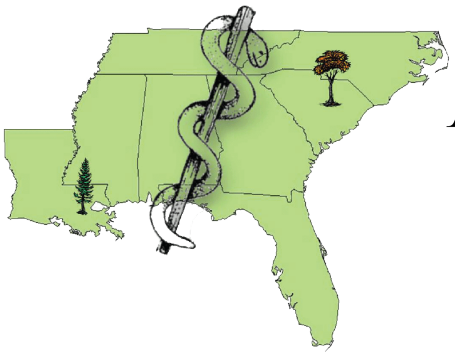

AUBURN UNIVERSITY
FOREST HEALTH COOPERATIVE

RESEARCH REPORT 19-04

INSECT DIVERSITY IN A LOBLOLLY PINE STAND
INFECTED WITH *LEPTOGRAPHIUM TEREBRANTIS*

by
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2.1. ABSTRACT

Ophiostomatoid fungi, such as *Leptographium terebrantis*, are vectored by root-feeding bark beetles and cause sap staining, reduced growth, and vascular occlusion in tree tissues they infect. To assess the impact this fungus has on insect diversity, especially root-feeding bark beetles and other insects of concern, we utilized pitfall and panel insect traps to sample these invertebrates in order to capture the widest variety possible. While undoubtedly a pathogen and stressor to trees, it is necessary to understand how it affects overall insect populations to make informed decisions regarding management. Of an initial two year study in a commercial loblolly pine stand, insect diversity varied between years. Pitfall traps captured more Hylobiini, while panel traps were more efficient at capturing ambrosia beetles. Despite this, average morphospecies capture, along with Shannon and Simpson indices, showed consistent diversity and species evenness across trap type. Panel traps were important in capturing different species along with consistent capture rates. Despite *L. terebrantis* inoculum, disparity of annual insect populations during the sampling period seemed due to seasonal variation.

2.2. INTRODUCTION

With a major stake in the southeastern United States' economy, loblolly pine is the tree staple due to its hardy nature and fast growth (Schultz 1997). However, despite this, it is not immune to pests and pathogens. While insects are innumerably beneficial, providing critical ecosystem services such as food, pollination, decomposition and nutrient recycling (Edmonds and Eglitis 1989; Ulyshen 2016), pest insects interfere with human desires for wood products, reducing wood quality through their habits, introducing pathogens, and impacting tree growth.

In forests, insects play host to microbes and fungi, acting as vectors and contributing to disease cycles (Manion 1981). Fungal species can alter tree tissue's nutrient content (Baker and Norris 1968; Beaver et al. 1989; Ayres et al. 2000), increase the insect's reproductive capacity (Eckhardt et al. 2004a), or interfere with insect development (Klepzig and Hofstetter 2011). Already, when

insects attack trees, they cause stress, affecting physiological processes – such as growth and defense – and reallocation of resources to those purposes (Christiansen et al. 1987). Weakened trees are further susceptible to more pests through the stress chemicals they release (Rudinsky 1966) and to disease (Manion 1981).

Root feeding bark beetles in particular vector ophiostomatoid fungi that stain wood and decrease tree growth in addition to their feeding habits that create galleries in wood and girdle trees (Repe and Jurc 2010). Beetles such as these include *Hylastes salebrosus* and *Hylobius pales*, and have been found associated with declining pine trees, carrying fungi such as *Leptographium* and *Grosmannia* (Klepzig et al. 1991; Jacobs and Wingfield 2001; Eckhardt et al. 2007; Matusick et al. 2013). Rane and Tattar (1987) showed that the infamous black turpentine beetle, *Dendroctonus terebrans*, was capable of picking up ophiostomatoid fungi by tunneling through infected tissues, further perpetuating the cycle.

As one such ophiostomatoid fungi in the southeastern United States, *Leptographium terebrantis* has been found to result in root damage and resin-filled lesions in the xylem, resulting in occlusion (Raffa and Smalley 1988; Matusick et al. 2012). Several species of pines – including shortleaf, slash, loblolly, longleaf, and red – have shown an association of decline with this fungus (Klepzig et al. 1991; Harrington and Cobb 1983; Jacobs and Wingfield 2001). With this study we were able to observe how increasing inoculum levels of this fungus affected insect diversity.

Biodiversity has been shown to be an instrumental factor in the invasion of non-native species in an area and enhances the spread of fungal diseases (Knops et al. 1999). Decreased insect species often corresponds to a decrease in plant diversity (Murdoch et al. 1972; Haddad et al. 2001), which in turn can increase pathogen infection (Ratnadass et al. 2012). Monocultures enriched with other plant species or genetic variants has potential to reduce diseases and pests though the increase of barriers and predatory and parasitic insects (Power 1987; Johnson et al. 2006; Ratnadass et al. 2012). Knowledge such as this is critical to developing management tools. This study was initiated to determine the impact *L. terebrantis* has on insect populations in association with varying inoculum levels and to test the hypothesis that root-feeding bark beetle pests increase with increasing inoculum.

2.3. MATERIALS AND METHODS

2.3.1. Study site and plot measurements

Fifteen plots were established on a loblolly pine, *Pinus taeda*, stand privately owned by a member of the Forest Health Cooperative (Auburn University, AL) in Eufaula, Alabama (Fig. 2.1). Prior to the setup, the land had undergone a third row thin, allowing for paired lined plots and double randomization. One line had five randomly chosen control trees and the second had another five chosen as treatment trees. These two lines together consisted of a single experimental plot and altogether all fifteen made up a split plot completely randomized design with three replicates per treatment (Fig. 2.2).

Trees at the time of selection were aged to be between twelve and thirteen years of age. Soil in the study area was identified mainly as Annemaine-Wahee complex (AwA) and minorly as Ocilla loamy fine sand (OcA) (Fig. 2.2).

2.3.2 Insect trapping

To monitor changes in insect populations over time, three different traps of two types – pitfall and panel – were used. Panel traps (Forestry Distributing Inc., Boulder, CO) (Fig. 2.3A) were made of black corrugated plastic and Lindgren funnels hung on metal poles and were designed to capture flying insects. Panel traps were installed on poles one meter above the ground and had a plastic cup attached to the bottom that contained a 2:1 mixture of water and antifreeze (Super Tech antifreeze, Bentonville, AR) to preserve captured insects.

Pitfall traps consisted of two types. One consisted of a 20-cm length of a 10-cm diameter polyvinyl chloride plastic pipe with eight holes spaced equally around the circumference (Klepzig et al. 1991) (Fig. 2.3B). Both ends of the pipe had caps that served as removable lids, with holes drilled into the bottom cap for drainage. Traps were buried into the soil and leaf litter until the entrance holes lined up at ground level. These traps' interiors were coated with a layer of liquid Teflon^{TM} (Northern Products Woonsockets, RI) to prevent insects from climbing out once inside. Each of these pitfall traps were baited with two 3 cm long by 1 cm diameter loblolly pine twigs placed in the bottom of the interior to provide refuge for captured bark beetles.

The second type of pitfall trap (15 cm diameter funnel-type traps, Multi-pher; Bio- Controle, Quebec City, Quebec) (Fig. 2.3C and D) allowed for the entry of larger ground based insects and each contained a 16 oz SOLO plastic cup filled with 50 mL of a 2:1 mixture of water and antifreeze (Super Tech antifreeze, Bentonville, AR) to preserve insects that fell inside. All types of insect traps had cups refilled and twigs replaced every two weeks during the collection period.

Insect collections were taken up every two weeks from February 2016 to March 2018. Each of the fifteen plots held two of the pitfall traps baited with twigs and one pitfall trap with an antifreeze mixture. Panel traps were placed with two on each of the sides and middle of the study area. Both twig-based pitfall traps and panel traps were established in 2016, one year prior to tree inoculation while antifreeze-based pitfalls were established February 2017 before inoculation. During collection periods, insects caught were placed in polyethylene cups and transported to the Forest Health Dynamics Laboratory at Auburn University (Auburn, AL, USA), placed in the cooler until time for processing, sorted, and identified.

2.3.3. Insect identification

Specimens captured were identified to family with taxonomic keys (Triplehorn and Johnson 2005) where possible, given a designated morphospecies name according to Ulyshen and Hanula (2009), and curated at the Forest Health Dynamics Laboratory at Auburn University. A voucher collection was also compiled for Louisiana State University. Species of concern were catalogued (Table 2.1) and also identified with taxonomic keys (Wood 1982).

2.3.4. Tree Inoculation

Tree inoculations took place in February of 2017. The fungal isolate of *L. terebrantis* was retrieved from the Forest Health Cooperative Laboratory at Auburn University after determining maximum virulence (Devkota et al. 2018b). Root-feeding beetles and weevils often inoculate the roots and lower bole of a tree with fungi, and previous studies showed that inoculation points utilizing the lower stem can have a similar pathogenicity (Matusick et al. 2016). Of the treatment plots (Fig. 2.2), inoculation procedures were done in a manner similar

to Devkota et al. (2018a). Toothpicks used for the experiment were previously sterilized at 121 ºC for sixty minutes, and fungal colonization was allowed for twenty-one days at 25 ºC.

