Evaluation of Strategies for Fire Blight Control in Organic Pome Fruit Without Antibiotics

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Abstract

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Apple and pear produced organically under the U.S. National Organic Program (NOP) standard can be treated with antibiotics for suppression of fire blight caused by *Erwinia amylovora*. Recent regulatory actions by the NOP, however, have lessened the likelihood of antibiotic use after the 2014 season. In response, western U.S. organic apple and pear stakeholders identified two immediate-need research objectives related to fire blight control: development of effective non-antibiotic control programs based on combinations of registered biological products; and, in apple, integration of these products with lime sulfur, which is sprayed at early bloom to reduce fruit load. In orchard trials in Oregon, increasing the frequency of treatment with biological products improved suppression of floral infection. In apple, fruit load thinning with 2% lime sulfur plus 2% fish oil (LS+FO) at 30 and 70% bloom

significantly ($P \leq 0.05$) reduced the proportion of blighted flower clusters in four of five orchard trials. Moreover, lime sulfur significantly ($P \leq 0.05$) suppressed epiphytic populations of *E. amylovora* after their establishment on apple flowers. Over four trials, treatment with *Aureobasidium pullulans* (Blossom Protect) after LS+FO reduced the incidence of fire blight by an average of 92% compared with water only; this level of control was similar to treatment with streptomycin. In three seasons, a spray of a *Pantoea agglomerans* product after the 70% bloom treatment of LS+FO established the antagonist on a significantly ($P \leq 0.05$) higher proportion of flowers compared with a spray of this bacterium before the thinning treatment. Consequently, in apple, biological treatments for fire blight control are not advised until after lime sulfur treatments for fruit load thinning are completed.

Organically certified pome fruit is produced on 9,000 ha in semiarid regions of the western United States, mostly in the state of Washington, with a spectrum of pear and apple cultivars that is similar to the spectrum grown with conventional methods. Within the region, the utilization of similar cultivars among organic and conventional crops creates marketing and horticultural efficiencies but has meant that producers of organic fruit must find novel solutions to disease and pest problems addressed in conventional systems with chemicals not approved for organic production. Fire blight, caused by the bacterial pathogen *Erwinia amylovora*, is a feared disease of both organic and conventional pome fruit orchardists because all commercial pear cultivars and most newer commercial apple cultivars are susceptible to this pathogen (33), and because major fire blight outbreaks have the potential to significantly damage orchards (32,33). Currently, antibiotics (streptomycin and oxytetracycline) are the most effective and efficient materials used to suppress floral infection by *E. amylovora* in both systems (1,19,28,29,33); however, a 2011 regulatory review by the United States Department of Agriculture National Organic Program (USDA NOP) established a 2014 phase-out of antibiotics in organic pome fruit production. Organic regulations in other countries, notably those in the European Union and Canada, prohibit the use of antibiotics for plant disease control.

In the last two decades, several non-antibiotic products for fire blight control have been developed, registered with the Environmental Protection Agency, approved for use in organic systems, and marketed to orchardists (Table 1). Although these biological products have found some acceptance by orchardists, most organic crops grown under the USDA NOP standard are still treated with antibiotics for fire blight during periods of elevated disease risk. In

general, the knowledge required to use antibiotics effectively for fire blight control is widespread among orchardists and their advisors. Conversely, there is greater uncertainty in how to confidently and effectively use the “softer” biological alternatives for suppression of this disease.

A recent scientific result that diminished some of this uncertainty was made by Stockwell et al. (28), who found that treating pear and apple flowers with a gram-negative bacterial antagonist and then following with a single application of oxytetracycline frequently provided better control of fire blight than two treatments of the antibiotic only. Previously, in large-scale plots in commercial orchards, Lindow et al. (14) demonstrated a benefit to fire blight control from the addition of bacterial antagonist treatments to regular, multiple-treatment antibiotic programs. This so-called “integrated strategy” (28) has resulted in the hypothesis that prevention of floral infection by *E. amylovora* can be viewed in two distinct phases: suppression the pathogen’s prerequisite epiphytic phase on floral stigmas (31)—most effectively accomplished by a competing nonpathogenic bacterium (6–8,22,27,34)—and suppression of infection by the pathogen via natural openings (nectar-thodes) on the hypanthium (20,35)—accomplished with antibiotics (1,28,29). Currently, the recommendation given to implement this integrated strategy initiates bacterial antagonist treatments early in bloom (30 to 80% bloom) followed by use of an antibiotic between full bloom and petal fall to protect the hypanthium if and when a temperature-based disease risk model (25) warns of an imminent infection event.

The purpose of this study was to investigate strategies of combining registered non-antibiotic materials for fire blight control in organic orchards based on the hypothesis that has emerged from results achieved with integrated biological and antibiotic programs (14,28). Specific questions addressed were (i) can the concept of the integrated strategy be adapted to non-antibiotic programs by utilizing specific combinations of biological materials; (ii) does frequency of treatment with non-antibiotic materials influence the degree of control; and (iii) can non-antibiotic biological materials be integrated with chemical fruit load (bloom) thinning treatments used in apple, which are often sprayed at similar stages of bloom?

