Use of Biological Controls and Sterilants as Alternatives to Streptomycin Against Fire Blight Blossom Infections in Apples

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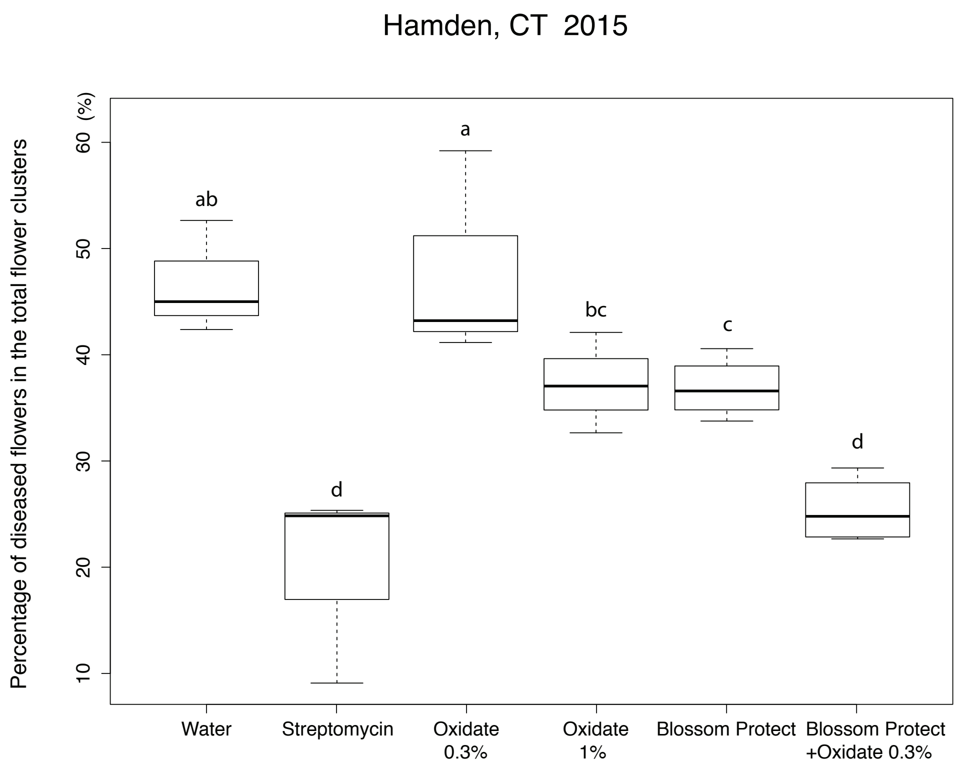
Fire blight, caused by the bacterial pathogen *Erwinia amylovora*, is a devastating disease of apples and pears. Application of the antibiotic streptomycin at bloom used to be the "silver bullet" for controlling fire blight. However, the intensive, long-term use of streptomycin not only leads to the evolution of streptomycin resistance in the pathogen population, but also raises concerns about its potential impact on the environment and human health. In 2014, the National Organic Standards Board (NOSB) terminated the use of streptomycin in organic fruit production in the U.S., making identification of effective, non-antibiotic control alternatives an urgent need for organic growers. At the same time, to reduce the reliance on streptomycin, effective alternatives need to be found for conventional growers as well.

Non-antibiotic control materials for fire blight generally fall into two categories: biological controls and non-antibiotic bactericides. Biological controls are non-pathogenic microorganisms that antagonistically inhibit pathogen growth, either by competing with the pathogenic bacteria for space and nutrients, or by producing antimicrobial compounds, or in some cases by stimulating apple tissue to be more resistant to infection. Examples of products based on microbes include *Pantoea agglomerans* (Bloomtime Biological), *Aureobasidium pullulans* (Blossom Protect) and *Bacillus amyloliquefaciens* (Double Nickel). Non-antibiotic bactericides are generally chemical toxins that kill bacteria, such as hydrogen peroxide + peroxyacetic acid (Oxidate) and copper octanoate (Cueva).

