Effect of Prohexadione-Calcium Dose Level on Shoot Growth and Fire Blight in Young Apple Trees

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ABSTRACT

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Prohexadione-calcium suppresses both shoot growth and fire blight in apple. In young apple orchards, there are conflicting requirements to control fire blight and allow sufficient tree growth for tree establishment. Application of prohexadione-calcium to various cultivars of orchard-grown apple trees ranging in age from newly planted to fifth-leaf trees indicated that fewer high-dose (125 or 250 mg·liter^{-1}) applications of prohexadione-calcium provided a better balance between fire blight control and growth in young orchards than multiple low-dose (30 or 63 mg·liter^{-1}) applications. The response of early-season shoot growth to prohexadione-calcium treatment dose was linear. However, trees that received high doses of prohexadione-calcium tended to grow more in the latter part of the season, resulting in little or no difference in total seasonal growth between trees that received a few high or multiple low doses of prohexadione-calcium. Enhancement of fire blight resistance by prohexadione-calcium was correlated with shoot growth suppression at the time of inoculation, and the resistance response to prohexadione-calcium treatment dose was linear. Fire blight management strategies that use prohexadione-calcium in young apple orchards are discussed.

Additional keyword: Apogee, *Erwinia amylovora, Malus* × *domestica*, plant growth regulator, shoot blight

Prohexadione-calcium (Phd-Ca) is a plant growth regulator that reduces longitudinal shoot growth by inhibiting gibberellin biosynthesis (4,16,20). On apple, controlling vegetative growth with Phd-Ca also reduces the incidence and severity of fire blight shoot infection caused by the bacterium *Erwinia amylovora* (3,10,12,15, 18,29). Phd-Ca does not have antibacterial activity against *E. amylovora* but increases host resistance by reducing plant vigor. In addition, treatment of apple with Phd-Ca results in alteration of phenylpropanoid biosynthesis pathways that may also enhance resistance (9,20–22).

Suppression of apple shoot growth by Phd-Ca requires application near petal fall as a single spray or as multiple sprays over an extended period (14). The growth response to Phd-Ca treatment is dependent on several factors, including total dosage applied, application timing, crop load, cultivar treated, and geographic region (14,26).

Fire blight in newly planted orchards can be particularly devastating because infections of young trees can result in complete tree death or significant reductions of bearing surface (18). Early productivity and economic success of high-density apple plantings is dependent upon rapid growth of young trees so that they fill their within-row space in the first few years after planting. Although Phd-Ca may limit fire blight damage in newly planted orchards, it may also have negative effects on young trees due to reduced canopy development. The use of reduced Phd-Ca doses has sometimes been recommended on young apple trees in an attempt to balance the benefit of shoot blight control against the drawback of reduced shoot growth (2).

The goal of this research was to determine if the use of reduced Phd-Ca dosage is a useful management strategy to provide protection against the shoot blight phase of fire blight on young apple trees.

MATERIALS AND METHODS

Experimental design and treatment application. Studies were conducted in 2001, 2002, and 2003 on orchard-grown trees of four apple cultivars that ranged in age from newly planted to trees in the fifth season of growth (referred to as “fifth-leaf” or 4-year-old trees) with a total of 10 cultivar-age treatment groups (Table 1).

In established trees, Phd-Ca treatments were applied as two high-dose or as multiple low-dose applications. The effective cumulative dose (ECD), defined as the sum of the individual spray applications in mg·liter^{-1} for a given treatment, was used to compare the magnitude of different Phd-Ca treatments. In 2001, the high-dose Phd-Ca treatment was 250 mg·liter^{-1} followed by a 125-mg·liter^{-1} application, and in 2002 and 2003, the high-dose treatment was two 125-mg·liter^{-1} applications. Low-dose treatment in 2001 was five applications of Phd-Ca at 63 mg·liter^{-1}, and in 2002 and 2003, the low-dose treatment was three applications at 30 mg·liter^{-1}. An additional low-dose Phd-Ca treatment of three applications at 63 mg·liter^{-1} was applied in 2002 to fifth-leaf ‘Royal Gala’ and ‘Ramey York’ trees. In established orchards, Phd-Ca was first applied when shoot growth averaged 5 to 8 cm, which corresponded to a growth stage ranging from late petal fall to 10 days after petal fall, and successive sprays were applied at approximately 2-week intervals (Table 1).

In the newly planted trees (‘Sun Fuji’) in 2002, a single application of Phd-Ca was made 37 days after planting. The high-dose treatment was 125 mg·liter^{-1}, and the low-dose treatment was 30-mg·liter^{-1}. In 2003, the high-dose treatment was 125 mg·liter^{-1} followed in 2 weeks by a 60-mg·liter^{-1} application. The low-dose treatment was two sprays of 30 mg·liter^{-1} each 14 days apart.

For all cultivar-age treatment groups, the negative control was a no-spray treatment. In 2001, streptomycin at 100 mg·liter^{-1} was applied 24 h before shoot inoculation and 72 h after inoculation. For each spray treatment in both established and newly planted orchards, trees were either not inoculated to evaluate the effect of treatment on growth or inoculated with *E. amylovora* (see below) to evaluate the effect of treatment on fire blight susceptibility, resulting in two experimental treatments for each spray treatment. Experimental treatments were assigned in a randomized complete block design with eight whole-tree replications.

The experiments were conducted in three orchards located at the USDA, ARS,

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Appalachian Fruit Research Station, Kearneysville, WV. Fourth- (2001) and fifth- (2002) leaf 'Royal Gala' and 'Ramey York' apple trees were planted in a single orchard on Malling 26 rootstock at a 2.4 × 6.1 m spacing, grown under recommended commercial orchard practices (2,19), and trained to a central leader form. Trees that received negative control and high-dose Phd-Ca treatments in 2001 received the same treatments in 2002; trees that received five applications of 63 mg·liter^{-1} Phd-Ca in 2001 received three applications of 63 mg·liter^{-1} Phd-Ca in 2002; and trees that received streptomycin treatment in 2001 received three applications of 30 mg·liter^{-1} Phd-Ca in 2002. Third- (2002) and fourth- (2003) leaf 'Ramey York' and 'Enterprise' apple trees were planted in another orchard on Malling Merton 111 rootstock with a Malling 9 interstem at a 6.7 × 4.9 m spacing and grown under recommended commercial orchard practices as freestanding central leader trees. A third orchard of 'Sun Fuji' on Budagovsky 9 rootstock was planted at a spacing of 1.5 × 6.1 m. At the time of planting, trees with no feathers (lateral shoots originating from the central leader) were cut back to approximately 70 cm above the graft union. Trees with at least three good feathers were cut back to approximately 30 cm above the topmost feather. After lateral buds began to grow, the first two to three shoots below the leader were removed.

