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Research

Insect-Microbial Interaction

Epizootiology of infections by *Batkoa major* (Entomophthorales: Batkoaceae) and *Beauveria bassiana* (Hypocreales: Cordycipitaceae) in spotted lanternfly (Hemiptera: Fulgoridae) populations

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The planthopper *Lycorma delicatula* (White) (spotted lanternfly; SLF) was introduced to North America from Asia. It was first found in southeastern Pennsylvania in 2014 and now, a decade later, has increased in abundance and spread into 18 eastern US states. To study naturally occurring fungal pathogens infecting SLF, eastern Pennsylvania sites were sampled every 1 to 2 wk in 2020 and 2021 during the adult life stage of *L. delicatula* to detect prevalence of infections by the fungi *Batkoa major* (Thaxt.) Humber (Entomophthorales: Batkoaceae) and *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae). To sample, living adult SLF were collected and reared and cause of death was diagnosed. In 2020, at the site hosting a co-epizootic of these 2 generalist pathogens in 2018, an epizootic caused by *B. major* was documented from 30 September to 21 October. Low levels of infection by both pathogens were detected at an additional 2020 site and both 2021 sites. Overall, there was a negative association of *B. major* infection with SLF density and no association with density for *B. bassiana*. Co-infections in individual SLF by both fungi were never documented, and there was an inverse relationship between prevalence of *B. major* and *B. bassiana* infections in the sampled populations. At the time that SLF for rearing were sampled, adult cadavers were also sampled. For *B. major*, infection rates of sampled cadavers and reared individuals were positively correlated, but higher infection rates were observed in cadavers than among reared SLF. For *B. bassiana*, no such pattern occurred.

Keywords: epizootic, entomopathogenic fungi, generalist pathogen, Lycorma delicatula, invasive species

Introduction

Entomopathogens persisting in the environment provide the inoculum necessary for disease transmission. When environmental conditions, hosts, and pathogens interact to provide the means for abundant transmission, epizootics can occur (Hajek and Shapiro-Ilan 2018). Numerous species of entomopathogenic fungi are competent for creating epizootic levels of infection. Among these, many species of Entomophthorales are known for their ability to create the high rates of infection characteristic of epizootics (Pell et al. 2001) and species of Hypocreales, such as *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae), can also cause epizootics (eg Feng et al. 1994). At times, fungal species co-occur and together cause high levels of mortality in host populations (eg Johnson et al. 1984, Clifton et al. 2019, Souza et al. 2021). The period of time after an epizootic in insect populations and before the next increase in infection has been called a post-epizootic or inter-epizootic. During this period for fungal entomopathogens, inoculum in the environment can be abundant initially but decrease over time until conditions occur for infections to increase again (eg Hajek et al. 2004). It remains difficult to predict ahead of time when an epizootic will occur in order to document the necessary conditions enabling abundant transmission and infection.

The invasive Asian planthopper *Lycorma delicatula* (White) (spotted lanternfly; SLF) was first reported from southeastern Pennsylvania in 2014 (Urban and Leach 2023). This invasive has spread relatively quickly and is now considered established

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in 18 eastern US states (Cornell IPM 2025). In early October 2018, co-epizootics were reported in dense populations in southeastern Pennsylvania (Clifton et al. 2019). Two native fungal pathogens causing fatal diseases were responsible for the co-epizootics. The entomophthoralean *Batkoa major* (Thaxt.) Humber (Entomophthorales: Batkoaceae) was the more abundant species (73%), while the hypocrealean *B. bassiana* killed 23% of infected SLF (Clifton et al. 2019). *Batkoa major* is a biotroph and *B. bassiana* is a hemibiotroph (Rehner et al. 2011, Hajek et al. 2025), and both are generalists, infecting broad ranges of arthropod hosts (Feng et al. 1994, Gryganskyi et al. 2022). *Beauveria bassiana* is common and occurs worldwide, persisting in the soil (eg Clifton et al. 2023). While *B. bassiana* primarily causes naturally occurring epizootics in insects not feeding on aerial parts of plants, epizootics in Hemiptera feeding on aerial plant parts have occasionally been reported (Wraight et al. 2018). Mechanisms for environmental persistence and information about epizootics by the more poorly understood *B. major* have not been elucidated.

We asked to what extent these 2 entomopathogens infected and impacted SLF populations after the 2018 epizootics. When the co-epizootics caused by *B. major* and *B. bassiana* were discovered in SLF populations in southeastern Pennsylvania in 2018 (Clifton et al. 2019), massive mortality had already occurred, leaving abundant sporulating cadavers in the environment. We continued investigating this system in 2020 and 2021 at the 2018 co-epizootic site and adding 2 similar sites. Because cadavers of SLF killed by *B. major* were often found on tree trunks in 2018, we evaluated whether sampling for this fungus should focus on tree trunks versus collecting SLF feeding near the ground. We report variability in levels of infection by *B. major* and *B. bassiana* across 2 autumns and investigate associations of infection with host density, relative abundances of the 2 pathogens, and association with environmental variables. To explore sampling, we also compare infection levels between adults collected as cadavers and those collected as adults and reared, both sampling methods being regularly used in studying prevalence of entomopathogens (Eilenberg and Pell 2007).

