

# What you eat is what we need: using ants to detect spotted lanternfly (*Lycorma delicatula*) DNA

Wei-Jiun Lin,<sup>a,b</sup> Fang-Ling Chloe Liu,<sup>b</sup> Xun-Yi Huang,<sup>a</sup>  
Alejandro I. Del Pozo-Valdivia,<sup>b,c</sup> Tracy C. Leskey<sup>d</sup> and  
Chin-Cheng Scotty Yang<sup>b\*</sup>



## Abstract

**BACKGROUND:** Early detection of invasive species such as the spotted lanternfly (SLF, *Lycorma delicatula*) is critical for effective management including eradication efforts and limiting further spread. SLF excretes honeydew containing detectable DNA, providing a unique opportunity to leverage environmental DNA (eDNA) for its detection. This study introduces the ant-derived DNA (antDNA) approach, utilizing ants as 'honeydew samplers' to detect SLF DNA. We validated the effectiveness of this method through three experiments.

**RESULTS:** Using SLF-specific polymerase chain reaction (PCR), we consistently detected SLF DNA in ants foraging or nesting near SLF infestations. We then showed that after a single honeydew meal, SLF DNA persisted in ants for at least 5 days, even when, subsequently, ants were fed plain honey solution. Lastly, ants collected from honey-baited lure stations along transects radiating from SLF infestations yielded positive detections up to 100 m from the core infestations, demonstrating the method's extensive detection range. These findings confirm that ants, through their active foraging and feeding on environmental honeydew and ability to retain the ingested material, are highly reliable SLF DNA samplers.

**CONCLUSION:** Combined with ants' ecological dominance and the ease and low cost of ant collection, the antDNA method offers a sensitive, efficient and practical alternative to traditional, labor-intensive approaches for detecting SLF and potentially other honeydew-producing insects.

© 2025 The Author(s). *Pest Management Science* published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.

Supporting information may be found in the online version of this article.

**Keywords:** ant-derived DNA (antDNA); environmental DNA (eDNA); spotted lanternfly (SLF); invasive species; pest detection and monitoring

## 1 INTRODUCTION

Early detection of invasive pest species is crucial to prevent additional spread and damage when they enter a new region.<sup>1</sup> Identifying invasive pests at the initial stages of invasion enables effective management including the potential for eradication.<sup>2,3</sup> However, detecting newly introduced species can be challenging, particularly when they are at low-densities, of small in size, or cryptic nature,<sup>4</sup> making them difficult to observe in the field. Recent advancements have focused on developing methods sensitive for low-density detection, including remote sensing technologies and environmental DNA (eDNA) analysis.<sup>5</sup> These approaches enhance detection accuracy in difficult-to-monitor environments, aiding in early intervention and improving invasive species management.

Recently, eDNA has emerged as a powerful tool for detecting invasive species primarily due to its sensitivity.<sup>6,7</sup> This method involves collecting and analyzing DNA that organisms shed into

their environment, such as water<sup>8</sup> or soil.<sup>9</sup> By extracting and identifying species-specific DNA fragments using molecular techniques such as endpoint polymerase chain reaction (endpoint

\* Correspondence to: C-CS Yang, Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA, E-mail: [scottyyang@vt.edu](mailto:scottyyang@vt.edu)

a Institute of Ecology and Evolutionary Biology, National Taiwan University, Taipei, Taiwan

b Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA

c Hampton Roads Agricultural Research and Extension Center, Virginia Polytechnic Institute and State University, Virginia Beach, VA, USA

d USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV, USA

PCR) or quantitative PCR (qPCR), multiple studies have demonstrated that eDNA can detect invasive species with high sensitivity, accuracy, and robustness.<sup>7,10</sup> The eDNA method for detecting invasive species is particularly advantageous in environments that are elusive or challenging to monitor using traditional methods, such as visual surveillance for an invasive insect in wooded areas, while minimizing potential ecosystem disturbance.<sup>11</sup> Despite its potential for providing valuable data for early detection, the application of eDNA for monitoring species in terrestrial systems has been relatively underutilized, which can be attributed to several challenges. Furthermore, eDNA is vulnerable to contamination and abiotic, environmental factors that facilitate DNA degradation. Indeed, external factors such as daily weather conditions, such as rainfall, have been known to affect eDNA concentrations.<sup>12,13</sup> Also, eDNA sampling must be conducted near the target species' location for accurate detection. These challenges center around obtaining sufficient amounts of eDNA from substrates that are difficult to collect from and effectively concentrate.<sup>14</sup> Given these challenges, developing innovative and efficient approaches for invasive detections remains crucial. As a result, novel eDNA sampling methods have been continually explored, including invertebrate-derived DNA (iDNA)<sup>15,16</sup> and spider webs.<sup>17</sup>

The spotted lanternfly (SLF, *Lycorma delicatula*) is a major invasive pest in the United States, posing serious threats to agriculture, forestry and other ecosystems.<sup>18,19</sup> Early SLF detection is an essential component to prevent its spread and provide early warning in newly invaded regions. Several SLF detection methods have been developed including an eDNA approach. Taking advantage of easily detectable SLF DNA in its honeydew, Valentin *et al.*<sup>20</sup> developed two innovative eDNA aggregation techniques to sample SLF DNA; they included 'spray aggregation' and 'tree rolling' techniques, which overcome limitations mentioned earlier. The 'spray aggregation' technique involves collecting eDNA from the surfaces of leaves, shrubs, and understory foliage by spraying water on target vegetation that may retain SLF honeydew, collecting and filtering the runoff to capture the eDNA. The second technique, tree rolling, is designed to gather eDNA by using a damp cotton roller applied against tree bark and main branches that is subsequently rinsed into a container for filtration. Both methods require large amounts of water for sampling, and extensive filtration, which limits scalability, especially in large or difficult-to-access areas. Furthermore, while the two methods are effective in both low- and high-SLF density areas, the optimal sampling frequency necessary for presence-absence decision-making remains elusive. These limitations highlight the need for a more efficient, and scalable eDNA method that reduces labor and sampling time, increases sampling flexibility, and maintains accuracy and sensitivity.

