Journal of Economic Entomology, 113(6), 2020, 2758–2771
doi: 10.1093/jee/toaa195
Advance Access Publication Date: 11 September 2020
Research

OXFORD

Forest Entomology

Sap Beetles (Coleoptera: Nitidulidae) in Oak Forests of Two Northeastern States: A Comparison of Trapping Methods and Monitoring for Phoretic Fungi

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Subject Editor: Christopher Fettig

Received 29 May 2020; Editorial decision 30 July 2020

Abstract

Oak wilt is slowly expanding in the northeastern United States. Several nitidulid beetle species are known vectors of the fungus [*Bretziella fagacearum* (Bretz) Z. W. De Beer, Marinc., T. A. Duong, and M. J. Wingf (Microascales: Ceratocystidaceae)] that causes this disease, acquiring spores from fungal mats on infected trees and transmitting them to uninfected trees. Survey and fungal isolation from captured nitidulid beetles could be an important tool for detecting the presence of this disease in a geographic area not previously known to have oak wilt. In preparation for monitoring activities in such areas, two trapping studies were conducted in the northeastern United States: 1) trap test comparing the efficacy of wind-oriented pipe, multiple-funnel, and modified pitfall traps for nitidulids and 2) wet and dry collection cup comparison. Lures were a combination of nitidulid pheromones and fermenting liquid. Results support the use of multiple-funnel traps over the other two trap types, for both targeted species-specific surveys and community sampling. More total nitidulids, *Colopterus truncatus* (Randall), and *Glischrochilus fasciatus* (Olivier) were captured in wet collection cups compared with dry cups. Twenty-seven fungal species were isolated, none of which were *B. fagacearum*. Many fungi isolated from beetles were plant pathogens, indicating that in addition to the oak wilt fungus, sap beetles may contribute to the spread of other plant diseases.

Key words: sap beetle, survey, multiple-funnel trap, detection, oak wilt

Oak wilt, a disease caused by the fungal pathogen *Bretziella fagacearum* (Bretz) Z. W. De Beer, Marinc., T. A. Duong, and M. J. Wingf (formerly known as *Ceratocystis fagacearum*) (Microascales: Ceratocystidaceae) (de Beer et al. 2017), was first detected in the northeastern United States in 2008 (Jensen-Tracy et al. 2009) and threatens the health of native oak forests. Oak wilt has two principal modes of transmission: local, underground spread by way of root grafting, and longer distance, overland spread by insect vectors (Gibbs and French 1980). Sap beetles (Coleoptera: Nitidulidae) are considered an important insect group in pathogen transmission (Dorsey and Leach 1956, Juzwik et al. 2004, Hayslett et al. 2008). Some sap beetles, as their common name implies, are attracted to and feed on sap created by wounds on trees. However, the feeding habits of nitidulids are diverse and include seeds, pollen, flower petals, beeswax and honey, carrion, and predatory on other invertebrates, with a majority living in decaying fruits, fermenting plant juices, and fungi (Parsons 1943). Oak wilt produces sporulating mats on the outer sapwood and inner phloem of infected trees (Gibbs and French 1980), which emit a volatile odor that attracts nitidulids (Lin and Phelan 1992). Beetles propagate the disease by then carrying spores from these mats to fresh wounds in otherwise healthy trees (Juzwik et al. 2004).

Trapping for free-flying oak wilt-associated nitidulid beetles is important in understanding the role nitidulid beetles play in spreading the pathogen (Juzwik and French 1983), seasonal dispersal patterns (Appel et al. 1986, Juzwik and French 1986, Jagemann et al. 2018), monitoring for the presence of the oak wilt pathogen at asymptomatic sites (Jagemann et al. 2018), and documenting the diversity of potential vectors in different parts of oak wilt's range (Appel et al. 1986). This study explores the diversity of spring flying nitidulid beetles and associated fungi isolated from them in the northeastern United States, while evaluating the efficacy of different types of traps.

Materials and Methods

Experiment 1: Trap Type Comparison (2017)

Two sites (Stid Hill Multiple Use Area, Connetquot River State Park Preserve) were chosen for their proximity to known oak wilt infection centers in New York (none of which contained any actively wilting trees during the experiments) and a third site, Casalis State Forest, was selected in a typical oak/pine forest in New Hampshire where the disease has not yet been detected. Stid Hill Multiple Use Area (42.76331° N, 77.37863° W) in South Bristol, Ontario County, New York, was a closed canopy mixed hardwood forest with an overstory comprised of northern red oak (*Quercus rubra* L.), sugar maple (*Acer saccharum* Marshall), and white oak (*Quercus alba* L.). Connetquot River State Park Preserve (40.75995° N, 73.15363° W) in Islip, Suffolk County, New York, was also a closed canopy mixed hardwood forest with scarlet oak (*Quercus coccinea* Muenchh.), white oak, pitch pine (*Pinus rigida* Mill.), and eastern white pine (*Pinus strobus* L.) present. Casalis State Forest (42.85003° N, 71.92901° W) in Peterborough, Hillsborough County, New Hampshire, had an overstory comprised of red oak, eastern white pine, red maple (*Acer rubrum* L.), and white ash (*Fraxinus Americana* L.). All three sites were chosen due to the large component of overstory tree cover in oak species.

Three trap types were evaluated during this study: 1) a wind-oriented pipe trap (Dowd et al. 1992), 2) modified pitfall trap (Heath et al. 2009), and 3) 12-unit multiple-funnel trap (Lindgren 1983). Although wind-oriented pipe traps are commonly referred to as wind-oriented funnel traps in most literature, we refer to them as wind-oriented pipe traps to avoid any confusion with multiple-funnel traps. Wind-oriented pipe traps have been used in sampling flying nitidulid beetles associated with oak wilt in other regions of North America (Kyhl et al. 2002, Ambourn et al. 2005, Hayslett et al. 2009, Jagemann et al. 2018). Our design followed that of Dowd et al. (1991), modified to attach a screw-on bottle containing a liquid fermenting bait (Fig. 1A). Wet collection cups were filled with propylene glycol antifreeze as a killing and preserving agent. Modified pitfall traps (Fig. 1B) have been successfully used to trap nitidulids associated with *Ceratocystis albifundus* Morris, De Beer, and M. J. Wingfield (Microascales: Ceratocystidaceae) in South Africa (Heath et al. 2009). These traps had a dry collection chamber and consisted of a 12-cm section of 5-cm-inside-diameter PVC pipe with a fine mesh screen glued to the bottom to keep insects from falling into

