State Horticultural Association of Pennsylvania **Research Grant Proposal**

Title: Assessing the short and long-term effects of persistent spotted lanternfly (*Lycorma delicatula*) feeding on young apple and peach trees

Personnel:

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Duration of Project: 3 years (May 1st, 2021-October 31st, 2024)

Justification:

Pest status. Spotted lanternfly (SLF), *Lycorma delicatula*, is an invasive insect species that is now established in nine states in the mid-Atlantic region since its first detection in Berks county, PA in 2014 (Barringer et al. 2015, Dara et al. 2015, NYS IPM 2019). Predictive models have shown that many regions of the USA are climactically suitable for SLF establishment (Jung et al. 2017, Wakie et al. 2019). Spotted lanternfly overwinter as eggs, hatch in the spring and begin feeding on over 70 host plants (Barringer and Ciafré. 2020). As they mature, their host range narrows to include a few key hosts including the invasive tree of heaven (TOH) *Ailanthus altisima*, black walnut and grape, but they have also been observed feeding on both peach and apple trees, along with some other tree fruit, during the adult stage in PA. Recent data acquired at the USDA-ARS shows that newly hatched nymphs can survive and develop through to second instar on peach trees alone and third instar on apple trees alone. The same study showed that both these cultivated hosts combined with TOH support the development of SLF through to the adult stages, and SLF were observed feeding on the apple and peach trees within these mixed host cages. In a recent preliminary study from Penn State where 4th instar SLF nymphs were placed on peach shoots bearing fruit, SLF feeding led to a marginal decrease in fruit size (by 8%) and an increase in fruit drop incidence (15%). Moreover, the effect of SLF on newly planted trees has not been evaluated.

Current and potential management strategies for SLF. Federal and State Departments of Agriculture have moved to quarantine and eradicate SLF as it has been detected in each state. Eradication processes have focused on TOH, a preferred host from their native range; removing this tree where possible and applying trunk treatments of systemic insecticides to remaining TOH in infested areas. The public are also encouraged to keep an eye out for SLF and dispose of the insects and egg masses whenever possible. Potential biological controls have already been identified for use on SLF populations.

An entomopathogenic fungus, *Beauvaria bassiana*, was identified in wild SLF populations and has been applied in VA and PA in 2019 and in 2020, though is largely still at the research stage. An egg parasitoid (*Anastatus orientalis)* is currently being evaluated for potential introduction into the U.S. for biological control of SLF, but efficacy and non-target effects are needed before any release is approved. Insecticide studies with foliar applications have shown that a wide range of compounds have excellent SLF kill, but only a few products within the Pyrethroid class provide residual activity (bifenthrin, beta-cyfluthrin, fenpropathrin). In PA vineyards, after an insecticide application is made, SLF present in the vineyard are easily killed by the spray application, but as with another invasive, brown marmorated stink bug, new adults continuously move into the crop from wild host habitat and begin feeding (Leach et al. 2019). Additionally, increased incidence of secondary pests including mealybug and leafhoppers have already been noted in vineyards treated for SLF.

Plant health decline. SLF is a phloem feeder; persistent feeding by dense populations of this species can lead to host plant health decline, and, in severe cases, plant death. There has been a broad range of host plants reported in the invaded range, including maples, wild and cultivated grapes, and fruit trees. In the case of cultivated grapes, this pest species has already caused serious losses in PA vineyards. In another invaded range, South Korea, decline of trees has also been reported. SLF feeding on grapevines has been associated with reduction in photosynthesis, sap flow, starch storage in roots, and reduced cold tolerance of buds. In other crops, reduced growth (brambles) and yield (cucumber, hops) has been noted. As well as the direct feeding effects on plant health, SLF can indirectly harm plants. SLF produce honeydew as they feed which coats surrounding foliage and propagates the growth of black sooty mold, inhibiting photosynthesis. The extent to which feeding (i.e., stylet penetration) mechanically damages the vascular system of the plant and may potentially increase disease susceptibility at sites of physical injury has not been evaluated to-date. Preliminary findings from an examination of tree cores in VA and PA suggests that SLF may exert stress leading to long term impacts on tree growth.

Establishment of young trees. Given their wide and fast spread in the Northeastern US, the establishment of SLF populations near fruit tree orchards is inevitable. However, the effects of SLF infestation on apple and peach, two of the predominant fruit crops in the mid-Atlantic, are unclear. Recently published results of grapevines suggest that by feeding on resources (carbohydrates) in the phloem, SLF may reduce winter hardiness of vines and thereby increase bud damage due to low temperatures (Leach and Leach, 2020). Therefore, whether SLF populations cause such effects and/or other damages in apple and peach trees awaits determination, particularly in young plantings, which are highly sensitive to cold injury. Even if populations can be managed to prevent persistent feeding, there are multiple risks posed by even initial feeding. Resource (i.e., carbohydrate) loss may cause prolonged stress conditions. This kind of stress has been shown to contribute to secondary problems in fruit trees including potential pathogen entry or attack by Ambrosia beetles as has been on the increase and associated with the "Rapid Apple Decline" syndrome.