Invertebrate-derived DNA (iDNA), an innovative extension of eDNA sampling, use terrestrial invertebrates as 'samplers' to detect vertebrate DNA from the organisms they feed on or interact with. Previous studies have shown that invertebrates such as leeches, mosquitoes, biting flies, and dung beetles can serve as effective iDNA samplers to monitor vertebrate populations<sup>21</sup> as these invertebrates exhibit flesh-eating, haematophagous (blood-sucking), coprophagous (feces-eating) or saprophagous (eating dead/decaying organic matter) behaviors.<sup>22</sup> Inspired by the iDNA concept, we propose the use of ant-derived DNA (antDNA) as a novel method to effectively detect SLF DNA in the environment. Given that SLF DNA is detectable in honeydew,<sup>20</sup> we hypothesized that it may remain detectable after being ingested by 'honeydew samplers'. Ants were selected as ideal honeydew samplers due to their abundance,

ease of collection, widespread occurrence across various habitat types,<sup>23</sup> and strong tendency to forage for and feed on honeydew produced by hemipterans, including SLF. Additionally, ants have extensive foraging ranges, increasing the size of the sampling area for detecting SLF DNA and thereby enhancing the probability of SLF detection.

To demonstrate that ants serve as a reliable sampler for SLF DNA detection, we conducted the following experiments: (1) detection of SLF DNA from ants in SLF-infested areas, (2) honeydew DNA degradation rates post-ingestion by ants, and (3) quantifying the detection rate and distance range of SLF DNA from ants in SLF-infested areas. Collectively, these experiments provide the basis for usage of antDNA as an innovative and promising tool to aid SLF detection and monitoring efforts.

## 2 MATERIALS AND METHODS

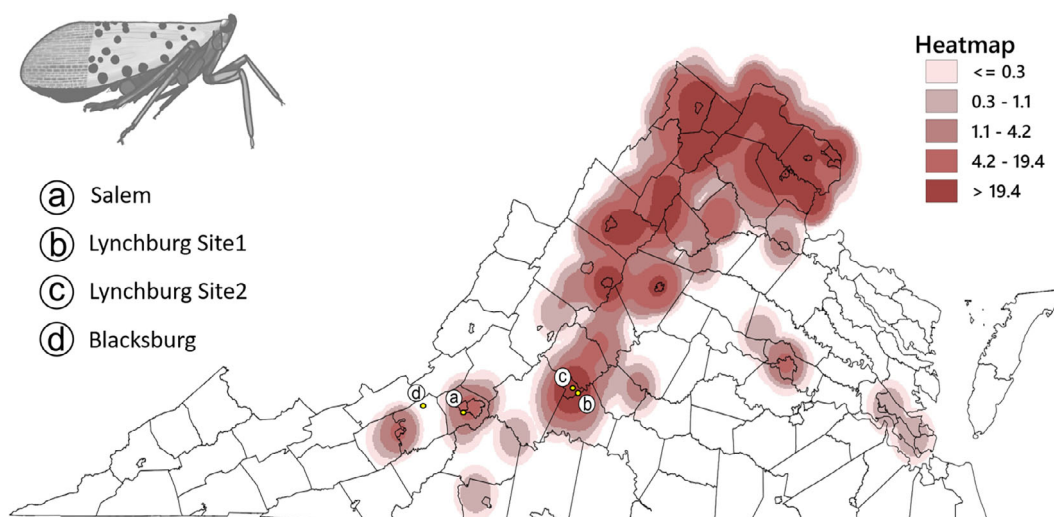
### 2.1 Study sites

We generated a density heatmap of SLF in Virginia, USA, to understand the insect's distribution pattern using a total of 1,487 Global Biodiversity Information Facility (GBIF) occurrence records from July 2019 to August 2024 (<https://doi.org/10.15468/dl.vurasf>) (Fig. 1). The heatmap was then used to validate whether our study sites were located in either an invasion front, core invasion area or non-invaded region. We selected three sites, including two in the city of Lynchburg, VA and one in the city of Salem, VA. Each site contained a single SLF-infested host tree with no other preferred host trees<sup>18</sup> (e.g., Tree of Heaven, *Ailanthus altissima* or black walnut, *Juglans nigra*) within a 200-m radius. This configuration minimized the potential effects of surrounding SLF infestations and allowed us to assess the detection rates and distance ranges of the antDNA method without introducing additional bias (see Section 2.2.4). The Salem site, located along the active SLF invasion front in southwest Virginia, was categorized as a low-density site with only six SLF adults found on one single tree after a thorough search. Lynchburg site 1 was a medium-density site, where 25–30 SLF adults were observed on one single, central tree. Lynchburg site 2 was a high-density site, where more than 100 SLF adults were found on a single, central tree. Both Lynchburg sites were located in the core invasion area in central Virginia (Fig. 1; Supporting Information, Table S1).