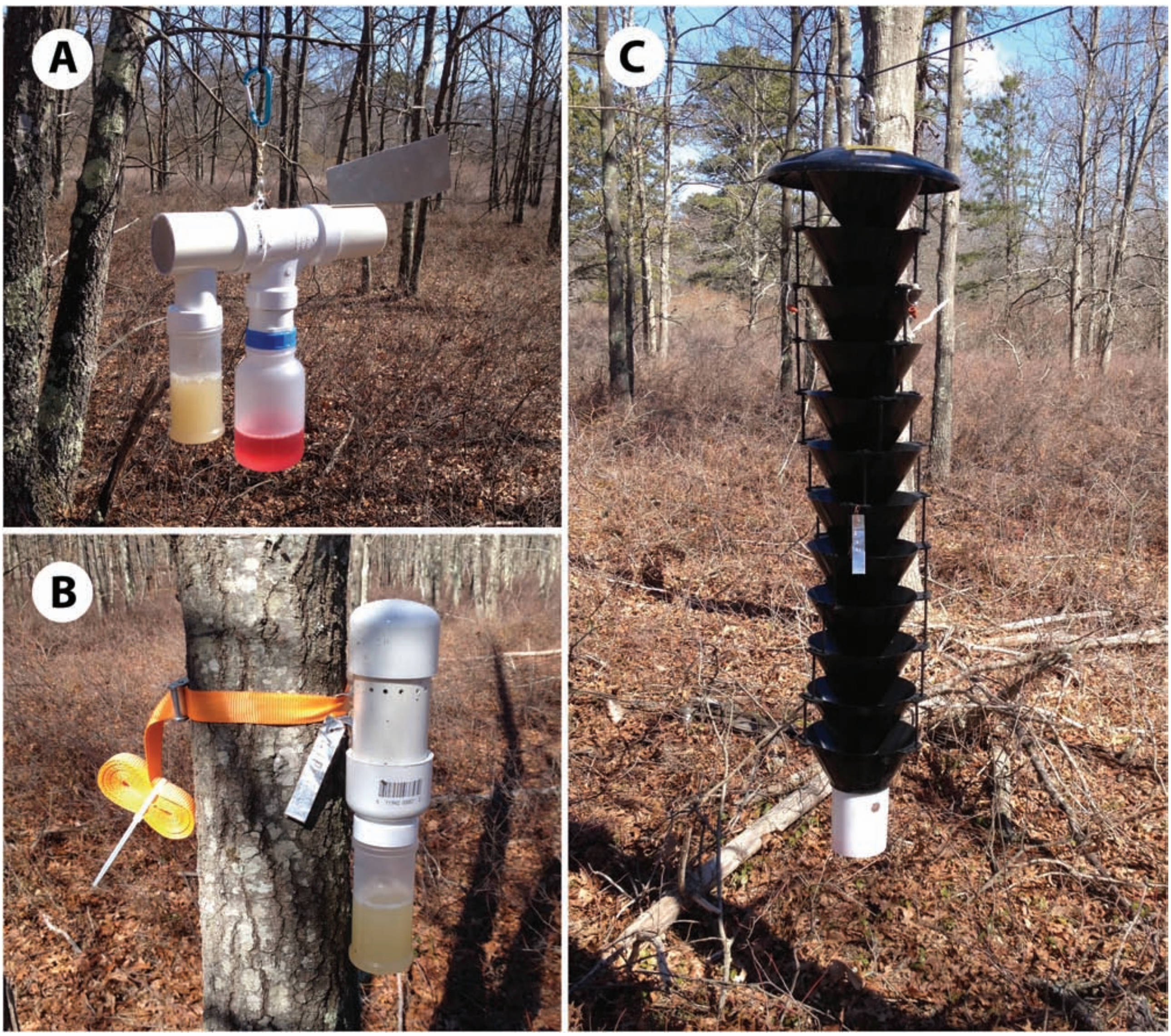

Fig. 1. Three trap types evaluated for efficacy of trapping nitidulid beetles in northeastern oak forests. A, wind-oriented pipe trap; B, modified pitfall trap; C, multiple-funnel trap.

the fermenting bait. Around the top of this cylinder, a series of entrance holes was drilled. Attached to the bottom was a standard PVC end cap, modified with a liquid lure jar that can be screwed on and off. Safety pins were glued to the inside of the bottom end cap to securely hold pheromone septa. Volatiles from the lures rise through the trap body and out the drilled holes to encourage beetles to climb into the trap and fall into the chamber. Preliminary testing revealed that insects could easily escape the trap, and so a 5 cm strip of Vaportape II (Hercon Environmental, Emigsville, PA) was included as a killing agent inside the trap. Attached to the top was an unmodified PVC end cap to protect the trap from collecting rain and debris. The final traps tested, 12-unit multiple-funnel traps (Fig. 1C; Synergy Semiochemicals Corp., Burnaby, BC, Canada), were selected because they are a common trap type that many natural resource agencies have experience with and are readily available. Nitidulid beetles are common by-catch in multiple-funnel trapping surveys for woodborers and bark beetles (Powell 2015). The top funnel was modified with a cutout to allow access to the center, and the first two funnels were shortened to create a wider opening to accommodate the liquid lure bottles that hung inside the traps (Miller et al. 2013). A cord was taped to the liquid lure bottle, so that it could be hung from the trap top through the modified top funnels. The center of the lure bottle cap was drilled out and replaced with a fine mesh screen glued in place to allow volatiles to escape, while preventing insects from entering the lure bottle. Safety pins were zip tied to funnel tabs around the midpoint of each trap to securely hold pheromone septa and allow easy access for lure replacement.

All traps were baited with a combination of fermenting liquid bait and available nitidulid pheromones. The fermenting bait was mixed and replaced every 2 wk during the collection cycle. It consisted of 240-g powdered malt extract and 7-g active dry yeast dissolved into 3.8 liter of water. This was enough fermenting bait to service all 21 traps at a site. Each ~236-ml lure bottle was filled about 2/3 full with fermenting liquid. Two pheromone types were used, high-dose *Carpophilus sayi* Parsons and *Colopterus truncatus* (Randall) commercial lures (Trécé Pherocon, Great Lakes IPM, Vestaburg, MI), each impregnated into rubber septa.

At each of the three sites, seven replicates (blocks) of three trap types were set up in a randomized complete block design throughout each forested area. Trap types within a block were separated by at least 10 m and each block was separated by at least 30 m. Wind-oriented pipe traps and multiple-funnel traps were hung with the top of the traps around 2 m off the ground from cord tied between two trees, and pitfall traps were strapped to an overstory oak tree bole at around breast height (1.3 m). Because transmission risk by sap beetles is lowest during summer and fall (Juzwik et al. 2006), we focused trapping efforts in spring and early summer. Traps were collected, and lures were replaced every 2 wk from mid-April to at least mid-July.

Experiment 2: Wet versus Dry Multiple-Funnel Trap Comparison (2018)

Casalis State Forest in NH was used to test wet versus dry collection cups for capturing nitidulid beetles. A randomized complete block design was established with six blocks and two treatments. The two treatments, a multiple-funnel trap with propylene glycol as the killing and preserving agent (wet cups) and a multiple-funnel trap with only a 5-cm strip of Vaportape II as the killing agent (dry cups), were established approximately 10 m apart from one another within each block. The blocks were established in the same area as the 2017 study and baited with the same lure combinations as described in experiment 1. Traps were collected weekly, and lures were replaced biweekly from late April to mid-June.

Sample Processing

For all trapping experiments at all sites, samples were strained through labeled paint strainers, placed into zip lock bags, and then stored in a freezer until processing. Samples were then thawed, and all adult nitidulid beetles were identified to species using available taxonomic resources (Parsons 1943, Connell 1977, Downie and Arnett 1996, Habeck 2002) and tallied. Voucher specimens of each species are deposited in the U.S. Forest Service Durham Field Office Forest Insect Collection, Durham, NH.

Statistical Analysis

For trap type comparisons (experiment 1), insect collections at each site were pooled by trap type over the entire season. Individual species that accounted for ≥1% of total catches were analyzed separately. Eleven species met this requirement. Data were analyzed as a multilocation randomized complete block design, with three sites (locations), seven replicates (blocks), and three trap types (treatments). Trap collections were compared using a generalized linear mixed model (Proc GLIMMIX, version 9.3; SAS Institute, Cary, NC) via maximum likelihood estimation with replicates as blocks. The negative binomial function with log link was used to analyze data. Sites and replicates were treated as random variables, with replicates nested within sites. For *Carpophilus brachypterus* (Say), *Colopterus unicolor* (Say), and *Glischrochilus obtusus* (Say), data analysis varied slightly due to zero or very low counts at some sites. In the case of *C. brachypterus*, very low numbers were captured in Connetquot and Casalis, so only collections from Stid Hill were used. *Colopterus unicolor* and *G. obtusus* trap catches were only analyzed from Connetquot, as Stid Hill and Casalis collections were too low to allow comparisons. Differences among treatments were tested with Tukey's honestly significant difference test.

Data from all sites were combined to investigate community metrics including species richness, Berger–Parker dominance, Simpson's diversity index, unique species, and singletons captured in each trap type. Those estimates, as well as individual-based rarefaction curves, were calculated using PAST software (Hammer et al. 2001). A similar approach was taken to investigate community metrics at each site. For these comparisons, all traps were pooled by site. In addition to the estimates mentioned above, similarity estimates based on relative species abundances (q = 1, Horn equal weight index) at each site were calculated using SpadeR (Chao et al. 2016).

Trap collections from wet and dry cups (experiment 2) were pooled by treatment over the entirety of the trapping season. Individual species that accounted for ≥1% of total catches were analyzed separately. Five species met this requirement. The experiment was designed as a randomized complete block design and analyzed with a generalized linear mixed model (Proc GLIMMIX, version 9.3; SAS Institute) via maximum likelihood estimation. Replicates were blocks, and cup type was the main effect. The negative binomial function with log link was used to analyze data.

Fungal Isolations from Nitidulids Captured in Wind-Oriented Pipe Traps (2018)

At six trapping sites in 2018 (Connetquot, Stid Hill, Casalis, Glenville, Sanders Preserve, and Indian Kill), between three and five wind-oriented pipe traps with a 5-cm strip of Vaportape II and crumpled paper in a dry collection cup were hung to collect beetles for fungal isolation. Traps were monitored biweekly, and any nitidulid

Table 1. Trap catches of nitidulid species collected from three trap types at three sites in the northeastern United States in 2017

