

WORK PLAN

Plant Protection and Quarantine, Science and Technology

Title: Establishing Parameters for Selecting Appropriate Spotted Lanternfly Biosurveillance Techniques

Prior Year FAIN: N/A

Period of Performance: September 1, 2026 – August 31, 2027.

I. Introduction

This Work Plan reflects a cooperative relationship between USDA-ARS (Cooperative PI Leskey), University of Maryland (PI Nixon) and the Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ). It outlines the project specific goals, objectives, and anticipated accomplishments as well as the approach for assessing spotted lanternfly biosurveillance methods and establishing best practices and costs, and the related roles and responsibilities of the parties as negotiated.

II. Background

Purpose The spotted lanternfly (SLF), *Lycorma delicatula*, (Hemiptera: Fulgoridae) is a phloem-feeding invasive, regulated insect native to China that has known, established populations in 18 Eastern US states. SLF poses a serious threat to grapevine crops, as our current research has demonstrated that SLF feeding significantly reduces yields on wine grape, *Vitis vinifera*, varieties after a single year. It is also a costly issue for the green industry in states which require full inspections for movement of plant material. Our proposed project aligns with Goal 3, Strategies 1 - to develop/improve all aspects of early detection technologies and resources; and 2 - to develop, validate, or improve diagnostic methods, including molecular-based assays and other technologies, to detect and/or identify plant pests. Specifically, our objective is to compare sensitivity, labor needs, and cost for biosurveillance techniques for SLF.

Overview Techniques that can be used to detect the presence of SLF include visual scouting, traps, and eDNA (environmental DNA). Each of these techniques have strengths and weaknesses. Visual scouting requires trained observers to search the landscape for SLF presence; this approach can be labor-intensive and less effective under low SLF relative densities. Trapping relies on circle traps deployed ~1m above the ground around the trunk of a host tree. While circle traps provide a mechanism to sample for SLF walking up host trees continuously, they should be checked on a weekly basis to maintain the integrity of the trap due to detritus and degradation of samples over time. More importantly, no reliable olfactory lures are available to enhance attraction to traps, reducing the likelihood of capture under low relative densities of SLF. Alternatively, eDNA can serve as a tool for detection under low relative densities but requires a large labor input as trees must be sampled with wet paint rollers to pick up the environmental DNA left behind by foraging insects, the rinsate filtered, DNA extracted, and analyzed using qPCR in the laboratory. Additionally, abiotic conditions such as rainfall or heavy dew can reduce the likelihood of detection using this method. Recently, we have developed an

alternative sampling method in which foraging ants are captured in falcon tubes baited with cotton balls containing honey or protein-based liquids. This technique relies on local ant species foraging on SLF honeydew and, therefore, retaining some SLF DNA; this reduces labor inputs as the method requires ~30 mins of sampling time before transport to the laboratory for DNA extraction and qPCR. Here, we aim to compare each of these sampling techniques (visual scouting, traps, eDNA and ant-sampled eDNA) for sensitivity, reliability, labor and cost to provide practitioners with strengths and weaknesses of all techniques to guide their biosurveillance efforts.

Literature Review and Prior Work of PIs Despite extensive studies identifying SLF attractants (Cooperband et al. 2019; Cooperband and Murman 2022), there is not a lure that reliably attracts SLF in the environment (Nixon et al. 2020). Several passive trap designs have been evaluated for efficacy, with the circle trunk trap being the most effective for capturing SLF whilst reducing non-target capture (Francese et al. 2020; Nixon et al. 2020). Deploying traps on the tree of heaven host is the most reliable for monitoring presence of SLF populations when compared to other secondary hosts (Nixon et al. 2023a, 2023b), however this is not reliable or sensitive enough for use in low population or frontier population regions. As such, eDNA methodology has been developed utilizing rinsate from host trees to detect SLF DNA left behind in honeydew excrement (Valentin et al. 2020; Patterson et al. 2022). Although this method detects SLF in an area prior to visual or trap confirmation (Allen et al. 2021), the sampling methods developed still rely on selecting the correct tree/s. A novel method for eDNA has been developed utilizing natural ant foraging; when ants forage on SLF honeydew, they retain SLF DNA for up to 5 days which can be extracted (Lin et al. 2025a). Field trials show that by using honey-baited lure stations, ants captured up to 100 m from known SLF populations retained SLF DNA (Lin et al. 2025a).

Site selection for SLF monitoring (independent of selected method) is generally dependent on presence of preferred host plants, e.g. tree of heaven, grapevines, black walnut, weeping willow (Uyi et al. 2021; Nixon et al. 2023a, 2023b). Despite initial reports of over 70 host plants in the US (Barringer et al. 2020), further research shows the selection of preferred and common host plants is much narrower (Nixon et al. 2022b), especially for reliable detection purposes (Nixon et al. 2023a, 2023b). Further evaluation of preferred hosts should continue to optimize site selection for biosurveillance.

PIs Leskey and Nixon developed trap designs and trapping protocol for SLF early in the US invasion (Nixon et al. 2020, 2023a) and have been actively involved in the progression and ground-truthing of eDNA sampling methods. Additionally, they have performed and published several studies on SLF host utilization, including greenhouse rearing, survivorship studies, and field behavioral trials (Elsensohn et al. 2023; Nixon et al. 2022a, 2022b, 2023b). Co-PI Yang developed the ant-sampled eDNA method, and has optimized field protocol in collaboration with PI Leskey (Lin et al. 2025a, b) and co-PI Gross.

