Effective Post-Infection Programs of Prohexadione-calcium for Reducing Shoot Blight and Preventing Fire Blight Canker Initiation on Apple Wood with Cost-Benefit Analysis

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One of the options to mitigate an already established fire blight outbreak is/are spray application/s of plant growth regulator (PGR) prohexadione-calcium (PCA, Apogee® by BASF Corporation; Kudos® 27.5 WDG Fine Americas Inc). By managing primarily shoot blight stage of this disease, PCA can mitigate an established fire blight outbreak in the current and can help prevent the infections from renewing in the next growing season. PCA acts as an apple shoot growth reducer through inhibiting gibberellin biosynthesis, which increases the thickness of plant cell walls on green apple tissues and results in reduced shoot susceptibility to fire blight (Evans et al., 1999; McGrath et al., 2009; Ramirez et al., 2005). Hence, PCA activates structural resistance to fire blight infection by thickening the plant cell walls (Sundin, 2014). Under natural infections, research has shown that preventive PCA spray applications reduce shoot blight incidence and severity, but can also prevent or reduce initiation of fire blight cankers on apple wood for 61 – 96 % (Norelli and Miller, 2004; Yoder, 2001; Yoder et al., 1999; Yoder and Marini, 2002). However, the ability of PCA to reduce the initiation of fire blight cankers on wood, which could kill young trees and serve as primary inoculum sources for flower infections in spring, has not been tested under controlled inoculations and by PCA applications after the fire blight infections have already established.

> **"We demonstrated that post-infection applications of prohexadione-calcium (Apogee) alone or in combination with acibenzolar-S-methyl (Actigard) initiated two days after the infection reduce shoot blight severity, but more importantly reduce or prevent fire blight canker initiation and development on perennial apple wood."**

Growers usually do not wish to use PCA for growth reduction on 1 to 6-yr old trees as their top priority is to promote growth to quickly fill up the row space and reach the top trellis wire. However, in years with highly favorable disease conditions when severe fire blight infections have already established in young or high-density orchards, this priority must change from horticultural goals to disease management practices that could prevent tree deaths. By preventing migration of fire blight bacterium *E. amylovora* from shoots into the trunk and/or rootstock, an emergency application of PCA on infected young or mature trees in high-density orchards might be the only option that could prevent or reduce large number of tree deaths resulting from initiation of deadly cankers. In young orchards, fire blight infections cannot be tolerated because they can easily kill smaller size trees due to high susceptibly of juvenile host tissues (Johnson et al., 2016). Therefore, a benefit of saving apple trees from dying after an unmanaged fire blight outbreak/s by post-infection applications of PCA could outweigh any disadvantages from tree growth reduction. On mature trees with classic training systems, PCA can reduce chances for onset of new shoot infections, prevent shoot blight severity from increasing on already infected shoots, and prevent an establishment of more cankers per tree that over time could lead to tree death. However, it is not clear at which rate, at which application time/s after the infection, and for how much, the PCA can prevent or reduce the initiation of fire blight cankers on wood that can kill trees.

In 2016, fire blight epidemic in northern New York led to initiation of fire blight cankers on scaffolds, trunk, and rootstock leading to tree deaths, with ca. 2,500 trees per farm removed in high- and mid-density apple orchards with spindle-shaped tree training systems. Cankers are the primary sites for overwintering of fire blight pathogen *E. amylovora* until the next growing season (Slack and Sundin, 2017; Sundin, 2014). This pathogen emerges in the form of dark orange ooze on the surface of overwintered cankers which is disseminated by wind, rain and insects to new flowers and shoots allowing infections. Infected flowers or shoots are possible sites for subsequent canker initiation on perennial wood. The thicker the apple branches or trunk are with fire blight cankers, the higher the chances are for pathogen to successfully survive in them until next spring (Van Der Zwet and Keil, 1979; Vanneste, 2000). Some estimates are that as few as 1 to 4 cankers/2.5 acres might be enough to allow renewal of fire blight outbreak next spring (Brooks, 1926; Tullis, 1929). To reduce chances for infection renewal, it is important to remove cankers by pruning (Schroth et al., 1974; Vanneste and Eden-Green, 2000). Reduction of primary fire blight inoculum by pruning of infected wood or removal of heavily blighted trees is one of the key management strategies of every fire blight management program (Slack and Sundin, 2017). Even though the pruning crews could remove most of the holdover cankers during summer or winter, some always remain on the trees until the next growing season (Aćimović et al. 2014; Slack and Sundin 2017). Depending on how well trained are the crews to recognize fire blight cankers, there can be 0 – 7 cankers/2.5 acres left after the pruning (Aćimović et al., 2014). Hence, developing a post-infection PGR spray programs that could stop *E. amylovora* migration from shoots into the wood and reduce or prevent initiation of fire blight cankers would help reduce the establishment of inoculum sources and reduce necessity for reconstructive pruning on infected scaffolds and trunk (Aćimović and Meredith, 2017; Johnson and Temple, 2015; Johnson et al., 2016).

