



Northern NY Agricultural Development Program 2018 Final Report

Continued Lab Detection of Fire Blight Bacterium *Erwinia amylovora* in Susceptible Apple Rootstocks in Commercial Apple Orchards Affected by 2016 Epidemic in Northern NY

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Collaborators:

- Dr. Ricardo Santander, Postdoctoral Associate, Acimovic Lab, HVRL
- Elizabeth Higgins, Business Management Specialist, HVRL

Cooperating Producers: Seven apple farms in NNY:

1. Forrence Orchards - Burrel

McIntosh Forrence
macforrence@msn.com
2731 Keeseville Rd
Peru NY 12972

3. Forrence Orchards - Valcour

Seth Forrence
forrencemac@aol.com
753 Telegraph Road
Peru NY 12972

5. Everett Orchards

Tom Everett
675 Calkins Rd.
Peru NY 12972

2. Forrence Orchards - Main

Mason Forrence
gaylewager@yahoo.com
2731 Rt22
Peru NY 12972

4. Hart Apple Farm LLC

Mr. Randy Hart
randy.hart1@gmail.com
2301 NY-22
Peru NY 12972

6. Northern Orchards

Jesse Mulbury
jam623@cornell.edu
537 Union rd. Peru NY 12972

7. Chazy Orchards Inc.

Mr. Jay Toohill

chazyorchards@westelcom.com

9486 U.S. 9

Chazy NY 12921

Background:

Fire blight is a devastating bacterial disease that infects apple flowers, shoots and sometimes entire trees, causing concern about the potential for economic damage to regional apple businesses. Unusually hot and humid weather with rains and hail at the end of apple bloom in 2016, favored serious fire blight epidemic in Northern New York (NNY). The epidemic caused severe losses in apple orchards, including yield reduction, tree death, and reduction of fruit budwood due to pruning removal of infections on mature trees. Losses in crop, trees and emergency management options amounted to more than \$14 million. Infection resulting in fire blight cankers on scaffolds and small diameter trunks and visible or latent fire blight infections of rootstocks led to rapid tree death.

Our previous research enabled detection of fire blight pathogen *E. amylovora* in rootstocks, aiming to help growers select infected trees to remove. The data confirmed that majority of young trees affected by the 2016 epidemic died due to rootstock fire blight infections. We detected *E. amylovora* in 76 - 94% of them. In contrast, on the same trees we sampled, we found much lower incidence of dead vs. alive trees of 0 - 35.4%, and of cankered vs. non-cankered rootstocks of 20.8 - 56.3%. Hence, we found a high presence of symptomless fire blight infections in the rootstocks.

In this project, our goal was to follow up this analysis and conduct rootstock tree sampling the second time in 2017, from the same orchards, and conduct one more round of detection of fire blight pathogen. This would help growers to determine if pathogen is still present in trees and select more trees for removal. Removal of infected trees and branches is important since it reduces the chance for successful overwintering of fire blight pathogen in cankers. Cankers are the main infection sources for fire blight epidemic renewal and spread in spring.

In 2017, the research team used infrastructure (on-farm monitoring, e-alerts and articles, and grower contacts and meetings) put in place by the NNYADP-funded precision apple management project the year before to help growers stay alert to fire blight. While 2017 saw less outbreaks of fire blight, significant tree losses continued to be reported. Although several reports of the infection in 2017 were identified as false alarms, in orchards where fire blight was confirmed present, growers effectively managed this devastating bacterial disease. Additional data collection is needed to detect regional and site-specific trends and to build a databank for calculating average emergence and activity of fire blight to help growers apply well-developed management strategies.

Methods:

In winter 2017-2018, we collected additional rootstock samples from the same or surrounding trees and sites sampled for the first time in 2016 on seven Northern New York apple farms listed above on pg. 1-2 badly hit with fire blight in 2016.

The project leader provided e-mail alerts with location-specific fire blight disease prediction interpretations based on RIMpro, which also provides an apple scab prediction model, and NEWA EIP fire blight model (Network for Environment and Weather Applications; Epiphytic Infection Protentional) with model use instructions, interpretations for each infection period, and pest management recommendations in 2018 to the participating and other NNY growers.

Notices to growers included the following Acimovic Lab Disease Management blogs:

1. We Continue HVRL & NY Apple Industry Partnership on Use of Disease Models in 2018: RIMpro (via RIMpro B.V.) and EIP (via Cornell's NEWA)
2. 2018 Spray Recommendations from Silver/Green Tip Onward
3. Copper Plus Oil Spray Cautions as Frosts Occurred This Week
4. It's Easy: View 15 min Video on How to Use RIMpro to Time Your Apple Scab Sprays
5. RainWise Weather Stations Need to Work Accurately for Models to Predict Scab Infections
6. Be Ready to Apply Fire Blight Sprays Based on Prediction Model Warnings (!);
2. Major Scab Infection is Coming 3-4 May
7. Fire Blight Spray Needed Today – 4 May 2018
8. Spray Strong: Apple Scab Infections Coming 10-13 May and Fire Blight Infections Possible Where Flowers Opened
9. Warning: Thunderstorm with Hail & Gusts Possible From 5 – 8 pm, 10 May
10. Spray Considerations Ahead of Rains Coming 15-16 May
11. Fire Blight Infection(s) Possible from 15-19 May (90% RH and Rain Coming)
12. First Cedar-Apple Rust Symptoms Visible at HVRL, Highland NY, & All Other Fruit Spray Recommendations and Mix Cautions
13. Scab Is Not Done Just Yet and Fire Blight Is a Threat Where Flowers are Still Open (May 20, 2018)
14. Strep Spray Requiring Fire Blight Risks Have and Will Continue to Occur at All East NY Locations – Where Flowers Open! 25 May 2018
15. Last Few Apple Scab Infection(s) on 31 May – 4 June; Fire Blight Visible in Hudson Valley Where Unsprayed
16. 2018 Primary Scab Season Over – However, Sprays to Continue If 2018 Scab Lesions Visible and Scab Was Issue Last Year; Fire Blight Visible; Start Thinking of SBFS
17. Warning: Hazardous Weather Conditions, Thunderstorm With Damaging Gusts Possible From 13-14 June – Concern for Fire Blight (!)
18. Warning II: Marginal Chance for Thunderstorm With Damaging Gusts Possible Tonight 18 June – Possible Concern for Fire Blight
19. Rain Comes Back: (I) Use NEWA SBFS Model, (II) Severe Thunderstorm Warning – Damaging Winds and Hail Possible – Fire Blight Risk (!)
20. Warning 27 July 2018: Thunderstorm, Strong Winds and Hail Possible in Southern Ulster County – Possible Concern for Fire Blight (!)
21. Warning 18 Jul 2018: Thunderstorm, Damaging Gusts, Hail Possible in Ulster, Dutchess, Greene, Columbia Counties – Concern for Fire Blight (!)
22. Warning – Updated 17 Jul 2018: Thunderstorm, Damaging Gusts, Hail Possible in Northeastern Ulster County 16 July – Concern for Fire Blight (!)
23. Warning: Thunderstorm, Damaging Gusts, Hail Possible in Northeastern Ulster &

- South-Central Greene County 14-15 July – Concern for Fire Blight (!)
24. Warning: Thunderstorm with Damaging Gusts and Hail Possible in Northeastern
Orange County 3-6 July – Concern for Fire Blight (!)