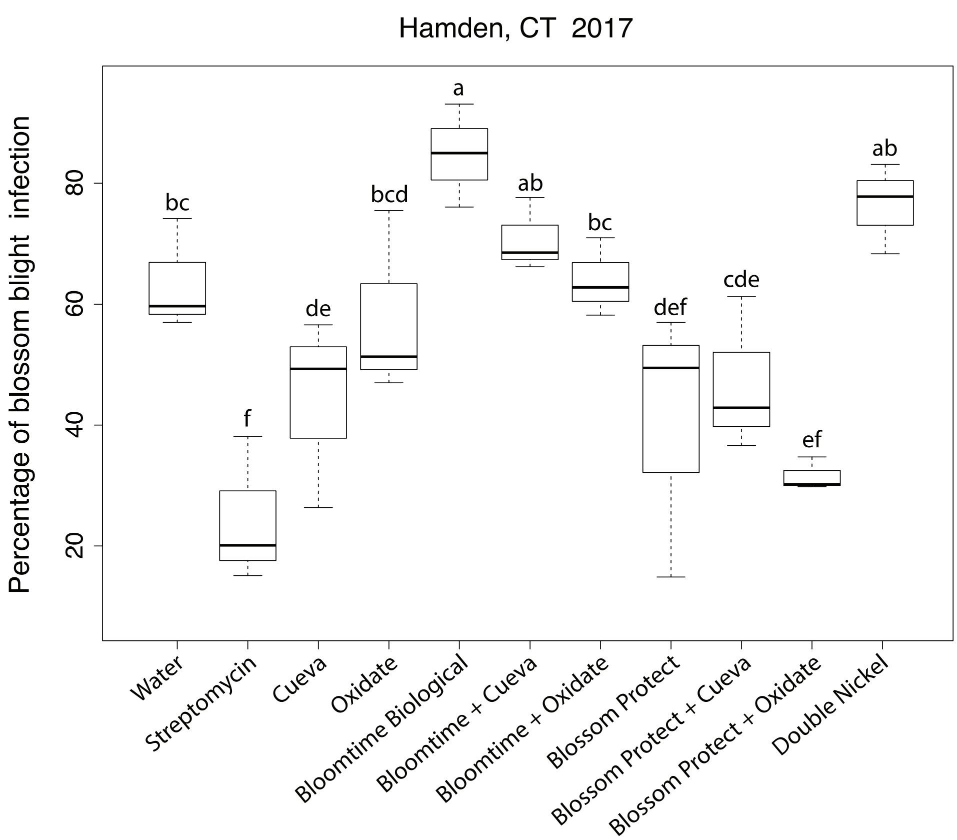
Although the use of biological controls and other non-antibiotic bactericides has been explored in fire blight management, efficacy is inconsistent and is largely affected by the climate and growing conditions of specific regions. The goal of this research is to evaluate the efficacy of non-antibiotic materials in controlling fire blight in the New England region by performing field trials at two locations (CT and MA) for multiple seasons (2015, 2017 and 2018).

Experiments

Apple trees were spray-inoculated with lab cultured *E. amylovora* cells at the concentration of 5×10^6 CFU/ml at 100% bloom. Each infected tree was treated with a biological control, or a non-antibiotic bactericide, or both. At least three trees were tested in each treatment, and trees receiving different treatments were organized in random blocks for statistical analysis. Biological control products were applied twice at 40% bloom, and again at 70% bloom. Non-antibiotic bactericides were applied two hours after *E. amylovora* inoculation unless otherwise specified. When a biological control was used in combination with a non-antibiotic bactericide, the biological control was applied first, at 40% and 70% bloom, followed by a one-time application of non-antibiotic bactericide two hours after pathogen inoculation. All pathogen inoculation and material applications were performed using a Solo motorized backpack sprayer (CT) or a hand-pumped Solo backpack sprayer (MA) to fully cover the flowers until drip. The blossom blight control efficacy was evaluated approximately two to three weeks after inoculation by calculating the percentage of infected flower clusters of the total flower clusters. The control efficacy of non-antibiotic materials was compared with water (negative control) and streptomycin (positive control) treatments.



