



A pair of native fungal pathogens drives decline of a new invasive herbivore

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Two North American fungal pathogens caused a coepizootic leading to localized collapse of an outbreak population of the newly invasive planthopper pest, the spotted lanternfly (*Lycorma delicatula*), in the eastern United States. The pathogens partitioned the habitat, with the majority of *L. delicatula* on tree trunks killed by *Batkoa major*, while cadavers of *L. delicatula* killed by *Beauveria bassiana* were usually on the ground. The future will show whether these pathogens will be drivers in boom–bust cycles or will result in recurrent low population densities of this new invasive species.

coepizootic | pathogen accumulation | invasive species | entomopathogenic fungi | spotted lanternfly

The outcome of incipient biological invasions is difficult to predict (1, 2), and while some species increase and then remain at high densities, others undergo boom–bust cycles (periods of rapid growth followed by precipitous decrease) or remain at lower densities after decreasing in abundance (3, 4). The success of invasive species has been attributed to various causes, including escape from natural enemies present in their native range (the enemy release hypothesis) (5). The causes of population collapse of invasive species are typically unknown, but collapse is often hypothesized as being caused by predators, pathogens, parasites, competitors, or declines in resources (4). Pathogen species can accumulate in invasive species based on the history of the invasion (6, 7), but whether pathogen activity can lead to recovery of native ecosystems due to suppression of invasive species is presently under discussion (2, 8).

The spotted lanternfly, *Lycorma delicatula*, is native to China, Taiwan, and Vietnam and has invaded South Korea and Japan (9). This univoltine, polyphagous planthopper feeds on 70 or more species of woody plants in 25 families and has caused damage to grape and apple crops (9). In 2014, *L. delicatula* was discovered in southeastern Pennsylvania where it now occurs in high densities and has subsequently spread to six additional eastern states (10). *L. delicatula* is only distantly related to hemipteran insects in the region, as there are no native species of Fulgoridae in northeastern North America (11). Few natural enemies have been reported attacking *L. delicatula* in Pennsylvania (12).

We report a coepizootic caused by two unrelated native fungal entomopathogens attacking an outbreak population of this new invasive insect. We identify and compare the two pathogens and present how they impacted the host population.

Results

The pathogens, both of which infect and kill insect hosts, are in the Division Zoopagomycota (Order Entomophthorales) and the Division Ascomycota (Order Hypocreales). For the entomophthoralean pathogen, the top-two GenBank references for 18S ribosomal RNA partial sequences were both *Batkoa major* with 98% identity (GenBank MK483702; February 11, 2019) (Figs. 1A–C); in addition, morphology matched descriptions (13). For the hypocrealean pathogen, the top-10 search results were all representative of isolates of *Beauveria bassiana* (GenBank MK574670; April 9, 2019) (Fig. 1D).

On October 9, 2018, dead *L. delicatula* adults were abundant throughout the site, on tree-of-heaven (*Ailanthus altissima*) (Fig. 1A), on neighboring trees and vines, and on the ground. Cadaver locations differed by fungal species (Fisher's exact test, $P = 0.0064$; Fig. 1E). Almost all dead *L. delicatula* adults on tree trunks were killed by *B. major* (97%) and attached by fungal rhizoids. On the ground was a mix of *L. delicatula* killed by *B. major* (51%) or *B. bassiana* (49%). A few *B. bassiana*-killed *L. delicatula* adults were collected from tree trunks (3%) but most were recovered from the ground. Across all collections, 73% of cadavers contained *B. major* and 27% contained *B. bassiana*. This coepizootic occurred when females were gravid but before most oviposition, and due to the massive *L. delicatula* mortality, only 12 egg masses were found.

Discussion

Invasive pests reach high densities because natural enemies are not attacking them (5). We report on the accumulation of two native fungal pathogens causing fatal diseases, leading to the sharp decline of a population of this new invasive pest—an example of biotic resistance (14). Neither pathogen has been previously reported as infecting *L. delicatula* in North America, despite intensive monitoring of this economically important insect, making these “emerging” pathogens. The probability of increasing disease prevalence generally increases with the age of an invasive infestation (2). The coepizootic described here is unusual because *L. delicatula* was discovered in North America only a few years before.

The ability of these pathogens to cause a coepizootic could in part be due to density-dependent transmission, host selection plasticity, or evolutionary adaptations (1, 6). *B. bassiana* principally lives as an arthropod pathogen, with a broad host range and a cosmopolitan distribution, and this pathogen is known to infect *L. delicatula* in China (15, 16). *B. bassiana* may also persist in the environment as an endophyte or saprotroph (16). In contrast, *B. major* is a poorly known obligate pathogen that infects a diversity of insects in the Americas, Europe, and Asia (13). This coepizootic provides an excellent opportunity to compare these pathogens and to study how they responded differentially to a high-density population of this new host. *B. major* forms rhizoids to hold cadavers in place and then actively ejects relatively short-lived primary and supernumerary infective spores. Conidia produced by entomophthoralean fungi can become airborne and travel longer distances (17). *B. bassiana* neither forms rhizoids nor actively ejects spores, but cadavers become densely covered with persistent spores that can be spread by direct contact and rainsplash (18). Thus, whether living *L. delicatula* planthoppers

