Effects of Prohexadione-calcium on Grape Yield Components and Fruit and Wine Composition

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Abstract: Prohexadione-calcium (prohexadione-Ca) was applied to field-grown Cabernet franc, Cabernet Sauvignon, Chardonnay, and Seyval to evaluate rates and timing effects on fruit yield components and on fruit and wine composition. Berries per cluster, berry weight, cluster weight, and clusters per shoot in the subsequent season were all decreased by multiple, prebloom plus postbloom, applications to Cabernet Sauvignon and Cabernet franc. Similar reductions in current season components of yield were observed with Seyval. Application (250 mg/L) to single clusters of Cabernet Sauvignon and Chardonnay at bloom, or in the one-to-two-week prebloom period decreased fruit set, whereas applications one to two weeks postbloom reduced berry weight, with no impact on fruit set. Berry weight reduction correlated to increased color intensity (420 nm + 520 nm), total anthocyanins, total phenols, and phenol-free glycosyl-glucose (PFGG) in Cabernet Sauvignon. In a separate experiment, prohexadione-Ca increased Cabernet franc must color intensity, total anthocyanins, and total phenols, despite having minimal effects on berry weight or crop yield. Aroma and flavor triangle difference tests did not distinguish treatment differences with young Cabernet franc wines. This study of prohexadione-Ca effects on grape reproductive development illustrated that berry set and berry weight were responsive to application timing, with the one-to-two-week period after bloom most sensitive to reductions in berry weight. The concurrent effects on fruit composition were generally positive, while the full impact on wine quality remains equivocal, but worthy of further evaluation.

Key words: Apogee, plant growth regulator, prohexadione-calcium

Grape flavor and aroma components are not uniformly distributed in the berry but rather are concentrated or compartmentalized in specific regions. Glucose and fructose are concentrated in the flesh, whereas inorganic ions, phenolics, and tartrate are concentrated in the skin (Coombe 1992, Coombe and McCarthy 2000). Phenols impart color and structure to wine, while nonphenolic compounds, such as monoterpenes and norisoprenoids, are largely responsible for aroma and flavor (Williams et al. 1992). Flavonoid phenols, particularly anthocyanins, are found in greater amounts in red cultivars than in white cultivars, primarily in the skins (Fernandez de Simon et al. 1993). Anthocyanins are found in the vacuoles of cells located a few cell layers below the epidermis (Roubelakis-Angelakis and Kliewer 1986). Nonflavonoid phenolic compounds such as hydroxycinnamates and nonphenolic monoterpenes such as geraniol and nerol are also more concentrated in the skin than in the flesh of the berry (Fernandez de Simon et al. 1993, Park and Noble 1993). A decrease in berry size would lead to an increase in the surface to volume ratio, theoretically increasing the proportion of these flavor and aroma precursors in the must and wine and potentially increasing wine quality. Smaller berries can be clonally selected and can be achieved by deficit irrigation (Coombe and McCarthy 2000, Esteban et al. 1999, Reynolds and Naylor 1994) in arid regions.

Aside from potential increases in wine quality, a reduction of berry size and cluster compactness may lessen the incidence and severity of botrytis and other bunch rots, particularly in humid environments. Vail and Marois (1991) found a significant correlation between cluster compactness and the susceptibility to bunch rots caused by *Botrytis cinerea*.

Prohexadione-calcium (prohexadione-Ca) (3-oxido-4-propionyl-5-oxo-3-cyclohexene-carboxylate) is a gibberellin biosynthesis inhibitor currently marketed by BASF as Apogee. Prohexadione-Ca is currently EPA-registered for apples (Byers and Yoder 1999, Owens and Stover 1999) to suppress vegetative growth. The mode of action of prohexadione-Ca differs from that of other gibberellin biosynthesis inhibitors currently in use in commercial agriculture. Many of these growth regulators, including the quaternary ammonium compounds, substituted pyrimidines, norbornenodiazetine derivatives, and triazole derivatives (Graebe 1987) function by interrupting the synthesis of gibberellin early in the biosynthetic pathway, specifically at the synthesis of ent-kaurene. Prohexadione-Ca is known to interfere with the 3-ß hydroxylation of GA_{20} to GA_1. The net effect is a reduction in immobile, biologically active GA_1 and an increase in the levels of mobile, but inactive GA_{20} (Evans et al. 1999, Graebe 1987).

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Manuscript submitted April 2003; revised September 2003

Our initial interest in prohexadione-Ca centered on its potential for suppressing vegetative growth of large, vigorous grapevines. Despite limited and insufficient reductions in shoot growth (Lo Giudice et al. 2003), we did observe that prohexadione-Ca reduced fruit set and berry size, depending upon the timing of application. These studies describe that reproductive response. Our objectives were to quantify the response of grapes to prohexadione-Ca and to determine whether reductions in berry size or crop translated to increased fruit and wine quality attributes.

Materials and Methods

General. Prohexadione-Ca was applied as Apogee (27.5% prohexadione-Ca) (BASF, Mount Olive, NJ) at variable rates in tap water, using a hand-held, backpack, or motorized sprayer. Rates of prohexadione-Ca given in treatments are as active ingredient. Except as indicated, all treatments included Regulaid surfactant (polyoxyethylene-polypropoxypropanol and alkyl 2-ethoxyethanol dihydroxy propane) (Kalo, Inc., Overland Park, KS) at 0.13% (v/v) and ammonium sulfate at 900 mg/L. Ammonium sulfate has been used to prevent the deactivation of prohexadione-Ca by calcium or other cations in hard water (Byers et al. 2000). Applications were made when there was no rain predicted for a minimum of 24 hr. Treatments were applied to both sides of the grapevine canopy, wetting the entire shoot including clusters to the point of runoff, unless otherwise noted. Control plots, including individual cluster treatments, were sprayed with aqueous solutions of the adjuvants at concentrations equal to those used in prohexadione-Ca treatment plots. Border plots or multiple border vines buffered treatment plots within the row, and considerations were given to wind speed and proximity of adjacent row plots to avoid unintended application.

