FINAL REPORT
WTFRC Project #: AH-02-208

Project title: Epiphytic bacteria in the shoot blight phase of fire blight

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Objectives:

1. Determine if fire blight bacteria can actively multiply on apple shoots (epiphytic) or if they can only survive on apple shoots for a limited period of time (resident).
2. Use resulting information to develop improved recommendations for the control of the shoot blight phase of fire blight.

Significant findings:

- The growth of the fire blight pathogen on the surface (epiphytic) of apple leaves does not play an important role in the shoot blight phase of the disease; rather it is the establishment of bacteria within the leaf that seems to be the critical event in the development of this destructive phase of the fire blight.
- Wetting events associated with high temperatures are conditions that can lead to the establishment of fire blight bacteria within the leaf and can favor shoot blight development.
- Apogee (prohexadione-calcium) can be used to manage shoot blight in young orchards where fire blight infections can be particularly devastating because infections of young trees often result in complete tree death. It was determined that fewer high-dose applications of Apogee (27.5 DF at 6 ounces per 100 gallons) provided good blight control, without unduly restricting young tree growth or impairing tree establishment, as opposed to multiple low-dose applications which did not effectively control fire blight.

Methods:

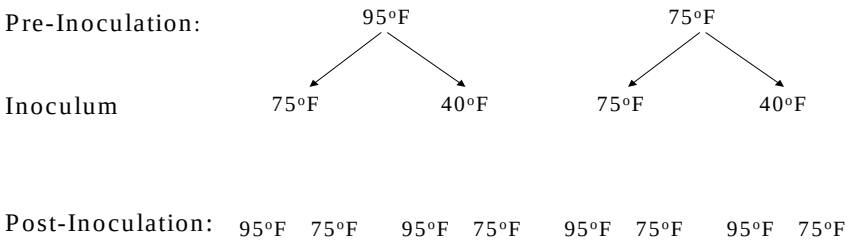
Growth chamber studies

Growth chamber studies were used to study the growth and survival of fire blight bacteria either on, or in apple leaves, under controlled environmental conditions. Shoots of 'Royal Gala' apple plants or shoots of M.26 rootstock were spray inoculated with the fire blight bacteria, *Erwinia amylovora*. Plants were incubated in a growth chamber at 75^{O} F (24^{O} C) or 95^{o}F (35^{o} C). Plant were kept in the growth chamber for up to 43 days after inoculation and observed for the development of fire blight symptoms.

At time intervals after inoculation, leaves were harvested and the populations of fire blight bacteria determined. Individual leaves were washed, or ground, and plated on a growth medium selective for the fire blight bacteria. To estimate the population size of fire blight bacteria on the surface of the leaf, leaves were first printed onto selective growth medium by briefly placing the leaves on the medium. Leaves were then removed from the medium, washed and washings will be plated as described above. The post-printing plating probably detected bacteria on the surface that were not removed by printing and bacteria within the leaf that were washed out of the leaf or released when the leaf was ground.

To determine if high temperatures or sudden temperature changes during wetting events could lead to establishment of fire blight bacteria in apple leaves plants were placed in growth chambers

at either 75°F or 95°F for approximately 4 days prior to inoculation with the fire blight bacteria. Plants were then spray inoculated with fire blight bacteria which were either warm (75° F, 24° C) or cold (40° F, 4° C). After inoculation, half of the plants were incubated at the initial incubation temperature and half of the plants were transferred to the alternate temperature.



Orchard populations

To monitor populations of fire blight bacteria on orchard grown trees during the summer, leaves were collected from 4-yr old (2002) or 5-yr old (2003) 'Gala' trees grown in a research orchard in West Virginia. Both trees with and without fire blight blossom infections were monitored. To distinguish between external and internal populations of the bacteria, leaves were printed, ground, and plated as described above. Five leaves were harvested and processed individually from each of 5 trees with fire blight and 5 trees without fire blight (50 samples per assay date). In 2002, assays were conducted prior to predicted rainfall and within 24 hours of rain events. In 2003, due to extensive rainfall assays were conducted at time intervals.