Our goal was to test whether PCA alone or in mix with activator of systemic acquired resistance (SAR) acibenzolar-S-methyl (ASM) could prevent or reduce the initiation of fire blight cankers on wood from the infected shoots. We evaluated the effect of different rates and number of post-infection spray applications of PCA through time, used alone or in mix with nonionic surfactant and/or ASM, on shoot blight severity and initiation of the resulting fire blight cankers on perennial apple wood. We intended to develop an effective post-infection spray program/s with PCA that growers could apply to reduce or prevent initiation of fire blight cankers from infected shoots and avoid or reduce excessive tree deaths in young and mature high-density apple orchards. We would recommend these PCA program/s for use after the fire blight prediction models indicated that a recent infection has occurred (NEWA, RIMpro), but was not timely prevented with an application of antibiotic/s in surfactant up to 24 h after the infection established on opened flowers.

Materials and Methods

An orchard experiment with five spray programs of Apogee (PCA 27.5%, BASF Corporation, Research Triangle Park, NC), Actigard (ASM 50%, Actigard 50 WG, Syngenta Crop Protection LLC, Greensboro, NC) and two untreated controls, was conducted in Highland, NY (Table 1). We applied these programs on 17-year-old apple trees of fire blight susceptible cv. 'Royal Cort' on M.9(337)/EMLA111 interstem, (Table 1). The experiment simulated emergency post-infection application of Apogee done immediately after fire blight infection/s were reported in the prediction models (Maryblyt, NEWA, Cougarblight, RIMpro), with the aim to provide the best chance for Apogee or Apogee + Actigard to reduce shoot blight severity and prevent the resulting canker development on perennial apple wood. Hence, spray programs started 1.5 – 2 days after the first shoot inoculation with *E. amylovora* and simulated the emergency Apogee application after fire blight model/s indicated that natural infections happened on open flowers and antibiotic application in surfactant up to 24 h after this event was not done (Steiner and Lightner, 1996). The experiment was based on the fact that in majority of severe fire blight years infections usually occur sometime during late bloom or petal fall when bacterial ooze first emerges from cankers and shoots start their growth. Each spray program consisted of three replicate trees assigned in a completely randomized design. To secure good coverage, all programs were sprayed dilute to drip (300 gal/A) using a tractor-carried Rear's Pak-Tank 100-gal sprayer, at 250 PSI, with a brass handgun. The application dates and growth stages are shown in Table 1.

Before inoculation, *E. amylovora* populations were adjusted to 7×10^8 CFU/ml on 15 May 2018 and 3×10^9 CFU/ml on 14 June 2018. These concentrations were chosen based on previous research on fire blight canker initiation on wood (Johnson and Temple 2015; Aćimović and Meredith 2017). Shoots were manually inoculated twice on 15 May at 1 to 2-inch shoot length and then on 14 June 2018 to increase the infection pressure which is more similar to natural conditions where multiple shoot infections are possible. Shoot inoculations

Table 1. Post-infection fire blight spray programs evaluated in 2018 with Apogee (prohexadione-calcium) and Actigard (acibenzolar-S-methyl), with or without surfactant Regulaid. for reduction of shoot blight and prevention of canker initiation on wood of 'Royal Cort' apple trees. Programs started 1.5 – 2 days after the first inoculation of shoots with *Erwinia amylovora* on 15 May 2018 (7 × 108 CFU/ml). Shoots were inoculated the second time on 14 June 2018 (3 × 109 CFU/ml).