E-mail alerts with proprietary location-specific disease prediction interpretations from RIMpro fire blight model, with instructions and disease management recommendations to affected NNY growers who were RIMpro Subscribers that used this model in 2018:

- 22 E-mails titled: 2018 for ENY Apple FARM group RIMpro Scab Model Commentary and Interpretations

Results:

Section 1: PCR (Polymerase Chain Reaction) diagnostics in apple rootstocks for tree removal decisions in NNY

By using a molecular method targeting specific “fingerprint” DNA regions indicative of species called Polymerase Chain Reaction (PCR) we were able to detect *Erwinia amylovora* in rootstocks from infection foci on all seven farms sampled in fall 2016. The sampling procedure is explained in Figure 1. Positive presence of *E. amylovora* in apple rootstocks ranged from 11-52% samples depending on specific farm (Table 1).

The visual ratings of focus edge trees in fall 2017 revealed that on the majority of farms there is a much lower percent of dead trees in comparison to the positive PCR diagnosis percent, i.e., pathogen detections (Table 1). On all farms except on the Forrence - Main farm, where these percents were equal, this indicated the large presence of latent infections of *E. amylovora* in rootstocks.

When we visually rated the same sampled rootstocks in fall 2017 for presence/absence of fire blight cankers, which is a different rating parameter in comparison to tree death, on five farms, there were more fire blight cankers found on rootstocks than the positive PCR detections of *E. amylovora* in them. Only two farms, Forrence - Burrell and Everett Orchard had lower percent of visible cankers on rootstock in comparison to the percent of positive detections, again confirming on the presence of latent infections of *E. amylovora* in rootstocks.

This could be explained by any of the three possible scenarios, from the most likely to the least likely:

- (1) Due to the visual rating being done roughly one year after the PCR diagnosis, the incidence of cankers on rootstocks could have increased over time in relation to the PCR detections (in other words, more infections that PCR did not detect expressed over time),
- (2) *E. amylovora* was not detected in more rootstocks with visible cankers as the concentration of *E. amylovora* live or dead cells was too low for PCR to detect (it was below PCR sensitivity for diagnostics),
- (3) The higher percentage of cankers was caused by other plant pathogens due to rainy conditions in 2017 (e.g. *Phytophthora spp.* cause of Phytophthora crown & root rot).

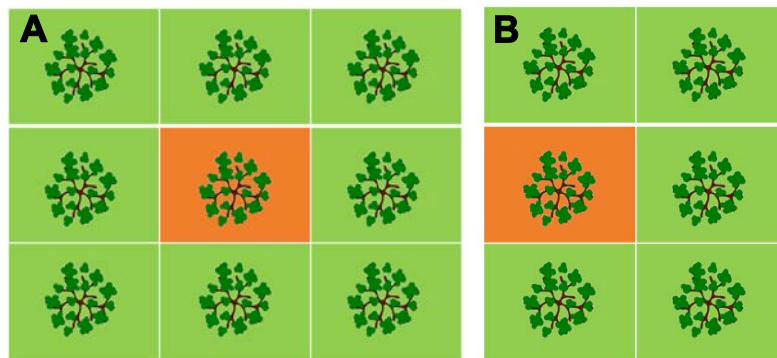


Figure 1. Rootstock sampling schematics for (A) complete fire blight infection foci inside the orchard consisting of one central and eight surrounding trees, and (B) partial fire blight infection foci on the orchard sides, consisting of one central and five surrounding trees. All the trees in each infection focus were sampled. Roughly 50 rootstock samples per each of seven farms were collected, making it around six infection foci selected and sampled per each farm. The central tree in the infection focus with orange background (A, B) was either dead, with visible fire blight strikes in the crown, or with visible fire blight canker on the rootstocks. We assumed that trees like this would be recognized and removed by farm staff. The trees surrounding the central tree are edge trees and have green background in the schematics.

Table 1. First round of PCR detection of *Erwinia amylovora* in 360 apple tree rootstock samples collected on 1 Nov 2016 with visual ratings of tree viability (live vs. dead) and rootstock health (canker present/absent) both conducted roughly a year later on 22 Oct 2017. PCR detection was done on both central tree and edge trees in each infection focus. Ratings of rootstocks and of the tree viability were only performed on edge trees in each focus as it was assumed that central trees would be removed by farm staff.

Apple farm with number of collected samples	1 Nov 2016		Edge trees in all foci	22 Oct 2017			
	PCR positive - <i>E. amylovora</i> detected (number of trees)	PCR negative - no pathogen detected (number of trees)		Edge trees dead	Edge trees alive	Edge rootstock cankered	Edge rootstock healthy
Northern Orchard (n=54)	35% (19)	65% (35)	n=48	2%	98%	56%	44%
Forrence Burrell (n=52)	52% (27)	48% (27)	n=45	4%	96%	26%	74%
Forrence Main (n=54)	35% (19)	65% (35)	n=48	35%	65%	42%	58%
Forrence Valcour (n=54)	33% (18)	67% (36)	n=48	6%	94%	40%	60%
Everett Orchard (n=63)	43% (27)	57% (36)	n=50	25%	75%	35%	65%
Chazy Orchard (n=55)	20% (11)	80% (44)	n=48	21%	79%	23%	77%
Hart Apple Farm (n=28)	11% (3)	89% (25)	n=24	0%	100%	21%	79%

One year after PCR diagnosis in fall 2016, namely in fall 2017, by following and rating the same trees, we detected that majority of the PCR positive trees and dead trees from fall 2016 were not removed by farm staff (Table 2) allowing us to sample rootstocks again in fall 2017 to repeat PCR diagnosis for *E. amylovora* presence in rootstocks. If in

some cases the edge trees in infection foci were removed, we would sample the ones next to them as explained in the Figure 2.

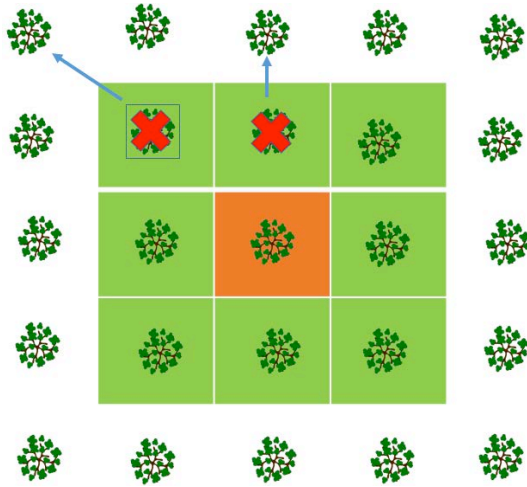


Figure 2. Rootstock sampling schematics on 16 Nov 2017 for the second round of PCR diagnosis for a complete fire blight infection focus inside the orchard consisting of one central and eight surrounding trees, where if any of the original foci edge trees was removed, the tree next to it would be sampled as indicated in the image.

