Final Report: NYS IPM Program Agricultural Grants

Project Type: Research & Development

Title: Statewide survey for streptomycin-resistant fire blight in orchards.

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Abstract: Streptomycin is the industry standard for managing fire blight on apples and pears. In 2002, streptomycin-resistance was confirmed in Wayne County and eradicated through prompt action. In October 2011, streptomycin-resistance was confirmed in four apple blocks in Wayne and Ontario Counties. We conducted a statewide survey in 2012 to locate and characterize any additional resistant bacterial populations. A total of 174 samples were collected. *Erwinia amylovora* was isolated from 129 of these and 15 of these isolates were resistant to streptomycin (11.6%). Preliminary phylogenetic results show that all streptomycin-resistant isolates carry the streptomycin resistance genes, strA and strB, on transposon Tn5393 which resides on the non-conjugative plasmid, pEa29, meaning that the isolates had to be spread physically. The survey found seven new locations in which resistant bacteria are present in Wayne, Ontario, Monroe, Orleans, and Niagara counties. These counties are now considered 'High Risk Areas' for streptomycin-resistant fire blight in 2013. To prepare for the 2013 growing season, we prepared new sample submission guidelines and fire blight best IPM practices for distribution by faculty and Extension educators, at the winter fruit schools, and at the Empire State Producers Expo.

Background and Justification: For more than 40 years, streptomycin has been the industry standard for preventing blossom blight infections of fire blight on apples and pears. Its utility is due to a high level of activity against *Erwinia amylovora*, the bacterium that causes the disease. Streptomycin, when properly applied and timed using a disease forecast model such as MaryBlyt (available as a free download) or Cougarblight (available on NEWA newa.cornell.edu), can provide close to 100% control of blossom blight. Preventing blossom blight is essential to successful management of fire blight.

In NY, streptomycin-resistant fire blight was identified in a Wayne County orchard ten years ago. This young orchard had been planted with trees from a Michigan nursery where streptomycin-resistance was known to be present. Prompt identification and cooperation between stakeholders and extension personnel ensured that the outbreak was eradicated. Annual assays for streptomycin-resistance were conducted in samples of fire blight collected from this region of NY to ensure continued absence of resistant bacterial strains.

In October 2011, streptomycin-resistance was found at one location in Wayne County and subsequently confirmed in three other locations in Wayne and Ontario Counties. To add to the seriousness of the findings, streptomycin-resistance was confirmed in a nursery and many apple growers in NY now propagate their own nursery stock. To successfully contain the spread of resistant fire blight bacteria we conducted a statewide survey, charted the locations of the resistant isolates, and phylogeneticaly characterized the resistant bacterial populations. Our statewide survey focused on newly planted orchards and existing fire-blight-prone orchards.

Unfortunately, alternatives to streptomycin often provide no more than 50% control of blossom blight. Thus, the loss of streptomycin would be disastrous for the NY apple and pear industry. Further, the spread of streptomycin-resistant bacteria could have been hastened through distribution of young trees from the affected nursery. Notwithstanding, it is possible for streptomycin-resistant *E. amylovora* (SR Ea) to be spread considerable distances (> 5 miles) in one season by both bees and wind-blown rain during storms. Hence, it is imperative to determine the prevalence and distribution of streptomycin-resistance and to distribute up-to-date IPM guidelines for suppressing it and preventing its spread.

The NY State Department of Agriculture and Markets has placed a high priority on determining the distribution of streptomycin-resistant fire blight in NY orchards. Fungicide and bactericide resistance management is number 3 and fire blight management number 4 on the NYS Fruit IPM Extension and Research Priorities list. The Lake Ontario Fruit Program Advisory Committee lists these as number 1 and number 2 priorities, respectively. This project addresses the high priority need, "Assessment of the distribution of streptomycin resistant *Erwinia amylovora* causing fire blight in New York orchards" identified in the NYSIPM Projects in Agriculture, 2012 RFA.

Objectives:

1. Determine the distribution and prevalence of streptomycin-resistant *E. amylovora* in NY.
2. Characterize the genetic nature of streptomycin-resistance.
3. Develop best IPM practices for streptomycin-resistant fire blight.
4. Project evaluation.