Treatment plots were designated three each as either control, wound, low, medium, or high. Control trees were untouched and wound trees had inoculum-free toothpicks inserted to simulate infection. Treatment corresponded to toothpick density inserted. Low, medium, high, and wound each had inoculations of two, eight, sixteen, and sixteen, respectively, per 6.35 cm of diameter at breast height (DBH) (Fig. 2.2).

2.4. STATISTICAL ANALYSIS

Species richness in the form of total number of morphospecies was conducted for twig- and antifreeze-based pitfalls with a repeated measures analysis of variance (ANOVA). The dependent variable was morphospecies while the independent variables were treatment and year. Compound symmetry (CS) was used for the covariance structure. These statistical analyses were run with the program SAS (SAS 9.4, 2013). Indices for Shannon and Simpson, Sørensen's coefficient, and species accumulation curves, were ran with the EstimateS program (EstimateS, 9.1.0, 2013; Colwell 2013) and Excel (2016). Transformed values for Shannon and Simpson were computed and repeated measures ANOVA ran with SAS (SAS 9.4, 2013).

2.5 RESULTS

A total of 9,748 insects divided into 16 orders, 149 families, and 676 different morphospecies were collected over the duration of this study (Table 2.2). Orders with the most to the least number of morphospecies were as listed: Coleoptera, 299; Diptera, 130; Hymenoptera, 120; Hemiptera, 47; Lepidoptera, 22; Collembola, 19; Trichoptera, 12; Psocodea, 11; Orthoptera, 4; Blattodea, 3; Neuroptera, 3; Microcoryphia, 1; Mecoptera, 1; Mantodea, 1; Phasmida, 1; Thysanoptera, 1; and unknown, 1.

Diversity was described in the form of species richness, defined as the number of morphospecies, the indices of Shannon and Simpson, and Sørensen's coefficient where applicable. Morphospecies captures were averaged for twig-based pitfalls and separated by year (Fig. 2.4), but species richness was only significant between years ($p \leq 0.0001$; Table 2.3). Similarly, antifreeze-based pitfall captures were averaged for the one year they were out (Fig. 2.5). However, results on total morphospecies were marginally significant among treatments ($p=0.0632$; Table 2.4). Simpson and Shannon indices for antifreeze-based pitfalls were calculated for pre- (Table 2.5) and post-inoculation periods (Table 2.6) by season and treatment, yet these transformed values were not significant among treatment for the year they were in place (Shannon, $p=0.0918$; Simpson, $p=0.2057$).

Averaged morphospecies for panel traps (Fig. 2.6) were also separated by year and totals were significant between years ($p=<0.0001$; Table 2.7). In the interest of comparing trends, separating total morphospecies caught per trap for year 1 (Fig. 2.7) and year 2 (Fig. 2.8) revealed seasonal variation. Shannon and Simpson index values for each panel trap were averaged according to season and for pre- (Table 2.8) and post-inoculation periods (Table 2.9) and transformed values supported initial significant results between years (Table 2.12; Table 2.13). Sørensen values were also averaged by season, showing – despite similar capture totals – most traps had less than 50% similarity of morphospecies for pre- (Table 2.10) and post-inoculation (Table 2.11). The three

orders of insects that made up over 80% of all the morphospecies documented – Coleoptera, Diptera, and Hymenoptera – were also separated by year in panel traps, revealing trends (Fig. 2.9, 2.10, 2.11) similar to the overall total (Fig. 2.6).

Species of concern (Table 2.1) by taxonomic group for the duration of the study were totaled (Fig. 2.9) and broken down further by trap type (Fig. 2.13). Panel traps captured overwhelmingly more ambrosia beetles, but accounted for few other species of concern. In contrast, pitfall traps captured most Hylobiini but few ambrosia beetles. After breaking down ambrosia beetles by species (Fig. 2.14), it can be seen that that *Xylosandrus germanus* Blandford, an introduced species originally from Asia, made up over sevenfold the next highest individual collected of a species. Indeed, even combining all species of concern caught, including ambrosia beetles, this species still made up over half of the total individuals of concern collected (Fig. 2.15).

Finally, species accumulation curves were created according to Colwell et al. (2012) for each trap type, divided by season, inoculation period where relevant, and extrapolated twofold. For twig-based pitfall traps (Fig. 2.16), morphospecies counts remained low and extrapolation revealed that additional traps of this type would gain few new species to warrant their placement. Antifreeze-based pitfalls (Fig. 2.17) revealed a similar trend, supporting the idea that enough traps are in place. Panel traps (Fig. 2.18) revealed sharper rates of increase, showing that, at least in warmer months, additional traps have the potential to catch two to four more species per trap.

2.6 DISCUSSION

In this 25 month period of insect collection, we compared insect species richness of plots infected with varying levels of *L. terebrantis* inoculum in a commercial loblolly pine stand. Species richness only varied significantly between years among twig-based pitfall and panel traps. Due to the shorter period of duration for antifreeze-based pitfalls but given the marginal significance of the period they were in place, these traps show promise for recording results in future years. As for the panel and twig-based traps, the fact that years differed but treatment does not warrant further monitoring. A drought that occurred during the winter of the first year could be an influencing factor between years (Table 2.14). While these events can stress plants, leading to more insect attacks due to the attracting semiochemicals trees emit (Mattson and Haack 1987; Ferrell 1996), prolonged periods can also affect insect survival (Schowalter et al. 1981). Insects in dormancy have both morphological and physiological methods to conserve water, but they are not invulnerable (Danks 2000). While insect diversity can be positively correlated with plant diversity (Haddad et al. 2001), decreasing pest populations (Power 1987), studies have suggested moisture can be a stronger influencing factor during certain parts of the year (Janzen 1972; Wenninger and Inouye 2008). Wood-boring beetles and hemipterans in particular have been shown to benefit from drought, taking advantage of lowered plant defenses (Mattson and Haack 1987; Ferrell 1996), concentrated nutrient content of tissues (Barras and Hodges 1969; Mattson and Haack 1987), elevated temperatures for development, and decreased parasite loads (Mattson and Haack 1987).

Panel traps, despite their differing placements around the study area, caught similar amounts of morphospecies, even across seasons (Fig. 2.7; Fig. 2.8), despite catching less than 50% of similar species the majority of the time (Table 2.10). However, this lower similarity of traps supports their separation and importance in recording diversity for the entire study area. Likewise, Shannon and Simpson index values supported individual trap's consistency in diversity and evenness (Table 2.8; Table 2.9). These same values showed higher diversity in the first year, supporting a

significant find in terms of species richness between years (Table 2.7). Seasonal trends shown by panel traps (Fig. 2.6) were similar in both pitfall based traps (Fig. 2.4; Fig. 2.5) with peaks in spring and early summer and decreases in winter. This suggests that perhaps, despite lower catch numbers in twig-based traps, insect species captured were a reflection of the larger community trends. Other studies have shown flight intercept traps, such as our panel traps, to be more efficient at capturing total species, though pitfall traps catch insects that flight intercept traps do not, supporting their use (Hyvärinen et al. 2006). Insects can often be attracted to certain colors, especially flower visiting insects, influencing captures of certain groups (Campbell and Hanula 2007) and some insect groups, despite being observed, may be excluded from traps due to sampling bias, reinforcing the need for multiple trap types (Su and Woods 2001).

Our species accumulation curves for both twig- and antifreeze-based pitfall traps provided low rates of new species discovered per additional trap. Williams et al. (2007) previously suggested a new species discovery rate of more than two for additional sampling to be considered worth the cost of time and effort. Both curves for pitfall traps provide support for the determination that sufficient traps are in place for the remaining duration of the study (Fig. 2.16; Fig. 2.17). However, in contrast to pitfall based traps, panel traps showed a possible greater collection rate of new species if more traps were deployed in the future (Fig. 2.18), especially during the warmer months. However, it is worth noting that the curve shows no near sign of leveling off as an asymptote, and therefore suggests sampling has not been sufficient (Williams et al. 2007). Nevertheless, with poorly described taxon such as in some insect groups, this result is not unexpected (Gaston et al. 1995).

Ambrosia beetles far outnumbered root-feeding bark beetles in this study (Fig. 2.12). Panel traps were by far the most efficient at recording these species, but ground based pitfalls were more efficient at capturing Hylobiini, possibly due to their root-feeding tendencies (Fig. 2.13). Pitfall traps have traditionally been used to sample ground-dwelling arthropods, especially those active at ground level (Prasifka et al. 2007) while ambrosia beetles, such as *X.germanus* or *Xyleborus glabratus*, have been shown to have better capture rates with airborne traps 0.5 and 1.5 meters off the ground (Reding et al. 2010; Hanula et al. 2011). Supporting this, more than one study has noted higher Hylobiini and *Hylastes* populations in areas of root mortality (Klepzig et al. 1991; Erbilgin and Raffa 2002; Eckhardt et al. 2007). The large number of invasive *X. germanus* (Fig. 2.14) may be an indicator that plant diversity is lacking (Power 1987; Knops et al. 1999) and may indicate a need for an increase in genetic diversity of plants currently present (Johnson et al. 2006). Invasive pests such as this can introduce and spread pathogens, their movements aided by increased spatial connectivity and concentration of resources (Ratnadass et al. 2012) and can outcompete native insects and disrupt pollination services (Kenis et al. 2009). Increasing plant diversity as a method to disrupt the spread of this species, dilute available resources, and facilitate predators and competitors may be an option (Johnson et al. 2006). As a polyphagous pest of hardwoods such as elm, oak, hickory, maple, and walnut, and even conifers such as red, white, and scotch pine (Buchanan 1941; Weber and McPherson 1983), this species has a wide variety of hosts and is a possible vector of *Ophiostoma ulmi*, the pathogen that causes Dutch elm disease, a forest health threat (Hoffmann 1941).