All Phd-Ca treatments were Apogee 27.5 DF (BASF Corp., Research Triangle Park, NC) applied at the specified concentration of active ingredient with a handgun piston-pump sprayer to wet the tree canopy to the point of spray drip. A nonionic adjuvant, Regulaid (Kalo Inc., Overland Park, KS), was included in all sprays at 0.125% (vol/vol) along with spray-grade ammonium sulfate as a water conditioner on a weight basis equivalent to the weight of Apogee in the spray solution. The final spray solution was adjusted to approximately pH 6.0 with distilled white vinegar (National Fruit Products Co., Winchester, VA). To control the blossom blight phase of fire blight, all established trees received a dormant copper spray (Tenn-Cop 5E, Griffin L.L.C., Valdosta, GA) applied at 3.5 liters·ha^{-1} followed by streptomycin (Agri-Mycin 17, Syngenta, Greensboro, NC) spray(s) as needed during bloom at 1.3 kg·ha^{-1}. The need for streptomycin sprays during bloom was based on the Maryblyt predictive model (1). Trees used in the 2001 experiment received a foliar boron (Solubor, U.S. Borax, Inc., Valencia, CA) spray at 4.5 kg·ha^{-1} at petal fall (7 May) and first cover (16 May). Calcium chloride (DowFlake, Manley Regan Chemicals, Middletown, PA) at 6.7 kg·ha^{-1} was included in all cover sprays beginning with the fourth (29 June) in 2001. Boron or calcium chloride was not applied to experimental trees in 2002 or 2003. Maintenance sprays for pest control were based on local recommendations for commercial apple orchards (19).

Evaluation of tree growth. The effect of Phd-Ca dose on season-long shoot growth and yield were determined on both inoculated and noninoculated trees. To determine the effect of Phd-Ca on shoot growth in 2001, 10 noninoculated terminal shoots were selected at random from the periphery of the tree on 30 October. In 2002 and 2003, mean shoot growth was determined on terminal shoots selected at random from the tree canopy and measured at the time of the initial spray application, and again at selected times during the growing season. Twenty shoots were measured on fifth-leaf 'Royal Gala' and 'Ramey York' at the time of the initial spray application and again on 6 June, 1 July, and 15 November. On third-leaf 'Ramey York' and 'Enterprise', 10 to 20 shoots, depending upon the number of terminal shoots available, were measured at the time of the initial Phd-Ca application and again on 6 June, 1 July, and 19 November. In the newly planted 'Sun Fuji' orchard, the length of the top five shoots (leader plus new growth of top four lateral shoots) was recorded on 12 June, 15 July, 14 August, and 13 November. Yield was determined by harvesting and weighing all fruits from each tree at the time of commercial harvest.

Results were subjected to an analysis of variance (SuperANOVA, Abacus Concepts, Berkeley, CA) and treatment means separated using Tukey's studentized range test. Linear regression analysis was used to quantify the effect of Apogee dose on shoot growth and yield.

In addition, the length of the current season's shoot growth was measured on inoculated shoots at the time of inoculation with *E. amylovora* (see below). Results were subjected to an analysis of variance and Tukey's studentized range test as described below.

Table 2. Effect of prohexadione-Ca (Phd-Ca) sprays on shoot growth of fourth-leaf 'Royal Gala' and 'Ramey York' apple trees in 2001

Treatment[x]	ECD[y] (mg·liter^{-1})	Shoot growth (cm) 'Royal Gala'	Shoot growth (cm) 'Ramey York'
No treatment	0	53.4 a[z]	53.7 a
Phd-Ca 63 mg·liter^{-1} × 5	315	27.4 b	38.6 b
Phd-Ca 250 + 125 mg·liter^{-1}	375	31.7 b	36.7 b
P values			
ANOVA		0.0001	0.0077
Linear regression		0.0001	0.0005

[x] Phd-Ca sprays applied 2 May ('Royal Gala') or 8 May ('Ramey York') when shoot growth averaged 5 to 8 cm; second and successive sprays applied at 2-week intervals. × followed by a number indicates number of treatment applications during the growing season.
[y] ECD = effective cumulative dose of Phd-Ca; i.e., the sum of concentrations of individual spray applications.
[z] Means within a column that are followed by the same letter did not differ significantly at the P = 0.05 level based upon Tukey's studentized range test.

Table 1. Date of treatment with prohexadione-calcium or streptomycin and inoculation with *Erwinia amylovora* in various cultivar-age treatment groups of apple trees used in study

Treatment[x]	'Royal Gala' 4th-leaf[y] 2001	'Royal Gala' 5th-leaf 2002	'Ramey York' 4th-leaf 2001	'Ramey York' 5th-leaf 2002	'Ramey York' 3rd-leaf 2002	'Ramey York' 4th-leaf 2003	'Enterprise' 3rd-leaf 2002	'Enterprise' 4th-leaf 2003	'Sun Fuji' 1st-leaf 2002	'Sun Fuji' 2nd-leaf 2003
1st Phd-Ca application	2 May	23 April	8 May	30 April	8 May	15 May	30 April	15 May	12 June	15 May
2nd Phd-Ca application	16 May	5 May	23 May	16 May	23 May	30 May	16 May	30 May	NA[z]	30 May
3rd Phd-Ca application	30 May	23 May	8 June	30 May	7 June	16 June	30 May	16 June	NA	NA
1st Strep application	4 June	NA	4 June	NA	NA	NA	NA	NA	NA	NA
Inoc. with *E. amylovora*	5 June	4 June	5 June	12 June	19 June	25 June	12 June	25 June	27 June	12 June
2nd Strep application	8 June	NA	8 June	NA	NA	NA	NA	NA	NA	NA
4th Phd-Ca application	13 June	NA	19 June	NA	NA	NA	NA	NA	NA	NA
5th Phd-Ca application	27 June	NA	29 June	NA	NA	NA	NA	NA	NA	NA

[x] Phd-Ca = prohexadione-Ca, Strep = streptomycin sulfate.
[y] Age of orchard (leaf = season of orchard growth).
[z] NA = not applied.