	Stid Hill, Naples, NY				Connetquot, Islip, NY				Casalis, Peterborough, NH				
	Funnel	Pitfall	Wind	Total	Funnel	Pitfall	Wind	Total	Funnel	Pitfall	Wind	Total	Grand Total
Amphicrossus ciliatus (Olivier)	—	—	—	—	101	—	—	101	—	—	—	—	101
Carpophilus antiquus Melsheimer	1	—	—	1	—	—	—	—	—	—	—	—	1
Carpophilus brachypterus (Say)	272	4	121	397	2	1	11	14	10	2	5	17	428
Carpophilus corticinus Erichson	584	22	104	710	34	4	17	55	201	19	39	259	1,024
Carpophilus dimidiatus (F.)	—	—	—	—	—	—	—	—	—	1	—	1	1
Carpophilus freemani Dobson	—	—	2	2	2	—	1	3	—	—	—	—	5
Carpophilus hemipterus (L.)	41	2	11	54	—	—	—	—	10	2	13	25	79
Carpophilus lugubris Murray	57	—	12	69	1	—	1	2	—	—	—	—	71
Carpophilus marginellus Motschulsky	1	—	—	1	—	—	—	—	—	—	—	—	1
Carpophilus sayi Parsons[a]	680	10	61	751	97	4	20	121	149	29	31	209	1,081
Colopterus maculatus (Erichson)[a]	—	—	—	—	76	2	23	101	—	—	—	—	101
Colopterus semitectus (Say)[a]	24	1	15	40	2	2	3	7	2	—	2	4	51
Colopterus truncatus (Randall)[a]	668	71	585	1,324	840	299	1,522	2,661	700	80	1,014	1,794	5,779
Colopterus unicolor (Say)	—	—	—	—	274	32	41	347	7	2	2	11	358
Cryptarcha ampla Erichson	156	4	10	170	112	7	3	122	37	12	3	52	344
Cryptarcha concinna Melsheimer[a]	19	—	—	19	14	4	1	19	2	—	—	2	40
Cryptarcha strigatula Parsons	35	1	—	36	69	3	—	72	1	—	—	1	109
Epuraea corticina Erichson	2	—	—	2	—	—	—	—	—	—	—	—	2
Epuraea peltoides Horn	48	—	3	51	20	1	—	21	3	10	—	13	85
Epuraea planulata Erichson	1	—	—	1	3	—	—	3	6	1	—	7	11
Epuraea rufomarginata (Stephens)	1	—	—	1	—	—	—	—	—	—	—	—	1
Epuraea sp.	—	—	—	—	3	—	—	3	—	—	—	—	3
Glischrochilus confluentus (Say)	1	—	—	1	—	—	—	—	—	—	—	—	1
Glischrochilus fasciatus (Olivier)	166	1	16	183	246	—	11	257	763	81	28	872	1,312
Glischrochilus obtusus (Say)	94	1	3	98	510	1	1	512	—	—	—	—	610
Glischrochilus quadrisignatus (Say)	374	8	24	406	21	1	1	23	4	1	2	7	436
Glischrochilus sanguinolentus (Olivier)	460	3	16	479	1,366	2	24	1,392	879	96	45	1,020	2,891
Glischrochilus siepmanni Brown	—	—	—	—	—	—	—	—	1	—	—	1	1
Glischrochilus vittatus (Say)	1	—	—	1	—	—	—	—	—	1	—	1	2
Lobiopa undulata (Say)	7	—	—	7	445	29	1	475	—	—	—	—	482
Lobiopa setosa Harold	—	—	—	—	3	—	—	3	—	—	—	—	3
Omosita nearctica Kirejtshuk	—	—	—	—	1	—	—	1	—	—	—	—	1
Phenolia grossa (F.)	—	—	—	—	2	—	—	2	2	—	—	2	4
Stelidota geminata (Say)	1	—	—	1	13	—	—	13	—	—	—	—	14
Total Beetles	3,694	128	983	4,805	4,257	392	1,681	6,330	2,777	337	1,184	4,298	15,433
Number of Species	24	12	14	25	25	15	16	25	17	14	11	19	34
Number of Unique Species	10	0	1	5	7	0	0	5	4	2	0	2	

[a]The species has been reported as a vector of the oak wilt fungus in literature.

beetles were placed in a sterile petri dish, taped closed, and placed in a freezer. Beetles were then identified to species and placed together in sterile vials (all conspecifics from a single collection date and trap together in the same vial) and remained in a freezer until processing.

Fungi on adult nitidulid beetles were isolated by serial dilution plating as described by Ambourn et al. (2005) and Jagemann et al. (2018). All assayed beetles were processed within 10 mo of collection. Up to three individuals of each nitidulid species were randomly selected from each collection date at each site. Each beetle was placed into 0.5-ml sterile distilled water and agitated with a vortex mixer for 1 min. The fungal propagules were then dislodged from the insect using a sonicator for 30 s. The resulting suspension of distilled water and fungal propagules was serially diluted (10–1 and 10–2), and 0.5-ml aliquots of each dilution was spread onto lactic acid-amended potato dextrose agar (PDA) plates and incubated for 7 d in darkness at 24°C. Individual fungal colonies were transferred onto PDA plates overlaid with a cellophane membrane and grown for 1 wk in darkness at 24°C. Fungal isolates were grouped by morphotype. DNA was extracted from representative isolates as described by Chi et al. (2009). Each PCR contained a total volume of 50 µl consisting of 25 µl of 2× MasterMix solution, 2 µl each of a 5-pmol concentration of each primer (ITS1 and ITS4), 19 µl of sterile deionized water, and 2 µl of DNA. The internal transcribed spacer (ITS1-5.8s-ITS2) region of the rDNA was amplified with ITS1 and ITS4 primers and sequenced in the forward direction (GENEWIZ, South Plainfield, NJ). Sequences

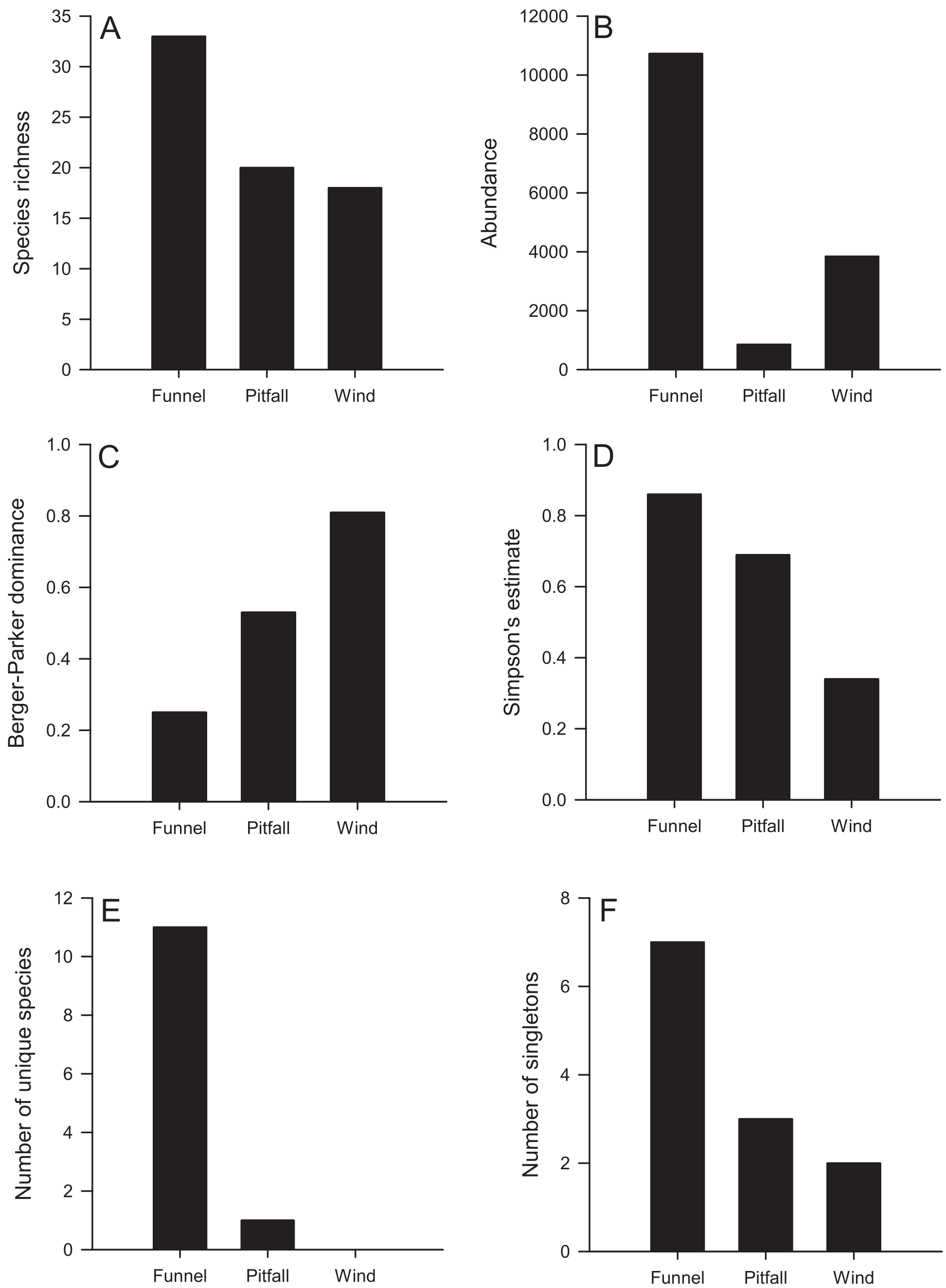

Fig. 2. Community metrics for nitidulids captured by three trap types in northeastern oak forests. A, Number of species captured; B, number of beetles captured; C, Berger–Parker dominance estimate; D, Simpson's estimate; E, number of unique species captured; F, number of singleton species captured.

were manually edited using BioEdit v7.2.5 and deposited in GenBank (Table 2). BLASTn searches in GenBank of the ITS-rDNA sequences that resulted in >97% homology to sequences from vouchered cultures or published sources were considered successful. Once fungi were identified, colony appearance and spore characteristics were used to identify morphotypes. Fungi were categorized into guilds (Nhu et al. 2016) or plant pathogens (Farr and Rossman 2020) where data were available.

