Evaluation of dormant copper sprays with bark penetrating surfactants in reduction of *Erwinia amylovora* in cankers and of low-rate copper sprays in blossom blight control

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This research was supported by the New York Apple Research and Development Program

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In 2016, fire blight caused severe losses in some New York (NY) apple orchards. Financial damage included apple yield reduction, tree death and costs of removal of infected trees and wood, and of orchard re-planting. Estimated crop losses ranged between $10,000 and $75,000 per farm. Between 150 and 2500 trees per farm were removed in the NY Champlain Valley apple growing region due to infections of susceptible M.9 rootstock. Tree removal continued into spring of 2017, increasing financial losses as latent fire blight infections on rootstocks were expressed as tree collapse with oncoming warmer weather. Approximately 20–40% of productive bud wood per farm was lost due to fire blight. Summer-long labor costs for fire blight removal ranged between $27,000 and $75,000 per farm. Cost of bactericides applied after infection was estimated at $25,000 to $55,000 per farm, with repeated applications increasing the potential risk of streptomycin resistance in *Erwinia amylovora* populations.

In contrast to historically cool weather during bloom, hot days in 2016 at the end of bloom and the start of the spring growth flush allowed fire blight to grow its population on the remaining opened flowers. With two warm rains, hail, and wind, fire blight spread quickly to intensively growing shoots. Climate change is predicted to trigger more extraordinarily warm springs, uncommon to cooler northern regions, but favoring fire blight infections. Use of fire blight prediction models available online will be essential for applying timely control sprays during bloom and curtailing future devastating epidemics.

Besides the warming climate, several other interacting factors put NY apple orchards at a high risk for future fire blight outbreaks. First, fresh fruit buyers favor fire blight-susceptible varieties that growers plant to fulfill this demand. Second, high demand for fire blight-resistant rootstocks of the Geneva series (G.11, G.16, G.30, G.41, G.202, G.210, G.214, G.890, G.935), coupled with the slow pace and/or low capacity of their production in nurseries, pushes growers to order new trees from nurseries on the more immediately available but blight-susceptible rootstocks such as M.9, M.9-337, M.9 Nic 29, and EMLA 26. Third, since new apple orchards are constantly planted in NY, higher susceptibility of young trees in comparison to mature trees puts apple production at a much higher risk from frequent and destructive fire blight epidemics that can kill many trees in one season, because infections in the young orchards can provide inoculum that spreads over an entire farm or region. Finally, in high-density plantings with spindle-shaped training systems, internal fire blight infections spread faster into the tree trunks because fruiting limbs are much shorter and thinner in comparison to thick, old limbs of classic training systems. Resulting cankers on small diameter trunks, and visible or latent fire blight infections of rootstock, lead to rapid tree death.

In the past, fire blight was a sporadic, relatively rare problem in northeastern NY. Cool weather during bloom did not favor epidemic disease development. However, several low-incidence fire blight outbreaks during late spring or early summer were recorded on several farms over the past several decades (D. Rosenberger, personal communication). Even though fire blight strikes and cankers were mostly removed by winter pruning at these farms, some fire blight cankers may have remained on old trees and served as primary sources for infection in 2016. The fire blight life cycle starts from overwintering cankers formed on perennial wood in a previous year's outbreak (Figure 1) or the pathogen is introduced on nursery stock material. Bacterial inoculum overwinters in cankers and emerges in spring with warm weather as orange ooze droplets or smears on canker edges, dry ooze strands, or oozless colonies (Figure 2). Carried by attracted insects, wind, and rain, ooze is disseminated to opened flowers, where bacteria multiply and increase their populations at warm temperatures (Figure 1) (Norelli et al. 2003). After intensive population growth on flower stigma surfaces, infections are established if rain or heavy dew events wash the bacteria down into the nectar glands (Figure 1).

