Project Title: Program for Control of Shoot Blight and Fire Blight Cankers on Pear

Report Type: Final Project Report

Primary PI: Srdjan G. Acimovic
Organization: Virginia Tech
Telephone: 540-232-6037
Email: acimovic@vt.edu
Address: 595 Laurel Grove Rd
Address 2: Alson H. Smith Jr. Agricultural Research and Extension Center
City/State/Zip: Winchester, VA 22602

Co-PI 2: Achala N. KC
Organization: Oregon State University,
Telephone: 541-772-5165 ext. 222
Email: achala.kc@oregonstate.edu
Address: 569 Hanley Rd
Address 2: Southern Oregon Research and Extension Center
City/State/Zip: Central Point, OR 97502

Cooperator: Kenneth Johnson
Organization: Oregon State University
Telephone: 541-737-5249
Address: Cordley Hall 4105, 2701 SW Campus Way
Address 2: Department of Botany and Plant pathology
Email: Kenneth.Johnson@oregonstate.edu
City/State/Zip: Corvallis, OR 97331

Project Duration: 2-Year

Total Project Request for Year 1 Funding: $20,513
Total Project Request for Year 2 Funding: $21,257

Other related/associated funding sources: Awarded
Funding Duration: 2022 - 2024
Amount: $286,650
Agency Name: USDA Crop Protection and Pest Management Program
Notes: Title “Creating Next-Gen controls for fire blight cankers, blossom and shoot blight with copper, PGR-s, plant activators and anti-biofilm enzymes”

Other related/associated funding sources: Awarded
Funding Duration: 2023 - 2027
Amount: $5.7 million
Agency Name: USDA Specialty Crop Research Initiative (SCRI) Program
Notes: Title “An all-stage fire blight control: remote sensing, DNA, enzyme and plant activator technologies for cankers, blossom blight and shoot blight”

WTFRC Collaborative Costs: N/A

Budget 1
Primary PI: Srdan G. Acimovic
Organization Name: Virginia Tech
Contract Administrator: Jessi King
Telephone: 540-231-7521
Blacksburg, VA 24061, Campus Mail Code: 0170
Contract administrator email address: Jessilp2@vt.edu
Station Manager/Supervisor: Lesley Mitchell
Station manager/supervisor email address: lesleyg@vt.edu

Item	02/01/2023	02/01/2024
Salaries (Graduate student /GRA/, 29% effort)	$9,165	$9,603
Benefits	$848	$884
Wages	-	-
Benefits	-	-
Equipment	-	-
Supplies	-*	-*
Travel	-	-
Miscellaneous	-	-
Plot Fees	-	-
Total	$10,013	$10,487

Footnotes:
Salaries: Salaries are requested for a Graduate Student (GRA Step 15) @ $2,554/month for 29% effort and benefits rate.
*Laboratory and field supplies will be covered from the above-mentioned USDA CPPM project.

Budget 2
Co PI 2: Achala N. KC
Organization Name: Oregon State University
Contract Administrator: Russ Karow
Telephone: 541-737-4066
Contract administrator email address: russell.karow@oregonstate.edu
Station Manager/Supervisor: Richard Roseberg
Station manager/supervisor email address: richard.roseberg@oregonstate.edu

Item	02/01/2023	02/01/2024
Salaries		
(FRA 1 month)	$3,750	$3,863
Benefits (FRA @ 79.99%)	$3,000	$3,090
Equipment	-	-
Supplies	$2,250	$2,318
Travel	-	-
Miscellaneous	-	-
Plot Fees	$1,500	$1,500
Total	$10,500	$10,770

Footnotes:
Salaries: Salaries are requested for a Graduate Student (FRA) @ $45,000/year for 1 month, and 80% benefit rate.
Supplies: Funding is requested for materials to collect and process samples, plates, and media to culture *E. amylovora*, labels and field supplies.
Plot Fees: Funding is requested for SOREC research plot fees for trials @ $3,000 per acre. We estimated that approximately ½ acre worth of trees and fruits will be used for this trial.
Funding request for year 2 includes additional 3% inflation.
[5] Funding request for year 2 includes 3% inflation

1. VIRGINIA

Objectives

With the goal to reduce or prevent shoot blight severity and prevent canker development on pear wood, our objectives are to:

(1) Determine Regalia efficacy on mature pear trees against shoot blight and canker development (OR) and compare it to the same effect on young trees (VA, OR),

(2) Determine if 153.6 fl oz/acre of Regalia applied in less numbers of treatments (one, two) can achieve the same effect on fire blight (VA, OR),

(3) Compare fruit russeting at harvest after Regalia, Actigard and Apogee and antibiotic spray programs (OR).

(4) Using the price lists from local pesticide distributors in WA, OR and VA, compare the cost of Regalia programs, select, and recommend the most effective and cost-beneficial program for pears that do not cause fruit russeting.

Deviations from the original objectives: fruit did not develop in sufficient numbers due to 3-hour frost during pear bloom in Winchester, VA (4/9/2023), so the russeting incidence was not rated. In Winchester, not enough pear fruit developed in 2024 in per tree basis to be able to fulfill statistical requirements, so the fruit russeting incidence was not rated in 2024.

Significant Findings

In 2023:

A) In Winchester, when compared to the untreated control with 47% shoot blight severity:

- The spray programs #2 (three spray applications of Regalia 32 fl oz/A) and #4 (one spray application of Regalia 153.6 fl oz/A) provided 100% and 78.3% shoot blight severity control, respectively.
- The spray program #7 (antibiotics with surfactant) and #8 (Apogee 6 oz/100 Gal + Cueva 120 fl oz) provided 100% and 89.4% shoot blight severity control, respectively.
- To our surprise spray program #1 (five spray applications of Regalia 30.72 fl oz/A) which is inconsistent with our previous 2-year results.

B) In Winchester, when compared to the untreated control with 44% canker incidence:

- The spray programs #2 (three spray applications of Regalia 32 fl oz/A) and #4 (one spray application of Regalia 153.6 fl oz/A) provided 100% and 76.8% canker incidence control, respectively.
- The spray program #7 (antibiotics with surfactant) and #8 (two spray applications of Apogee 6 oz/100 Gal + Cueva 120 fl) oz provided 100% and 88.6% canker incidence control, respectively.
- Five spray applications of Regalia 30.72 fl oz/A failed to control canker incidence.

In 2024:

A) In Winchester, when compared to untreated control with 47.1% shoot blight severity:

- The spray program #7 (two applications of FireWall+FireLine+Regulaid) at BL, then ~24 h after shoot inoculation, provided 100% control of shoot blight, canker incidence and canker length. This is consistent with the 2023 results of the same spray program.
- In comparison to 2023 results, the only other spray program that was numerically consistent in comparison to 2024 results was #2 (three spray applications of Regalia 32 fl oz/A) which gave 67.1% control of shoot blight severity, albeit not significantly different from #9 Untreated Control (Fig. 6). This consistency was also seen in canker incidence and length reduction (Fig. 7, 8)
- Surprisingly but unfortunately, the spray programs #4 (one spray application of Regalia 153.6 fl oz/A) and #8 (two spray applications of Apogee 6 oz/100 Gal + Cueva 120 fl), which were effective in 2023, failed to provide even numerical fire blight disease reduction in 2024.
- Finally, spray program #1 (five spray applications of Regalia 30.72 fl oz/A) was the closest to the success in fire blight control of the spray program #7 (two applications of FireWall + FireLine + Regulaid) in 2024, which is glaringly inconsistent with the 2023 results.

Methods

Cultivar. 7-yr-old (2023) and 8-year-old (2024) Bartlett trees, planted at 10 ft between trees and 16 ft between rows. Trees were assigned in a completely randomized design.

Spray equipment in 2023 and 2024. Spray programs were spray-applied to 4 trees for each spray program (4 replicates per treatment). Spray was applied dilute at 100 gal/A to drip using a tractor-carried sprayer using a brass 'Friend' handgun connected to Pak-Blast 100-gal sprayer, with diaphragm pump pressure at 250 PSI (Rear's Manufacturing, Coburg, OR) at 11.7 gal/min output to secure good spray coverage.

