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Vegetative Response of *Vitis vinifera* to Prohexadione-calcium

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Abstract. **Prohexadione-calcium (prohexadione-Ca) was evaluated for its ability to suppress vegetative growth of grapevines (*Vitis vinifera* L.) under field conditions. Two or three applications of 250 mg·L^{-1} prohexadione-Ca reduced primary shoot growth of 'Cabernet Sauvignon', but had little effect on other canopy characteristics or cane pruning weights. The reduction of shoot growth was not persistent and shoot hedging was ultimately needed to avoid canopy shading. Similarly, three applications of either 125, 250, or 375 mg·L^{-1} prohexadione-Ca reduced 'Cabernet franc' shoot growth, but again did not eliminate the need for shoot hedging. Cane pruning weights of 'Cabernet franc' were unaffected by treatment, and canopy characteristics were generally not improved. Two prebloom and one postbloom application of 250 mg·L^{-1} prohexadione-Ca were evaluated on 'Cabernet franc' and 'Chardonnay' in separate field experiments. The prebloom treatments retarded shoot growth of 'Chardonnay', but had no effects on 'Cabernet franc' shoot characteristics. To retard shoot growth, prohexadione-Ca had to be applied prior to bloom; however, prebloom applications had the potential for severe reductions in crop yield.**

Excessive canopy shade can reduce grape and wine quality due to reductions in some of the light-dependent constituents of fruit (Dokoozlian and Kliewer, 1995, 1996). In addition, dense canopies may increase the incidence of fruit rots favored by increased humidity, leaf wetness, and reduced wind speeds measured within canopies (English et al., 1989). Current industry canopy management practices for large, vigorous grapevines prescribe conversion to more elaborate trellis systems to expose more of the foliage and fruit to sunlight, and use of remedial procedures such as shoot and leaf thinning to avoid shade (Dokoozlian and Kliewer, 1995; Smart and Robinson, 1991; Zabadal and Dittmer, 1998).

Gibberellin biosynthesis inhibitors have been evaluated since the early 1960s for their ability to restrict vegetative growth and to achieve the goals currently accomplished by manual canopy manipulation (Coombe, 1967; Kumar et al., 1998; Reynolds et al., 1992). Quaternary ammonium compounds such as chlormequat chloride (Cyclocel™ or CCC) and triazole-type compounds such as paclobutrazol were also extensively evaluated (Coombe, 1967; Kumar et al., 1998; Reynolds et al., 1992; Wolf et al., 1991). Although these compounds were never registered for use 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.

Prohexadione-calcium (prohexadione-Ca) (3-oxido-4-propionyl-5-oxo-3-cyclohexene-carboxylate) is a gibberellin biosynthesis inhibitor with a different mode of action from that of quaternary ammonium compounds and triazole-type growth regulators (Evans et al., 1999; Graebe, 1987; Hedden, 1988; Rademacher, 2000). Previous studies in Virginia indicated that prohexadione-Ca suppressed vegetative growth of apple trees (*Malus* ×*domestica* Borkh.) when applied at petal fall and reapplied at 14- to 21-d intervals (Byers and Yoder, 1999). The ability of prohexadione-Ca to suppress vegetative growth of apple (Owens and Stover, 1999; Yoder et al., 1999) and pear (*Pyrus communis* L.) (Costa et al., 2001) has led to its use in the control of fire blight, caused by *Erwinia amylovora*. Timing of application and choice of cultivar had significant effects on vegetative development in apple. Positive results from these and other studies (Guak et al., 2001; Unrath, 1999) led to the registration of a commercial formulation of prohexadione-Ca, "Apogee"®, on apple.

Virginia's climate and soils favor sustained and often excessive vegetative growth of grapevines (Wolf and Poling, 1995). Considerable manual effort, including selective leaf removal and repeated shoot hedging, is expended in altering canopy architecture to minimize mutual leaf shading and fruit shading (Smart and Robinson, 1991). The objective of the experiments reported here was to determine if prohexadione-Ca could effectively suppress vegetative growth of vigorous *V. vinifera* cultivars. To our knowledge, this is the first formal report of the evaluation of prohexadione-Ca on grape.

