PennState
College of Agricultural Sciences

PROPOSAL TRANSMITTAL

Date: 12/19/2018 **PSU Ref. No: 206033**

Title: Optimizing Fire Blight Control Alternatives: Non-antibiotic Blossom Protection and Full Season Shoot Blight Management

Submitted to: Patti Keller

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Proposed Project **05/01/2019 - 04/30/2020** **Total Project Request: $12,344**

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2019 RESEARCH GRANT PROPOSAL
STATE HORTICULTURAL ASSOCIATION OF PENNSYLVANIA

Title: **Optimizing Fire Blight Control Alternatives: Non-antibiotic Blossom Protection and Full Season Shoot Blight Management**

Personnel:
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Co-PI: Dr. Tim McNellis, Associate Professor, Department of Plant Pathology & Environmental Microbiology, The Pennsylvania State University

Project Duration: May 1, 2019 – April 30, 2020

Amount requested: $12,344
Peter: $4,088
McNellis: $8,256

Justification

Fire blight, caused by *Erwinia amylovora*, is a persistent problem faced by apple growers each year. Due to favorable conditions season-long, fire blight management is a full season affair for apple growers, especially on the East Coast. Another concern is the life span of streptomycin to control blossom blight: either due to bacterial resistance or potential future regulations prohibiting/limiting antibiotic use in agriculture. Thanks to funding by SHAP and the Maryland State Horticultural Society the last few years, we have been able to be proactive in addressing these concerns. Our overall goal is to develop novel strategies to control fire blight in apple trees using plant immune stimulation and bacterial antagonists, with the objective of providing growers with sustainable options for seaon-long disease control.

Plant immune stimulation and low-dose plant growth regulators

Part of a plant's defense against pathogens involves stimulation of defense mechanisms. This stimulating usually requires initiation of infection by a plant pathogen or an injury to the plant. Pathogens that haven't evolved with a plant tend to have the ability to overcome the plant's defense mechanism because they can often avoid detection, cause infection faster than the plant can activate its defense mechanisms or suppress the activation of plant immune responses during the infection process. *E. amylovora* can cause rapid infections in cultivated apples and pears, often leading to death of the plant. If it doesn't kill its host, *E. amylovora* can become systemic and persist in the tree and symptoms can reoccur.

Standard control methods of applying streptomycin at bloom to prevent flower infection, if applied at the proper timing and at close intervals, can be effective. However, during seasons where conditions remain ideal for bacterial multiplication and spread, outbreaks of shoot blight can occur. Once this happens, little can be done to slow or suppress infections. Use of the plant growth regulator prohexadione calcium (ProCa; Apogee, Kudo) is excellent for limiting shoot blight since it hardens off the shoots, making it difficult for the bacteria to enter the plant. However, ProCa suppresses growth, even at very low rates, and there is reluctance to use this product on growing trees actively filling their tree space. One strategy for safe and effective disease control, which does not suppress growth, is through controlled activation of the plant immune system by foliar sprays containing plant defense activating compounds. Modern research has revealed some of the plant molecules that serve to activate plant

immunity, including salicylic acid and methyl jasmonate. Chemicals that mimic salicylic acid, such as acibenzolar-S-methyl (Actigard), have been used commercially to boost plant immunity and make plants resistant to pathogens to which they might normally be susceptible. Glycerol-3-phosphate is known to be an intermediate in the activation of a plant defense. Our previous research suggests that glycerol, which has the component glycerol-3- phosphate, reduces the severity of fire blight. A few plant extracts which are marketed as plant defense activators, such as Regalia and Vacciplant, have also shown some positive, yet inconsistent, results. Regalia is also described to increase lignification (strengthen cells walls).

Promising results from the 2018 season: Data from our greenhouse timing trials showed Actigard to be effective if applied seven days before the onset of infection,but had little to no effect if applied after infection occurred (**Fig. 1**). Surprisingly, Actigard shows there is still some efficacy persisting when it had been applied 10 and 14 days prior to infection (**Fig. 1**). For our field trials, combining Cueva with Glycerol reduced fire blight severity more than Cueva or glycerol alone in field trials (**Fig. 2**). In our Apogee trials at the WMREC in Keedysville, MD (funded by MSHS), we saw significantly less fire blight severity on two different cultivars (2010 planting, different Geneva rootstocks) when Apogee (2 oz/A) and Regalia were applied together, as opposed to Apogee by itself (**Figs. 3 and 4**). It is hypothesized that there is a synergy when mixing the two products considering Regalia is known to strenthen plant cell walls.

It takes time and is challenging to understand the nuances of all these alternative products; however, we feel we are getting closer to finding the "sweet spot" of usage. For 2019, we wish to build on our results from 2018 by understanding timing applications for optimal disease control for glycerol, Vacciplant, and Regalia in the greenhouse. From these results, we wish to take our programs to field and build on last year's data by evaluating products as standalone and in combination, to determine if there are additive effects, on three different aged tree plantings.

