Dissemination and Vectoring of the Fire Blight Pathogen (*Erwinia amylovora*) by the Potato Leafhopper (*Empoasca fabae*)

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Fire blight, caused by the bacterium *Erwinia amylovora*, is a serious disease of pome fruits worldwide, which occurs only intermittently in the northeast U.S., but causes severe losses of trees and sometimes of entire plantings when it does occur. The disease overwinters within an infected tree and is disseminated in the spring by wind, rain, and insects. If environmental conditions are favorable during bloom, blossoms become infected, and the infection can spread to the rest of the tree. In early summer, shoot infections may appear; in some cases these are simply an expression of a systemic infection, but in others they are an indication that a new infection has occurred. These new shoot infections may be caused by wounds, such as hail or wind injury, or may be caused by insects feeding.

The epidemiology of the "blossom blight phase" is fairly well understood, but that of the "shoot blight phase" is less clearly delineated. The role of insects in this phase of the disease is one area where more understanding is needed. As growers move away from broad-spectrum insecticides toward softer, more narrow-spectrum insecticides, the question of which insects – if any – are involved in fire blight dissemination becomes increasingly critical. Several studies have implicated the potato leafhopper, a migratory pest of apple in the Northeast, as possibly playing a significant role in the shoot blight phase, and preliminary work that we did in 2002 tends to support that idea. Growers can manage potato leafhoppers with soft insecticides and even with a plant growth regulator, thus a greater understanding of this insect and its role in fire blight transmission might lead to better control with minimal insecticide.

Cages will be constructed in a cooperating orchard in a location where they will not present a threat to the rest of the orchard. The grower will assist with maintenance of the trees in the cages, while the project director, Leahy, will perform the bacterial inoculations and other work directly related to the research. Drs. Greene and Cooley will provide guidance and expertise on horticulture, plant pathology and laboratory methods.

One-year-old 'Gala' apple trees on M.7 rootstock will be placed in 15 L containers in a mixture of soil, peat, and sand. The trees will be placed in cages measuring 1.25 m x 2.5 m x 2.0 m and covered with white Lumite fabric. Trees will be headed at 50 cm above the graft union and given moderately high nitrogen to encourage vigorous lateral shoot growth. Trickle irrigation will be installed at the time of planting, and the need for water will be measured with lysimeters in the containers. Vigorous shoot growth should help to predispose the trees to fire blight. Normal disease control measures directed against fungal pathogens will be done as needed prior to the start of the treatments, while insect pests should be adequately excluded by the cages.

The first experiment will assess the facilitation of fire blight by potato leafhoppers, and try to establish both a threshold population density and a plateau density beyond which fire blight damage does not increase. Twenty-four individually caged trees will be misted with a 1×10^6 cfu

suspension of *Erwinia amylovora*. Following *E. amylovora* inoculation, potato leafhoppers collected from nearby alfalfa fields will be introduced into half the cages in incrementally dense populations, starting at 0 and increasing to 100 in multiples of 10. In the other half of the cages, leafhopper density will increase in multiples of 20, starting at 120 and ending with 360. The experiment can be repeated at different densities to revise or refine the leafhopper threshold.

Potato leafhopper density will be assessed daily using a visual scale of 0-5, with 0 = no injury and 5 = severe marginal burn and leaf curling. Fire blight strikes per tree will be assessed daily, beginning at 57DD > 12.7EC from the initial inoculation. Assessment will continue for 30 days to assure full manifestation of infections. Temperature, humidity, and leaf wetness will be monitored in four cages using electronic environmental monitoring equipment. Data will be subjected to regression analysis.

The second experiment will assess the possibility of the insects' vectoring the disease by carrying it to new trees on their bodies. Full expression of infection from such vectoring may not occur at the levels possible under such conditions, but we will also look at the process incrementally.

At the conclusion of the first experiment, the trees will be removed and replaced with a pair of similar trees in each cage, separated by approximately 1 m. Treatments will be applied in a fully factorial design, with potato leafhoppers and *E. amylovora* as the two treatments. In treatments involving *E. amylovora*, a 10^6 cfu suspension of *E. amylovora* will be applied to one tree in each cage using an atomizer. In treatments with potato leafhoppers, the number of leafhoppers released will be the same in each cage, and will be determined based upon the results obtained in the first experiment.

Both trees in the cage will be assessed for fire blight incidence daily for a minimum of 30 days. Leafhoppers will be collected at release and at one, three, seven, and 14 days after release. They will be placed in 2 ml micro-centrifuge tubes and inactivated using CO_2 and ethanol, and assayed for *E. amylovora*, after which they will be crushed and shaken in phosphate buffer for 30 minutes. They will then be centrifuged, and the supernatant will be dilution-plated on CCT medium amended with 100 Φg of rifampicin ml^{-1} and 100Φg of cycloheximide ml^{-1}. The plates will be incubated for 48 hours at 28EC and bacterial colonies will be counted to determine population levels.

To assess the level of bacteria delivered to non-treated trees by the insects, leaves on these trees will also be collected and evaluated. On the day that *E. amylovora* is applied to treated trees, eight new leaves, representing about one leaf per shoot, will be collected from the non-treated trees. Each leaf will be individually placed in a large tube containing sterile phosphate buffer and agitated for 30 minutes on a rotary shaker. An aliquot of the liquid will be dilution-plated on a modified CCT medium as described above for potato leafhoppers. This process will be repeated at one, three, seven, and 14 days after inoculation. Temperature, humidity, and leaf wetness will be monitored as previously described. Data will be subjected to analysis of variance as a 2 x 2

factorial.