Ooze on cankers can also reach young shoots directly (Slack

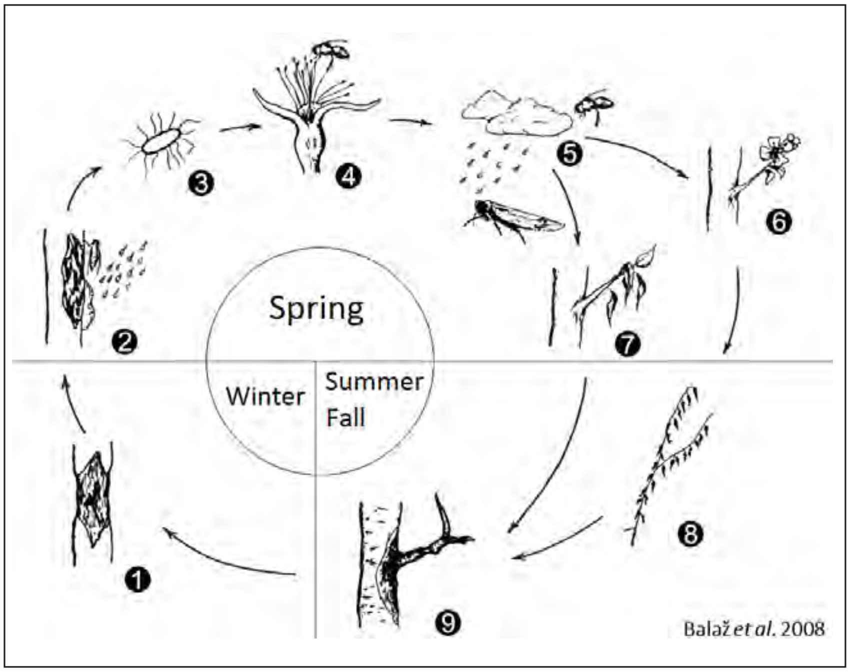

Figure 1. Life cycle of fire blight bacterium *Erwinia amylovora:* 1. Overwintering in cankers on wood, 2. Emergence of bacteria on the bark surface at canker edges (oozeless colonies, ooze droplets, dry ooze strands), 3. Dissemination of bacteria on flowers by insects, rain and wind, 4. Bacterial population growth on flowers and insect vector exposure, 5. Further dissemination of bacteria to flowers and shoots by rain, wind and insects, 6. Blossom blight development, 7. Shoot blight development, 8. Branch death, 9. Formation of new cankers and overwintering.

Figure 2. Fire blight canker around a pruning cut on 'Rome' apple limb with damp bacterial ooze emerging from the canker edge (lower left corner).

and Sundin 2017), leading to shoot blight infections after skipping population enlargement on flowers, or after being successfully controlled with antibiotics at bloom. Based on well investigated fire blight biology and ecology during the spring and early summer, key management practices target controlling the bacteria on the plant surfaces with bactericides, before they move into the plant and multiply internally. Once bacteria enter and establish in the flowers, shoots, and wood tissue, sprayed bactericides have no effect (Sundin 2014).

In contrast to what we know about infection processes during spring, our understanding of fire blight biology and ecology during the late summer, fall, and winter is limited. Little is known about cankers as primary sources of infection and survival of *E. amylovora* populations inside different tree hosts. Even though survival and population growth of *E. amylovora* on flowers and in water has been researched (Pusey and Smith 2007; Santander et al. 2014), population dynamics, ecology, and survival rate of *E. amylovora* in cankers are largely uninvestigated. The efficacy and benefit of delayed dormant copper sprays in the reduction of fire blight populations from cankers has not been demonstrated with quantitative data. In addition, evaluation of the efficacy of low-rate copper sprays for blossom blight management and their relationship to fruit russetting has received limited attention. Only limited comparisons have been made between older copper formulations and the newer low-rate copper formulations.

