

Evaluation of newer biologicals and the SAR-activator candidate Regalia in fire blight control applied by spraying or trunk injection

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The 2016 fire blight epidemic caused severe losses in young apple orchards in northeastern and western New York (NY). Based on studies associating global warming

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with changes in plant pest ranges and degree of infestations, we predict that years with very favorable weather for fire blight epidemics will become more frequent in cool climate regions of the US and the rest of the world (Pautasso et al. 2012; Bebbler et al. 2013; Rosner

2015). Besides losses in yield, young trees, and costs of removal of dead trees and wood, plus orchard re-planting costs, some farms in NY applied several bactericide sprays long after the infections had become established on opened flowers at the end of bloom. Even though this practice can prevent further pathogen spread to new shoots, surface-sprayed bactericides are ineffective once the fire blight bacterium *Erwinia amylovora* enters the apple xylem or bark. In cases where, instead of copper, streptomycin was used after the infections became visible, repeated applications of this antibiotic might have increased the risk of streptomycin resistance acquisition in *E. amylovora* populations. Resistance to streptomycin can lead to blossom blight control failures due to loss of antibiotic efficacy. This would require use of a more expensive but very effective antibiotic such as kasugamycin, or less effective alternatives under high-disease pressure, such as biologicals, SAR compounds, and growth reducers such as prohexadione-calcium (Sundin 2014). Streptomycin efficacy should be preserved by applying it only when fire blight prediction models indicate that flower infections can occur (e.g., NEWA's EIP and Cougarblight, Maryblyt, RIMpro). After the bloom is over, streptomycin should be applied only following hail events when streptomycin is needed, to prevent severe trauma blight infections through hail injury wounds on trees.

Due to prevalent use of antibiotics in animal production and occurrence of antibiotic resistance in clinical pathogens in hospitals, antibiotics are not considered to be as acceptable for use in agriculture as they were in the past. Even though the mechanisms for transferring the resistance genes are distinct between human and plant pathogens (Sundin 2002), fear of potential transfer of antibiotic resistance from environmental bacteria to clinical pathogens promotes scrutinized use of antibiotics in all agriculture. As of 2014, use of antibiotics is prohibited in organic apple production. In addition, effective bactericides for plant protection are becoming very rare and difficult to get approved by EPA. Finally, due to toxic effects of metallic copper on soil fauna, per-acre yearly limits on spray-use of copper products are currently being reconsidered. However, the main benefit of copper as a bactericide is that low rates can be used to prevent spread of secondary shoot blight infections during the summer. In search of alternative plant protection materials for fire blight, previous research on biologicals, plant-growth reducers, activators of systemic acquired resistance (SAR), and copper, has shown medium to low efficacy under high disease pressure and inconsistent results, depending on location-specific climate conditions and tree age.

The complex status of products available to control fire blight led us to determine the efficacy of newer biological control products Prestop (*Gliocladium catenulatum* strain J1446, 10^8 CFU/g), MBI-110 AF5 (*Bacillus amyloliquefaciens* strain F727), and an SAR-activator candidate Regalia/MBI-10612 (5% and 12% extract of plant *Reynoutria sachalinensis*) for blossom and shoot blight management (Table 1). The fungus *G. catenulatum* is a known fungal antagonist that primarily inhabits soils. The strain J1446 of this fungus in Prestop was isolated from Finnish field soil and has been tested or shown some efficacy in foliar, flower/fruit, and root diseases on tomato, strawberry and raspberry (Karise et al. 2016). On apples, preliminary trials show that Prestop reduces incidence of core rot of apple caused by flower infections of *Fusarium avenaceum* and *Botrytis cinerea*. Primary modes of action of this biocontrol fungus consist of its strong ability to colonize roots, foliage, and flowers, and outcompete the plant pathogenic fungi. Some tests showed that *G. catenulatum* parasitizes certain plant pathogenic fungi, has antagonistic enzyme activity against fungi, and could trigger induction of plant resistance after root treatments.