Evaluation of fire blight resistance. The effect of Phd-Ca on fire blight resistance was evaluated based upon the severity of infection resulting from inoculation of shoots with *E. amylovora*. Inoculum consisted of 18-h-old shake cultures of *E. amylovora* strain Ea273 (24) grown in Kado 523 broth (11) at 28°C. Inoculum concentration was estimated by absorbance at 620 nm using a standard curve and adjusted to a concentration of 1×10^9 CFU·ml^{-1} by dilution with sterile 0.05 M potassium phosphate buffer, pH 6.5. Inoculum was maintained on ice and was used for plant inoculation within 4 h of dilution. Shoots were inoculated by transversely bisecting the two youngest leaves on the shoot with scissors dipped in the suspension of *E. amylovora*. In 2001 and 2003, shoots were selected at random from the periphery of the tree at the time of inoculation. In 2002, terminal shoots were selected for inoculation in the spring prior to bud break and marked for subsequent inoculation 12 days after the final application of Phd-Ca (Table 1). The numbers of shoots inoculated per tree were: 2001, 10 shoots per tree; 2002, 10 shoots per tree on fifth-leaf 'Royal Gala' and 'Ramey York', 5 shoots on third-leaf 'Ramey York' and 'Enterprise', and 1 shoot on first-leaf 'Sun Fuji'; 2003, 5 shoots per tree on fourth-leaf 'Ramey York' and 'Enterprise', and 2 shoots on second-leaf 'Sun Fuji'. The length of fire blight lesions on inoculated shoots, the age of fire blight infected tissue on inoculated shoots measured in years of growth (0 = no stem infection, 1 = current season's growth, 2 = previous season's growth, etc.), and the number of fire blight strikes per tree occurring on noninoculated shoots (disease spread) were determined after fire blight lesions on inoculated shoots had ceased extension as determined by a clear demarcation between diseased and healthy tissue.

The percent fire blight control and the percent shoot growth suppression were calculated as 100% × (lesion length or growth without treatment minus lesion length or growth with Phd-Ca treatment) divided by lesion length or growth without treatment, respectively. Differences between treatments were determined by an analysis of variance and Tukey's studentized range test (SAS Institute Inc., Cary, NC). To determine if the data satisfied test assumptions, SAS Univariate procedure was used to verify independence between residuals and predicted values, and to confirm a normal distribution of residuals. The response of fire blight resistance to ECD of Phd-Ca was tested by linear regression (SAS Institute).

RESULTS

Effects of Phd-Ca on tree growth and yield. The effects of Phd-Ca dose on season-long shoot growth and yield were determined on both inoculated and noninoculated trees. Because the treatment effects on shoot growth and yield were similar on both sets of trees, results are reported for noninoculated trees only.

In 2001, Phd-Ca applied as a two-spray high-dose (250 + 125 mg·liter^{-1}) treatment or in a five-spray low-dose (63 mg·liter^{-1}) treatment was equally effective in reducing the current season's terminal shoot growth in fourth-leaf 'Royal Gala' and 'Ramey York' apple trees (Table 2). Shoot growth decreased linearly with increasing Phd-Ca ECD for both 'Royal Gala' (r^2 = 0.62) and 'Ramey York' (r^2 = 0.44). Mean yield per tree did not differ among treatments for 'Ramey York' (9.6 kg per tree) or 'Royal Gala' (23.7 kg per tree).

In 2002, three sprays of PhD-Ca at doses of 30 or 63 mg·liter^{-1}, or two sprays at 125 mg·liter^{-1} to fifth-leaf 'Royal Gala' and 'Ramey York' reduced early-season shoot growth measured on 6 June, 5 to 6 weeks after the initial application (Table 3). In 'Ramey York' trees, the three-spray treatment at 63 mg·liter^{-1} per spray was as effective in suppressing early (30 April to 6 June) shoot growth as two sprays at 125 mg·liter^{-1}. Both higher ECD treatments (189 and 250 mg·liter^{-1}) provided better early-season shoot growth control than the 90 mg·liter^{-1} ECD treatment (Table 3). Similarly, in fifth-leaf 'Royal Gala' trees, two sprays at 125 mg·liter^{-1} suppressed

Table 3. Effect of prohexadione-calcium (Phd-Ca) dose on incremental and total seasonal shoot growth of apple trees in their third- (2002), fourth- (2003), or fifth- (2002) leaf