Hamden, CT 2015
Percentage of diseased flowers in the total flower clusters (%)
10
20
30
40
50
60
ab
d
a
bc
c
d
Water
Streptomycin
Oxidate 0.3%
Oxidate 1%
Blossom Protect
Blossom Protect +Oxidate 0.3%



Hamden, CT 2017
Percentage of blossom blight infection
20
40
60
80
bc
f
de
bcd
a
ab
bc
def
cde
ef
ab
Water
Streptomycin
Cueva
Oxidate
Bloomtime Biological
Bloomtime + Cueva
Bloomtime + Oxidate
Blossom Protect
Blossom Protect + Cueva
Blossom Protect + Oxidate
Double Nickel

2015

The field trial in 2015 was performed on 35-year-old 'Golden Smoothie' apple trees at the Lockwood Farm of the Connecticut Agricultural Experiment Station, Hamden, CT. We tested one biological control, Blossom Protect (Westbridge Inc), and one non-antibiotic bactericide Oxidate 2.0 (Biosafe Inc). Biological control alone, non-antibiotic bactericide alone and combined application of biological control and non-antibiotic bactericide were included in this year's testing. The Cougarblight disease risk level was "Extreme high" and "High" during the infection period.
Forty six percent of flower clusters from trees treated with water developed blossom blight symptoms. Effective control was achieved when trees were treated with antibiotic streptomycin; only 20% of flowers showed blossom blight symptoms. Among the non-antibiotic treatments, the best control was achieved when Blossom Protect was used in combination with 0.3% Oxidate 2.0., with 25% of flower clusters infected. When applied alone, Blossom Protect can still provide some level of control (37% of infection), although the efficacy is significantly lower than when used in combination with Oxidate 2.0. No significant reduction in blossom blight infection was observed when Oxidate 2.0 was used by itself (either at 0.3% or at 1%) compared to the water treatment.

2017

In 2017, a field trial was performed on 20-year-old 'Red Delicious' apple trees at the Lockwood Farm of the Connecticut Agricultural Experiment Station, Hamden, CT. We expanded our testing to three biological controls, Blossom Protect, Bloomtime Biological (Northwest Agri Products Inc), and Double Nickel (Certis USA Inc); and two non-antibiotic bactericides Oxidate 2.0 and Cueva (Certis USA Inc). We also compared the efficacy of biological control application alone, non-antibiotic bactericide application alone, with combined applications of biological control and non-antibiotic bactericide. The Cougarblight risk level during infection period was "Extreme high" during the infection period.

Among the three biological control products tested, Blossom Protect exhibited the highest control efficacy. Forty eight percent of flowers treated with Blossom Protect was infected with fire blight, which is significantly lower than the water treated control (60%). Between the two non-antibiotic bactericides tested, Cueva (49%) performed better than Oxidate 2.0 (53%) when used alone. Notably, combined use of non-antibiotic bactericides (Cueva or Oxidate 2.0) with Blossom Protect provided better protection than when the products were used alone. The highest control efficacy was achieved when Blossom Protect was used in combination with Oxidate 2.0, which resulted in 31% of control efficacy.