Several issues have propelled our research into options for increasing the efficacy of delayed dormant copper sprays and increasing the use of copper for blossom blight management. Occurrence of *E. amylovora* resistance to streptomycin can lead to blossom blight control failures with this antibiotic. Loss of streptomycin efficacy due to resistance would require growers to use more expensive antibiotics or less effective alternatives. Scrutiny of the use of antibiotics in agriculture is growing, due to fears of potential transfer of antibiotic resistance from environmental bacteria, including plant pathogens, to clinical pathogens. Highly effective bactericides for conventional fruit production are becoming extremely rare and difficult to get approved by the EPA. Per-acre yearly limits on the use of copper products are currently being reconsidered due to high copper toxicity on soil fauna. Lowering the current limits on the amount of copper that can be applied each year could severely affect conventional and limit organic apple production. Most of the available fire blight control materials for organic use are of mediocre efficacy. The main benefit of copper as a bactericide is that low rates can be used for shoot blight control during the summer. Streptomycin use for the same purpose is not advised, to avoid occurrence of resistance in environmental bacteria as well as *E. amylovora* to this antibiotic. It should be reserved only for application after hail injury to prevent severe trauma blight infections.

In this paper, we quantified *E. amylovora* populations in fire blight cankers after dormant sprays of copper applied in mixes with bark penetrating surfactants, and we evaluated blossom and shoot blight control after two preventive sprays of a low-rate copper (2 x 0.196 lb of metallic copper equivalent/A).

Can Dormant Copper Sprays Mixed with Bark Penetrating Surfactants Affect Fire Blight in Cankers?

The delayed dormant copper spray is one of the pillars of our current fire blight management program. Metallic copper ions released from different copper compounds kill bacteria on plant surfaces. Copper products differ widely in the availability of free copper ions released on moist plant surfaces (Zitter 2012). For example, copper sulfate crystals are highly soluble in water, thus readily freeing copper ions and having the highest potential to cause phytotoxicity. Usually, highly soluble copper compounds are mixed with lime or gypsum to help bind copper ions and slow their release over time (Shane 2011). Fixed copper compounds, such as copper hydroxide, are less soluble in water. Copper products are sprayed as a water suspension of copper active ingredient particles, which persist on plant surfaces after the spray dries. With each wetting of plant surfaces, from either rain or dew, active copper ions are gradually released from copper deposits. Slow release of copper from the copper deposits provides residual protection against fire blight. However, with 3 inches of rain or more, almost all copper residues are washed off the plant. Copper products are applied late in tree dormancy, from bud break up to the 1/4- to 1/2-inch green bud stage, if the label permits, with the goals of (a) reducing fire blight populations on the plant surface originating from overwintered cankers, including bacteria in oozeless colonies on the bark, in ooze droplets, and in dry ooze strands (Van Der Zwet and Keil 1979; Vanneste 2000); (b) securing enough copper deposit to remain on tree surfaces until bacteria emerge from cankers with warm weather, and (c) avoiding phytotoxic effects of copper on developing green tissue, including the tissue at the base of the flower buds that will eventually develop into fruit. Depending on weather conditions, one or more of these goals might not be reached, reducing the effectiveness of the copper spray or risking injury if applied too late or at excessive rates. Spring rains can wash off all copper residues if 3 inches of rain or more fall between spray application and pink bud stage (Rosenberger 1992). If only low copper residues remain at the time when bacteria emerge from cankers, and if these residues are additionally diluted by bud development between green tip and bloom, they might not be sufficient to kill all the bacteria. Finally, in most years, bacteria emerge from cankers at the end of bloom and start of shoot growth, when temperatures are higher and sap flow intensifies with green tissue growth. By then, little if any copper residue is likely to remain on the bark. Furthermore, copper deposits on the bark are probably not effective in killing the bacteria in emerging ooze droplets or dry ooze strands (Steiner 1998). Ooze droplets or dry strands are forced through the bark cracks, reaching out and beyond the two-dimensional copper deposit film on the bark surface. Steiner (1998) argues that delayed dormant copper sprays should provide an inhibitory barrier over bark and

Table 1. Dormant copper treatments applied on 6 Dec 2016 to reduce overwintering populations of fire blight *E. amylovora* in wood cankers on 'Cortland' apple trees. *71.1% basic bopper sulfate. **94.33% basic copper sulfate.