Results and discussion:

Summary

The goals of this research project were to identify the sources of fire blight bacteria that initiate the shoot blight phase of the disease and to define the conditions necessary for infection to occur. In addition, control strategies for the shoot blight phase of the disease were developed.

- Initial studies at conditions considered favorable for the epiphytic growth of the fire blight bacteria (constant 75° F, high relative humidity) suggested that the bacteria are not multiplying epiphytically on the surface of apple leaves, but rather in association with plant infection.

- Monitoring orchard populations of fire blight bacteria on apple leaves during June and July 2002 suggested that rapid changes in temperature during a summer storm were associated with the establishment of fire blight bacteria within apple shoots.

- The effect of high temperature and rapid temperature change during inoculation were studied under controlled environmental conditions. Surprisingly, a post-inoculation incubation temperature of 35°C resulted in more shoot infection than incubation at 24°C. Pre-inoculation incubation temperature did not have a significant effect on shoot infection. Inoculating plants

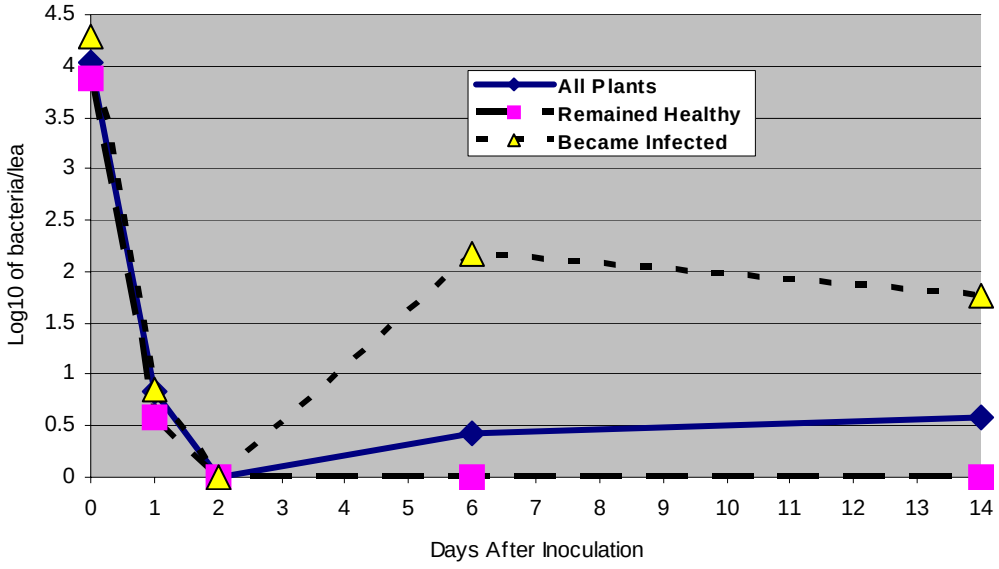
with bacteria at 4°C resulted in more shoot infection than with bacteria at 24°C. When plants were inoculated with cold bacteria (4°C) and incubated at high temperature (35°C), fire blight bacteria quickly became established within young leaves but rapidly declined on the surface of older leaves. These results suggest that rapid temperature changes during summer storms or during summer overhead irrigation can lead to the establishment of fire blight bacteria within the leaf and can favor shoot blight development.

- When Apogee (prohexadione-calcium) was applied to orchard-grown apple trees ranging in age from newly planted to fifth season of growth (4-year-old orchards) it was found that two high dose applications provided a better balance between fire blight control and growth in young orchards than multiple low dose applications. The results indicate that one to two Apogee applications at 6 ounces per 100 gallons can be used to manage fire blight in the fourth to sixth season of growth when there is a high risk of fire blight.