Vine and pest management was typical for Virginia (Wolf and Poling 1995). Shoot density was adjusted to 10 to 14 shoots per m of canopy before bloom, and primary shoots were trimmed one or two times per season to prevent shoot tops from obstructing the fruit zone of the vertically shoot-positioned canopies.

Cabernet Sauvignon, 2000. Nine-year-old Cabernet Sauvignon (Foundation Plant Materials Service [FPMS] clone #7) vines, grown at the Alson H. Smith Jr. Agricultural Research and Extension Center (AHS-AREC) in Winchester, VA, were used to evaluate a single rate of prohexadione-Ca at variable times. Vines were cordon-trained and spur-pruned on an open-lyre trellis system. Row and vine spacing were 3.7 and 2.1 m, respectively. The experimental design was completely randomized with five replicates of three-vine plots.

Treatments consisted of a control and prohexadione-Ca (250 mg/L) applied either two (2xP-Ca) or three (3xP-Ca) times. The 2xP-Ca and 3xP-Ca treatments were applied prebloom (15 and 31 May), while 3xP-Ca comprised an additional postbloom application (30 June). Full bloom occurred 5 June. Spray volumes and active ingredient applied per plot in this and following experiments are given in Lo Giudice (2002).

Fruit was harvested on 20 October, at which time clusters and total fruit weight were recorded by vine. Individual vine data were averaged by plot for statistical analyses. Fifty-berry samples were collected from each treatment replicate immediately before harvest to determine average berry weight. Berry sampling in all experiments consisted of a random selection of clusters, but a methodical sampling of individual berries. Berries were collected by sequentially sampling the back (toward center of row) of a cluster, then the front (toward canopy exterior) of the next cluster, the next cluster's lateral shoulder region, the next cluster's tip region, and so on. This strategy lessened the likelihood of disproportionately sampling one region of a cluster over another. The opposing sides of the divided canopy (lyre-trained) vines were equally sampled (25 berries/side). Primary shoots and their flower clusters were counted on treatment vines in 2001, before shoot thinning, to determine if there were any second-year effects from prohexadione-Ca applications made during 2000.

Cabernet franc, 2000. Four-year-old Cabernet franc vines, grown at Indian Springs Vineyard near Woodstock, VA, were used to evaluate varied rates of prohexadione-Ca. Vines were cordon-trained and spur-pruned and the canopy was vertically shoot-positioned (VSP). Row and vine spacing was 2.7 and 2.1 m. The experimental design was completely randomized, and each treatment consisted of 10 single-vine replicates. Treatments were 125, 250, and 375 mg/L prohexadione-Ca. To determine if ammonium sulfate itself affected components of yield or fruit composition, two controls were included; one control included ammonium sulfate and Regulaid, while the other control included only Regulaid. All vines were sprayed to the point of drip once prebloom (18 May), and twice postbloom (1 and 30 June). Full bloom occurred 26 May.

Clusters and total fruit weight per vine were determined at harvest on 11 October. Fifteen-berry samples were collected from each vine immediately before harvest and were pooled to provide each treatment with two 45-berry samples and one 60-berry sample for sufficient sample volume. Average berry weight was calculated from these replicates. Primary shoots and their flower clusters were counted on treatment vines in 2001, before shoot thinning, to determine if there were any second-year effects from prohexadione-Ca applications made during 2000.

Seyval, 2000. Multiple applications of a single rate of prohexadione-Ca were evaluated with own-rooted, 10-year-old Seyval (interspecific hybrid) vines, grown at the AHS-AREC. Vines were cordon-trained and spur-pruned with a VSP canopy. Row width and in-row vine spacing were 3.7 and 2.1 m, respectively. The experimental design was completely randomized with five replicates of three-vine plots. Treatments consisted of three applications of prohexadione-Ca (250 mg/L) and a control. The initial application was made

prebloom on 15 May, the second on 31 May (full bloom), and the third on 30 June.

Fruit was harvested on 6 September. Fifty-berry samples were randomly collected from each of the five treatment replicates immediately before harvest and frozen for later analysis. Clusters per vine and total fruit weight per vine were recorded. Clusters were visually inspected as harvested, and those with >5% rot were recorded as being rot affected. No effort was made to characterize rot; however, the signs and symptoms suggested that most rot was due to *Botrytis cinerea*, to which Seyval is susceptible. Rot severity was also recorded by estimating the percentage of rot-affected berries in each cluster and calculating as:

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

Berry weights and basic fruit composition were determined from berry samples. After thawing at room temperature, samples were homogenized and the juice was separated from pulp with a filter bag (model 400; Stomacher Labsystem, Seward Ltd., London, UK) and centrifuged at 17,000 rpm at 10°C for 30 min. Immediately following processing, juice soluble solids were measured with a temperature-compensated refractometer (model 10430; Reichert Scientific Instruments, Depew, NY), pH with a Fisher Accumet pH meter (model 815MP; Pittsburgh, PA), and titratable acidity via procedures of Zoecklein et al. (1999).

Cabernet Sauvignon individual cluster experiment, 2001. Individual Cabernet Sauvignon clusters were treated with prohexadione-Ca during 2001 to more precisely gauge treatment effects on berry development at different phenological stages. Vines were in the same block of Cabernet Sauvignon as described above, but had not been previously treated with prohexadione-Ca.