Materials and Methods

Spotted Lanternfly Biology

Spotted lanternflies are univoltine in the United States, overwintering as eggs that hatch from May to June (Urban and Leach 2023). First through third-instar nymphs are polyphagous, feeding on tender plant tissue while fourth instars and adults feed on phloem through woody plant tissues, with more restricted host ranges, often being found on invasive tree of heaven, *Ailanthus altissima* (Mill.) Swingle (Sapindales: Simaroubaceae). In Pennsylvania, eclosion to the adult stage begins in late July, most mating occurs from September to mid-October, and oviposition begins in mid-September to early November with adult populations normally declining from late October when freezing temperatures begin to occur (Cooperband and Murman 2022, Urban and Leach 2023). Dispersal behavior by flying adults is poorly understood but often occurs at unpredictable intervals from early September through late October (Urban and Leach 2023).

Study Sites for Disease Prevalence

Disease prevalence studies were conducted in wooded sites hosting mature *A. altissima* trees in Berks and Monroe Counties, Pennsylvania (Table 1). One site that was sampled in both 2020 and 2021 (Angora Fruit Farm; AFF) was the location where the *B. major* and *B. bassiana* co-epizootic occurred in early October 2018 (Clifton et al. 2019). Another site sampled in only 2020 (Conrad Rd.; CR) was also the site of a co-epizootic in 2018. The third site for this study (Glen Run; GR) was sampled only in 2021. SLF densities and infection prevalence were quantified at each site every 1 to 2 wk from September to early November in 2020 and 2021, when virtually all SLF were in the adult stage. Each site was sampled 5 to 9 times per year. All rearing and fungal identification were conducted at the Sarkaria Arthropod Research Laboratory, Cornell University in Ithaca, New York under USDA APHIS permits #P526-18-02512 and #P526P-21-02895.

Table 1. Study sites in eastern Pennsylvania for naturally occurring *Batkoa major* and *Beauveria bassiana* infections in *Lycorma delicatula* populations, 2020 to 2021

Site name	Pennsylvania County	Coordinates[a]	Elevation (meters)	Years pathogens sampled
Angora Fruit Farm	Berks	40°21′30.9″N 75°52′59.9″W	237	2020, 2021
Conrad Road residence[a]	Berks	40°26′50.7″N 75°37′20.3″W	273	2020
Glen Run	Monroe	40°53′11.0″N 75°11′30.3″W	184	2021

[a]For this site located on private property, coordinates are provided for a nearby intersection.

Quantification of SLF Population Densities

Spotted lanternfly population density was quantified at each site when adults were sampled. Five mature *A. altissima* trees were randomly selected for each site. Densities were recorded for 5 to 6 dates between 14 September 2020 and 3 November in 2020 and 8 to 9 dates between 7 September and 25 October in 2021. For each tree, 4 sides at 2 m height on the tree trunk were marked with Markal B Paintstik crayons. SLF populations were only quantified on tree trunks between the ground and 2 m height, to provide relative density measures across space and time. During each visit, we photographed the 4 marked areas of each tree and composite images were loaded into DotDotGoose version 1.5.2 (DotDotGoose; amnh.org) to visually count SLF adults. After photographs were taken, each tree was checked for dead SLF on the quantified area of tree trunk and SLF numbers were adjusted accordingly so that only living SLF were included in counts. During these same sampling times, all SLF cadavers (including those emptied by scavengers and those with *B. major* or *B. bassiana* outgrowth) were counted within 1 m^2 areas around the bases of each tree.

Quantifying Infection Prevalence

In 2020, living adult SLF were collected from 5 *A. altissima* in the same tree stands where densities were quantified but not using the same trees. We hypothesized that infection levels would be greater for those SLF found feeding at or near the root collar, as they were near the ground where chances of encountering inoculum could be greater. Therefore, 30 to 35 living adults were collected from root collars and exposed roots near ground level and from 1 to 2 m high on tree trunks on the same 5 sample dates used for SLF density quantification. Since

no pattern was found in 2020, in 2021, 29 to 33 living adults were randomly collected on quantification dates from 5 *A. altissima* not used for density quantification. In both years, the living adults that were collected were reared for determination of infection by *B. major* or *B. bassiana*.

Living SLF were transported to the SARL quarantine where they were reared on potted tree of heaven in cages in a walk-in growth chamber and monitored daily for mortality for 14 days (see Clifton and Hajek 2022 for rearing methods). A total of 1,132 SLF were reared over both years. Any living adults that died were surface sterilized (Hajek et al. 2023) and then enclosed within 60 mm diam. petri dishes containing 1.5% water agar. Cadavers were monitored daily for 3 d to detect *B. major* outgrowth and thereafter occasionally for the following 11 days to detect *B. bassiana* outgrowth. Identifications of these 2 pathogens were based on morphology of fungal outgrowth from cadavers (Humber 2012) and experience with these species (eg Gryganskyi et al. 2022, Clifton et al. 2023). Only infections by *B. major* and *B. bassiana* were used for this study, and no additional entomopathogenic fungal species were included, such as those occurring infrequently that we reported previously (Hajek et al. 2023).