Growth of fire blight bacteria on apple leaves at 75° F

When 'Royal Gala' apple shoots were inoculated with the fire blight pathogen and incubated at 75° F (24.5° C), bacterial populations dropped below detectable limits within 48 hrs. Low populations were detected 6 and 14 days after inoculation (Fig. 1, all plants). However, when plants that developed fire blight symptoms 28 days after inoculation were analyzed separately from those that remained healthy, no bacteria were detected 6 and 14 days after inoculation on plants that remained healthy, while bacteria were usually detected in those that eventually became infected (Fig. 1).

Figure 1. Recovery of *E. amylovora* from apple leaves following inoculation. Data is presented for the mean of all inoculated plants (All Plants), those that did not develop fire blight symptoms 28 days after inoculation (Remained Healthy) and those that had developed fire blight symptoms 28 days after inoculation (Became Infected).



These data indicate that inoculation of apple shoots with fire blight pathogen resulted either in eventual shoot infection or death of the bacteria, and suggest that fire blight bacteria are not multiplying on the surface of apple leaves, but in association with plant infection. They also suggest that shoot blight infections should be associated with wetting events (rain or overhead irrigation) and close proximity to infected material.

Orchard populations

In general, fire blight bacteria were detected during June 2002 after rain events and were short lived on leaves (Table 1, see 6/17 to 6/25). The results of leaf prints and leaf washings in June are consistent with the concept that fire blight bacteria do not grow, or grow poorly, on apple leaves and wetting events spreading bacteria from infection sites to the leaf surface (Table 1). For example, after the rain of 13 June bacteria were found at more sites on the leaf and bacteria were recovered from trees without fire blight infections that were in close proximity to trees with infections. By 25 June, fire blight bacteria could not be detected (Table 1).

Table 1. Populations of fire blight bacteria detected by printing and washing leaves of orchard grown 'Gala' trees. Leaf prints indicate the number of colonization sites on exterior of leaf (both top and bottom), while leaf washings indicate the number of bacteria extractable from both on or within the leaf.

Sample	Rain	Trees with fire blight[2]		Trees without fire blight	
Date	Event[1]	Print #	Wash #	Print #	Wash #
6/12		5	218	<1	0
	6/13, 1.3"				
6/14		12	38	1	3
	6/17, trace				
6/18		<1	38	<1	0
6/25		0	0	0	0
	6/26, 1.0"				
6/27		<1	0	0	0
	7/9, 0.9"				
7/10		<1	5	0	0
7/15		0	198	0	18
	7/16, 1.5"				
7/17		134	2363	39	513

[1] Date of rainfall and amount in inches.

[2] Twenty-five leaves were sampled from each of 5 trees with fire blight blossom infections and 5 trees that were free of fire blight. The same leaf was printed and then washed.

However, the increase in population of fire blight bacteria observed in July 2002 is quite different from the population dynamics observed in June 2002 (Table 1). The July observations suggest that fire blight bacteria became internal within the leaf after, or during, the rain on 9 July. On that day the maximum temperature of 35.5°C dropped approximately 5.5°C as thunderstorms associated with a cold front passed through the area. Six days latter on 15 July bacteria were not detected on the leaf surface by leaf printing but were detected by washing, suggesting that the bacteria were within the leaf (Table 1). Following the rains of 16 July, high numbers of bacteria were detected on all trees by both leaf printing and washing, suggesting that bacteria were washed from the leaf and dispersed during the rain.