Besides sprays, we also tested two formulations of Regalia applied via trunk-injection(s). Our previous research on mature

Table 1. Treatments with sprayed biologicals and SAR-activator candidate Regalia/MBI-10612, alone or in combination with copper, used for blossom and shoot blight control on ‘Honeycrisp’ apple trees. Treatments in bold were trunk-injected.

No.	Product	Active ingredient	Times applied and amount per acre	Metallic copper per amount of product	Metallic copper equivalent sprayed in lb/A
1	Prestop WG	<i>Glucadium catenulatum</i> Strain J1446	2X 35 oz / A	/	/
2	Prestop WG	<i>Glucadium catenulatum</i> Strain J1446	2X 70 oz / A	/	/
3	CS2005 + Regalia	19.8% copper sulfate pentahydrate + 5% extract of plant <i>R. sachalinensis</i>	2X 16 fl oz / A + 32 fl oz / A	0.418 lb/gal	2 x 0.052 lb/A
4	CS2005 + Regalia	19.8% copper sulfate pentahydrate + 5% extract of plant <i>R. sachalinensis</i>	1X 47.7 fl oz / A + 95.4 fl oz / A	0.418 lb/gal	1 x 0.160 lb/A
5	Regalia	5% extract of plant <i>R. sachalinensis</i>	2X 64 fl oz / A	/	/
6	Regalia	5% extract of plant <i>R. sachalinensis</i>	3X 64 fl oz / A	/	/
7	MBI-10612	12% extract of plant <i>R. sachalinensis</i>	2X 32 fl oz / A	/	/
8	MBI-110 AF5	<i>B. amyloliquefaciens</i> strain F727	2X 64 fl oz / A	/	/
9	MBI-10612 Trunk-injection	12% extract of plant <i>R. sachalinensis</i>	2X 32 fl oz / A	/	/
10	Regalia Trunk-injection	5% extract of plant <i>R. sachalinensis</i>	2X 76.8 fl oz / A	/	/
11	MBI-10612 Trunk-injection	12% extract of plant <i>R. sachalinensis</i>	4X 32 fl oz / A	/	/
12	Harbour + Regulaid	17% streptomycin + 90.6% 2-butoxyethan- ol, poloxalene, monopropylene glycol	2X 1.5 lb / A + 3 pts	/	/
13	Fireline 17 WP	17% oxytetracycline	2X 1 lb / A	/	/
14	Untreated Control	/	/	/	/

‘Gala’ apple trees demonstrated that trunk-injected phosphites (Phospho-Jet) and acibenzolar-S-methyl, (Actigard), a known SAR-activator, induce plant defense response in apple leaves (Aćimović et al. 2015). SAR is a broad-spectrum defensive resistance response towards plant pathogens that is induced in plants by a localized exposure to a pathogen or after spraying with a synthetic or natural compound, known as an SAR inducer or activator (Hammerschmidt 2007). SAR induction results in a myriad of synthesized and accumulated defensive compounds, including antimicrobial proteins, that help the plant to suppress the disease. In our trial, Regalia was also evaluated mixed with a copper product CS2005, in the hope that this combination would provide better efficacy (Table 1). Besides efficacy, we also evaluated the potential of these eco-friendly and/or organically acceptable products to cause fruit russet. In this paper, we present this data along with the fruit russet data for low-rate copper treatments applied at bloom and published in a previous Fruit Quarterly article (Aćimović and Meredith 2017).

Can Preventive Applications of Prestop, MBI-110 AF5, and Regalia/MBI-10612 Suppress Blossom and Shoot Blight Under High Disease Pressure?

On 26 Apr 2017, ‘Honeycrisp’ trees were at pink growth stage. On 27 Apr, bloom reached 63% due to extremely warm weather. On 29 Apr, when Honeycrisp trees were at 65–70% king bloom opened, treatments in Table 1 and Figure 1 were applied using a gas-powered backpack airblast sprayer delivering 50 gal/A (Solo 451 Mist Blower, 3 gal). This volume was used and the treatments were timed to avoid slow-drying conditions that could promote