On the same sample dates, cadavers of recently killed SLF were collected from the ground, within 30 cm of the bases of trees. These "fresh" cadavers were from individuals that had died recently and had no external fungal growth, no consumption by fungivores and scavengers, and no natural decomposition. We estimate that these SLF had died in the previous 24 to 48 h (= "fresh cadavers"), based on cadaver moisture content. Numbers of cadavers collected per sample date depended on the numbers found within 30 cm of tree bases, with a maximum of 30 for earlier collections. At the end of October and beginning of November, sometimes cadavers were so numerous that the maximum collected per sample date was increased to 50 (2021). All cadavers were surface sterilized and incubated on water agar and checked for outgrowth by *B. bassiana* and *B. major* as with reared SLF. A total of 519 field-collected cadavers were processed during this study.

Data Analysis

A generalized linear mixed model (GLIMMIX; SAS 2021) was used to compare the proportions of living adults infected with *B. major* that had been collected on tree trunks versus near ground level across the 5 sample dates at the two 2020 sites, with site as a random variable. GLIMMIX was also used to analyze the association of infection rate of reared samples with host density (SLF number/m^2 on tree trunks), including all site-years. Each pathogen was analyzed separately, with site-year as a random variable.

Season-long mortality was calculated by aggregating mortality across weekly or biweekly collections. Marginal mortality rates for each pathogen were calculated under the assumption of proportional hazards (Elkinton et al. 1992). Cumulative mortality was calculated for *B. major, B. bassiana* and for both fungi together as one minus the product of weekly or biweekly proportions surviving (1-marginal mortality rates; Varley et al. 1973).

Weather data for the 4 site-years were obtained from PRISM Climate Group (2025). Rainfall was evaluated for September and October of both years when sampling occurred but also during August, when initial infection cycles would have occurred before development of an epizootic (Supplementary Material S1A and B). Preliminary analyses did not demonstrate associations of rainfall or temperature with infection prevalence for either pathogen. More in-depth linear regression analyses evaluated the association of rainfall 7, 10, or 14 days before sample dates with infection of reared insects in the epizootic site-year (AFF2020).

Association of abundances of the 2 fungal species with each other was evaluated using data from living SLF plus cadavers of recently dead adults collected on the same sample dates, using simple regression (JMP 2023). Only those sample dates on which at least one of the pathogens had caused infection were included ($n = 21$ sample dates).

To compare the rate of fungal infection of SLF samples collected live with samples collected as cadavers, we used linear regression (JMP 2023). For each pathogen separately, we regressed the fraction of the sample collected as cadavers against the fraction collected live the previous week. The weeks were offset because those cadavers that were infected were likely infected close to the same time as the living individuals collected during the previous week. Numbers of cadavers that were present at any sampling date varied, and earlier during our fall collections, numbers of cadavers collected were frequently low (Supplementary Material S2). Therefore, only those sampling dates for which more than 10 cadavers were collected were included.

Results

Rearing to Detect Infections: Tree Trunk Versus Near the Ground

Among the 617 SLF that were collected and reared during 2020, *B. major* was by far the more abundant pathogen. The rate of *B. major* infections of reared SLF collected from tree trunks versus those collected near the ground, often feeding on exposed roots did not differ (tree 11.6±1.1%, roots 9.9±1.0%) ($F_{1,17} = 0.64$; $P = 0.4361$). Among these collections, *B. bassiana*-infected SLF only occurred 5 times (3 of these were collected near the ground and 2 were collected from tree trunks).

Epizootiology

Epizootic levels of infection by *B. major* occurred in SLF populations at AFF in 2020 among the reared SLF, with higher infection levels extending through October (Fig. 1A). The cumulative analysis of mortality due to *B. major* at this site, across sample dates was 65% (Table 2). This trend was confirmed by high levels of infection among "fresh" cadavers collected on October 1 and 8 (64% and 72%, respectively) and the fact that the percent of *B. major* infection among cadavers from the remaining collection dates before 3 November ranged from 27% to 37% (Supplementary Material S2). Season-long *B. major* infection was 14% at CR2020, the same year as the AFF2020 epizootic (Fig. 1B). Sampling at AFF in 2021, the year after the 2020 *B. major* epizootic, no *B. major* infections were found among reared insects (Fig. 1C), while GR2021 had minimal *B. major* season-long infection (6%) (Fig. 1D) (Table 2).

Surprisingly, infections by *B. bassiana* did not occur among reared samples from AFF in 2020 (Fig. 1A). In contrast, among the other site-years, *B. bassiana* was always present among reared SLF, with season-long infection levels of 32% for both AFF2021 and GR2021 and 8% at CR2020 (Table 2). Percent