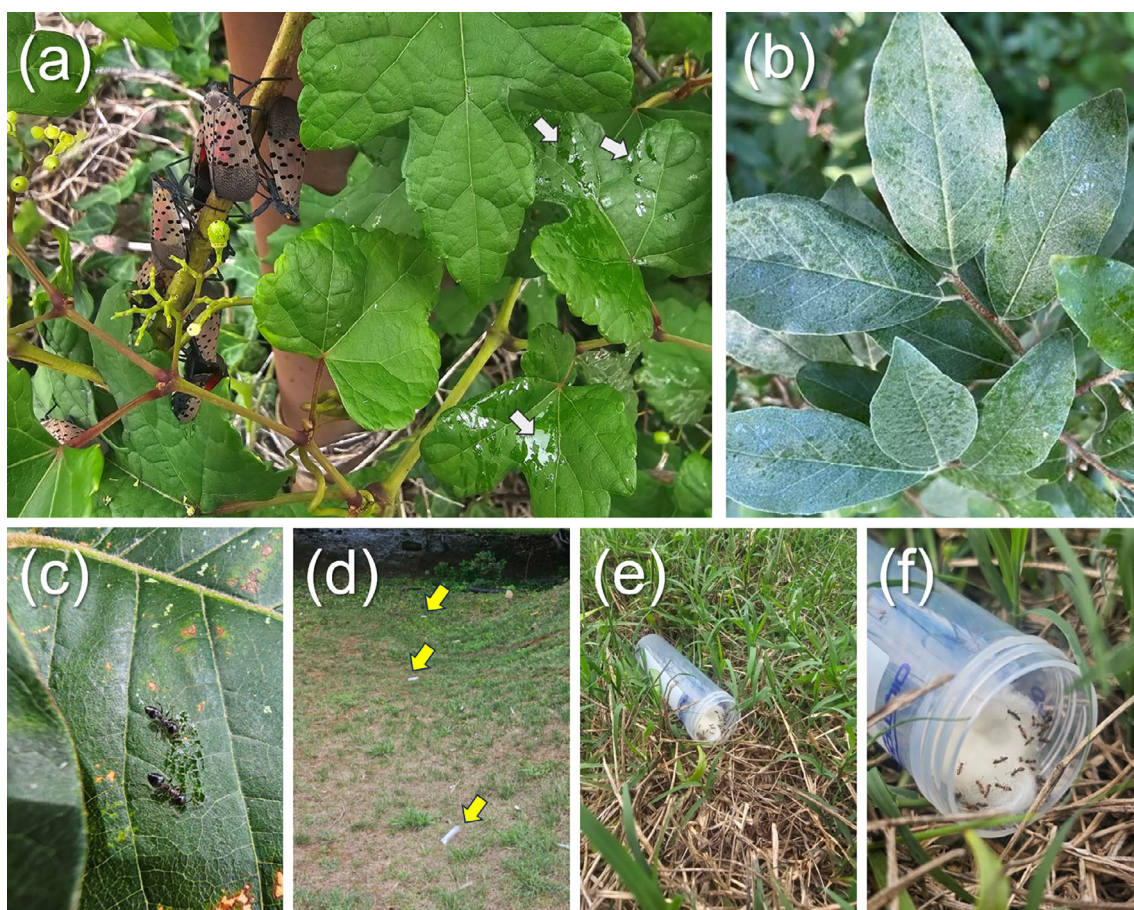
### 2.2 Sample collection

#### 2.2.1 Honeydew solution sample

Instead of freshly excreted honeydew, leaves partially or fully covered with SLF honeydew were collected from beneath the infested tree at each of the three study sites (Fig. 2(a)). Our field observation indicates that ants commonly feed on honeydew found on the leaves of infested trees, where honeydew is most likely a mixture of freshly excreted and older honeydew (Fig. 2(b)). Since freshly excreted SLF honeydew contains detectable DNA,<sup>20</sup> we aimed to determine if dried, 'older' honeydew also serves as a viable source for SLF DNA. Collected leaves were placed in sterilized zip lock bags and returned to the laboratory. Honeydew was rinsed by placing the leaves in a 50 mL Falcon tube with 2 mL of molecular biology grade double-distilled water, followed by vortexing for 5 s. The resulting 'honeydew solution' was then transferred to another sterile 50 mL Falcon tube and either stored at  $-20^{\circ}\text{C}$  for SLF honeydew DNA extraction or at  $4^{\circ}\text{C}$  for ant feeding experiments (see Section 2.2.3.).



**Figure 1.** Density heatmap of spotted lanternfly across the state of Virginia. QGIS 3.26.3 was used to plot the density distribution map utilizing the styled heatmap function (kernel density estimation) in the density analysis tool, with the kernel distance set to 20 km for the density estimation.



**Figure 2.** (a) Fresh spotted lanternfly honeydew on a leaf surface indicated by white arrows; (b) dried and older spotted lanternfly honeydew on a leaf surface; (c) *Crematogaster ashmeadi* workers feeding on spotted lanternfly honeydew on a leaf surface; (d) honey-baited lure stations, indicated by yellow arrows, along a transect established in Lynchburg site 1. Each station was spaced 10 m from each other; (e) lure station setup included a 50 mL Falcon tube containing a cotton ball soaked in 10% honey solution; (f) worker ants attracted to the honey solution-soaked cotton ball in a honey-baited lure station. All photos by Wei-Jiun Lin.

### 2.2.2 Field worker ant samples

To determine whether SLF DNA could be detected in foraging ants or ants collected from nearby nests, ants actively foraging on (Fig. 2(c)) or around the infested tree in Lynchburg site 1 and site 2 (Table S1), as well as those nesting within 5–10-m radius of the infested tree, were collected using aspirators and immediately preserved in absolute ethanol. As control, we also collected foraging ants from an additional site in Blacksburg, VA, where SLF has not been reported (Fig. 1 and Table S1), ensuring these ants had no prior SLF exposure.