fruit russetting. Fireline was delivered in 100 gal/A and 1X CS2005 + Regalia in 150 gal/A. To apply the same amount of each tested product as one apple tree would receive from a tractor airblast sprayer in a high-density apple orchard, we divided the treatment rates per acre by 940 trees/A, which is the lower limit for the number of planted trees in a high-density orchard. We used timed applications with the backpack sprayer to apply the appropriate amounts of product per tree (Table 1). In the treatments with two sprays (Table 1), applications on 29 Apr were ~15 min apart because the flower opening was so rapid that sprays could not be applied as planned at 20 and 50% king bloom for all the treatments, except at 50, 80, 100% king bloom for the 2X CS2005 at 16 fl oz + Regalia 32 fl oz treatment and for the 2X Regalia 64 fl oz treatment, and at 50, 80% king bloom, petal fall, first cover for the 2X MBI-110 AF5 at 64 fl oz treatment and the 2X MBI-10612 at 32 fl oz treatment (Table 1). The 3X Regalia 64 fl oz treatment was sprayed the first time on 28 Apr, and the second and third time both on 29 Apr, ~15 min apart, instead of the planned three sprays at pink, 20%, and 50% king bloom (Table 1). Applications for the trunk injection treatments in Table 1 were made at half-inch green (16 Apr) and at pink (24 Apr) for 2X MBI-10612 at 32 fl oz and for 2X Regalia at 76.8 fl oz, while for 4X MBI-10612 at 32 fl oz applications were made at half-inch green (16 Apr), pink (24 Apr), petal fall (7 May), and at first cover (17 May).

We inoculated flowers on 30 Apr by misting entire ‘Honeycrisp’ trees with a water suspension of 3×10^6 CFU/ml of *E. amylovora*, at 80% king bloom (GroundWork® rolling cart sprayer, 30 PSI, 3 gal). We used a slightly higher amount of inoculum than the usual 1×10^6 CFU/ml due to the low average daily temperature

of 52.7°F. First fire blight symptoms developed on 17 May. RIMpro fire blight forecast model connected to the on-site NEWA weather station in Highland, NY, where the experiment was conducted, issued a prediction on 29 Apr at 7:30 pm that natural fire blight infections were possible on 30 Apr and 2 May, based on the weather forecast.

We rated blossom and shoot blight incidence on 27 May and 3 Jun, respectively (Figure 1A & B). Going around the crown, we randomly chose 100 flower clusters per tree and counted the number of diseased and healthy clusters in that sample. Flower infections migrated into the shoots. We randomly chose 100 shoots per tree and counted the number of infected and healthy shoots in that sample. Blossom and shoot blight were calculated as blossom and shoot blight percent on a per-tree basis. Mean percent of blossom and shoot blight incidences were calculated for each treatment from 4 single-tree replicates.

Overall, under high disease pressure, Prestop sprays during rapid bloom provided poor but statistically significant blossom blight reduction of 15.5%, while the higher rate provided 8.6% reduction, which was not different from the untreated control. This could be explained by the fact that in some species of biocontrol fungi, like *G. catenulatum* strain J1446, inhibition of their mycelial growth can occur if there are too many spores germinating at the same time. It therefore seems that there could be a trend in some biocontrol materials that “less is often more” in terms of the effectiveness. MBI-110 AF5 provided no blossom blight control. This indicates that more preventive sprays applied farther apart are probably needed during bloom to allow better growth and establishment of *B. amylovora* F727 populations. This biocontrol bacterium releases antibacterial metabolites that reduce *E. amylovora* populations on the flower stigma and thus reduce fire blight infection.

A similar trend in poor control was visible for shoot blight incidence (Figure 1B). However, exceptions were 20% shoot blight control with 2X CS2005 + Regalia, 19% shoot blight control with sprayed 2X MBI-10612, and 35.7% shoot blight control with the highest rate of trunk-injected 2X Regalia treatment (Figure 1B). The fact that the highest rate of trunk-injected Regalia reduced shoot blight incidence by 36% implies that this plant extract product probably activates SAR defense response in apple trees, which leads to disease reduction. However, until the pathogenesis-related (PR) gene expression assays that prove the SAR response are conducted, the true mode of action, i.e., mechanism of Regalia activity, remains unconfirmed (Aćimović et al. 2015). In conjunction with the high rate, delivery of this extract into the plant by trunk injection seems to increase the efficacy of Regalia in shoot blight control in comparison to spraying. However, to prove this, the same rate should be applied by spraying and trunk injection. MBI-10612 or Regalia alone, or mixed with CS2005, did not control blossom blight. This indicates that the copper rates were probably too low to kill *E. amylovora* and that Regalia, an SAR-activator candidate, is probably not activating SAR in the green flower parts. When we tested the SAR