Table 2. The tree removal status in sampled infection foci roughly one year after rootstock sampling and PCR diagnosis for fire blight infection. Note that the PCR positive detections of *E. amylovora* in Table 1 above (orange highlighted) consisted of different number of Ea-positive non-removed trees and dead trees in Table 2.

Apple farm with number of collected samples	22 Oct 2017		
	<i>Ea</i> -positive non-removed trees	<i>Ea</i> -positive removed trees	Dead trees
Northern Orchard (n=54)	14	0	7
Forrence Burrell (n=52)	25	0	7
Forrence Main (n=54)	8	0	22
Forrence Valcour (n=54)	14	0	8
Everett Orchards (n=63)	14	0	16
Chazy Orchard (n=55)	5	0	16
Hart Apple Farm (n=28)	2	0	3 (central)

In the second round of PCR diagnosis on samples collected on 16 Nov 2017, we could not detect fire blight pathogen in rootstocks (Table 3). This could be explained by several possible scenarios:

- (1) *E. amylovora* cells died over time in wood and the DNA needed for detection of pathogen degraded in wood beyond possibility for PCR detection,
- (2) Due to environmental factors, the concentration of live cells of this pathogen

decreased below the PCR sensitivity level for positive diagnostics so it was not detected even if present in rootstocks, or

- (3) Due to bacterial pathogen presence, the natural plant defenses, namely systemic-acquired resistance aka SAR, could have been triggered and cumulatively increased over time in rootstock wood leading to *E. amylovora* death and reduction of cell concentration and DNA degradation below level of PCR detection.

Roughly a year after, namely on 22 Sep 2018, when we rated trees for tree death and rootstock canker presence/absence (Table 3), we detected very little change in symptoms (only on one farm, Everett Orchards, was there slightly more dead trees in comparison to 22 Oct 2017 in Table 1).

Table 3. Second round of PCR detection of *Erwinia amylovora* in 360 apple tree rootstock samples collected on 16 Nov 2017 with visual ratings of tree viability (live vs. dead) and rootstock health (canker present/absent) both conducted roughly a year later on 22 Sep 2018. PCR detection was done on both central tree and edge trees in each infection focus. Ratings of rootstocks and of the tree viability were only performed on edge trees in each focus as it was assumed that central trees would be removed by farm staff. If any of the original foci edge trees was removed, the tree next to it would be sampled as indicated in the Fig. 2 above.

Apple farm with number of collected samples	16 Nov 2017		Edge trees in all foci	22 Sep 2018			
	PCR positive - <i>E. amylovora</i> detected (number of trees)	PCR negative - no pathogen detected (number of trees)		Edge trees dead	Edge trees alive	Edge rootstock cankered	Edge rootstock healthy
Northern Orchard (n=54)	0%	100%	n=48	2%	98%	56%	44%
Forrence Burrell (n=52)	0%	100%	n=45	4%	96%	26%	74%
Forrence Main (n=54)	0%	100%	n=48	35%	65%	42%	58%
Forrence Valcour (n=54)	0%	100%	n=48	6%	94%	40%	60%
Everett Orchard (n=63)	0%	100%	n=50	27%	73%	35%	65%
Chazy Orchard (n=55)	0%	100%	n=48	21%	79%	23%	77%
Hart Apple Farm (n=28)	0%	100%	n=24	0%	100%	21%	79%

One year after the PCR diagnosis in fall 2017, namely on 22 Sep 2018, by following and rating largely the same trees, due to no detection of *E. amylovora* by PCR in rootstocks (Table 3) we speculate that the previously *Ea*-positive non-removed trees, i.e., rootstocks, were no longer infected with this pathogen with reservation. There are two possibilities that can occur and why we claim this reservation:

- (1) *E. amylovora* cell populations naturally decline in plant tissues over time as cells die due to unfavorable environmental conditions and accumulating plant defense responses, but their DNA can still remain in the plant and be detected by PCR if it is present in an undegraded state and at a concentration of or above 1000 DNA copies per unit of tissue weight (the PCR detection limit) (EPPO 2013). But in that case, the PCR would give a false positive detection

for actually dead cells of the pathogen with just DNA present in the plant tissue.

In our case we did not get positive detection at all in year two samples, and, in that case, this can mean that trees are no longer infected with present live cells of *E. amylovora*, i.e., that DNA from dead cells is degraded due to considerable time that passed and PCR cannot amplify it; and

- (2) Since the number of live pathogen cells after the infection of the tissue naturally declines over time, and this decline can go below the limit of detection of PCR (minimum of 1000 cells present in the tissue), live populations of bacteria in rootstocks either:
 - (a) might not be present due to complete population die-off or
 - (b) they are present and alive but are at a very low number, which is beyond the detection capability of PCR to find them (pathogen is present at numbers below 1000 live cells per tissue weight).

This is why the PCR results of pathogen detection presence must be taken with reservation as some limited number of trees might still have a low concentration of *E. amylovora* which PCR did not detect since they were below the limit of positive detection (Table 4).

Knowing that we had plant samples that are older and that we probably had low bacterial concentrations in them, to improve the sensitivity of PCR to 100-1000 cells (i.e., to lower the limit of detection) we performed an enrichment step for all samples in year two in CCT medium as per instructions in European and Mediterranean Plant Protection Organization (EPPO) 2013 standards PM 7/20 for *E. amylovora* Diagnostics, Bulletin. 43:21–45. Since we used this last option i.e., enrichment step for rootstock samples collected in year two, before we did PCR, and we did not detect pathogen in any of the samples, we are speculating that the previously *Ea*-positive non-removed trees, i.e., rootstocks were no longer infected with this pathogen. The probability is very low for pathogen to be present in them.

We detected more tree removal by farm staff on 22 Sep 2018 (Table 4) in comparison to 22 Oct 2017, with only four farms removing almost all dead trees (in bold cursive and underline in Table 4). On some farms we detected several new tree deaths, possibly indicating an expression of pathogen from latently infected rootstocks (Table 4).

Table 4. The tree removal status on 22 Sep 2018, with data from 22 Oct 2017 for comparison, in sampled infection foci roughly one year after rootstock sampling on 16 Nov 2017 and PCR diagnosis.