#	Program and product/s	Active ingredient	Number of spray applications, amount per 100 gal or per acre	Growth stage of application*
1.	Inoculated UTC**	/	/	/
2.	Two Apogee low + Regulaid***	Prohexadione-calcium 27.5%	2 X 6 oz + 32 fl oz	1-3-inch, 14 days
3.	One Apogee high		1 X 12 oz	1-3-inch shoots
4.	Two Apogee high		2 X 12 oz	1-3-inch shoots, 14 days
5.	Two Apogee low + Actigard + Regulaid	Prohexadione-calcium 27.5% + Acibenzolar-S-methyl 50%	2 X 6 oz + 2 oz/A + 32 fl oz	1-3-inch shoots, 14 days
6.	Three Apogee low + Actigard		3 X 6 oz + 2 oz/A	1-3-inch shoots, 14 days, 14 days
7.	Non-inoculated UTC**	/	/	/

* The dates of application/s were: 1-3 -inch shoot – 17 May, 14 days after previous application – 31 May, 14 days after previous application – 15 June.

** UTC – untreated control.

*** 90.6% 2-butoxyethanol, poloxalene, monopropylene glycol, Kalo Inc., Overland Park, KS.

were done by horizontally cutting and removing the upper one-third of the leaf blade of the second or third youngest unfolded leaf on succulent shoot tip using sterile scissors previously dipped into the *E. amylovora* suspension (Koczan et al. 2009; McGhee and Sundin 2011). A total of 10 selected shoots per each tree in each spray program were inoculated, while an additional 10 shoots on the same tree replicate were selected as a negative control and wounded with sterile scissors like the inoculated shoots but dipped in distilled water instead.

We calculated shoot blight severity percent for each inoculated shoot by multiplying the ratio of necrotic shoot length i.e. fire blight lesion length (cm) to the total shoot length (cm) by 100. Only the total shoot length was measured for the negative control shoots. The baseline total shoot length measurements were done on 21 May 2018, after the inoculation but before the shoot blight expression. The total shoot length, shoot blight lesion length, and the fire blight canker length and thus initiation (i. e. incidence) on perennial wood were recorded on 18 June, 11 July and 17 September 2018. The latter two ratings done after the terminal bud set on shoots, which usually occurs in July, were done to record fire blight canker initiation on perennial apple wood which occurs late in the growing season (Aćimović et al., 2014; Johnson and Temple, 2015). Each shoot blight severity mean per tree was calculated from 10 shoots and then these tree means were averaged across the three time points of 18 June, 11 July and 17 September 2018 when shoots were rated. The grand mean shown in each graph bar was calculated from the three replicate tree means (Figure 1) . Mean percent of cankers initiated on perennial apple wood (i.e. canker incidence) originating from inoculated shoots was calculated from 30 shoots in each spray program. The disease control percents discussed in the results and discussion were calculated as percent of decline of shoot blight severity and of canker percent incidence in comparison to the program 1 i.e. inoculated untreated control or UTC (Table 1). The shoot blight severity data were analyzed using MIXED procedure in SAS Studio (SAS Institute Inc., Cary, NC). The data on canker percent incidence on perennial apple wood were analyzed using JMP Pro 14 (SAS Institute Inc., 2018).

Results

The Post-infection Programs of Apogee Reduce Shoot Blight Severity. As per Figure 1, in comparison to the inoculated UTC (program 1), the best shoot blight severity control of 89.5% was achieved with the One Apogee high (program 3), followed by the 78.8% control provided with Two Apogee high (program 4), 72% control with Three Apogee low + Actigard (program 6) and 62.6% control with Two Apogee low + Actigard + Regulaid (program 5). The Two Apogee low + Regulaid (program 2) reduced shoot blight severity significantly for 50.8%.