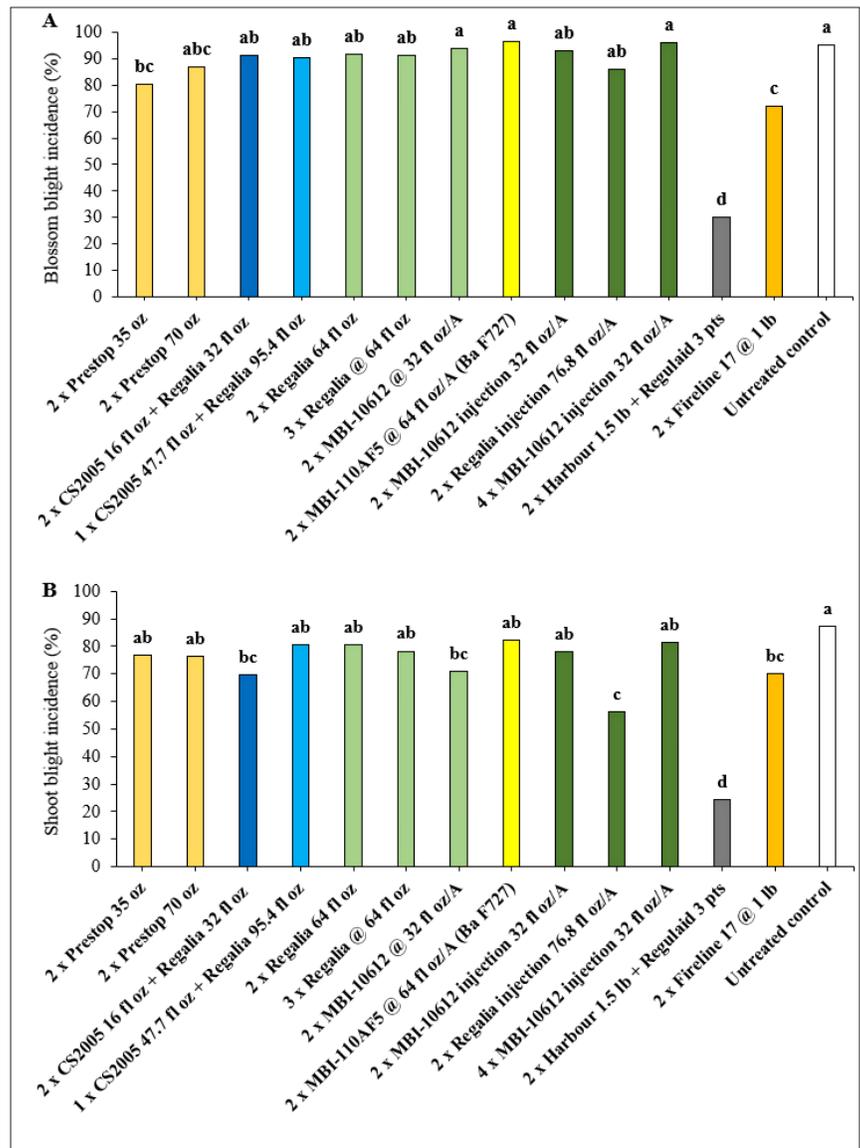


Figure 1. (A) Blossom blight and (B) shoot blight incidence on ‘Honeycrisp’ after preventive sprays of biologicals and sprays or trunk injections of SAR-activator candidate Regalia/MBI-10612. Trees were inoculated with fire blight bacterium *E. amylovora* at 80% king bloom opened (3×10^6 CFU/ml). Different letters above bars, within each graph, indicate significant differences between treatments ($P < 0.05$).

activator Actigard we did not detect the PR gene expression in green flower parts (Aćimović et al. 2015). Finally, it seems that there are no synergistic efficacy interactions between CS2005 and Regalia. Harbour provided blossom and shoot blight control of 68.5% and 72%, respectively.