Spray dates. Due to the uneven onset of growth stages in young trees of the pear block in Winchester, VA, the spray application dates for each spray program in 2023 were:

#	Number of spray applications, materials, and amount	Applied at pear growth stage (spray timing)	Dates of applications
1	5 X Regalia 30.72 fl oz/A	BB, GC, WB, PF, FS	3/26, 4/2, 4/5, 4/13, 4/19
2	3 X Regalia 32 fl oz/A (lower label rate)	WB, PF, FS	3/26, 4/9, 4/13
3	2 X Regalia 76.8 fl oz/A	PF, FS	4/13, 4/19
4	1 X Regalia 153.6 fl oz/A	FS	4/19
5	2 X Regalia 76.8 fl oz/A + Apogee 10 oz/100 gals	PF, FS	4/13, 4/19
6	**2 X** Apogee 10 oz/100 gal*	PF, FS	4/13, 4/19
7	**2 X** Agri-Mycin 16 oz/A + FireLine 16 oz/A + Regulaid 32 fl oz/100 gals	BL, 24 h before shoot inoculation	4/13, 4/23
8	**2 X** Apogee 6 oz/100 Gal* + Cueva 120 fl oz (2 oz metallic copper/A)	1 to 3-inch new shoot growth, 14 days after	4/13, 4/23
9	Untreated control	-	-

Spray dates. For each spray program in 2024 were:

#	Number of spray applications, materials, and amount	Applied at pear growth stage (spray timing)	Dates of applications
1	5 X Regalia 30.72 fl oz/A	BB, GC, WB, PF, FS	3/7, 3/14, 3/19, 4/5, 4/16
2	3 X Regalia 32 fl oz/A (lower label rate)	WB, PF, FS	3/29, 4/16, 4/22
3	2 X Regalia 76.8 fl oz/A	PF, FS	4/16, 4/22
4	1 X Regalia 153.6 fl oz/A	FS	4/22
5	2 X Regalia 76.8 fl oz/A + Apogee 10 oz/100 gals	PF, FS	4/16, 4/22
6	**2 X** Apogee 10 oz/100 gal*	PF, FS	4/16, 4/22
7	**2 X** Agri-Mycin 16 oz/A + FireLine 16 oz/A + Regulaid 32 fl oz/100 gals	BL, 24 h before shoot inoculation	4/10, 4/29
8	**2 X** Apogee 6 oz/100 Gal* + Cueva 120 fl oz (2 oz metallic copper/A)	1 to 3-inch new shoot growth, 14 days after	4/22, 5/6
9	Untreated control	-	-

Maintenance sprays. 2023: Thiram 4/19/2023 and 5/5/2023 as deer repellent. **2024:** none.

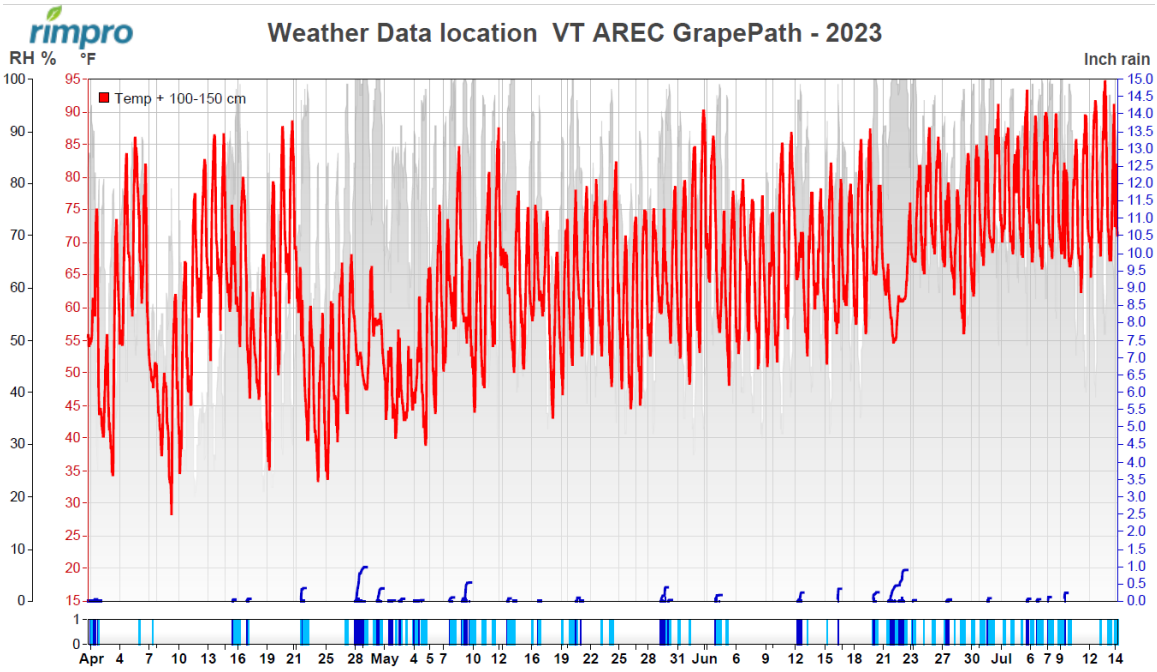


Figure 1. Weather conditions in 2023 during the fire blight experiment at Winchester, VA. Source: RIMpro B.V., Netherlands via NEWA, Cornell University, NY, U.S.A.

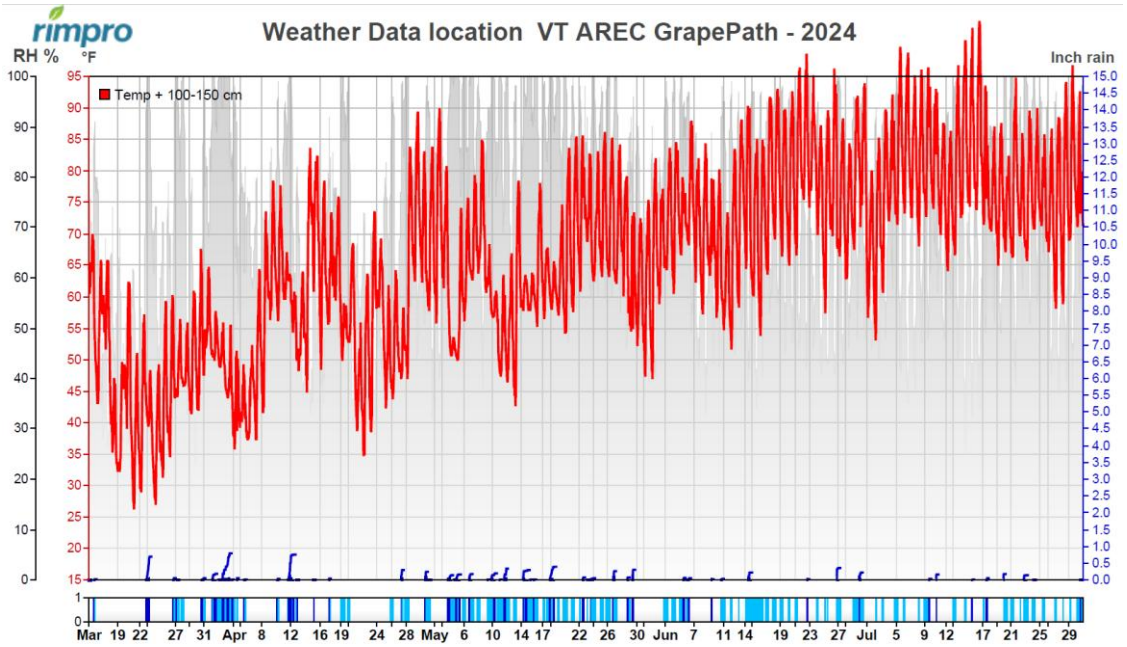


Figure 2. Weather conditions in 2024 during the fire blight experiment at Winchester, VA. Source: RIMpro B.V., Netherlands via NEWA, Cornell University, NY, U.S.A.

2023 Inoculation and disease rating. A 10 'Bartlett' shoots per tree were inoculated on **4/25/2023**. Bloom was lasting from 4/4/2023 to 4/11/2023. We used *E. amylovora* suspension of strain 110 (2 x 10^8 CFU/ml). We inoculated shoots by making a slanted sleeve incision 1 to 2 inches below the shoot tip by cutting into the soft stem tissue with a sterile scalpel. We delivered 40 microliters of *E. amylovora* cell suspension by a micropipette into the sleeve incision. For each inoculated shoot, we calculated shoot blight severity percent by multiplying the ratio of necrotic shoot length i.e. fire blight lesion length (cm) to the total shoot length (cm) by 100. We repeatedly measured the shoot blight severity, canker incidence, and canker length on the same shoots on 23 May, 23 June, and 23 July 2023 and conducted repeated measures statistical analysis accounting for time as the factor (Figures 3 – 5) Mean shoot blight severity percent, mean percent of initiated cankers on perennial pear wood (canker incidence), and mean canker length on wood per each replicate tree was calculated from 10 shoot replicates. Mean shoot blight severity, mean canker incidence, and mean canker length on perennial apple wood for each program was calculated from the four replicate tree means (Figures 3 – 5).