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Materials and Methods

Evaluated materials. Biological and antibiotic materials evaluated in this study are listed in Table 1. Prior knowledge of the specific properties of the biological materials was used in the development of integrated strategy non-antibiotic programs for evaluation in orchard trials. For example, the stigma-colonizing abilities of the gram-negative bacterial biocontrol agents used for fire blight control (e.g., *Pantoea* spp.) are well documented (6–8,21,22,27,31,34). Furthermore, after initial establishment, these gram-negative bacterial biocontrol agents readily disperse to pear and apple flowers that open after the time of treatment (10,15), which favors treatment in early bloom (9). Previous investigators (11,23,29; K. Johnson *unpublished data*) have observed that preformed antimicrobial compounds within the commercially formulated Serenade Max (produced during fermentation of *Bacillus subtilis* strain QST 713) are a major component of the product's effectiveness; therefore, this material was considered only as a direct substitute for antibiotics and applied later in bloom. Yeasts have been shown to be excellent colonizers of the hypanthium (20,21); thus, treatments that included the yeast product Blossom Protect were skewed toward timings later in bloom. We addressed the question of the effect of frequency of treatment with non-antibiotic materials on disease incidence with a *Pantoea vagans* (BlightBan C9-1) or *P. agglomerans* (Bloomtime Biological) product early in bloom, and Serenade Max late in bloom; an integrated treatment program that used each of these materials once (two-spray program) was compared with a treatment program that used each of these materials twice (four-spray program). An integrated program of a *Pantoea* sp. product followed by oxytetracycline, one or two treatments of streptomycin, and two treatments with water only were included as comparative controls. Fruit load (bloom) thinning was done with a mixture of calcium polysulfide (Rex Lime Sulfur Solution 28%; OR-CAL Inc.) and fish oil (Crocker's Fish Oil, Inc.) with both materials in a 2% (vol/vol) suspension. Thinning treatments were made at 20 to 30% bloom and repeated at 70% bloom (3,18).

Orchards and experimental protocol. Non-antibiotic treatments for fire blight control were evaluated from 2009 to 2012 in experimental orchards located at Oregon State University's, Botany and Plant Pathology Field Laboratory near Corvallis, OR: a 0.5-ha block of 'Bartlett' pear (*Pyrus communis* L., Bartlett seedling rootstock, 49 years old in 2009), a 0.4-ha block of 'Golden Delicious' apple (*Malus × domestica* Borkh., EMLA 26 rootstock, 30 years old), a 0.5-ha block of 'Gala' apple (EMLA 111 rootstock, 10 years old), and a 0.4-ha block of 'Rome Beauty' apple (EMLA 7 rootstock, 51 years old). All cultivars are moderately to highly susceptible to fire blight (33). Because of the rainy spring climate

of western Oregon, apple scab was suppressed in the apple orchards with two prebloom treatments of mancozeb (Dithane DF at 4.5 kg/ha; Dow AgroSciences) applied with an air-blast sprayer (935 liters/ha at 862 kPa) at the "green tip" and "pink" stages of growth; no insecticides were applied and no conventional fungicides were applied during or after the bloom period.

Treatments were assigned to individual trees (single-tree plots) in a randomized complete block design with four replications; trees were grouped into blocks based on similarity of prebloom counts of flower cluster number per tree and secondarily on tree location in the orchard. An exception was an apple experiment in 2009 when very low flower densities restricted the number of trees available for experimentation; consequently, treatments in this experiment were replicated three times with two experimental blocks located in the Gala orchard and the third block located in the Golden Delicious orchard.

Biological and antibiotic materials applied to trees were pre-mixed in 1-liter Nalgene bottles filled partially with water and shaken vigorously. These suspensions were poured into an 11.5-liter volume of water into hand-pumped backpack sprayers (model 475; Solo). Material suspensions were applied under pressure (345 kPa) to run-off to near runoff (3 to 4 liters per tree) with a hand wand attached to the sprayer. Thinning treatments of lime sulfur and fish oil (LS+FO) were also applied with a backpack sprayer or, in a few trials, with a motorized 100-liter tank sprayer and attached hand wand pressurized at 414 kPa. All treatment sprays were made in early morning (6:00 to 8:00 a.m.) when wind conditions were calm. Dates of treatments applications with corresponding bloom stages are shown in Table 2.

Streptomycin-sensitive *E. amylovora* strain 153N (6,8,9) was inoculated onto the experimental trees near full bloom (Table 2). Freeze-dried inoculum of the pathogen was first resuspended in a 1-liter Nalgene bottle, and then poured into a 100-liter volume of water in the motorized tank sprayer to achieve a final concentration of 5×10^5 to 1×10^6 CFU/ml. The pathogen suspension (2 liters) was misted onto each tree with the motorized tank sprayer. Inoculations were made under calm wind conditions either in the evening between 8:00 and 9:30 p.m. or in the morning between 6:00 and 7:30 a.m.

