A dissertation overview: Molecular and behavioral interactions between *Erwinia amylovora* and potential insect vectors

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Introduction

Erwinia amylovora is the bacterial agent of fire blight in pome fruits, eliciting a burned-like appearance in foliage and causing severe damage and loss in orchards across 47 countries. Bacteria ooze from woody tissue in the spring, where insects acquire it and can transmit it to new hosts. The ooze (Fig.1A,B) has three components: plant sap; bacteria-generated exopolysaccaride (EPS); and high concentrations of *E. amylovora* cells, and is hypothesized to attract insects, namely dipterans (Fig. 1C). Fire blight colonizes new hosts when it is washed from the stigma into the floral cup or through damaged tissue caused by weather events or insect feeding.

Though sporadically occurring, fire blight can have resounding economic impacts on apple orchards worldwide, resulting in 100% loss in some cases. The New York apple industry is worth nearly $250 million annually, ranking second in the nation, and requires intensive monitoring and control programs to protect orchards from fire blight. To date, growers in the New York State and elsewhere depend on streptomycin applications to control fire blight, but continued streptomycin use is not sustainable given risks for resistance. Indeed, this risk has recently become a reality, leading to an urgent need for new fire blight control methods.

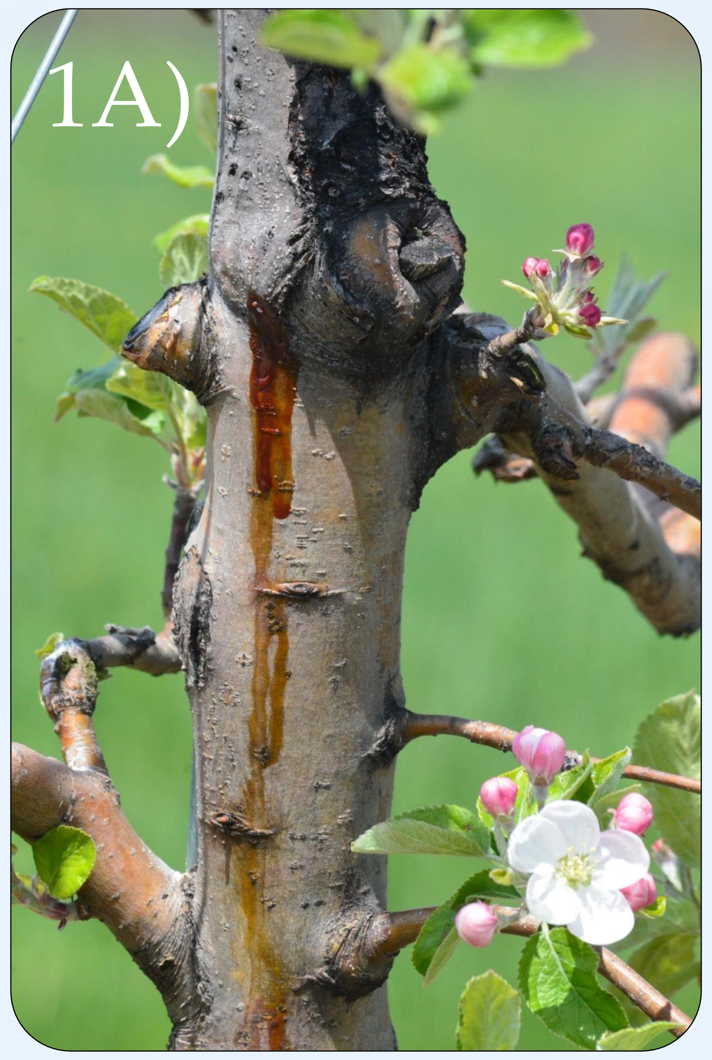

Figure 1: A) Bacterial ooze exuding from a canker margin in early spring. **B)** Fire blight infected fruitlet oozing in mid-June. **C)** Dipteran feeding on an oozing fruitlet.

Purpose

Fire blight is a major impediment to profitable apple production in the New York State and requires significant attention to reduce its regional and global impact. To this end, a large body of research implicates insects as important vectors of fire blight, but the potential benefits of managing vectors as part of a fire blight management program have not been previously investigated. Impeding this management approach is a lack of in depth knowledge regarding interactions between *E. amylovora* and potential vectors at molecular, behavioral, and ecological levels.

Our primary goal is to address host-microbe-vector interactions at each of the above mentioned levels of biological organization to increase the efficacy of including vector management into fire blight control. We will start by answering three key questions.

Question 1: Who are the primary vectors?