2024 Inoculation and disease rating. A 10 'Bartlett' shoots per tree were inoculated on **4/30/2024**. We used a stem sleeve cut inoculation method on 30 April 2024 by delivering 40 µL of *E. amylovora* (Ea110) distilled water suspension per shoot adjusted to 2 x 10^8 CFU using a micropipette. A total of 10 selected and labeled shoots per apple tree in each treatment were inoculated with E. amylovora. For each inoculated shoot, we calculated shoot blight severity percent by multiplying the ratio of necrotic shoot length i.e. fire blight lesion length (cm) to the total shoot length (cm) by 100. We repeatedly measured the shoot blight severity, canker incidence, and canker length on the same shoots on 24 May, 24 June, and 24 July 2024 (Figs 6, 7, 8). Shoot blight severity is shown in Fig. 6. Canker incidence is shown in Fig. 7 and canker length on perennial wood originating from blight lesion expansion from the inoculated shoots in Fig. 8. Mean shoot blight severity percent and mean percent of initiated cankers on perennial apple wood for each replicate tree (canker incidence) was calculated from 10 shoot replicates. Mean shoot blight severity and the mean percent of initiated cankers on perennial apple wood for each program was calculated from the four tree replicate means.

Problems or limitations that were encountered in 2023 (Virginia). Fruit did not develop in sufficient numbers due to 3-hour frost during bloom (4/9/2023) in Winchester, VA, so the russeting incidence was not rated. **Types and timing of anticipated results.** We plan to repeat the same trial in Winchester in 2024.

Problems or limitations that were encountered in 2024 (Virginia). Limited fruit which developed in 2024 on young pear trees was not sufficient in number in per tree basis to be able to fulfill statistical requirements, so the fruit russeting incidence was not rated.

Results and Discussion

VIRGINIA 2023:

Summary graphics.

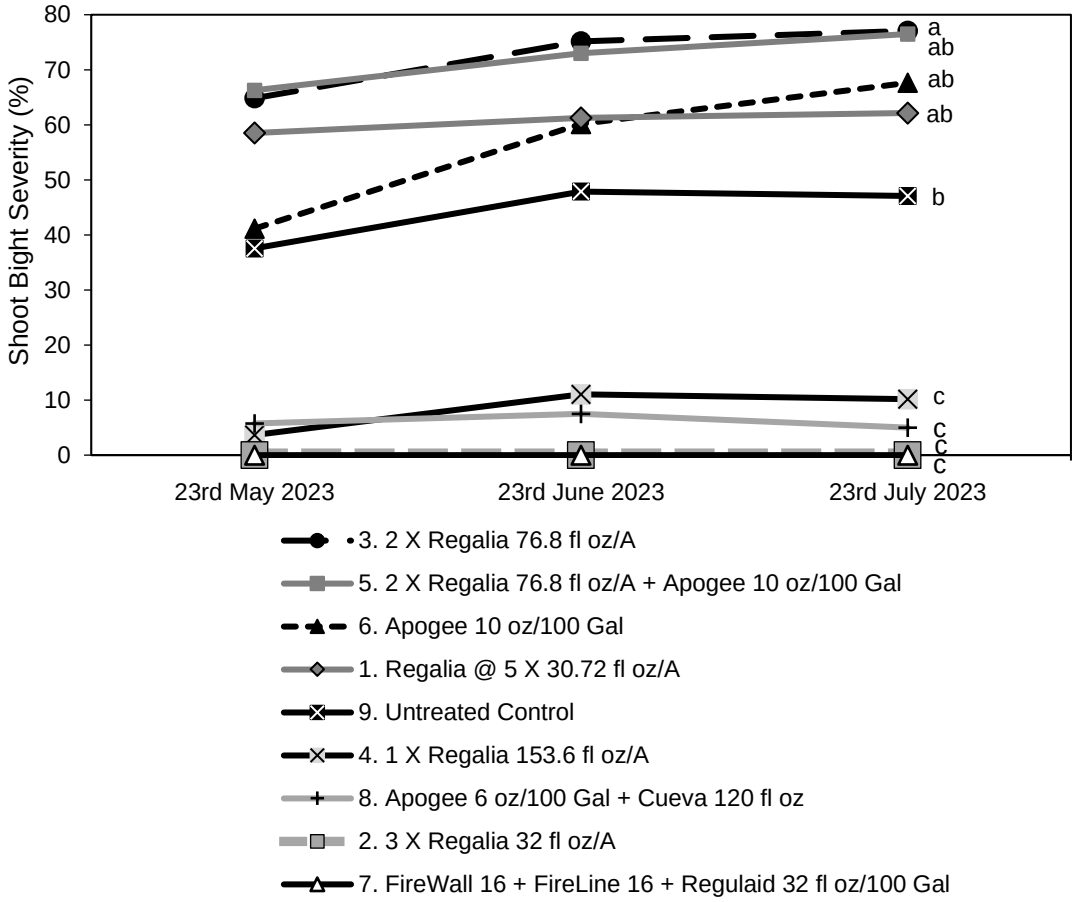


Figure 3. Shoot blight severity on pear cultivar 'Bartlett' from infected shoots after preventive spray treatments in 2023. Shoots were inoculated on 25 April at 2.5- to 5-cm shoot size with *Erwinia amylovora* (2 × 10^8 CFU/ml). Treatment lines followed by different letters are significantly different (repeated measures *t*-tests, $P < 0.05$). Each mean consists of four trees, each with a tree mean consisting of 10 shoots per tree.

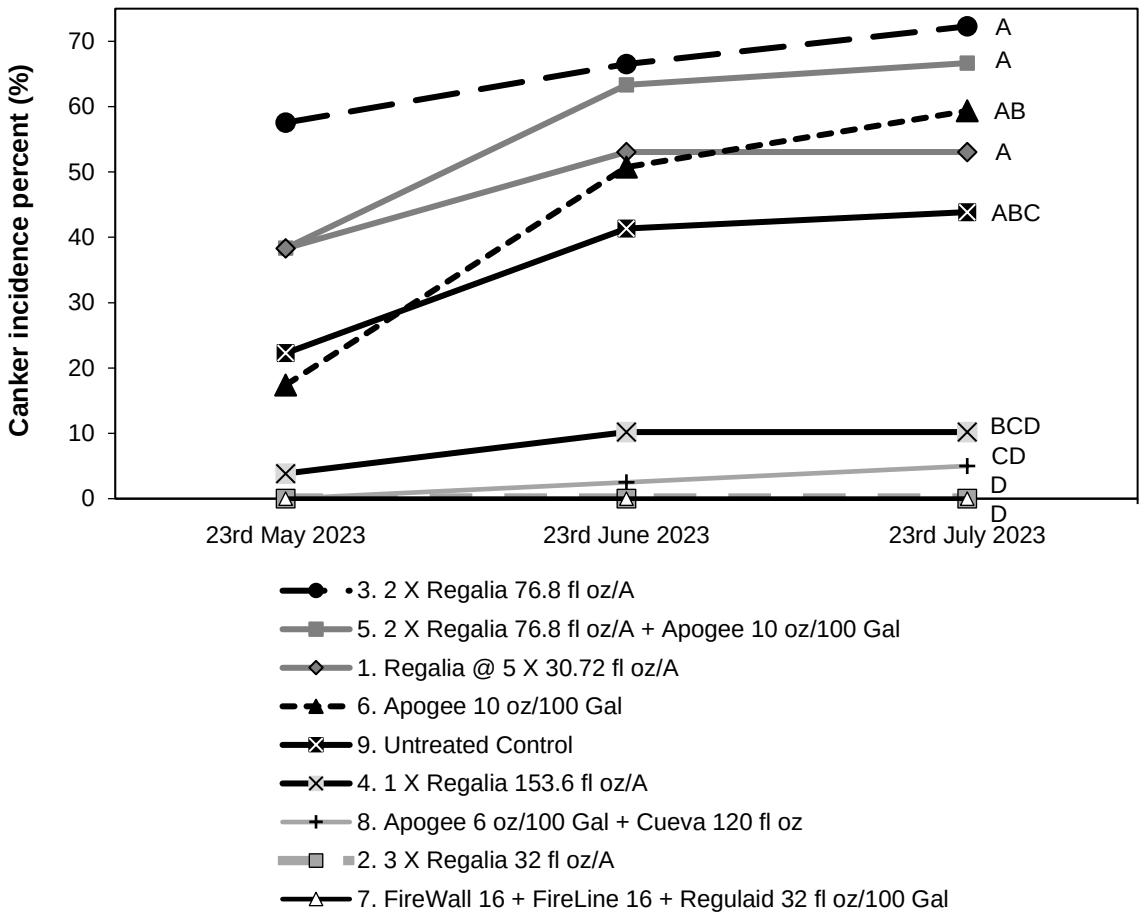


Figure 4. Canker incidence on pear cultivar 'Bartlett' from infected shoots after preventive spray treatments in 2023. Treatment lines followed by different letters are significantly different (repeated measures *t*-tests, $P < 0.05$). Each mean consists of four trees, each with a tree mean consisting of 10 shoots per tree.