Apple farm with number of collected samples	22 Oct 2017		22 Sep 2018		
	<i>Ea</i> -positive non-removed	Dead trees	<i>Ea</i> -positive non-removed	Removed trees	Dead trees

	trees		trees		
Northern Orchard (n=54)	14	7	0	1	6
Forrence Burrell (n=52)	25	<u>7</u>	0	<u>7</u>	1 (new)
Forrence Main (n=54)	8	<u>22</u>	0	<u>21</u>	2 (new)
Forrence Valcour (n=54)	14	8	0	4	2 (new)
Everett Orchards (n=63)	14	16	0	6	1 (new)
Chazy Orchard (n=55)	5	<u>16</u>	0	<u>16</u>	0
Hart Apple Farm (n=28)	2	<u>3</u> (central)	0	<u>3</u>	0

Section 2: Identification and characterization of *Erwinia amylovora* strains from rootstocks from Champlain and Hudson valleys and comparison to other NY strains

Besides PCR detection, more necessary identification assays were performed to confirm *Erwinia amylovora* in rootstock samples. We isolated bacteria from more than several rootstocks and sampled farms. Out of a group of many *E. amylovora* isolates we selected 12 (Table 5) for further identification and characterization to increase our understanding of why the rootstock infections were so dominant after the fire blight epidemic in Champlain Valley in 2016.

By characterizing the isolates, we can learn their virulence, which can differ among strains of this pathogen, thus explaining the ability to infect on a large scale and be devastating, as with the epidemic in the Lake Champlain Valley. It has been shown before that usually only several dominant *Erwinia amylovora* strains are involved in fire blight epidemics in the USA (Zeng et al. 2017: Comparative genomics of Spiraeoideae infecting *Erwinia amylovora* strains provides novel insight to genetic diversity and identifies the genetic basis of a low-virulence strain, Molecular Plant Pathology: <https://doi-org.proxy.library.cornell.edu/10.1111/mpp.12647>).

It is important to characterize strains to explain the disease incidence. Characterization of strains can also inform us about susceptibility/resistance to streptomycin or copper bactericides which gives insight into how effective are the past, current, and future disease management practices used in this region.

Isolation of *E. amylovora* from apple samples and strain selection for further identification and characterization

- Most of the isolates come from apple trees showing typical fire blight symptoms (cankers, necrosis and/or exudate).

Table 5. *E. amylovora* isolates stored at HVRL and used in identification characterization. Isolates in dark red rectangle boxes originated from the seven sampled farms from the Lake Champlain Valley participating in this NNYADP-funded project.

1 CFBP 1430	Type strain	25 CM26c1-D7	Apple cv. Cortland (canker); Peru, NY
5 Ea88-100	Type strain (SmR)	26 CM26c1-D9	Apple cv. Cortland (canker); Peru, NY
6 ATCC 49946	Type strain	27 CM26c2-A1	Apple cv. Cortland (canker); Peru, NY
7 Deborah Breth (DB)	Koru, Orchard Dale Farms, Waterport, NY	28 CM26c3-A1	Apple cv. Cortland (canker); Peru, NY
8 DB	Koru, Or. Dale Farms, Waterport, NY	29 CM26c3-D5	Apple cv. Cortland (canker); Peru, NY
9 DB2	Koru, Or. Dale Farms, Waterport, NY	30 HM9c2-1	Apple cv. Honeycrisp (canker); Peru, NY
10 DB3	Koru, Or. Dale Farms, Waterport, NY	31 HM9c3-1	Apple cv. Honeycrisp (canker); Peru, NY
11 DB8	Koru, Or. Dale Farms, Waterport, NY	32 RCM9r1	Apple cv. Royal Cortland (rootstock; M9)
12 ODF7	Apple cv. Koru, Orchard Dale Farms, Waterport, NY	33 F19.6r1	Apple cv. Royal Cortland (rootstock; M9)
13 ODF8	Apple cv. Koru, Orchard Dale Farms, Waterport, NY	34 Ts-1	Apple cv. Tyderman (shoot); CB, Campbell Hall, NY
14 ODF11	Apple cv. Koru, Orchard Dale Farms, Waterport, NY	35 RFs-1	Apple cv. Ruby Frost (shoot); CB, Walden, NY
15 ODF12	Apple cv. Koru, Orchard Dale Farms, Waterport, NY	36 JMs-1	Apple cv. Jonamac (shoot); CB, Campbell Hall, NY
16 ODF13	Apple cv. Koru, Orchard Dale Farms, Waterport, NY	37 RDs-1	Apple cv. Red Delicious (shoot); CB, Campbell Hall, NY
17 ODF14	Apple cv. Koru, Orchard Dale Farms, Waterport, NY	38 GGs-1	Apple cv. Ginger Gold (shoot); CB, Campbell Hall, NY
18 Crist Bros (CB) 15	Royal Red Honeycrisp (Pajam 2); Walden, NY	39 HCs-1	Apple cv. Royal Red (Honeycrisp) (shoot); Walden, NY
19 CB 17	Royal Red Honeycrisp (Pajam 2); Walden, NY	40 HVRLs-1	Apple cv. Unknown (shoot); CB, Walden, NY
20 Red Crabapple 13	Tennessee (nursery), Lowes	41 SDs-1	Apple cv. Snapdragon NY-1 (shoot); Indian Lader Farm, NY
21 White Crabapple 123	Tennessee (nursery), Lowes	42 SDs-2	Apple cv. Snapdragon NY-1 (shoot); Indian Lader Farm, NY
22 CM9c2-1	Apple cv. Cortland (canker); Peru, NY	43 SDs-3	Apple cv. Snapdragon NY-1 (shoot); Walden, NY
23 CM9c3-D7	Apple cv. Cortland (canker); Peru, NY	R2 HCr-1	Apple cv. Honeycrisp (rootstock); CB, Schoonmaker Farm, NY
24 CM26c1-A1	Apple cv. Cortland (canker); Peru, NY	K HCr-1	Apple cv. Honeycrisp (canker); CB, NY

* The first 4 strains are reference strains used for comparative purposes

- Isolation was conducted according to the EPPO standards PM 7/20 for the isolation of *E. amylovora* from plant material (EPPO, 2013). Briefly, plant material showing symptoms is selected, weighted and processed by hammering inside of a plastic bag containing antioxidant maceration buffer (AMB) in a ratio 1:50 (w/v). The resulting plant macerates and serial tenfold dilutions of plant macerates are spread-plated on KB, SNA and/or CCT, the *E. amylovora*-like colonies are selected and the bacterial species is confirmed by PCR (2 regular PCRs, also by dPCR) and pathogenicity tests on immature fruits, shoots, or leaves.

A total of 39 isolates were obtained from different apple varieties (cv) and/or different locations in NY State (Table 5).

- Part of the isolates were obtained using a selective medium developed at the Plant Pathology Lab at the HVRL (Fig. 3.).