Materials and Methods

General. Prohexadione-Ca was applied as Apogee® (27.5% prohexadione-Ca) (BASF Corp., Research Triangle Park, N.C.) to 'Cabernet Sauvignon', 'Cabernet franc', and 'Chardonnay' grapevines during the 2000 and 2001 seasons. Except as indicated, all treatments included Regulaid® (KALO, Overland Park, Kans.) surfactant (polyoxyethylenepolypropoxypropanol and alkyl 2-ethoxethanol dihydroxy propane) at 0.13% (v/v) and ammonium sulfate, $(NH_4)_2SO_4$, at 900 mg·L^{-1}. Ammonium sulfate has been used to prevent the deactivation of prohexadione-Ca by calcium or other cations in hard water (Byers et al., 2000). Treatments were applied to both sides of the canopy to point of runoff. Spray volume increased with increasing vegetative growth to ensure complete coverage. For each application date, spray volume was uniform between treatments. Aqueous solutions applied to the control plots included the two adjuvants in concentrations equal to those in the treatment applications.

'Cabernet Sauvignon' 2000. Nine-year-old 'Cabernet Sauvignon' (FPMS clone #7) vines, grown at the Alson H. Smith Jr. Agricultural Research and Extension Center (AHSAREC) in Winchester, Va., were used. Vines were spaced 2.1 m apart in rows 3.7 m wide and were cordon-trained (quadrilateral) and spur-pruned on a lyre, divided canopy trellis system. Treatments consisted of an untreated control and prohexadione-Ca applied twice (2×P-Ca) or three times (3×P-Ca). The 2×P-Ca treatment was sprayed prebloom (15 and 31 May). The 3×P-Ca treatment included those two prebloom applications plus a postbloom application on 30 June. Full bloom occurred 5 June. Applications were made with a hand-held pressure sprayer. Treatments were applied to three-vine plots, each replicated five times in a completely randomized design. Treatment plots were bounded by buffer plots within the row, and care was taken to avoid inter-row spray drift. Given the spray volume, we calculated that each vine received 315 mg a.i. on 15 May and 505 mg a.i. on 31 May and 30 June.

Shoot length and leaf area, point quadrat analyses (PQA) of canopies, photosynthetic photon flux density (*PPFD*) within fruit zones, and cane pruning weights were measured as vegetative response variables. For shoot length measurements, 24 shoots of uniform length, and representative of the average canopy height, were selected per treatment plot prior to the first treatment application. These shoots were equally divided between the east and west canopies of the north–south oriented rows. Their lengths were measured on 16 May, 30 May, and 14 June. Shoot length measurements were discontinued after 14 June due to the need to hedge the shoots to keep shoot tops from shading the basal portions of canopies.

The PQA for quantifying canopy density was similar to that described by Smart and Robinson (1991). A total of 21 measurements in the fruit zone, each 8 cm apart, were taken for each of the two canopies (east and west) of each plot. The frequency of probe contacts with fruit and leaves, as well as canopy gaps, was recorded, from which leaf layer number, percent exterior fruit, and percent gaps were calculated.

Photosynthetic photon flux density measurements were made with a 1.0-m line quantum sensor (model LI-191SB; LI-COR, Lincoln, Nebr.) held in the fruit zone. Measurements were made between 1100 and 1600 EDT on 17 Aug. Sky conditions were clear, but hazy. Due to the structure of the lyre canopy, six readings were taken within each three-vine

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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. 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 fruit zone light readings were divided by the ambient readings to provide a value for the percentage of ambient light in the fruit zone.

Leaf area was measured between 23 Aug. and 12 Sept. To calculate leaf area, two shoots of similar and representative length, one from each side of the horizontally divided canopy, were selected per vine (30 shoots per treatment). Leaf area was recorded with an area meter (LI-3000, LI-COR). Specifically, the number of primary nodes, primary leaves, lateral shoots, lateral leaves, and primary and lateral leaf area were recorded for each shoot. Cane pruning weights were recorded by vine at dormant pruning.