Disease assessment. Incidence of fire blight was determined by counting the number of blighted flower clusters (i.e., strikes) on each tree during inspections made from mid-May through June; blighted flower clusters were pruned from trees as they were counted. Incidence was determined by dividing the sum of diseased flower clusters on each tree by the prebloom count of total flower clusters per tree. Incidence values for each replicate tree were transformed arcsine ($\sqrt{x}$) prior to analysis of variance

Table 1. Materials evaluated in this study for fire blight control with approval for use under the 2012 United States Department of Agriculture National Organic Program standard

Material	Manufacturer and location	Active ingredient	Rate	
			379 liters (per 100 gal)	In suspension ^x
Biological products				
BlightBan C9-1 ^y	Nufarm AgroSource, Cranford, NJ	Lyophilized <i>Pantoea vagans</i> strain C9-1S (4,10,28)	142 g (5 oz)	4 × 10 ⁶ CFU/ml
Bloomtime Biological	NAP, Pasco, WA	Lyophilized <i>P. agglomerans</i> strain E325 (22)	142 g (5 oz)	4 × 10 ⁶ CFU/ml
Blossom Protect ^z	Bio-Ferm, Tulln, Austria	Air-dried spores of <i>Aureobasidium pullulans</i> strains DSM 14940 and DSM 14941 (11–13)	570 g (1.3 lb)	1 × 10 ⁶ CFU/ml
Serenade Max	AgraQuest, Davis, CA	Air-dried spores of and antimicrobial lipopeptides from <i>Bacillus subtilis</i> QST 713 (29)	1,362 g (3 lb)	3 × 10 ⁷ CFU/ml
Antibiotics				
Mycoshield FireLine	Nufarm AgroSource, Cranford, NJ	17% oxytetracycline	454 g (16 oz)	200 µg/ml
Agri-Mycin 17 FireWall	Nufarm AgroSource, Cranford, NJ	17% streptomycin sulfate	227 g (8 oz)	100 µg/ml

^x Based on concentration (CFU/g) stated on the product label.

^y Registered with the Environmental Protection Agency but not marketed currently to pome fruit growers.

^z This product is applied with a proprietary citric acid buffer at a rate of 4 kg per 379 liters (8.75 lb per 100 gal).

(ANOVA). ANOVA was conducted with the GLM procedure in SAS (version 9.2; SAS Institute Inc.).

Lime sulfur effects on microbial populations. Because timings of lime sulfur for fruit load thinning (3,18) coincide with recommended timings for treatment with bacterial antagonists (9,28), experiments were designed to compare treatment with Bloomtime Biological (*Pantoea agglomerans* strain E325) applied at 50% bloom—and, therefore sandwiched between LS+FO treatments at 20 to 30 and 70% bloom—to treatment with Bloomtime Biological delayed until after the second LS+FO treatment. Methods for spray application of the materials were as described above. In 2010, eight Golden Delicious apple trees were sprayed with LS+FO applied at 30 and 70% bloom (16 and 19 April, respectively) (Table 2); Bloomtime Biological was applied to four LS+FO-treated trees at 50% bloom (17 April) and to the other four LS+FO-treated trees at full bloom (22 April). Similarly, in 2011 and 2012, LS+FO were applied at 30 and 70% bloom (Table 2) to 8 and 12 Gala apple trees, respectively. Bloomtime Biological was applied at 50% bloom to four LS+FO-treated trees (5 May 2011 and 26 April 2012) and at 80% bloom to four (7 May 2011) or eight LS+FO-treated trees (28 April 2012).

Between full bloom and petal fall, 8 (2010) or 10 (2011 and 2012) flowers with mature (dark) anthers were sampled haphazardly from each replicate tree (each flower from a different cluster) on two or three sampling dates and washed individually to enumerate populations of *P. agglomerans* strain E325 on the floral stigmas. Styles with attached stigmas were removed from each flower with a forceps, transferred into 1 ml of sterile phosphate buffer, and sonicated for 3 min. After vortexing, a 10-μl sample of the flower wash and two 1:100 dilutions were spread on *Pseudomonas* agar F (Difco Laboratories) amended with streptomycin (50 μg/ml), rifampicin (50 μg/ml), and cycloheximide (50 μg/ml) (PAF). Colonies with size and morphology consistent with *P. agglomerans* strain E325 on reference controls of PAF were counted after 3 days of incubation at 20°C.

In 2011, additional sprays of Blossom Protect (*Aureobasidium pullulans*) at full bloom and prior to petal fall (Table 2) were superimposed onto the Gala apple trees that had received Bloomtime Biological at 50 or 80% bloom. Similarly, in 2012, Blossom Protect sprays at full bloom and prior to petal fall were superimposed onto four of the eight trees that received Bloomtime Biological at 80% bloom. Flowers sampled to enumerate populations of *P. agglomerans* also were used to enumerate populations of *A. pullulans*. For stigmas, the same wash was dilution plated onto potato dextrose agar (Difco Laboratories) amended with kanamycin (150 μg/ml) (PDA). In addition, the lower portion of each flower (hypanthium with sepals but stamens and petals removed) was similarly washed and plated onto PDA and PAF to enumerate *A. pullulans* and *P. agglomerans*, respectively. Plates were incubated for three days (20°C), after which time colonies consistent with the sizes and morphologies of *A. pullulans* and *P. agglomerans* on reference controls were counted. Occasionally, recovered colonies of *A. pullulans* were verified by polymerase chain reaction (16).