No.	Treatment	Amount per Acre	Metallic copper / lb of product	Metallic copper equivalent sprayed lb/A
1	Untreated Control	/	/	/
2	Cuprofix Ultra 40 Disperss* + lime + Regulaid	(31.8 lb copper sulfate + 31.8 lb lime + 100 gal water) + 2 pts/A	0.40 lb / lb	12.72
3	Cuprofix Ultra 40 Disperss + lime + Pentrabark	(31.8 lb copper sulfate + 31.8 lb lime + 100 gal water) + 32 fl oz / 100 gal	0.40 lb / lb	12.72
4	Cuprofix Ultra 40 Disperss	31.8 lb copper sulfate + 31.8 lb lime + 100 gal	0.40 lb / lb	12.72
5	Basic Copper 53** + Regulaid	15 lb/A + 2 pts/100 gal	0.53 lb / lb	7.95
6	Basic Copper 53 + Pentrabark	15 lb/A + 32 fl oz/100 gal	0.53 lb / lb	7.95
7	Basic Copper 53	15 lb / A	0.53 lb / lb	7.95

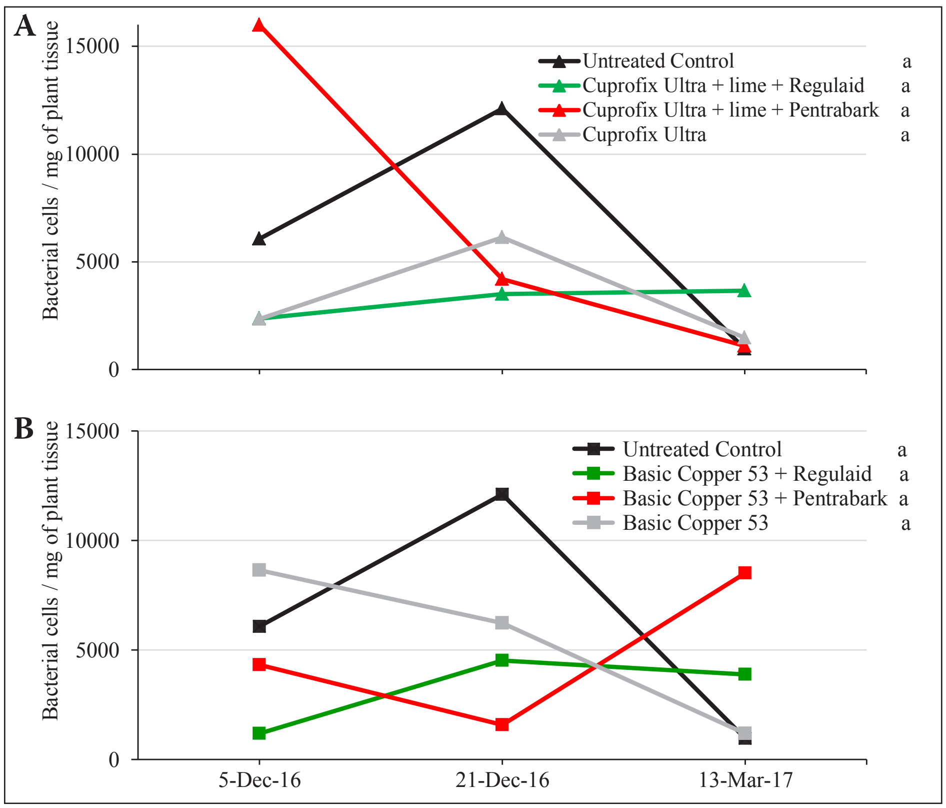

Figure 3. Number of live bacterial cells of *Erwinia amylovora* detected with digital PCR in the edges of fire blight wood cankers on apple cv. 'Cortland' before and after application of (A) Cuprofix Ultra 40 Disperss + lime, and (B) Basic Copper 53, alone or mixed with bark penetrating surfactants (Regulaid, Pentrabark). Treatments were sprayed on 6 Dec 2016. Number of detected live *E. amylovora* cells is equal to the number of PCR-amplified copies of specific chromosomal DNA target in *E. amylovora* (Gottsberger 2010). Mean at each time point consists of 3-single tree replicates. Three cankers were collected from each tree replicate at each time point. In each graph, treatments followed by the same letters are not significantly different ($P<0.05$). The last two time points of each treatment line in both graphs are not on a continuous scale.

Table 2. Low-rate copper treatments used for blossom blight control on 'Honeycrisp' apple trees. Treatments in bold provided either a higher or lower amount of metallic copper than 0.196 lb/A.

No.	Treatment	Active ingredient	Amount per acre	Metallic copper per amount of product	Metallic copper equivalent sprayed in lb/A