What is the Potential of Prestop, MBI-110 AF5, and Regalia/MBI-10612 to Cause Fruit Russet?

In addition to fire blight control, we rated the fruit russet incidence for the treatments in Table 1 and for the copper spray treatments reported previously (Aćimović and Meredith 2017). Fruit russet forms if epidermal cells are damaged, sometimes during the first 30–40 days after petal fall. When fruit epidermis is damaged, a brown layer of cork cells forms in the lower epidermis and pushes outward, becoming visible on fruit surface as it matures (Pscheidt 2015). Cool weather in conjunction with a wet fruit surface, especially from pink growth stage until 3 weeks after petal fall, is conducive for russetting.

The 2017 weather conditions at the experiment location in Highland, NY, during the treatments and just after the applications, did not promote slow-drying of spray deposits that would allow fruit russet development. However, frequent rains in 2017 with cooler weather and high relative humidity, which lasted from 30 Apr to 15 May, and then from 19 May to 7 Jun, could have promoted russet formation in conjunction with the dried spray deposits (Figure 2). The percent incidence of fruit russet was calculated from the number of fruit with russet symptoms versus the russet-free fruit on 25 randomly selected fruit clusters per tree, summing up to 50 fruits per replicate. Mean percent of fruit russet incidences were calculated for each treatment from 4-single tree replicates.

Prestop and Harbour treatments showed the least fruit russet and did not differ from the untreated control (Figure 3A). 2X MBI-110 AF5 showed significantly higher russet compared with these treatments. Surprisingly, the high rate 2X Regalia trunk injection showed the next highest fruit russet incidence, followed by the 2X CS2005 + Regalia. This is unexpected, since 2 x Regalia in this treatment was not deposited on the surface of the flowers or fruit, but delivered through the apple xylem. If this plant extract is highly soluble in water, it is possible that it accumulated in immature fruit during development and size enlargement. However, since the incidence was not statistically different from the untreated control, it is difficult to speculate whether and how the trunk-injected Regalia reached and affected the fruit epidermis cells to cause russet.

Unexpectedly, there was no difference in russet incidence among the low-rate copper treatments, antibiotics, and the untreated control (Aćimović and Meredith 2017), leading us to believe that cool and rainy weather conditions during fruit growth

contributed to russet development across all treatments (Figure 3B). Nevertheless, many copper treatments resulted in numerically more russet in comparison to the untreated control.

Conclusion

Our evaluations of the newer biologicals Prestop, MBI-110 AF5, and an SAR-activator candidate Regalia/MBI-10612 showed overall poor control of blossom and shoot blight under the high disease pressure that evolved in our trial and under the rapid bloom scenario in 2017. Part of the reason for such results could be that, with the abrupt applications governed by the rapid flower opening, too short a time was available for these materials to trigger their respective modes of action, such as establishing larger populations for biocontrol of *E. amylovora* or inducing an SAR defense response, if that is Regalia's mode of action. Efficacy of the same treatments might be better in years with conditions less conducive to fire blight establishment and/or with flowers exposed to lower levels of inoculum. Results on fruit russet incidence indicate that even on 'Honeycrisp', this disorder can occur when weather conditions are favorable and/or spray product formulations contain damaging active ingredients or adjuvants.