Treatments consisted of a single prohexadione-Ca application on one of 13 separate dates that progressed weekly (T_1 through T_{13}) and that were compared with an untreated control (T_0). The subscripts indicate the week during which the prohexadione-Ca (250 mg/L) treatment was applied, with T_1 applied one month after budbreak and T_3 applied at 50% bloom. The Eichhorn and Lorenz (E-L) growth stage (Coombe 1995) was recorded at each treatment to further define the phenological stage of development. Treatments were applied to individual clusters (experimental units). Clusters were located on 10 vines and each vine constituted a complete block on which all treatments, including T_0, were included. The treatment vines were randomly arranged in the vineyard and were uniform in canopy characteristics and vine size. Treatments were randomly assigned to clusters on a given vine so that no treatment had a favored position on the vine. Treatments T_0, T_2, T_5, and T_8 were chosen for midseason destructive sampling in order to chart the development of treated clusters. Consequently, these treatments were randomly applied to 20 additional clusters (5 per treatment vine) to provide an adequate sample size for those dates. The experimental units were the basal fruit clusters of shoots that only bore two clusters. Treatments were applied with a hand-held spray bottle. Each cluster was sprayed four times from different angles to completely wet the cluster. Plastic bags were used to isolate the target cluster during applications to avoid spraying other clusters or leaves. The first treatment (T_1) was sprayed one month after budbreak at E-L 18.

The four midseason sampling dates were 21 June, 13 July, 2 August, and 14 September. Five clusters were collected from T_0, T_2, T_5, and T_8 on each date. Berries were removed from the cluster, counted, weighed, and average berry weight was calculated for each cluster. Final harvest of all 10 clusters for all treatments was on 12 October 2001. Total and average berry weight was determined for each cluster. Berries were then frozen, later thawed, and processed for fruit composition. For each cluster, 80 berries were randomly selected for compositional analyses. When clusters had fewer than 80 berries, all berries from the cluster were used. After thawing at room temperature, samples were homogenized and the juice was separated from pulp with a filter bag and centrifugation, as described above. Juice soluble solids, titratable acidity, and pH were measured as described above. Samples were then refrozen at -37°C.

After thawing, a 10-mL sample was removed for juice color estimation and for a phenol-free glycosyl-glucose (PFGG) assay. Color was measured with a Spectronic Genesys model-5 spectrophotometer (Thermo Spectronic US, Rochester, NY). Juice absorbance values were measured at 280, 420, and 520 nm. Color intensity, anthocyanins, ionized anthocyanins, and total phenols were estimated from absorbance values (Zoecklein et al. 1999).

The PFGG analysis procedures were described by Zoecklein et al. (2000), modified by Whiton and Zoecklein (2002), and were a modification of previous methods to determine glycosides in juice and wine samples by measuring glucose (Iland et al. 1996, Williams et al. 1995). Briefly, must samples were raised to pH 13 to exclude phenolic glycosides, extracted on solid-phase, and eluted with ethanol. Elutions were hydrolyzed for 1 hr, neutralized, and glucose was enzymatically determined (Roche Diagnostics, Basel, Switzerland) with a Thermo Labsystems spectrophotometer (Multiskan MCC/340, Franklin, MA).

Chardonnay individual cluster experiment, 2002. Individual Chardonnay clusters were treated with prohexadione-Ca during 2002 to gauge effects on berry development at different phenological stages. The experiment was similar in purpose and methods to the above 2001 Cabernet Sauvignon experiment. Eleven-year-old Chardonnay (FPMS clone #4) vines grown at the AHS-AREC were used. Vine spacing and training were identical to that described for the Cabernet Sauvignon.

Treatments consisted of five separate dates (weekly) of prohexadione-Ca treatment (T_1 through T_5) and a control (T_0). Treatment rate, method of application, replication, and arrangement of replicates on the vines were identical to the Cabernet Sauvignon individual cluster experiment. Treatments T_0, T_2, and T_4 were chosen for two midseason

destructive samplings of five clusters per treatment in order to chart the development of treated clusters. Consequently, those treatments were randomly applied to 10 additional clusters per treatment among the test vines. The first treatment (T_1) was sprayed at bloom (E-L stage 23), with subsequent treatments applied at one-week intervals.

The two midseason sampling dates were 1 July and 1 August, at which time five clusters were collected from T_0, T_2, and T_4 treatments. Berries were removed from the cluster, counted, weighed, and average berry weight calculated. Final harvest of 10 clusters per treatment was on 13 September 2002, with berries per cluster and average berry weight obtained. Fruit soluble solids at harvest ranged from 20.3 to 21.1 Brix.

Cabernet franc, 2001. Prohexadione-Ca was applied to Cabernet franc in 2001 with the goal of further evaluating the timing of application, as well as evaluating the effects on fruit and wine composition. Five-year-old Cabernet franc vines, grown at Indian Springs Vineyard and described above, were used. Test vines had not been previously treated with prohexadione-Ca.

Prohexadione-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 uniformly wetted. The experiment comprised two controls and three treatments, and the experimental design was completely randomized with five replicates of four-vine plots.

The three prohexadione-Ca treatments consisted of two prebloom applications (2prb) (7 and 23 May), or a single postbloom application (1pstb) (19 June), or two postbloom applications (2pstb) (19 June and 3 July). Bloom occurred 6 June. The E-L stages at treatment applications were 15 and 17 (prebloom) and 29 and 31 (postbloom).

Because prohexadione-Ca had the potential to reduce crop, any evaluation of fruit color, aroma, or other quality component had the confounding influence of differential crop level. We therefore included two sets of controls, one crop-thinned, the other not. On 18 May, shoots and clusters on all vines were counted and thinned to 16 shoots/meter of canopy length. In late July, one of the controls was cluster-thinned to an anticipated prohexadione-Ca induced crop level based on midseason cluster weights and cluster counts for all treatment plots. Due to the slight difference in average cluster weight between 2prb, 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.

Fruit was harvested on 10 October and both clusters and total fruit weight per vine were recorded. Fifty-berry samples were collected from each treatment plot immediately before harvest. These samples were processed and analyzed in a manner identical to that described for the Cabernet Sauvignon individual cluster experiment.