### 2.2.3 Worker ants fed with SLF honeydew and tissue

To confirm that SLF honeydew is a primary source of DNA, we collected colonies of a common ant species, *Lasius americanus*, from Blacksburg, VA. Three colony fragments, consisting of at least 30 workers each, were prepared and starved for 72 h (as starvation generally induces constant foraging of worker ants), after which three workers were collected from each fragment as pre-feeding controls. The remaining workers were then fed 1.5 mL of SLF honeydew solution, prepared from leaves, through a 1.7 mL microcentrifuge tube for 24 h. A 10% plain honey solution was then provided after removing the SLF honeydew solution. Another three worker ants were sampled immediately after the plain honey solution was provided (day 1) and every 24 h up to 120 h (day 5). To compare DNA detectability from alternative sources, additional *Lasius americanus* colony fragments were prepared and fed SLF tissue (six legs from freshly killed SLF adults) instead of honeydew solution. Sampling procedures were identical to those used for SLF honeydew solution-fed ants. All sampled ants were preserved in absolute ethanol and stored at  $-20^{\circ}\text{C}$  until molecular analysis.

### 2.2.4 Collection of ant samples from honey-baited lure transects

To validate the detection rates and distance ranges of the antDNA method, we established one to three transects of honey-baited lure stations radiating outward from the infested tree at each site (three at Salem site, two at Lynchburg site 1, and one at Lynchburg site 2). Each lure station consisted of a 50 mL tube (Fig. 2(d)) containing a cotton ball soaked in 10% honey solution. Lure stations were spaced at 5-m intervals at the low- (Salem site) and medium-density site (Lynchburg site 1) and 10-m intervals at the high-density site (Lynchburg site 2) along each transect (Fig. 2(d)). After 30 min, the lure stations were retrieved, and ants were collected, preserved in absolute ethanol, and stored at  $-20^{\circ}\text{C}$  for molecular analysis (Fig. 2(e),(f)). The entire process, from honey-baited lure station deployment to ant specimen preservation, took approximately 40 min to complete per site.

## 2.3 DNA extraction

For SLF DNA detection in honeydew solutions, we followed the protocol described in Laube *et al.*<sup>24</sup> Briefly, the collected honeydew solution was heated at  $65^{\circ}\text{C}$  for 30 min and then centrifuged at  $2400 \times g$  for 30 min. The supernatant was discarded, and the remaining pellet was processed for DNA extraction using the E.Z.N.A. Tissue DNA Kit (Omega Bio-tek, Norcross, GA, USA). For worker ant samples from the various collections outlined earlier, three to ten individual ants of the same species were pooled for DNA extraction, with the number of ants adjusted based on body size. For larger species, such as *Camponotus*, three individuals were used, while for smaller species, such as *Monomorium*, ten individuals were pooled. To prevent contamination from external DNA, all ant specimens underwent surface decontamination

using a bleaching method described in Huszarik *et al.*<sup>25</sup> DNA extraction from these pooled ant samples was carried out using the E.Z.N.A. Tissue DNA Kit following the manufacturer's instructions.

## 2.4 SLF DNA amplification and sequencing

Kim *et al.*<sup>26</sup> developed two primer sets specific to SLF, with one targeting the *NADH dehydrogenase subunit 2* (ND2) and the other targeting the *NADH dehydrogenase subunit 6* (ND6).<sup>26</sup> Our preliminary tests showed that the ND6 primer set (393 bp) consistently outperformed the ND2 primer set (552 bp) in amplifying SLF DNA from ant samples, although both primer sets successfully amplified their respective target genes from SLF tissues. Based on these results, all subsequent endpoint PCRs were performed using the ND6 primer. PCR cycling conditions followed the protocol described in Kim *et al.*<sup>26</sup> except with the annealing temperature adjusted to  $56^{\circ}\text{C}$  to ensure amplification specificity. Endpoint PCR products generated using the ND6 primer set were Sanger sequenced and subjected to BLAST analysis. All resulting sequences matched SLF sequences recovered from GenBank with greater than 99% identity, confirming the specificity and reliability of the ND6 primers for SLF detection. To validate DNA extraction and account for false-negative results, all honeydew solutions and ant samples were also subjected to mitochondrial DNA barcoding PCR using either LCO1490F/HCO2198R<sup>27</sup> or LepF1/LepR1.<sup>28</sup>

# 3 RESULTS

## 3.1 Detection of SLF DNA in field ant samples

Worker ants of nine different species were collected from the SLF-infested tree at Lynchburg site 1 and site 2. Endpoint PCR analysis with the ND6 primer successfully detected SLF DNA in nearly all samples, regardless of whether the ants were collected directly on the tree, foraging near the tree, or from nearby nests. The sole exception was a *Formica pallidefulva* worker foraging near the SLF-infested tree (Table 1). Detection rates were consistently high across species. Foraging ants collected directly on the infested tree exhibited a 100% detection rate, whereas ants foraging on the ground showed detection rates ranging from 75% to 100% (Table 1). In contrast, none of the ant specimens collected at the Blacksburg, VA control site harbored SLF DNA (Table S2).

## 3.2 Honeydew-fed ants retained SLF DNA

The SLF honeydew solution prepared from honeydew-covered leaves showed apparent SLF DNA signals, consistent with the findings of Valentin *et al.*<sup>20</sup> This also confirmed that the honeydew solution used to feed ants in this experiment contained detectable SLF DNA. Colony fragments of *Lasius americanus* fed either SLF honeydew solution or SLF tissue retained detectable SLF DNA signals throughout the 5-day observation period. Ants fed SLF tissue exhibited apparent SLF DNA signals on day 1, which began to decline by day 2 but remained detectable through day 5, as inferred from the electrophoresis gel results (Supporting Information, Fig. S1(b)). In contrast, ants fed SLF honeydew solution demonstrated relatively stable and persistent DNA signals throughout the 5-day period, with slightly stronger bands on the gel observed on day 5 compared with SLF tissue-fed ants (Fig. S1(c)). Furthermore, the provision of plain honey solution following SLF honeydew or tissue feeding did not interfere with the detection of SLF DNA, particularly for honeydew solution-fed ants. These findings indicate that SLF honeydew serves as a more reliable and stable source of SLF DNA for longer-term detection (at least up to 5 days) in ants compared with SLF tissue.