Based on the 2023 data from Virginia and when compared to Oregon data from 2023 (see below), it seems that Regalia is more effective on younger pear trees (Virginia) in comparison to mature trees (Oregon). In Virginia, the most effective treatments were #2, #4, #7, and #8 (Figures 2-4). Furthermore, five spray applications of Regalia 30.72 fl oz/A seems to be inconsistent with the previous results we reported in Borba et al. (2023). We have met in person with ProFarm Group (formerly known as Marrone Bio Innovations) to record the container batch number of the Regalia (5%) used in our trial and inform us if any issues were associated with formulating this product potentially leading to poor results. At the time of creation of this report we have not heard back from ProFarm Group.

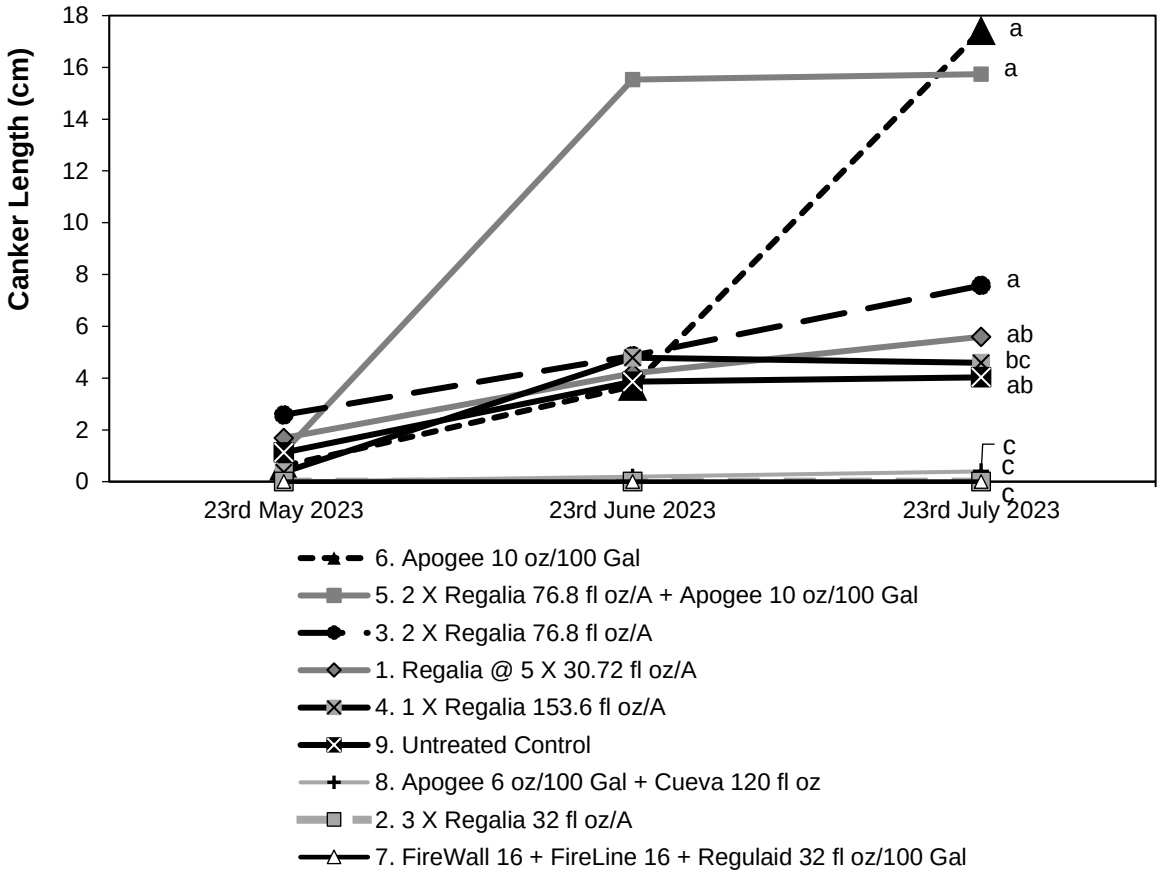


Figure 5. Length of fire blight cankers on perennial wood of pear cultivar 'Bartlett' from infected shoots after preventive spray treatments in 2023. Treatment lines followed by different letters are significantly different (repeated measures *t*-tests, $P < 0.05$). Each mean consists of four trees, with each tree mean consisting of 10 shoots per tree.

***VIRGINIA 2024*:**

Summary graphics.

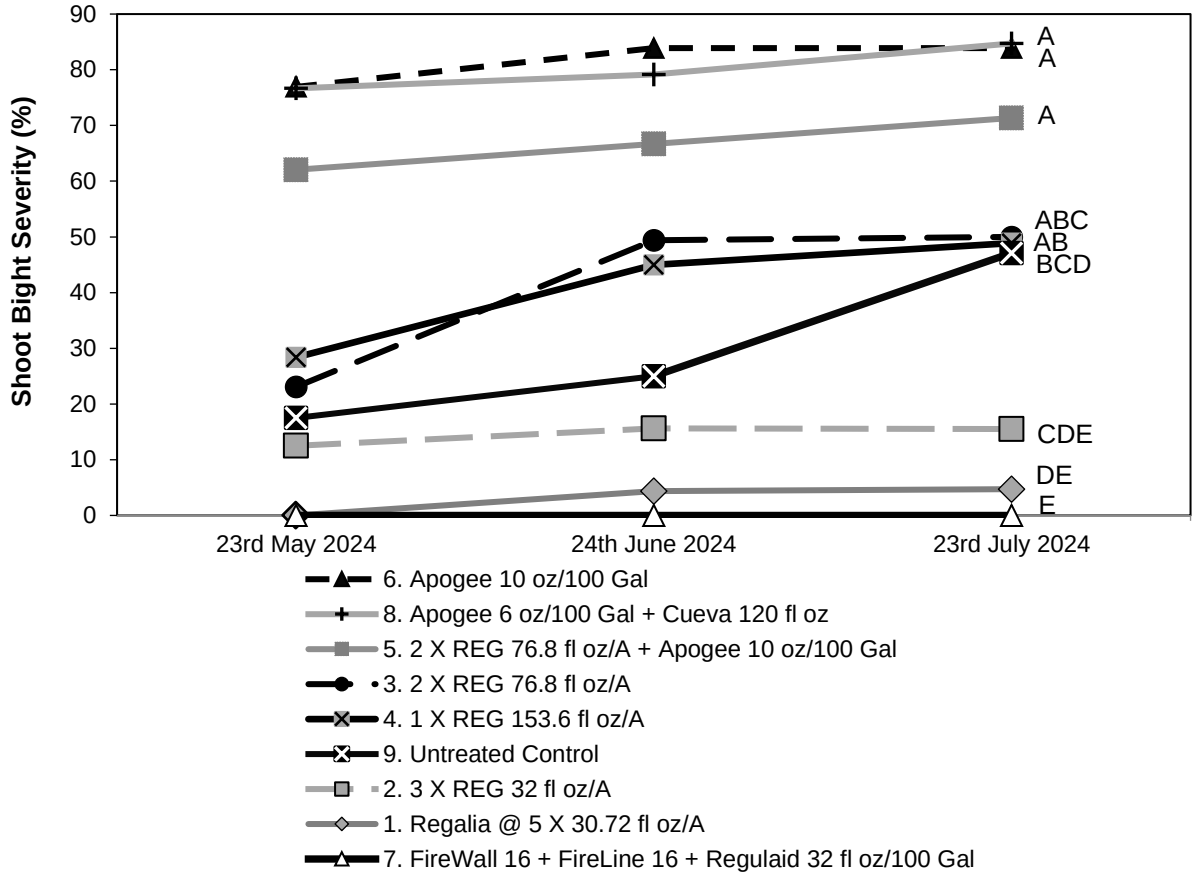


Figure 6. Shoot blight severity on pear cultivar 'Bartlett' from infected shoots after preventive spray treatments in 2024. Shoots were inoculated on 30 April at 2.5- to 5-cm shoot size with *Erwinia amylovora* (2 × 10^8 CFU/ml). Treatment lines followed by different letters are significantly different (repeated measures *t*-tests, $P < 0.05$). Each mean consists of four trees, each with a tree mean consisting of 10 shoots per tree.