Detailed fruit sampling, including crop yield and fruit composition, was also performed (Lo Giudice, 2002), but will be reported elsewhere; however, crop per vine is included here.

'Cabernet franc' 2000. Four-year-old 'Cabernet franc' vines, grown at Indian Springs Vineyard in Woodstock, Va., were used. Vines were cordon-trained and spur-pruned with the canopy vertically shoot positioned (VSP) in an upward fashion with the aid of foliage catch wires. Row × vine spacing was 2.7 × 2.1 m. The experimental design was completely randomized, with each treatment consisting of 10 single-vine replicates, each bounded by two or more buffer vines within the row. Treatments included two controls and prohexadione-Ca applied at either 125, 250, or 375 $mg·L^{-1}$. To determine the effect of ammonium sulfate in the spray mixture, one control was treated with ammonium sulfate and Regulaid, the other with Regulaid only, at the rates indicated under the *General* heading. Treatments were applied once prebloom (18 May) and twice postbloom (1 and 30 June). Full bloom occurred 26 May.

Vegetative response variables included shoot length measures, canopy density, and cane pruning weights. Shoot length was measured on two representative shoots per vine on 18 May and 1 and 15 June. After 15 June, length measurements were discontinued due to summer pruning. The procedures used for the PQA were similar to those described for 'Cabernet Sauvignon', except that only six, regularly spaced probes were made per vine. Cane pruning weights were recorded at dormant pruning. Crop was harvested by vine at the time of commercial harvest in the host vineyard.

'Cabernet franc' and 'Chardonnay' 2001. 'Cabernet franc' and 'Chardonnay' experiments were conducted identically, except where noted. The experiments used 5-year-old, spur-pruned, cordon-trained 'Cabernet franc' and 4-year-old 'Chardonnay', both grown at Indian Springs vineyard in Woodstock, Va.. Vines were spaced 2.1 m apart in 2.7-m-wide rows and were cordon-trained with VSP canopies. Treatments for each cultivar included a control and three prohexadione-Ca treatments. Treatment 2×-prebloom comprised two prebloom (7 and 23 May) applications of prohexadione-Ca. Treatment 1×-postbloom entailed a single postbloom application (19 June). Bloom (≈75%) occurred on 7 June with 'Cabernet franc' and on 5 June with 'Chardonnay'. Excepting the control, prohexadione-Ca was applied at 250 $mg·L^{-1}$ for all treatments, and Regulaid and ammonium sulfate were included with all treatments at rates indicated above. Experimental units consisted of four-vine plots, replicated five times in a completely randomized design, with buffer panels. Spray applications were made to both sides of the canopy and were concentrated on the fruit zone, an area defined, at its lowest point, by the cordon wire and extending vertically upwards 0.5 m. The first prebloom application covered the canopy in entirety due to the short length of the shoots. The amount of prohexadione-Ca a.i. per vine per application date was 47.4 mg for each of the two prebloom applications, and 71.2 mg for each of the two postbloom applications. Shoot lengths were measured on 3 July for the 2×-prebloom, the 1×-postbloom, and the control. Two shoots were selected per vine, one from each arm of the bilateral cordon. Nodes per shoot were also recorded to determine average internode length.

Data analysis. 'Cabernet Sauvignon' PQA, *PPFD*, leaf area, and pruning weight data were evaluated with single degree of freedom contrasts following analysis of variance (ANOVA). 'Cabernet franc' PQA values were evaluated with single degree of freedom contrasts as well as linear regression analysis. Shoot growth for the 2000 experiments was analyzed using repeated measures ANOVA. The 'Cabernet franc' and 'Chardonnay' 2001 shoot growth data were evaluated using single degree of freedom contrasts. All analyses were performed using SAS, v. 8e software (SAS Institute, Cary, N.C.).

Results and Discussion

'Cabernet Sauvignon' 2000. Prohexadione-Ca inhibited 'Cabernet Sauvignon' shoot growth in the second, but not the first measurement period of 2000. Shoot length of control shoots averaged 55 cm on 16 May, 94 cm on 30 May, and 147 cm on 14 June. By contrast, shoots of vines treated with two applications of 250 $mg·L^{-1}$ proxhexadione-Ca averaged 54, 90, and 117 cm on those same dates. There was a significant ($P \leq 0.05$) treatment effect observed with the above growth rates for the period from 30 May to 14 June. Because the 3×P-Ca treatment was made 2 months after the primary shoot measurements stopped, no assessment of effects on shoot growth was made. Prohexadione-Ca did not reduce the need for summer pruning or shoot hedging with these vigorous grapevines.