Even though eco-friendly and/or organically acceptable options for management of fire blight are much more available today than before, their efficacy is still not as reliable as the efficacy of streptomycin and kasugamycin (Sundin 2014). This is especially true in years with high disease pressure due to very favorable conditions for fire blight development. In these situations, when infections can be predicted by disease prediction models, fire blight can become very destructive, leading to yield reduction and tree

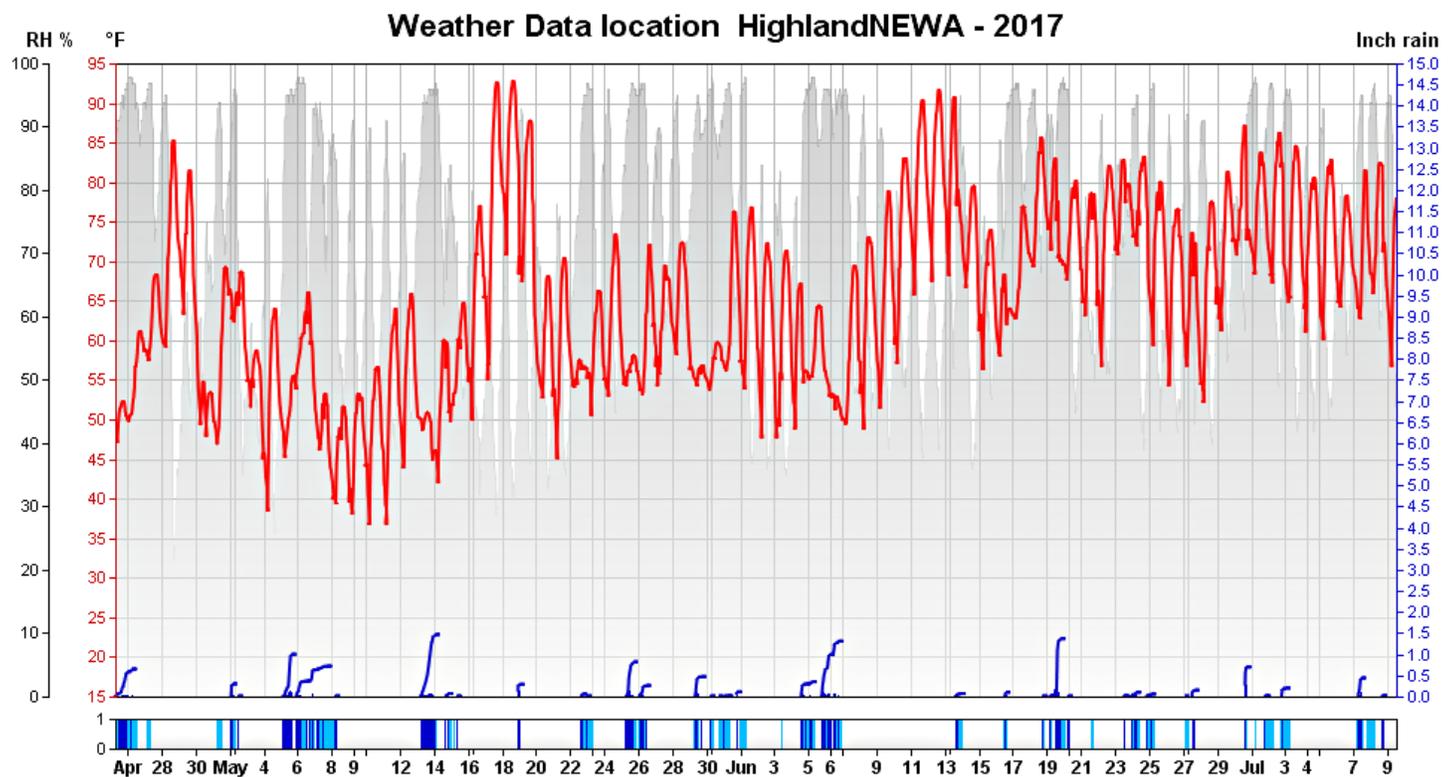


Figure 2. Summary of weather conditions from 27 Apr to 9 Jul 2017 from an on-site NEWA weather station in Highland, NY, during the fire blight efficacy trial on 'Honeycrisp'. Data are obtained from RIMpro Cloud Service, an interactive Decision Support System for pest and disease prediction and management in fruit and grape production. The upper main graph: red line represents temperatures (left y-axis in red), blue line/s are rain events indicating amount in inches (right y-axis in blue), gray background peaks show relative air humidity (RH) in % (far left y-axis in black). The bottom small graph with dates indicates the length of periods of rain (dark blue bars) and of wetting afterwards (light blue bars).

death if highly effective control materials are not applied. With the current technology used in apple production, including fire blight-susceptible varieties and rootstocks, spindle training systems with young fruiting limbs, high-density plantings, and the warming climate, it will be increasingly difficult to control fire blight. Until safe (i.e. non-russetting) and effective replacements for plant protection antibiotics are developed and registered, fire blight management will continue to be a challenge.