Results

Experiment 1: Trap Type Comparison (2017)

In total, 15,433 nitidulids from 34 species were captured in 2017 from the three sites (Table 1). *Colopterus truncatus* (37.4%), *Glischrochilus sanguinolentus* (Olivier) (18.7%), *Glischrochilus fasciatus* (Olivier) (8.5%), *C. sayi* (7.0%), and *Carpophilus corticinus* Erichson (6.6%) were the most common species captured during the experiment. Seven species were captured only one time. In Casalis, a total of 4,298 nitidulids from 19 species were captured. *Colopterus truncatus* (41.7%), *G. sanguinolentus* (23.7%), and *C. corticinus* (6.0%) were the most common species found at the site. Four species were captured only once. From Connetquot, a total of 6,330 nitidulids from 25 species were captured. *Colopterus truncatus* (42.0%), *G. sanguinolentus* (22.0%), *G. obtusus* (8.1%), *Lobiopa undulata* (Say) (7.5%), and *C. unicolor* (5.5%) were the most common species found at the site. Only one species was captured once. In Stid Hill, a total of 4,805 nitidulids from 25 species were captured. *Colopterus truncatus* (27.6%), *C. sayi* (15.6%), *C. corticinus* (14.8%), *G. sanguinolentus* (10.0%), *Glischrochilus quadrisignatus* (Say) (8.4%), and *C. brachypterus* (8.3%) were the most common species found at the site. Seven species were captured only once.

Where pooled data were used to assess trap effectiveness for sampling the nitidulid beetle community, multiple-funnel traps captured more species than modified pitfall or wind-oriented pipe traps (Fig. 2A). Abundance, Simpson's estimate, unique species, and singletons were also higher in multiple-funnel traps compared with modified pitfall and wind-oriented pipe traps (Fig. 2B, D, E, and F, respectively). Berger–Parker dominance estimates were highest for wind-oriented pipe traps compared with other trap types, suggesting these traps were dominated by only a few species (Fig. 2C). Individual-based rarefaction curves showed that multiple-funnel traps captured the most species, followed by modified pitfall and then wind-oriented pipe traps (Fig. 3).

Multiple-funnel traps captured significantly more total nitidulid beetles than wind-oriented pipe and modified pitfall traps ($F_{2,4}$ = 49.31, P = 0.0015; Fig. 4A). *Carpophilus corticinus* ($F_{2,4}$ = 28.9, P = 0.004) and *C. sayi* ($F_{2,4}$ = 14.7, P = 0.01) responded to traps in a similar manner, with more beetles captured in multiple-funnel traps compared with modified pitfall and wind-oriented pipe traps for both species (Fig. 5A and E, respectively). *Colopterus truncatus* ($F_{2,4}$ = 30.8, P = 0.004; Fig. 5C) was caught significantly less in modified pitfall traps compared with other traps. *Carpophilus brachypterus* was captured in greater numbers in multiple-funnel and wind-oriented pipe traps than modified pitfalls ($F_{2,12}$ = 26.3, P < 0.0001; Fig. 5B). *Glischrochilus sanguinolentus* ($F_{2,4}$ = 13.3, P = 0.02), *G. fasciatus* ($F_{2,4}$ = 43.1, P = 0.01), *G. obtusus* ($F_{2,12}$ = 35.6, P < 0.0001), *G. quadrisignatus* ($F_{2,4}$ = 12.4, P = 0.02), *L. undulata* ($F_{2,4}$ = 14.1, P = 0.02), *C. unicolor* ($F_{2,12}$ = 15.1, P = 0.0005), and *Cryptarcha ampla* Erichson ($F_{2,4}$ = 16.1, P = 0.01) were all captured in higher numbers in multiple-funnel traps compared with modified pitfall and wind-oriented pipe traps, whereas wind-oriented pipe traps and modified pitfall traps did not differ from each other for any of these species (Figs. 4B, C, D, E, F and 5D and F, respectively).

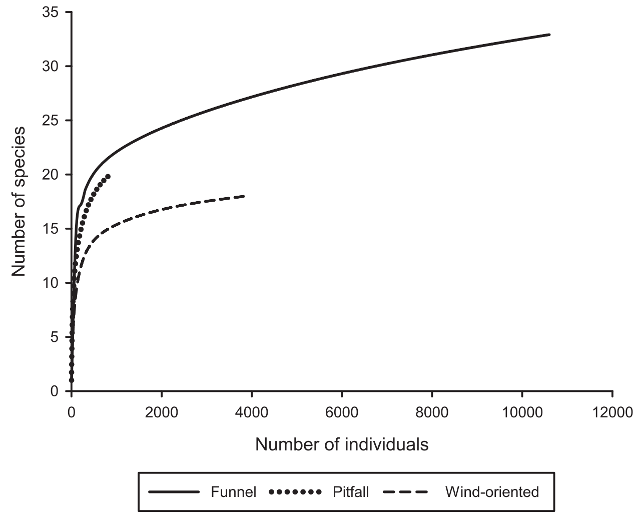

Fig. 3. Individual-based rarefaction curves for three trap types deployed at three sites in the northeastern United States.

Community metrics by site (Fig. 6) show that the largest number of nitidulids were captured at Connetquot, followed by Stid Hill, and Casalis. The same number of species and unique species were captured in Connetquot and Stid Hill and these were both higher than those reported for Casalis. Simpson's estimate was highest at Stid Hill compared with Connetquot and Casalis. This was reversed for Berger-Parker estimates, with the lowest occurring in Stid Hill and highest at the two other sites. Numbers of singletons were highest in Stid Hill, followed by Casalis and Connetquot where there was only one. Individual-based rarefaction curves showed that the most species were captured at Stid Hill, followed closely by Connetquot, with Casalis lower than both sites (Fig. 7). Overall, similarity among the sites was 0.78 ± 0.005, with Connetquot and Casalis the most similar (0.81 ± 0.007), followed by Stid Hill and Casalis (0.79 ± 0.008). Stid Hill and Connetquot were the least similar (0.7 ± 0.009).

Dispersal Phenology (2017)

Colopterus truncatus and *C. sayi* were captured at all three sites in sufficient numbers to report phenological data for 2017 (Fig. 8). At all three sites, *C. truncatus* was captured during the first 2-wk collection period, indicating that flight may have started before trapping began. Collections during this first collection period were relatively low at all three sites. Peak captures of *C. truncatus* occurred during the collection periods ending 26 April at Connetquot, 9 May at Stid Hill, and 25 May at Casalis. *Carpophilus sayi* was not captured during the first collection period at any of the three sites. The first captures of *C. sayi* occurred during 24 April–10 May at all three sites. The number of captures of *C. sayi* peaked during the period ending 24 May at Connetquot, whereas peaks at the other two sites occurred later during the periods ending 7 June and 9 June for Stid Hill and Casalis, respectively. Although peaks in flight had occurred, individuals of both species continued to be captured in smaller numbers until trap collections ended.

Experiment 2: Wet versus Dry Multiple-Funnel Trap Comparison (2018)

More total nitidulids were captured in wet collection cups compared with dry cups ($F_{1,5}$ = 17.3, P = 0.009; Fig. 9A) in funnel traps at Casalis State Forest. This pattern was the same for *C. truncatus* ($F_{1,5}$ = 46.4, P = 0.001) and *G. fasciatus* ($F_{1,5}$ = 11.2, P = 0.02) where more were captured in wet cups compared with dry cups