Table 2.1. Species of concern captured and sorted by taxonomy.

Taxonomy Classification	**Species Collected**
Hylastes	*Hylastes salebrosus*
Hylobiini	*Hylobius pales, Pissodes nemorensis*
Ips	*Ips avulsus*
Ambrosia	*Xyleborus pubescens, Xyleborus ferrugineus, Orthotomicus caelatus, Xyleborinus saxeseni, Xylosandrus crassiusculus, Xylosandrus germanus, Monarthrum fasciatum, Gnathotrichus materiarius, Xyleborus affinis*

Table 2.2. Totals of insect families collected over the entire two-year sampling period (25 months). Of 9,748 total insects collected in 16 orders, there were 676 morphospecies in 149 families.

Coleoptera		Oedemeridae	1	Reduviidae	4	Psocodea	1	Hybotidae	1	Encyrtidae	1
Cerylonidae	1	Silvanidae	4	Miridae	1	Psocoptera	8	Tipulidae	1	Colletidae	1
Ciidae	3	Tenebrionidae	12	Nabidae	1	**Diptera**		UNK Diptera	70	Megaspilidae	1
Sphindidae	2	Zopheridae	2	Cixiidae	1	Bibionidae	1	**Phasmida**		Scelionidae	4
Byrrhidae	1	Trogossitidae	1	Psylloidea	1	Cecidomyiidae	6	Pseudophasmatidae	1	Chrysididae	1
Rhysodidae	1	Throscidae	1	Plataspidae	1	Chironomidae	4	**Thysanoptera**		Pompilidae	7
Carabidae	20	Phalacridae	1	Rhyparochromidae	1	Dryomyzidae	1	Thysanoptera	1	Platygastridae	1
Cerambycidae	2	Cleridae	1	Thyreocoridae	1	Simuliidae	1	**Microcoryphia**		Proctotrupidae	3
Colydidae	2	Endomychidae	5	Tingidae	1	Tabanidae	1	Machilidae	1	Halictidae	1
Curculionidae	41	Derodontidae	1	Pentatomidae	2	Sciomyzidae	1	**Mecoptera**		Vespidae	6
Cryptophagidae	4	Dytiscidae	2	Aphididae	1	Anthomyiidae	1	Mecoptera	1	UNK Hymenoptera	18
Tetratomidae	1	Coccinellidae	1	Issidae	1	Scathophagidae	1	**Mantodea**		**Collembola**	
Chrysomelidae	5	Catharidae	1	Largidae	2	Sphaeroceridae	4	Mantidae	1	Smithuridae	1
Dermestidae	4	Nitidulidae	5	Ceratocombidae	1	Tachnidae	1	**Hymenoptera**		Isotomidae	7
Elateridae	18	Anobiidae	1	UNK Hemiptera	7	Fannidae	2	Apidae	3	UNK Collembola	11
Eucinetidae	1	Leiodidae	5	**Orthoptera**		Dolichopodidae	3	Bethylidae	4	**Lepidoptera**	
Melandryidae	1	Hydrophilidae	1	Gryllidae	1	Sciaridae	6	Chalcoidea	1	Geometridae	1
Histeridae	7	Ptilodactylidae	1	Gryllinae	1	Empididae	1	Chalcidoidea	4	UNK Lepidoptera	21
Geotrupidae	3	Ptinidae	1	Rhaphidophoridae	1	Chloropidae	1	Diapriidae	6	**Blattodea**	
Scarabaeidae	6	Anthicidae	1	UNK Orthoptera	1	Drosophilidae	4	Braconidae	6	Rhinotermitidae	1
Scarabaeoidae	15	Latridiidae	4	**Trichoptera**		Phoridae	6	Formicidae	32	Blatellidae	2
Scirtidae	2	Lycidae	2	Psychomyiidae	2	Syrphidae	1	Sircidae	1	**Neuroptera**	
Silphidae	2	UNK Coleoptera	45	Calamoceratidae	2	Asilidae	3	Sphecidae	4	Hemerobiidae	1
Staphylinidae	49	**Hemiptera**		Hydroptilidae	1	Sarcophagidae	1	Evaniidae	1	Chrysopidae	2
Erotylidae	1	Achilidae	1	UNK Trichoptera	7	Culicidae	5	Scoliidae	1	**UNKNOWN**	
Passandridae	1	Aradidae	4	**Psocodea**		Chaoboridae	1	Crabronidae	4	UNK	1
Laemophloeidae	1	Cicadellidae	14	Lepidopsocidae	1	Mycetophilidae	1	Ichneumonidae	3		
Mordellidae	6	Cercopidae	2	Psocidae	1	Elasminae	1	Mutilidae	6		

*UNK stands for unknown family.

Table 2.3. Repeated measures ANOVA results for twig-based pitfall traps with years 1 and 2 from February 26, 2016 to March 9, 2017 and March 23, 2017 to March 10, 2018, respectively.

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	4	815	0.33	0.8609
Year	1	815	22.10	<.0001
Treatment*Year	4	815	1.04	0.3846

Table 2.4. Repeated measures ANOVA results for antifreeze-based pitfall traps for one year from February 23, 2017 to March 10, 2018.

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	4	413	2.25	0.0632

Table 2.5. Pre-inoculation averages for diversity indices, Shannon's and Simpson's Inverse, for antifreeze-based pitfall traps according to imminent treatment type and season, where n corresponds to the number bimonthly sampling periods used for each season.

	Winter 2 n = 3		**Spring 2** n = 3	
	Shannon	Simpson	Shannon	Simpson
Control	1.73	4.83	1.43	3.82
Wound	1.27	3.17	1.2	2.76
Low	1.63	4.34	0.95	2.59
Medium	1.56	4.02	1.01	3.11
High	1.88	5.8	1.22	3.22

Table 2.6. Post-inoculation averages for diversity indices, Shannon's and Simpson's Inverse, for antifreeze-based pitfall traps according to imminent treatment type and season, where n corresponds to the number of bimonthly sampling periods used for each season.

	Spring 2		**Summer 2**		**Fall 2**		**Winter 3**		**Spring 3**	
	n = 18		n = 18		n = 21		n =18		n = 18	
	Shannon	Simpson	Shannon	Simpson	Shannon	Simpson	Shannon	Simpson	Shannon	Simpson
Control	1.78	5.28	1.75	5.13	1.41	3.76	1.07	2.94	0.92	2.05
Wound	1.62	4.58	1.66	4.67	1.33	3.81	0.81	2.35	0.7	2.17
Low	1.45	4.1	1.6	4.48	1.38	4.01	0.99	2.81	0.92	2.3
Medium	1.23	3.41	1.61	4.32	1.37	3.31	0.95	2.71	0.68	1.98
High	1.77	5.13	1.71	4.9	1.33	3.69	0.82	2.33	1.3	4.05

Table 2.7. Repeated measures ANOVA results for panel trap species richness for Year 1 and Year 2.

Effect	Num DF	Den DF	F Value	Pr > F
Trap	5	312	1.04	0.3964
Year	1	312	32.90	<.0001
Trap*Year	5	312	0.43	0.8300

Table 2.8. Pre-inoculation averages for diversity indices, Shannon's and Simpson's Inverse, according to panel trap and season, where n corresponds to the number of sampling events.

	Winter 1 n = 1		**Spring 1** n = 6		**Summer 1** n = 6		**Fall 1** n = 7		**Winter 2** n = 6		**Spring 2** n = 1	
	Shannon	**Simpson**	**Shannon**	**Simpson**	**Shannon**	**Simpson**	**Shannon**	**Simpson**	**Shannon**	**Simpson**	**Shannon**	**Simpson**
1A	*	*	2.39	9.64	1.8	5.5	1.78	4.98	0.9	2.51	1.099	3
1B	*	*	2.26	9.43	2.1	7.28	1.67	5.97	0.71	2.46	0	1
2A	*	*	1.88	7.56	1.82	5.73	1.18	2.66	0.69	1.58	*	*
2B	1.46	3.31	2.38	9.3	1.81	6.18	1.57	4.95	1.16	3.92	1.386	4
3A	1.47	3.76	2.31	9.04	1.62	5.07	1.34	3.98	0.97	2.63	0	1
3B	1.63	4.5	2.27	9.54	1.53	5.12	1.29	3.77	0.98	2.54	1.386	4

*Traps captured no insects.