A slight increase in leaf layer number due to prohexadione-Ca was measured, with no differences in the percentage of exposed fruit, canopy gaps, or between frequency (2× vs. 3×) of prohexadione-Ca application (Table 1). Similarly, no differences in *PPFD* values were measured as a function of treatment. The leaf layer numbers for all treatments were within the range of "acceptable" values (Smart, 1985), but somewhat greater than the optimum (one layer) proposed by Smart and Robinson (1991). Two possible explanations for the increase in fruit zone leaf layer number were increased lateral leaves and leaf area per shoot (Table 2), and decreased primary shoot internode length. Reduced internode length was observed in other studies where gibberellin biosynthesis inhibitors were evaluated on grapes (Coombe, 1967). Dormant cane prunings were similar among treatments (Table 1).

Both prohexadione-Ca treatments substantially reduced crop per vine (Table 1), which was due to a reduction in berry weight and berries per cluster (Lo Giudice, 2002). Given the lack of pruning weight differences, crop load decreased in tandem with decreased crop per vine (Table 1). The 2× prohexadione-Ca treatment reduced crop more than the 3× treatment did; however, we do not have a meaningful explanation for that response.

Primary leaf *number* per shoot was not influenced by prohexadione-Ca, but primary leaf *area* per shoot, and per leaf, were both reduced by prohexadione-Ca (Table 2). Lateral leaf area per leaf was also reduced by prohexadione-Ca (Table 2). Interestingly, the total number of lateral leaves per primary shoot was increased

Table 1. 'Cabernet Sauvignon' canopy characteristics, cane pruning weights and crop per vine in response to two (2×P-Ca) and three (3×P-Ca) 250 $mg·L^{-1}$ prohexadione-Ca applications during the 2000 season. Leaf layer number, percent external fruit, and percent canopy gaps were calculated from point quadrat analyses (PQA) of canopy fruit zones.

	PQA				Cane pruning wt		
Treatment	Leaf layer no.	External fruit %	Canopy gaps %	Fruit zone *PPFD* % of ambient light ($\mu mol·m^{-2}·s^{-1}$)	Cane pruning wt (kg/vine)	Crop load (kg crop/ kg pruning wt)	Crop per vine (kg)
Control	1.8	52	3	2.9	4.6	2.0	9.24
2xP-Ca	2.3	64	3	3.4	4.3	0.7	2.75
3xP-Ca	2.1	54	2	3.1	4.4	0.9	3.71
			Significance of contrast[z] *(P-value)*				
Control vs. mean of 2× and 3×P-Ca	0.010	0.448	0.866	0.444	0.250	<0.0001	<0.0001
2×P-Ca vs. 3×P-Ca	0.120	0.325	0.769	0.448	0.750	0.087	0.024

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

Table 2. ‘Cabernet Sauvignon’ primary and lateral leaf a areas affected by two (2×P-Ca) and three (3×P-Ca) applications of prohexadione-Ca during the 2000 season.

Treatment	Primary leaves/shoot	Primary leaf area/leaf (cm^2)	Primary leaf area/shoot (cm^2)	Lateral shoots/shoot	Lateral leaves/ primary shoot	Lateral leaf area/leaf (cm^2)	Lateral leaf area/ primary shoot (cm^2)
Control	14	125	1691	8.5	44	43	1889
2×P-Ca	13	109	1397	10.2	72	32	2358
3×P-Ca	12	112	1283	9.0	62	33	2117
			Significance of contrast[z] *(P-value)*				
Control vs. mean of 2x and 3x P-Ca	0.263	0.062	0.003	0.152	0.044	<0.0001	0.340
2×P-Ca vs. 3×P-Ca	0.322	0.731	0.349	0.218	0.469	0.762	0.599

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

by prohexadione-Ca. Thus, the total lateral leaf area was almost double the primary leaf area per shoot for the prohexadione-Ca treatments.