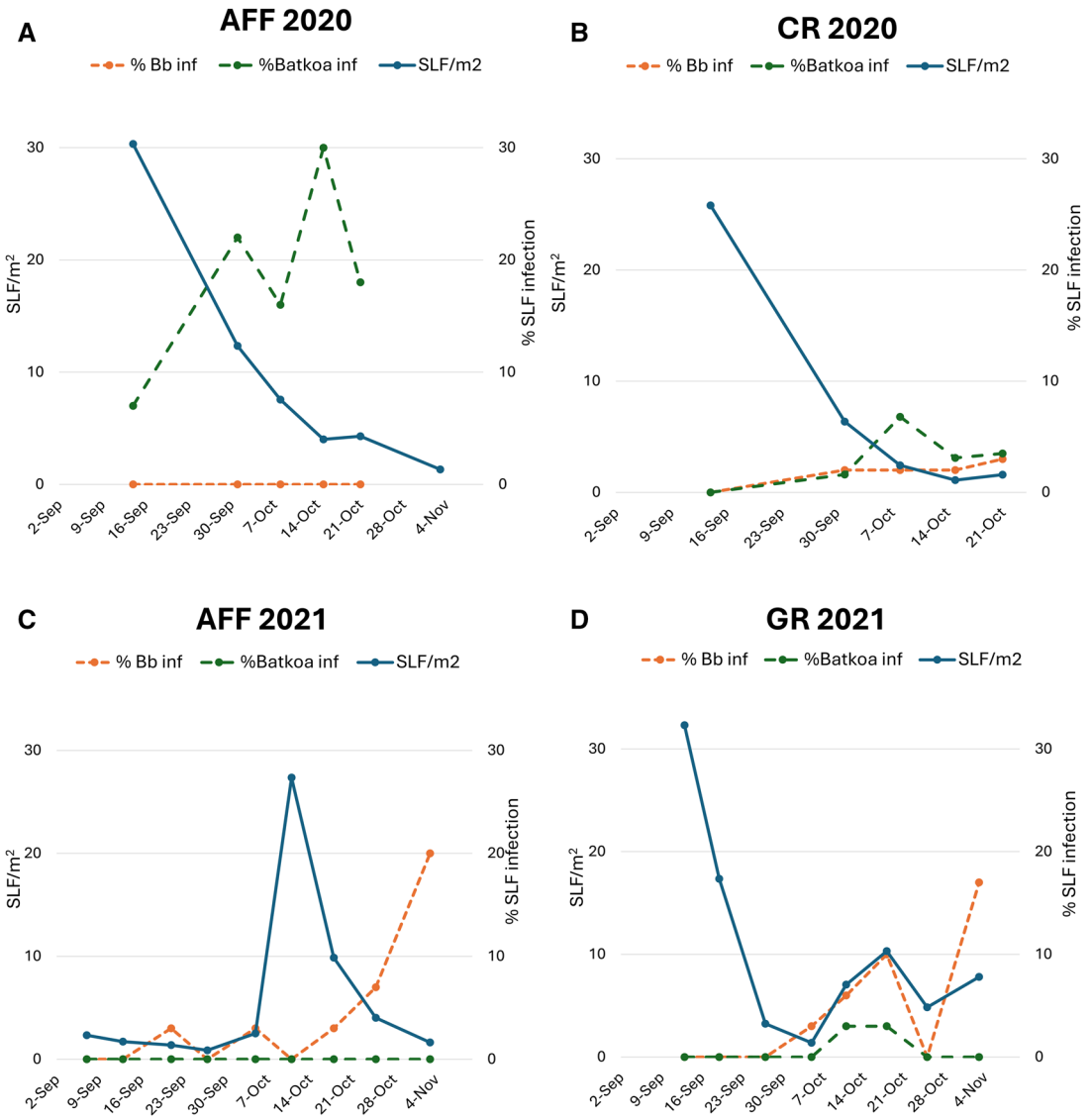

Fig. 1. Weekly or biweekly percent infection from September to early November by *Batkoa major* or *Beauveria bassiana* among *Lycorma delicatula* adults collected and reared in the quarantine lab. A) Angora Fruit Farm, 2020 (AFF2020), B) Conrad Road, 2020 (CR2020), C) Angora Fruit Farm, 2021 (AFF2021), and D) Glen Run, 2021 (GR2021).

Table 2. Cumulative mortality across each sampling period caused by *Batkoa major*, *Beauveria bassiana*, and both fungi together for the 4 site-years

	Batkoa major	*Beauveria bassiana*	Both fungi
AFF2020	65.0%	0.0%	65.00%
AFF2021	0.0%	32.3%	32.60%
CR2020	14.2%	8.1%	21.40%
GR2021	6.5%	32.0%	36.90%

B. bassiana infection from reared SLF ranged from 3% to 20% on 5 of the 9 collection dates at AFF in 2021 (Fig. 1C).

Percent infection by *B. major* was greater with lower SLF density ($F_{1,22} = 8.56$; $P = 0.0078$). Percent infection by *B. bassiana* also trended toward being negatively associated with SLF density ($F_{1,22} = 3.32$; $P = 0.0821$). Rainfall amounts and frequencies were highly variable among the site-years and did not display any pattern associated with infection by either pathogen in analyses of rainfall 7, 10, and 14 days before SLF collection in the epizootic site-year (AFF2020). Temperatures were similar across site-years, with maximum temperatures at 27 °C for August to October (Supplementary Material S1A and B).

Relative Abundance of *B. major* Versus *B. bassiana*

Coinfections of individual SLF with *B. major* and *B. bassiana* were never found. Over the 4 site-years, among the weekly or biweekly reared samples plus cadavers, collected when at least one pathogen was present, there was a negative association of rate of infection by *B. major* and *B. bassiana* ($F_{1,19} = 0.0319$) (Fig. 2).