Separate wines were made from each of the Cabernet franc treatments. Clusters were destemmed and after a 48-hr cold soak (7°C) the must was warmed and inoculated with 3.8 g/L yeast (Lalvin BM45, Lallemand, Montréal, PQ). Caps were punched down three times per day until the must fermented to dryness (2.0 g/L residual sugar) at a fermentation temperature of 65°C. The treatments were then pressed and racked into glass containers. Titratable acidity, pH, ethanol, absorbance at 280, 420, and 520 nm, and phenol-free glycosides were measured after fermentation. Color intensity, anthocyanins, ionized anthocyanins, hue, PFGG, and total phenols were estimated as described above.

Sensory analysis. A triangle difference test (Larmond 1982) was performed on Cabernet franc wines approximately four months after completion of fermentation. Sensory panelists were students selected from a collegiate course on wine appreciation and technology. At each flight, panelists (n = 29 or 30) were presented with three opaque, coded glasses of wine and told that one differed from the other two. They were then asked to smell the wines and indicate the different wine, after which they were presented with three different wines for taste evaluation. Treatment wines were always compared to untreated controls. Because the unthinned control had berry weights closest to those of the treatments, the unthinned control was chosen as the comparison wine.

Data analysis. Data analysis of variance (ANOVA) was conducted with GLM or *t* test functions of SAS (version 8.2; SAS Institute, Inc., Cary, NC), and means compared with either single degree-of-freedom contrasts or Duncan's multiple range test (DMRT). Individual vine data from multivine plots were averaged by plot prior to ANOVA. Correlations between berry weight and fruit composition in the Cabernet Sauvignon individual cluster experiment were also evaluated with SAS procedures. Statistical analyses were not performed on wine chemistry data because wine lots were not replicated. Mean values for prefermentation fruit chemistry analyses were calculated from the five treatment replicates, analyzed with ANOVA procedures, and means separated with DMRT. 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, crop per vine, and clusters per shoot in the subsequent season were all decreased by applications of 250 mg/L prohexadione-Ca (Table 1). Berries per cluster and crop per vine were both greater for the 3xP-Ca treatment than for the 2xP-Ca treatment. Reductions in berries per cluster were due to abortion and withering of individual florets.

Cabernet franc, 2000. There were few differences noted between the two controls, one exception being a slight decrease in berry weight with the +N control compared to the -N control (Table 2). Because of these small differences,

prohexadione-Ca treatments were compared to the control that included ammonium sulfate. Prohexadione-Ca reduced clusters per vine, cluster weight, berries per cluster, berry weight, crop per vine, and clusters per shoot in the subsequent season. A negative linear relationship was observed between prohexadione-Ca concentration and clusters per vine, berries per cluster, berry weight, and clusters per shoot, while a quadratic relationship was observed for cluster weight and crop per vine. Again, the reduction in berries per cluster appeared due to senescence of individual florets; however, the reductions in clusters per vine were due to a senescence of the entire cluster similar to that described for inflorescence necrosis.

Seyval, 2000. Cluster weight, berries per cluster, berry weight, and crop weight per vine were all decreased by prohexadione-Ca (Table 3). The rot severity of fruit from prohexadione-Ca-treated vines (3.5%) was comparable to that of control vines (5.6%). No differences in juice soluble solids (19.5 Brix for control versus 18.6 Brix for prohexadione-Ca) or pH (3.3 for both) were observed. Seyval berry skins, normally greenish-gold, acquired a reddish cast on vines treated with prohexadione-Ca.

Cabernet Sauvignon individual cluster experiment, 2001. Prohexadione-Ca applications before and during bloom (T_{1-3}) severely reduced fruit set (Table 4 and Figure 1). The minimal variation in berry weight observed for T_2 and T_3 reflects the almost complete loss of fruit set with clusters of those two treatments. Clusters from T_2 and T_3 applications were so severely affected it was impossible to gather any fruit chemistry results. Prohexadione-Ca ceased to affect fruit set beyond T_3 (E-L stage 26); in fact, T_4 had more berries per cluster at harvest than any other treatment, including the control (Table 4). Vines at T_4 responded to treatment with a 51% reduction in berry weight. A reduction in berry weight was evident for applications through T_6, although this response was less intense after T_4 (Table 4). Treatments T_4 and T_5 (E-L stages 26 and 27) were most effective in reducing berry weight without adversely affecting berry set; however, there was also a certain amount of variation in berry

Table 1 Cabernet Sauvignon yield components in 2000 and flower clusters per shoot counted in spring of 2001, in response to two (2xP-Ca) or three (3xP-Ca) applications of prohexadione-Ca at 250 mg/L in 2000.

	2000 season measures					
Treatment	Clusters/ vine	Cluster wt (g)	Berries/ cluster	Berry wt (g)	Crop/ vine (kg)	Clusters/ shoot 2001
Control	72.7	127.03	92	1.38	9.24	1.5
2xP-Ca	75.3	36.11	46	0.83	2.75	1.4
3xP-Ca	76.1	48.41	58	0.81	3.71	1.4
	Significance of contrast[a] (*p* value)					
Control vs mean of treatments	0.243	<0.001	<0.001	<0.001	<0.001	0.003
2xP-Ca vs 3xP-Ca	0.781	0.063	0.046	0.523	0.024	0.389

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

Table 2 Cabernet franc yield components in 2000 and flower clusters per shoot counted in spring of 2001, in response to three rates of prohexadione-Ca (125, 250, and 375 mg/L) and two controls, either with (+N) or without (-N) ammonium sulfate, in 2000.