Procedures:

Objective 1. We conducted a statewide survey for fire blight. The survey focused on locations where streptomycin resistance was confirmed in 2011, as well as newly planted orchards, and existing fire-blight-prone orchards. Rosenberger took the lead for surveys in the Hudson Valley with assistance, as needed, from Fargione. Iungerman took the lead for surveys in Northeastern NY. Breth took the lead for surveys in the Lake Ontario region. Carroll surveyed for fire blight in six orchards as part of the CAPS Orchard Survey. Collected, suspect samples were shipped express mail or hand-delivered to the Plant Pathology and Plant-Microbe Biology Dept. in Geneva, NY for analysis. Isolation of bacteria, identification of *E. amylovora*, characterization of the pathogenicity, relative virulence, and streptomycin resistance of the *E. amylovora* isolates and their genetic characterization was done in Aldwinkle's lab with assistance from the Cox lab. The GPS coordinates of the streptomycin resistant fire blight samples were obtained and mapped to determine the counties in the "High Risk Areas".

Objective 2. Streptomycin-resistance in *E. amylovora* was confirmed and genetically characterized using diagnostic techniques already in use in Aldwinkle's lab. A phylogeny of the resistant bacterial isolates is being established and may elucidate possible common sources of

streptomycin resistance and geographic spread within NY. Genetic characterization is ongoing to determine if the resistance basis is chromosomal or plasmid borne. Records of the resistance genotype of the bacterial isolates will be maintained.

Objective 3. We developed plans for curtailing the further spread of streptomycin resistance and informed and continue to inform growers in at-risk locations about best IPM practices. Based on the distribution of streptomycin-resistant fire blight in NY and the phylogeny of the resistant bacteria, a probable pattern of resistance spread may be ascertained. The 2011 IPM guidelines to use oxytetracycline in combination or rotation with streptomycin and apply Apogee to reduce shoot blight susceptibility were revised and updated based on the findings.

Objective 4. We evaluated the project based on grower cooperation with the survey efforts, enumerating farms where sampling was conducted. The project will be considered successful if we are able to develop a reasonable estimate of the distribution of streptomycin-resistant fire blight across NY. A benchmark of ten fire blight sample submissions from each of the major regions was used as a basis to provide a reasonable perspective of distribution. One potential pitfall was the occurrence of a growing season not very conducive to fire blight. However, samples of fire blight were collected and analyzed. Our research laid the groundwork for developing the best IPM practices for managing streptomycin-resistant fire blight.

Results:

The 2012 season did not prove to be a disaster with heavy economic impact caused by fire blight. The bloom period was relatively cool and the summer relatively dry with areas in western NY experiencing drought. The majority of samples (150 out of 174) were collected from the Lake Ontario region, in order to focus on areas near the resistant fire blight findings in 2011. Twenty samples were collected from the Hudson Valley and four from the Lake Champlain apple-growing regions.

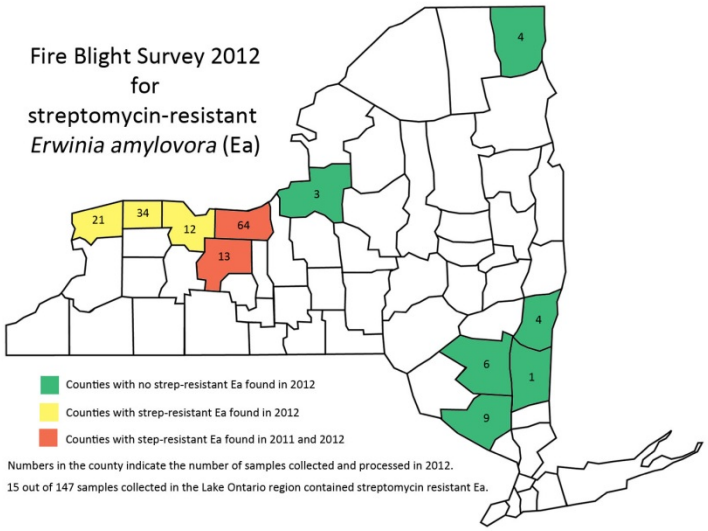

Figure 1. Distribution of streptomycin-resistant fire blight in NY. In red are the counties in which streptomycin-resistant *E. amylovora* (SR Ea) was found in both 2011 and 2012. In yellow are the counties in which SR Ea was newly found in 2012. In green are the counties in which Sr Ea was not found in the survey.