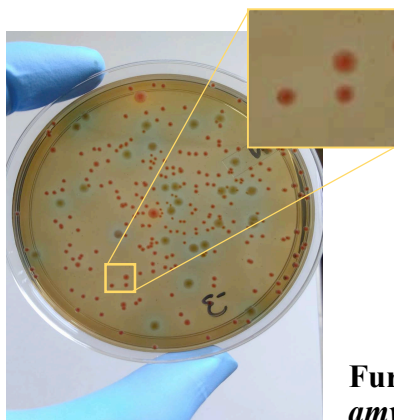


Figure 3. *E. amylovora* morphology in a new selective-differential medium being developed at Cornell's HVRL Plant Pathology Lab.

Further identification and characterization of *E. amylovora* isolates from rootstocks and comparison to other isolates and referent strains of this pathogen

- All the isolates carried the almost ubiquitous plasmid pEA29, detected by a species-specific PCR (Bereswill *et al.*, 1992) (Fig. 4).



Figure 4. Positive detection of plasmid pEA29 in different *E. amylovora* isolates. Amplicon size 900-1100 bp (Bereswill et al., 1992: Sensitive and species-specific detection of *Erwinia amylovora* by polymerase chain reaction analysis, Applied and Environmental Microbiology, Nov, 58(11):3522-6.

- All the strains were sensitive to Streptomycin (50–100 µg/mL).
- All the strains were pathogenic on apple (cv. Cortland) and pear (cv. Bartlett) leaves, and pear slices (cv. Bartlett) (Fig. 5).

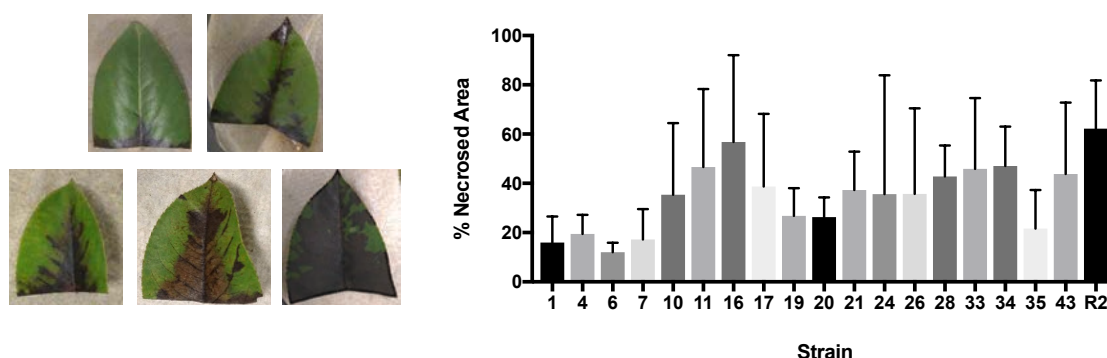


Figure 5. Different degrees of leaf blight symptoms (pear cv. Bartlett) (left) and virulence analysis of a representative number of *E. amylovora* NY isolates.

- The strains showed variable EPS (exopolysaccharide) production on KB agar (King et al., 1954) (Fig. 6).
- Some of the isolates were unable to grow on RESC medium (KB amended with 1.5 mM CuSO₄) (Ordax et al., 2012, Improved recovery of *Erwinia amylovora*-stressed cells from pome fruit on RESC, a simple, rapid and differential medium, Trees, Volume 26, Issue 1, pp 83–93) (Fig. 6).
- Although Copper (Cu) is a powerful bactericide, some media for the *E. amylovora* isolation contain sublethal Cu concentrations, enhancing a characteristic and differential ooze production, yellow pigmentation, acting as a selective agent against other bacteria, and favoring the recovery of stressed cells (Bereswill et al., 1998; Ordax et al., 2012).

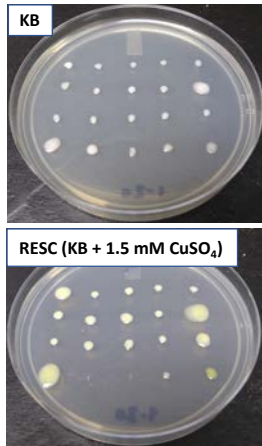


Figure 6. Variable EPS production, pigmentation and Cu sensitivity on KB and RESC medium.

- The analyzed strains also showed different amplicon sizes of the spacer arrays CR1 and 2, from the CRISPR locus (Fig. 7, 8). CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats are short DNA repeat sequences, separated by non-repetitive spacer sequences which in combination with Cas proteins are thought to work as an adaptive immune system against invading DNA.

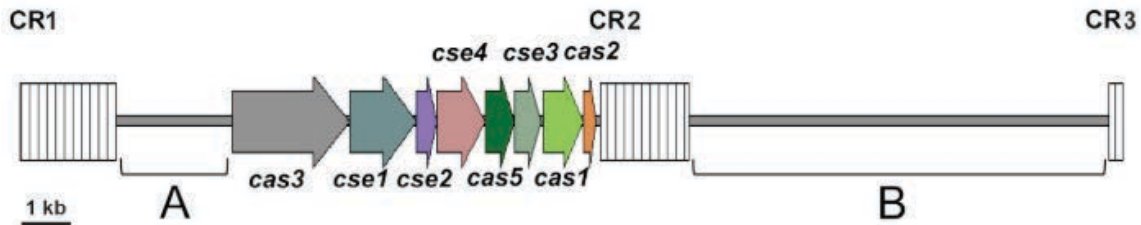


Figure 7. Genetic map of the CRISPR locus of *E. amylovora* ATCC 49946 showing the location of *cas* and *cse* genes and the spacer arrays CR1, CR2, and CR3. Sequences denoted by brackets and designated “A” and “B” contain housekeeping genes apparently unrelated to CRISPR function (McGhee et al., 2012).

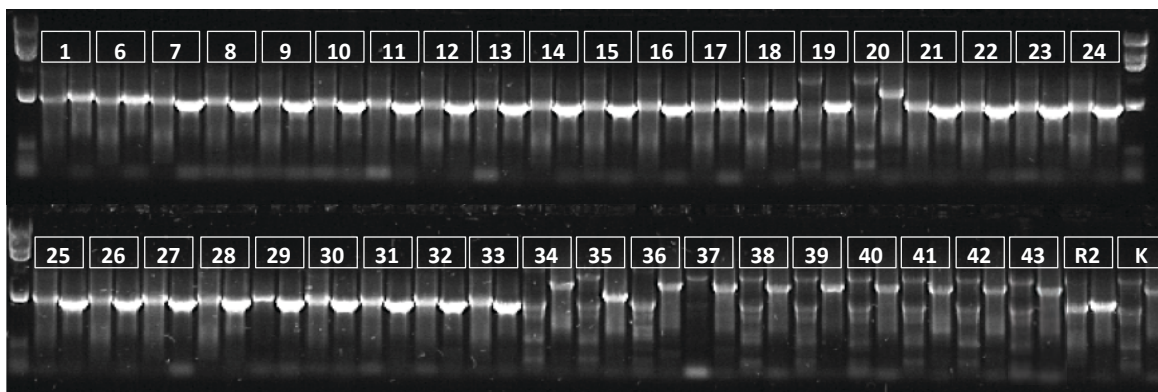


Fig. 8. PCR amplification of the CRISPR spacer arrays CR 1 and 2. Each number represents a strain, and the amplicons on the left and the right, show spacers CR1 and CR2, respectively.

