Fire blight, a devastating disease of apple and pear, is caused by the bacterium *Erwinia amylovora* which is present in the natural population of epiphytes in the orchard, but overwinters in cankers formed during the preceding season on apple and pear trees. In the spring, insects and rain can spread this bacterium from the cankers that ooze in warm weather to open flowers. *E. amylovora* colonizes and multiplies on the sugary stigma of open blossoms when temperatures exceed 65 °F. Infection occurs when the multiplied bacteria are washed into floral cup (hypanthium) and enter into the natural floral nectaries. Following infection, flowers will wilt over the course of 14 days then become blackened and necrotic (Figure 1A). If temperatures are warm and humidity is high, wilting blossoms may show signs of amber colored ooze, which is composed of *E. amylovora* (Figure 1B). The bacterial ooze resulting from blossom blight may be spread via insects, wind, and may cause infections in young shoot tissue when wounding occurs during insect feeding, hail, or wind storms. In addition, the bacteria can spread internally throughout the vascular system of the plant, and cause or initiate infections in susceptible rootstocks, resulting in death of the tree (Figure 2). Hence, controlling epiphytic populations of *E. amylovora* during bloom is essential for effectively managing this disease.

Current management practices for fire blight are focused on removing infection sources, such as cankers during winter and spring pruning, and also protecting blossoms with applications of bactericides at bloom. Streptomycin is the most effective and widely used bactericide for controlling blossom blight. Several other antibiotics, growth regulators, and biological control materials have been shown to be effective against blossom blight, particularly in the semi-arid apple production regions of the western United States. They are not nearly effective as streptomycin for controlling blossom blight in the wet temperate climates of NY and New England.

Although streptomycin is crucial for many apple growers managing fire blight in NY and New England, reports of streptomycin resistance throughout the 20th and 21st centuries raise concern.

"Strains of the Fire blight pathogen with streptomycin resistance have been found in orchards in 6 Western NY counties. However, there have been no devastating losses linked to these occurrences. To limit the appearance of streptomycin resistance, it is important that streptomycin be applied only for blossom blight in conjunction with disease forecast models, or after a hail storm."
about the sustainability of streptomycin use for fire blight management. Resistance to streptomycin was first discovered in California in 1971 followed by discoveries in Washington and Oregon in 1972. Streptomycin resistant E. amylovora are now widespread on the west coast and in certain areas of Michigan and Missouri (McManus, et al., 2002). Russo et al. (2002) published the first report of streptomycin resistance in New York, and this study described two bacterial isolates originating from two adjacent orchards with fire blight outbreaks in Wayne County. These orchards were promptly removed to prevent spread of resistant bacteria and routine surveys the following year showed no recovery of streptomycin resistant E. amylovora (Russo, et al., 2008; Russo and Aldwinckle, 2009).

There are two known mechanisms behind streptomycin resistance in E. amylovora. The first mechanism of resistance is the presence of a point mutation in the rpsL gene, which codes for the ribosomal protein S12 (Chiou and Jones, 1995). In a sensitive strain of E. amylovora, streptomycin binds S12 on the ribosome to block protein synthesis, killing the bacterium. The point mutation prevents the binding of streptomycin, making the antibiotic ineffective. This is the most common form of resistance in E. amylovora in the United States. Fortunately, strains with this type of resistance are rare in apple orchards east of the Mississippi (McManus, et al., 2002). A second mechanism of resistance is the presence of two streptomycin resistance genes, strA and strB, on transposable element on a plasmid specific to E. amylovora (Chiou and Jones, 1993). These genes encode for aminoglycoside phosphotransferase enzymes that confer resistance to streptomycin when expressed in E. amylovora (Chiou and Jones, 1995). The strA, strB gene pair was the basis of streptomycin resistance for isolates reported by Russo et al. in 2002. The most interesting part about this gene pair is the fact that it currently resides on a plasmid that cannot be transferred between strains, and as such, bacteria with the strA, strB gene pair must be physically moved to new locations, which underscores the importance of avoiding on-farm nursery operations for new propagative materials.

Since the first discovery in New York, surveys have been conducted for streptomycin resistant E. amylovora (SmR Ea) throughout the apple growing regions of the state. As a result of these surveys, several new isolates of streptomycin resistant E. amylovora were found in four additional apple production operations in western New York in 2011. Hence, it is apparent that SmR Ea is present in Western NY beyond the sites of original detection in 2002. Currently, little can be said about the origin of the new strains. Moreover, the prevalence of SmR Ea and the resulting threat to the NY apple industry is largely unknown. In order to address these concerns, intensive surveys were conducted in the last two years to detect and characterize SmR Ea in New York.

**Methods**

Suspected fire blight samples from shoots, trunks, and rootstocks were collected and sent to the NYSAES in Geneva, NY. Collections were a cooperative effort between NYSAES, LOFT, NYS-IPM and Eastern NY regional extension programs. Samples were cut into 2-3 cm long pieces near the margin of an actively growing lesion, surfaced sterilized in 10% Chlorox solution for ten minutes, and then rinsed in distilled water. Bark was removed from the samples and samples were cut into approximately 1 cm cambium sections. The sections were subsequently placed on Cross-Goodman (CG) media at 28°C for 2-5 days to promote bacterial growth. The resulting bacteria were streak plated on CG to obtain individual colonies. Colonies with a cratered appearance on CG were considered putative E. amylovora isolates. Isolates were grown in Luria Bertani broth liquid media and stored at -80°C for later genetic analysis.