Comparing Data from Rearing Versus Collecting Cadavers

There was a significant positive association of *B. major* infection among hosts collected live or as cadavers ($R^2 = 0.501$; $F_{1,11} = 11.03$, $P = 0.0068$). However, the rate of infection of cadavers was higher than infection of hosts collected live (Fig. 3). There

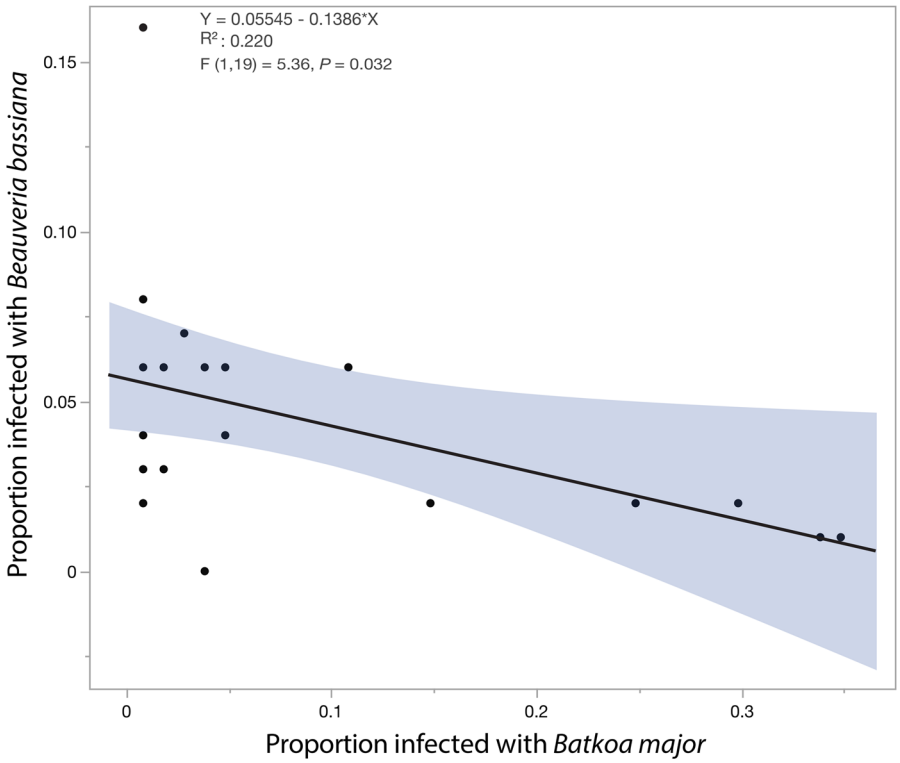

Fig. 2. Association of proportion infection by *Batkoa major* versus *Beauveria bassiana* among *Lycorma delicatula* living adults plus cadavers sampled on the same dates in fall, 2020 and 2021. The black line represents the linear regression. The shaded area is the 95% confidence interval around it.

was no significant association between infection rate by *B. bassiana* in hosts collected live versus those collected as cadavers (R^2= 0.028; $F_{1,11}$ = 0.32, P = 0.5850).

Discussion

An epizootic caused by *B. major* occurred in the SLF population at AFF in 2020 (Fig. 1A), with infection levels building from mid-September through early October. This is the same site where a co-epizootic occurred in 2018, when *B. major* infections were more abundant than *B. bassiana* (Clifton et al. 2019). It is rare to document the start of an epizootic but we were lucky to begin sampling when infection levels were increasing in mid-September. Le Rû and Iziquel (1990) distinguished 2 stages to an epizootic: (i) the initiation of infections to produce inoculum for initial horizontal transmissions; this stage is dependent on host age, and spatial organization (= "population structure") as well as host density and (2) a period of horizontal transmission in the host population that is independent of host density and population structure. The epizootic documented in AFF2020 could be thought to follow this pattern as the mid-September *B. major* infection level was lower when SLF population density was high; through the seasons for all site-years, host density was negatively associated with *B. major* and not associated with *B. bassiana*. This latter result is consistent with other systems where host density through the season was not associated with fungal infection levels (Vandenberg and Soper 1978, Le Rû and Iziquel 1990, Galaini-Wraight et al. 1991, Leite et al. 2002). In this study, the other 3 site-years all displayed enzootic levels of infection by *B. major* and *B. bassiana* (Fig. 1B–D), except the early November samples from both sites in 2021 when *B. bassiana* increased to above 10% infection. These November increases in infection are consistent with results from laboratory bioassays showing that older SLF adults (present in November) were weak and very susceptible to *B. bassiana* infection compared with younger and mid-stage adults (Clifton and Hajek 2022).