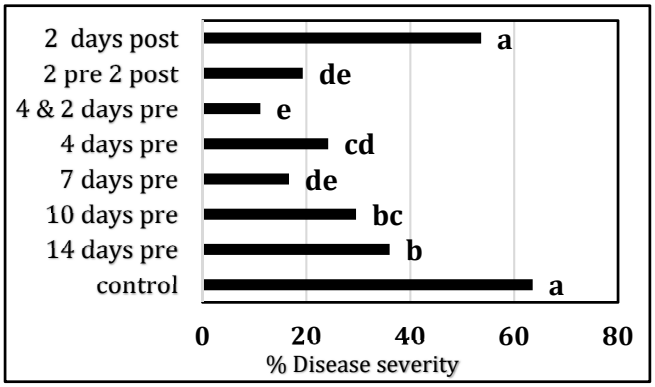

Figure 1. Timing of Actigard treatments for fire blight in GH.

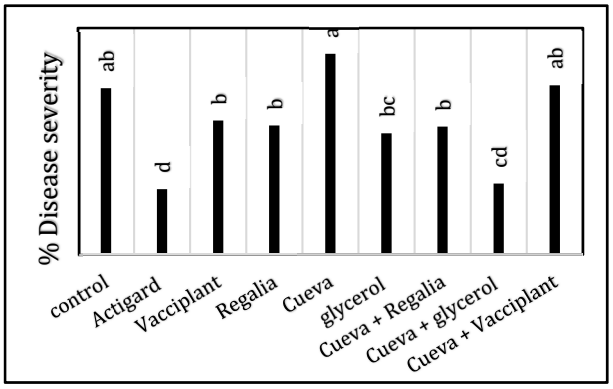

Figure 2. Products used on 8 year-old Gala trees in the field.

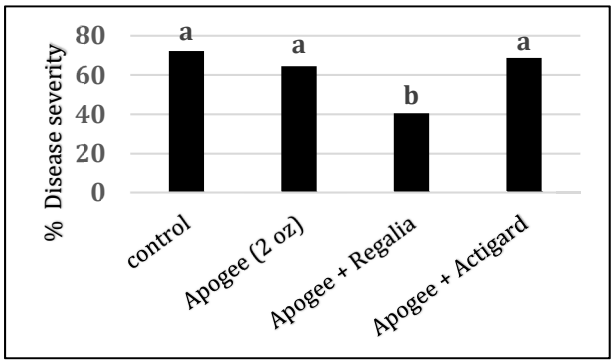

Figure 3. Severity of shoot blight on 'Brookfield Gala.'

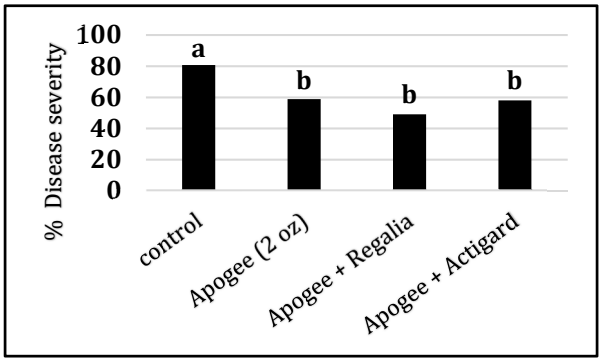

Figure 4. Severity of shoot blight on 'Cripp's Pink.'

Bacterial Antagonists

This proposal aims to further develop and understand the approach of using non-pathogenic *E. amylovora* strains to compete with virulent *E. amylovora* cells in the flower environment to slow bacterial growth and potentially reduce fire blight disease incidence or development as an alternative for blossom blight management. Because of SHAP funding in 2016, 2017, and 2018, we have tested and confirmed that application of high concentrations of *E. amylovora* to apple, crabapple, and hawthorn flowers will inhibit the growth of virulent *E. amylovora* cells by two or three orders of magnitude. So far, this activity has been specific to the flower tissue environment, since we found in 2018 that this inhibitory effect does not occur in wounded immature apple fruit tissues. It is likely that the non-pathogenic *E. amylovora* strains compete for limited resources on the apple flower exterior, thereby limiting the growth of the virulent *E. amylovora* cells. However, it is also possible that the non-pathogenic *E. amylovora* cells elicit plant immunity within the flower tissues and inhibit the growth of the virulent *E. amylovora* cells indirectly. In any case, we now wish to determine whether the inhibition of growth of the virulent *E. amylovora* cells is sufficient to reduce fire blight disease incidence or severity during blossom blight. In 2018, we applied for a USDA-APHIS permit to apply the non-virulent *E. amylovora* strain as a biocontrol at a PSU FREC orchard block. However, the application was not approved, with a request to determine whether the non-pathogenic *E. amylovora* strains could cause disease in apple flowers and pear fruits. Better characterization of the non-pathogenic *E. amylovora* strain behavior on host plants will improve the likelihood of approval by the USDA, and they were supportive of re-application for a permit. The present proposal aims to provide more information about non-pathogenic *E. amylovora* strain behavior on apple flowers and pear fruits, as well as to better understand the mechanism underlying the inhibition of virulent *E. amylovora* growth by high concentrations of non-pathogenic *E. amylovora*. This information will improve chances for approval of field testing. It is also possible that high concentrations of natural isolates of non-pathogenic, epiphytic bacteria, such as *Pantoea agglomerans,* can also inhibit the multiplication of virulent *E. amylovora* cells on apple flowers. Testing for this will provide additional insight into the potential mechanism of action of inhibition of virulent *E. amylovora* growth by floral epiphytes, and if successful, may provide an alternative fire blight management approach.