	2000 season measures					
Treatment	Clusters/ vine	Cluster wt (g)	Berries/ cluster	Berry wt (g)	Crop/ vine (kg)	Clusters/ shoot 2001
-N	53.5	100.8	58	1.74	5.4	1.6
+N	46.8	105.4	69	1.55	4.9	1.6
125 mg/L	34.6	68.0	48	1.44	2.4	1.3
250 mg/L	31.0	42.5	38	1.11	1.4	1.3
375 mg/L	19.1	25.4	26	0.95	0.5	1.1
	Significance of contrast[a] (*p* value)					
+N vs -N	0.054	0.530	0.083	0.001	0.173	0.711
+N vs mean of treatments	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic	0.950	0.052	0.295	0.544	0.001	0.103

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

Table 3 Seyval yield components as affected by three applications of prohexadione-Ca (P-Ca) in 2000.

Treatment	Clusters/ vine	Cluster wt (g)	Berries/ cluster	Berry wt (g)	Crop/vine (kg)
Control	48.8	198.0	79	2.53	9.8
P-Ca	45.5	131.8	63	2.10	6.0
	Probability > | t |				
t test	ns[a]	0.004	0.047	<0.001	0.020

[a]ns: not significant.

weight with those samples, as illustrated in Figure 1. Berry development of the control (T_0) and T_8 vines was nearly identical over the four sampling dates (Figure 2), with T_5 and T_2 exhibiting correspondingly lower terminal berry sizes and development curves.

The immediate postbloom application (T_4) had no impact on soluble solids (Brix), pH, or titratable acidity (Table 5); however, the T_4 treatment did increase phenols, total and ionized anthocyanins, and color intensity (420 + 520 nm). Phenol-free glycoside concentration was highest for the T_4 treatment and similar among other treatments.

Berry weight was inversely and significantly correlated to juice spectral quality. The correlation coefficients between absorbance at 280, 420, and 520 nm and berry weight, across all treatments, ranged from -0.55 to -0.58, all highly significant. The strength of those correlations increased to -0.67 to -0.77, still highly significant if examined for T_4 treatment only. Prohexadione-Ca was ineffective in reducing berry weight when applied after T_6 (Table 4). Accompanying this return to "normal" berry weight was a return to absorbance and PFGG values that were comparable to those of the control (Table 5).

Chardonnay individual cluster experiment, 2002. The bloom (T_3) prohexadione-Ca application significantly reduced both berry weight and berries per cluster (Table 4), while berry weight, but not berry set, continued to be negatively affected by treatments made at T2 (E-L 25) and T3 (E-L 29). The rates of berry development over time appeared similar among the control, T_2, and T_4 treatments (data not shown); however, T_2 had consistently smaller berries than the other two treatments.

Cabernet franc, 2001. Although the crop-thinned vines had significantly fewer clusters than did the unthinned treatment, that did not affect cluster weight or crop per vine (Table 6). Due to the lack of significant differences between the controls, treatments were compared to unthinned treatment. Only the 2pstb treatment differed significantly from either of the controls in terms of components of yield. Two postbloom prohexadione-Ca applications reduced crop per vine without any significant reduction in cluster weight or berries per cluster. The reduction in crop was principally due to a reduction in clusters.

Prohexadione-Ca did not affect fruit soluble solids, but all treatments increased pH (Table 7).

Table 4 Eichhorn and Lorenz (E-L) stage at treatment, berries per cluster, and berry weight of Cabernet Sauvignon and Chardonnay clusters treated with 250 mg/L prohexadione-Ca. Bloom (50% cap-fall, or E-L stage 23) is T_3 for Cabernet Sauvignon and T_1 for Chardonnay.

	Cabernet Sauvignon (2001)			Chardonnay (2002)		
Treatment[a]	E-L stage	Berries/ cluster	Berry wt (g)	E-L stage	Berries/ cluster	Berry wt (g)
T_0	na[b]	127.5 bc[c]	1.42 abc	na	198.5 a	1.68 a
T_1	18	44.1 d	1.25 c	23	89.7 c	1.08 c
T_2	21	0.2 e	0.86 e	25	139.1 ab	1.22 bc
T_3	23	4.5 e	0.32 g	29	173.7 ab	1.33 b
T_4	26	166.2 a	0.69 f	32	143.0 ab	1.62 a
T_5	27	132.0 abc	1.09 d	33	150.7 ab	1.79 a
T_6	29	133.4 abc	1.30 bc			
T_7	32	136.4 ab	1.47 ab			
T_8	33	108.1 bc	1.48 ab			
T_9	33	128.6 bc	1.48 ab			
T_{10}	33	131.7 abc	1.48 ab			
T_{11}	33	98.3 c	1.52 a			
T_{12}	34	108.8 bc	1.46 ab			
T_{13}	37	98.0 c	1.46 ab			

[a]Subscripts indicate week at which treatment was applied.
[b]na: not applicable.
[c]Means within columns followed by the same letter do not differ at $p \leq 0.05$.

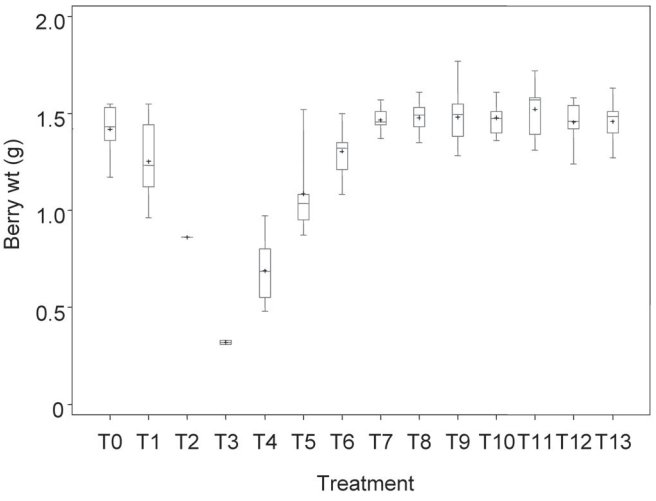

Figure 1 Cabernet Sauvignon berry weight at harvest as affected by single applications of 250 mg/L prohexadione-Ca to individual clusters at weekly (T value) intervals in 2001. Box whiskers indicate extreme values, the box bottom and top define the first and third data quartiles, the cross bar indicates the median, and + indicates the sample mean. T_0 is the untreated control, T_1 was applied one month after budbreak, and T_3 corresponds to 50% bloom.