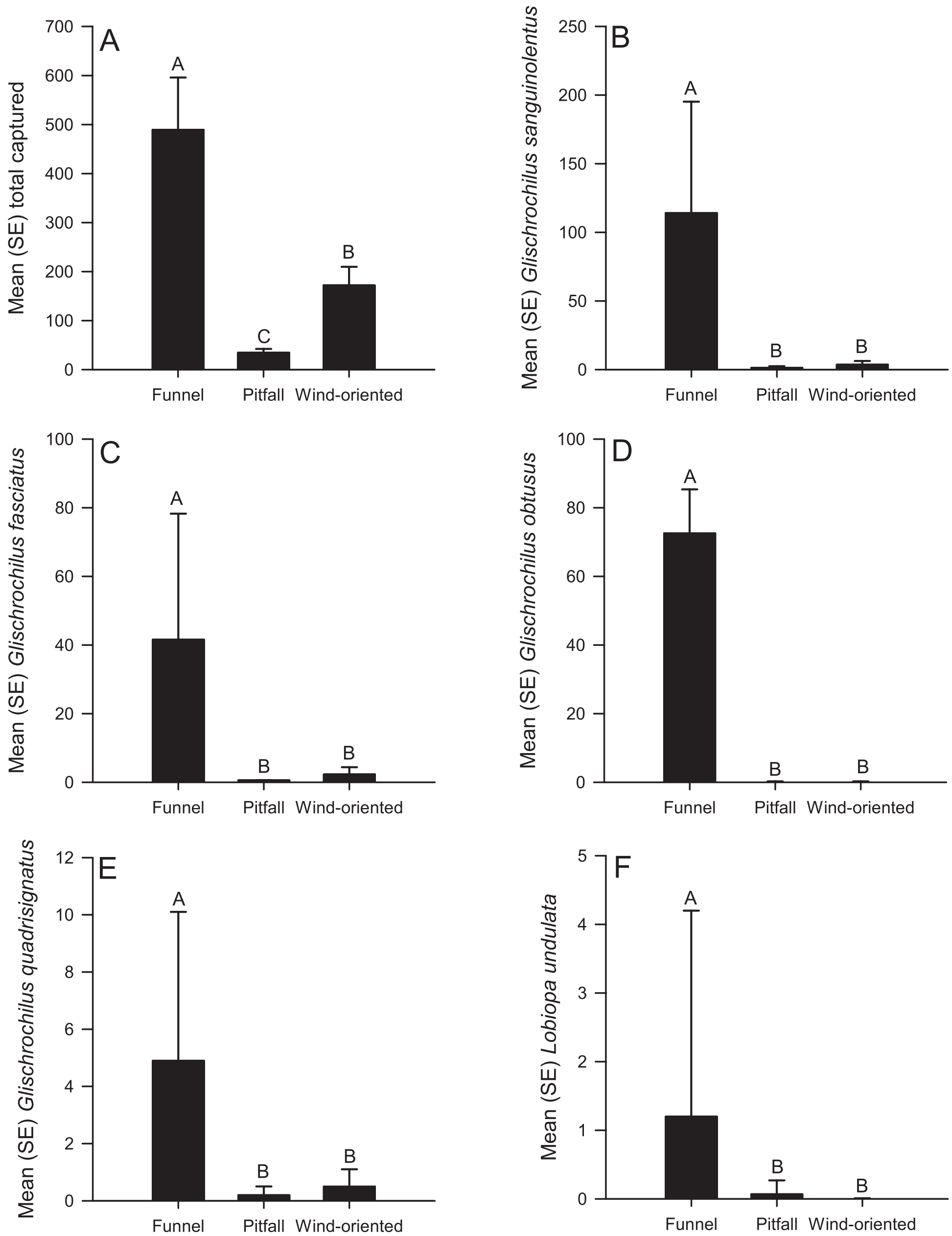

Fig. 4. Comparison of mean number of beetles captured by three trap types in northeastern oak forests. Means of columns sharing the same letter label were not significantly different. A, Total nitidulid beetles; B, *Glischrochilus sanguinolentus*; C, *Glischrochilus fasciatus*; D, *Glischrochilus obtusus*; E, *Glischrochilus quadrisignatus*; F, *Lobiopa undulata*.

(Fig. 9B and D, respectively). There were no differences in catches of *C. corticinus* ($F_{1,5}$ = 2.4, P = 0.2), *C. sayi* ($F_{1,5}$ = 0.00, P = 0.96), and *G. sanguinolentus* ($F_{1,5}$ = 1.7, P = 0.2) in wet and dry cups (Fig. 9C, E, and F, respectively).

Fungal Isolations from Nitidulids Trapped in Wind-Oriented Pipe Traps (2018)

In total, 140 beetles were collected in wind-oriented pipe traps in 2018. Of these, 102 were assayed for fungi, and 73 beetles produced identifiable cultures (mean number of unique cultures per beetle = 1.4, range 1–4), none of which were the oak wilt pathogen. Twenty-seven fungal species were isolated (Table 2), of which *Penicillium glandicola* (Oudemans) Seifert and Samson, *Penicillium paneum* Frisvad (Eurotiales: Aspergillaceae), and *Cladosporium* Link spp. (Capnodiales: Cladosporiaceae) were the most ubiquitous. Most fungal species, however, were only isolated once.

Discussion

Semiochemical trapping of nitidulids coupled with improvements in pathogen isolation provide a strong survey tool for oak wilt detection efforts as the disease continues to expand across North America. Nitidulid beetles are a primary vector (Gibbs and French 1980) of this pathogen and targeted trapping surveys with subsequent pathogen screening will provide natural resource managers with an important tool to confront the challenges of oak wilt in a new environment. Oaks are ubiquitous overstory trees in urban and natural forests throughout

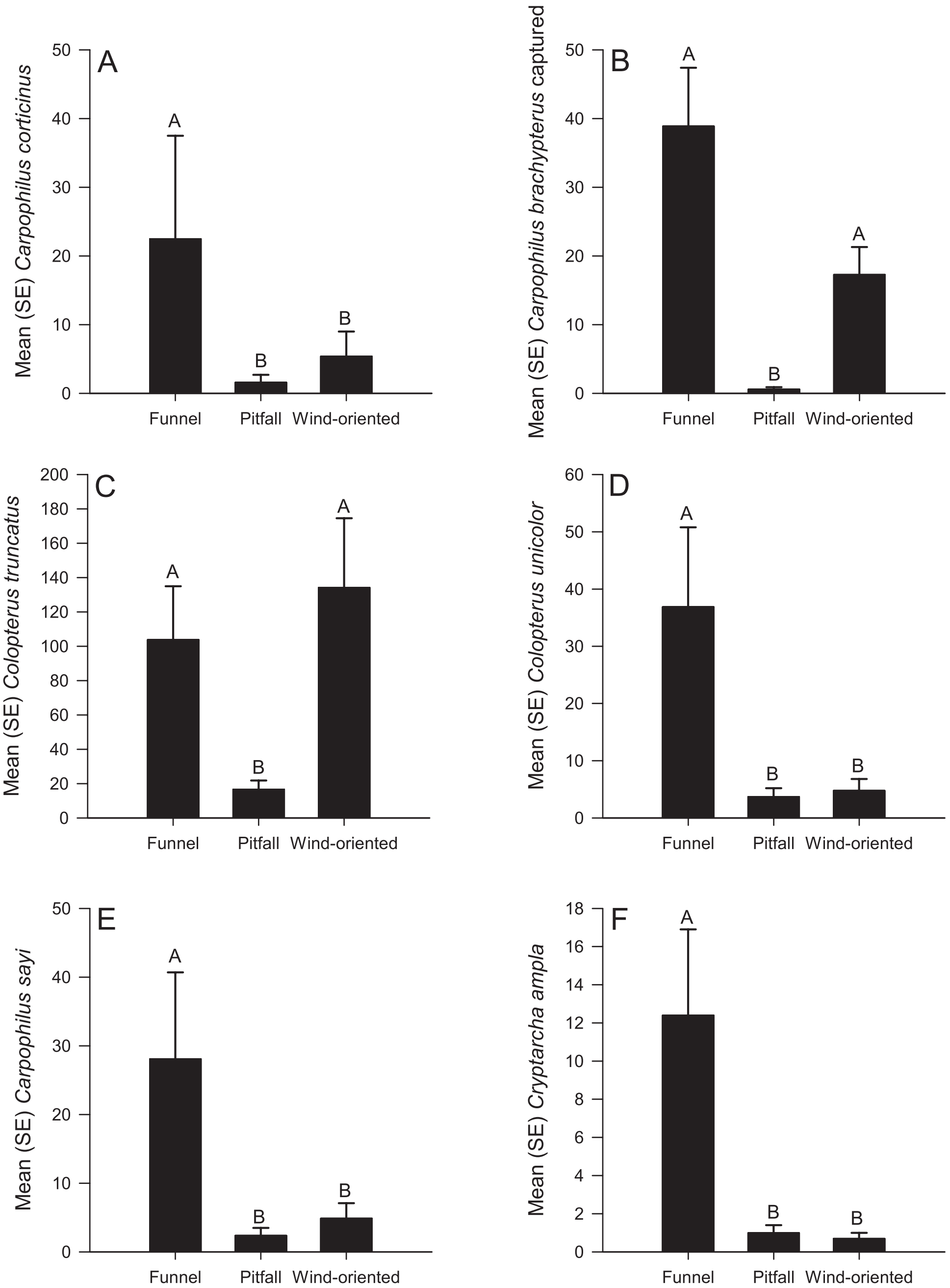

Fig. 5. Comparison of mean number of beetles captured by three trap types in northeastern oak forests. Means of columns sharing the same letter label were not significantly different. A, *Carpophilus corticinus*; B, *Carpophilus brachypterus*; C, *Colopterus truncatus*; D, *Colopterus unicolor*; E, *Carpophilus sayi*; F, *Cryptarcha ampla*.

the northeastern Unites States, and establishment and expansion of oak wilt presents a serious economic concern (Haight et al. 2011). Understanding factors that influence nitidulid capture, and subsequent fungal isolation, are critical to developing an effective survey and monitoring plan for northeastern forests.

