North American Bramble Growers Research Foundation
2016 Report

Fire Blight: An Emerging Problem for Blackberry Growers in the Mid-South

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A poster with many of these initial findings was presented at the North American Berry Conference in Grand Rapids, MI in December 2016

Background and Initial Rationale:

Fire blight, caused by the bacterial pathogen *Erwinia amylovora*, infects all members of the family Rosaceae and is considered to be the single most devastating bacterial disease of apple. *Erwinia amylovora* was first isolated from blighted blackberry plants in Illinois in 1976, from both mummified fruit and blighted canes (Ries and Otterbacher, 1977). These symptoms had been sporadically reported previously in both blackberry and raspberry, but the causal agent had never been identified. Since this first report, the disease has been found throughout the blackberry growing regions of the United States, but is generally not considered to be a pathogen of major concern (Smith, 2014; Clark, personal communication). However, with the advent of primocane fruiting plants, a significant increase in disease incidence has been witnessed in Arkansas (Garcia, personal communication) and fruit loss of up to 65% has been reported in Illinois (Schilder, 2007). Fire blight is a disease that is very environmentally dependent, and warm, wet weather at flowering is most conducive to serious disease development. The Arkansas growing conditions are ideal for disease development, and the shift in production season with primocane fruiting moves flowering time to a time of year with temperatures cool enough for bacterial growth (Smith, 2014). This puts us at the forefront of a potentially devastating epidemic, as resistant varieties are unknown and very few chemical controls are labeled for use on blackberry and raspberry.

After Arkansas blackberry growers began reporting a new disease in early 2015, it was reported as increased incidence of fireblight based on classical presentation of symptoms (Fig. 1), beginning with shepherd's crooking, blossom blight, and ending with general cane collapse from the shoot tips downwards. As this disease is relatively rare in blackberry, samples were collected from infected blackberry plants at several growing sites in Northwest Arkansas for further study. Bacteria were isolated from these samples for identification. Of the bacterial samples collected from symptomatic blackberry tissue, 100% were identified as species other than *Erwinia amylovora* via DNA testing. One related species, *Pantoea agglomerans*, appeared more frequently than any other. Our objectives have shifted slightly to take into account these initial findings.

Initial Objectives:

1. Survey *Erwinia amylovora* strains isolated from *Malus* spp. and *Rubus* spp. to identify potential sources of initial inoculum
2. Review strength of host specificity between *Malus* spp.- and *Rubus* spp.-infecting strains
3. Clarify the mechanisms of survival and transmission of the pathogen in blackberry

New Objectives:

1. Identify bacterial strains isolated from symptomatic *Rubus* spp.
2. Review strength of host specificity of *P. agglomerans* isolates collected from *Malus* spp. and *Rubus* spp.
3. Clarify the mechanisms of survival and transmission of the pathogen in blackberry

Figure 1. Left: Shepherd's crooking (top) and blossom blight (bottom) in blackberry infected with *Pantoea agglomerans*
Right: Shepherd's crooking (top) and blossom blight (bottom) in apple infected with *Erwinia amylovora*

Procedures and Initial Results:

Study 1. Isolate bacteria from *Rubus* spp. and identify causal agent of fireblight-like symptoms

Bacteria was isolated from a number of primocane fruiting blackberry cultivars growing at the Arkansas Agriculture Research and Extension Center in Fayetteville, AR and from the University of Arkansas Fruit Experiment Station in Clarksville, AR. Bacterial DNA was sent for 16s rRNA sequencing in order to accurately identify these bacterial isolates. Of bacterial isolates collected from symptomatic blackberry plants, none were identified as Erwinia amylovora and 90% were identified as a *Pantoea* spp., with *Pantoea agglomerans* appearing as the most positively identified species.

Greenhouse plants were wound inoculated with *E. amylovora* and *P. agglomerans* to verify the comparative virulence of this newly identified pathogen (Figs 2,3,4).
All results support the hypothesis that *P. agglomerans* is a causal agent of the blackberry disease that had initially been reported.