For all 4 site-years, the season ended with lower density SLF populations. The only site-year that started with a low-density population was AFF2021, the site where the *B. major* epizootic had occurred the previous year during mating and oviposition times. Entomopathogenic fungal infections impact infected females not only via mortality but also in lower fecundity before death (eg Hajek et al. 2008, Baker et al. 2018, Portilla et al. 2022). Therefore, although the population decline associated with the epizootic in 2020 (Fig. 1A) was not faster than the population declines at non-epizootic sites (Fig. 1B and D), the low SLF density at AFF2021 beginning in September 2021 would be consistent with an effect of infection decreasing reproduction during 2020, thus leaving fewer viable eggs to overwinter. Unfortunately, we did not quantify egg masses at the end of the seasons. However, we hypothesize that the population would not have decreased between egg hatch and early September at AFF in 2021 (when eclosion to adult was occurring) due to (i) emigration, as predominantly nymphs would be present during this time (from 2021 hatch to early September) and nymphal dispersal is usually not far (Keller et al. 2020), (ii) predation, as predation of spotted lanternflies was not observed during the 4 to 5 yrs that we sampled these sites, or (iii) fungal infection, as we did not find infected eggs or nymphs at these sites (EHC and DCH, unpublished data), At AFF2021, a large population increase occurred in mid-October, presumably due to the arrival of a dispersal flight of adults.

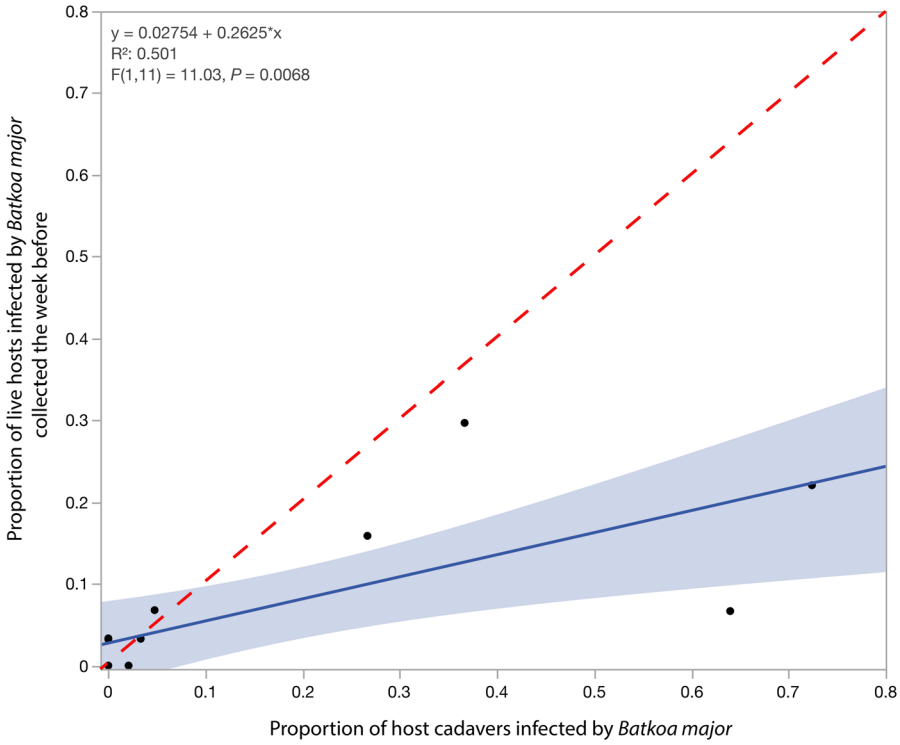

Fig. 3. Comparison of the proportion *Batkoa major* infection in reared adult *Lycorma delicatula* versus in cadavers of adults that had died recently and were collected during the next sampling date. The black line represents the linear regression. The shaded area is the 95% confidence interval around it. The dashed, red line shows what the 1:1 relationship would be if the sampling methods were equivalent.

However, that population quickly decreased afterward, with increasing *B. bassiana* infections (Fig. 1C).

In 2020 at the epizootic site, rainfall was not associated with infection levels. Among entomophthoralean fungi, conidial production, survival, and germination all require moist conditions and, correspondingly, epizootics caused by entomophthoralean fungi are known to be associated with moist environmental conditions (Carruthers and Soper 1987, Ment et al. 2018). Some studies of Entomophthorales have found rainfall to be significantly associated with development of epizootics (eg Wilding 1975, Leite et al. 2002). However, studies positively linking moisture levels with epizootics have often focused on positive associations with periods of relative humidity or surface moisture like leaf wetness (Le Rû and Iziquel 1990, Galaini-Wraight et al. 1991, and see Carruthers and Soper 1987). In at least one study, rainfall was not associated with infection prevalence while other moisture variables were highly significant (Galaini-Wraight et al. 1991). Unfortunately, in our study only daily rainfall data were available. Quantification of moisture within each 24 h period probably would have been needed to correctly evaluate these relations. We encourage further studies of the epizootiology of entomophthoralean fungi to quantify environmental factors and microclimatic conditions that can affect transmission from host to host, at intervals of less than a day.