Treatment[v]	ECD[w] (mg·liter^{-1})	Seasonal shoot growth (cm)[x] Early	Mid	Late	Total
Treatment group = 2002, 'Ramey York' fifth-leaf[y]					
No treatment	0	24.6 a[z]	5.8 a	0.5 a	30.9 a
Phd-Ca 30 mg·liter^{-1} × 3	90	15.9 b	1.1 b	1.1 a	18.0 b
Phd-Ca 63 mg·liter^{-1} × 3	189	10.3 c	0.8 b	5.7 a	16.7 b
Phd-Ca 125 mg·liter^{-1} × 2	250	8.4 c	1.4 b	5.6 a	15.4 b
ANOVA *P* value		0.0001	0.0001	0.0178	0.0005
Linear regression *P* value		0.0001	0.0005	0.0032	0.0001
Treatment group = 2002, 'Royal Gala' fifth-leaf					
No treatment	0	24.9 a	2.3 a	0.8 b	28.0 a
Phd-Ca 30 mg·liter^{-1} × 3	90	14.4 b	1.1 a	2.0 b	17.4 b
Phd-Ca 63 mg·liter^{-1} × 3	189	13.4 bc	0.9 a	5.0 ab	19.4 b
Phd-Ca 125 mg·liter^{-1} × 2	250	10.8 c	1.0 a	8.7 a	20.5 b
ANOVA *P* value		0.0001	0.0941	0.0029	0.0014
Linear regression *P* value		0.0001	0.0828	0.0008	0.0331
Treatment group = 2002, 'Ramey York' third-leaf					
No treatment	0	21.6 a	10.7 a	21.6 a	53.9 a
Phd-Ca 30 mg·liter^{-1} × 3	90	15.0 b	4.9 b	20.5 a	40.4 ab
Phd-Ca 125 mg·liter^{-1} × 2	250	8.1 c	2.1 b	25.7 a	35.9 b
ANOVA *P* value		0.0001	0.0001	0.7104	0.0319
Linear regression *P* value		0.0001	0.0001	0.4808	0.0457
Treatment group = 2002, 'Enterprise' third-leaf					
No treatment	0	21.3 a	6.0 a	29.3 a	56.6 a
Phd-Ca 30 mg·liter^{-1} × 3	90	14.0 b	6.7 a	35.5 a	56.2 a
Phd-Ca 125 mg·liter^{-1} × 2	250	6.0 c	5.2 a	32.2 a	43.4 b
ANOVA *P* value		0.0001	0.5011	0.2775	0.0127
Linear regression *P* value		0.0001	0.4406	0.6201	0.0072
Treatment group = 2003, 'Ramey York' fourth-leaf					
No treatment	0	24.9 a	14.6 a	0.0 a	39.5 ab
Phd-Ca 30 mg·liter^{-1} × 3	90	23.7 a	16.1 a	2.5 a	42.2 a
Phd-Ca 125 mg·liter^{-1} × 2	250	10.2 b	9.3 b	9.2 a	28.6 b
ANOVA *P* value		0.0001	0.0048	0.0756	0.0235
Linear regression *P* value		0.0001	0.0072	0.0143	0.0221
Treatment group = 2003, 'Enterprise' fourth-leaf					
No treatment	0	15.3 a	16.0 a	4.0 a	35.3 a
Phd-Ca 30 mg·liter^{-1} × 3	90	14.1 a	17.7 a	8.5 a	32.4 a
Phd-Ca 125 mg·liter^{-1} × 2	250	6.3 b	15.5 a	3.5 a	33.1 a
ANOVA *P* value		0.0001	0.5385	0.0601	0.6159
Linear regression *P* value		0.0001	0.2788	0.0412	0.4288

[v] Phd-Ca sprays were applied when shoot growth averaged 5 to 8 cm, and successive sprays were applied at 2-week intervals. × followed by a number indicates number of treatment applications during the growing season.

[w] ECD = effective cumulative dose of Phd-Ca; i.e., the sum of concentrations of individual spray applications.

[x] Mean length of shoot growth: In 2002, early = from first Phd-Ca application to 6 June; mid = 7 June to 1 July; late = 2 July to 15 November; total = from first application to 15 November. First Phd-Ca applications were made 'Ramey York' fifth-leaf: 30 April; 'Royal Gala' fifth-leaf: 23 April; 'Ramey York' third-leaf: 8 May; and 'Enterprise' third-leaf: 30 April. In 2003, early = 14 May (first Phd-Ca application 15 May) to 24 June; mid = 25 June to 29 July; late = 29 July to 3 December; total = from first application to 3 December.

[y] Age of orchard (leaf = season of orchard growth).

[z] Means within a treatment-group column that are followed by the same letter did not differ significantly at the P = 0.05 level based upon Tukey's studentized range test.

early-season shoot growth more than three sprays at 30 mg·liter^{-1}. All Phd-Ca treatments continued to suppress midseason (6 June to 1 July) shoot growth of fifth-leaf 'Ramey York' trees, with no differences detected among individual Phd-Ca dose treatments (Table 3). After 1 July, when terminal buds normally set and shoot growth ceases, the growth of fifth-leaf 'Ramey York' trees treated at an ECD of 189 and 250 mg·liter^{-1} appeared to resume and exceeded that of control trees or trees treated at the lowest Phd-Ca dose (ECD 90 mg·liter^{-1}), although mean differences were not significant based on Tukey's studentized range test (Table 3). In fifth-leaf 'Royal Gala', Phd-Ca had no effect on midseason shoot growth (Table 3). As with the fifth-leaf 'Ramey York' trees, late-season (1 July to 15 November) growth appeared to be stimulated by the higher dose sprays in the fifth-leaf 'Royal Gala' trees (Table 3). All Phd-Ca spray treatments were equally effective in suppressing the total season-long (April to November) shoot growth in both cultivars (Table 3). In both fifth-leaf orchards, shoot growth response to Phd-Ca was linear over the ECD range of 0 to 250 mg·liter^{-1} for all dates and both cultivars except 'Royal Gala' between 6 June and 1 July (midseason) (Table 3). Mean yields did not differ among treatments for fifth-leaf 'Royal Gala' (10.6 kg per tree) or 'Ramey York' (15.7 kg per tree).

In 2002, Phd-Ca applied to third-leaf 'Ramey York' or 'Enterprise' apple trees as three low-dose (30 mg·liter^{-1}) or two high-dose (125 mg·liter^{-1}) sprays reduced early-season shoot growth when measured 4 to 5 weeks after the initial spray was applied (Table 3). Early shoot growth decreased linearly with increasing Phd-Ca ECD for third-leaf 'Ramey York' (r^2 = 0.88) and 'Enterprise' (r^2 = 0.83). The high-dose treatment reduced shoot growth more than the low-dose treatment in both cultivars at the early date. However, it should be noted that only two sprays of the low-dose treatment had been applied to the 'Ramey York' trees at the time of the early growth measurement. Further, in the third-leaf 'Enterprise' trees, the third (final) spray preceded the early growth measurement by only 1 week. Absorption of Phd-Ca is generally considered complete within 8 h of application (5), with effects on shoot growth measurable within 10 to 14 days after treatment (6). Thus shoot growth recorded on 6 June is the result of two and not three sprays. Midseason shoot growth (6 June to 1 July) of third-leaf 'Ramey York' was reduced by both Phd-Ca treatments, but growth of the 'Enterprise' trees was not affected during this time period by either Phd-Ca treatment. A second cycle (or flush) of growth occurred after 1 July as a result of abundant rainfall and ideal growing conditions. Late-season (1 July until 19 November) shoot growth did not differ among treatments. Only the ECD 250 mg·liter^{-1} treatment provided season-long growth suppression on the third-leaf 'Ramey York' and 'Enterprise' trees (Table 3, Fig. 1). Mean yields were low for both third-leaf 'Ramey York' (1.41 kg per tree) and 'Enterprise' (2.15 kg per tree), with no significant differences among treatments.