Microbial population data were graphed with time on the x-axis and with the y-axis as either incidence of detection of *A. pullulans* or *P. agglomerans*, or mean log₁₀ (population size) of each microbe on the flower part on which they were detected (detection limit of the

plating method was 1 × 10² CFU/ml of wash suspension; individual samples with no detection of a microbe were not included in the calculation of population size). The summary variables relative area under incidence curve (*A*_{inc}) and relative area under population size curve (*A*_{pop}) were computed for each replicate tree:

$$A_{inc \text{ or pop}} = \sum_{i=1}^{n-1} \left\{ \left[(y_i + y_{i+1}) / 2 \right] \cdot (t_{i+1} - t_i) \right\} / (t_n - t_1)$$

where *y* and *t* are the values of the response variable and days after inoculation, respectively, for the *i*th sample date, and *n* is the total number of sample dates. Values of *A*_{inc} and *A*_{pop} were subjected to ANOVA to test for differences in the timing of Bloomtime Biological treatment and to compare relative establishment of *A. pullulans* and *P. agglomerans* on the treated flowers.

In 2012, an additional experiment evaluated the effect of lime sulfur oversprays on established epiphytic populations of *A. pullulans*, *P. agglomerans*, and *E. amylovora*. The experiment was conducted once in the Golden Delicious apple orchard and repeated in the Rome Beauty apple orchard. Treatments were arranged as a split-plot design with three replications, five single-tree whole plots per replication, and three subplots per tree. At 60% bloom, each subplot was sprayed to runoff with a suspension of Blossom Protect, Bloomtime Biological, or *E. amylovora* (1 × 10⁶ CFU/ml). The following day (70% bloom), whole plots received 2% lime sulfur treatment with 2% fish oil; or 3, 6, or 9% lime sulfur alone; or untreated control. All treatments were applied with backpack sprayers as described above. After the lime sulfur treatment, for each of two sampling dates, five flower clusters were haphazardly sampled from each subplot (approximately 25 flowers), washed in bulk, sonicated in 25 ml of water, and dilution plated onto culture media appropriate for each microbe (as described above, for *E. amylovora* [6]). In Golden Delicious apple, the microbe inoculation, lime sulfur treatment, and two sampling dates corresponded to 27, 28, and 29 April and 2 May, respectively; in Rome Beauty apple, the respective dates were 1, 2, 5, and 10 May. Data were subjected to ANOVA and graphed.

Results

Effect of treatment frequency. A spray program beginning with a *Pantoea* sp. product (BlightBan C9-1 or Bloomtime Biological) followed by Serenade Max was used to evaluate the effect of treatment frequency on control of fire blight with a non-antibiotic integrated strategy. Compared with the water-treated control, one treatment with a *Pantoea* sp. (70% bloom) followed by one treatment of Serenade Max (full bloom) provided a significant (*P* ≤ 0.05) reduction in fire blight incidence in three of four orchards trials (Table 3). Similarly, also compared with the water control, a *Pantoea* sp. followed by oxytetracycline provided significant (*P* ≤ 0.05) control of fire blight in five of five orchard trials. Moreover, in three of four orchard trials, the degree of incidence suppression obtained with the two-spray program of *Pantoea* sp. and oxytetracycline was significantly (*P* ≤ 0.05) superior to the two-spray program of a *Pantoea* sp. product and Serenade Max.

Overall, the high-frequency, four-spray non-antibiotic program (a *Pantoea* sp. at 30 and 70% bloom and Serenade Max at full

Table 2. Dates in 2009 to 2012 corresponding to bloom stages of pear and apple trees when fruit-thinning and biological and antibiotic treatments for fire blight control were applied in experimental orchards located near Corvallis, OR

Year	Orchard	Cultivar	Bloom stage				
			30%	70%	Inoculation ²	Full bloom	Prior to petal fall
2009	Pear	Bartlett	16 April	18 April	19 April	21 April	25 April
	Apple	Golden Delicious and Gala	25 April	29 April	30 April	4 May	8 May
2010	Apple	Golden Delicious	16 April	19 April	21 April	22 April	26 April
2011	Apple	Golden Delicious	5 May	7 May	9 May	11 May	13 May
2011	Apple	Gala	4 May	6 May	9 May	10 May	13 May
2012	Apple	Gala	25 April	27 April	28 April	1 May	5 May

² The pathogen, *Erwinia amylovora* stain Ea153N, was misted onto the trees (5 × 10⁵ to 1 × 10⁶ CFU/ml) 24 to 84 h prior to the full bloom biological or antibiotic treatment.

bloom and prior to petal fall) provided a degree of control intermediate to the two-spray non-antibiotic program and the two-spray program of a *Pantoea* sp. followed by oxytetracycline (Table 3). The four-spray non-antibiotic program was significantly ($P \leq 0.05$) better than the water-treated control in four of four trials and significantly ($P \leq 0.05$) better than the two-spray program in two of four trials. Averaged over all trials, the four-spray non-antibiotic program suppressed fire blight incidence by $70.5 \pm$ (standard error) 3.4% , compared with $47.5 \pm 4.7\%$ for the two-spray non-antibiotic program, and $82.6 \pm 1.0\%$ for the program of a *Pantoea* sp. followed by oxytetracycline. In four of four trials, streptomycin applied once or twice was significantly ($P \leq 0.05$) better than the four-spray program of a *Pantoea* sp. product and Serenade Max; the average degree of suppression from streptomycin was $93.4 \pm 2.1\%$.