Table 2.9. Post-inoculation averages for diversity indices, Shannon's and Simpson's Inverse, according to panel trap and season, where n corresponds to the number of sampling events.

	Spring 2 n = 6		**Summer 2** n = 6		**Fall 2** n = 7		**Winter 3** n = 6		**Spring 3** n = 1	
	Shannon	**Simpson**	**Shannon**	**Simpson**	**Shannon**	**Simpson**	**Shannon**	**Simpson**	**Shannon**	**Simpson**
1A	1.88	6.14	1.07	3.25	0.82	2.53	0.98	2.54	2.16	8.33
1B	1.9	6.59	1.09	3.62	0.92	2.74	0.74	1.95	1.79	5.99
2A	1.44	4.86	1.45	4.31	0.72	2.12	0.75	2.05	1.61	5
2B	1.94	7.47	1.41	4.78	1.08	2.92	1.19	3.21	1.61	5
3A	1.81	6.63	1.34	4.09	0.43	1.19	0.81	2.71	1.39	4
3B	2.03	6.86	1.78	6.39	0.75	2.54	0.76	2.14	1.61	5

Table 2.10. Pre-inoculation averages of the Sørensen coefficient per panel trap pair according to season, where n corresponds to the number of sampling events.

	Winter 1	**Spring 1**	**Summer 1**	**Fall 1**	**Winter 2**	**Spring 2**
	n = 1	n = 7	n = 6	n = 7	n = 6	n = 1
1A - 1B	0.222	0.275	0.26	0.112	0.269	0
1A - 2A	0.5	0.237	0.099	0.283	0.216	0
1A - 2B	0.4	0.258	0.188	0.193	0.337	0
1A - 3A	0.5	0.231	0.133	0.219	0.309	0
1A - 3B	0.444	0.272	0.117	0.225	0.292	0
1B - 2A	0.285	0.205	0.116	0.103	0.15	0
1B - 2B	0.153	0.311	0.228	0.133	0.233	0
1B - 3A	0.363	0.23	0.272	0.235	0.2	0
1B - 3B	0.166	0.233	0.145	0.439	0.255	0.4
2A - 2B	0.25	0.231	0.131	0.134	0.185	0
2A - 3A	0.333	0.197	0.207	0.308	0.197	0
2A - 3B	0.285	0.191	0.234	0.307	0.197	0
2B - 3A	0.5	0.193	0.259	0.161	0.246	0
2B - 3B	0.461	0.244	0.164	0.192	0.272	0.25
3A - 3B	0.545	0.26	0.286	0.155	0.193	0

Table 2.11. Post-inoculation averages of the Sørensen coefficient per panel trap pair according to season, where n corresponds to the number of sampling events.

	Spring 2	**Summer 2**	**Fall 2**	**Winter 3**	**Spring 3**
	n = 6	n = 6	n = 7	n = 6	n = 1
1A - 1B	0.224	0.03	0.067	0.401	0
1A - 2A	0.153	0.163	0.129	0.408	0.285
1A - 2B	0.245	0.085	0.086	0.368	0.142
1A - 3A	0.118	0.084	0.036	0.369	0
1A - 3B	0.216	0.096	0	0.447	0.142
1B - 2A	0.144	0.13	0.088	0.331	0
1B - 2B	0.215	0.032	0.052	0.439	0.181
1B - 3A	0.104	0.077	0.032	0.181	0
1B - 3B	0.226	0.091	0.048	0.314	0.363
2A - 2B	0.158	0.098	0.179	0.321	0.4
2A - 3A	0.121	0.133	0.036	0.255	0.222
2A - 3B	0.144	0.073	0	0.365	0.2
2B - 3A	0.144	0.063	0.079	0.229	0
2B - 3B	0.229	0.141	0.105	0.305	0
3A - 3B	0.193	0.22	0	0.224	0

Table 2.12. Repeated measures ANOVA results for log transformed Shannon index values for panel traps for the two year collection period.

Effect	Num DF	Den DF	F Value	Pr > F
Trap	5	277	1.08	0.3728
Year_character	1	277	5.84	0.0164

Table 2.13. Repeated measures ANOVA results for Arcsine squared transformed Simpson index values for panel traps for the two year collection period.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Year_character	1	0.43560882	0.43560882	4.42	0.0364
Trap	5	0.30511971	0.06102394	0.62	0.6855

Table 2.14. Average temperature in Fahrenheit and total rainfall in inches for each month of the study, separated by year.

Year	Month	Average Temperature (°F)	Precipitation (Inches)
2016	February	49.49	0.16
	March	60.56	0.14
	April	63.64	0.22
	May	70.32	0.04
	June	78.64	0.18
	July	80.87	0.08
	August	80.62	0.13
	September	77.02	0.01
	October	66.06	0.00
	November	54.67	0.05
	December	51.84	0.17
2017	January	52.03	0.31
	February	55.27	0.20
	March	58.17	0.06
	April	66.37	0.11
	May	70.37	0.18
	June	75.91	0.18
	July	79.77	0.16
	August	78.34	0.18
	September	74.02	0.14
	October	65.82	0.10
	November	54.22	0.03
	December	48.23	0.09
2018	January	43.80	0.09
	February	61.95	0.17
	March	57.55	0.09

Figure 2.1 Location of study site in Eufaula, AL in relation to Auburn, AL.

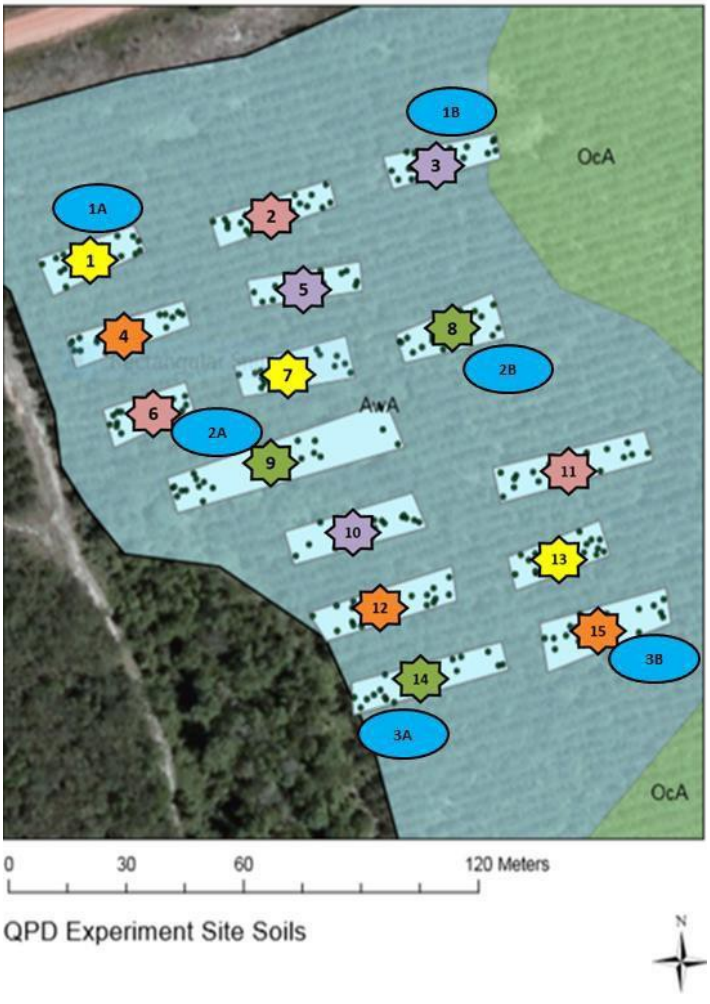

Figure 2.2. Fifteen plots of the study site in Eufaula, AL, with stars corresponding to plots, denoted by number, and ovals demonstrating the location of panel traps and their associated label. Treatment levels were assigned by color where green is control, orange is low, yellow is medium, high is pink, and wound is purple.

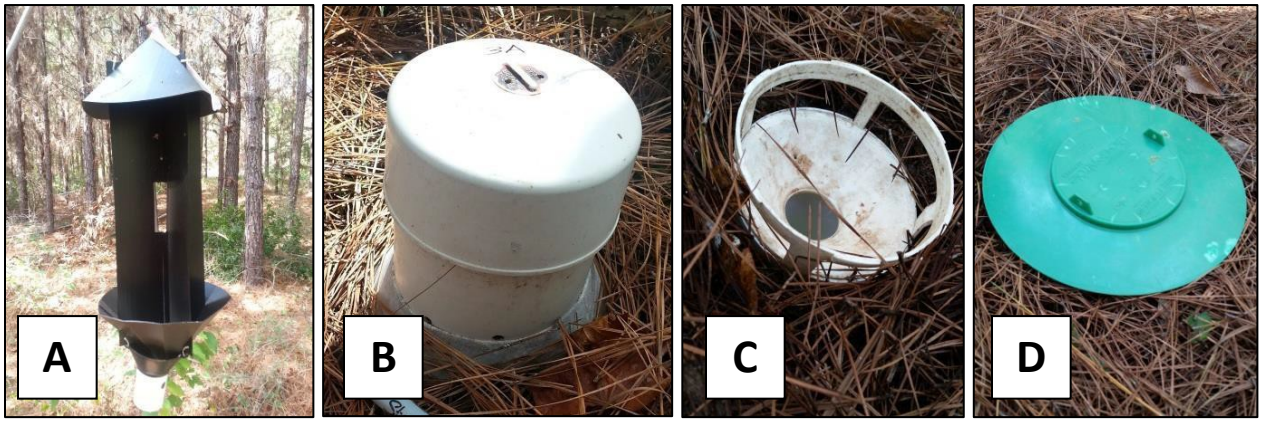

Figure 2.3. (A) Panel trap, (B) pitfall trap to be baited with twigs, and pitfall trap without top (C) and pitfall trap with top (D).