Objectives:
This project builds on previous SHAP funded projects with the aim of the objectives to address the 2019 SHAP priorities under the Plant Pathology section: **Alternative Disease Control Strategies** and **Disease Resistant Management for Key Diseases:**

Objective #1: Evaluate applications of glycerol, Vacciplant, and Regalia on potted trees in the greenhouse to understand optimal timing for usage to achieve effective fire blight disease control.

Objective #2: Evaluate applications on different aged trees in the field of Actigard, Apogee, Cueva, Regalia, and Vacciplant, as standalone and in combination, to understand potential additive effects to manage shoot blight severity.

Objective #3: Test whether non-pathogenic *Erwinia amylovora* proposed biocontrol strains can block fire blight disease symptom development caused by normal *E. amylovora* on flowers on potted apple trees in the greenhouse.

Objective #4: Test whether non-pathogenic *Erwinia amylovora* proposed biocontrol strains can cause disease on pear fruits.

Objective #5: Test whether high concentrations of natural, epiphytic bacterial strain *Pantoea agglomerans* can block *E. amylovora* growth and fire blight disease development on apple flowers, both detached flower and flowers on trees growing in the greenhouse.

Objective #6: In the case of favorable results from Objectives 1 & 2, Dr. McNellis will reapply for a USDA-APHIS permit for field testing of non-pathogenic *E. amylovora* strains for biocontrol of fire blight blossom blight.

Procedures:

Objective #1 Procedures (Peter Program): In the greenhouse, applications of glycerol, Regalia, and Vacciplant will be tested alone at different timings to determine which application timing would be most effective at reducing shoot blight. Treatments will be applied 14 days pre-inoculation, 10 days pre-, 7 days pre-, 4 days pre-, 4 and 2 days pre-, 2 pre- and 2 days pre- and post-inoculation, and 2 days post-inoculation. Four shoots per tree will be inoculated by wounding shoot tips with a scalpel dipped in a suspension of *Erwinia amylovora*, strain Ea273, adjusted to 10^7 cfu/mL (Fig 2). Shoots will be evaluated for severity of fire blight approximately 7 and 14 days after inoculation. Severity will be assessed as a percent of blight symptoms down vs total shoot length as well as with a rating scale of 0-7, with 0 being no infection and 7 being the most severe.

Objective #2 Procedures (Peter Program): In the field, applications of Actigard, Apogee (2 oz/A), Cueva, Regalia, and Vacciplant will be tested alone or in combination to determine what would be most effective at reducing shoot blight in a field setting. Treatments will be applied 4 and 2 days pre-inoculation and shoot inoculations will be carried out with the same method as the greenhouse study. To determine effectiveness of the products on a newly established block vs. an established block, treatments will be applied in a 2018 planted Gala/M.9 block; 2015 planted Gala/M.7 block; and a 2011 Gala/M.9 block. Shoot inoculations will be carried out two to three weeks after bloom. Programs: 1) Untreated; 2) Actigard (2 oz/A); 3) Apogee (2 oz/A); Regalia; Apogee + Regalia; 4) Glycerol; 5) Glyecerol + Cueva; 8) Vacciplant.