Methodology: Vectors will be identified through sampling of an orchard experimentally inoculated with fire blight. Targeted pollinator sampling from bloom to petal fall (Fig. 2A, B) and when high levels of ooze are detectable in the orchard. Deploy yellow sticky cards throughout the season to monitor the key insects in the community (Fig. 3). Samples will be tested for fire blight via PCR and confirmed as vectors through acquisition and transmission experiments.

Predictions: Flies landing on ooze, pestiferous insects such as potato leafhopper and tarnished plant bug, and pollinators such as honey bees are hypothesized to be key fire blight vectors at different periods during the growing season.

Figure 2: A) Targeted sampling of pollinators during bloom. **B)** Honey bees are considered a passive fire blight vector, transporting inoculated pollen from blossom to blossom.

Figure 3: Yellow sticky cards are deployed on a weekly basis in the canopy and at the trunk of 20 trees.

Question 2: Does *E. amylovora* alter the behavior of its vector?

Methodology: Choice and non-choice preference assays for fire blight inoculated media, fruits, and oozing fruitlets collected from the field (Fig. 4). Volatile collections of diseased tissue coupled with bioassays to identify chemical blends associated with vector behavior changes.

Predictions: We hypothesize that vectors are highly attracted to diseased tissue, preferring it to non-oozing, potentially less nutritious tissue, and these interactions can be exploited in vector monitoring/control.

Question 3: What is the molecular mechanism mediating vector-phytopathogen interactions?

Methodology: qPCR and bioassays using mutant *E. amylovora* lacking a type III secretion system hypothesized to mediate interactions between vectors and insect guts.

Predictions: Mutants lacking this type III secretion system will be unable to replicate/interact within a vector, reducing the overall transmissibility of the pathogen and providing an avenue for future research into sustainable fire blight control.

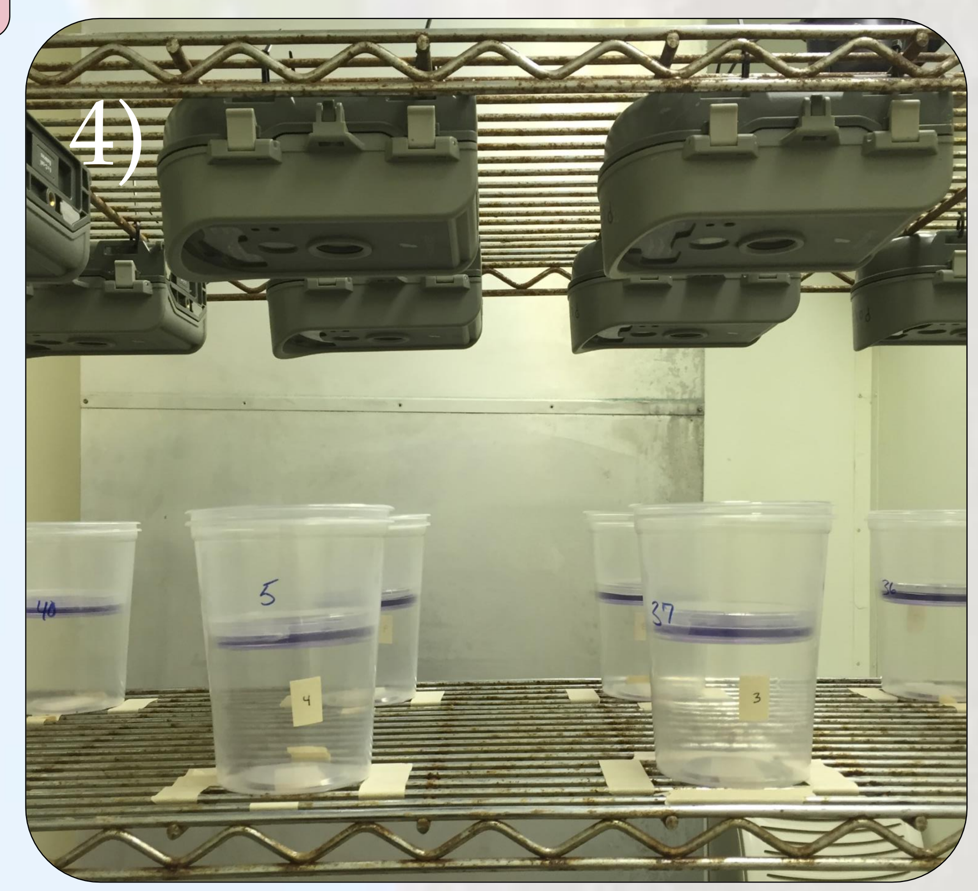

Figure 4: Field images capture location of insect within arena every 30 seconds to monitor whether or not *Drosophila melanogaster* prefers media inoculate with fire blight over fire blight free media.

Acknowledgements

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