In 2003, the low-dose (90 mg·liter^{-1} ECD) Phd-Ca spray treatment had no effect on early-season shoot growth in the fourth-leaf 'Ramey York' or' Enterprise' trees (Table 3). The high-dose treatment

Table 4. Effect of prohexadione-Ca (Phd-Ca) spray on incremental and total seasonal shoot growth of first- and second-leaf 'Sun Fuji' apple trees

Treatment[v]	ECD[w] (mg·liter^{-1})	Seasonal shoot growth (cm)[x] Early	Mid	Late	Total
Treatment group = 2002, first-leaf[y]					
No treatment	0	7.4 a[z]	15.6 a	19.9 a	62.6 a
Phd-Ca 30 mg·liter^{-1}	30	5.6 ab	15.1 a	11.3 b	54.5 a
Phd-Ca 125 mg·liter^{-1}	125	2.0 b	16.7 a	17.4 ab	55.8 a
ANOVA *P* value		0.0161	0.8479	0.0147	0.4111
Linear regression *P* value		0.0088	0.6078	0.9947	0.4806
Treatment group = 2003, second-leaf					
No treatment	0	18.3 a	22.4 a	11.4 a	69.8 a
Phd-Ca 30 mg·liter^{-1} × 2	60	17.9 a	21.4 a	9.1 a	67.8 a
Phd-Ca 125 + 60 mg·liter^{-1}	185	11.4 b	22.7 a	15.0 a	69.5 a
ANOVA *P* value		0.0150	0.8633	0.1207	0.9316
Linear regression *P* value		0.0038	0.6802	0.2359	0.9941

[v] In 2002, Phd-Ca applied on 12 June. In 2003, Phd-Ca applied on 15 and 30 May. × followed by a number indicates number of treatment applications during the growing season.

[w] ECD = effective cumulative dose of Phd-Ca; i.e., the sum of concentrations of the individual spray applications.

[x] Mean length of shoot growth: In 2002, early = from 12 June (Phd-Ca application) to 15 July; mid = 16 July to 14 August; late = 15 August to 13 November; total = total seasonal shoot growth (includes shoot growth prior to Phd-Ca application on 12 June). In 2003, early = from 13 May (Phd-Ca application) to 18 June; mid = 19 June to 24 July; late = 25 July to 17 November; total = total seasonal shoot growth (includes shoot growth prior to Phd-Ca application on 13 May).

[y] Age of orchard (leaf = season of orchard growth). Trees planted on 6 May 2002.

[z] Means within a treatment-group column that are followed by the same letter did not differ significantly at the *P* = 0.05 level based upon Tukey's studentized range test.

Fig. 1. Effect of prohexadione-calcium (Phd-Ca) on third-leaf 'Ramey York' apple trees. **A,** Untreated control tree. **B,** Tree treated with three low-dose (30 mg·liter^{-1}) Phd-Ca spray applications. **C,** Tree treated with two high-dose (125 mg·liter^{-1}) applications. First application of Phd-Ca was made 8 May 2002, and subsequent applications were made at approximately 2-week intervals; photograph was taken 17 September 2002. Each section of height marker is 30.5 cm.

(250 mg·liter^{-1} ECD) reduced shoot growth, similar to the effect in 2002. High-dose Phd-Ca treatment on 'Ramey York' reduced midseason growth in the fourth-leaf; however, the same treatment had no effect on 'Enterprise'. Late-season growth was not affected by either the high- or low-dose Phd-Ca treatments in the fourth-leaf (2003) trees. In general, total shoot growth in fourth-leaf (2003) 'Ramey York' and 'Enterprise' trees was less than total growth obtained in these cultivars in the third-leaf (2002). Conditions in 2003 did not appear to favor strong late-season growth (Table 3). Only the 'Ramey York' trees exhibited a total season growth response to Phd-Ca treatment in the fourth-leaf, and this occurred in the high-dose treatment (Table 3). Mean yields for fourth-leaf Phd-Ca–treated 'Ramey York' and 'Enterprise' trees did not differ from the untreated control trees in 2003.

On newly planted 'Sun Fuji', mean shoot growth of the top five shoots 5.5 weeks after planting was 20.6 cm when a single Phd-Ca spray was applied 12 June. Phd-Ca treatment at 125 mg·liter^{-1} reduced shoot growth from 12 June to 15 July during the first 4.5 weeks after treatment (early season) compared with the untreated control (Table 4). Phd-Ca treatment at 30 mg·liter^{-1} produced a slight but nonsignificant reduction in shoot growth when measured on 15 July. There were no treatment differences in the incremental midseason shoot growth produced between 15 July and 14 August (midseason) (Table 4). Late-season shoot growth between 14 August and 13 November was significantly less for trees treated at 30 mg·liter^{-1} than for untreated control trees or trees treated with 125 mg·liter^{-1} (Table 4). Total seasonal shoot growth did not differ among treatments in 2002. 'Sun Fuji' treated with Phd-Ca in the second-leaf (2003) at 60 mg·liter^{-1} ECD (low dose) or 185 mg·liter^{-1} ECD (high dose) affected shoot growth similarly to the low- (30 mg·liter^{-1} ECD) and high-dose (125 mg·liter^{-1} ECD) treatments in the first-leaf (Table 4), except that there was no difference in late-season growth among treatments.