Yeast as a component of integrated control. In 2010 and 2011, a four-spray program utilizing two treatments of Bloomtime Biological (*P. agglomerans*) in early bloom followed by Blossom Protect (*A. pullulans*) at full bloom and prior to petal fall significantly ($P \leq 0.05$) reduced fire blight by an average of 86% compared with trees that received only water (Table 3); the level of control from this four-spray non-antibiotic program was similar statistically to treatment with *P. agglomerans* once followed by oxytetracycline once, and to treatment with streptomycin once. Also in 2011, a

four-spray program that reversed the order of the Blossom Protect and Bloomtime Biological treatments provided a 70% reduction in incidence of blighted flower clusters (*data not shown*).

Integrated program of lime sulfur followed by yeast biocontrol. Compared with the water-treated control, treatment of apple trees with LS+FO at 30 and 70% bloom significantly ($P \leq 0.05$) reduced the incidence of blighted flower clusters in four of five orchard trials (Table 4); the mean level of incidence suppression achieved by LS+FO over all trials was $48 \pm 10\%$. In the same orchard trials, two treatments of Blossom Protect (*A. pullulans*) following the bloom-thinning protocol and pathogen inoculation reduced the incidence of fire blight by an average of $91 \pm 1\%$ compared with trees treated with water only (Table 4). However, within this four-spray program, the addition of Bloomtime Biological (*P. agglomerans*) to the full bloom Blossom Protect treatment did not improve control beyond that achieved by LS+FO followed by Blossom Protect alone (Table 4). Overall, two applications of Blossom Protect after LS+FO provided a reduction of disease incidence that was similar statistically to the level provided by the integrated program of *P. agglomerans* followed by oxytetracycline, and also to a single spray of streptomycin.

Lime sulfur effects on microbial populations. For three seasons (2010 to 2012), apple flowers treated with Bloomtime Biological at 50% bloom, which occurred between LS+FO treatments

Table 3. Incidence of fire blight on pear and apple flower clusters as affected by integrated biological and antibiotic treatments in orchard trials conducted near Corvallis, OR from 2009 to 2011^v

Treatment	Incidence (%) ^w				
	2009		2010	2011	
	Pear	GD & Gala	GD	GD	Gala
Water	44.7 a (485)	56.4 a (132)	32.5 a (261)	34.9 a (183)	14.1 a (78)
<i>Pantoea agglomerans</i> twice, then Blossom Protect twice	...	...	3.6 cd	...	2.5 b
<i>Pantoea</i> sp. once, then Serenade Max once ^x	19.1 b	27.5 b	25.5 ab	17.0 b	...
<i>Pantoea</i> sp. twice, then Serenade Max twice	10.6 c	31.8 b	11.9 bc	9.1 c	...
<i>Pantoea</i> sp. once, then oxytetracycline once ^y	5.1 c	11.2 bc	6.8 cd	7.4 cd	1.4 b
Streptomycin sulfate once or twice ^z	1.1 d	2.3 c	2.5 d	4.2 d	2.1 b

^v Single-tree plots were arranged in a complete randomized block design with three (apple in 2009 only) to four replications per treatment. For treatments with a total of two material applications, the first application was applied at 70% bloom and the second at full bloom. For treatments with a total of four material applications, the first two treatments were made at 30 and 70% bloom, and the second were made at full bloom and prior to petal fall.

^w Year of trial and orchard cultivars: 'Bartlett' pear (Pear), 'Golden Delicious' apple (GD), and 'Gala' apple (Gala). Within a column, means followed by the same letter do not differ significantly according to Fischer's protected least significance difference at $P = 0.05$. The arc-sine square root transformation was applied to incidence data prior to analysis of variance; nontransformed means are shown. Numbers in parentheses are the mean number of diseased flower clusters on water-treated trees. Incidence on each tree was computed by dividing the number of diseased flower clusters by the prebloom count of total clusters.

^x BlightBan C9-1 (*P. vagans*) in 2009; Bloomtime Biological (*P. agglomerans*) in 2010 and 2011.

^y Mycoshield in 2009; FireLine in 2010 and 2011.

^z Agrimycin 17 applied at 70% and full bloom in 2009; FireWall applied at full bloom in 2010 and 2011.