**Table 1.** Ant species collected at all spotted lanternfly (SLF) infestation sites and their SLF DNA detection rates

Species name	Number of ants tested positive for SLF DNA/number of ants collected <sup>†</sup>
<i>Aphaenogaster fulva</i>	A: 3/4 (75%)
<i>Tapinoma sessile</i>	A: 3/4 (75%), O: 2/2 (100%), N: 2/2 (100%)
<i>Monomorium minimum</i>	A: 3/5 (60%)
<i>Camponotus pennsylvanicus</i>	A: 2/2 (100%), O: 3/3 (100%)
<i>Camponotus castaneus</i>	A: 1/2 (50%), O: 2/2 (100%)
<i>Camponotus subbarbatus</i>	A: 2/2 (100%), O: 2/2 (100%)
<i>Formica pallidefulva</i>	A: 0/1 (0%), O: 1/1 (100%)
<i>Formica fusca</i>	A: 2/3 (66.7%)
<i>Crematogaster ashmeadi</i>	O: 3/4 (75%)

<sup>†</sup> Worker ants collected on (O) or around (A) the infested trees. Worker ants collected from nests (N) nearby the infested trees.

### 3.3 Detection of SLF DNA in ant samples from lure stations along transects

To evaluate detection rates and range, transects with honey-baited lure stations were established at varying distances from SLF-infested trees. Most honey-baited lure stations contained ants of a single species (Fig. 2(f)), and when multiple species were present, there was always a dominant species (Table S3), which was selected for subsequent endpoint PCR analysis. Across all sites, ant detection rates at lure stations were exceptionally high, ranging from 88.9% to 100%, with each station attracting between several dozen to several hundred ants. This highlights the effectiveness of honey-baited lure stations in sampling ants in the field. SLF DNA detection rates varied across sites. At the low-density site (Salem site), 46.7% of ant samples tested positive for SLF DNA, compared with detection rates of 62.1% and 62.5% at the medium- and high-density sites, respectively (Fig. 3). Positive SLF DNA detection was observed in ant samples collected from lure stations located up to 100 m from the infested trees at the medium- and high-density sites (Fig. 3).

## 4 DISCUSSION

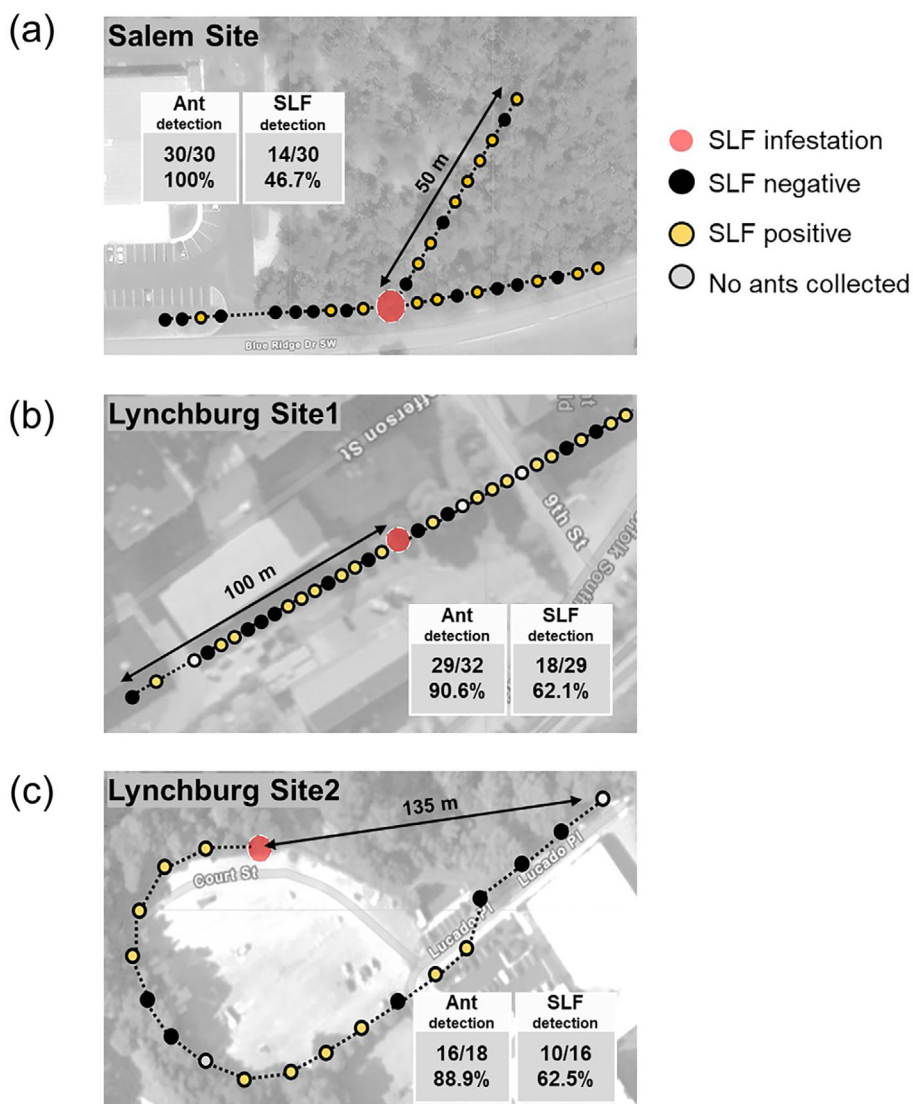
We developed and validated a novel method to detect SLF by using ants as 'honeydew samplers' to capture SLF DNA from honeydew. In our transect experiment, 93% of lure stations (75/80 across three sites) attracted ants after 30 min, reinforcing that ants can easily and quickly be sampled. More than 50% of collected ant samples (42/75) tested positive for SLF DNA, demonstrating the method's sensitivity for SLF detection. Additionally, SLF DNA was detectable up to 100 m from a single SLF-infested tree, highlighting the capability of this method to extend the SLF detection perimeter to considerable distances from the core infestation. Note our approach used endpoint PCR rather than qPCR for SLF DNA detection. While qPCR enables DNA quantification, endpoint PCR offers a more cost-effective alternative for simple presence-absence detection, making it more accessible in resource-limited settings.<sup>29</sup> Despite its lack of quantification power, endpoint PCR proved highly effective in detecting SLF DNA in our study.