Effects of Phd-Ca on fire blight resistance. The severity of fire blight infection resulting from shoot inoculation was evaluated based upon the length of fire blight lesions, the proportion of the current season's shoot length blighted, and the maximum age of fire blight infected tissue measured in seasons of growth. Initial analysis indicated that the residuals for the proportion of the current season's shoot length blighted were not normally distributed because infections had progressed beyond the current season's shoot length for some treatments. There was very limited secondary spread of fire blight shoot infections from inoculated shoots, and the number of secondary infections was too low to allow valid statistical analysis.

Table 5. Effect of prohexadione-calcium (Phd-Ca) on growth of apple shoots at time of inoculation with *Erwinia amylovora* and resulting severity of fire blight determined by lesion length and maximum age of tissue infected

Treatment[v]	ECD[w] (mg·liter^{-1})	Shoot growth (cm)	Lesion length (cm)	Infected tissue age (years)[x]
Treatment group = 2001, 'Royal Gala' fourth-leaf[y]				
No treatment	0	37.7 a[z]	18.8 a	1.0 a
Phd-Ca 63 mg·liter^{-1} × 5	126	17.5 b	0.7 b	0.1 b
Phd-Ca 250 and 125 mg·liter^{-1}	375	18.2 b	0.0 b	0.0 b
Streptomycin 100 mg·liter^{-1} × 2	0	38.1 a	17.4 a	1.0 a
ANOVA *P* value		<0.0001	<0.0001	<0.0001
Linear regression *P* value		<0.0001	<0.0001	<0.0001
Treatment group = 2001, 'Ramey York' fourth-leaf				
No treatment	0	29.6 b	65.1 a	2.2 a
Phd-Ca 63 mg·liter^{-1} × 5	126	19.8 c	34.8 b	1.7 b
Phd-Ca 250 and 125 mg·liter^{-1}	375	15.4 d	1.5 c	0.4 c
Streptomycin 100 mg·liter^{-1} × 2	0	32.6 a	65.3 a	2.2 a
ANOVA *P* value		<0.0001	<0.0001	<0.0001
Linear regression *P* value		<0.0001	<0.0001	<0.0001
Treatment group = 2002, 'Royal Gala' fifth-leaf				
No treatment	0	29.1 a	35.7 a	1.5 a
Phd-Ca 30 mg·liter^{-1} × 3	90	20.6 b	23.7 b	1.3 a
Phd-Ca 63 mg·liter^{-1} × 3	189	16.1 c	15.4 bc	0.8 b
Phd-Ca 125 mg·liter^{-1} × 2	250	17.4 c	13.3 c	0.7 b
ANOVA *P* value		<0.0001	<0.0001	<0.0001
Linear regression *P* value		<0.0001	<0.0001	<0.0001
Treatment group = 2002, 'Ramey York' fifth-leaf				
No treatment	0	32.3 a	58.8 a	2.0 a
Phd-Ca 30 mg·liter^{-1} × 3	90	23.4 b	36.7 b	1.5 ab
Phd-Ca 63 mg·liter^{-1} × 3	189	15.2 c	15.1 c	0.9 b
Phd-Ca 125 mg·liter^{-1} × 2	250	13.2 c	12.2 c	1.3 ab
ANOVA *P* value		<0.0001	<0.0001	0.0072
Linear regression *P* value		<0.0001	<0.0001	0.0054
Treatment group = 2002, 'Ramey York' third-leaf				
No treatment	0	31.0 a	67.9 a	1.9 a
Phd-Ca 30 mg·liter^{-1} × 3	90	23.9 b	55.1 a	1.8 a
Phd-Ca 125 mg·liter^{-1} × 2	250	15.3 c	25.0 b	1.2 b
ANOVA *P* value		<0.0001	<0.0001	<0.0001
Linear regression *P* value		<0.0001	<0.0001	<0.0001
Treatment group = 2002, 'Enterprise' third-leaf				
No treatment	0	24.9 a	2.4 a	0.20 a
Phd-Ca 30 mg·liter^{-1} × 3	90	20.9 b	0.8 a	0.15 ab
Phd-Ca 125 mg·liter^{-1} × 2	250	16.8 c	0.0 a	0.00 b
ANOVA *P* value		<0.0001	0.3873	0.0362
Linear regression *P* value		<0.0001	0.2084	0.0180
Treatment group = 2003, 'Ramey York' fourth-leaf				
No treatment	0	37.0 a	62.7 a	2.35 a
Phd-Ca 30 mg·liter^{-1} × 3	90	37.5 a	66.0 a	2.25 a
Phd-Ca 125 mg·liter^{-1} × 2	250	25.3 b	44.9 b	2.00 b
ANOVA *P* value		<0.0001	<0.0001	0.0315
Linear regression *P* value		<0.0001	<0.0001	0.0359
Treatment group = 2003, 'Enterprise' fourth-leaf				
No treatment	0	31.7 a	12.1 a	1.05 a
Phd-Ca 30 mg·liter^{-1} × 3	90	29.5 a	7.6 ab	0.77 b
Phd-Ca 125 mg·liter^{-1} × 2	250	21.1 b	0.8 b	0.50 c
ANOVA *P* value		<0.0001	0.0158	0.0005
Linear regression *P* value		<0.0001	0.0045	<0.0001
Treatment group = 2002, 'Sun Fuji' first-leaf				
No treatment	0	27.5 a	32.6 a	1.6 a
Phd-Ca 30 mg·liter^{-1} × 1	30	25.1 ab	29.9 a	1.6 a
Phd-Ca 125 mg·liter^{-1} × 1	125	17.9 b	14.0 a	1.1 a
ANOVA *P* value		0.0411	0.0182	0.2454
Linear regression *P* value		0.0081	0.0213	0.1376
Treatment group = 2003, 'Sun Fuji' second-leaf				
No treatment	0	36.1 a	77.1 a	2.5 a
Phd-Ca 30 mg·liter^{-1} × 1	30	38.1 a	62.2 ab	2.4 a
Phd-Ca 125 mg·liter^{-1} × 1	125	33.5 a	52.9 b	2.1 a
ANOVA *P* value		0.3892	0.0104	0.4238
Linear regression *P* value		0.3000	0.0051	0.1788

[v] Phd-Ca sprays were applied when shoot growth averaged 5 to 8 cm and successive sprays were applied at 2-week intervals. × followed by a number indicates number of treatment applications during the growing season. Streptomycin was applied 24 h prior to inoculation and 72 h after inoculation.