Table 4. Incidence of fire blight on apple flower clusters as affected by the fruit load thinning treatment, lime sulfur plus fish oil, and subsequent biological treatments in orchard trials conducted near Corvallis, OR from 2009 to 2011^v

Treatment ^x	Incidence (%) ^w				
	2009	2010	2011		2012
	GD & Gala	GD	GD	Gala	Gala
Water	56.4 a (132)	32.5 a (261)	34.9 a (183)	14.1 a (78)	41.1 a (215)
LS+FO twice	16.1 b	27.3 a	15.5 b	8.9 b	16.7 b
LS+FO twice, then BP twice	...	3.2 b	3.7 cd	0.9 c	4.5 d
LS+FO twice, then Combined once, then BP once	...	3.1 b	1.5 d	1.7 c	4.0 d
Bloomtime Biological once then FireLine once ^y	...	6.8 b	7.4 c	1.4 c	9.3 c
Streptomycin sulfate once or twice ^z	2.3 c	2.5 b	4.2 cd	2.1 c	5.1 cd

^v Single tree plots were arranged in a complete randomized block design with three (2009 only) to four replications per treatment. The bloom thinning treatment, lime sulfur (2%) plus fish oil (2%), was sprayed at 30 and 70% bloom. The biological materials following LS+FO were applied at full bloom and prior to petal fall.

^w Year of trial and apple orchard cultivars 'Golden Delicious' (GD) or 'Gala'. Within a column, means followed by the same letter do not differ significantly according to Fischer's protected least significance difference at $P = 0.05$. The arc-sine square root transformation was applied to incidence data prior to analysis of variance; nontransformed means are shown. Numbers in parentheses are the mean number of diseased flower clusters on water-treated trees. Incidence on each tree was computed by dividing the number of diseased flower clusters by the prebloom count of total clusters.

^x LS+FO = lime sulfur plus fish oil, BP = Blossom Protect, and Combined = Bloomtime Biological combined with BP.

^y Bloomtime Biological was applied at 70% bloom and FireLine was applied at full bloom (28).

^z Agrimycin 17 applied at 70% and full bloom in 2009; FireWall applied at full bloom in 2010 and 2011.

at 20 and 30 and 70% bloom, showed reduced incidence of *P. agglomerans* on stigmas compared with flowers treated with Bloomtime Biological at 80% to full bloom, which was after the last bloom-thinning treatment (Fig. 1A–C). This difference in incidence of *P. agglomerans* was reflected in the values of the summary statistic, A_{inc} : 0.50 ± 0.12 (2010), 0.15 ± 0.05 (2011), and 0.37 ± 0.09 (2012) for the 50% bloom timing, compared with $A_{inc} = 0.88 \pm 0.08$ (2010), 0.63 ± 0.04 (2011), and 0.70 ± 0.06 (2012) for the 80% to full timing. Within each season, the differences in A_{inc} among treatment timings were significant ($P < 0.05$).

In contrast, differences in the population size of *P. agglomerans* on stigmas on which it was detected were not consistent with the timing of the Bloomtime Biological treatment. In 2010 and 2012, the population sizes of *P. agglomerans* measured on flowers that received the 80% or full-bloom timing were approximately a half log unit greater than on those from the 50% bloom timing (Fig. 1D and F) whereas, in 2011, the populations of the antagonist were approximately a log unit higher for the 50% bloom timing compared with the later timing (Fig. 1E).

In 2012, compared with the untreated control, lime sulfur oversprays onto preestablished epiphytic microbe populations on Golden Delicious flowers (Fig. 2) reduced significantly ($P < 0.05$) the population sizes of *E. amylovora*, *P. agglomerans*, and *A. pullulans* regardless of sampling date or the rate of lime sulfur applied. Similarly, for the first sampling date (7 May) from Rome Beauty apple, epiphytic populations of *E. amylovora* and *A. pullulans* were significantly ($P < 0.05$) reduced by all rates of lime sulfur but the effects of lime sulfur on the population size of *P. agglomerans* were inconsistent (Fig. 2). On 10 May, only the *A. pullulans* populations on the Rome Beauty flowers were suppressed significantly ($P < 0.05$) by the lime sulfur treatments made on 3 May.

Colonization of flowers sprayed with both *P. agglomerans* and *A. pullulans*. In both 2011 and 2012, overspray of the 80% bloom Bloomtime Biological treatment with Blossom Protect at full bloom and prior to petal fall revealed differences in the colonization of the flowers by *P. agglomerans* and *A. pullulans*. For washed stigmas, the incidence of both microbes was high (2011: $A_{inc} = 0.63 \pm 0.03$ and 0.98 ± 0.01 for *P. agglomerans* and *A. pul-*

lulans, respectively; 2012: $A_{inc} = 0.71 \pm 0.07$ and 0.94 ± 0.04 for *P. agglomerans* and *A. pullulans*, respectively) (Fig. 3A and B), with population size on styles and stigmas on which they were detected averaging $4.44 \pm 0.16 \log_{10}$ (CFU per flower) for the bacterium and $3.24 \pm 0.06 \log_{10}$ (CFU per flower) for the yeast. In contrast, for washed hypanthia, the yeast was again recovered from 98% (2011) to 100% (2012) of sampled flowers whereas *P. agglomerans* was detectable on 9% and 18% of washed hypanthia in 2011 and 2012, respectively (Fig. 3C and D). For both seasons, differences in hypanthial A_{inc} values among *P. agglomerans* and *A. pullulans* were significant at $P < 0.01$. Measured population sizes of the bacterium and the yeast on hypanthia on which they were detected averaged 2.60 ± 0.21 and $3.18 \pm 0.18 \log_{10}$ (CFU per flower), respectively.