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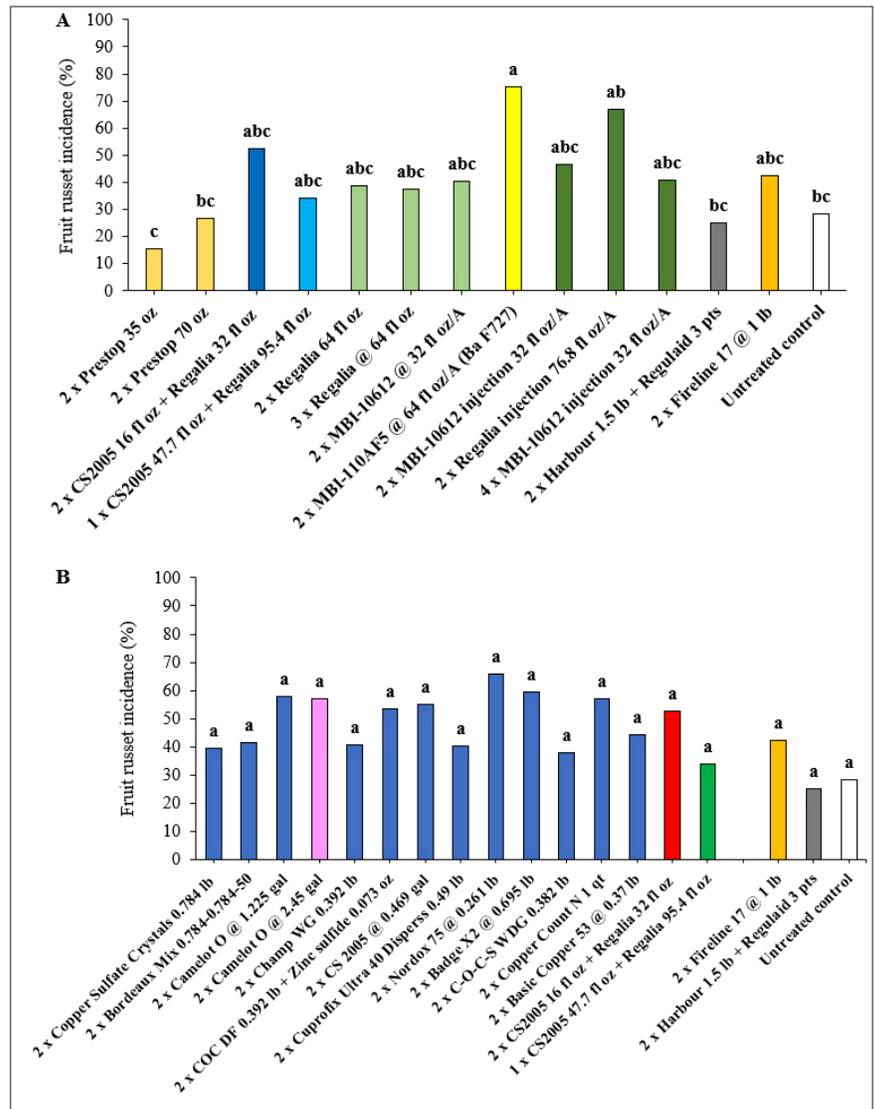


Figure 3. Fruit russet incidence on 'Honeycrisp' apple trees after (A) preventive sprays of biologicals and sprays or trunk injections of SAR-activator candidate Regalia-10612, and (B) after preventive sprays of copper products during bloom. Russet data in graph (B) is related to the copper sprays presented in Ćimović and Meredith (2017). Different letters above bars, within each graph, indicate significant differences between treatments ($P < 0.05$).

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