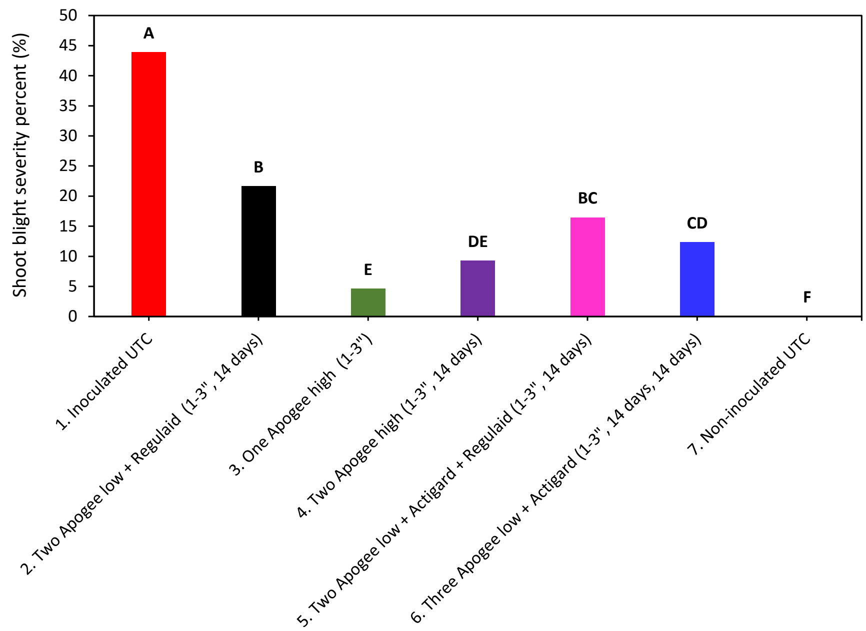

Figure 1. Reduction of shoot blight severity on 'Royal Cort' after post-infection spray programs of Apogee (prohexadione-calcium) and Actigard (acibenzolar-*S*-methyl) applied in 2018, with or without surfactant Regulaid. Shoots were inoculated with *Erwinia amylovora* on 15 May at 1 to 2-inch shoot size (7 × 10^8 CFU/ml) and on 14 June 2018 (3 × 10^9 CFU/ml). Bars with different letters are significantly different (LSD test, *P* < 0.05). Each spray program was replicated on three trees and each tree had 10 inoculated shoots rated for blight severity. Each mean per tree was calculated from 10 shoots and then these tree means were averaged across the three time points of 18 June, 11 July and 17 September 2018 when shoots were rated. Then the grand mean shown in each graph bar was calculated from the three tree means.