There are funded SLF projects aiming to define short term feeding effects on a variety of economically important tree species, including apples and peaches. However, these projects are focused on quarantine laboratory settings or with potted plants, and do not evaluate the effects of persistent population pressure, impacts to nutrient availability, the longer-term effects of feeding, or potential interactions with other pests or pathogens. Certainly, these types of studies are incapable of being measured in a quarantine laboratory and can be challenging in the field as well. With the recent and unfortunate spread of SLF populations in VA and WV, this enables us to study longer-term impacts on young fruit trees in orchards.

The research outlined herein is justified based on a need to establish if: 1) SLF populations can persistently feed and survive on peach and apple trees; 2) this feeding has short-and long-term consequences on young tree survival and growth; 3) there is a particular SLF life stage that is more destructive. ***Ultimately, it is critical to establish the risks that SLF poses to mid-Atlantic fruit growers.***

Objective(s):

Years 1-2

1. Track survivorship of SLF on single tree host diets (apple and peach) in the field on small potted trees and use these data to establish their pest status on these crops.

2. Establish if short-term, acute effects of SLF feeding occur on young peach and apple trees, including growth, fruit quality and retention, nutrient availability, and tissue damage at feeding sites.

Years 2-3

3. Assess longer-term effects of SLF feeding on young apple and peach trees under field conditions.

Procedures:

Study Phases. This study will be conducted in three phases. *PHASE ONE* will begin in the early spring of 2021. Young, fruiting apple and peach trees will be potted in 25-liter pots containing soil during the early spring and transported to the quarantine zone. Cultivars and rootstock combinations will be chosen based on availability and among those commonly planted in the mid-Atlantic. Plants will be fertilized, watered and maintained according to guidelines for fruiting trees in 2021 Spray Bulletin for Commercial Tree Fruit Growers. *PHASE TWO* will begin after SLF hatch from egg masses. For *PHASE TWO*, different lifestages and durations of SLF feeding exposure will be imposed on potted trees. Following these exposure periods, *PHASE THREE-WV* will then begin for WV. At the end of each exposure interval, trees will be treated with a knockdown insecticide (e.g. carbaryl) to kill any remaining SLF. Post-exposure trees will all be maintained with irrigation in their pots until they are transplanted into the ground in November for *PHASE THREE-WV*. In November, trees will then be inspected by a certified inspector and moved to a final location for planting to measure short- and longer-term impacts in 2022 and 2023. As this funding is approved on an annual basis, we acknowledge that longer-term measurements proposed here will still require review and authorization. However, we foresee PHASES ONE and TWO (conducted in Year 1) producing data that will help define the risk of SLF feeding within young orchards. In PA, PHASE ONE and TWO will be the same as described above. However, because the research location in PA does not allow for long-term in-ground planting of trees, *PHASE THREE-PA* will be the sampling of plant tissues (shoots, trunk, roots) for nutrient analysis to be conducted at AFRS. Fed-upon tissue will also be sampled (leaves, stems, trunk) to analyze the presence of mechanical feeding injury. Microscopic examination of fixed tissues will reveal punctures or breakage in membranes in various plant cell types; staining of salivary sheaths deposited as SLF fed will reveal how SLF penetrates various tissues and differentially accesses plant resources across life stages.

Objective 1. Track survivorship of SLF on single tree host diets (apple and peach) in the field on small potted trees and use these data to establish their pest status on these crops.

In 2021, potted peach and apple trees will be transported to Winchester, VA and Reading, PA and placed in individual mesh structures (75 x 75 x 115 cm). Wild collected SLF will be introduced into the cages and, twice weekly, the number of dead SLF will be recorded and replaced with an equal number of live SLF. Densities evaluated will include 50 nymphs and 30 adults; these relative densities are intended to represent those observed in vineyards. In PA, additional levels will be tested (100 nymphs and 60 adults) to focus on more extreme population pressure. Lifestage cohorts and exposure periods evaluated include: 1st and 2nd instars for four weeks, 3rd and 4th instars for four weeks, adults for four weeks, and all lifestages season-long (see Table 1 for experimental timeline).

Objective 2: Establish if short-term, acute effects of SLF feeding occur on young peach and apple trees.

To establish immediate effects of SLF feeding and evaluate the impact it could have on young trees, peach and apple trees exposed to SLF in Objective 1 will be assessed and compared to the controls (trees with no SLF exposure). Prior to and during exposure to SLF (*PHASES ONE* and *TWO*, respectively), growth parameters (trunk diameter, plant height, and canopy size) and physiological attributes (leaf chlorophyll readings, photosynthesis rate, and water status) of all trees will be evaluated on a weekly basis, with leaves collected for non-structural carbohydrate analysis. Additionally, visual assessment of foliage wilt, gummosis at feeding sites, etc. will be recorded. *PHASE THREE-WV* will then begin as trees will then be treated with insecticide to kill all SLF following their exposure interval. Trees will continue to be maintained in the same conditions (being potted in cages at the field site with drip irrigation) until all exposure treatments are completed. *PHASE THREE-PA* will begin at the end of the season with destructive sampling of trees for tissue analysis and mechanical feeding injury analysis. For analysis of micro and macro-nutrients, tissue samples will be sent to a PSU lab service. For mechanical feeding impacts, tissue samples will be sectioned, fixed, stained, and examined at Penn State's microscopy core facility.