‘Cabernet franc’ 2000: Shoot growth rate exhibited a negative, linear response to increasing prohexadione-Ca concentration, but only in the second period of measurement (Fig. 1). The 375 mg·L^{-1} concentration caused the greatest reduction in growth, an average of 20 cm of shoot length by 15 June ; however, as was the case with the ‘Cabernet Sauvignon’, the reduction was not sufficient to eliminate the need for shoot hedging of these vines by early July. That is, shoot tops had exceeded the height of the trellis and had begun to cascade over the vertically shoot-positioned portion of the canopy.

Leaf layer number was unaffected by treatment (Table 3). Although a quadratic response to increased prohexadione-Ca rate was observed for the percent exposed fruit and for canopy gaps, those responses were not readily interpretable. Ideal canopies are considered to have 20% or more canopy gaps (Smart and Robinson, 1991). The lack of external fruit contacts with the 375 mg·L^{-1} rate had more to do with the reduction in cluster number with that treatment (Lo Giudice, 2002), than with cluster shade. Cane pruning weights varied little among treatments (Table 3) and, although prohexadione-Ca decreased primary shoot growth (Fig. 1), there was no corresponding decrease in pruning weight. By contrast, we reduced the cane pruning weights of ‘Seyval’ (interspecific hybrid) grapevines from 0.9 kg/vine for controls to 0.7 kg/vine (P < 0.012) for vines treated three times with 250 mg·L^{-1} prohexadione-Ca (Lo Giudice, 2002). There are several potential explanations why prohexadione-Ca did not reduce vine size with the *V. vinifera* cultivars. First, reductions in early-season shoot growth might have been insufficient to affect dormant pruning weight given the uniform shoot hedging that vines received, and the subsequent lateral shoot growth. Another possible reason for a lack of decreased pruning weight is the reduction in crop yield per vine. ‘Cabernet franc’ crop yield was reduced by prohexadione-Ca, with the 375 mg·L^{-1} treatment eliciting the greatest reduction (Table 3). Because a reduction in crop can stimulate vegetative development (Howell, 1999; Miller et al., 1993), any depressive effects of prohexadione-Ca on pruning weight might have been countered by a stimulation of shoot growth due to a lighter crop (Lo Giudice, 2002); however, it should be emphasized that pruning weights were very similar (1.2–1.4 kg/vine) across all treatments. The reduction in crop per vine was substantial and was due to a combination of decreased berry size and berries per cluster, but also a direct loss of clusters with the highest rate of prohexadione-Ca (Lo Giudice, 2002).

The addition of ammonium sulfate as a spray conditioner had no influence on any of the responses reported here. Ammonium sulfate has been used in apple studies to prevent the deactivation of Apogee by calcium in hard water (Byers et al., 2000). The cation content

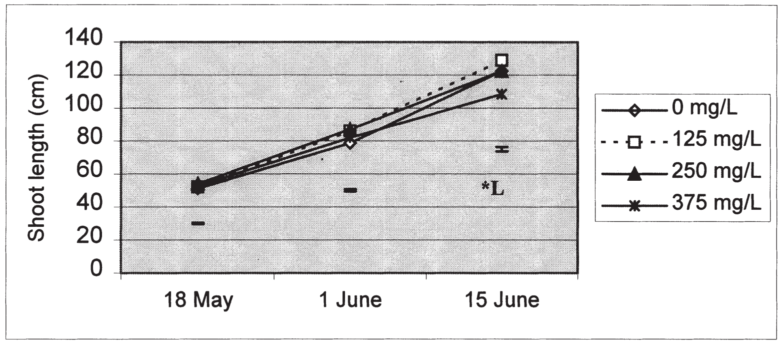

Fig. 1. Primary shoot length of ‘Cabernet franc’ subjected to four rates of prohexadione-Ca in 2000. Shoot extension decreased linearly with increased prohexadione-Ca concentration in the period from 1 to 15 June. Vertical bars are ±SE for combined treatments at each date.