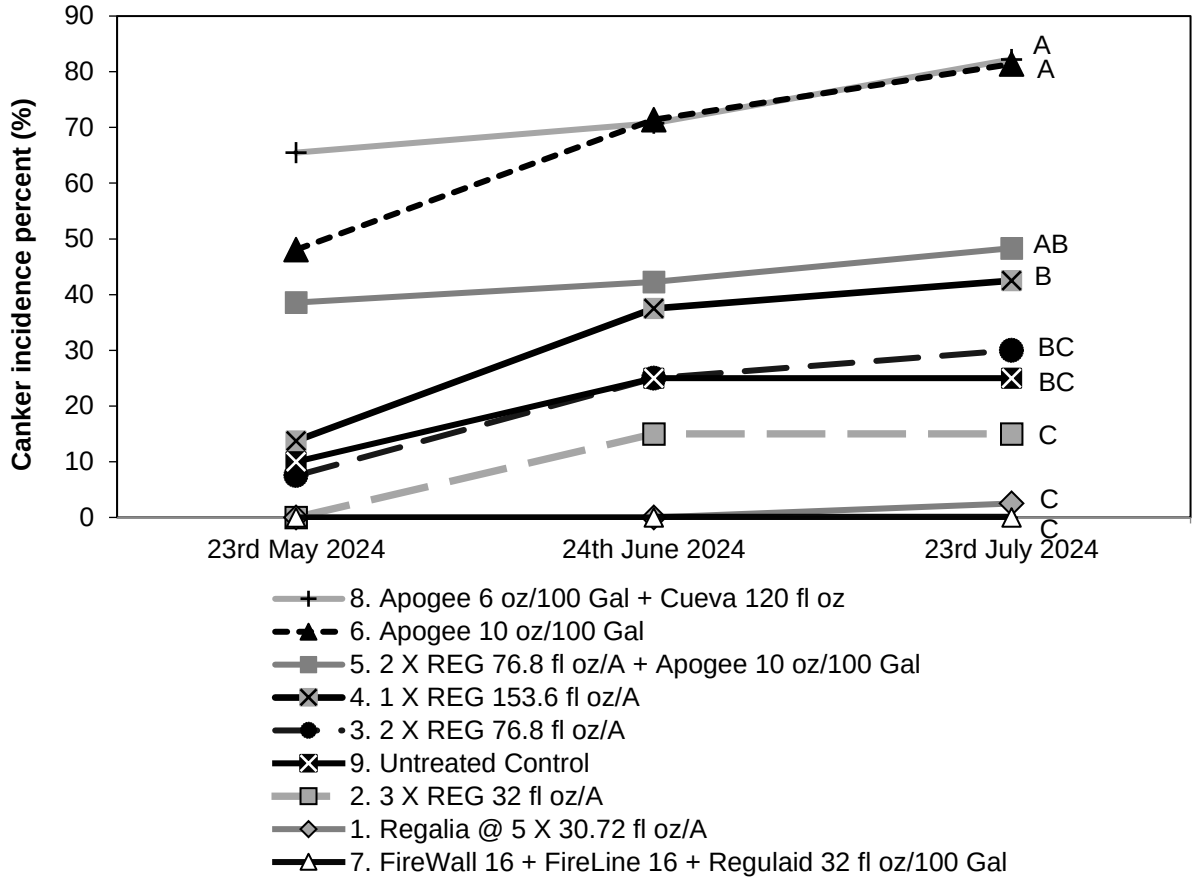


Figure 7. Canker incidence on pear cultivar 'Bartlett' from infected shoots after preventive spray treatments in 2024. Treatment lines followed by different letters are significantly different (repeated measures *t*-tests, $P < 0.05$). Each mean consists of four trees, each with a tree mean consisting of 10 shoots per tree.

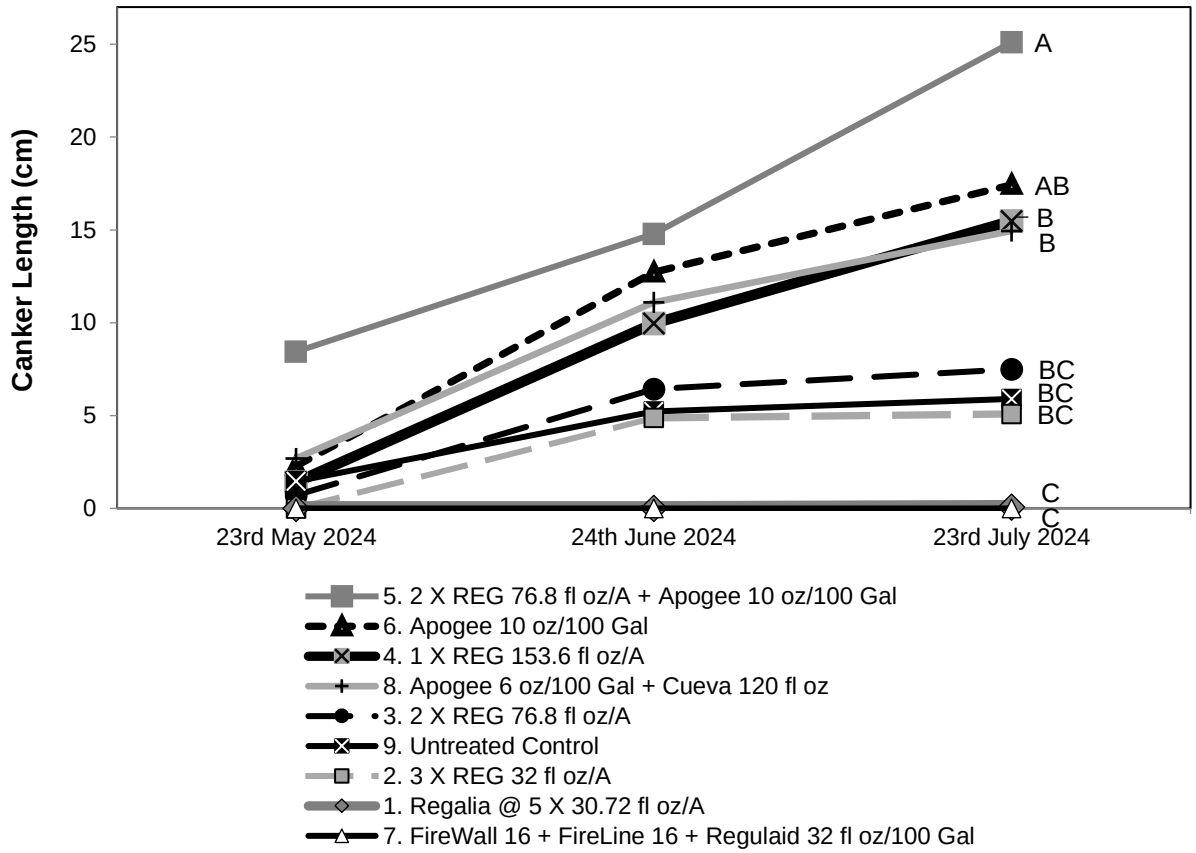


Figure 8. Length of fire blight cankers on perennial wood of pear cultivar 'Bartlett' from infected shoots after preventive spray treatments in 2024. Treatment lines followed by different letters are significantly different (repeated measures *t*-tests, $P < 0.05$). Each mean consists of four trees, with each tree mean consisting of 10 shoots per tree.

Based on younger pear trees we used in Virginia the most effective treatment for controlling shoot blight severity for #7 FireWall + FireLine (Figure 4). However, this significant effect of treatment #7 has not stayed stable and did not control canker incidence and canker size (no significant differences in comparison to #9 Untreated Control. We are not sure why the fire blight did not advance more in the #9 Untreated Control, which would be expected. We are unaware of any reasons for this #9 Untreated Control failure.

Since no significant differences were visible in 2024 data set between Regalia and #9 Untreated Control, we cannot make a sound conclusion on Regalia's effectiveness on pear shoots and cankers, except that we find Regalia to be more and more inconsistent across many years of our and others research. We believe that Regalia is likely highly dependent from unknown optimal environmental, spray water, or formulation conditions to confer its desired effect. We have not tested the pH of water we used and we have not investigated the effect of environmental factors on the Regalia efficacy. However, we did hear that Regalia's formulation has undergone a change to reduce side effect of apple/pear flower phytotoxicity. This information was conveyed to use unofficially. The inconsistency in the efficacy from year to year is also in comparison to our previous results we reported in Borba et al. (2023) in Virginia, and in comparison to the results from Yan et al. (2023) in Michigan on apple, and from Boeckman et al. (2024) in Virginia on apple. Our discussions with ProFarm Group (formerly known as Marrone Bio Innovations) who produce Regalia have not

continued after we provided them with the container batch number of the Regalia (5%) used in our trials. They committed to informing us if there were any issues associated with the formulation of this product potentially leading to our poor results in 2024 when compared to earlier years, but we doubt they will contact us. At the time of creation of this report we have not heard back from ProFarm Group.