Previous efforts have investigated survey methods for nitidulid beetles with various trap types used in different environments (Peng and Williams 1991, Dowd et al. 1992, Williams et al. 1993, James et al. 1996). Commercially available traps repurposed for nitidulids, including Magnet funnel traps, bucket traps, and fruit fly traps, as well as traps designed specifically for nitidulid beetles (Dowd et al. 1992), have been tested for abundance-based comparisons. Of the three trap types we tested, multiple-funnel traps were consistently the best trap for catching nitidulid beetles, regardless of species, or variable considered. This is consistent with previous work where multiple-funnel traps were one of the best traps for total nitidulid beetles captured, as well as for *Carpophilus lugubris* Murray, *G. quadrisignatus*, and *G. fasciatus* (Peng and Williams 1991). The wind-oriented pipe traps used in our study also performed well for some species, including *C. truncatus*, *C. brachypterus*, and *C. sayi*. Modified pitfall traps were the least effective trap when considering average abundance and were never the most effective trap for a given species. Multiple-funnel traps have a much larger surface area than the two other traps, and wind-oriented pipe traps only had a narrow

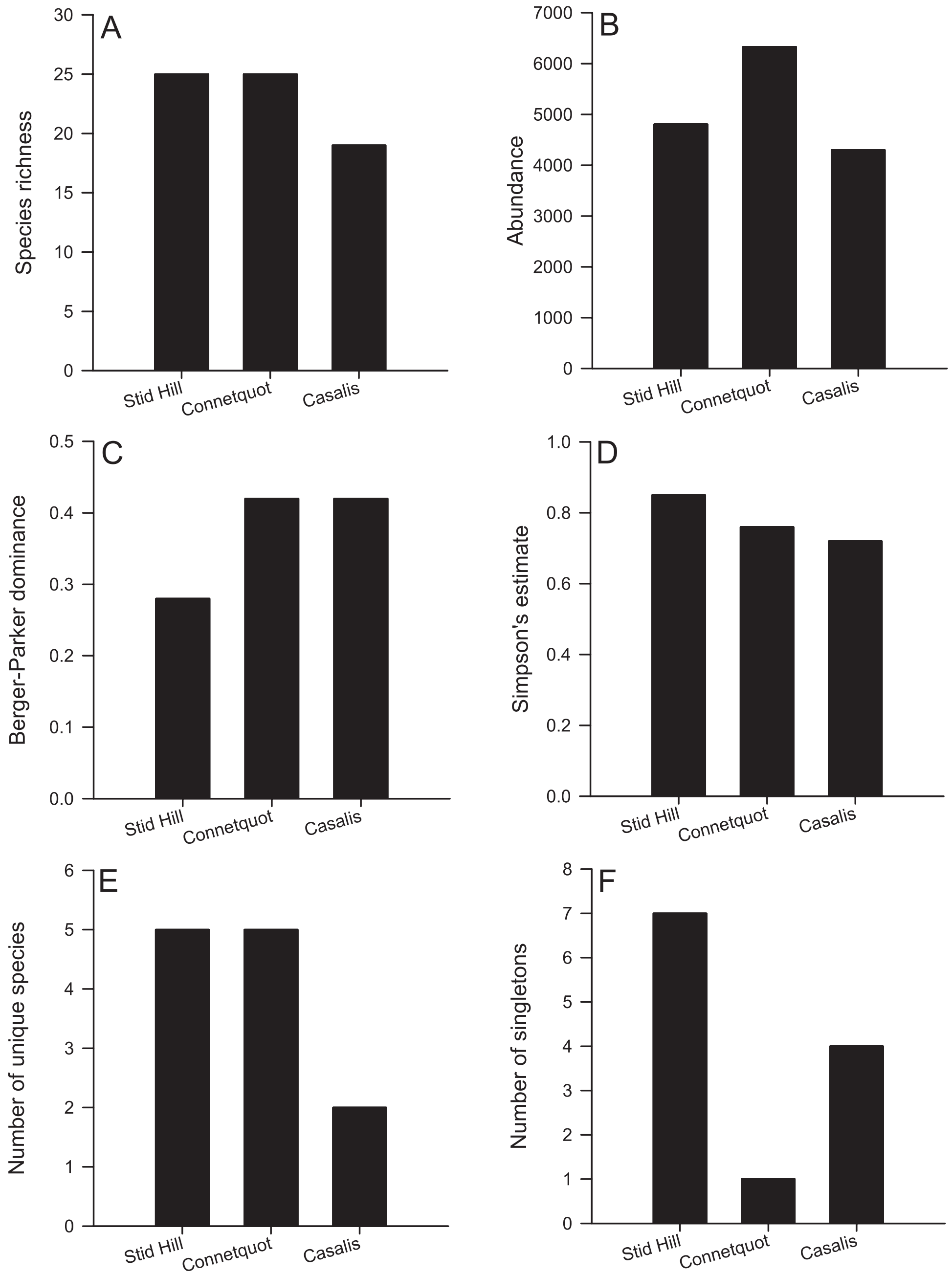

Fig. 6. Community metrics by site for nitidulids captured in northeastern oak forests. A, Number of species captured; B, number of beetles captured; C, Berger–Parker dominance estimate; D, Simpson's estimate; E, number of unique species captured; F, number of singleton species captured.

entrance for beetles attracted to the lures, and this undoubtedly factors into trap efficacy. Modified pitfall traps are tied directly to potential habitat selection and/or resources, potentially resulting in lower visitation rates from the population at large.

Although the lures contained pheromones targeting two species, cross attraction of these compounds has also been documented for *C. corticinus* and *C. brachypterus* (Bartelt et al. 2004), both caught at relatively high numbers during this study, and likely other species in those genera. Fermenting liquid also served as a synergist for these pheromones (Bartelt et al. 2004) and generic attractant, allowing for assessment of the broader nitidulid community present at each site. In terms of community metrics, multiple-funnel traps were superior to other traps, capturing higher total abundance, species diversity, singletons, and unique species. Rarefaction indicated that multiple-funnel traps captured the highest number of species where abundance was equal for traps, while also not reaching an asymptote suggesting sampling was incomplete. Modified pitfall traps were more effective at catching higher numbers of species and unique species than wind-oriented pipe traps. Modified pitfall traps also contained more singletons than wind-oriented pipe traps.

There was a relatively high degree of similarity among the three sites surveyed for nitidulid beetles, with Connetquot on Long Island and Casalis in New Hampshire the most similar, even though forest composition, latitude, and elevation differed the most between those two stands. Stid Hill and Connetquot had higher abundance, species richness, Simpson's estimates, and number of unique species

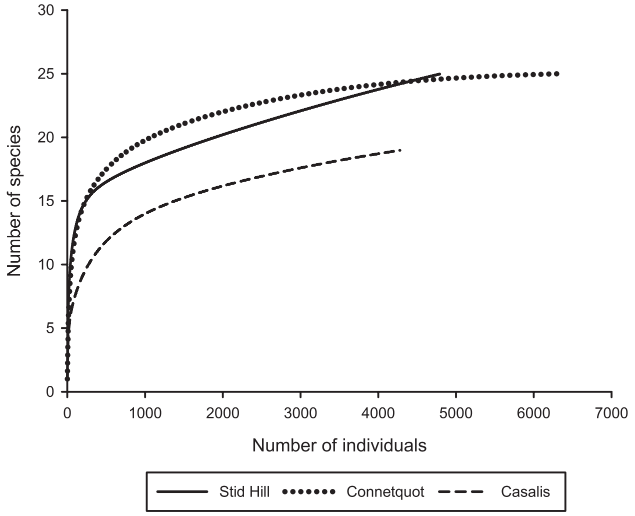

Fig. 7. Individual-based rarefaction curves for three sites in the northeastern United States.