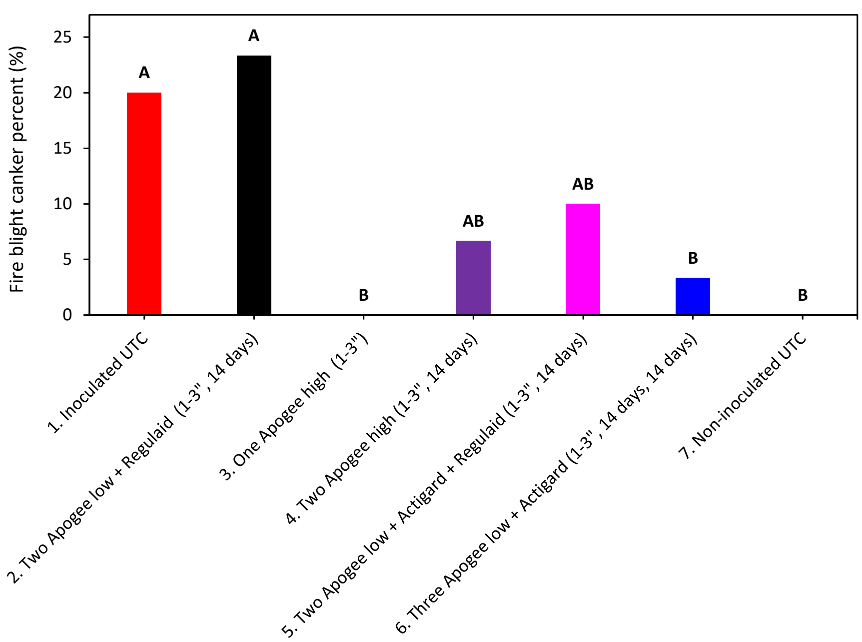

Figure 2. Fire blight cankers on perennial apple wood of 'Royal Cort' on 17 September 2018 developed from inoculated shoots in post-infection spray programs of Apogee (prohexadione-calcium) and Actigard (acibenzolar-S-methyl) with or without surfactant Regulaid. Shoots were inoculated with *Erwinia amylovora* on 15 May at 1 to 2-inch shoot size (7 × 10^8 CFU/ml) and on 14 June (3 × 10^9 CFU/ml). Bars with different letters are significantly different (Wilcoxon nonparametric each pair comparisons, *P* < 0.05). Each mean consists of 30 shoots rated for canker initiation .

The High-Rate Post-infection Programs of Apogee Reduce or Prevent Fire Blight Canker Initiation on Apple Wood. The best prevention or reduction of canker initiation on perennial wood for 100% and 83.5% was achieved by the spray programs 3 and 6, i.e. with One Apogee high and Three Apogee low + Actigard, respectively (Figure 2). Even though canker initiation was reduced numerically in programs 4 and 5, these programs were not different from the inoculated UTC (Figure 2).

Economic Viability of Post-Infection Apogee Programs. While efficacy matters, growers also need to consider the cost-effectiveness of decisions compared to other management options. In the case of the post-infection applications of Apogee, growers will consider the direct costs of additional materials applications (materials and labor) compared to the cost of alternative management practices. Two concerns for growers are pruning labor right after the fire blight outbreak to contain the disease and tree loss and continuing disease pressure from canker development.

Table 2. Assumptions used in the economic viability analysis of post-infection Apogee spray programs.

#	Program and product/s	Materials cost per acre	Labor cost for spraying per acre	Total treatment cost per acre	Reduction in pruning labor per acre
1	Inoculated UTC	$ -	$ -	$ -	0%
2	Two Apogee low + Regulaid	$ 67.01	$ 18.00	$ 85.01	50.80%
3	One Apogee high	$ 46.57	$ 9.00	$ 55.57	89.50%
4	Two Apogee high	$ 97.14	$ 18.00	$ 115.14	78.80%
5	Two Apogee low + Actigard + Regulaid	$ 235.90	$ 18.00	$ 253.90	62.60%
6	Three Apogee low + Actigard	$ 326.19	$ 27.00	$ 353.19	72%