Figure 1 shows the counties from which samples were collected. The majority of the affected farms were in Monroe, Ontario, and Wayne Counties, and there were no instances of SR Ea in samples from Oswego County or Eastern NY, though fewer samples were submitted from these regions as a whole. In the Lake Ontario apple-growing region, 33% of the isolates collected in Ontario County were SR Ea, 16% in Wayne County, 11% in Monroe County, 7% in Orleans County, and 8% in Niagara County. Although the proportion is high in Ontario County, many samples were collected from a farm in which SR Ea had been confirmed in 2011 in an effort to understand within-farm distribution patterns.

Of the 174 samples, 129 yielded *E. amylovora* and 15 of these isolates were streptomycin resistant, 11.6% of the total isolated in the 2012 statewide survey. The 15 SR Ea isolates were

from nine different production operations. Interestingly, locations with confirmed SR Ea also had samples infected with streptomycin-sensitive Ea in similar or in greater abundance.

The cultivars most frequently submitted for analysis and confirmed to have fire blight infection included 'Gala' with 20%, 'NY2' with 12%, 'Ginger Gold' with 11% and 'Idared' with 9% of samples with confirmed fire blight (Figure 2). Cultivars from which SR Ea was isolated included 'Gala'(3 samples), 'NY2' (3 samples), 'Idared' (2 samples), and 'Aztec Fuji', 'Cameo', 'Ginger Gold', 'Lady', 'SweeTango', 'Twenty Ounce', and M.26 rootstock (1 sample each) (Figure 2). The 14 scions from which SR Ea was isolated were grafted onto either an undetermined rootstock (6 samples), B.9 (4 samples), M.9 (3 samples), and M.26 (1 sample). Six samples were submitted from pear, quince and crabapple and although *E. amylovora* was isolated from each, none of the isolates was resistant to streptomycin.