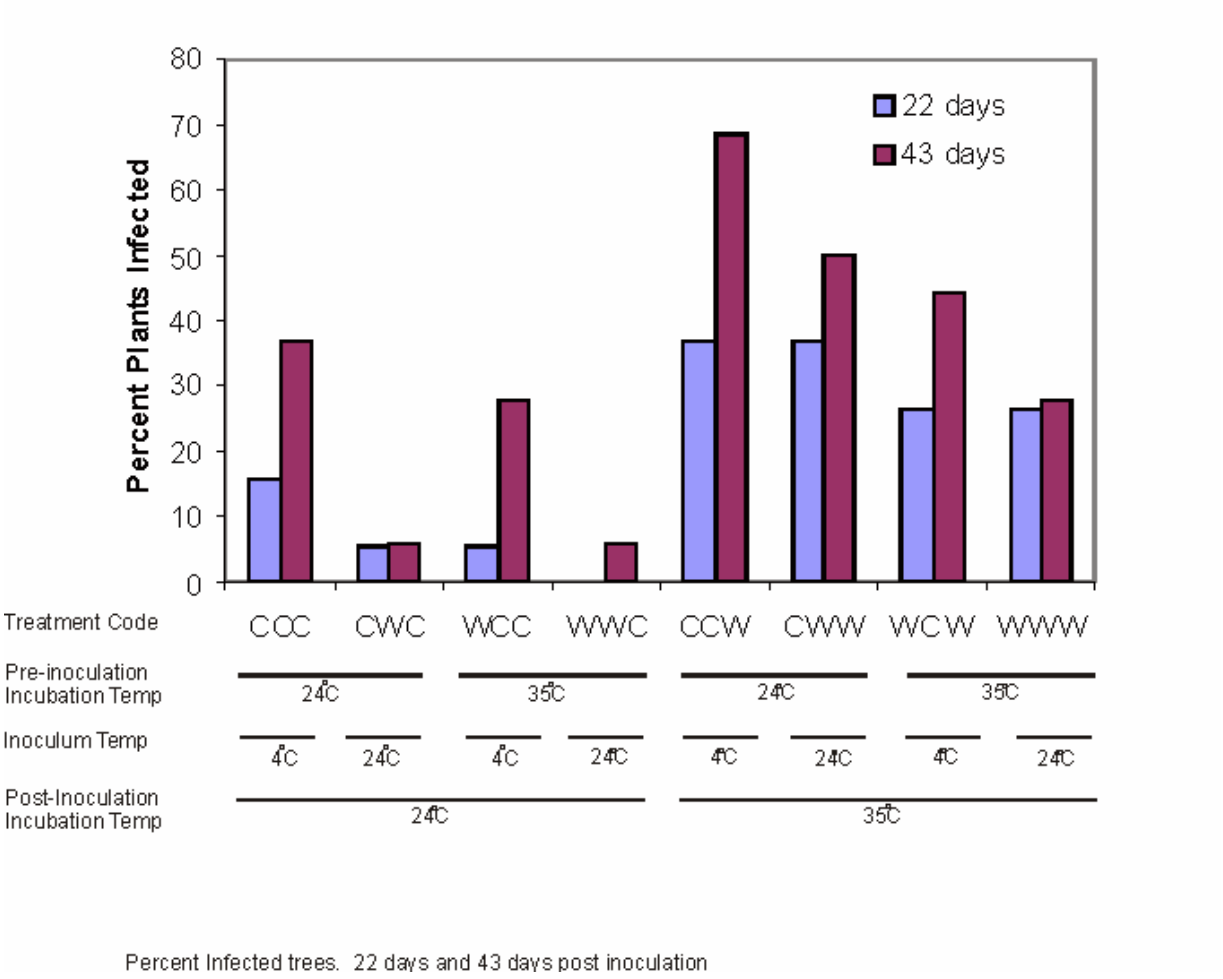
Effect of high temperature and wetting events during high temperatures on shoot blight

The effect of high temperature and wetting events during high temperatures on the development of shoot blight was studied in growth chambers. Single-shoot plants of Malling 26 apple rootstock were placed at 75° F (24°C in Fig. 2) or 95°F (35° C in Fig. 2) (pre-inoculation incubation). Plants were spray inoculated with fire blight bacteria in a cold buffer suspension at 40° F (4°C in Fig. 2) or a

warm buffer suspension at 75° F (24°C) (inoculum temperature). Following inoculation, plants were incubated at 75° F (24°C) (relative humidity ca. 94%) or 95°F (35° C) (relative humidity ca. 86%) (post-inoculation incubation) for 43 days.