Figures 2 and 3. Each series of photos pictures PrimeArk 45 blackberry plantlets following inoculation with (left) sterile H_20, (center) *Pantoea agglomerans*, (right) *Erwinia amylovora.* Pictures are 10 days post inoculation. Younger tissues (Figure 2 to left) produced lesions when inoculated while older tissues (Figure 3) produced cankers. (Inoculation sites indicated with arrows.)

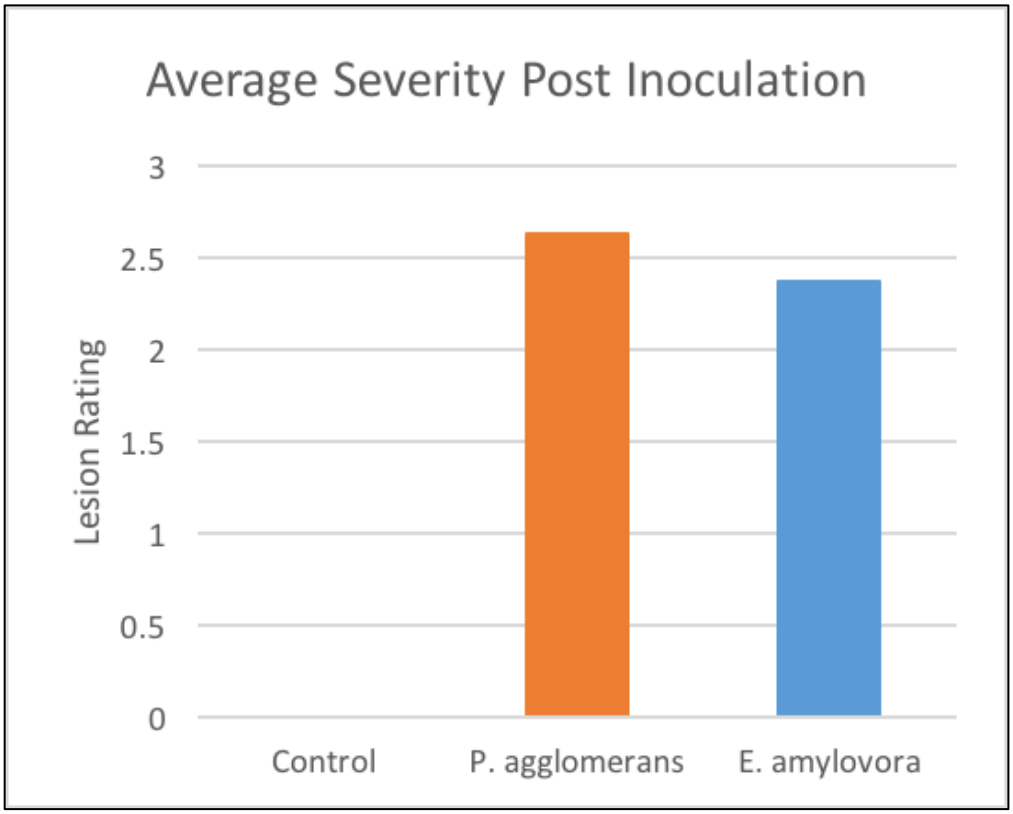

Figure 4. Statistical evaluation of comparative lesion severity following three replicated greenhouse trials. PrimeArk 45 plantlets were wound inoculated and lesions were evaluated 10 days post inoculation. Using Tukey's HSD test, no significant difference was found in the severity of the lesions caused by *Pantoea agglomerans* and those caused by *Erwinia amylovora*.

Study 2. Test for host specificity of *P. agglomerans* isolated from *Malus* spp. and *Rubus* spp. in order to identify sources of initial inoculum

In addition to collecting bacteria from blackberry plants exhibiting fireblight-like symptoms, isolates were also collected from a number of symptomatic apple trees. The University of Arkansas apple breeding program has maintained a selection block at the Arkansas Agriculture Research and Extension Center in Fayetteville since 2004. These trees have exhibited significant fireblight incidence the past two flowering seasons and are an excellent source of *Malus* spp.-infecting isolates. Several *Erwinia amylovora* isolates were collected from this block, along with a few *Pantoea* spp. isolates. Although *Pantoea* spp. is not reported to be a pathogen of apple, it was not surprising to isolate this organism from this block as it is a ubiquitous epiphyte (Walterson and Stavrinides, 2015). However, the presence of these isolates led to concern about whether epiphytic *Pantoea* spp. on neighboring plants could serve as a source of inoculum for neighboring blackberry plants and whether, under ideal conditions, these epiphytic bacteria could become pathogenic on apple.