Table 3. Leaf layer number, percent external fruit, percent canopy gaps, and cane pruning weights of ‘Cabernet franc’ as affected by three rates of prohexadione-Ca (P-Ca) and controls that either contained (+N) or did not contain (–N) ammonium sulfate during the 2000 season.

	PQA					
Treatment	Leaf layer no.	External fruit (%)	Canopy gaps (%)	Cane pruning wt (kg/vine)	Crop load (kg crop/ kg pruning wt)	Crop per vine (kg)
–N	2.5	45	0	1.2	4.5	5.4
+N	2.7	28	0	1.2	4.0	4.9
P-Ca 125 mg·L^{-1}	2.2	33	0	1.3	1.9	2.4
P-Ca 250 mg·L^{-1}	2.6	40	0	1.3	1.1	1.4
P-Ca 375 mg·L^{-1}	2.6	0	7	1.4	0.4	0.5
			Significance of contrast[z] *(P-value)*			
+N vs. –N	0.547	0.277	1.000	0.718	0.154	0.173
+N vs. mean of treatments	0.987	0.095	0.233	0.272	0.000	0.000
Linear	0.698	0.130	0.011	0.320	<0.0001	0.000
Quadratic	0.251	0.046	0.052	0.610	0.001	0.001

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

Table 4. ‘Cabernet franc’ and ‘Chardonnay’ shoot characteristics on 3 July 2001 as affected by two prebloom or one postbloom application of 250 mg·L^{-1} prohexadione-Ca, compared to a control.

	‘Cabernet franc’			‘Chardonnay’		
Treatment	Shoot length (cm)	Node no.	Internode length (cm)	Shoot length (cm)	Node no.	Internode length (cm)
Control	120.9	21.9	5.3	116.6	21.4	5.2
2×-prebloom	117.4	22.6	5.0	69.6	14.5	4.5
1×-postbloom	139.7	24.2	5.7	100.5	20.4	4.6
			Significance of contrast[z] *(P-value)*			
2×-prebloom vs. control	0.750	0.560	0.241	<0.0001	<0.0001	0.028
1×-postbloom vs. control	0.096	0.066	0.179	0.167	0.481	0.077
2×-prebloom vs. 1×-postbloom	0.047	0.196	0.013	0.009	<0.0001	0.658

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

of the water used in the 'Cabernet franc' study was not assessed, but the results suggest that high calcium concentration was not an issue.

'Cabernet franc' and 'Chardonnay', 2001. There was no difference in shoot length, internode length, or node number between the control and either the 2× prebloom or the single postbloom treatments for 'Cabernet franc' (Table 4); however, the postbloom treatment had greater shoot and internode length on 3 July compared to the 2× prebloom treatment. The 2× prebloom treatment reduced 'Chardonnay' shoot length, node number, and internode length, relative to the control, when measured on 3 July (Table 4). The single postbloom application, by contrast, had no impact on these responses. Despite the early-season retardation of growth, most of the shoots of the 2× prebloom treatment, like the controls, required hedging in late-July.

Our experiments demonstrated that while prohexadione-Ca does have the potential to reduce primary shoot growth, the growth reduction was insufficient to eliminate the summer pruning normally practiced with vertically shoot-positioned training under Virginia conditions. The greatest reduction in growth occurred when vines were treated prebloom, but that timing also resulted in substantial crop loss (Lo Giudice, 2002). Cultivars of *V. vinifera* differed somewhat in their response to prohexadione-Ca in terms of shoot growth and canopy architecture. In apple, prohexadione-Ca was more effective at suppressing vegetative growth with low-vigor, heavily cropped trees, compared to high-vigor trees, and peach trees were relatively unresponsive (Byers, unpublished, personal communication; Byers and Yoder, 1999). Grapevines, and specific grapevine cultivars, may be less responsive to prohexadione-Ca than apple in terms of vegetative growth suppression. Besides the influence of cultivar, frequency and rate of application also impacted grape vegetative development. Higher rates and greater frequency of applications might produce greater reductions in shoot growth; however, it will be essential to fully evaluate a range of prebloom applications on grape yield as well.

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