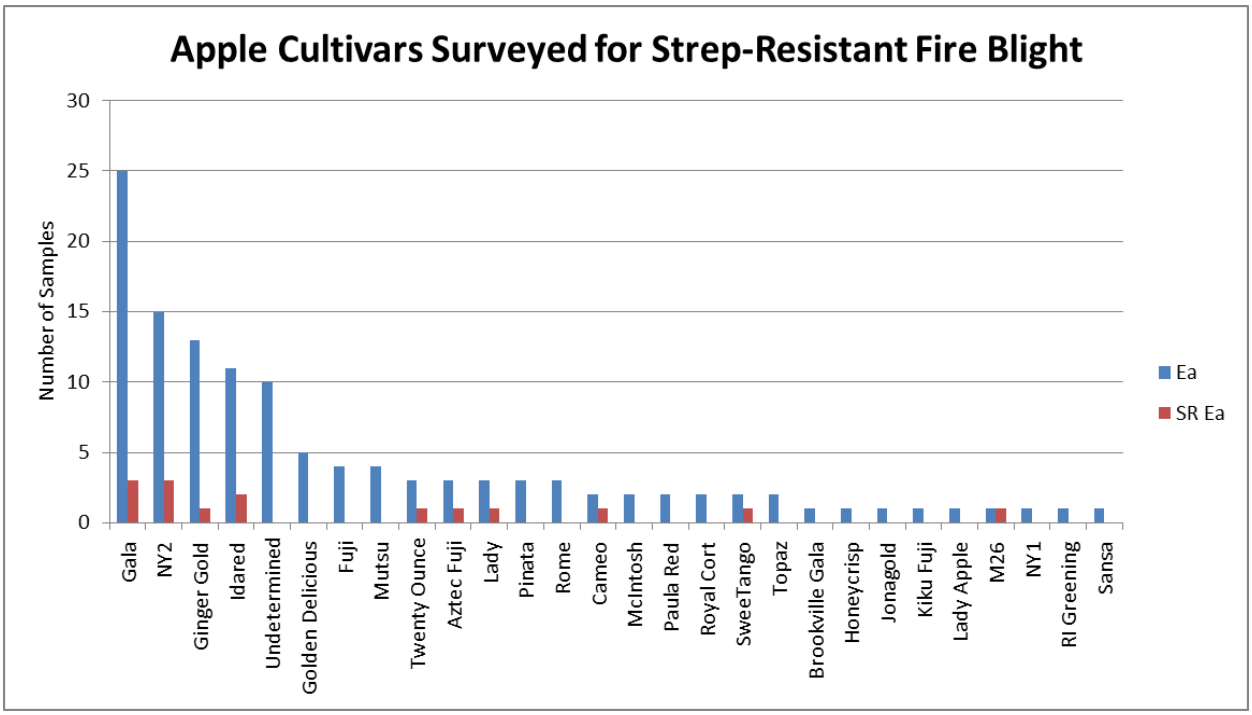

Figure 2. Apple cultivars from which samples were submitted for analysis in the survey. The blue bars show the total number of samples from which *E. amylovora* (Ea) was isolated. The red bars show the number of samples out of the total that were positive for streptomycin-resistant *E. amylovora* (SR Ea).

Genetic characterization of the 15 SR Ea isolates recovered in the survey is ongoing. Currently, all streptomycin-resistant isolates carry the streptomycin resistance genes, strA and strB, on transposon Tn5393 which resides on the non-conjugative plasmid, pEa29, meaning that the isolates had to be spread physically. A non-conjugative plasmid cannot pass from one bacterial cell to another through conjugation, the non-sexual union between bacterial cells in which they exchange and share DNA on plasmids. The majority of the SR Ea isolates recovered from NY in 2012 seem to be from one of the two lineages documented in Michigan. By no means does this phylogenetic information imply that isolates came from Michigan to NY. Several isolates need additional investigation as they may be newly arisen. Furthermore, the limited number of samples obtained in 2012 precluded the ability to determine a pattern of distribution and spread of streptomycin-resistant fire blight in NY. Although the phylogenetic characterization of the 15 bacterial isolates is ongoing, this number of isolates in insufficient to determine a probable pattern of resistance spread with any level of certainty.

In 2011, the guidelines for managing fire blight were to use oxytetracycline in combination or rotation with streptomycin and to apply Apogee to reduce shoot blight susceptibility to reduce selection pressure for streptomycin resistance. The antibiotic kasugamycin (Kasumin) would give excellent blossom blight control of SR Ea as well as streptomycin-sensitive fire blight. Kasugamycin is in the pipeline for EPA Section 3 registration and it may be available in 2013. Fire blight best IPM practices were developed for control of fire blight without and with use of kasugamycin. For the "High Risk Areas" of Wayne, Ontario, Monroe, Orleans, and Niagara Counties, including sites with known populations of SR Ea, the IPM guidelines are as follows.

GUIDELINES FOR HIGH RISK AREAS (with confirmed SR Ea)

1. All fire blight cankers should be removed during winter pruning. Remove all trees with fire blight on the central leader or main trunk. Infected wood should be removed from the orchard.
2. Copper sprays should be applied at green tip.
3. CCE alerts and disease model forecasts for fire blight infection periods should be heeded, and suggested materials sprayed promptly.
4. Never apply streptomycin without another active ingredient effective against fire blight. When blossom infection is forecast, apply a tank mix of either
    a. oxytetracycline* in combination with streptomycin at highest labeled rates,
    b. or, the highest labeled rate of streptomycin in combination with a bloom time rate of a registered copper** product,
    c. or, kasugamycin (Kasumin), if registered.
5. Prohexadione-Calcium (Apogee) sprays should be applied at the highest labeled rate at 1-3 inches shoot growth. Apogee will not be effective if applied after you see fire blight symptoms.
6. Fire blight strikes should be pruned out promptly and destroyed.
7. If severe blossom blight occurs regardless of the timing of a streptomycin application, contact CCE for SR Ea testing, listed under "Sample Submission" below.
8. If you need to interplant apple trees in existing orchards where fire blight was observed; wait until late fall, so the bloom on the new trees will be synchronized with the established trees.
9. If fire blight symptoms appear, collect samples for streptomycin resistance screening so you can plan your management program. Contact CCE for SR Ea testing, listed under "Sample Submission" below.
10. No quarantine will be imposed if SR Ea is found in your orchard.