2. OREGON 2023 & 2024

Significant Findings

Other than the commercial standard of fire blight management on pears using antibiotics, no significant differences among treatments were observed for shoot blight and canker management.

Methods

The 'Bartlett' trees at Southern Oregon Research and Extension Center, in Central Point, OR, are planted at 14.8 ft. between rows and 9 ft between trees within rows. Each spray program (treatment) listed in Table 1 were applied to three replicate trees in Oregon. Trees were assigned in a completely randomized design. Inoculation of 15 shoots per tree were done after application of spray programs with *E. amylovora* suspension of 2 x 10^8 CFU/ml on May 19, 2023. Similarly, 10 shoots per tree were inoculated on May 7, 2024 for second year trial.

Treatment #	Number of spray applications, materials, and amount	Applied at pear growth stage (spray timing)	Dates of treatment application (2023)	Dates of treatment application (2024)
1	5 X Regalia 30.72 fl oz/A	BB, GC, WB, PF, FS	04/05; 04/18; 04/24; 05/09; 05/18	03/18; 03/21; 03/25; 04/10; 05/06
2	3 X Regalia 32 fl oz/A (lower label rate)	WB, PF, FS	04/24; 05/09; 05/18	03/25; 04/10; 05/06
3	2 X Regalia 76.8 fl oz/A	PF, FS	05/09; 05/18	04/10; 05/06
4	1 X Regalia 153.6 fl oz/A	FS	05/18	05/06
5	2 X Regalia 76.8 fl oz/A + Apogee 10 oz/100 gals	PF, FS	05/09; 05/18	04/10; 05/06
6	2 X d'Anjou pear rate for Vegetative Growth Control and Reduced Latent Bloom: Apogee 10 oz/100 gal*	PF, FS	05/09; 05/18	04/10; 05/06
7	2 X Pear Grower Standard in PNW: Agri-Mycin 16 oz/A + FireLine 16 oz/A + Regulaid 32 fl oz/100 gals	BL, 24 h before shoot inoculation	05/01; 05/18	04/06; 05/06
8	2 X d'Anjou pear Grower Standard for Shoot Blight: Apogee 6 oz/100 Gal* + Cueva 120 fl oz (2 oz metallic copper/A)	1 to 3-inch new shoot growth, 14 days after	05/09; 05/23	04/22; 05/10
9	Untreated control	-	-	-

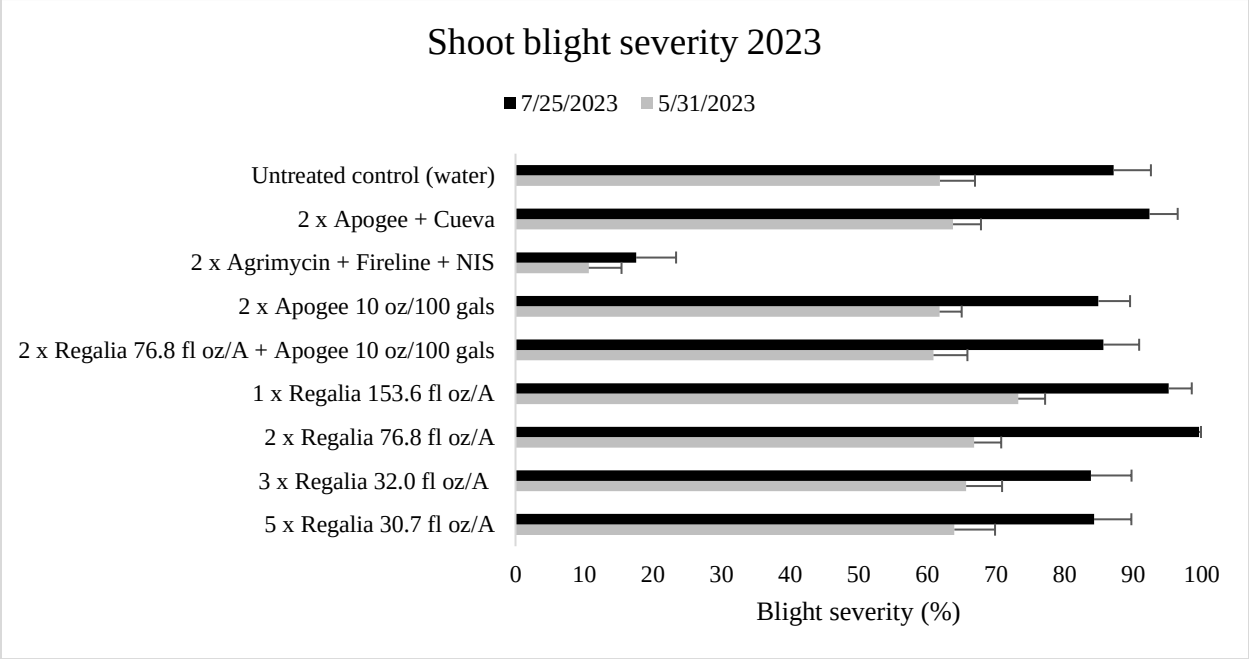
Data on total shoot length and lesion length were measured on May 31, and July 25, 2023. Similarly, data on total shoot length and lesion length were measured on May 17, and June 6, and July 17, 2024. Cankers were noticed on secondary and tertiary branches during July 25 and 17 data collection in 2023 and 2024 respectively. The total branch length and canker length were measured.

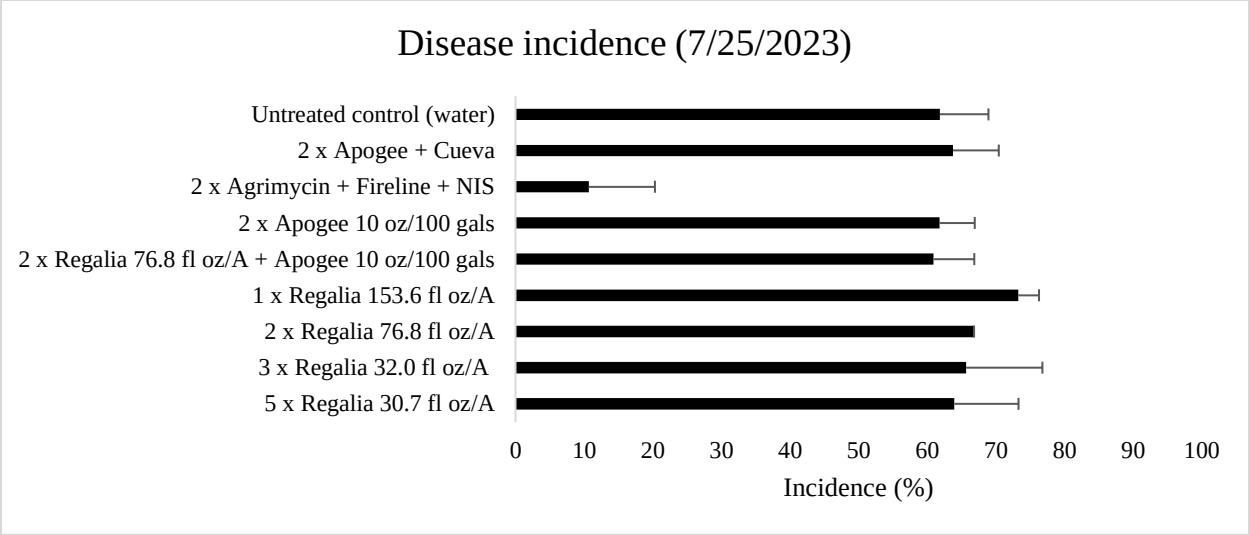
For shoot blight analysis, severity was calculated as ratio of lesion length and total shoot length expressed as percentage. Similarly, for canker severity analysis, ratio of canker length and total branch length was expressed as percentage. The number of blighted shoots were counted and disease incidence was calculated as the ratio of blighted shoots to the total number of inoculated shoots. Significance of treatment application on shoot blight and canker severity was analyzed using ANOVA and the treatment means were compared using Fisher's protected LSD ($P<0.05$).

Data on fruit russet was collected during Bartlett harvest in Southern Oregon on August 3, 2023 and August 6, 2024. Forty fruit per tree was harvested and russet on individual fruit surface was rated using modified Horsfall-Barratt rating scale and converted to the midpoint category to obtain percentage of severity. The percent severity data was analyzed using ANOVA and the treatment means were compared using Fisher's protected LSD ($P<0.05$).