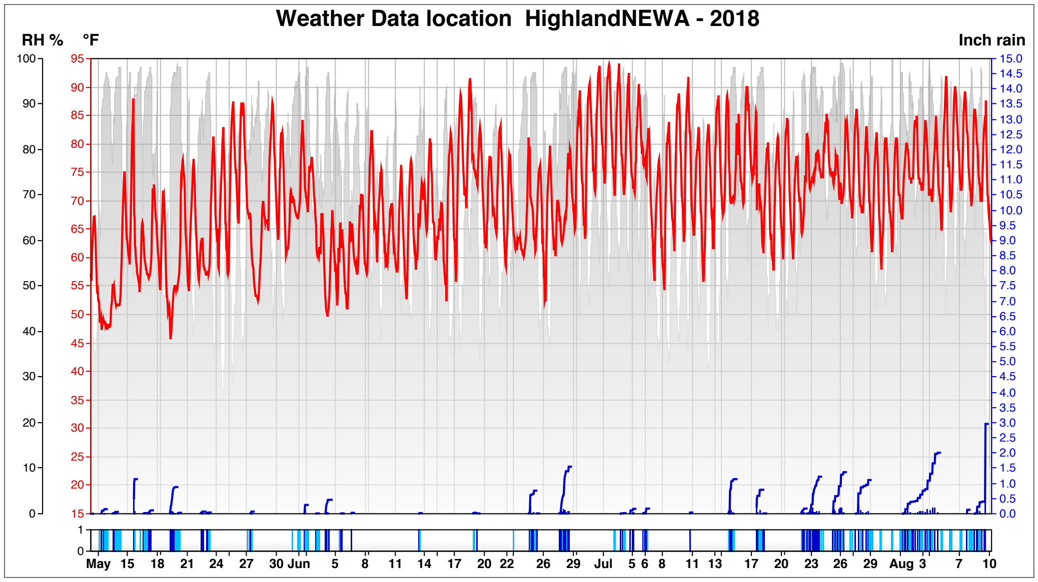

Figure 3. Weather conditions during the Apogee experiment recorded by an on-site NEWA weather station in Highland, NY. Top graph: red line shows temperatures (left y-axis in red), blue curved lines show rain lengths and amounts in inches (right y-axis in blue), grey background represent relative air humidity (RH) in % (far left y-axis in black). Bottom graph with dates shows the length of rain (dark blue) and of the wetting periods after the rain stopped (light blue). Source: RIMpro Cloud Service (RIMpro B.V., Zoelmond, Netherlands).

In the first season, the immediate economic benefit of Apogee applications would be to reduce the volume of fire blight strikes needed to be pruned out, reducing labor costs. So, the first question is whether or not an Apogee program reduces the additional pruning costs from fire blight strikes enough to justify the added expense of application. The short run economic viability assessment assumes a mature orchard where fire blight shoot strikes, even at a high pressure, are unlikely to result in the need for wide-spread tree removal. In a young orchard tree loss is a significant concern and higher short-run management costs are more likely to be justified.

Without Apogee, in the experiment, the inoculated trees had over 50% of the shoots with fire blight strikes. We assumed that the reduction in the labor cost of pruning was proportional to the reduction in fire blight strikes – i.e. if the number of strikes was reduced by 80% by an Apogee application, then labor pruning costs were also reduced by 80%, assuming that the workers could get through the orchard much more quickly. The Apogee application costs per acre were based on the actual materials costs from the experiment and estimated labor application cost (Table 2, assumed 30 min per acre for each application and labor costs of about $18 per hour based on a sprayer rate of 1.5 MPH and a 4 x 12 foot planting distance in a high density orchard).

For each program we calculated the break-even labor cost of pruning fire blight strikes from an untreated orchard. $X-tX=PCAc$ where X is equal to the break-even cost of labor of pruning fire blight strikes in an untreated orchard, tX is the expected reduction in labor costs due to the predicted percent reduction in fire blight strikes and PCAc is the cost of the Apogee program.

If a farm's actual expected labor costs (aX) are higher than the break-even labor price (X), then the Apogee program is cost effective, if the farm's expected labor costs are below the break-even labor price then the Apogee program is not cost effective (for pruning removal of shoot strikes). For farm's who are unsure of their per acre labor costs for pruning, Matt Wells of the CCE Lake Ontario Fruit Team developed a spreadsheet (available on-line) where you can put in a farm's information and come up with a per acre estimate. Pennsylvania State University (2018) estimated pruning costs per tree of $0.55 or $500 for a season in a 908 tree per-acre orchard.

Given these assumptions, in the short run, Apogee program 3 (One Apogee high) is the least cost option (Table 2). As long as a farm's actual cost for pruning labor per-acre, for fire blight strikes (when 50% of shoots are affected) is more than $62 dollars an acre (Figure 4), applying the Apogee treatment saves money in the first year. Programs 2 and 4 are also likely to have positive returns compared to the control. Programs 5 and 6 are the highest cost options, despite the effectiveness of program 6 in reducing strikes, as their per acre materials and application costs are high, even with the assumed reduction in strikes.