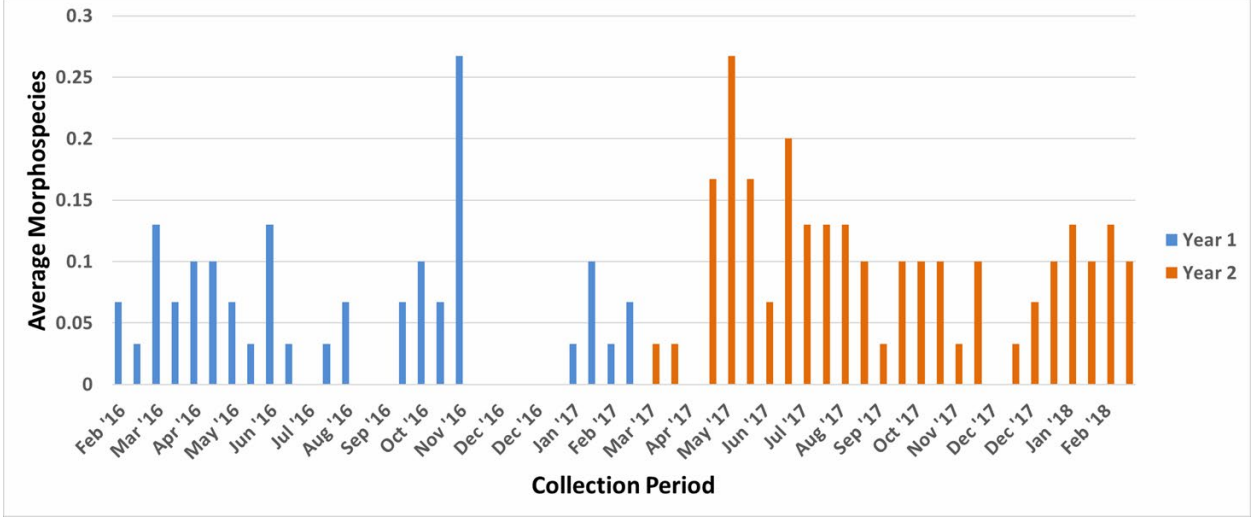

Figure 2.4. Average number of morphospecies collected in twig-based pitfall traps for both sampling years, with two-week sampling periods between February 26, 2016 to March 10, 2018.

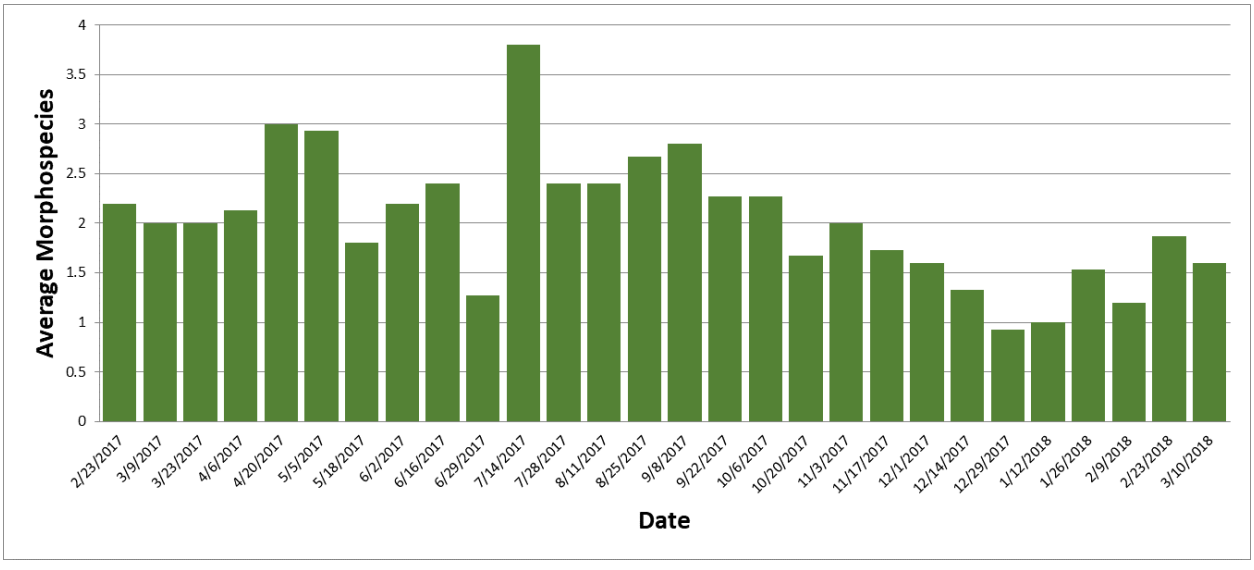

Figure 2.5. Average number of morphospecies collected in antifreeze-based pitfall traps according to sampling period.

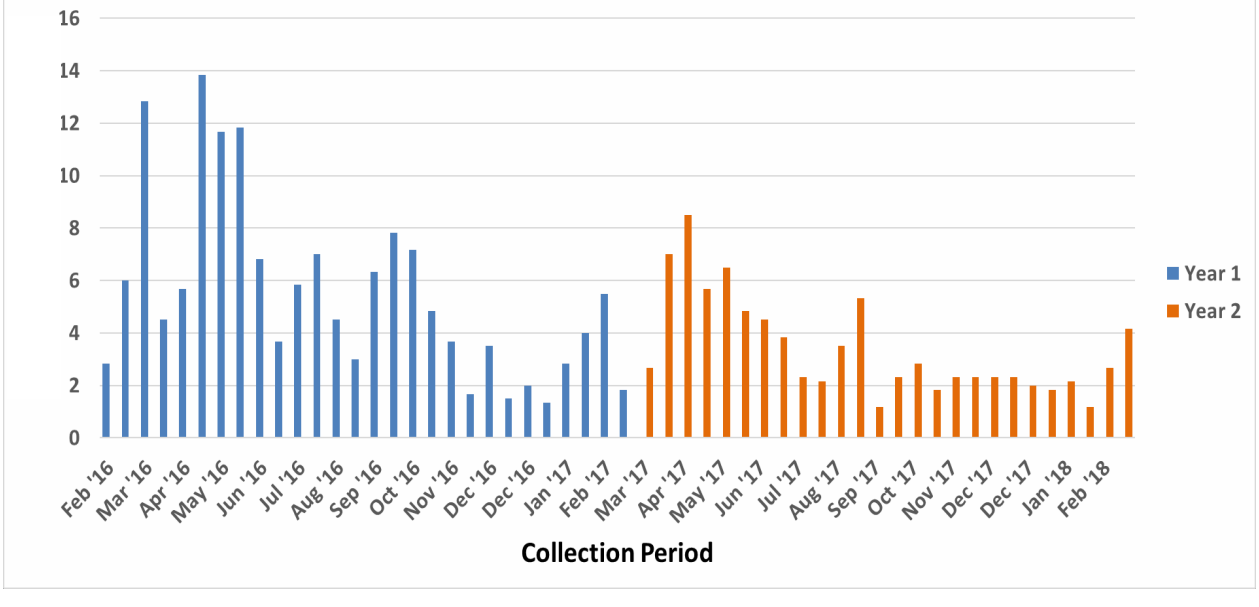

Figure 2.6. Average number of morphospecies collected in panel traps for both sampling years, with two-week sampling periods between February 26, 2016 to March 10, 2018.