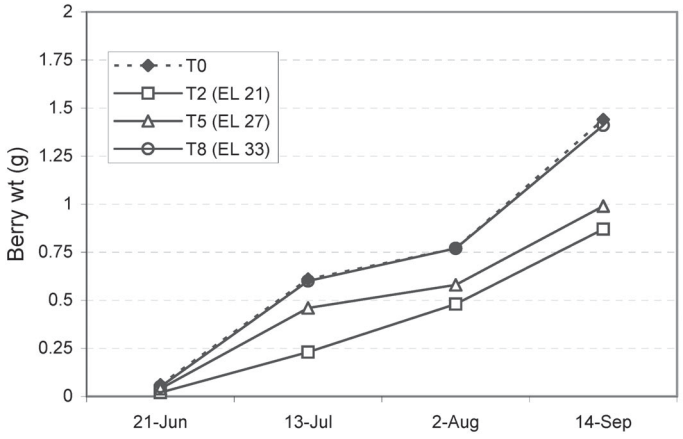

Figure 2 Average berry weights of 2001 Cabernet Sauvignon clusters treated with 250 mg/L prohexadione-Ca. T numbers indicate week of treatment application, followed by Eichhorn-Lorenz (E-L) stage at treatment. T_0 is the untreated control.

Table 5 Must soluble solids (Brix), pH, titratable acidity (TA), hue, total anthocyanins (anth), ionized anthocyanins, total phenols, color intensity, and phenol-free glycosyl-glucose (PFGG) for Cabernet Sauvignon individual clusters to which applications of 250 mg/L prohexadione-calcium were made in 2001.

Treatment	Brix	pH	TA (g/L)	Hue (420/520 nm)	Anth (mg/L)	Ionized anth (mg/L)	Total phenols (au)	Intensity (420+520 nm)	PFGG (μM)
T_0	21.1a-d[a]	3.87a	5.4a-d	0.74ab	95bc	16.5c	4.8bc	1.6bc	289.2bc
T_1	21.5ab	3.73b	5.4b-d	0.70b	103a-c	20.5ab	6.2b	2.0b	318.3bc
T_2	na[b]	na	na	na	na	na	na	na	na
T_3	na	na	na	na	na	na	na	na	na
T_4	21.0a-d	3.72b	6.1a	0.69b	139a	22.8a	10.3a	3.2a	542.6a
T_5	21.6a	3.82ab	5.6a-d	0.75ab	109ab	17.8bc	6.2b	2.1b	333.4b
T_6	20.6c-e	3.78ab	5.8ab	0.78ab	82bc	17.8bc	4.5bc	1.5bc	347.1b
T_7	20.5c-e	3.79ab	5.5a-d	0.70ab	83bc	18.0bc	3.6bc	1.5bc	219.3bc
T_8	20.2e-f	3.77ab	5.6a-c	0.77ab	80bc	16.4c	3.5bc	1.3bc	242.1bc
T_9	20.0ef	3.81ab	5.5a-d	0.75ab	83bc	15.0c	3.6bc	1.3bc	334.2b
T_{10}	20.9a-d	3.79ab	5.3b-d	0.78ab	72bc	14.4c	3.1bc	1.3bc	299.1bc
T_{11}	19.6f	3.80ab	5.1cd	0.78ab	69c	16.5c	3.2bc	1.1c	284.9bc
T_{12}	20.4c-e	3.86a	5.1cd	0.80ab	64c	15.8c	2.7c	1.1c	179.4c
T_{13}	20.7b-e	3.85a	4.9d	0.84a	70bc	16.2c	3.3bc	1.1c	292.5bc

[a]Means within columns followed by the same letter do not differ at $p \leq 0.05$.
[b]na: data not available.

Table 6 Components of yield for Cabernet franc treated with two prebloom (2prb), one postbloom (1pstb), or two postbloom (2pstb) applications of 250 mg/L prohexadione-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[a] (*p* value)					
UT vs T	<0.001	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

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

Titratable acidity was generally not affected with the exception of a decrease caused by the two postbloom applications. Two prebloom applications or a single postbloom application increased total anthocyanins, total phenols, and color intensity. Aside from pH and TA, fruit chemistry for 2pstb was not affected by treatment. There were no significant differences for PFGG with any treatment.

Wine analyses were similar among treatments and controls for ethanol, titratable acidity, and pH (Table 8). Prohexadione-Ca increased wine total anthocyanins, total phenols, and color intensity, particularly with the postbloom applications. Lacking replication, however, it is uncertain whether those differences were significant. The wine chemistry data are somewhat consistent with the must data (Table 7), one difference being the relative differences between the single and double postbloom

Table 7 Cabernet franc must soluble solids (Brix), pH, titratable acidity (TA), hue, total anthocyanins (anth), ionized anthocyanins, total phenols, color intensity, and phenol-free glycosyl-glucose (PFGG) as affected by two prebloom (2prb), one postbloom (1pstb), or two postbloom (2pstb) applications of 250 mg/L prohexadione-calcium during the 2001 season, compared to an unthinned (UT) and a thinned (T) control.