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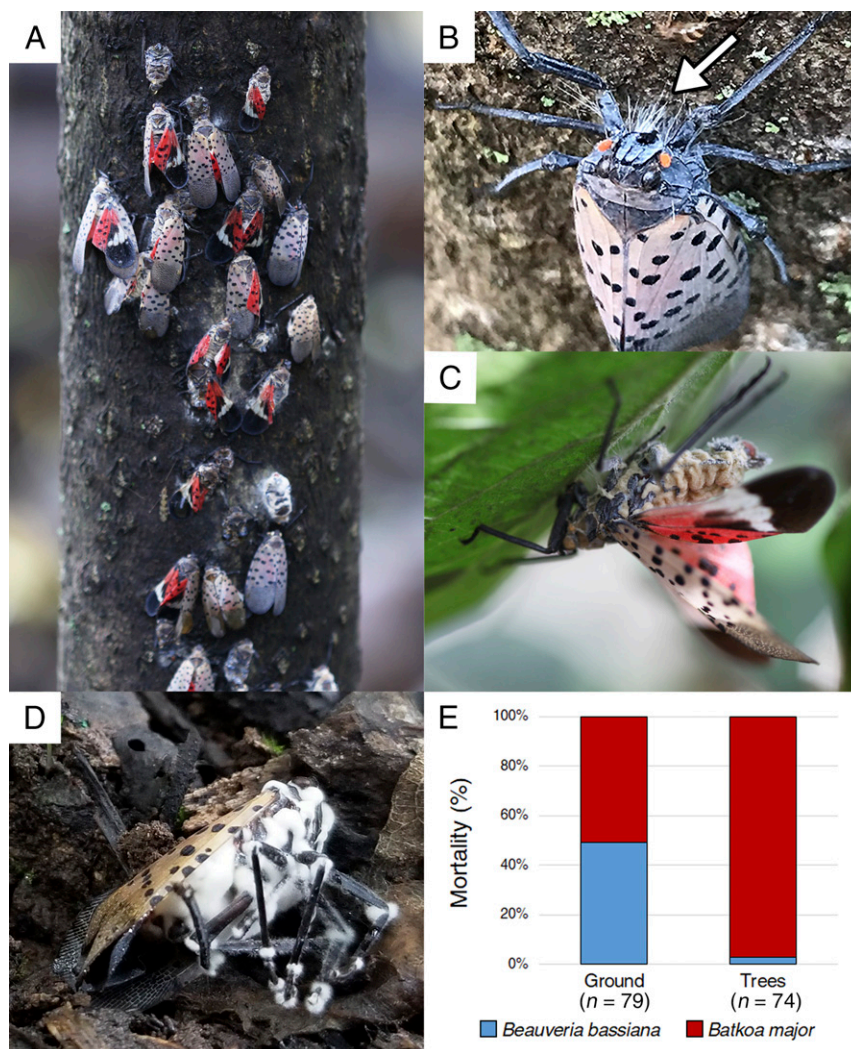


Fig. 1. Two fungal pathogens causing a coepizootic in an *L. delicatula* population. (A) *L. delicatula* during the epizootic: only one of these adults was alive and the remainder had been killed by *B. major*. A similar degree of mortality was observed on trees throughout the site. (B) Rhizoids from *B. major* (arrow) attaching a dead adult to a tree. Image courtesy of Kelly Murman (photographer). (C) Adult cadaver with wings and legs extended outward as *B. major* conidia are released from the abdomen. (D) Adult killed by *B. bassiana*. (E) Percent *L. delicatula* killed by either pathogen on the trees or surrounding ground. Sample size is below each bar.

were present on tree trunks, tree bases, or the ground, they were exposed to infective spores produced by one or the other of these fungal pathogens, resulting in this coepizootic.

Epizootics observed in insect populations usually include host species principally being attacked by a dominant pathogen species [e.g., western tent caterpillars (*Malacosoma californicum pluviale*) by the nucleopolyhedrovirus McplNPV (19) and manuka beetles (*Pyronota* spp.) by *Rickettsiella pyronotae* (20)]. Therefore, this coepizootic is uncommon, in that significant levels of infection were caused by two unrelated entomopathogenic fungi within the same host population. We hypothesize that due to the coepizootic, increased densities of these pathogens persisting in the environment would impact native communities of insects due to spillover events. However, the increased pathogen reservoir would also provide inocula for future infection of this invasive herbivore (7, 8).

Materials and Methods

On October 9, 2018, an epizootic caused by two fungal pathogens was observed in a high-density *L. delicatula* population (Fig. 1A) in a wooded area (690 m², 161 trees, and 43 shrubs) alongside an apple orchard at Angora Fruit Farm (40°21'30.6"N, 75°53'00.4"W), near Reading, Pennsylvania. Abundant

A. altissima trees were used as a basis for sampling (72% of the trees in these woods were *A. altissima*). Two species of entomopathogenic fungi that cause fatal diseases were identified and isolated from *L. delicatula* cadavers that contained fungal cells internally or externally.

For isolation of the entomophthoralean pathogen, primary conidia were obtained via the "ascending conidia" showering method, and hyphal growth occurred in cell culture media (21). *Beauveria* was isolated using selective media (22).

Fungal Identification. To identify both pathogens, sequencing with subsequent comparison with the National Center for Biotechnology Information nucleotide database was used as well as morphological evaluation (13, 16). For the entomophthoralean species, primers NS1/NS4 (23) amplified a region of the 18S ribosomal DNA (rDNA), and primers LROR/LR5 (24, 25) amplified 28S rDNA. For both primers, two phase autoextension protocols were used. For the hypocrealean isolate, nucleotide sequences were determined for the B locus nuclear intergenic region using primers B22Udg/B3.3R following PCR conditions described by Rehner et al. (16).

Describing the Epizootic. To compare the locations of cadavers by fungal pathogen, ~180 *L. delicatula* cadavers were collected from three randomly chosen canopy-height *A. altissima* trees. For each tree, 30 cadavers were collected from the lower 2 m of the tree trunk and 30 cadavers were collected from the ground around the tree base.

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