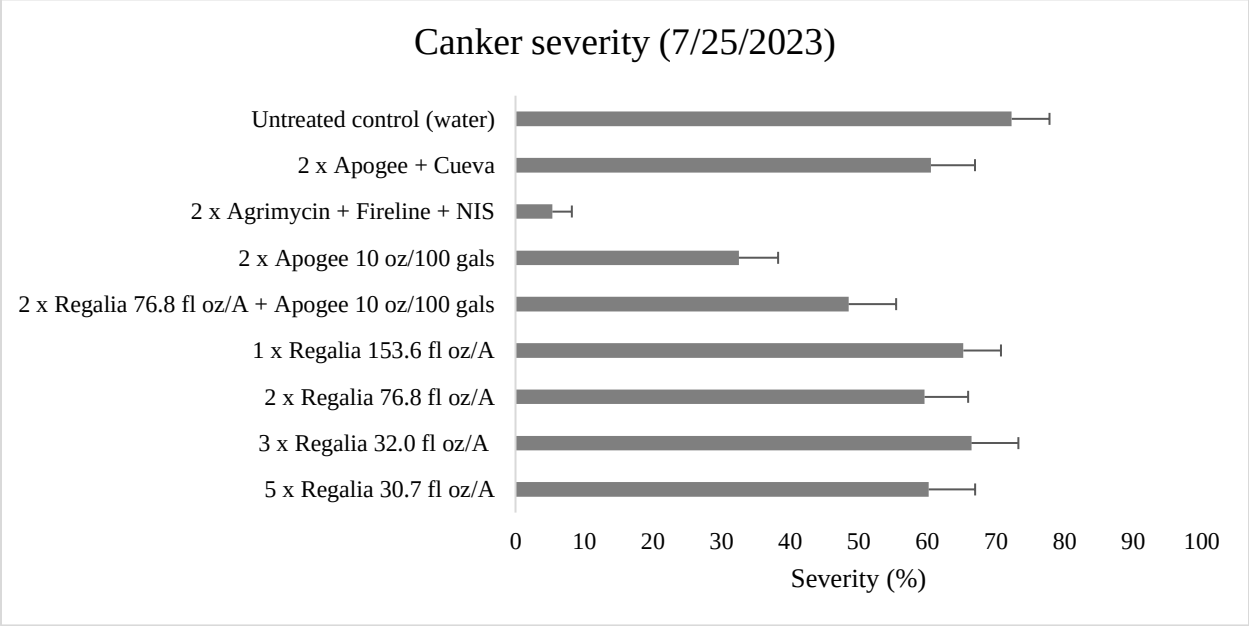
Results and Discussion

Year 1:

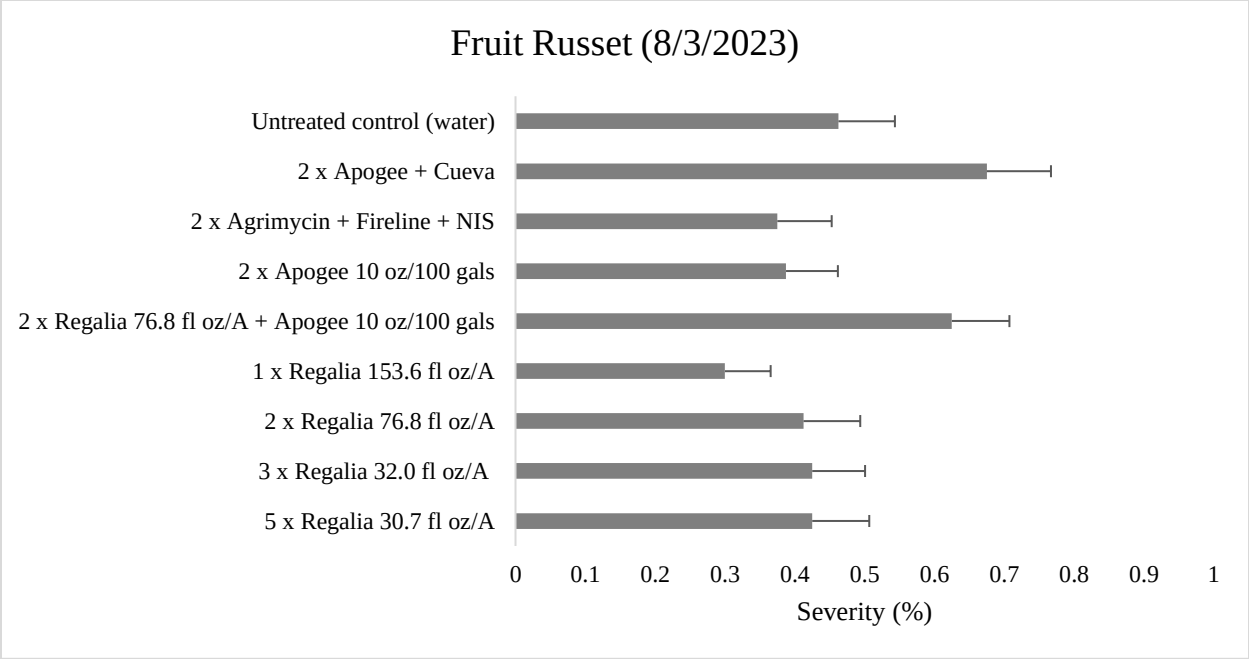




Only the commercial standard, where antibiotics were used during full bloom and one day before shoot inoculation significantly reduced the shoot blight severity and disease incidence. The severity in rest of the treatments were not significantly different from the non-treated controls.