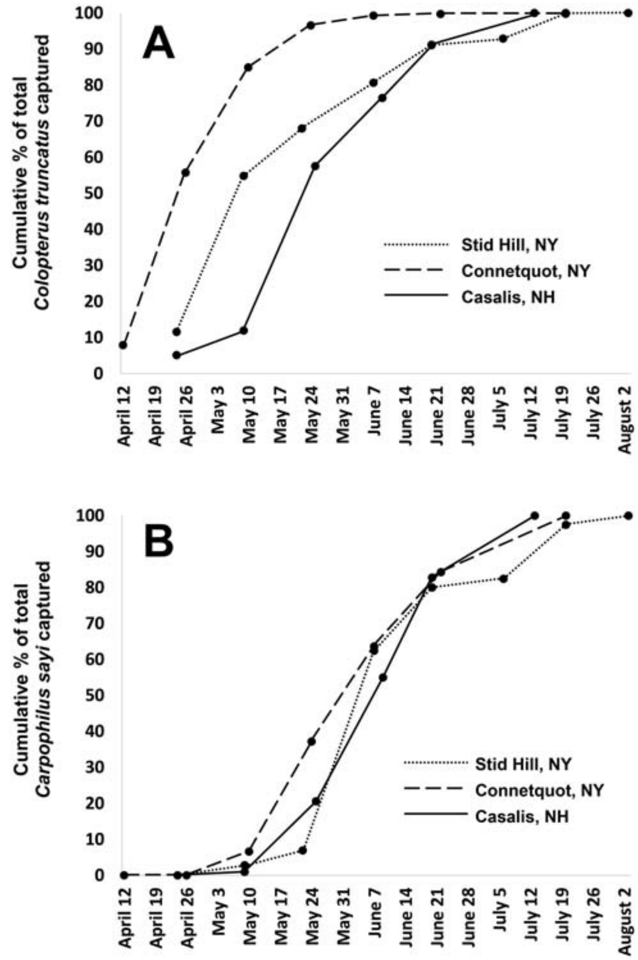

Fig. 8. Cumulative percentage of the total number of beetles collected during spring and early summer of 2017 at three sites in the northeastern United States. A, *Colopterus truncatus*; B, *Carpophilus sayi*.

compared with Casalis. Rarefaction curves also suggested that species richness was lowest in Casalis. Connetquot rarefaction curves were close to an asymptote, suggesting that sampling was nearly complete at this site. Stid Hill had higher species richness that the other sites, with more species likely available to sample.

Glischrochilus spp. were captured more often in multiple-funnel traps than any other trap during our experiment. *Glischrochilus fasciatus* and *Glischrochilus vittatus* (Say) have been reported as predaceous on the bark beetles, *Trypodendron lineatum* (Olivier) and *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae: Scolytinae), respectively (Chamberlin 1918, 1939), and *Glischrochilus* spp. are known scolytine predators in Europe (Parsons 1943). This may drive them more strongly to a trap with a strong vertical silhouette shape mimicking a host tree, an attribute of multiple-funnel traps that is also attractive to bark and ambrosia beetles (Lindgren et al. 1983). The pheromone and fermenting lures may serve as the general attractant to the trap vicinity, but the actual size and shape of the trap could influence host-locating behavior once beetles are in the area.

Spring flight patterns were compared among the three sites in the trap type experiment (2017) for *C. truncatus* and *C. sayi*, both captured in high numbers at all three sites. Early season visitation by these species to fresh wounds on asymptomatic trees can be an important factor in the transmission of the oak wilt pathogen. Risk from this mode of transmission is highest from April through June in the Great Lakes region (Ambourn et al. 2005, Juzwik et al. 2006), and reducing the number of fresh wounds created from activities such as pruning and cutting during times of peak nitidulid flight is the recommended management guideline (Juzwik et al. 2011). Flight of *C. truncatus* showed a similar pattern at the two New York sites, with a slightly delayed peak in New Hampshire likely corresponding to cooler spring temperatures. *Carpophilus sayi* began its spring flight later in the spring, with flight beginning in early May and peaking in late May to early June. This is comparable to flight phenologies reported for Wisconsin (Jagemann et al. 2018) and Minnesota (Ambourn et al. 2005) where peak flight was early to mid-spring for *C. truncatus*. Both studies also reported a second peak of *C. sayi* in late summer to early fall, which was outside the seasonal window of our trapping effort. Based on these results, a similar strategy of limiting pruning and cutting activities from April through June would also be practical for management of oak wilt transmission in New York and southern New England as oak wilt continues to establish in the region.

Wet collection cups captured more total nitidulid beetles, as well as more *C. truncatus* and *G. fasciatus*, whereas no differences were found between cup type for *C. corticinus*, *C. sayi*, and *G. sanguinolentus*. Wet cups are generally better at capturing arboreal insects and have been tested for many species when used with multiple-funnel traps (Miller and Duerr 2008). With molecular methods for detecting fungal DNA becoming more sensitive, and successful detection of fungal DNA reported from beetles collected from propylene glycol funnel traps (Moore et al. 2019), wet collections of nitidulids in funnel traps may soon become a more practical monitoring tool. Tremblay et al. (2019) demonstrated that many phytopathogenic fungal species may be detected in discarded collection fluid using high-throughput sequencing technology.

Although this study does not define nitidulid beetle species that are implicit in vectoring *B. fagacearum* in the northeastern United States, we provide baseline information on the community of nitidulid beetles dispersing in spring, a first step in identifying insect species that may be important vectors as the disease range continues to expand. Several species that have been reported as possible or likely vectors in other parts of the country (*C. truncatus* and *C. sayi*

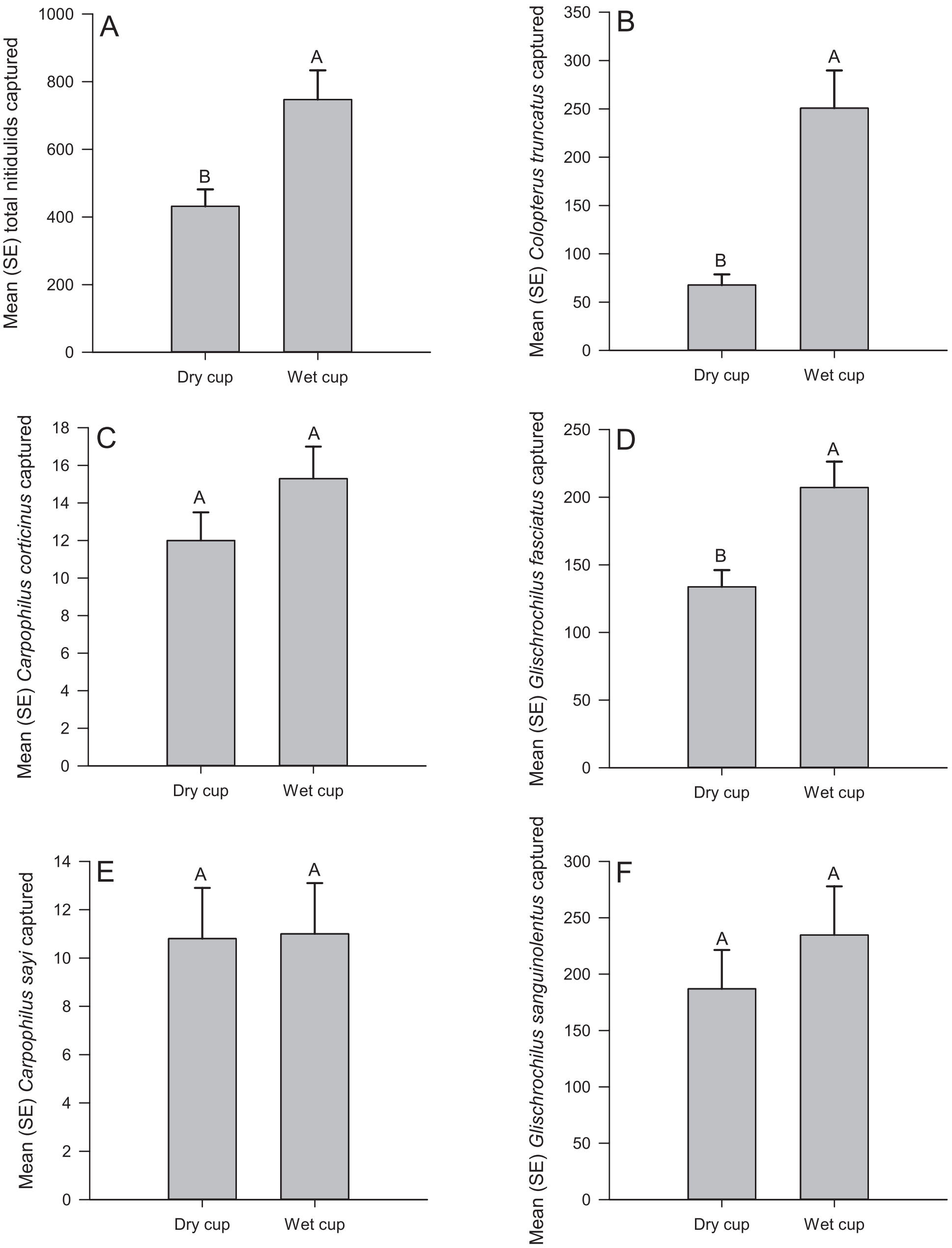

Fig. 9. Comparison of mean number of beetles captured in multiple-funnel traps with dry versus wet collection cups in Casalis State Forest, New Hampshire. Means of columns sharing the same letter label were not significantly different. A, Total nitidulid beetles; B, *Colopterus truncatus*; C, *Carpophilus corticinus*; D, *Glischrochilus fasciatus*; E, *Carpophilus sayi*; F, *Glischrochilus sanguinolentus*.

in Minnesota, Wisconsin, and Missouri; *Colopterus semitectus* (Say) in Missouri; *Colopterus maculatus* (Erichson) and *Cryptarcha concinna* Melsheimer in Texas; Appel et al. 1990, Cease and Juzwik 2001, Ambourn et al. 2005, Hayslett et al. 2008) were captured at some of our sites, with *C. truncatus* and *C. sayi* captured consistently and abundantly at all three sites. Further research to determine which species visit both sporulation mats on infected trees and fresh wounds on uninfected trees is necessary to determine species involved in pathogen transmission in the northeast United States.