[w] ECD = effective cumulative dose of Phd-Ca up to time of inoculation; i.e., the sum of concentrations of sprays applied at least 7 days prior to inoculation.

[x] Mean maximum age of tissue infected, where 0 = no infection, 1 = infection of current season's growth, 2 = infection progressing into the previous season's wood, etc.

[y] Age of orchard (leaf = season of orchard growth).

[z] Treatments within a treatment-group column that are followed by the same letter did not differ significantly at the $P = 0.05$ level based upon Tukey's studentized range test.

Therefore, the effect of Phd-Ca treatment on fire blight resistance was determined based upon the length of fire blight lesions and the age of fire blight infected tissue.

The application of Phd-Ca to various cultivars of orchard-grown apple trees ranging in age from first-leaf to fifth-leaf indicated that enhancement of fire blight resistance by PhD-Ca was linear over the ECD ranges tested and resistance was correlated with growth suppression at the time of inoculation (Table 5, Fig. 2). On fourth-leaf 'Royal Gala' trees in 2001, both the high-dose Phd-Ca treatment (250 + 125 mg·liter^{-1}) and the low-dose treatment (five applications of 63 mg·liter^{-1}) resulted in equivalent degrees of growth suppression at the time of inoculation, and both treatments resulted in excellent control of fire blight (Table 5). However, on fourth-leaf 'Ramey York' in 2001, trees that received the low-dose Phd-Ca treatment (163 mg·liter^{-1} ECD) resulted in an intermediate level of growth suppression and also resulted in an intermediate level of fire blight resistance (Table 5). Similarly, in 2002 and 2003, on fifth-leaf 'Royal Gala' and 'Ramey York' (2002), third-leaf 'Ramey York' (2002), and fourth-leaf 'Ramey York' and 'Enterprise' (2003), low-dose Phd-Ca treatment (90 mg·liter^{-1} ECD) resulted in reduced growth suppression and reduced fire blight resistance (Table 5). Due to the low severity of fire blight on the disease-resistant cultivar Enterprise in 2002, significant growth reduction by Phd-Ca treatment did not result in significant decrease in fire blight severity when measured by the length of fire blight lesions; however, an increase in fire blight resistance was observed when measured by the mean age of tissue infected (Table 5). This relationship was less evident on 'Sun Fuji' trees in their first- (2002) and second- (2003) leaf. In 2002, there was a significant linear response for both growth suppression and fire blight suppression to increasing Phd-Ca ECD treatment (Table 5). Although in 2003 shoot growth at the time of inoculation was not significantly affected by Phd-Ca treatment on second-leaf 'Sun Fuji', high dose Phd-Ca treatment (250 mg·liter^{-1} ECD) significantly reduced the severity of fire blight, and there was a significant linear response between increasing Phd-Ca ECD and fire blight suppression.

The effects of Phd-Ca on growth suppression and fire blight control were clearly correlated (Fig. 2). There was a significant linear correlation between the percent growth suppression at the time of inoculation and the percent fire blight control based upon the length of fire blight lesions resulting from Phd-Ca treatment of all cultivar-age treatment groups (r = 0.7787, df = 17, $P < 0.01$). Because 'Enterprise' is highly resistant to fire blight and had little or no infection, it was considered an outlier and removed from the analysis, resulting in a correlation coefficient of $r = 0.8977$, df = 15, $P < 0.001$.

In 2001, treatment of 'Royal Gala' and 'Ramey York' shoots with streptomycin before and after inoculation with a strain of *E. amylovora* sensitive to streptomycin had no effect on growth or fire blight suppression.

DISCUSSION

The results of this study indicate that the use of reduced Phd-Ca doses is not an effective solution to the conflicting requirements to control fire blight and allow sufficient tree growth in young apple orchards. The level of fire blight control obtained in young orchards was correlated to the level of growth suppression resulting from Phd-Ca treatment (Fig. 2). When Phd-Ca dose was lowered sufficiently to reduce growth suppression, fire blight control was also reduced (Table 5). Among the 10 cultivar-age treatment groups in this study, there was no case where a reduced Phd-Ca dose allowed significant increases in the growth of young trees and provided a level of fire blight control equivalent to that of the higher dose of Phd-Ca.

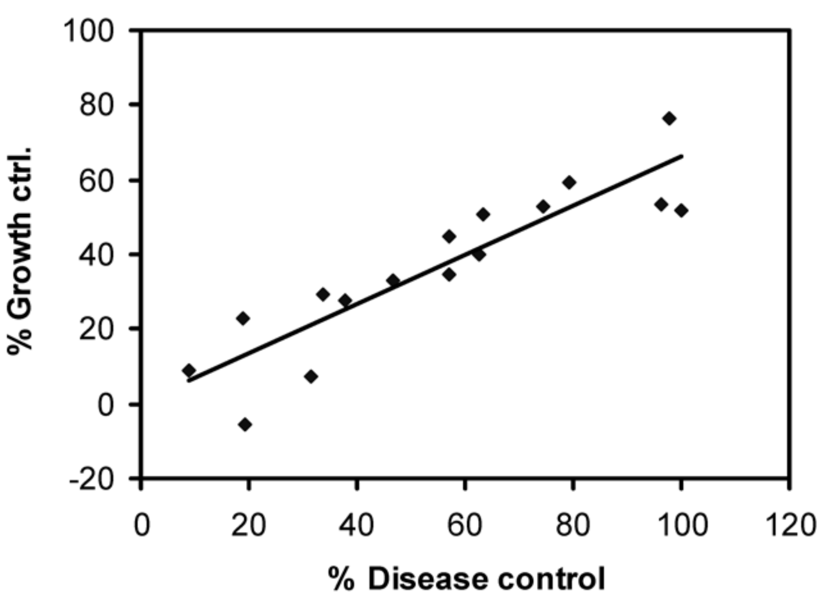

Fig. 2. Linear correlation between percent fire blight control based upon length of fire blight lesions and percent shoot growth suppression resulting from treatment with prohexadione-calcium (Phd-Ca) ($r = 0.8977$, df = 15, $P < 0.001$). Correlation analysis included all Phd-Ca treatments of 'Royal Gala', 'Ramey York', and 'Sun Fuji', but did not include treatment of 'Enterprise', which was considered an outlier due to its high level of fire blight resistance.