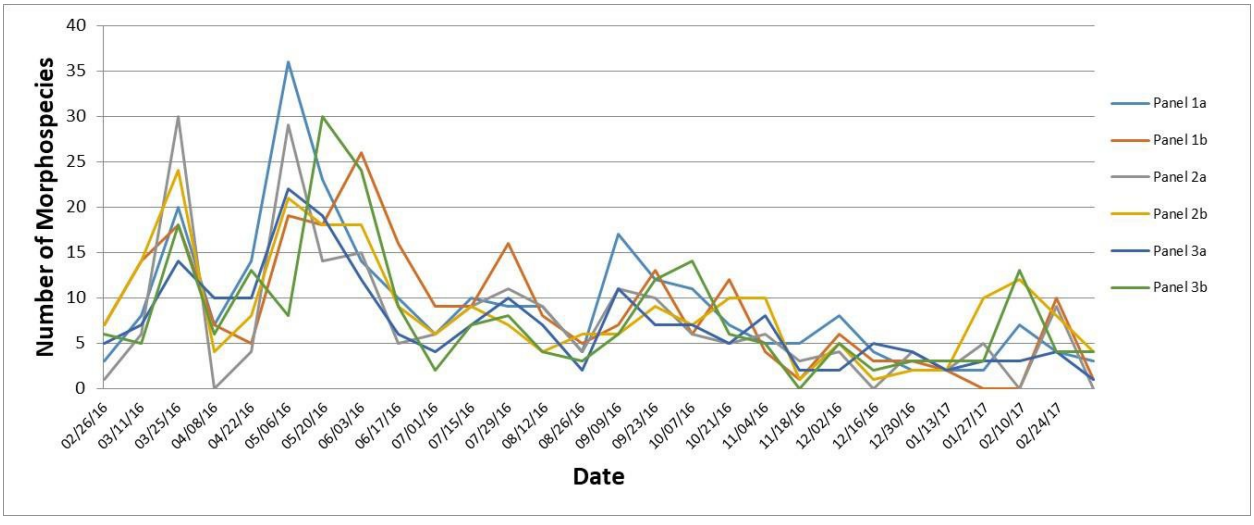

Figure 2.7. Number of morphospecies caught per panel trap according to sampling period for year 1.

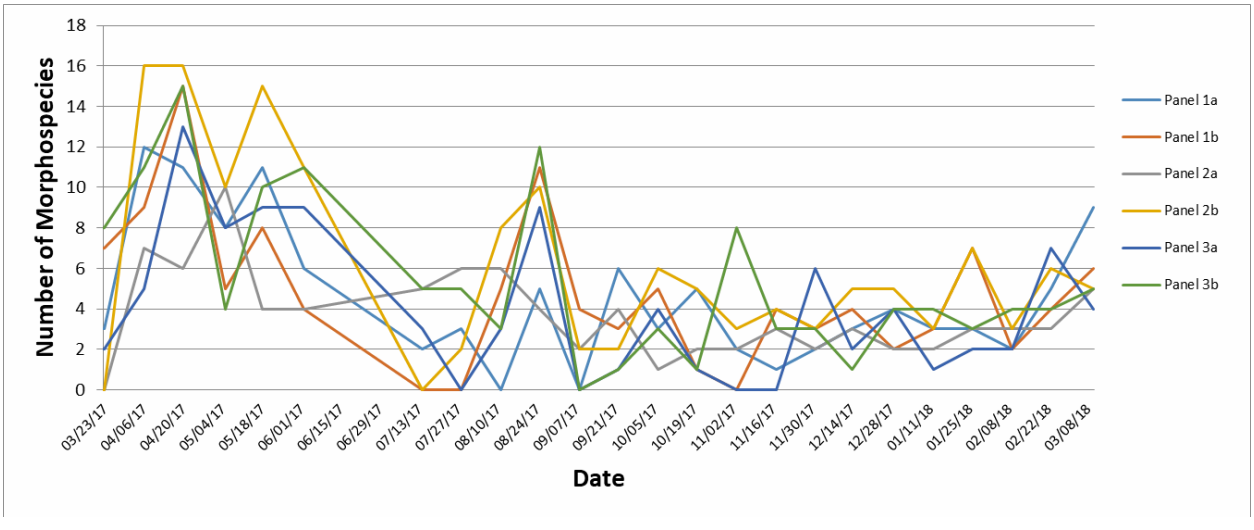

Figure 2.8. Number of morphospecies caught per panel trap according to sampling period for year 2.

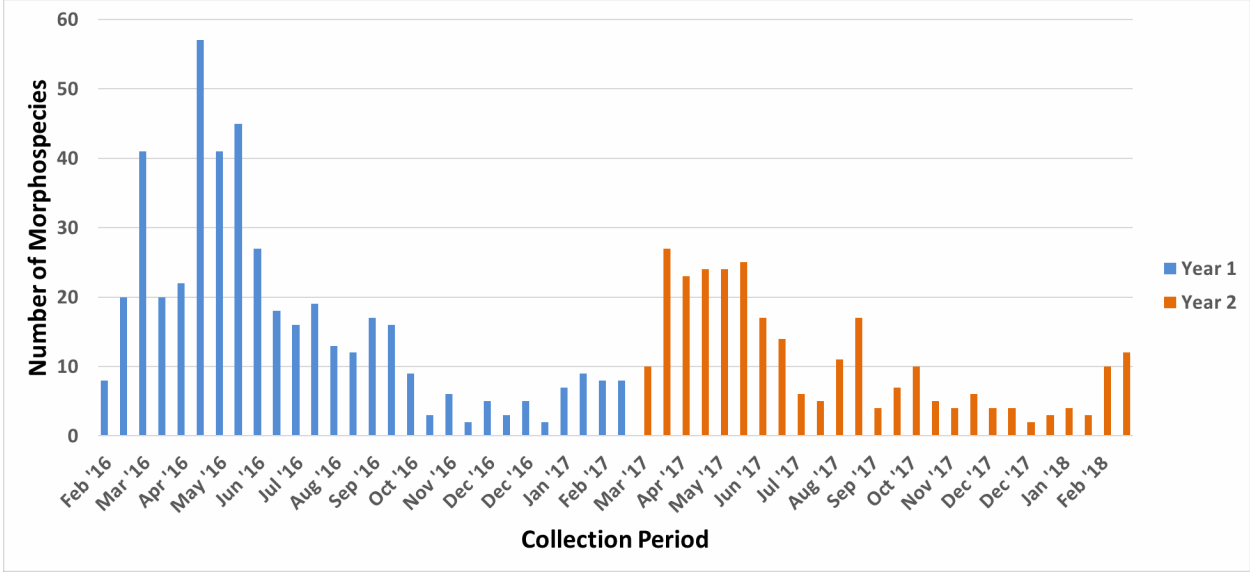

Figure 2.9. Total morphospecies of Coleoptera caught in panel traps during the study period.

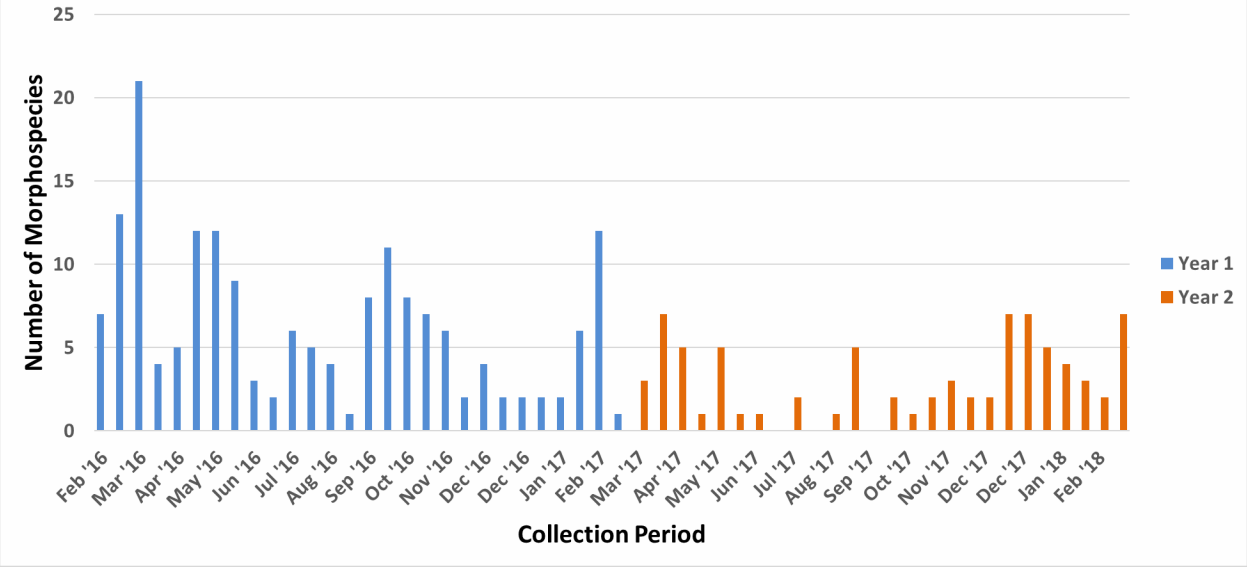

Figure 2.10. Total morphospecies of Diptera caught in panel traps during the study period.