Discussion

Biological control of fire blight has been researched for decades (6–9,14,15,20–22,27–29,34) but this effort is the first to examine the integration of biological products for enhanced disease control, and to integrate fire blight control with lime sulfur, which is a common fruit-load-thinning treatment used in both organic and conventional apple orchards located in western North America (3,18). Our research effort was initiated at stakeholder request after regulatory actions by the USDA NOP had established a phase-out date for use of antibiotics in organic pome fruit production. The degree of infection suppression obtained by certain specific combinations of biological products (Tables 3 and 4) are among the highest and most consistent ever observed for biological control of fire blight in inoculated orchard trials (6,19,28,29).

The integrated biological and chemical approach demonstrated by Stockwell et al. (28) utilized a gram-negative bacterial antagonist (e.g., *Pantoea* spp. or *Pseudomonas fluorescens*) to suppress the epiphytic phase of *E. amylovora* on floral stigmas followed by an oxytetracycline treatment later in bloom to prevent infection in the hypanthium. A non-antibiotic program modeled on this strategy involved substitution of the biological product Serenade Max for oxytetracycline. In this role, the integrated program with Serenade Max proved to be suppressive of fire blight; however, obtaining a

Fig. 1. A–C, Incidence of detection and D–F, $\log_{10}$ (population size) of *Pantoea agglomerans* on floral stigmas by date of sampling from 'Golden Delicious' (2010) and 'Gala' apple (2011 and 2012) trees treated with Bloomtime Biological at either 50% (solid arrow and squares) or 80% to full bloom (open arrow and squares) in experimental orchards located near Corvallis, OR. The fruit-load-thinning treatment lime sulfur plus fish oil (2%:2% [vol/vol]) was applied to trees at 30 and 70% bloom (small arrows). In 2011, Blossom Protect also was applied to all trees at the timings of full bloom and prior to petal fall. For each sampling date, incidence of *P. agglomerans* was determined by dilution plating dissected stigmas from 8 (2010) or 10 (2011, 2012) flowers from each of four replicate trees. Mean population size of *P. agglomerans* includes only those flowers on which the antagonist was detected. Error bars associated with each point represent $\pm$ one standard error of the mean.

degree of control closer to that achieved with oxytetracycline after a bacterial antagonist required doubling the frequency of application of the biological products (Table 3). The result indicates that non-antibiotic programs that utilize only biological products will likely be more expensive than programs utilizing antibiotics because satisfactory disease control will require more sprays. Spraying more often also means that orchardists will need to be more preventative in their approach to fire blight control (9) as opposed to reactive, where a single antibiotic spray could be applied based on an imminent infection event forecasted by a disease warning model (25).

The yeast product Blossom Protect provided outstanding disease control when used either as part of an integrated program following *Pantoea agglomerans* or following bloom-thinning treatments of LS+FO. In organic pome fruit production, the former program would have higher value in pear, where bloom thinning is not practiced routinely, whereas yeast treatments after LS+FO (or lime sulfur alone) would be more practical in apple. The excellent disease suppression obtained with the yeast after LS+FO raises the question of the value of gram-negative bacterial antagonists (e.g., *P. agglomerans*) in non-antibiotic programs for apple. The experiment with Bloomtime Biological timed either before or after the second bloom-thinning treatment demonstrated that an overspray of LS+FO onto *P. agglomerans* was detrimental to the establishment of the antagonist on flowers compared with delaying the antagonist treatment until after the LS+FO treatments were complete (Fig. 1). However, delaying the *P. agglomerans* treatment moved the use of this material into the period of bloom that was adequately protected by the *A. pullulans* treatments (Table 4). Moreover, based on the microbial populations measured on flowers (Fig. 3), *A. pullulans* appeared to be an excellent colonist of both the stigma and the hypanthium, whereas *P. agglomerans* colonized stigmas but was detected on the hypanthium infrequently. Although not proven in this study, the ability of *A. pullulans* to colonize the hypanthium may be a primary mechanism by which this organism provides outstanding fire blight suppression when applied to trees at middle to later stages of bloom. Certainly, for yeasts used as biocontrol agents of postharvest fruit rots (5,24), the effectiveness of these antagonists is commonly attributed to an ability to rapidly utilize nutrients available at the site of infection.