The observed high SLF detection rates using antDNA are likely attributable to the feeding biology of ants. Ants can store substantial amounts of liquid in their crops<sup>30,31</sup> due to the presence of

proventriculus that segregates a communal food supply in the crop from the worker's personal supply. SLF honeydew thus can be retained rather than rapidly digested, making it possible to detect DNA from honeydew for extended periods post-ingestion. Furthermore, honeydew stored in an ant's crop can be shared with nestmates via trophallaxis,<sup>32,33</sup> potentially spreading SLF DNA among other workers which further increases the likelihood of detection. This is further supported by our laboratory results, where worker ants in colony fragments that did not directly feed on SLF honeydew still tested positive for SLF DNA. Although our data showed that both SLF honeydew and tissue can serve as sources of SLF DNA for ants, SLF DNA detection rates may vary by source after ingestion. Our data also showed that SLF DNA in honeydew appears to persist longer in ants, which aligns well with the fact that adult worker ants are incapable of processing or storing solid food particles,<sup>34</sup> while liquid food, such as honeydew, could be stored and retained in their crops for up to 14 days after single meal.<sup>35</sup>

Ant foraging strategies likely play a significant role in the extended distance detection range of the antDNA method. When foraging, dispersing colony members form multi-branched networks, allowing them to search multiple locations simultaneously.<sup>36</sup> This foraging pattern increases the likelihood of food encounter and provides broad spatial coverage, especially when resources are scattered (for instance, SLF distribution is often not evenly distributed across the landscape, but aggregated on acceptable hosts particularly for the adult stage).<sup>37</sup> SLF honeydew often falls directly to the ground,<sup>20</sup> potentially providing accessible cues that attract ants.<sup>38</sup> This process increases the likelihood of non-arboreal ant species, typically less adapted at climbing trees, encountering and consuming SLF honeydew.<sup>23</sup> Such interactions further expand the utility of ants as effective samplers for SLF DNA detection. Our results show that SLF DNA was detected in ant samples collected up to 50 m away from a single infested tree with a low density of SLF adults (Salem site, Fig. 3), suggesting that ant foraging behavior effectively aggregates SLF DNA from across the landscape into ant nests and significantly enhances the detection range of the antDNA approach. Furthermore, half of the ant samples along the transects in the same site were positive for SLF DNA, underscoring the method's detectivity for low-density SLF infestations. These two features are particularly advantageous when detecting low density SLF populations in recently invaded areas or at sites with limited access due to rough terrain or land ownership issues. However, we suspect that detection range may vary with SLF life stage: Adult SLFs, with their greater dispersal capacity due to flight, likely extend the DNA detection radius to longer distances compared with nymphs, which are wingless and typically do not disperse over long distances.<sup>39</sup> Further research is warranted to explore the maximum detection range of the antDNA method across different SLF life stages (i.e., adults *versus* nymphs).

Several advantages make antDNA an effective and practical alternative to other SLF detection methods, including existing eDNA sampling techniques.<sup>20</sup> Key benefits of the antDNA approach include, but not limited to: (1) establishing ant lure transects is simple and requires minimal technical skill, which is accessible even for non-professionals and easy to implement in various field settings; (2) the honey-baited lure station for ant collection involves only a few simple steps, including station deployment, a brief ant collection period, and then station retrieval, making it more time-efficient than other detection techniques; (3) materials for honey-baited lure stations, such as tubes, cotton balls, and 10% honey solution, are inexpensive, widely available, and scalable, making this method economically viable for large-scale sampling; (4) unlike other



**Figure 3.** Distribution of honey-baited lure stations along the transects at the three field study sites, showing ant detection rates (number of lure stations with ants/total number of lure stations) and SLF DNA detection rates (number of lure stations with ants positive for SLF DNA/total number of lure stations with ant presence). At Lynchburg site 2 (c), the transect was curved along the edge of the parking lot due to traffic constraints.

terrestrial eDNA methods for molecular analysis,<sup>40</sup> antDNA sampling does not require large or concentrated samples since ants gather SLF DNA while foraging (our results show that bulk DNA extraction from as few as 6–10 ants consistently yielded detectable SLF DNA); (5) while most eDNA sampling methods require a cold chain for sample preservation,<sup>41</sup> antDNA sampling is less reliant on specialized cooling equipment (ant specimens can be preserved in absolute ethanol without the need of cold storage, thus reducing logistical demands); (6) unlike other terrestrial eDNA sampling methods that require water for sampling followed by filtration,<sup>42</sup> ant samples can be directly processed for molecular assays, eliminating additional filtration and preparation steps; (7) antDNA is less vulnerable to abiotic conditions such as rainfall and ultraviolet (UV) exposure. These advantages underscore the substantial potential of antDNA method to be an efficient, cost-effective, scalable tool for SLF detection, especially for surveillance programs delineating the invasive range of SLF into a new area.