In addition, low-dose Phd-Ca applications in young plantings usually did not provide a significant growth advantage over higher dose applications. Although a reduced Phd-Ca dose usually resulted in greater early season growth, there was often no difference in total seasonal growth between high- and low-dose Phd-Ca applications because trees receiving high doses tended to grow more in the latter part of the season (Table 3). There was no significant difference in total seasonal growth between high- and low-dose Phd-Ca applications in 8 of 10 cultivar-age treatment groups (Tables 2 to 4).

The results reported here on the effect of Phd-Ca on tree growth are consistent with those previously reported on mature trees. Previous studies have demonstrated a Phd-Ca rate response for early-season growth suppression in mature trees (6,14). Resumption of shoot growth in mid- to late-season has been reported for Phd-Ca–treated trees (8,14,26), and a slight increase in the shoot growth on 'Law Rome' apple trees in the year following a high-dose (178 g·ha^{-1}) Phd-Ca treatment was reported (14). Increased shoot growth was also reported in the year after the growth regulator, daminozide, was applied to apple (7,25). Similarly, our observation that growth control by Phd-Ca did not affect yield is consistent with earlier reports where either higher rates or multiple (three or more) low-dose sprays on apple (6,14,26) and pear (3) did not affect yield.

Several researchers have reported that Phd-Ca treatment will reduce the severity of fire blight shoot infection on apple (12,15,28,29). On pear, four Phd-Ca applications at 100 mg·liter^{-1} were more effective for reducing the incidence of shoot infection than four 50 mg·liter^{-1} applications, but there was not a significant effect of dose on fire blight severity (3). Some reports on Phd-Ca as a potential agent against fire blight shoot infection include a streptomycin treatment; in contrast to the present study, streptomycin significantly reduced the incidence and severity of shoot infection on apple (3,15,28,29).

To determine the effect of Phd-Ca treatments on fire blight resistance, trees were inoculated with *E. amylovora* in June

(early season), when shoot blight is usually most severe and most likely to occur. Because high-dose PhD-Ca treatment resulted in less early-season growth and greater late-season growth than reduced-dose treatment, early-season inoculation may have favored the evaluation of fire blight resistance toward high-dose treatment. Late-season epiphytotics of shoot blight are rare but have been reported to occur (27), and under these conditions trees treated with high doses of Phd-Ca may be more susceptible to shoot blight if high-dose treatment results in a flush of late-season growth. The short half-life and nonpersistent nature of Phd-Ca (4) is in contrast to some other growth regulators applied to apple, such as daminozide and paclobutrazol (13), and could present a problem if late-season growth is challenged by *E. amylovora*. However, the inoculations conducted in these experiments are a valid evaluation of protection against early-season shoot blight, which is usually most important.

In northern growing regions with shorter seasons, such as New York and Michigan, a single application of 250 mg·liter^{-1} or two applications of 125 mg·liter^{-1} are recommended for both growth and fire blight control (10,23). In North Carolina, multiple low-rate sprays were reported to be more effective for season-long growth suppression than a single, high-rate spray treatment (26). The high rate of Phd-Ca used in our 2001 trials (250 mg·liter^{-1} followed by 125 mg·liter^{-1}) provided excellent fire blight control (96 to 100%), but would probably be considered an excessive rate of application in the mid-Atlantic region. Growers may be reluctant to use Phd-Ca at rates above that needed to gain growth control due to cost of Phd-Ca treatments. However, our data indicate that for fire blight control in young plantings, the most effective strategy may be to apply one or two high-dose Phd-Ca treatments to provide fire blight protection early in the growing season and then encourage growth later in the season when there is a lower risk of fire blight. Late-season growth will also be dependent upon tree vigor, fruit load, and water availability, and may not be desirable in northern regions where late-season growth can result in reduced winter hardiness. In addition, these results could vary in other geographic regions due to the environment-dependent nature of tree response to Phd-Ca treatment.

Management of the shoot blight phase of fire blight has been hampered by a lack of effective control treatments. The development of Phd-Ca has been a significant advance in our ability to manage shoot blight in mature apple orchards, but there are constraints associated with its use both in mature and young orchards. To be effective, Phd-Ca must be applied 2 to 3 weeks before the normal period of shoot infection and before the effectiveness of blossom blight control sprays can be evaluated, so the expense of Phd-Ca applications may not be recovered in years when fire blight is ultimately not a significant problem. In mature orchards, the economic benefits of Phd-Ca as an orchard management tool may be realized even in the absence of fire blight due to its effect on growth suppression (reduced pruning costs, improved fruit quality, etc). However, in young orchards there is no economic benefit to suppressing shoot growth, and the practice may result in economic loss. Here we demonstrate that effective fire blight control by Phd-Ca requires suppression of shoot growth at the time of infection. Therefore, in young orchards the use of Phd-Ca should be considered only when the risk of fire blight shoot infection clearly outweighs the negative effects of growth suppression. Losses in the 2000 Michigan epidemic were greatest in plantings in their fourth, fifth, and sixth season of growth, and few losses were seen in plantings in their first season of growth (18). Similarly, in controlled orchard studies in New York, trees in their third-leaf were found to be significantly more susceptible to rootstock infection following a blossom blight epiphytotic than trees in their first- or second-leaf (17). The data presented in this study regarding the efficacy of Phd-Ca to control fire blight on first- and second-leaf trees is inconclusive. However, because newly planted trees appear to be at lower risk of tree loss due to fire blight and vigorous tree growth is critical to the establishment of the orchard, Phd-Ca should probably not be used in the first 2 years after planting. In this study, treatment of trees with Phd-Ca in their fourth season of growth did not negatively impact yield the following year, which suggests that use of one or two high-dose Phd-Ca applications may be justified in the fourth to sixth season of growth when there is a high risk of shoot blight.

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