1	Copper Sulfate Crystals	99% copper sulfate pentahydrate crystals	2 x 0.784 lb/A	0.25 lb/ lb	2 x 0.196 lb/A
2	Bordeaux Mixture	99% copper sulfate pentahydrate crystals + hydrated lime + water	2 x 0.784-0.784-50 (0.784 lb copper sulfate + 0.784 lb lime + 50 gal water/A)	0.25 lb/ lb	2 x 0.196 lb/A
3	Camelot O	10% copper octanoate (copper soap)	2 x 1.225 gal /A	0.16 lb/ gal	2 x 0.196 lb/A
4	**Camelot O**	**10% copper octanoate (copper soap)**	**2 x 2.45 gal/A**	**0.16 lb/ gal**	**2 x 0.392 lb/A**
5	Champ WG	77% copper hydroxide	2 x 0.392 lb/A	0.50 lb/ lb	2 x 0.196 lb/A
6	COC DF + ZnS	84.04% copper oxychloride + 97% zinc sulfide	2 x 0.392 lb/A + 0.073 oz zinc sulfide/A	0.50 lb/ lb	2 x 0.196 lb/A
7	CS 2005	19.8% copper sulfate pentahydrate	2 x 0.469 gal/A	0.418 lb/gal	2 x 0.196 lb/A
8	Cuprofix Ultra 40 Disperss	71.1% basic bopper sulfate	2 x 0.49 lb/A	0.40 lb/ lb	2 x 0.196 lb/A
9	Nordox 75 WG	83.9% cuprous oxide (Cu_2O)	2 x 0.261 lb/A	0.75 lb / lb	2 x 0.196 lb/A
10	Badge X2	23.82% copper oxychloride + 21.49% copper hydroxide	2 x 0.695 lb/A	0.282 lb/ lb	2 x 0.196 lb/A
11	C-O-C-S WDG	73.49% copper oxychloride + 13.39% basic copper sulfate	2 x 0.382 lb/A	0.5125 lb/ lb	2 x 0.196 lb/A
12	Copper Count N	27.15% copper diammonia diacetate complex	2 x 1 qt/A	0.773 lb/ gal	2 x 0.196 lb/A
13	Basic Copper 53	94.33% basic copper sulfate	2 x 0.37 lb/A	0.53 lb/ lb	2 x 0.196 lb/A
14	**CS2005 + Regalia**	**19.8% copper sulfate pentahydrate + 5% extract of plant *R. sachalinensis***	**2 x 16 fl oz/A + 32 fl oz/A**	**0.418 lb/gal**	**2 x 0.052 lb/A**
15	**CS2005 + Regalia**	**19.8% copper sulfate pentahydrate + 5% extract of plant *R. sachalinensis***	**1 x 47.7 fl oz/A + 95.4 fl oz/A**	**0.418 lb/gal**	**1 x 0.160 lb/A**
16	Fireline 17 WP	17% oxytetracycline	2 x 1 lb/A	/	/
17	Harbour + Regulaid	17% streptomycin + 90.6% 2-butoxyethan- ol, poloxalene, monopropylene glycol	2 x 1.5 lb/A + 3 pts/ 100 gal	/	/
18	Untreated Control	/	/	/	/