We evaluated the pathogenic potential of these isolates by inoculating greenhouse plants. This work was conducted in a greenhouse at the Rosen Alternative Pest Control Center at the University of Arkansas in Fayetteville. Forty 'PrimeArk 45' plantlets were maintained in the greenhouse to prevent secondary infection. Two *Erwinia amylovora* isolates from *Malus* along with two *Pantoea* spp. isolates from *Malus* and four *Pantoea* spp. isolates from *Rubus* were selected. For each isolate, five plantlets were wound inoculated at three individual points with a bacterial suspension. Five plantlets were inoculated with sterile water as a control. The plants were maintained in the mist chamber for 24 hours to mimic an infection period and then moved back into the greenhouse and monitored for disease development for 10 days. Plants were rated based on lesion size and presence of cankering. As was found in our initial study, there was no significant difference between the severity of lesions on plantlets inoculated with *Pantoea* versus those inoculated with *Erwinia*. The source of the *Pantoea* isolate also had no effect. Apple isolates were equally pathogenic on blackberry. (Fig. 5.)

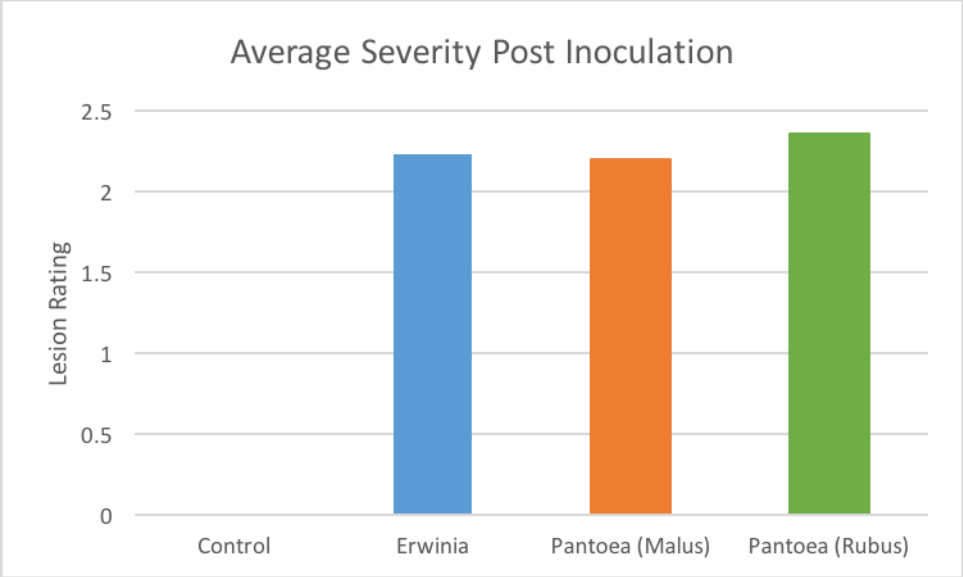

Figure 5. Statistical evaluation of comparative lesion severity following greenhouse trials. PrimeArk 45 plantlets were wound inoculated and lesions were evaluated 10 days post inoculation. Using Tukey's HSD test, no significant difference was found in the severity of the lesions caused by *Pantoea* isolates from apple or blackberry and those caused by *Erwinia amylovora*.

In order to determine whether *Pantoea* isolates from either apple or blackberry can be pathogenic on apple fifteen Jonathan seedlings have been moved into the greenhouse. Once these seedlings develop new leaves they will be wound inoculated in a similar fashion and the lesions will be rated. (Contact the authors in early spring for final results.)