## AUTHOR CONTRIBUTIONS

W-JL and C-CSY conceived the study; AIDP-V, TCL, and C-CSY secured funding and oversaw the project; W-JL, F-LCL and C-CSY collected the data; X-YH constructed the density heatmap; W-JL and C-CSY analyzed the data and wrote the manuscript. AIDP-V, TCL and C-CSY edited, reviewed and finalized the manuscript.

## ACKNOWLEDGEMENTS

The authors thank two anonymous reviewers for their constructive comments. Thanks also go to Aaron Gross, Mark Johnson, Tom Kuhar and Doug Pfeiffer for comments on earlier versions of the manuscript. The authors also appreciate Yu-Chieh Sammi Chou, Lucie Cho, Yun-Syuan Amber Yang, Tsu-Yi Austin Yang, Hui-Wen Ranra Yang, Yu-Hsueh YangTsai, Cheng-Lin Yang, and Cheng-Chun Yang for field assistance. This work is partially supported by the Graduate Students Study Abroad Program, National Science and Technology Council, Taiwan to W-JL (113-2917-I-002-032), the

Virginia Tech New Faculty Start-up Funds to C-CSY, and the USDA-ARS Areawide Pest Management Program to TCL and C-CSY.

## CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this article.

## DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

## REFERENCES

- Larson ER, Graham BM, Achury R, Coon JJ, Daniels MK, Gambrell DK *et al.*, From eDNA to citizen science: emerging tools for the early detection of invasive species. *Front Ecol Environ* **18**:194–202 (2020).
- Dejean T, Valentini A, Miquel C, Taberlet P, Bellemain E and Miaud C, Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. *J Appl Ecol* **49**:953–959 (2012).
- Williams KE, Huyvaert KP, Vercauteren KC, Davis AJ and Piaggio AJ, Detection and persistence of environmental DNA from an invasive, terrestrial mammal. *Ecol Evol* **8**:688–695 (2018).
- Kheirodin A, Simmons AM, Legaspi JC, Grabarczyk EE, Toews MD, Roberts PM *et al.*, Can generalist predators control *Bemisia tabaci*? *Insects* **11**:823 (2020).
- Morisette J, Burgiel S, Brantley K, Daniel WM, Darling J, Davis J *et al.*, Strategic considerations for invasive species managers in the utilization of environmental DNA (eDNA): steps for incorporating this powerful surveillance tool. *Manag Biol Invasions* **12**:747 (2021).
- Valentin RE, Fonseca DM, Nielsen AL, Leskey TC and Lockwood JL, Early detection of invasive exotic insect infestations using eDNA from crop surfaces. *Front Ecol Environ* **16**:265–270 (2018).
- Saccò M, Guzik MT, van der Heyde M, Nevill P, Cooper SJ, Austin AD *et al.*, eDNA in subterranean ecosystems: applications, technical aspects, and future prospects. *Sci Total Environ* **820**:153223 (2022). <https://doi.org/10.1016/j.scitotenv.2022.153223>.
- McDonald R, Bateman PW, Cooper C, van der Heyde M, Mousavi-Deramhaleh M, Hedges BA *et al.*, Detection of vertebrates from natural and artificial inland water bodies in a semi-arid habitat using eDNA from filtered, swept, and sediment samples. *Ecol Evol* **13**:e10014 (2023).
- Andersen K, Bird KL, Rasmussen M, Haile J, Breuning-Madsen H, Kjaer KH *et al.*, Meta-barcoding of 'DIRT' DNA from soil reflects vertebrate biodiversity. *Mol Ecol* **21**:1966–1979 (2012).
- Wood SA, Pochon X, Laroche O, von Ammon U, Adamson J and Zaiko A, A comparison of droplet digital polymerase chain reaction (PCR), quantitative PCR and metabarcoding for species-specific detection in environmental DNA. *Mol Ecol Resour* **19**:1407–1419 (2019).
- Kyle KE, Allen MC, Dragon J, Bunnell JF, Reinert HK, Zappalorti R *et al.*, Combining surface and soil environmental DNA with artificial cover objects to improve terrestrial reptile survey detection. *Conserv Biol* **36**:e13939 (2022).
- Troth CR, Sweet MJ, Nightingale J and Burian A, Seasonality, DNA degradation and spatial heterogeneity as drivers of eDNA detection dynamics. *Sci Total Environ* **768**:144466 (2021).
- Curtis AN, Tiemann JS, Douglass SA, Davis MA and Larson ER, High stream flows dilute environmental DNA (eDNA) concentrations and reduce detectability. *Divers Distrib* **27**:1918–1931 (2021).
- Bell KL, Campos M, Hoffmann BD, Encinas-Viso F, Hunter G and Webber BL, Environmental DNA methods for biosecurity and invasion biology in terrestrial ecosystems: Progress, pitfalls, and prospects. *Sci Total Environ* **926**:171810 (2024).
- Fernandes K, Bateman PW, Saunders BJ, Bunce M, Bohmann K and Nevill P, Use of carrion fly iDNA metabarcoding to monitor invasive and native mammals. *Conserv Biol* **37**:e14098 (2023).
- Drinkwater R, Jucker T, Potter JH, Swinfield T, Coomes DA, Slade EM *et al.*, Leech blood-meal invertebrate-derived DNA reveals differences in Bornean mammal diversity across habitats. *Mol Ecol* **30**:3299–3312 (2021). <https://doi.org/10.1111/mec.15724>.
- Gregorič M, Kutnjak D, Bačnik K, Gostinčar C, Pecman A, Ravnikar M *et al.*, Spider webs as eDNA samplers: biodiversity assessment across the tree of life. *Mol Ecol Resour* **22**:2534–2545 (2022).