bud surfaces to prevent *E. amylovora* from colonizing these areas.

The second pillar of the fire blight management program is reduction of bacterial inoculum by pruning. Fire blight cankers on large branches and trunks have the highest chance to produce viable bacterial ooze or colonies for flower infections (Figure 2).

Removal of cankers as the main sources of primary inoculum for flower infections is an essential practice that limits the chance for severe fire blight outbreaks in future growing seasons. However, no matter how meticulous the pruning crews are in removing cankers during the winter, some cankers remain unpruned on old branches or on the trunk. Carried over into the future growing seasons, cankers pose a constant threat for development of new epidemics. Old literature sources report that just 1 to 4 cankers per 2.5 acres is enough to allow fire blight infection renewal in the spring (Brooks 1926; Tullis 1929).

We believe that development of new spray options that could kill *E. amylovora* cells overwintering in cankers, perhaps via an increase in the efficacy of dormant copper sprays, is essential for reduction or eradication of primary fire blight inoculum. In previous work, we demonstrated that copper chelate injected into the xylem of apple tree trunks provided blossom blight control of 15.5–17.8% and shoot blight control of 24.5–33.9% (Aćimović et al. 2015). We assumed that if copper ions helped by bark penetrating surfactants can penetrate into the compromised bark near fire blight canker edges, then the copper ions might reduce the number of overwintering cells of *E. amylovora*.

On 25 June 2016, we inoculated shoots on mature 'Cortland' trees, using a water suspension of 1 x 10^9 colony forming units (CFU/ml) of *E. amylovora,* with the objective of generating fire blight cankers for use in studying the effect of dormant copper sprays in the overwintering phase of fire blight. After collecting untreated fire blight cankers on dormant trees on 5 Dec 2016 for analysis of pre-treatment bacterial populations, we sprayed two copper products mixed with two commercial surfactants on 6 Dec (Table 1). The 7.95 lb/A rate of metallic copper used is the maximum allowed rate per application per acre during dormancy for fire blight (Table 1). The 12.72 lb/A rate of metallic copper used was chosen as an experimental extreme spray for comparison purposes and is not allowed by EPA. Each treatment was sprayed on 3 single-tree replicates. We applied the sprays in early December to avoid extremely low winter temperatures that could potentially reduce bacterial populations in cankers. Treatments were sprayed dilute (300 gal/A) to drip using a tractor-carried handgun sprayer (Rears Pak-Tank 100 gal sprayer, 250 psi).

After extracting total DNA of both plant and *E. amylovora* cells from live wood tissue around the fire blight canker edges, we detected and quantified live *E. amylovora* cells using digital (d)PCR. dPCR is a new molecular diagnostic method for identification and absolute quantification of microorganism propagules based on a specific DNA target of choice. Results revealed that on 21 Dec 2016 and 13 Mar 2017, there was no significant reduction of bacterial populations in cankers by any of the copper products mixed with either Pentrabark or Regulaid, as compared with either the untreated control or the two copper products used alone (Figures 3A, B).