Studies of epizootiology stress that for an epizootic to occur, the correct conjunction of hosts, pathogens, and environmental conditions must occur (Hajek and Shapiro-Ilan 2018). It is uncommon that the density of entomophthoralean pathogens prior to or at the beginning of an epizootic has been quantified. We hypothesize that for AFF2020, *B. major* inoculum could have persisted in the environment from the 2018 epizootic. In additional studies, bioassays with *Galleria mellonella* (L.) in fall 2020 demonstrated that both pathogens were present in the leaf litter and in the surface soil (Supplementary Material S3) and *B. bassiana* was also confirmed from soil samples (Supplementary Material S4). However, low levels of infection by both pathogens occurred at the other 3 site-years, some of which also had host densities on par with the initial host density at AFF2020. In contrast, when the living SLF at the AFF epizootic site were sampled in 2021, no *B. major* was detected although *B. major* had killed 2 of 48 SLF collected as cadavers on 25 October 2021 (Supplementary Material S2).

One more environmental condition that could impact the activity of these pathogens could be the host plants being eaten by SLF. Nymphal SLF have more diverse host plant ranges than adults (Kim et al. 2011, Liu 2019). While our study only included adult SLF collected on *A. altissima*, there is the possibility that host plants previously eaten by nymphs could impact infection of adults. For the entomophthoralean aphid pathogen *Pandora neoaphidis* (Remaud. & Hennebert) Humber, transferring aphids from one host plant species to another affected infection (Tkaczuk et al. 2007). The potential influence of host plants on fungal infections deserves further study.

In 2018, SLF cadavers were randomly collected at both AFF and CR on October 9. While *B. major* infections among cadavers at the AFF site were greater than *B. bassiana* ($n = 153$; 73% vs. 27%, respectively), at the CR site, where the host density was lower and fewer cadavers were available to collect ($n = 38$), percentages of the 2 pathogens were much more similar (*B. major* 53%, *B. bassiana* 47%). In 2020, SLF collected and reared in the quarantine laboratory also displayed more *B. bassiana* at CR than at AFF. Surprisingly, in 2018, *B. major* was more abundant in cadavers collected from tree trunks than *B. bassiana* and cadavers with these 2 pathogens on the ground were found in

Fig. 4. A and B) *Lycorma delicatula* adults feeding on root collars and exposed roots. C) Groups of *L. delicatula* adults near the ground.

equal numbers. This can in part be explained by the fact that *B. major* produces rhizoids that attach cadavers to surfaces such as tree bark (Hajek and Harris 2023). In 2020, equal percentages of reared SLF infected by *B. major* occurred on tree trunks versus feeding on exposed roots (Fig. 4), and *B. bassiana* was not detected among reared SLF. Thus, these data do not suggest a preference among SLF infected with *B. major* to remain higher on tree trunks.

During 2020 to 2021, sampling was principally conducted in September and October based on the timing of the 2018 epizootic which was discovered on 9 October 2018. During occasional sampling in Pennsylvania throughout late summer and through autumn from 2018 to 2021 (EHC and AEH, unpublished data), *B. major* infection was never seen in nymphal stages and naturally occurring *B. bassiana* infections in nymphs were not common. Laboratory bioassays demonstrated that nymphs as well as adults are susceptible to both pathogens, under optimal conditions (Clifton and Hajek 2022, Hajek et al. 2022). However, we hypothesize that infections and epizootics occurred among adults rather than nymphs at least in part because nymphs are in the plant canopy while adults are often found nearer to the leaf litter and soil (Fig. 4) where fungal inoculum occurs. In addition, adults are often found in groups (Fig. 4C), which would facilitate horizontal transmission. In this study, we were could not sample adults higher on trees than 2 m. Insects dying from infections by some entomophthoralean species are known to climb upwards before dying (Roy et al. 2006), but we were unable to evaluate whether SLF infected with *B. major* climbed above 2 m before dying.

Multiple types of parasites present in a host population can have an additive effect on host mortality or even increase it further through facilitation (ie one parasite enabling another; eg Hajek and van Nouhuys 2016). However, 2 species that use a resource in a very similar way can interfere with one another, even leading to competitive exclusion (McPeek 2014, Hajek and van Nouhuys 2016). Our data demonstrate that there is a negative interaction (Fig. 2) between *B. major* and *B. bassiana*, supported by the absence of coinfection of SLF individuals and paucity of co-occurrence of these 2 pathogens at our study sites. The lack of co-infections is consistent with experimental studies of co-infections by the entomophthoralean *Zoophthora radicans* (Bref.) A. Batko and *B. bassiana* in diamondback moth (*Plutella xylostella* (L.)) larvae; Furlong and Pell (2001) never found that both pathogens sporulated from the same *P. xylostella* cadaver. Simultaneous inoculation of larvae with these 2 pathogens reduced mortality from either pathogen but with greater reduction for *B. bassiana* and, in adults, *B. bassiana* severely inhibited development of *Z. radicans* (Furlong and Pell 2001). These experimental results are consistent with our study. In 2018, at AFF, *B. major* was the more abundant pathogen but *B. bassiana* occurred too (Clifton et al. 2019). Other studies on the prevalence of entomopathogens have reported on co-occurrence of hypocrealean fungi with entomophthoralean fungi. In one study, the infections by the entomophthoralean *Zoophthora phytonomi* (Arthur) A. Batko were more abundant than *B. bassiana* infections (Johnson et al. 1984). Another study reported that when *Batkoa* sp. was more abundant, the co-occurring *Metarhizium brasiliense* Kepler, S.A. Rehner & Humber was much less abundant (Souza et al. 2021).