- A total of 18-23 isolates showing different profiles of CRISPR spacers CR1 and CR2 and/or EPS production, copper sensitivity or virulence were selected for further characterization.
- Most of the strains (>71%) showed positive reaction for the Voges-Proskauer (VP)

test (production of acetoin as a sub-product of glucose digestion), fermentation of Glucose (Glu), Mannitol (Man), Sorbitol (Sor), and Sucrose (Sac). About 48% and 14% of the strains were additionally able to use arabinose (Ara) and Inositol (Ino) as carbon sources, respectively. About 29% of the strains were positive for gelatin hydrolysis (Gel) and just 5% of the strains possessed arginine dihydrolase activity (ADH) (Fig. 9).

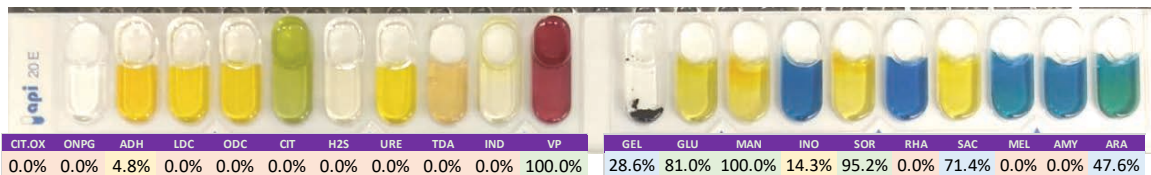


Figure 9. Different profiles of the *E. amylovora* isolates on API 20E Strips. Main differences found on Arginine dihydrolase activity (ADH), gelatinase activity (GEL) and fermentation of Inositol (INO), sucrose (SAC) and Arabinose (ARA).

- The selected strains showed variable production of the EPS amylovoran and levan (Fig. 10; Table 6).

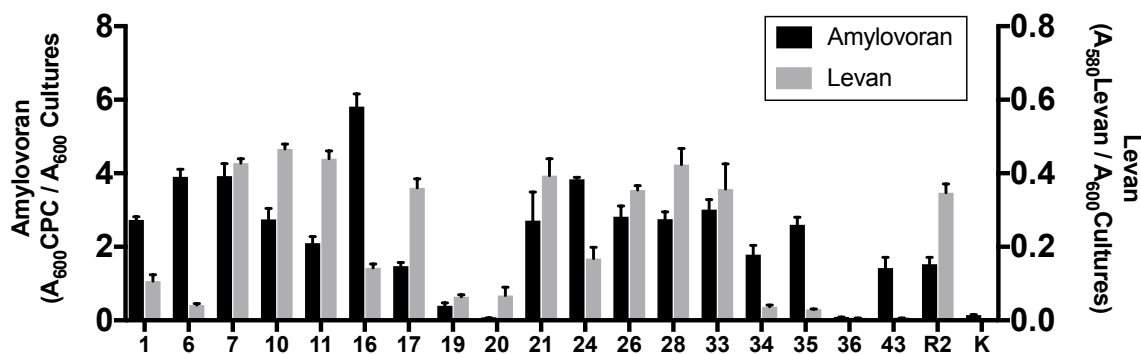


Fig. 10. Relative amylovoran and levan quantification in overnight cultures grown at 28°C in MBMANic and NB liquid media, respectively, by a procedure described elsewhere (Santander et al., 2014).

Table 6. Classification of the *E. amylovora* isolates into low, intermediate and high EPS producers.

AMYLOVORAN										LEVAN										
Low producers [0-2]										Low producers [0-0.2]										
20	36	K	19	43	17	R2	34			K	36	43	35	34	6	19	20	1	16	24
Intermediate [2-4]										Intermediate [0.2-0.4]										
11	35	21	1	10	28	26	33	24	6	7	R2	26	33	17	21					
High producers [4-6]										High producers [0.4-0.6]										
16										28	7	11	10							

- Based on preliminary results (see Fig. 6), some of the *E. amylovora* isolates showed a characteristic Cu sensitivity at concentrations usually employed in selective/differential media for the isolation of the pathogen. To quantify Cu sensitivity, we compared the ability to form colonies of the different isolates on KB and KB amended with 1.5, 2.5, 3.5 and 5 mM CuSO₄ (Fig. 11; Table 7).

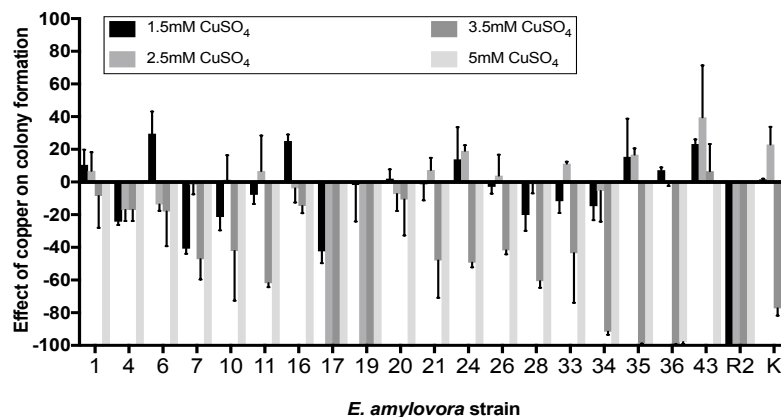


Figure 11. Effect of CuSO₄ on *E. amylovora* CFU numbers on KB. The percentage of growth enhancement (positive values) or inhibition (negative values) was calculated as:

$$((KB_{CFU} - KB_{CuCFU})/KB_{CFU}) * 100.$$

Table 7. Percentage of *E. amylovora* isolates showing Cu-induced growth enhancement or inhibition.

	[Cu]			
	1.5 mM	2.5 mM	3.5 mM	5 mM
% <i>E. amylovora</i> strains showing Cu-induced growth enhancement	42.86	47.62	4.76	0.00
% <i>E. amylovora</i> strains showing Cu-induced growth inhibition	57.14	52.38	95.24	100.00
Average % of growth enhancement	15.42	13.15	5.81	0.00
Average % of growth Inhibition %	-24.25	-32.04	-56.77	-99.98

- Copper had a partial or total inhibitory effect on *E. amylovora* growth in most of the tested strains. On average, this effect was stronger than the growth enhancement observed in some of the tested strains.
- EPS have been described as an indirect mechanism for the protection of bacteria against Cu. The exposure of the isolates to sublethal CuSO₄ concentrations induced EPS production (Fig. 10). Strains that were particularly sensitive to Cu (R2, 17, 19) (Fig. 11) showed reduced amylovoran production but similar or higher EPS production (Fig. 10, Table 6) than other strains that showed a better tolerance to Cu (e.g. 6, 16, 43) (Fig. 10).