*Oxytetracycline must be applied before infection occurs. Therefore, monitor fire blight forecasts and heed CCE alerts carefully when using oxytetracycline. Data from university field research trials suggest that different formulations of the same antibiotic active ingredient may perform differently in the field. Consult with specialist before choosing the product for your operation.

**Copper must be applied before infection occurs. Therefore, monitor fire blight forecasts and heed CCE alerts carefully when using copper. Copper may cause fruit russet. Hydrated lime may be used to saften copper. An example would be Badge SC at rate of 0.75 to 1.75 pints /acre buffered with 1-3 lbs. of hydrated lime for every 2 pints of Badge to minimize fruit finish damage.

In addition to established orchards, fire blight IPM guidelines were developed for nurseries and new plantings. The key aspects of the fire blight IPM guidelines include emphasizing suitable scouting, sanitation and horticultural practices, and, in established orchards, the use of alternative antibiotics or safe rates of copper in lieu of or in tank mixture with streptomycin for blossom blight and Apogee for shoot blight, and, in new plantings and nurseries, safe rates of copper in lieu of or in tank mixture with streptomycin.

Based on independent grower participation with the survey efforts, the project was not as successful as we would have hoped, given that only eight of the submitted samples were from two growers and two consultants. However, grower cooperation with our survey efforts was successful. Forty-four farms participated in sampling for streptomycin-resistant fire blight. We were able to develop a reasonable estimate of the distribution of streptomycin-resistant fire blight in the Lake Ontario region of NY. However, the benchmark of 'ten fire blight samples submitted' was not achieved in the Lake Champlain region or in each of the Hudson Valley counties in the survey. Therefore, the 2012 data collected may not provide sufficient evidence for developing a reasonable perspective of distribution of SR Ea in these areas. The growing season was not very conducive for fire blight development across NY and this likely contributed to the low number of samples submitted. Our research laid the groundwork for developing the best IPM practices for managing streptomycin-resistant fire blight. Growers are interested in cooperating on the fire blight survey work and this bodes well for continuing to monitor for streptomycin resistance in fire blight in NY in the coming years.

Impact: New York ranks second in the nation in apple and fourth in pear production, with 42,000 acres of apples and 1200 acres of pears. Annual crop value (2011) was $227 million and $4.3 million, respectively. The outcomes from this project will benefit all NY growers with orchards of fire blight susceptible apple and pear varieties. Apple farms number 700 with 9.5 million trees and there are 255 pear farms with 150,000 trees. Since most varieties and rootstocks in existing and newly planted orchards are susceptible to fire blight, crop and tree losses would be severe in a normal year and catastrophic ($5-10M) in a year favorable for fire blight. Complete control of fire blight without streptomycin is nearly impossible. Therefore, everything we can do to improve the IPM guidelines for fire blight that will minimize the risk of streptomycin resistance developing in the bacterial pathogen will have a large impact in terms of tree longevity, crop yield, pesticide mitigation and environmental stewardship, given the size of the apple and pear industries in NY.

The intensive sampling and diagnostic work undertaken have resulted in better understanding of streptomycin resistance outbreaks. This has paved the way for improved fire blight management and prevention of antibiotic resistance. Efforts to curtail the spread and development of resistance will be beneficial by providing needed data and time for registration of an alternative antibiotic, kasugamycin, nearly as effective as streptomycin, currently in the pipeline for EPA Section 3 registration. Growers will benefit directly from detection of streptomycin-resistance in their orchards and the associated fire blight IPM education regarding managing fire blight in the face of antibiotic resistance.

Project location(s): The project took place in the three major apple-growing regions of NY. The IPM guidelines for fire blight are applicable in NY, the Northeast and the Great Lakes regions.

Samples of resources developed:

K. Cox, H. Aldwinckle, D. Breth, J. Carroll. 2012. 2013 Guidelines for Fire Blight Management in New York. 3 pp. - pdf

K. Cox, H. Aldwinckle, D. Breth, J. Carroll. 2012. Where is strep-resistant fire blight? Sample Submission Guidelines. 2 pp. - pdf

Photos - several photos of fire blight strikes, sample collection and summer technicians in the orchard are available from J. Carroll.