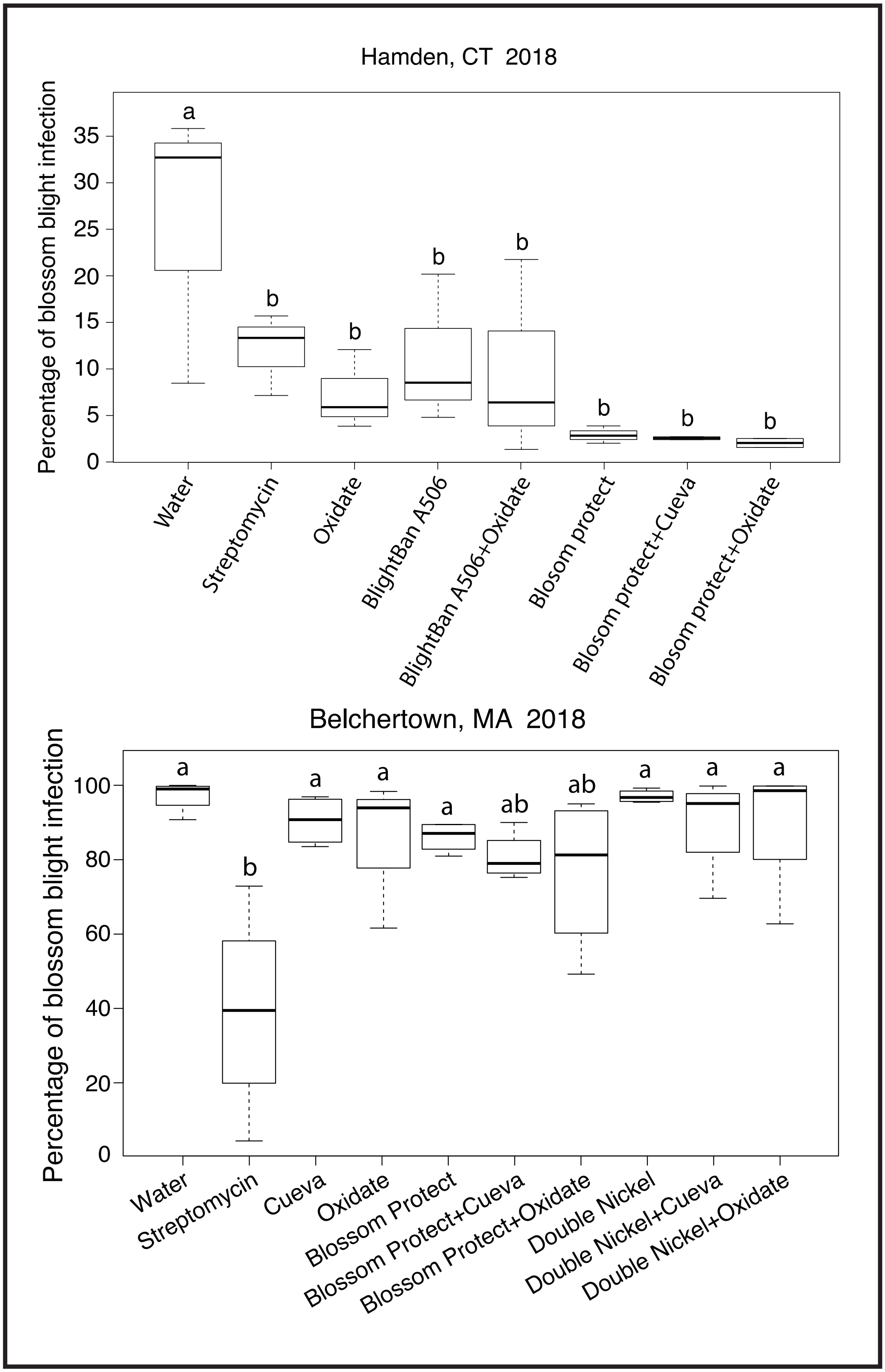
A similar test in Massachusetts was unsuccessful as cold temperatures during bloom prevented infection, even at high levels of bacterial inoculum (5 X 10^7 CFU/ml). Cougarblight risk level prior to and following inoculation was "Low".

2018

Field trial of 2018 was performed at two separate locations, one at the Lockwood Farm of the Connecticut Agricultural Experiment Station, Hamden, CT and the other one at the Cold Spring Orchard of University of Massachusetts, Belchertown, MA. Thirty-five year-old 'McIntosh' apple trees and thirty-year-old 'Jonagold' apple trees were used in CT and MA, respectively. Two non-antibiotic bactericides Oxidate 2.0 and Cueva were tested at both locations. Two biological controls, Blossom Protect, BlightBanA506 were tested at the CT location and Blossom Protect and Double Nickel were tested at the MA location. Biological controls alone, non-antibiotic bactericides alone, and combined application of biological controls and non-antibiotic bactericides were tested. The Cougarblight risk level was "Low" and "Extreme high" during the infection period at CT and MA locations respectively.