Treatment	Brix	pH	TA (g/L)	Hue (420/520 nm)	Anth (mg/L)	Ionized anth (mg/L)	Total phenols (au)	Intensity (420+520 nm)	PFGG (μM)
UT	22.3	3.44	5.35	0.85	108	11.9	17.9	5.5	298
T	22.6	3.51	5.50	0.97	107	4.3	17.5	5.2	577
2prb	23.0	3.49	5.38	0.76	153	4.1	21.6	6.9	289
1pstb	22.5	3.50	5.10	0.68	179	4.1	22.4	7.6	340
2pstb	22.6	3.51	4.86	0.84	113	4.0	18.8	5.6	233
Significance of contrast[a] (*p* value)									
UT vs T	0.212	0.035	0.425	0.428	0.976	0.134	0.836	0.634	0.190
UT vs 2prb	0.368	0.046	0.867	0.170	0.057	0.123	0.059	0.038	0.967
UT vs 1pstb	0.469	0.023	0.195	0.070	0.005	0.121	0.025	0.003	0.847
UT vs 2pstb	0.337	0.007	0.018	0.415	0.830	0.118	0.636	0.884	0.764

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

Table 8 Cabernet franc wine alcohol (ETOH), titratable acidity (TA), pH, total anthocyanins (anth), ionized anthocyanins, total phenols, color intensity and phenol-free glycosyl-glucose (PFGG) as affected by prohexadione-Ca treatments in 2001. Treatments included unthinned (UT) and crop-thinned (T) controls, and prohexadione-Ca applied twice prebloom (2prb), once postbloom (1pstb) or twice postbloom (2pstb) at 250 mg/L.

Treatment	ETOH (%)	TA (g/L)	pH	Anth (mg/L)	Ionized anth (mg/L)	Total phenols (au)	Intensity (420+520 nm)	PFGG (µM)
UT	11.30	7.48	3.40	377	20.7	28.6	6.3	104
T	11.95	7.63	3.47	390	22.5	31.3	7.1	110
2prb	11.70	7.50	3.43	384	22.8	30.3	6.9	101
1pstb	11.80	7.44	3.43	424	24.7	33.7	8.4	102
2pstb	12.00	7.68	3.44	473	37.6	40.8	13.4	113

Table 9 Cabernet franc triangle test of wine aroma and flavor comparing an unthinned control (UT) with either two prebloom applications (2prb), one postbloom application (1pstb), or two postbloom applications (2pstb) of 250 mg/L prohexadione-Ca in 2001.

Comparison	Panelists (n)	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

applications. Despite fruit and potential wine chemistry differences between the treatments, sensory panels could not distinguish differences among the wines in triangle tests (Table 9).

Discussion

Prohexadione-Ca had the potential to severely reduce fruit set and crop yield. This was particularly evident with prebloom (E-L stages 18 to 21) and bloom (E-L stage 23) applications. Reduced set was observed in *V. vinifera* cultivars as well as the interspecific cultivar Seyval. Reduced set appeared to result from destruction of developing flowers; the prebloom treatments caused browning and abscission of flowers and, at higher rates, abscission of entire clusters at the peduncle. No corresponding necrosis or other lesions were observed on foliage or other tissues, indicating that the effects of prohexadione-Ca were localized in reproductive tissues. Prohexadione-Ca inhibits the conversion of aminocyclopropanecarboxylic acid into ethylene, and its application *reduces* ethylene-induced senescence in several plant systems (Rademacher et al. 1992). The senescence and abscission of grapevine reproductive structures, therefore, is not easily explained as a response to reduced ethylene. The only prebloom applications that failed to reduce set were the two prebloom applications made to Cabernet franc in 2001, at E-L stages 15 and 17. Why those treatments failed to reduce set is uncertain. The prebloom treatment of Cabernet franc in 2000 was about one week before bloom and set was reduced. The second of the two prebloom treatments to Cabernet franc in 2001 was about two weeks before bloom. Thus, it might be that the Cabernet franc clusters were not yet sensitive to the prohexadione-Ca in the 2001 experiment.

Reduction in fruit set may have two benefits. One potential benefit would be a purposeful reduction in crop to increase fruit and wine quality. Because crop thinning typically is done manually, it is expensive. Hence, a chemical means of thinning could save money. The lack of berry set reductions with the 2001 Cabernet franc experiment suggests, however, that timing of prohexadione-Ca application is critical to achieve a predictable thinning effect.

A second potential benefit of reduced set might be realized as a reduction in bunch rots with rot-prone cultivars. We did not observe such a reduction during the course of these experiments; however, the overall incidence of rot was very low. Further investigations of the effects of prohexadione-Ca on berry set, berry size, and bunch rot susceptibility are warranted under more disease-conducive conditions.

The single-cluster Cabernet Sauvignon and Chardonnay experiments provided a more precise measure of when the clusters were sensitive to prohexadione-Ca application. The most significant effects were reduced fruit set for applications made before and during bloom, and reduced berry weight for the immediate postbloom applications. Variation in berries per cluster and berry weight might have been due to uneven distribution of spray material on the clusters, asynchrony of flower and berry development on a given cluster, or a combination of those factors. Because the single-cluster experiments targeted clusters, it is apparent that the clusters can directly absorb the prohexadione-Ca and that the effect is not mediated by foliar absorption. The severity of fruit-set reductions that we observed with prebloom and bloom applications was unexpected. The literature describing the effect of other 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 (Coombe 1965). To our knowledge, this is the first formal report of the effects of prohexadione-Ca on grape berry development, and information on the reproductive response with other fruits is meager. Prohexadione-Ca applications suppressed vegetative growth of apple tree shoots but had no effect on fruit, other than a slight increase in red coloration, which was attributed to increased light interception (Byers and Yoder 1999).

Only recently has the change in concentration of bioactive gibberellins during the course of berry development been studied. New literature describes a sharp increase in GA_3 levels 21 and 65 days after anthesis in seeded cultivars of *V. vinifera* (Perez et al. 2000). The reductions in berry weight observed with the immediate postbloom applications of prohexadione-Ca may reflect a depression of bioactive GA_3 in berries.