III. Goals and Objectives

Objective 1. Compare sensitivity, labor needs and cost for biosurveillance techniques for SLF.

Goals: To generate science-based recommendations for spotted lanternfly biosurveillance by comparing the sensitivity, reliability, labor requirements, and costs of visual surveys, trapping, environmental DNA, and ant-sampled eDNA detection methods.

1-a. Evaluate and compare the detection sensitivity and reliability of visual surveys, circle traps, environmental DNA (eDNA), and ant-sampled eDNA methods for spotted lanternfly surveillance across sites with varying infestation levels.

1-b Quantify and compare the labor requirements and operational costs associated with visual surveys, circle traps, environmental DNA (eDNA), and ant-sampled eDNA methods to identify practical surveillance options for different management scenarios.

IV. Milestones

Objective 1: Compare sensitivity, labor needs and cost for biosurveillance techniques for SLF.

Milestone	Quarter					Performance Measure
	4 (FY 26)	1 (FY 27)	2 (FY 27)	3 (FY 27)	4 (FY 27)	
Identify low and high relative density SLF sample sites	x	x				Study sites selected
Deploy trapping at sites prior to SLF hatch			x			Set up circle traps, measure and flag scouting area
Weekly (visual scouting and circle traps) and Biweekly site visits for data collection (eDNA and ant-sampled eDNA) samples collected				x	x	Prepare all eDNA sample collection materials, collect samples, visual scout counts, trap checks
Analyses of all eDNA samples				x	x	Extract samples from eDNA collection, preserve ant samples, ship to VT, full DNA extraction and analysis procedure
Prepare manuscript					x	Collate all data throughout experiment, perform analyses, write manuscript

V. Methods

Obj. 1. Compare sensitivity, labor needs and cost for biosurveillance techniques for SLF.

Methods. We will conduct sampling in two locations – one with known high relative densities of SLF and a second with probable SLF presence but likely harboring low relative densities. At each location, five sites (~ 4 m²) containing at least two SLF host trees will be selected. In early April, a circle trap will be deployed on one of the host trees in each site. The additional tree in each site will be designated for sampling eDNA (both standard and ant-sampled eDNA methods). Weekly, circle traps will be checked for presence of SLF nymphs and adults, and time required for total sampling and trap maintenance will be recorded. Additionally, a 5-minute visual survey will be conducted in each site noting presence/absence sampling, as well as total number of nymphs and adults observed. Every two weeks, standard eDNA and ant-sampled eDNA samples will be taken. The designated host trees will be sampled separately using a wetted sterile paint roller, which will then be rinsed with deionized water and the rinsate filtered using 10-micron polycarbonate track-etched filters and a peristaltic pump. DNA on filters will be fixed *in situ* with 100% ethanol for transport. DNA from filters will then be extracted using commercially available kits, amplified with SLF-specific primer, and analyzed in triplicate using SLF-specific qPCR to inform presence/absence of SLF. Total time required to collect and process samples will be recorded. Finally, ant-sampled eDNA also will be collected. For ant-sampled eDNA, three 15-meter transects of honey-baited lure stations will be deployed, radiating from the eDNA-designated tree. Each transect will include three lure stations, spaced 5 meters apart. Ants collected at each station will be preserved in 100% ethanol and transported to the Gross Lab and Yang Lab for DNA extraction and SLF-specific qPCR analysis in triplicate. To evaluate reproducibility, samples will be distributed between the two laboratories using a rotating cross-lab design. During each sampling event, one site from each location will be selected as a subset for cross-laboratory processing, while the remaining samples will be processed by the assigned laboratory. For example, Yang Lab will process 18 lure samples plus a subset of ants from nine additional lures from one location, while Gross laboratory will process the reciprocal set from the second location. Laboratory assignments will be alternated among sampling events so that each location is processed by both laboratories over time. Virginia Tech will receive samples from Nixon and Leskey Labs and coordinate sample dispatch between the Gross and Yang laboratories. Again, the total time required for samples to be collected and processed will be recorded. Sampling will occur until late October at both locations. Data on total cost, labor requirements, and sampling sensitivity will be compared among the four treatments using appropriate statistical methods.

Outcomes. These data will contribute to Goal 3/Strategies 1 and 2 to enable practitioners to better select biosurveillance tools based on available labor and funding levels, as well as overall sensitivity of the technique.

VI. Deliverables

- Sensitive and streamlined biosurveillance protocol for spotted lanternfly detection will be identified
- Experiments in Objective 1 and 2 will be published as one full manuscript (1) and one short communication (2) in peer reviewed scientific journals

- A one-pager will be produced from the results of Objective 1 on the sensitivity and costs of different biosurveillance methods for stakeholders and regulatory personnel to consult

APHIS has the right and hereby requests review of ALL manuscripts/reports/presentations developed from this agreement prior to their release/publication. The cooperator is responsible for submitting the materials to the ADODR, allowing a reasonable amount of time (2 weeks minimum) for the review. The ADODR is responsible for following the appropriate review PPQ processes to obtain clearance.

VII. Budget – [Complete S&T Cooperative Agreement Budget Worksheet](#) The completed excel spreadsheet should be submitted along with this document.

VIII. Data – Note: All data will be made available to USDA APHIS upon request.

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