However, in the case of fire blight management on apples, the impact of management choices extends beyond the immediate costs during the outbreak. The impacts of management decisions carry over into future years. Build-up of fire blight pathogen in the orchard results in higher susceptibility and higher future management costs. One way to estimate the economic impact of Apogee programs on tree and yield loss is to consider reduction in canker development. Trees with more cankers are more likely to result in more severe tree pruning and, in younger orchards, more tree loss. Tree loss is significant, not only because of the direct cost of tree replacement, but because of the multi-year impact of lost revenue. These losses will vary based on the price of apples and the expected yield or life expectancy of the tree. Matt Wells of the CCE Lake Ontario Fruit Program developed a spreadsheet that calculates the net present value of a lost tree under different apple prices and orchard systems (Table 3). A single lost tree in a high-density orchard can have a net present value of over $60. In the table 3 below, using the spreadsheet we calculated the net present value of the loss of 2, 5 and 10 trees per acre in a 908 tree per acre orchard over a 14-year period, directly due to fire blight cankers at different expected rates of return per bushel.

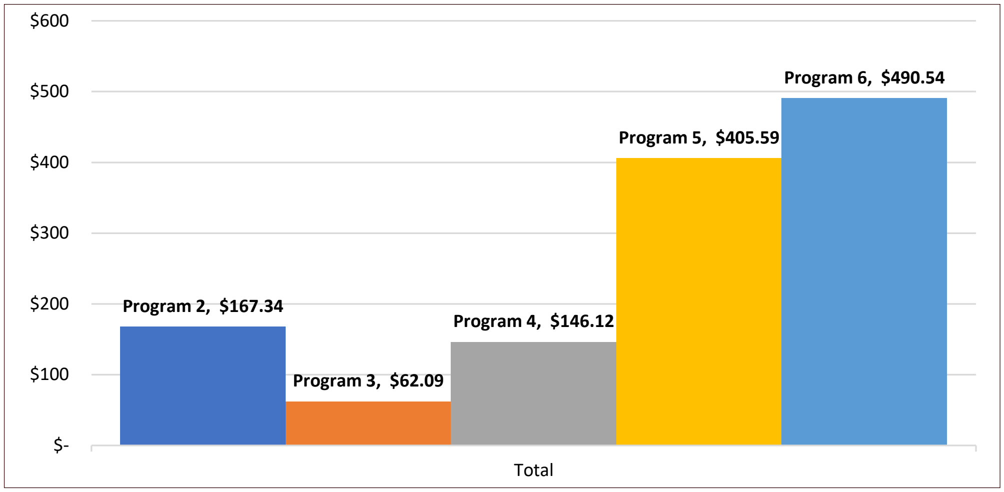

Figure 4. Break-even fire blight strike pruning cost per acre for tested post-infection Apogee programs.

Table 3. Net present value of apple tree loss per acre under different apple prices and orchard systems.

Net Present Value of Tree Loss per Acre			
	2 trees per/A	**5 trees per /A**	**10 trees per /A**
Average Yield per Acre	1000/bu	1000/bu	1000/bu
Tree Replacement Cost	$ 8.00/ea	$ 8.00/ea	$ 8.00/ea
Average Returns	**Net Present Value of Loss (per Acre)**		
Average $8/bu	$ 87.00	$ 217.00	$ 434.00
Average $10/bu	$ 102.00	$ 256.00	$ 512.00
Average $12/bu	$ 118.00	$ 295.00	$ 591.00
Average $14/bu	$ 134.00	$ 335.00	$ 669.00
Average $16/bu	$ 150.00	$ 374.00	$ 747.00

Apogee program 3 and program 6 both had statistically significant reductions in canker development. Program 3 has an $55.6 per acre cost. If the treatment prevented more than 2 trees per acre from being lost, regardless of reduced pruning costs, it would pay for itself. At $353 per acre, program 6 is likely to be economically viable if it prevents the loss of more than 5 trees per acre. Although expensive compared to the other treatments, given its relatively high level of effectiveness in both preventing strikes and cankers, program 6 might be an option to consider in younger orchards where tree loss is a high risk. Overall, Apogee program 3, because of its strong performance in both canker reduction and its cost effectiveness in shoot blight control would seem to be the economically the best option for most growers.