- Urban JM and Leach H, Biology and management of the spotted lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae), in the United States. *Annu Rev Entomol* **68**:151–167 (2023).
- Urban JM, Perspective: shedding light on spotted lanternfly impacts in the USA. *Pest Manag Sci* **76**:10–17 (2020).
- Valentin RE, Fonseca DM, Gable S, Kyle KE, Hamilton GC, Nielsen AL *et al.*, Moving eDNA surveys onto land: strategies for active eDNA aggregation to detect invasive forest insects. *Mol Ecol Resour* **20**:746–755 (2020).
- Abrams JF, Hörig LA, Brozovic R, Axtner J, Crampton-Platt A, Mohamed A *et al.*, Shifting up a gear with iDNA: from mammal detection events to standardised surveys. *J Appl Ecol* **56**:1637–1648 (2019).
- Calvignac-Spencer S, Leendertz FH, Gilbert MTP and Schubert G, An invertebrate stomach's view on vertebrate ecology: certain invertebrates could be used as "vertebrate samplers" and deliver DNA-based information on many aspects of vertebrate ecology. *Bioessays* **35**:1004–1013 (2013).
- Carroll CR and Janzen DH, Ecology of foraging by ants. *Annu Rev Ecol Evol Syst* **4**:231–257 (1973).
- Laube I, Hird H, Brodmann P, Ullmann S, Schöne-Michling M, Chisholm J *et al.*, Development of primer and probe sets for the detection of plant species in honey. *Food Chem* **118**:979–986 (2010).
- Huszarik M, Röder N, Eberhardt L, Kennedy S, Krehenwinkel H, Schwenk K *et al.*, External DNA contamination and efficiency of bleach decontamination for arthropod diet analysis. *Environ DNA* **5**:540–550 (2023).
- Kim H, Kim M, Kwon DH, Park S, Lee Y, Huang J *et al.*, Molecular comparison of *Lycorma delicatula* (Hemiptera: Fulgoridae) isolates in Korea, China, and Japan. *J Asia Pac Entomol* **16**:503–506 (2013).
- Folmer O, Black M, Hoeh W, Lutz R and Vrijenhoek R, DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* **3**:294–299 (1994).
- Ward DF, Building a DNA barcode reference collection of Hymenoptera in New Zealand. *Biodivers Data J* **12**:e131701 (2024).
- Xia Z, Johansson M, Gao Y, Zhang L, Haffner GD, MacLissac HJ *et al.*, Conventional versus real-time quantitative PCR for rare species detection. *Ecol Evol* **14**:11799–11807 (2018).
- Greenwald EE, Baltiansky L and Feinerman O, Individual crop loads provide local control for collective food intake in ant colonies. *Elife* **7**:e31730 (2018).
- Fujioka H, Marchand M and LeBoeuf AC, Diacamma ants adjust liquid foraging strategies in response to biophysical constraints. *Proc R Soc B* **290**:20230549 (2023).
- Nelson AS and Mooney KA, The evolution and ecology of interactions between ants and honeydew-producing hemipteran insects. *Annu Rev Ecol Evol Syst* **53**:379–402 (2022).
- Meurville M-P and LeBoeuf AC, Trophallaxis: the functions and evolution of social fluid exchange in ant colonies (Hymenoptera: Formicidae). *Myrmecol News* **31**:1–30 (2021).
- Casadei-Ferreira A, Fischer G and Economu E, Evidence for a thoracic crop in the workers of some neotropical *Pheidole* species (Formicidae: Myrmicinae). *Arthropod Struct Dev* **59**:100977 (2020).
- Howard DF and Tschinkel WR, The effect of colony size and starvation on food flow in the fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). *Behav Ecol Sociobiol* **7**:293–300 (1980).
- Lanan M, Spatiotemporal resource distribution and foraging strategies of ants (Hymenoptera: Formicidae). *Myrmecol News* **20**:53–70 (2014).
- Dechaine AC, Sutphin M, Leskey TC, Salom SM, Kuhar TP and Pfeiffer DG, Phenology of *Lycorma delicatula* (Hemiptera: Fulgoridae) in Virginia, USA. *Environ Entomol* **50**:1267–1275 (2021).
- Del-Claro K and Oliveira PS, Honeydew flicking by treehoppers provides cues to potential tending ants. *Anim Behav* **51**:1071–1075 (1996).
- Keller JA, Johnson AE, Uyi O, Wurzbacher S, Long D and Hoover K, Dispersal of *Lycorma delicatula* (Hemiptera: Fulgoridae) nymphs

- through contiguous, deciduous forest. *Environ Entomol* **49**:1012–1018 (2020).
- 40 Lentendu G, Bruni EP, Ah-Peng C, Fujinuma J, Kubota Y, Lorite J *et al.*, Soil filtration-sedimentation improves shelled protist recovery in eukaryotic eDNA surveys. *Mol Ecol Resour* **23**:1361–1371 (2023).
- 41 Mahon A, Rohly A, Budny M, Elgin E, Jerde C and Chadderton W, Environmental DNA monitoring and surveillance: standard operation procedures. Report to the United States Army Corps of Engineers, Environmental Laboratories, Cooperative Environmental Studies Unit, Vicksburg, Mississippi (2010).
- 42 Goldberg CS, Pilliod DS, Arkle RS and Waits LP, Molecular detection of vertebrates in stream water: a demonstration using Rocky Mountain tailed frogs and Idaho giant salamanders. *PLoS One* **6**: e22746 (2011).