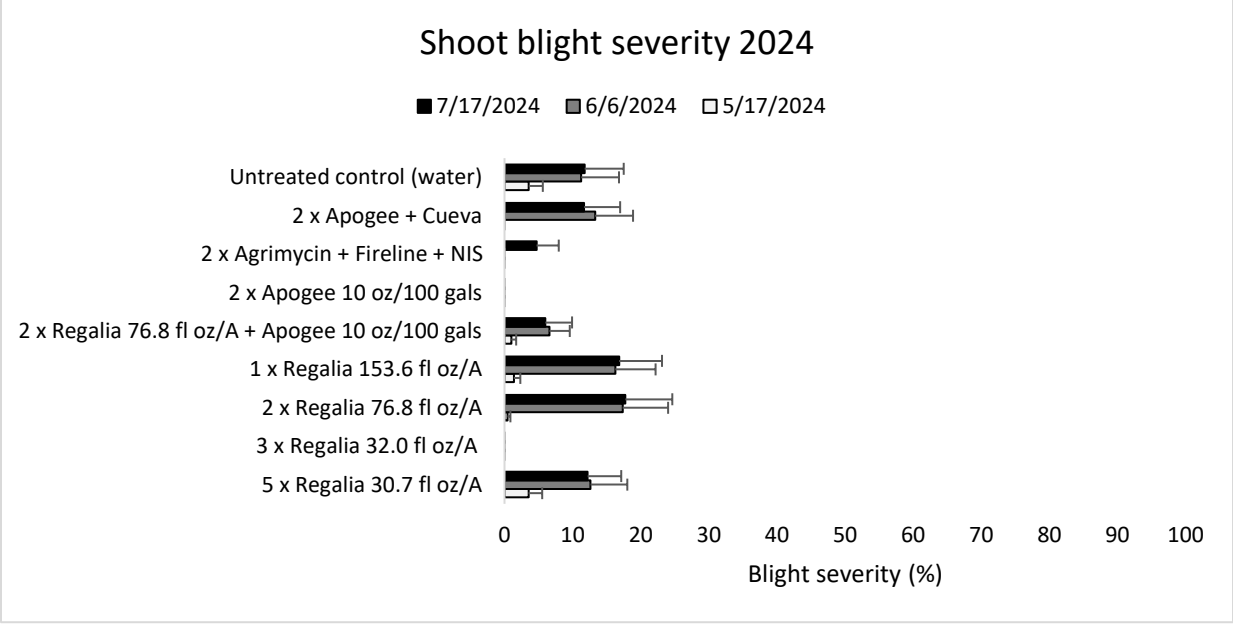


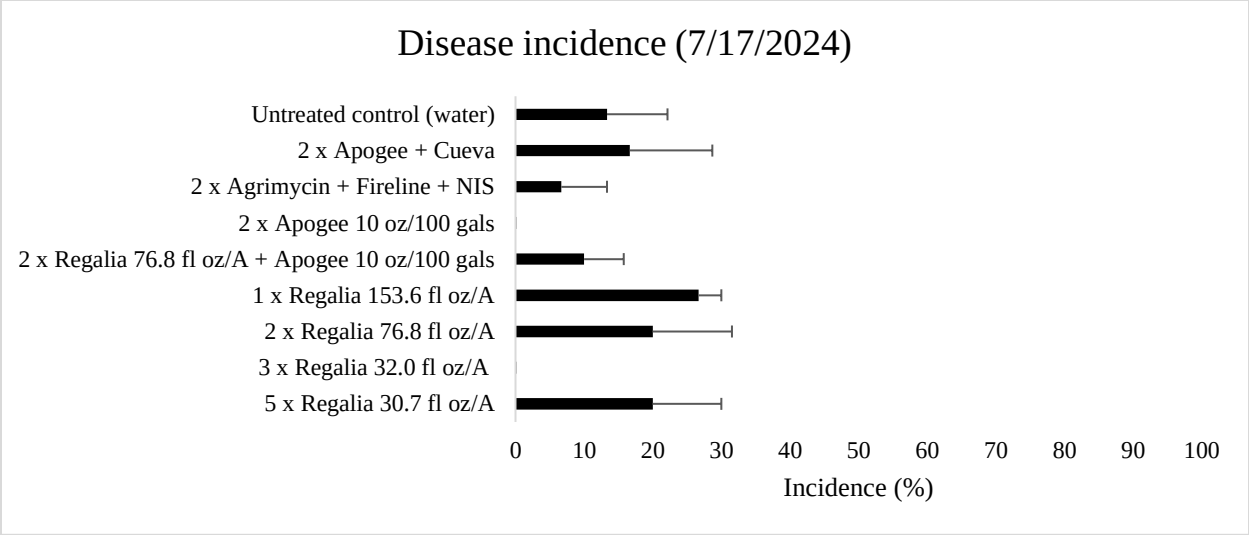
Similar to shoot blight severity, only the commercial standard with antibiotics significantly reduced the canker severity. The severity in rest of the treatments were not significantly different from the non-treated controls.



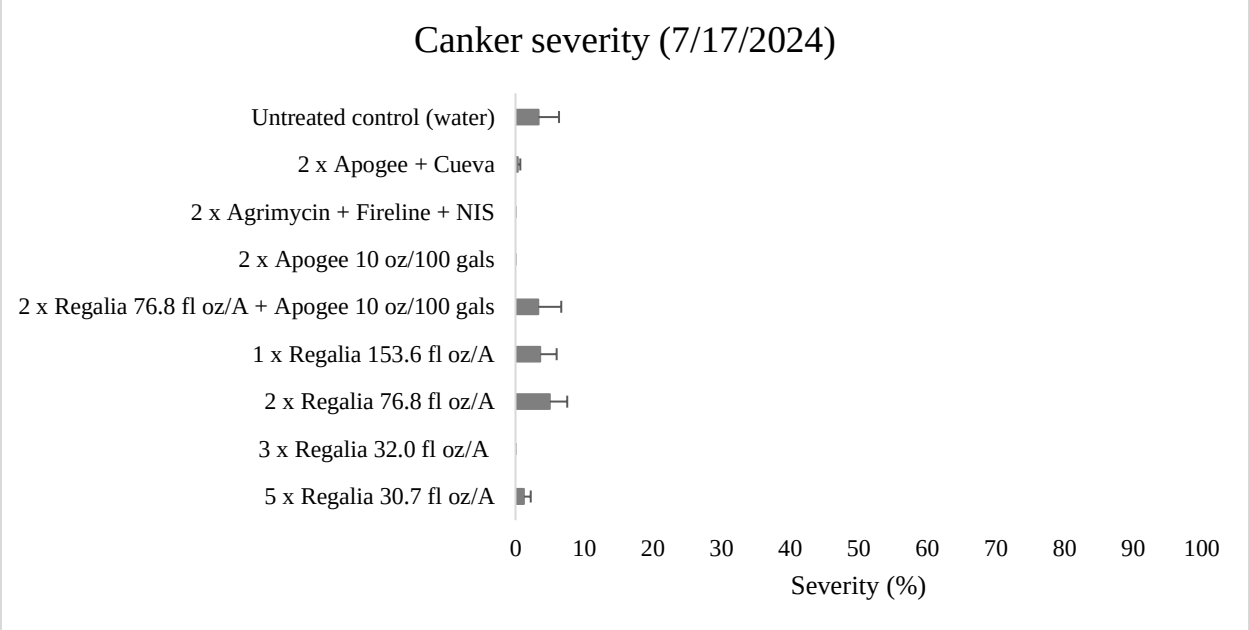
Different fruit russet response were observed on the treated fruits, however these differences were not statistically significant ($P<0.05$). The average russet were less than 1% with range of 0 to 3% russet on these fruits.

Year 2:



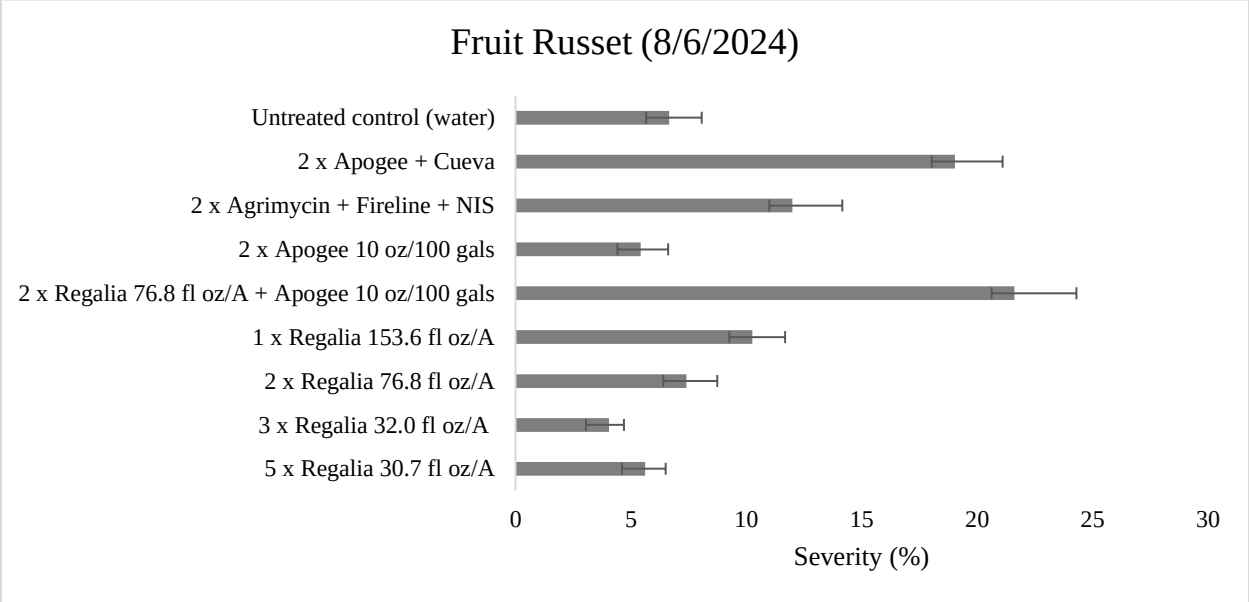


In the second year of the study, the overall fire blight incidence in inoculated shoots were very low. Even in non-treated control trees, only 13% of the inoculated shoots developed shoot blight. There was no blight in the shoots where Regalia was applied three times during WB, PF, and FS @ 32.0 fl oz/A (treatment #2); and Apogee applied two times during petal fall and fruit set (treatment #6). The highest incidence was observed on one time Regalia treated trees during fruit set @ 153.6 fl oz/A (treatment #4). Due to low disease incidence and higher variability between the treatment means, no statistically significant differences were observed among the treatments (*p= 0.204*)



Similar to shoot blight incidence, the canker incidence in the second year was low. Even though 7% of the inoculated shoots developed blight in antibiotics treated shoots (treatment #7), they did not develop any canker. Similarly, two times Apogee and Cueva applied during 1 to 3-inch new shoot growth, and 14 days after the shoot growth (treatment # 8) resulted in less than 1% canker even

though 17% of the inoculated shoots developed shoot blight. However, none of these differences are statistically significant due to low disease incidence and higher variability between the treatment means (*p= 0.449*)



Interestingly, fruit russet was the highest in second year of this trial and the treatment means were significantly different ((*p<0.001*). The highest russet was observed when Apogee was mixed with Cueva (treatment #8) and Regalia (treatment #5) and these results were similar to previous year's russet evaluation. The russet was observed in all other treatments; however these differences were not statistically different from non-treated water sprayed control.

Literature

Borba M. C., Meredith C. L., Dhar B. C., Aćimović S. G. (2023): Proof of concept for management of shoot blight and fire blight cankers on pear with preventive spray applications of giant knotweed extract. ***Frontiers in Horticulture, 1:1082284. pg. 1-14.***

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