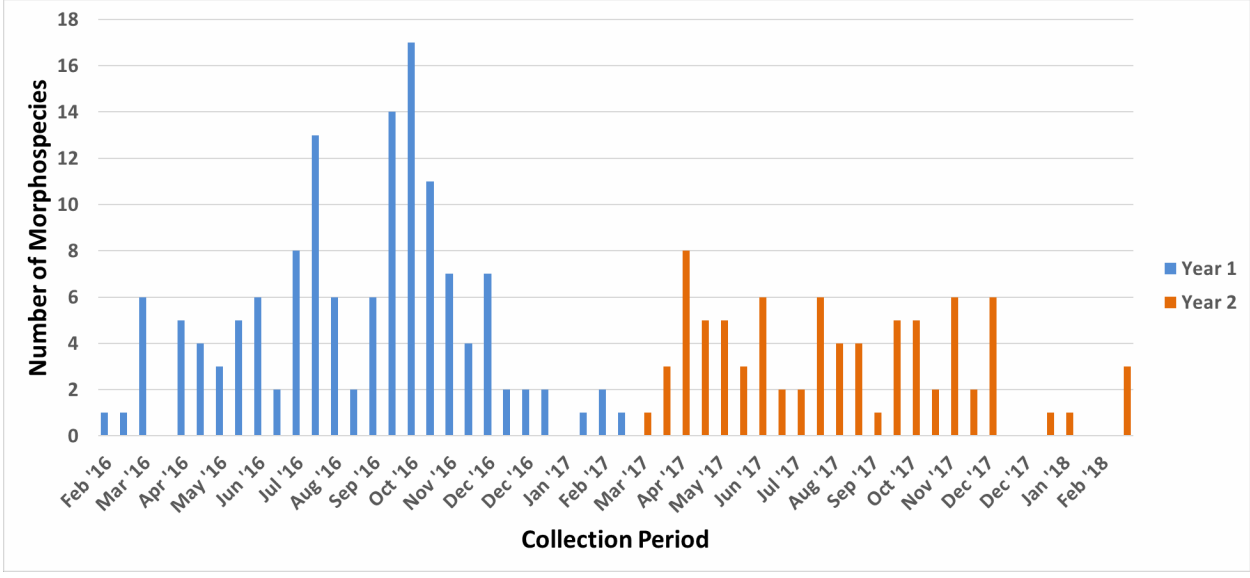

Figure 2.11. Total number of morphospecies of Hymenoptera caught in panel traps during the study period.

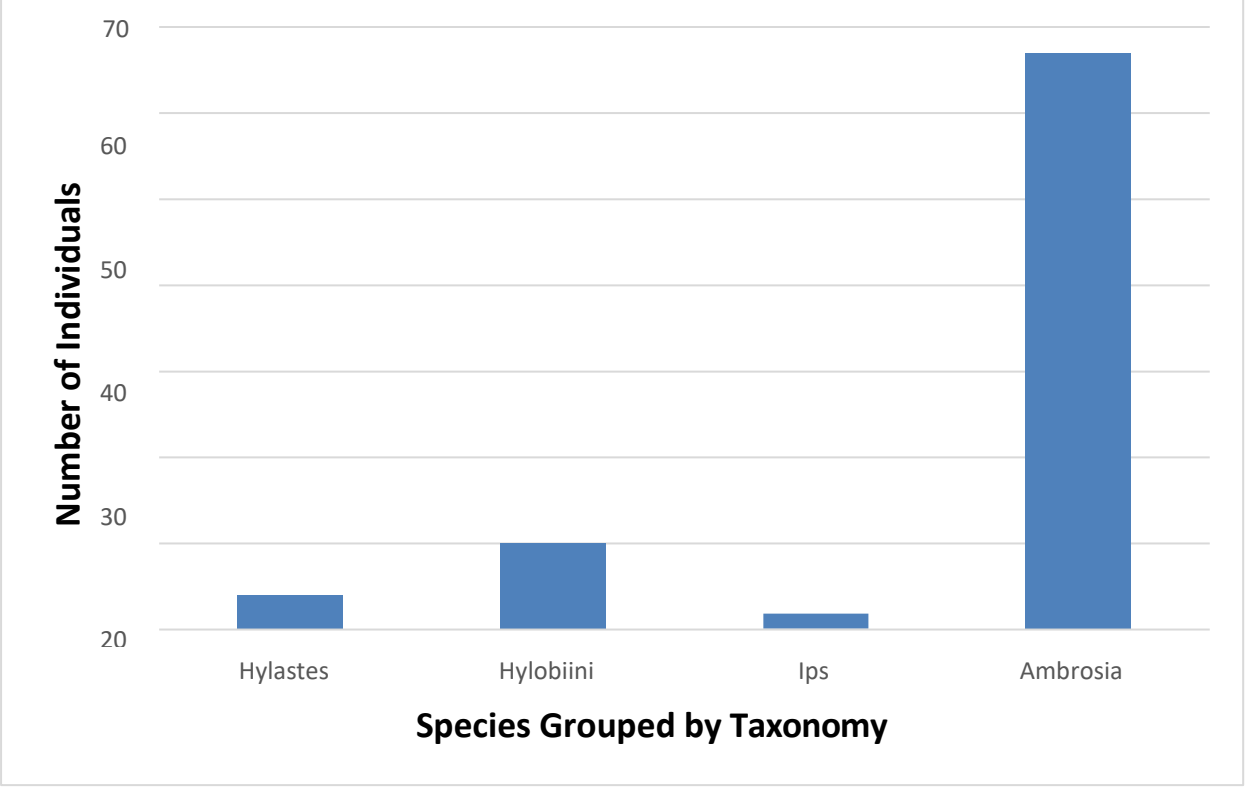

Figure 2.12. Total individuals of concern captured, as sorted by taxonomy.

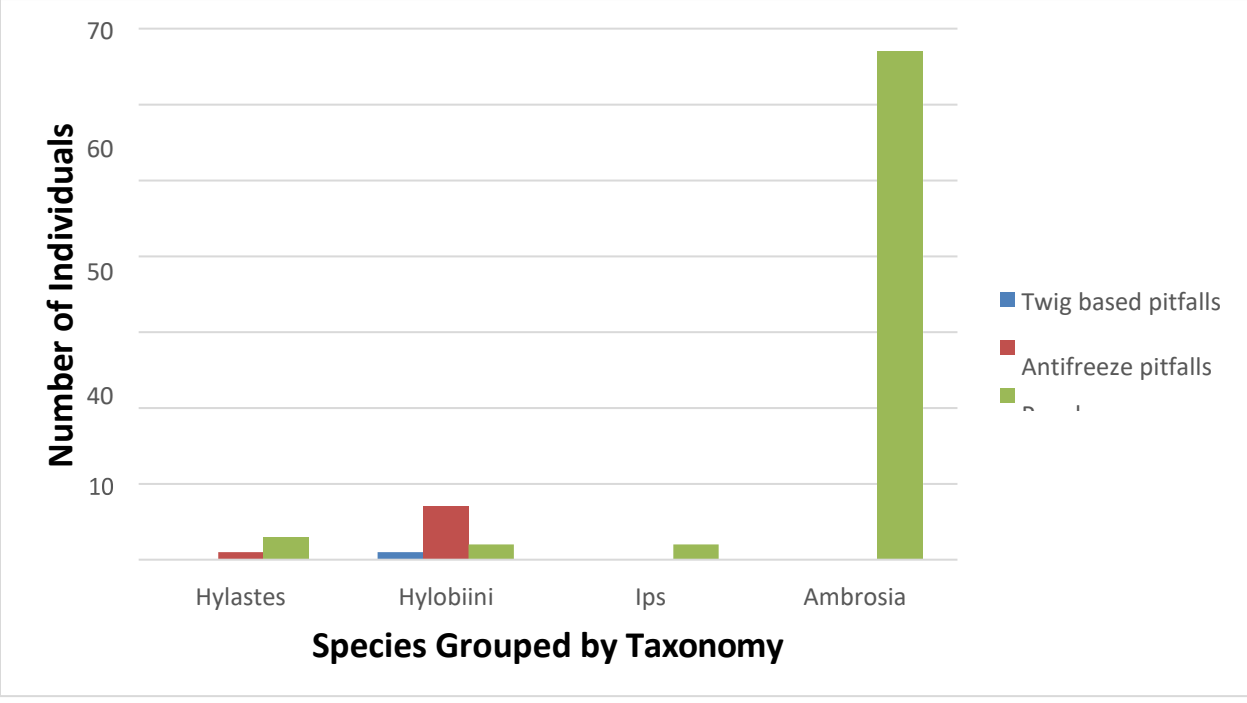

Figure 2.13. Total number of individuals of species of concern captured per trap type.

