Marois. Grape cluster architecture and the susceptiblity of berries to Botrytis cinerea. Phytopath. 81:188-191 (1991).

Weaver, R.J. and S.B. McCune. Effects of giberellin on seedless *Vitis vinifera*. Hilgardia. 29(6):247-271 (1959).

Williams, P.J., M.A. Sefton, and I. L. Francis. Glycosidic precursors of varietal grape and wine flavor. *In* Flavor Precursors. R. Teranishi, G.R. Takeoka, and M. Guntert (Eds.), pp. 74-86. Am. Chem. Soc., Washington, D.C. (1992).

Williams, P.J., W. Cynkar, I. L. Francis, J.D. Gray, P.G. Iland, and B.G. Coombe. Quantification of glycosides in grapes, juices, and wines through a determination of glycosyl glucose. J. Agric. Food Chem. 43:121-128 (1995).

Zoecklein, B.W. L.S. Douglas, and Y.W. Jasinski. Evaluation of the phenol-free glycosyl-glucose determination. Am. J. Enol. Vitic. 51:420-423 (2000).

Zoecklein, B.W., K.C. Fugelsang, B.H. Gump, and F.S. Nury. Wine Analysis and Production. Aspen Publishers Inc. Gaithersburg, Maryland. (1999).

Zoecklein, B.W., T.K. Wolf, J.E. Marcy, and Y. Jasinski. Effect of fruit zone leaf thinning on total glycosides and selected aglycone concentrations of Riesling. (Vitis vinifera L.) grapes. Am. J. Enol. Vitic. 49(1):35-42 (1998).

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