Streptomycin resistance testing was completed using five different concentrations of 0, 50, 100, and 1000 ppm of streptomycin. Single colonies of the isolates were grown in 1 ml of LB broth for 24 hours. Approximately 100 ul of the solution was plated on LB media and allowed to dry. Subsequently, filter paper discs of each streptomycin concentration were placed on each plate. Sensitivity to streptomycin was evaluated by observing the zone of inhibition around streptomycin discs after 48 hours.

The identity of putative Ea isolates were confirmed by PCR. Each isolate was plated on CG to obtain single colonies for PCR-based identification. They were subsequently tested for the presence of pEa29, a ubiquitous non-conjugative plasmid known only to be found in E. amylovora, and the ans gene, which encodes an exopolysaccharide, and is found on the chromosome of E. amylovora. The primers developed by Bereswill et al. (1992) used for the PCR detection of pEa29, and the AMSK/AMSJ primer pair was used for detection of the ans gene (Bugert and Geider, 1995). Following confirmation that the SmR strains were indeed E. amylovora, additional genetic characterization was performed to test of the
mechanism imparting streptomycin resistance. The presence of strA and strB genes was determined by PCR the using primers and methodology developed by Chiou and Jones (1993). Additionally, we tested for the rpsL gene mutation using PCR as described by Chiou and Jones (1995) followed by traditional Sanger sequencing. Sanger sequencing was performed at the Cornell Biotechnology Resource Center, and resulting sequences were subject to bioinformatics analysis to screen for the point mutation.

Results And Discussion

One hundred and seventy five E. amylovora isolates were collected from 43 orchards in 2012, and 320 E. amylovora isolates were collected from 32 orchards in 2013. Collectively, 24 isolates from 16 orchards in 6 counties were found to be resistant to streptomycin (Figure 3). No streptomycin resistant isolates were found in the Hudson Valley region or other apple production areas in eastern New York. When characterized in terms of the mechanism of resistance it was found that 24 of these isolates contained the strA and strB genes. Interestingly, two isolates had the mutation in the rpsL gene. It is important to note that this is the first report of this resistance mechanism in New York. The point mutation in the rpsL gene fairly common in isolates from the western United States, but is rarely found in the eastern United States (McManus, et al., 2002).

Although the surveys prior to 2012 weren’t as extensive, it is likely that SmR Ea has become more widespread in New York since the first discovery in 2002. Currently, these strains are confined to western New York near the outbreaks of 2002 and 2011. Although 16 operations were found to have resistance, these are not necessarily the result of a streptomycin control failure. Indeed, none of the samples were from blossom blight infections following streptomycin use. Indeed, all samples were shoot blight infection during terminal elongation. At this time streptomycin is not used for fire blight management, unless there is a trauma event. In orchards where resistant isolates were detected in 2012, growers were instructed to use tank mixes of streptomycin and oxytetracycline in 2013. Surveying of these orchards in 2013 resulted only in the recovery of streptomycin sensitive E. amylovora (SmS Ea). However, it cannot be determined if our failure to recover SmR Ea was a result of tank-mixing products.

Currently, it is also unclear whether new occurrences of SmR Ea in newly reported orchards are a result of spread from a single point of origin or if they are the result of local selection or new introductions of SmR Ea infected material into New York. While the current work focuses on continued surveying of orchards for SmR Ea, we are also beginning to employ strain tracking methods for SmR Ea strains. Strain tracking will aid in determining the geographical origin of SmR Ea in sampled orchards. This effort is not purposed to be used as criticism, but rather to provide information on potential sources in hopes of improved management and screening of materials.

While many locations in New York have been shown to harbor streptomycin resistant E. amylovora, there have been no devastating losses linked to these occurrences. It will be imperative to continue surveying New York orchards to assess how SmR Ea may affect losses due to fire blight. Future use of streptomycin to manage fire blight in New York is inevitable due to the relatively reduced efficacy of other potential controls. Evaluating how streptomycin use in New York will affect E. amylovora populations and resistance will require further research. Based on surveys in the western United States it is evident that frequent and prolonged use of streptomycin is correlated with an increased incidence SmR

Figure 3. Map of showing the prevalence of streptomycin resistant strains of Erwinia amylovora by county. Counties colored red had more than 10 strains, counties colored orange had 5-10 strains, and counties colored yellow had less than 5 strains.
For now it is important that streptomycin be applied only when necessary (i.e. for blossom blight control in conjunction with disease forecast models) and used in an integrated manner with other approved control methods.

**Acknowledgements**

The authors would like to thank apple producers for assistance in identifying and collecting survey samples, and industry stakeholders for supporting and assisting with survey efforts. We would to acknowledge the funding support of this project provided by New York State IPM, New York State Department of Agriculture & Markets - Specialty Crop Block Grant Program, and the Apple Research and Development Program.

**Literature Cited**


Kiersten Bekoscke is a graduate student who works with Kerik Cox; Deborah Breth is a senior extension associate who leads the Lake Ontario regional extension team; Shirley Kuehne is a research support specialist who works with Herb Aldwinckle; Ewa Borejsza-Wysocka was a research support specialist who worked with Herb Aldwinckle; Herb Aldwinckle is a emeritus professor of plant pathology who led Cornell’s research program on fire blight. Sara Villani is a graduate student who works with Kerik Cox, and Kerik Cox is a research and extension professor of plant pathology who leads Cornell’s program in disease control of tree fruits and berries.