Study 3. Investigate the possibility of systemic infection

In recent years, incidence of fireblight-like symptoms on *Rubus* spp. has been increasing with the widespread use of primocane fruiting blackberry cultivars. These plants have a secondary

flowering season in the late summer and fall, exposing them to a new potential infection period.

One of the points of confusion about the infection cycle in this pathosystem is what the initial source of inoculum is each season (Schilder, 2007), as a common management practice for blackberry production involves cutting canes down to the crown at the end of the season, removing existing cankers. In apple and pear, *E. amylovora* is known to overwinter in cankers on the trunk and limbs of the tree. Given the similarities between *P. agglomerans* and *E. amylovora*, we will investigate whether it is possible that in blackberry the infection is systemic to the point that the crown and root tissue left in the soil is serving as a reservoir. Crown tissue was collected from cut-back canes in a heavily infested stand at the Arkansas Agriculture Research and Extension Center in Fayetteville. This crown tissue was surface sterilized and rubbed onto LB plates to test for presence of the pathogen in the crown. The pathogen was present in all samples collected from all three of the blackberry cultivars tested.

Having found evidence to support the movement of bacteria into the crown tissue, we now aim to demonstrate that these bacteria will recolonize new tissue in the spring. This work will be done in a greenhouse at the Rosen Alternative Pest Control Center at the University of Arkansas in Fayetteville. 'PrimeArk-45' plantlets have been grown in pots and wound inoculated with either *E. amylovora*, *P. agglomerans*, or sterile water (5 replicates per treatment). 10 days after foliar symptoms developed, plants were cut back to the crown. New growth will be collected and plated onto LB agar to test for survival and movement of the pathogen. (Contact the authors in early spring for final results.)

Study 4. Examine the potential role of mite feeding/mechanical damage in disease development

A correlation between fireblight-like symptoms and broad-mite feeding has been noted in multiple locations by a number of growers and scientists (Clark, Garcia, Johnson, personal communications). We aim to verify and explain this correlation with a number of controlled studies.

Vector Potential

With a correlation between pest feeding and disease severity, the first question is whether the pest is acting as a vector. In order to rule out this possibility, we are collaborating with an entomology lab working extensively with broadmites.

A group of 'PrimeArk 45' plants have been maintained in an experimental plot at the University of Arkansas Southwest Research and Extension Center in Hope, AR. These plants are heavily infested with broadmites and over the course of the last season a number of these plants developed fireblight-like symptoms. We verified that these plants are infested with *Pantoea agglomerans*. Our collaborators in entomology are in the process of extracting DNA from the broadmites present on the symptomatic plants to test for the presence of *P. agglomerans* in the mite's gut and mouthparts. These data will provide insight into whether the broadmites likely vector the pathogen, or play a more indirect role in exacerbating the disease. (Contact the authors in early spring for results.)

Exacerbation of Systemic Infection

Fifteen nursery-produced 'PrimeArk 45' plantlets of a susceptible blackberry cultivar have been produced in a greenhouse to prevent secondary inoculation. Ten plants have been wound-inoculated with a *Pantoea agglomerans* suspension. Five have been inoculated with sterile water. The plants have been maintained in the greenhouse to allow systemic infection to develop. Five systemically infected plants and five control plants will be exposed to mite feeding experiments at the University of Arkansas Southwest Research and Extension Center in Hope, AR. Disease development will be monitored for up to 2 months. (Contact the authors in early spring for results.)

Discussion:

With these initial findings we have provided significant evidence that fireblight symptoms on Arkansas blackberry is not primarily caused by *Erwinia amylovora* and is likely caused in part by the ubiquitous *Pantoea agglomerans*. *Pantoea* isolates originating from other host species are equally pathogenic on blackberry, indicating that the potential reservoir of inoculum could be very large. There is evidence that this pathogen moves into the crown tissue, meaning that cutting back of canes in the winter does not ensure eradication of the pathogen.

Our ongoing studies hope to clarify some of these concerns, but there are still many questions. Future work will focus primarily on methods of control.

References:

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