The 2000 Cabernet franc experiment illustrated that yield components declined linearly with increasing concentration of prohexadione-Ca. Ammonium sulfate alone might have caused some decrease in berry weight; however, other components of yield were unaffected by the addition of this water conditioner. In light of the improved response of apple to prohexadione-Ca when ammonium sulfate was added to the spray solution (Byers et al. 2000), we felt that it was prudent to use the conditioner until further evaluations could be conducted on grape.

The 375 mg/L treatment caused a particularly severe crop reduction with Cabernet franc in 2000. Surprisingly, a 250 mg/L rate of prohexadione-Ca application did not reduce Cabernet franc yield components in 2001. A possible reason for this discrepancy is that the vines in the 2000 experiment received three applications of prohexadione-Ca, compared to only 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 inconsistency of effects between years may also relate to the fact that the timing of the prebloom applications differed slightly between the two seasons. A further possibility is that environmental conditions (such as temperature and sunlight conditions) differed between seasons and mediated the treatment effects.

Prohexadione-Ca unexpectedly depressed subsequent season shoot fruitfulness. The clusters of a given season originate from anlagen of the previous season that differentiated as either tendril or inflorescence primordia (Mullins 1986). Cytokinins favor the differentiation of anlagen into cluster primordia and regulate the formation of individual florets on the developing cluster primordia in the year of anthesis (Mullins 1986). Many of the nitrogen-containing, heterocyclic growth regulators cause elevated levels of cytokinins in plant tissues (Rademacher 2000), so one might suspect that prohexadione-Ca would lead to increased cytokinins and possibly increased fruitfulness. In contrast, gibberellin applications during the differentiation of anlagen cause anlagen to form tendril primordia rather than inflorescence primordia (Srinivasan and Mullins 1980). Again, one might therefore expect that the application of a gibberellin biosynthesis inhibitor to the differentiating anlagen in 2000 would have increased the cluster number in 2001. We do not have an explanation for the reduced fruitfulness observed in the second season.

Prohexadione-Ca had minimal effects on fruit soluble solids, pH, or titratable acidity. Direct effects of prohexadione-Ca on starch, sugar, and color in apple also seem to be minimal or lacking (Byers and Yoder 1999).

In contrast to the minimal effects on fruit primary metabolites, prohexadione-Ca did appear to have some effects on the concentrations of some secondary metabolites. For example, increased levels of PFGGs were significantly and negatively correlated with berry weight in the Cabernet Sauvignon individual cluster experiment. Others have found sunlight exposure to affect fruit quality. For instance, leaf thinning to increase fruit-zone light exposure increased total glycosides 17 to 29% in Riesling (Zoecklein et al. 1998). Bureau et al. (2000) found that light exposure could increase the concentration of C_{13}-norisoprenoids approximately 40%. We believe, however, that the increased concentration of PFGG is more likely due to reduced berry size than to increased sunlight exposure, because we were unable to alter the light environment of grapevine canopies with prohexadione-Ca applications (Lo Giudice et al. 2003).

Our results suggest that anthocyanins might also be increased by prohexadione-Ca, and two possible mechanisms are offered. The more obvious explanation would be an increase in surface to volume ratio, resulting in an increase in anthocyanins on a per berry basis. A second possibility is that anthocyanin production is directly affected by alterations in the flavonoid biosynthetic pathway. While not measured, we did note an increased red coloration of Seyval (but not Chardonnay) berries that had been treated with prohexadione-Ca. Countering the direct effect hypothesis are reports that prohexadione-Ca and related growth retardants inhibit the formation of anthocyanins in some other crops (Rademacher et al. 1992, 2000). The basis of that inhibition appears to be impairment of flavanone 3-hydroxylase (FHX), a key enzyme in the synthesis of dihydroquercetin, leucocyanidin, and other flavonoids (Rademacher 2000). The inhibition of FHX in apple (*Malus* sp.) leads to a buildup of the novel flavonoids, luteoliflavan and eriodictyol (Roemmelt et al. 1999). Detailed analyses of the biosynthetic activity of prohexadione-Ca in grape have yet to be pursued.

Unlike the individual cluster treatments used with Cabernet Sauvignon, the color response of Cabernet franc must to prohexadione-Ca appeared unrelated to berry weight. For example, the two prebloom and the single postbloom applications were associated with increased absorbance values and increased total phenols, even though the berry size was not significantly reduced. Cabernet franc wine color appeared to be greatest for the two postbloom applications. These results may be due to the existence of a direct treatment effect independent of berry weight, as discussed above. It is also possible that slight variations in fermentation conditions affected the wine chemistry results. The use of replicated winemaking lots in the future may help identify whether the observed effects are reproducible.

Despite some differences in juice and wine composition associated with prohexadione-Ca treatments, the sensory

panelists could not distinguish Cabernet franc control from treatment wines. Wines were very young when judged and subtle differences may not have emerged or been perceptible to an untrained panel.

Conclusion

Prohexadione-Ca reduced fruit set and berry weight in a rate- and timing-dependent fashion. With the exception of Cabernet franc in 2001, prebloom and bloom applications had a particularly depressive effect on fruit set, while immediate postbloom applications had less effect on fruit set but a more depressive effect on berry size. Prohexadione-Ca may therefore provide a useful means of reducing berry size or reducing fruit set where those responses are desired.

The reductions in berry weight corresponded to higher phenol-free glycosyl-glucose (PFGG) concentrations, greater total phenols, and greater color intensity of Cabernet Sauvignon must. Similar responses were measured with Cabernet franc musts, although PFGG concentrations were insignificantly affected. Many aroma and flavor precursors are glycosidically bound in must and young wine. Although an increase in must PFGG might lead to improved wine sensory characteristics, we were unable to demonstrate sensory improvements from prohexadione-Ca with our panelists in young Cabernet franc wines. Because Cabernet franc fruit and wine chemistry results were independent of berry weight, the possibility of a direct treatment effect, unrelated to berry size, is plausible.

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