Significantly more plants incubated at 95°F (35° C) after inoculation became infected than those incubated at 75° F (24°C) (Fig. 2, bottom line). Pre-inoculation incubation temperature did not have a significant effect on the incidence of infection. Although the effect of inoculum temperature was not as great as that of post-inoculation temperature, significantly more plants inoculated with cold bacteria (4°C inoculum) became infected than those inoculated with warm bacteria at 75° F (24°C inoculum, Fig. 2 middle line).

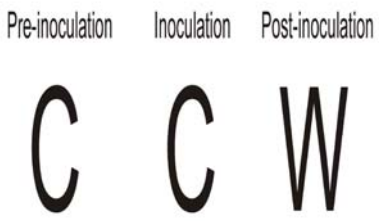
Figure 2 of Percent Plants Infected with Treatment Code



Percent Infected trees. 22 days and 43 days post inoculation

(See explanation of treatment code on the next page)

Treatment Code:



1st letter indicates pre-inoculation incubation temperature (C = 24°C, W = 35°C);
2nd letter indicates inoculum temperature (C = 4°C, W = 24°C);
3rd letter indicates post-inoculation incubation temperature (C = 24°C, W = 35°C).

At 2 h, 24 h and 132 h after inoculation the youngest leaf (leaf 1) at the time of inoculation and the fifth leaf from the shoot apex (leaf 5) at the time of inoculation were harvested from a subset of the plants in Figure 2. The populations of fire blight bacteria on the leaf surface and within these leaves indicated that when plants were inoculated with cold bacteria and incubated at high temperature, fire blight bacteria quickly became established within young leaves but rapidly declined on the surface of older leaves. The number of fire blight bacteria recovered 24 hours after inoculation from the youngest leaf of plants incubated at 95°F (35° C) was higher than that isolated from older leaves at 24 hours. In the young leaves of these plants most bacteria were recovered by plating the ground leaf, not by printing, indicating that the bacteria had become established within the leaf. In older leaves similar numbers of bacteria were recovered by plating and printing, indicating that most of the bacteria were probably on the outer surface of the leaf. For example, among the plants inoculated with cold inoculum and incubated at the high temperature (treatments xCW) the log10 of bacteria recovered 24 hours after inoculation from young leaves by grinding and printing were 4.2 and 2.3, respectively; but for older leaves were 1.0 and 1.6, respectively. These plants were also the most likely to develop shoot infections.

In contrast, on plants inoculated with warm inoculum 75° F (24°C) and incubated at cooler temperatures 75° F (24°C), fire blight bacteria appeared to survive better on the leaf surface but did not become established within the plant and was less likely to cause infection. For example, among these plants (treatments xWC) the log10 of bacteria recovered 24 hours after inoculation from young leaves by grinding and printing were 3.4 and 3.7, respectively; and similarly on older leaves were 3.5 and 3.9, respectively.

Using Apogee in young apple orchards to control shoot blight

Apogee (prohexadione-calcium, BASF) suppresses both shoot growth and fire blight in apple. In young apple orchards there are conflicting requirements to control fire blight and allow sufficient tree growth for tree establishment. When Apogee was applied to orchard-grown apple trees ranging in age from newly planted to fifth season of growth (4-year-old orchards) it was found that two applications of Apogee 27.5 DF at 6 ounces per 100 gallons of dilute spray provided a better balance between fire blight control and growth in young orchards than three applications of 3 or 1.5 ounces per 100 gallons. Although the high rate of Apogee suppressed early season shoot growth more than the lower rates, trees that received the high rate of Apogee tended to grow more in the latter part of the season resulting in little or no difference in total seasonal growth between trees that received two high or three low rate applications of Apogee. Fire blight control with Apogee required shoot growth suppression early in the growing season and Apogee DF at 6 ounces per 100 gallons often provided significantly better fire blight control than treatment at lower rates. Poor fire blight control occurred

when the rate of Apogee was lowered sufficiently to allow greater early season growth. The results indicate that one to two Apogee applications 6 ounces per 100 gallons can be used to manage fire blight in the fourth to sixth season of growth when there is a high risk of shoot blight.