Compared with research on gram-negative bacterial antagonists of *E. amylovora*, the ability of *A. pullulans* (and other yeasts) to control fire blight control is a relatively recent observation (11,12,21). Consequently, there are numerous questions regarding the implementation of yeasts in commercial fruit production. Foremost among these is the effect of the orchard environment on the yeast's ability to colonize flowers and suppress *E. amylovora*. Most organic pome fruit in the United States is produced in semiarid regions where little rainfall occurs during the spring and summer. In contrast, the *A. pullulans* strains in Blossom Protect were first selected in Germany to control postharvest fruit rots (13), then later evaluated for fire blight control (11,12) in a region where rain events occur frequently during bloom and fruit development. It is unknown whether *A. pullulans* will establish consistently in semiarid environments, although survey data from semiarid regions of Washington indicate that yeasts are relatively common on pome fruit flowers (21) and research data show that yeast epiphytes tolerate high osmotic pressures better than bacterial epiphytes (20). Furthermore, *A. pullulans* (Blossom Protect) is applied in combination with an acid buffer that aids establishment of the yeast by lowering the pH of the host surfaces (11,12). A relatively large quantity of acidic buffer (9.9 kg/ha) is applied in tank mix with Blossom Protect and it is unknown whether this rate can be reduced in a drier climate where less wash off could be expected. In wetter climates, a negative aspect of *A. pullulans* growing on flowers is a causal association with skin russet on the surfaces of developing fruit (12,17,26), which we also observed in our 2011 Gala apple trial when frequent rains occurred in Corvallis after primary bloom. Russetting of fruit surfaces can greatly reduce crop value; thus, an understanding of this potential in semiarid climates more

typical of western pome fruit production should be obtained before this product is used extensively.

That LS+FO were partially effective for fire blight control (Table 4) simplifies non-antibiotic programs in organic apple. The reason LS+FO suppresses fire blight is likely twofold: (i) lime sulfur is directly toxic to epiphytic microbes on plants (2) and (ii) the treatment causes flower abscission (36), which reduces the number of flower clusters that become diseased. Recent surveys that we made on the detectability of epiphytic *E. amylovora* in pear and apple flowers sampled from commercial orchards (30) found that likelihood of a positive detection is relatively small from early to mid-bloom but increases several-fold by petal fall. Consequently, because of its antibacterial properties, LS+FO (and lime sulfur alone; Fig. 2A) is likely sufficient in most orchards to delay or suppress the epiphytic increase of *E. amylovora* in early bloom, and the biological materials specifically registered for fire blight control can be implemented after the bloom-thinning protocol is completed. The deleterious effects of LS+FO oversprays onto biological antagonists (Fig. 2B and C) also indicate that antagonist treatments should be delayed until after the bloom-thinning protocol is complete.

Fig. 2. Log₁₀ (population size) of A, *Erwinia amylovora*; B, *Pantoea agglomerans*; and C, *Aureobasidium pullulans* on apple flowers sprayed to runoff with the fire blight pathogen (1×10^6 CFU/ml) or with Bloomtime Biological or Blossom Protect at 60 to 70% bloom (27 April and 2 May 2012 for 'Golden Delicious' and 'Rome Beauty', respectively) in experimental orchards located near Corvallis, OR. On the following day, inoculated trees were oversprayed to runoff with the fruit crop load-thinning treatment lime sulfur (LS) (3, 6, or 9% [vol/vol]), or with a mixture of lime sulfur and fish oil (LS+FO; 2%:2% [vol/vol]). Each point is the mean of three replications of five bulked flower clusters (approximately 25 flowers per replicate) washed and dilution plated onto a selective culture medium; standard errors for the points averaged $0.28 \pm$ (SD) 0.21. Sample dates shown in the legend are 1 and 4 days after lime sulfur in Golden Delicious (squares) and 4 and 7 days after lime sulfur in Rome Beauty (triangles). UTC is the untreated control with respect to the fruit crop load-thinning treatment.

Fig. 3. Incidence of detection of *Pantoea agglomerans* (open squares) and of *Aureobasidium pullulans* (gray diamonds) on **A** and **B**, floral stigmas and **C** and **D**, hypanthia by date of sampling from 'Gala' apple trees treated with Bloomtime Biological at 80% bloom (open arrow) and with Blossom Protect at full bloom and prior to petal fall (hatched arrows) in an experimental orchard located near Corvallis, OR in 2011 and 2012. On each sampling date, incidence was determined by dilution plating dissected stigma and hypanthium subsamples from 10 flowers from each of four replicate trees. Error bars associated with each point represent $\pm$ one standard error of the mean.

In summary, this study demonstrates that effective, non-antibiotic integrated programs for fire blight control in organic apple and pear can be developed from registered biological materials available for this purpose. Because the biological products are inherently less inhibitory (softer) to *E. amylovora* than are antibiotics, effective programs in commercial orchards will require more frequent treatment, which will result in a greater cost for the overall program. In apple, however, because fruit load thinning with lime sulfur is a common practice and because this practice provides partial fire blight control, effective non-antibiotic programs can be achieved with a relatively small increase of treatments compared with bloom thinning followed by an antibiotic. The outstanding control obtained from programs that included the yeast material Blossom Protect (*A. pullulans*) was surprising to us and, given that this technology was only recently developed (11,12), further research is needed to fully understand its potential and possible limitations. An additional question that requires further study involves the translation of results from small-plot research, like that conducted in this study, to the larger-scale commercial setting. That is, can organic orchardists with their larger orchards, sprayers, and constraints on time and economics achieve levels of floral establishment by antagonists and of fire blight suppression comparable with those we achieved in experimental plots? A positive answer to this question would further reduce the uncertainty in how to confidently and effectively use the softer non-antibiotic alternatives for suppression of this disease.

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