Compared to the high disease risks in previous years, low disease risk in CT in 2018 resulted in overall control success of all materials tested. This emphasizes that the biocontrol agents and non-antibiotic bactericides are particularly effective in controlling blossom blight under low disease pressure.

In contrast, the infection risk in MA was extreme, and almost all flower clusters on the water treated trees were infected. This is much higher than the average level of infection in typical inoculated experiments, where 30-60% of flower clusters are infected in water treated controls. Under this circumstance, the only treatment that caused a statistically significant difference from the water treatment was streptomycin. Despite this, we still observed a similar trend of the non-antibiotic bactericides as observed in previous years. Blossom Protect in combination with Cueva or Oxidate provided


Hamden, CT 2018
Percentage of blossom blight infection
0
5
10
15
20
25
30
35
a
b
b
b
b
b
b
b
Water
Streptomycin
Oxidate
BlightBan A506
BlightBan A506+Oxidate
Blosom protect
Blosom protect+Cueva
Blosom protect+Oxidate
Belchertown, MA 2018
Percentage of blossom blight infection
0
20
40
60
80
100
a
b
a
a
a
ab
ab
a
a
a
Water
Streptomycin
Cueva
Oxidate
Blossom Protect
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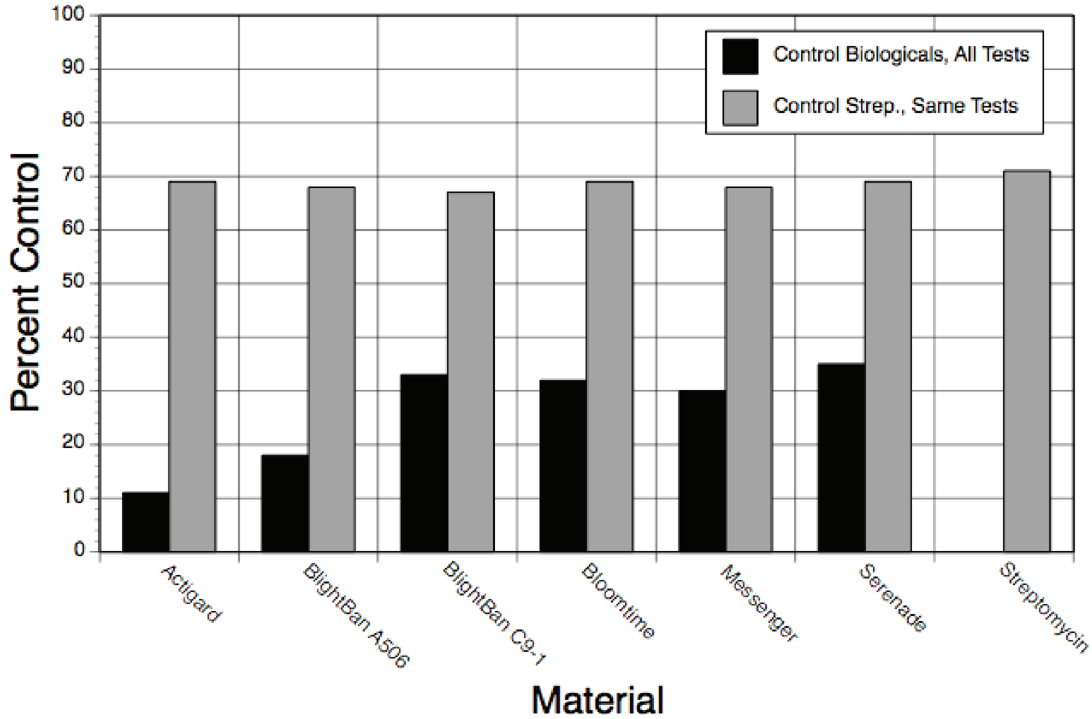
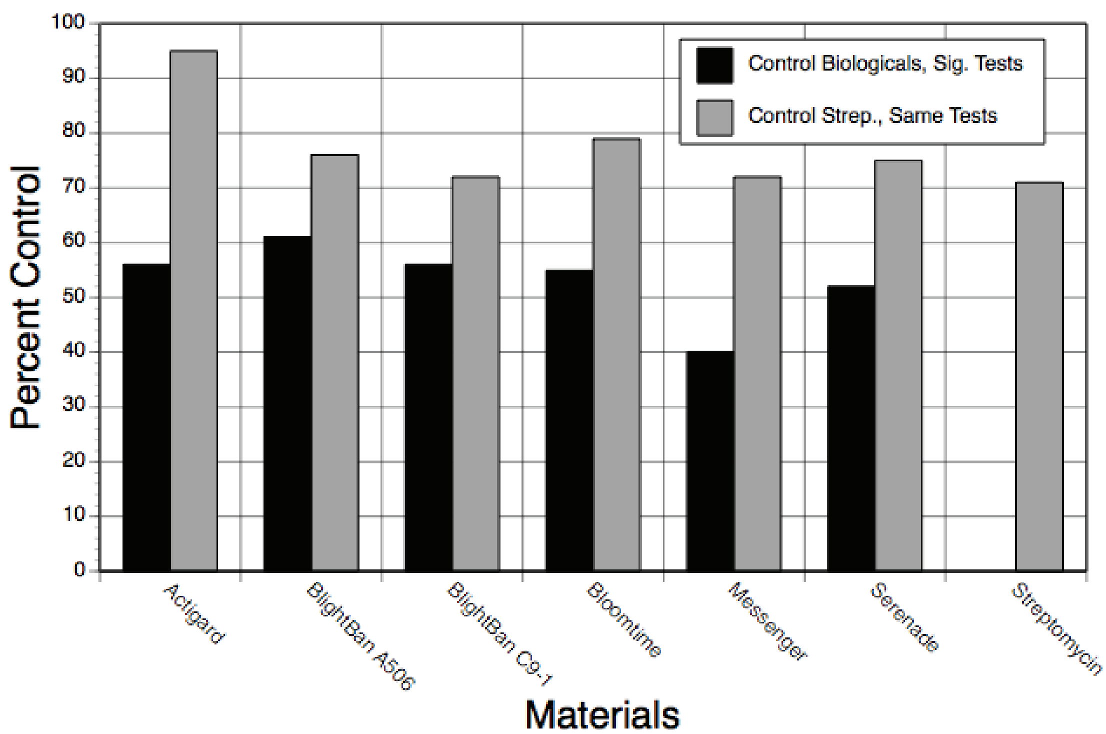
Double Nickel
Double Nickel+Cueva
Double Nickel+Oxidate





The top graph shows percent blossom blight control for twenty-five tests from 2000-2007. The bottom graph shows blossom blight control for just those tests in which the biological controls significantly decreased blossom blight relative to checks.

reduced blossom blight symptoms than water treated control, although the difference is not statistically difference.

Analysis and Recommendations

Over the three years, the biological control Blossom Protect in combination with Oxidate 2.0 provided the most consistent, high level of control against blossom blight. The control from the combined biological control plus non-antibiotic bactericide treatment comes in two ways. Blossom Protect, a yeast (*Aureobasidium pullulans*) competes with the pathogen for space and nutrients in apple flowers, and prevents the *Erwinia amylovora* bacteria from establishing and growing. The Oxidate 2.0 acts as a surface sterilant, destroying any *E. amylovora* that might be in flowers when it is applied.