Calculating prevalence of insect infection with entomophthoralean species has often been accomplished either by

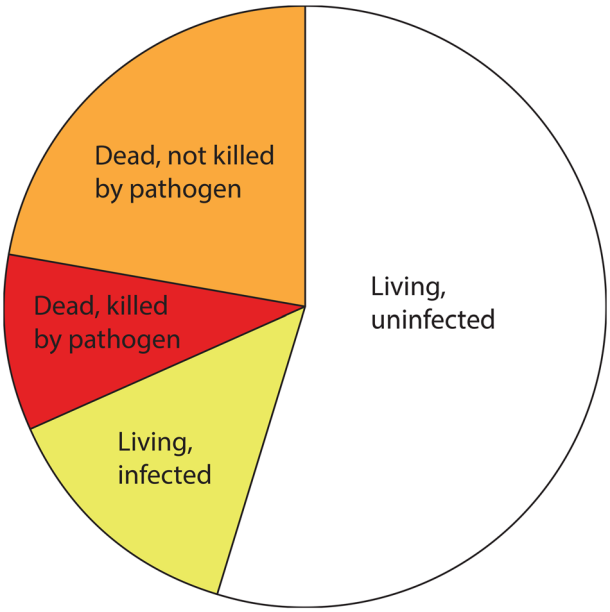

Fig. 5. Conceptual model illustrating disease occurrence in an insect population to be sampled. Based on this figure, we see that sampling only dead insects will give a different percent infection compared with sampling only living insects (after Eilenberg and Pell 2007). The relative sizes of the pie slices provide one potential scenario of relative abundances of the different groups (living vs. dead and infected vs. not infected) but this would vary with time and conditions in different systems. Our figure is based on the assumption that death due to the pathogen of interest can always be determined (regardless of the time since death of the insect).

collecting and diagnosing living insects, collecting dead insects for diagnosis, or combinations of these methods (Eilenberg and Pell 2007; Fig. 5). While this last option would be most representative of natural conditions, usually only living insects or cadavers are sampled and not both. The ability or ease of collecting data from one or the other of course depends on attributes of specific host/pathogen systems. However, the type of sampling is also dependent on levels of infection, as with low infection levels, few cadavers may be available to collect (eg as when sampling earlier in the season in CR2020, AFF2021, GR2021; Supplementary Material S2).

In this study, we collected samples for data on fungal infection from both living as well as recently dead SLF for diagnosis of both pathogens. We found that for *B. major* percent infection among cadavers and reared insects were positively correlated, but the infection rate of cadavers was greater than infection rate of reared insects (Fig. 3). In comparison, studies conducted on *Entomophaga maimaiga* Humber, Shimazu & R. S. Soper epizootics in *Lymantria dispar* (L.) populations also reported that percent infection from cadavers was greater than percent infection from reared insects (Hajek et al. 1996). While in this study only cadavers of very recently dead SLF were collected, in the *E. maimaiga* study, the time since death for cadavers was not mentioned. In both cases, percent infection among cadaver collections were only based on individuals that had died, while percent infection among living individuals was based on all individuals living during the period of rearing (see Fig. 5). Future studies in epizootiology of entomopathogenic fungi should consider this aspect of sampling during research planning, as sampling only cadavers could provide different results for disease prevalence than sampling living insects (eg as in this study where higher levels of infection were found when sampling cadavers compared with rearing living insects).

Conclusions

An epizootic caused by *B. major* occurred in a spotted lanternfly population 2 yr after a prior co-epizootic at that site. Sampling at additional sites demonstrated that *B. bassiana* was constantly present at enzootic levels at other sites and years. Infection by both pathogens was not related to rainfall when collections had been made or positively related to host density. A negative trend occurred toward levels of coexistence of these 2 fungal pathogens among sampled sites, which is supported by lack of coinfection among reared individuals. For *B. major*, sampling based on collecting cadavers overestimated percent infection when compared with rearing living SLF. However, for *B. major*, comparing percent infection from reared SLF versus cadavers, a positive association was seen (ie more infection from cadavers was matched by more infection from living SLF that were reared in the quarantine lab to detect infections). These same associations were not seen when comparing *B. bassiana* from reared SLF versus cadavers.

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Author Contributions

Eric H. Clifton (Conceptualization [lead], Data curation [lead], Formal analysis [supporting], Investigation [lead], Methodology [lead], Project administration [equal], Supervision [supporting], Visualization [supporting], Writing—original draft [supporting], Writing—review & editing [supporting]), Saskya van Nouhuys (Formal analysis [equal], Methodology [equal], Visualization [equal], Writing—original draft [supporting], Writing—review & editing [supporting]), David Harris (Data curation [supporting], Investigation [supporting], Methodology [supporting], Writing—review & editing [supporting]), and Ann E. Hajek (Data curation [supporting], Formal analysis [equal], Funding acquisition [lead], Project administration [equal], Resources [lead], Supervision [supporting], Writing—original draft [lead], Writing—review & editing [equal])

Supplementary Material

Supplementary material is available at *Environmental Entomology* online.

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Conflicts of Interest

None declared.

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