Significance to the Industry

Cost of fire blight: The danger of fire blight in apple orchards has increased to unprecedented levels due to the adoption of high-density orchard systems and recent planting of fire blight susceptible cultivars and rootstocks. A 10% incidence of rootstock blight in a 4-year-old high-density planting can result in losses up to $3,500 per acre when the costs of tree replacement, lost investment in tree establishment and maintenance, and reduced yields over several years are considered. A single fire blight epidemic in southwest Michigan in 2000 resulted in the death of over 220,000 trees with a total economic loss estimated at $42 million. Annual losses to fire blight and costs of control in the United States are estimated at over $100 million.

Apogee: Management of the shoot blight phase of fire blight has been hampered by a lack of effective control treatments. The development of Apogee has been a significant advance in our ability to manage shoot blight in mature apple orchards, but there are constraints associated with its use both in mature and young orchards. To be effective Apogee must be applied two to three weeks before the normal period of shoot infection and before the effectiveness of blossom blight control sprays can be evaluated, so the expense of Apogee applications may not be recovered in years when fire blight is ultimately not a significant problem. In mature orchards the economic benefits of Apogee as an orchard management tool may be realized even in the absence of fire blight due to its effect on growth suppression (reduced pruning costs, improved fruit quality, etc). However, in young orchards there is no economic benefit to suppressing shoot growth and the practice may result in economic loss. Effective fire blight control by Apogee requires suppression of shoot growth at the time of infection. Therefore, in young orchards the use of Apogee should be considered only when the risk of fire blight shoot infection clearly outweighs the negative effects of growth suppression. Losses in the 2000 Michigan epidemic were greatest in plantings in their fourth, fifth and sixth season of growth and few losses were seen in plantings in their first season of growth. Similarly in controlled orchard studies in New York, trees in their third leaf were found to be significantly more susceptible to rootstock infection following severe blossom blight infection than trees in their first or second leaf. This suggests that Apogee should probably not be used in the first two years after planting when trees are at lower risk of tree loss due to fire blight and vigorous tree growth is critical to the establishment of the orchard. In this study, treatment of trees with Apogee in their fourth season of growth did not negatively impact yield the following year and suggests that use of one to two high-dose Apogee applications may be justified in the fourth to sixth season of growth when there is a high risk of shoot blight.

Note: Mention of trade names or commercial products in this report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

More information:

Norelli, J. L., Jones, A. L., and Aldwinckle, H. S. 2003. Fire blight management in the 21st century: using new technologies that enhance host resistance in apple. Plant Disease 87: 756-765.

Norelli, J. L., Holleran, H. T., Johnson, W. C., Robinson, T. L., and Aldwinckle, H. S. 2003. Resistance of Geneva and other apple rootstocks to *Erwinia amylovora.* Plant Disease 87:26-32.

Norelli, J. L. and Miller, S. L. 2004. Effect of prohexadione-calcium dose level on shoot growth and fire blight in young apple trees. Plant Disease (submitted for publication).

Budget Summary:

Project title: Epiphytic bacteria in the shoot blight phase of fire blight
PI: Jay Norelli
Duration: 2002-2003
Current year: 2003
Total (2 years): **$20,455**
Current year request: project completed.

Item	Year 1 (2002)	Year 2 (2003)
Total	$10,000	$10,455

Item	Year 1 (2002)	Year 2 (2003)
Salaries	$7,000	$7,350[1]
Benefits (30%)	2,100	2,205
Equipment	0	0
Supplies	900	900[2]
Travel	0	0
Miscellaneous	0	0
Total	$10,000	$10,455

[1] 25% of a technician salary to assist in conducting experiments and in production of plant material to be used for the experiments.
[2] Supplies to determine bacterial populations, produce plant material, and greenhouse supplies.

Other funding sources: New York State Apple Research and Development Program provided $10,000 to support this research project.