Trap selection may ultimately depend on the availability of trap types and the overall objectives of the trapping effort. We have demonstrated that multiple-funnel traps were as good or better than the other trap types, whereas wind-oriented pipe traps also performed relatively well for a few species. If objectives include general monitoring for diversity, abundance, and flight patterns, then multiple-funnel traps with a wet collection cup would be the most effective trap, and potentially very cost effective if the traps are already on hand. If objectives include using beetles to check for the genetic presence of fungal pathogens, a wet collection may not be ideal. Even dry cups on multiple-funnel traps collected weekly were often filled with rainwater and forest debris, likely contaminating the sample for genetic pursuits. The collection cup on wind-oriented pipe traps, however, are more protected from the rain, and contained only dead beetles even after 2 wk in the field.

The oak wilt pathogen, *Bretziella fagacearum*, was not isolated from nitidulid beetles trapped in wind-oriented pipe traps, which was expected given that the incidence of oak wilt fungus isolation from *C. truncatus* and *C. sayi* trapped in Wisconsin sites where

Table 2. Fungi isolated from nitidulid beetles captured in spring 2018 from wind-oriented pipe traps

Fungus	Accession no.	Beetle spp.[a,c]	Location[b,c]	Guild[d]	Type of plant disease[e]
Alternaria alternata	MT355154	**GS**, CC	**Ca**	Animal Pathogen–Endophyte–Plant Pathogen–Wood Saprotroph	Foliar
Alternaria tenuissima	MT362612	**GF**	**Co**	Endophyte–Plant Pathogen	Foliar
Aspergillus insuetus	MT361321	**CS**	**Ca**	Animal Pathogen	Foliar
Aureobasidium pullulans	MT363099	**GQ**, CA, CS, GF, GS	**S**, Co	Animal Pathogen–Endophyte–Epiphyte–Plant Pathogen	Foliar
Bjerkandera fumosa	MT359331	**CT**	**Ca**	Wood Saprotroph	Wood decay
Cladosporium cladosporioides	MT359892	**CT**	**Ca**	Endophyte–Epiphyte–Plant Pathogen	Foliar
Cladosporium pseudocladosporioides	MT362705	**CC**	**Co**	No data	Foliar
Cladosporium ramotenellum	MT360380	**GS**	**Ca**	No data	Postharvest
Cladosporium ramotenellum	MT361323	**CS**	**Co**	No data	Postharvest
Cladosporium sp.		CA, GQ	S, G	Animal Pathogen–Endophyte–Lichen Parasite–Plant Pathogen–Wood Saprotroph	Foliar and postharvest
Cordyceps farinosa	MT361315	**GF**	**C**	Animal Pathogen–Clavicipitaceous Endophyte–Fungal Parasite	No data
Devriesia sp.	MT360263	**GF**	**Ca**	Plant Pathogen	Foliar and postharvest
Diplodia corticola	MT337404	**GS**	**Ca**	Epiphyte–Plant Pathogen–Wood Saprotroph	Canker
Fomes fomentarius	MT355083	**GS**	**Ca**	Wood Saprotroph	Wood decay
Nemania sp.	MT361320	**CS**	**Co**	Undefined Saprotroph	Foliar
Penicillium fellutanum	MT362922	**CT**	**Co**	No data	Foliar
Penicillium glandicola	MT355155	**CT**, CA, CC, CS, GF, GS	**Ca**, Co, G, S	No data	No data
Penicillium paneum	MT360564	**CB**, CC, CS, CT, CU, GF, GS	**Ca**, Co, G, S	No data	Postharvest
Pezizomycetes gen. sp.	MT360640	**GF**	**Ca**	No data	No data
Plicaturopsis crispa	MT363254	**CS**	**Ca**	No data	Wood decay
Ramularia unterseheri	MT363782	**CT**	**S**	Plant Pathogen	No data
Rhodotorula colostri	MT362984	**CS**, GQ	**S**, G	No data	Foliar
Roussoellaceae sp.	MT361641	**CS**	**Co**	No data	No data
Sporothrix eucastanea	MT364280	**CT**	**S**	No data	Sap stain
Sporothrix inflata	MT360679	**GF**	**Ca**	Animal Pathogen	Sap stain
Sporothrix prolifera	MT353979	**GS**	**Ca**	No data	Sap stain
Tympanis sp.	MT359319	**CU**	**Ca**	Undefined Saprotroph	Canker
Umbelopsis isabellina	MT362054	**GF**	**Ca**	Undefined Saprotroph	Foliar

[a]*Cryptarcha ampla* (CA), *Carpophilus brachypterus* (CB), *Carpophilus corticinus* (CC), *Carpophilus sayi* (CS), *Colopterus truncatus* (CT), *Colopterus unicolor* (CU), *Glischrochilus fasciatus* (GF), *Glischrochilus quadrisignatus* (GQ), *Glischrochilus sanguinolentus* (GS).

[b]Casalis (Ca), Connetquot (Co) Glenville (G), Stid Hill (S).

[c]Beetle species and location in bold correspond to the sequence submitted to GenBank.

[d]http://www.funguild.org/.

[e]https://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm

oak wilt has been detected since the 1940s ranged from 5 to 8% (Jagemann et al. 2018). In addition, oak wilt-positive trees from New York sites had been removed prior to these studies, reducing the likelihood that fungal mats were available for nitidulid beetles to acquire fungal propagules. Most fungi isolated from nitidulid beetles were known plant pathogens, suggesting that in addition to *B. fagacearum*, these beetles could potentially serve as vectors other plant pathogens. For example, *Diplodia corticola* A.J.L. Phillips, A. Alves & J. Luque (Botryosphaeriales: Botryosphaeriaceae) causes oak cankers and *Bjerkandera fumosa* (Pers.) P. Karst. (Polyporales: Meruliaceae) and *Fomes fomentarius* (L.) Fr. (Polyporales: Polyporaceae) are wood decay fungi. We did not perform any replicated experiments investigating the role nitidulid beetles play in transmitting any of the isolated fungi to healthy plants, and many were isolated only once. Therefore, additional experiments exposing nitidulid beetles to fungal cultures and releasing them onto host plants in a replicated manner, as was done with *Glischrochilus quadrisignatus*, which vectors the *Fusarium* stalk rot of corn pathogen (Attwater and Busch 1983), would be an avenue for future research. Given the potential of nitudulid beetles vectoring plant pathogens, it would also be interesting to sample a greater number of nitiduid beetles with high-throughput sequencing technology to explore their microbiome. Endophytes were also isolated (U'Ren et al. 2012). The fungal species found most often were in the genera *Cladosporium* Link and *Penicillium* Link, which is not surprising since fungi in these genera are some of the most ubiquitous, occurring worldwide and from nearly any environmental source (Bensch et al. 2012, Visagie et al. 2014). Some fungal species isolated from nitidulid beetles, such as *Sporothrix* Hektoen & C.F.Perkins spp. (Ophiostomatales: Ophiostomataceae), have been associated with bark and ambrosia beetles (Zhou et al. 2004, Jankowiak et al. 2019). Others, such as the marine fungus Roussoellaceae gen. sp. at

Connetquot, were unexpected, providing a fascinating glimpse of potential nitidulid beetle habitat and, thus, behaviors.

Acknowledgments

We thank the following individuals for assistance with site selection and access: Bob Spoerl and Ingeborg Seaboyer (New Hampshire Division of Forests and Lands), Robert Cole, Mark Gooding, Brice June (New York State Department of Environmental Conservation), and Annie McIntyre (New York State Parks). Robert Cole, Kelsey McLaughlin, Tom Schmeelk, and Liam Somers (New York State Department of Environmental Conservation) helped with field, laboratory, and identification work. Andrew Cline (California Department of Food and Agriculture) and Gareth Powel (Brigham Young University) provided valuable taxonomic assistance for difficult specimens. John Stanovick (USDA Forest Service) provided advice on statistical methods. We also thank Jennifer Juzwik, Melanie Moore, Danielle Martin (USDA Forest Service), Cynthia Ripoll (Macherey-Nagel), and Nichole Broderick (University of Connecticut) for their technical assistance concerning fungi isolation from beetles. We also thank two anonymous reviewers for their valuable comments and suggestions that greatly improved the manuscript.

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