Discussion

We demonstrate that post-infection applications of prohexadione-calcium (Apogee) alone or in combination with acibenzolar-S-methyl (Actigard) initiated two days after the infection reduce shoot blight severity, but more importantly reduce or prevent fire blight canker development on perennial apple wood. In a controlled inoculation study on mature apple trees, we show that the most effective programs for shoot blight severity reduction and canker initiation prevention or reduction were one spray application of Apogee at 12 oz rate and the three applications of Apogee at 6 oz + Actigard at 2 oz. Fire blight cankers on the limbs, trunk, and rootstock can kill young or columnar high-density trees due to the greater susceptibly of young tissues and/or small tree sizes and can serve as sources of inoculum for infection renewal on mature trees. Hence, we recommend these two programs as viable post-infection spray options to reduce or prevent the destructive initiation of fire blight cankers on perennial wood.

The Apogee rate of 12 oz applied two times after inoculation provided 78.8% reduction of shoot blight severity, while the same rate applied once gave 89.5% severity reduction. Previous study on cv. Idared and cv. Freedom showed that Apogee application/s before infection can reduce shoot blight severity for 44.9 – 95.7% at 7, 14 and 21 days after the inoculation (Bubán et al., 2004). Preventive Apogee 6 oz application once, during shoot growth, provided 63.9% control of shoot blight severity on Idared inoculated on 10 June (Cox et al., 2015). On Idared, with shoot inoculations done on 2 June, two preventive spray applications of Apogee on 13 and 20 May at 12 oz, applied at 1 –

5 inch and then 6 – 8 inch shoot sizes, provided 85.9% shoot blight severity reduction (Momol et al., 1998). In an experiment with inoculation of fourth-leaf Royal Gala, a preventive application of a 12 oz followed by a 6 oz appliation, provided fire blight severity reduction of 96 – 100% on (Norelli and Miller, 2004). These results after preventive Apogee applications at a similar rate were either very similar, or if the rate was lower, weaker in comparison to severity reduction we achieved in our post-infection programs on 'Royal Court' with Apogee 12 oz rate applied one or two times.

A 100% and 83.5% reduction in canker initiation on perennial apple wood was achieved with one Apogee application at 12 oz and the three Apogee applications at 6 oz + Actigard 2 oz/A, respectively. Previous studies show that preventive Apogee application at 18 oz in Regulaid at 0.125%, allowed inoculated shoots to developed tip shoot blight but significantly fewer number of these had the necrosis lesion expansion to the perennial wood to form a canker (Yoder et al., 1999). This directly supports canker reduction we achieved in our post-infection program with three Apogee applications at 6 oz + Actigard 2 oz/A, delivering a total of 18 oz of Apogee. In spray trials under natural fire blight infections, it was found in autumn that two preventive applications of Apogee at 6 oz + Choice 0.25% + LI 700 0.15% on 1 May and 29 June (total 12 oz Apogee), significantly reduced the incidence of cankers on perennial wood of limbs or trunks that were initiated from shoot blight: 70% reduction on cv. Northwestern Greening and 61 and 96% on apple cv. York Imperial (Yoder and Marini, 2002). These preventive Apogee applications directly support canker reduction we achieved on 'Royal Cort' after single post-infection application of Apogee at 12 oz.

Our spray programs applied two days after shoot inoculation can reduce canker initiation on perennial wood. This offers evidence that Apogee used at a high rate is a potent option for post-infection management of fire blight with the aim to stop pathogen progression into the wood. Similar research on spray programs with PGRs and SARs for management of fire blight is important as it has promising potential to lower the devastating effect of unmanaged epidemics of this disease through reduction of canker-related tree deaths and a need for substantial and time-consuming reconstructive pruning to remove cankers on scaffolds and trunk (Aćimović and Meredith, 2017; Johnson and Temple, 2015; Johnson et al., 2016). If fire blight infections have established based on disease models and antibiotic applications were not done on time, applications of Apogee or Apogee + Actigard as soon as possible are the best and cost effective practices that can reduce or avoid significant tree deaths in young and mature high-density apple orchards.

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