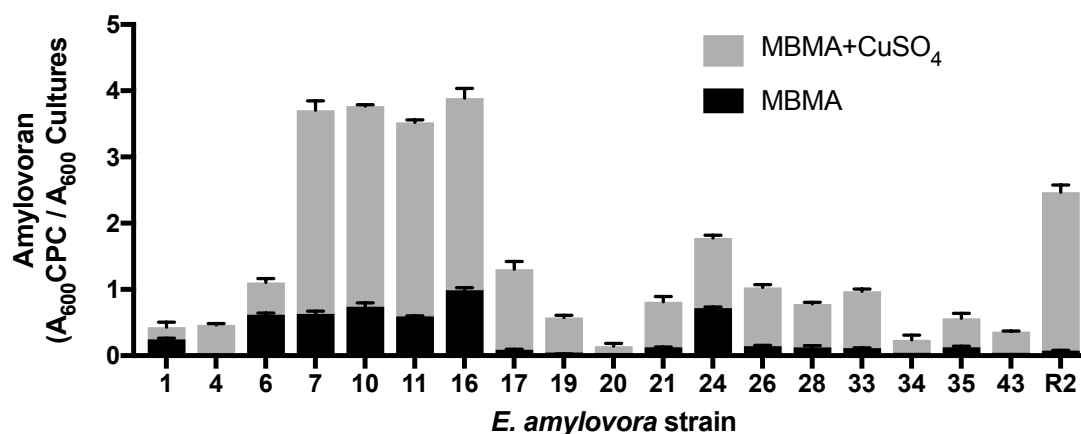


Figure 12. Amylovoran induction by 1.5 mM CuSO₄ (0.0117 mM in the case of strain R2).

- These results suggest that amylovoran could potentially contribute to the protection against Cu. However, other strains classified as low amylovoran producers (e.g., 36 and 43) based on Table 7 showed a great copper resistance compared to the other low amylovoran producers. This apparent weak correlation was absent in the case of levan, although a deeper analysis is required to demonstrate this hypothesis.
- Based on Table 7, all the strains that are very sensitive to copper are also low amylovoran producers (but not all the low amylovoran producers are copper sensitive).

SECTION 3. Present more of the two-year data results to apple growers and scientific communities

- We will be writing two articles for publishing in either *Plant Health Progress* or *Fruit Quarterly* (first) and in *Plant Disease* (second). We will present the data from this project (PCR data from 2017 and ratings from 2018) at the 2019 Eastern NY Fruit and Vegetable Conference on Feb 19, 2019 at the Desmond Hotel in Albany.

Conclusions/Outcomes/Impacts:

Our data show that the majority of young NNY orchard trees affected by the 2016 epidemic died due to rootstock fire blight infections and that fire blight pathogen strains differed widely in virulence and biochemical characteristics. This indicated that there is quite a diversity in strains in growers' orchards. This data will guide future projects that aim to improve fire blight management recommendations in terms of strain differences.

We provided the growers with results of our *E. amylovora* detection in rootstocks as a guide for them to select infected trees to remove. Our project data served as a disease management tool for the reduction of inoculum sources and helped determine which orchard blocks need tree re-planting.

State-of-the art fire blight management recommendations provided through Acimovic Lab blogs and e-mails with disease predictions and model interpretations (RIMpro, NEWA's Marybly/EIP) helped growers make educated, science-based management decisions and encouraged IPM on their farms by applying pesticides only when pathogen infections are possible.

The impact of this research on harvest/economics is depicted in the fact that growers did not have any new fire blight epidemics in 2017 and 2018 in the same or nearby orchards nor new tree and fruit losses as in 2016 from fire blight. Their fruit harvests and the fact that there were no fruit losses on their farms in 2017 and 2018 are the result of disease management recommendations for fire blight and apple scab made available through Acimovic Lab blogs and e-mails based on disease model prediction interpretations from models like RIMpro and NEWA's Marybly/EIP. Hence, through these blogs, fire blight disease predictions from the proprietary RIMpro model were made available free of charge to all the Champlain Valley apple growers.

The Acimovic Lab organized three growers (one in Chazy, two in Peru) in the Champlain Valley to subscribe to RIMpro to obtain the model outputs to support fire blight spray warnings to all growers in this region via Acimovic Lab Blogs.

Outreach:

The project leader S. G. Ćimović spoke at Extension educational meetings and published and distributed the following instruction tutorials, handouts, articles, instructional videos and surveys for fire blight prediction model use, interpretation, and management recommendations for fire blight in 2018:

Talks at Petal Fall Grower Meetings:

- Champlain Valley Thinning Meeting, Peru, NY: Current Status on Fruit Tree Diseases and Future Disease Model Predictions, 46 participants
- Capital Region Petal Fall Meeting, Bowman Orchards, Rexford, NY: Current Status on Fruit Tree Diseases and Future Disease Model Predictions, 23 participants
- Northern Hudson Valley Thinning Meeting, Columbia-Greene Cornell Cooperative Extension, Hudson, NY: Current Status on Fruit Tree Diseases and Future Disease Model Predictions, 24 participants
- Southern Hudson Valley Thinning Meeting, DuBois Farms. Highland, NY: Current Status on Fruit Tree Diseases and Future Disease Model Predictions, 42 participants

Tutorials:

- Tutorial #1. How to create account in RIMpro cloud service and join ENYApple Farm User Group. Ćimovic Lab, Vol. 2017 Growing Season, pg. 1-10.
- Tutorial #2. BIOFIX parameters settings in RIMpro cloud service. Ćimovic Lab, Vol. 2017 Growing Season, pg. 1-2.
- Tutorial #3. Find Your and Other NEWA Stations in ENYApple Farm User Group and Their Apple Scab Predictions in RIMpro. Ćimovic Lab, Vol. 2017 Growing Season, pg. 1-2.
- Tutorial #4. Accessing fire blight RIMpro graphs on other NEWA stations than your own in ENY apple farm user group in RIMpro. Ćimovic Lab, Vol. 2017 Growing Season, pg. 1-6.

Handouts:

- Handout for thinning meeting 2018 – Champlain Lake Valley. Ćimovic Lab. Vol. 2018 Growing Season, pg. 1-2.
- Handout for thinning meeting 2018 – Capitol Region. Ćimovic Lab. Vol. 2018 Growing Season, pg. 1-2.
- Handout for thinning meeting 2018 – South and North Hudson Valley. Ćimovic Lab. Vol. 2018 Growing Season, pg. 1-2.