Objective #3 Procedures (McNellis Program): Third leaf 'Fuji' trees on M.9-Nic29 rootstocks will be potted and grown in the greenhouse at Penn State University Park. Flowers will be sprayed with 10^{10} cfu/ml of non-pathogenic *E. amylovora*, either the Δ*argD* mutant, which is unable to synthesize the essential amino acid arginine, or a Δ*leuB* mutant, which is unable to synthesize the essential amino acid leucine. After 4 hours, all flowers in an individual cluster will be inoculated with about 2,000 cells of virulent *E. amylovora* by applying a droplet of 20 µL of 10^5 cfu/ml of the bacteria to the base of the flower hypanthium or to the stigma using a micropipette. Control flowers will include mock-inoculated flowers, flowers inoculated with virulent *E. amylovora* without pre-treatment, and untouched flowers. Flowers in one cluster will receive only one treatment type. Incidence of blossom blight within each flower cluster will be monitored for two weeks after inoculation. This will include observation and rating for degree of necrosis in the flowers. This experiment will tell us whether the non-pathogenic *E. amylovora* strains selected as potential biocontrols will produce any blossom blight disease on intact trees, which will be important information for application for field testing of this as a potential fire blight biocontrol.

Objective #4 Procedures (McNellis Program): Immature pear fruitlets will be collected from FREC trees and inoculated using standard procedures. Pear fruitlets will be inoculated with both the Δ*argD* and Δ*leuB* mutants, since preliminary data suggests that the Δ*leuB* mutant may be more completely compromised in virulence on pear than the Δ*argD* mutant. Starting bacterial concentrations will be approximately 2,000 cells per fruit. Fruits inoculated with virulent, wild-type *E. amylovora* and mock-inoculated fruits will be used for controls. Disease severity will be monitored over the course of 7 days, and disease severity will be measured using a quantitative disease rating scale that we developed for apple fruits.

Objective #5 Procedures (McNellis Program): Detached apple and crabapple flowers collected at University Park and at Rock Springs orchards nearby to University Park will be spray-coated with 10^{10}

cfu/ml concentration of a known, non-virulent, epiphytic *P. agglomerans* strain available in Dr. McNellis' lab. After 4 hours, the flowers will be inoculated with about 2,000 cells of virulent *E. amylovora* by applying a droplet of 20 µL of 10^5 cfu/ml of the bacteria to the base of the flower hypanthium or to the stigma using a micropipette. Growth of the virulent *E. amylovora* cells will be quantitatively measured at 2 days post inoculation by grinding up the apple flowers and serial dilution plating of the extracts on LB medium plates. Controls will include flowers inoculated with virulent *E. amylovora*, but without *P. agglomerans* pre-treatment as a negative control, and flowers inoculated with non-virulent *E. amylovora* as a positive control.

Objective #6 Procedures (McNellis Program): If results are favorable from Objectives 1 & 2, that is, if the Δ*argD* and Δ*leuB* mutants do not cause significant or detectable disease in pears or apple flowers, Dr. McNellis will submit an application to the USDA-APHIS for approval of field testing of non-pathogenic *E. amylovora* strains for fire blight biocontrol. This objective does not depend on whether fire blight disease inhibition is observed in Objective #1; the only way to really know whether biocontrol is effective in the field will be to do a field test under natural infection conditions.

Peter Budget:

Wages for part-time seasonal employee ($9.23/hr, 20 h/wk, 13 weeks):	$2,400
Wages fringe benefits (7.81%)	$188
Materials and supplies:	$500
Travel	$1,000
Total requested:	**$4,088**

McNellis Budget:

Summer 2019 graduate student stipend (1/2 time, grade 12)	$6,730
Graduate student fringe benefits (7.81%):	$526
Laboratory supplies:	$1,000
Total requested:	**$8,256**

Total amount requested: $12,344

Budget Justification:

Salaries/Wages - $9,130: Funds are requested for 2019 summer support for a graduate student, who will conduct the McNellis Lab portion of the project; and a seasonal technician to assist with the Peter Lab portion of the project.

Fringe Benefits - $714: Fringe benefits are computed using the fixed rates of 38.97% applicable to Category I Salaries, 14.74% applicable to Category II Graduate Assistants, 7.81% applicable to Category III Salaries and Wages, 0.18% applicable to Category IV Student Wages, and 25.34% for Category V, Postdoctoral Scholars and Fellows, for fiscal year 2018 (July 1, 2018, through June 30, 2019). If this proposal is funded, the rates quoted above shall, at the time of funding, be subject to adjustment for any period subsequent to June 30, 2019, if superseding Government approved rates have been established. Fringe benefit rates are negotiated and approved by the Office of Naval Research, Penn State's cognizant federal agency.

Materials and Supplies - $1,500: Funds are requested for microbiological media, plates, laboratory disposables, and antibiotics for the flower biocontrol research. Expenses for materials were determined from catalog prices using Penn State's eBuy. These funds will also cover the costs of trees, potting mix, pots, fertilizer, and greenhouse other greenhouse related costs.

Travel - $1,000: Funds are requested to support travel (transportation, hotel, meals) to present this research at regional and national meetings. All travel will be in accordance with University travel regulations and mileage will be charged at the current rate on the date of travel.