Low-Rate Copper Sprays at Bloom Provide Poor Protection under High Fire Blight Pressure

On 26 Apr 2017, 'Honeycrisp' trees were at pink bud stage. On 27 Apr, bloom reached 63%, due to extremely warm weather. On 29 Apr, when 'Honeycrisp' trees were at 65–70% bloom, treatments listed in Table 2 and Figure 4 were applied using gas-powered backpack airblast sprayer, delivering 50 gal/A (Solo 451 Mist Blower, 3 gal). The treatments were timed to avoid slow-drying conditions that could promote fruit russetting. Fireline was delivered at 100 gal/A and 1 x CS2005 + Regalia at 150 gal/A. To apply the same amount of copper that one apple tree receives from a tractor airblast sprayer in a high-density apple orchard, we divided the metallic copper rate per acre with 940 trees/A planted in a high-density system, and used timed applications with our backpack sprayer to apply the appropriate amounts of copper per tree (Table 2). We sprayed 0.196 lb/A of metallic copper twice (0.392 lb/A), which is the lowest labeled rate for a single application of Copper Count N for apples in bloom. The two sprays in each treatment were ~15 min apart because initially planned first application at 20% and second at 50% king bloom could not be conducted in a timely manner due to rapid flower opening. We used copper sulfate crystals, expecting better efficacy due to their high water solubility and thus high copper activity. We mixed COC DF with zinc sulfate to match a unique Cuprablau Z 35WP formulation of copper oxychloride, containing zinc sulfide as a synergist to the copper's bactericidal effect (Cinkarna Celje, Slovenia). This product is registered in the EU for blossom blight control and has some efficacy against *E. amylovora*. We hoped that Regalia, as a biological product, would provide positive interactions with the copper sulfate in CS2005.

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 CFU amount than usual, due to the low average daily temperature of 52.7°F during inoculation. The first fire blight symptoms developed on 17 May. We rated blossom and shoot blight incidence on 27 May and 3 Jun, respectively (Figure 4). Going around the crown, we randomly chose 100 flower clusters per tree and counted the number of diseased and healthy clusters. Flower infections migrated into the shoots. We randomly chose 100 shoots per tree and counted infected and healthy shoots. Blossom and shoot blight were calculated as blossom and shoot blight percent on a per tree basis. Mean percent blossom and shoot blight incidences were calculated for each treatment from 4 single-tree replicates. Overall, under high disease pressure, two low-rate copper sprays during bloom provided poor blossom blight control ranging from 3.9% for 2 x CS2005 + Regalia, 6% for Copper Count N, to 25.1% for Copper Sulfate Crystals. Similarly, shoot blight control ranged from 3.3% for Nordox 75 WG, 5.8% for Cuprofix Ultra 40 Disperss, to 27.7% for Copper Sulfate Crystals (Figure 4). In contrast, Harbour provided blossom and shoot blight control of 68.5% and 72%, respectively. The effects of the copper treatments on fruit russetting had not yet been evaluated at the time this paper was compiled.