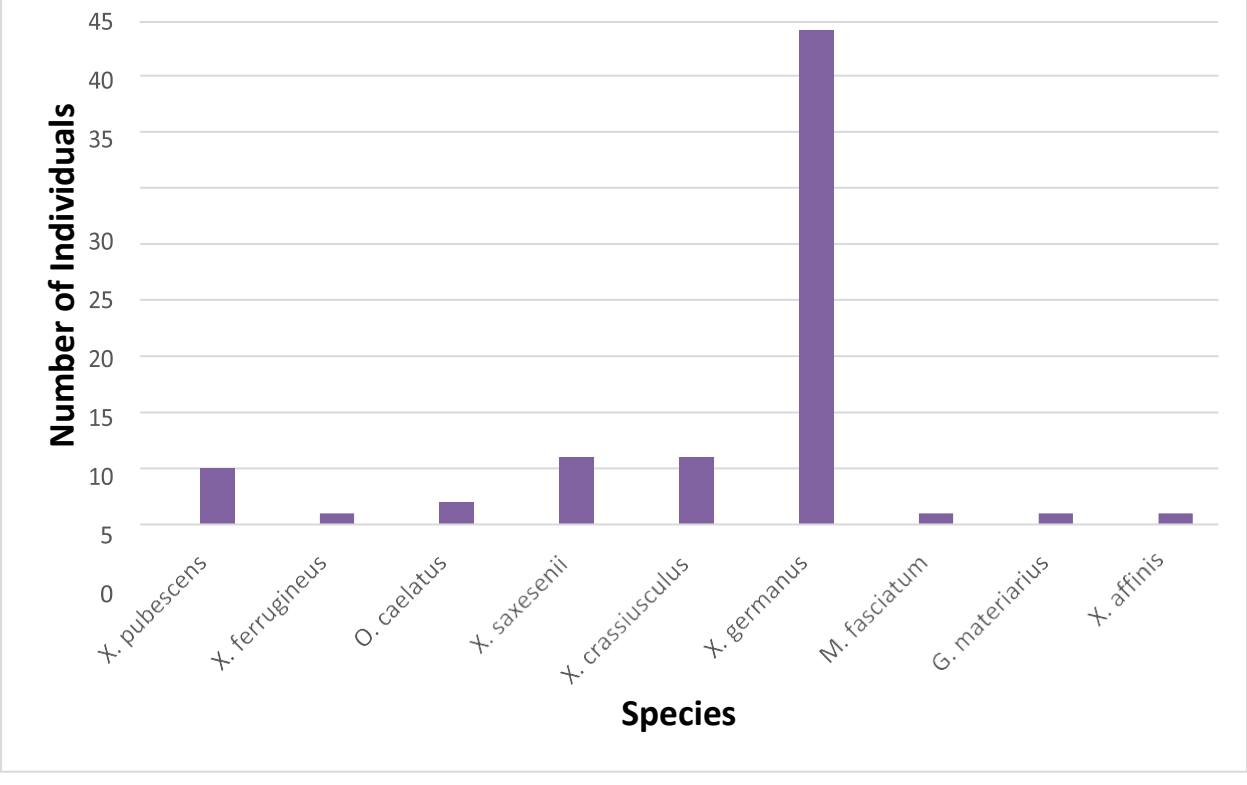

Figure 2.14. Total individuals of ambrosia (Scolytinae) beetle captured by species.

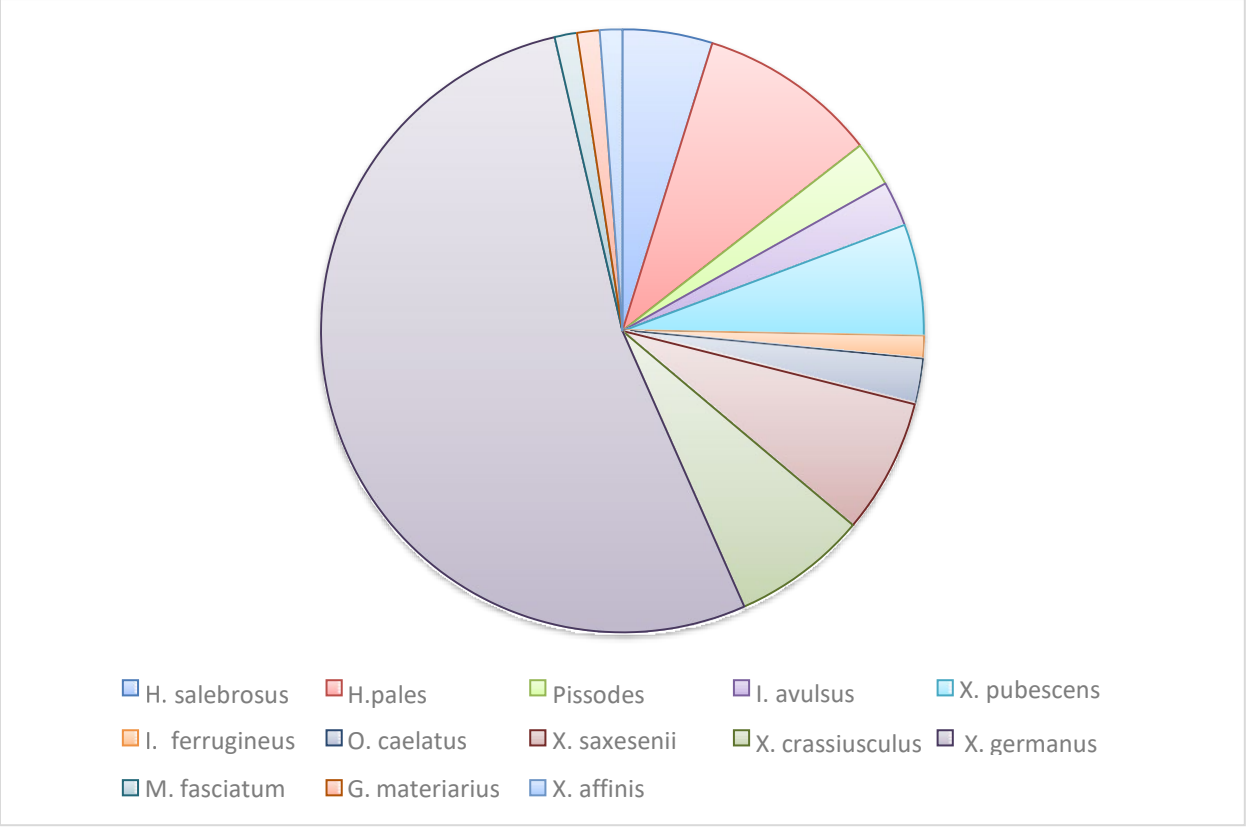

Figure 2.15. Pie chart representing the proportion of individuals caught by species.

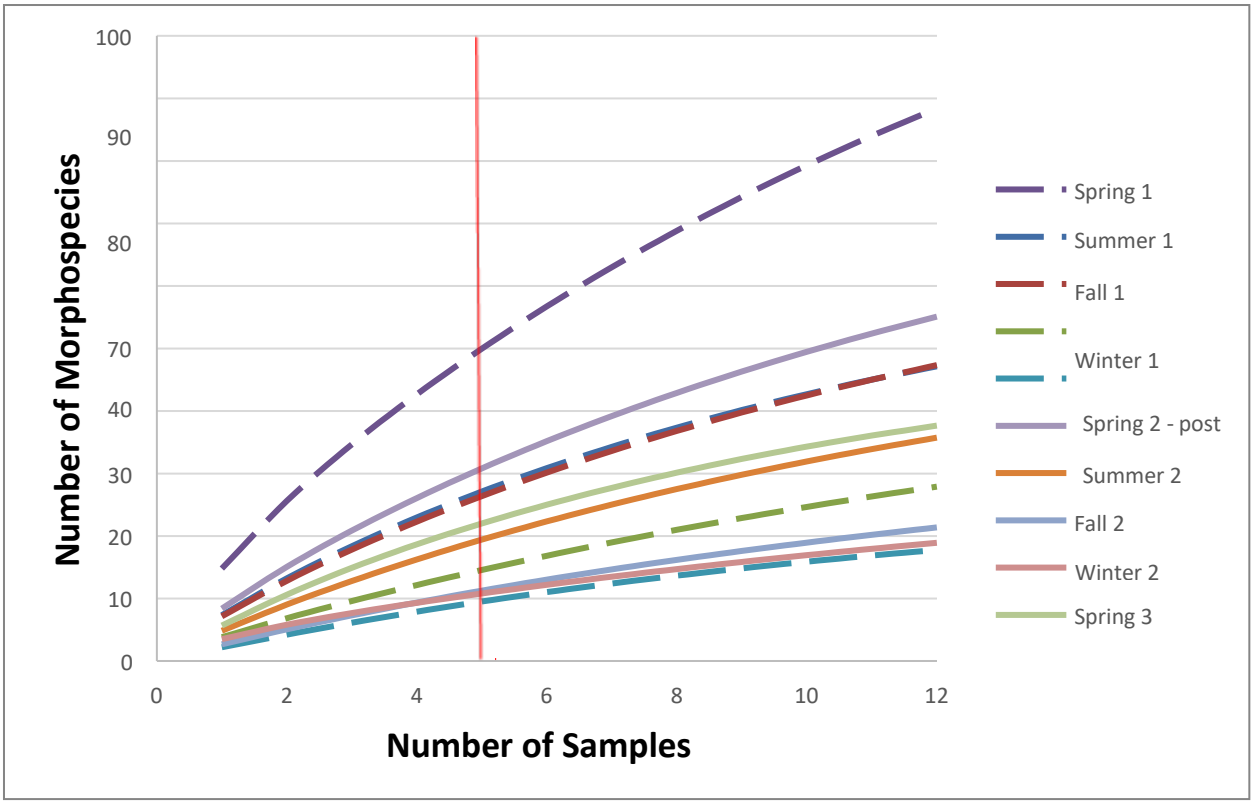

Figure 2.18. Species curves for panel traps based on season, with dotted lines denoting the pre-inoculation period and full lines corresponding to the post-inoculation period, each extrapolated past the number of samples by two-fold as defined by the vertical line.