Instructional Videos and Surveys:

- Ćimović, S. G., Jentsch, P. (2018). Show and Tell: How to Use RIMpro Apple Scab Model. Ćimovic Lab. Vol. 2017 Growing Season, 15 min.
- Ćimović, S. G. (2018) Current Status on Fruit Tree Diseases and Future Disease Model Predictions. Talk at The Champlain Valley Thinning Meeting, Peru, NY, 25 May 2018, 29 min.
- Ćimović, S. G., Rosenberger D. A., RIMPRO USE SURVEY FOR 2017 – HVRL PARTNERSHIP. Ćimovic Lab. Vol. 2017 Growing Season, pg. 1-2.

Articles on Fire Blight Management:

- S. G. Ćimović, D. A. Rosenberger (2018): An Introduction to the RIMpro Apple Scab Prediction Model. Scaffolds Fruit Journal, Vol. 27, No. 5, 23 April 2018.
- S. G. Ćimović (2018): Rootstock Blight - The Killer of Trees. Tree Fruit News, Vol. 6, Issue 2, pp. 3-4. May 3. Cornell Cooperative Extension - Eastern NY Commercial Horticulture Program.

The project leader S.G. Ćimović and his research team made the following presentations at regional grower meetings and published the following fact sheets:

January 10, 2018: 37th Annual Long Island Agricultural Forum, Riverhead, NY”

- “Apple Rootstock Infections After 2016 Fire Blight Epidemic in Northern New York and Efficacy of Low-rate Coppers, Biologicals and Regalia & SDHI Fungicides in Apple Scab Efficacy Trial with Widespread Max and LI700,” 45-min. presentation, 29 fruit growers, nursery owners, extension specialists, scientists, and private fruit pest consultants for apple growers

February 20-22: 2018 Eastern New York Fruit and Vegetable Conference (formerly Fruit Schools), Albany, NY:

- "Evaluation of Blossom and Shoot Blight Control with Different Copper Formulations, Apogee, Actigard, and Newer Biologicals," 45-min. presentation for fruit growers, nursery owners, extension specialists, scientists, and private fruit pest consultants for apple growers, 300 participants
- “Asymptomatic Fire Blight Infections of Apple Rootstocks After 2016 Epidemic in NE New York and Implications for Apple Growers in NY Champlain and Hudson Valleys,” 30-min. presentation, 120 participants
- “Biology of the Fire Blight and Virulence,” 45-minute presentation, 300 participants
- “Managing Fire Blight: A Cost/Benefit Analysis,” 30-min. presentation, 120 participants
- “Crop Insurance,” 30-min. presentation, 300 participants

March 16, 2018: Hudson Valley Research Lab Annual Members Meeting, Highland NY:

- “An Overview of Plant Pathology Program at HVRL,” 25-min. presentation, 23 fruit growers, extension specialists, scientists, and private fruit pest consultants

July 10: 2018 Annual Summer Meeting of the Massachusetts Fruit Growers’ Association, Belchertown, MA:

- "New Rules for Apple Scab and Fire Blight," 45-min. presentation, 120 growers, nursery owners, plant health company representatives, extension specialists, scientists, and private fruit pest consultants for apple growers

December 12, 2018: Cornell AgriTech New York State Apple Research and Development Program Reporting Session, Geneva, NY:

- “Continued Lab Detection of Fire Blight Bacterium *Erwinia amylovora* in Susceptible Apple Rootstocks in NY Orchards and HVRL Efficacy Trials” and “Characterization of *E. amylovora* strains from NY State,” 40 min. presentation

January 29, 2019: Cornell Hudson Valley Research Lab Apple Forum, Highland NY:

- “Multifaceted Extension Support to the Eastern NY Apple Industry, Conducting and Publishing Funded Programmatic Research & Running Plant Disease Efficacy Trials – Focus on Where the Problems Are,” 45-min. presentation

Fire Blight Fact Sheet:

S. G. Acimović (2018): Fire Blight Disease Fact Sheet, Cornell University, Hudson Valley Research Laboratory, Highland, NY: <https://cpb-us-e1.wpmucdn.com/blogs.cornell.edu/dist/7/7077/files/2017/02/Fire-Blight-Disease-Fact-Sheet-Acimovic-2018-z0a739.pdf>

Acimovic Lab Blog Fire Blight Postings:

- Jan-Mar 2018: 1,871 page views, 850 visits, 365 unique visitors
- Mar-Jun 2018: reached up to 3000 visitors
- June 1, 2017-June 30, 2018: 2,189 unique visitors, 13,624 blog views (Fig. 1)
- July-Sept 2018: 2,190 page views, 1299 visits, and 691 unique visitors.

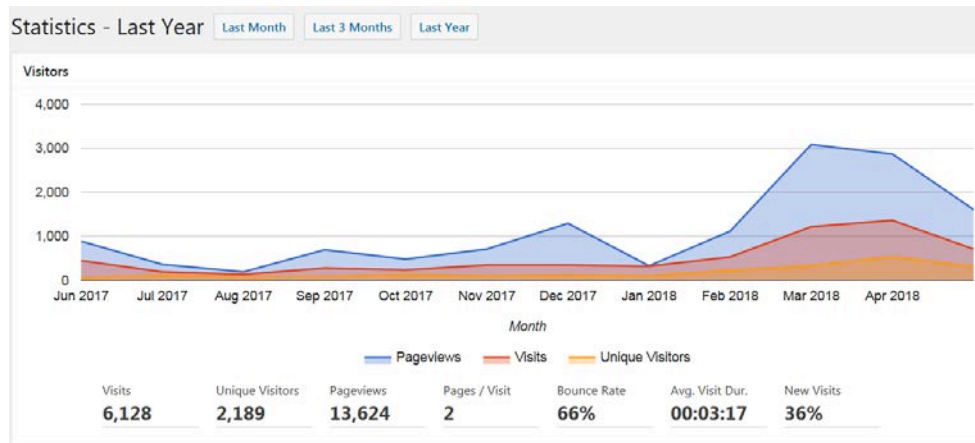


Figure 12.
Acimovic

Lab Disease Management blog statistics for 2018 – Blog visits 6-30-2018 for the period Mar – June. This image shows PD's lab blog visit history with number of reached growers.

Next Steps:

The next step in research or actions to help NNY apple growers will be to continue to advance disease management decisions, in particular for fire blight and apple scab by using RIMpro and NEWA disease prediction models so that new devastating outbreaks of fire blight do not happen again. In particular, research in 2019 will provide answers from other research projects in NNY based on the epidemic in 2016, and related to (1) efficacy of dormant copper applications in mix with bark penetrating surfactants (two years of data), and (2) population dynamics of *E. amylovora* in fire blight cankers over the summer, fall, winter and spring (two years of data). Both experiments will be evaluated by v-dPCR (viability digital PCR) for detection and quantification of live *E. amylovora* cells in fire blight cankers. Data is yet to come on two years of research providing Apogee programs for post-infection management of fire blight based on the epidemic that happened in NY.

Acknowledgments:

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