The different modes of action of the two products makes appropriate timing of each one critical. To allow ample time for the biological control microorganisms to multiply on the apple flowers and exert their antagonistic effect, biocontrols need to be applied early in bloom, at 30-70% bloom. The surface sterilants need to be applied at a later stage of bloom, 90-100%, which will enable it to clean up any pathogen population that survived the biocontrols. Research has shown that flowers at petal fall or later are not likely to be infected by *E. amylovora*.

The New England climate imposes additional challenges to effective use of biological controls in managing fire blight. In the Pacific Northwest, average temperatures during bloom are often low, 50º to 60º F. This results in a prolonged bloom period of 10 to 14 days or more, and does not favor pathogen growth. It gives ample time to not only apply the biological controls, but also to let the biological controls multiply during flowering. In New England, mean temperatures during bloom are usually higher, as high as 70º to 80º F. At these temperatures, a flower can quickly progress from freshly open to petal fall within two to four days. This short window makes it challenging to apply biocontrol products at early bloom, and give them enough time to establish in the flower before the pathogen arrives. Higher temperatures favor *E. amylovora* growth, allowing the pathogen to compete more successfully against the biocontrol. When temperatures are high, the fireblight model Cougarblight indicates a high disease risk, biological controls' performance decreases.

A review of twenty-five efficacy tests for biological controls around the U.S. from 2000 to 2007 showed that streptomycin was consistently much more effective than biologicals for control of blossom blight in apples. In some tests, the biological controls had no effect compared to controls. However, in many tests, the biological controls did significantly reduce blossom blight. If just these tests are combined, the performance of the biologicals approaches that of streptomycin. What's the difference? Disease pressure. Weather, primarily temperature, and other factors such as the cultivar combine to generate higher or lower risk of infection. Where risk is high enough to cause some disease, but not extremely high, biologicals can significantly reduce fire blight.

Given these facts, we recommend using streptomycin rather than biological controls or sterilants to control blossom blight when the mean temperature during bloom is above 70ºF. These temperatures typically give Cougarblight risk levels of "High" or "Extreme". Biocontrols and sterilants are more suited for controlling fire blight under moderate to low disease pressure, Cougarblight ratings of "Low" or "Caution", when temperature is below 70º F.

Since both products are allowed in organic production, the combined application of Blossom Protect plus Oxidate 2.0 may also give conventional growers an alternative to streptomycin when disease risk is relatively low. Conventional growers could use this combination, and perhaps other biological controls in applications timed around streptomycin sprays. For example, early in bloom growers might apply the biological, and if risk reaches a "High" rating in Cougarblight, apply streptomycin. Reducing streptomycin use can help growers reduce the risk of developing streptomycin resistance.

For organic growers, there really is no alternative. The Blossom Protect plus Oxidate 2.0 can be used in conjunction with other OMRI materials. It is particularly important for all growers to put on a green tip copper spray with a relatively high concentration copper. Organic growers can use, for example, Nu-Cop 50DF. In addition to the biological controls and Oxidate, growers can use low concentration copper products such as Cueva, or compounds that stimulate resistance, such as Actinovate or Regalia.

Acknowledgements

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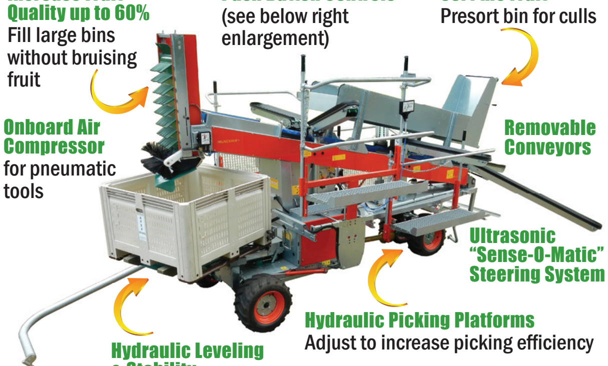

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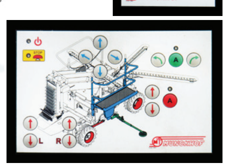

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