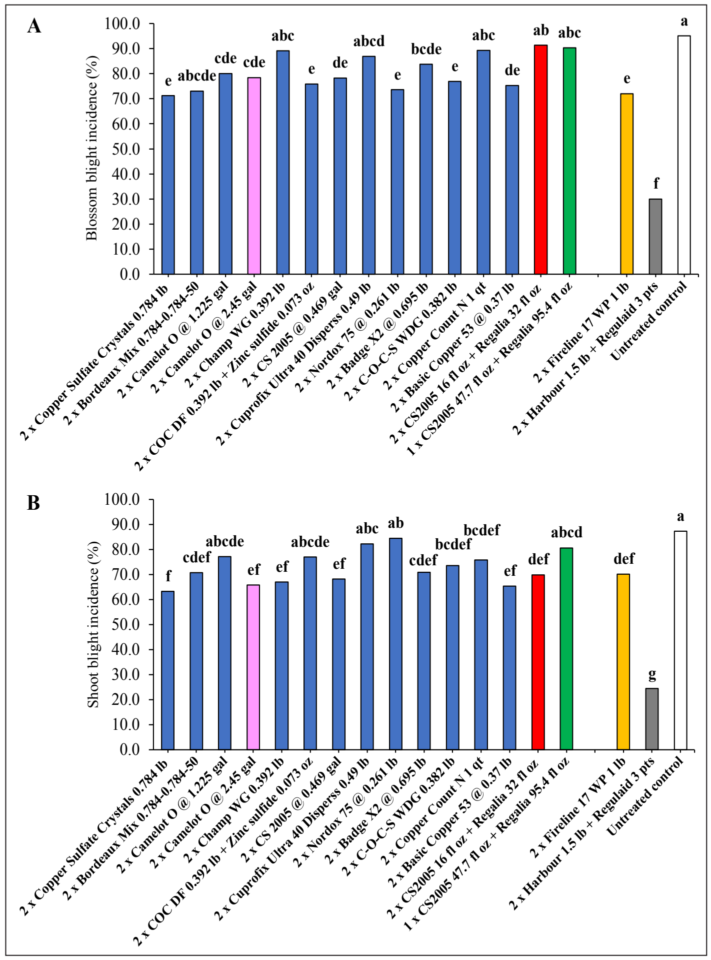

Figure 4. (A) Blossom blight and (B) shoot blight incidence on 'Honeycrisp' after preventive sprays of copper products. Trees were inoculated with fire blight bacterium *E. amylovora* at 80% king bloom (3 × 10^6 CFU/ml). Two sprays in each treatment were made one day before inoculation, each providing 0.196 lb/A of copper, except in 2 x Camelot O at 2.45 gal/A where 2 x 0.392 lb/A of copper was used (pink) and 2 x CS2005 + Regalia where 2 x 0.052 lb/A of copper was used (red). Only one spray in 1 x CS2005 + Regalia delivered 1 x 0.160 lb/A of copper (green). Different letters above bars, within each graph, indicate significant differences between treatments (*P*<0.05).

Conclusions

Our initial attempts to increase efficacy of dormant copper sprays by applying the copper with Regulaid and Pentrabark were not successful. However, it is possible that other compounds, such as bark penetrating oils, might enable copper penetration into the bark, and are the subject of our current research. It is also possible that better copper penetration into cankers might be possible if copper sulfate, which is more soluble than the fixed coppers used in this trial, were mixed with penetrants and acidifiers, given that the latter can further increase solubility of copper in aqueous solutions. Further work is also needed to generate and/or identify cankers that carry more uniform populations of *E. amylovora*. In our study, the variability of bacterial numbers among cankers in the same treatments was very high, and probably precluded the detection of statistically significant differences among treatments.

In our evaluations of low-rate copper sprays applied during bloom to control blossom blight, we found that 0.392 lb of metallic copper provided poor control of blossom and shoot blight under the high disease pressure that evolved in our trial. Efficacy of the same treatments might be better in years with lower disease pressure (i.e., in years with conditions less conducive to blossom blight and/or with flowers exposed to lower concentrations of inoculum).

Fire blight management is complicated by a warming climate, high susceptibility of planted varieties, use of spindle training systems, and lack of availability of resistant rootstocks. In addition, highly effective bactericides for plant protection are diminishing, and the existing ones are being scrutinized and limited due to potential resistance issues or toxicity to non-target fauna. Copper bactericides are an essential part of the overall fire blight management program on pome fruit and may become more important if we can apply lower doses in ways that improve the safety and efficacy of the copper sprays. The presented results are preliminary and serve as a long- and short-term basis necessary for improving existing and proposing new options for fire blight management, both during bloom and dormancy. Our goal is to expand spray options that can reduce or kill fire blight populations on or in apple trees.

Acknowledgements

We thank New York Apple Research and Development Program for their financial support of this research. We thank Dr. David Rosenberger for valuable opinion exchange, advice, and manuscript review, and the faculty and staff of the Hudson Valley Research Laboratory for providing research plots for this experiment and contribution in their maintenance.

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