Objective 3: Assess long-term effects of feeding on transplanted apple and peach trees and inspect for any symptoms of stress and decline.

Trees will be double inspected by a certified plant inspector to ensure no SLF are present and allow legal transport of the trees. All trees will be transported to and planted at the Appalachian Fruit Research Station in Kearneysville, WV in late September and early November, respectively. Trees will be planted in a randomized block design to ensure there are no plot location effects. In 2022 and 2023, horticultural parameters of peach and apple trees will be assessed, including times of bud break and bloom in spring, growth and physiological measurements throughout the growing season (described in Objective 2), and yield and fruit quality at harvest. To assess the effect of SLF exposure on cold hardiness of buds, shoots with buds will be sampled during the dormancy period. Visual inspection for common stress effects, such as leaf wilt, dieback and presence of Ambrosia beetle, will be carried out monthly. Should SLF population spread into Jefferson County, WV, presence of SLF feeding on these blocks will also be monitored.

Overall Budget: **Total= $47,315**

Year 1 **Total = $24,785**

Salaries	$1,790	Supplies	$6,860 (Total)
Hourly Wages	$7,625	Peach trees	$1,500
Fringe Benefits	$3,700	Apple trees	$1,500
Travel	$0	Cage supplies	$2,500
Miscellaneous	$4,810 (Total)	Plant pots (25l)	$110
Microscopy time	$250	Plant tissue prep.	$750
Laboratory analysis	$4,560	Histology supplies	$500

Year 2 **Total = $11,265**

Salaries	$1,830	Supplies	$0
Hourly Wages	$5,865		
Fringe Benefits	$3,570		
Travel	$0		
Miscellaneous	$0		

Year 3 **Total = $11,265**

Salaries	$1,830	Miscellaneous	$0
Hourly Wages	$5,865	Supplies	$0

Fringe Benefits $3,570
Travel $0
Other Support: Post-doctoral salary and labor associated with tree planting and maintenance will be associated with USDA-NIFA SCRI grant.

Table 1 Experimental design and timeline for SLF exposure to young potted peach and apple trees. Tree numbers will be split equally between WV and PA trials.

Year	# Apple trees	# Peach trees	Set up date	SLF #	Treatment	Length of exposure
2021	40	40	13-May	0	Control	Season-long (12 weeks)
	40	40	13-May	50	1st-Adult	Season-long (12 weeks)
	40	40	20-May	50	1st/2nd instar	4 weeks
	40	40	24-Jun	50	3rd/4th instar	4 weeks
	40	40	5-Aug	30	Adults	4 weeks

Literature Cited

Barringer, L.E., Donovall, L.R., Spichiger, S.E., Lynch, D. and Henry, D., 2015. The first new world record of *Lycorma delicatula* (Insecta: Hemiptera: Fulgoridae). *Entomological news*, *125*(1), pp.20-24.

Barringer, L.E. and Ciafré, C.M., 2020 Worldwide feeding host plants of spotted lanternfly, with significant additions from North America. *Environmental Entomology, 49(5)*, pp. 999–2011

Dara, S.K., Barringer, L. and Arthurs, S.P., 2015. *Lycorma delicatula* (Hemiptera: Fulgoridae): a new invasive pest in the United States. *Journal of Integrated Pest Management*, *6*(1), p.20.

Jung, J.M., Jung, S., Byeon, D.H. and Lee, W.H., 2017. Model-based prediction of potential distribution of the invasive insect pest, spotted lanternfly *Lycorma delicatula* (Hemiptera: Fulgoridae), by using CLIMEX. *Journal of Asia-Pacific Biodiversity*, *20*(4), pp.532-538.

Leach, H., Biddinger, D.J., Krawczyk, G., Smyers, E. and Urban, J.M., 2019. Evaluation of insecticides for control of the spotted lanternfly, *Lycorma delicatula*, (Hemiptera: Fulgoridae), a new pest of fruit in the Northeastern US. *Crop Protection*.

Leach, A. and Leach, H., 2020. Characterizing the spatial distributions of spotted lanternfly (Hemiptera: Fulgoridae) in Pennsylvania vineyards. *Scientific Reports*. 20, 20588.

New York State Integrated Pest Management 2019 https://nysipm.cornell.edu/environment/invasive-species-exotic-pests/spotted-lanternfly/

Wakie, T.T., Neven, L.G., Yee, W.L. and Lu, Z., 2019. The establishment risk of *Lycorma delicatula* (Hemiptera: Fulgoridae) in the United States and globally. *Journal of Economic Entomology*.