Colonization of Artificially Stressed Black Walnut Trees by Ambrosia Beetle, Bark Beetle, and Other Weevil Species (Coleoptera: Curculionidae) in Indiana and Missouri

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ABSTRACT Thousand cankers disease (TCD) is a new disease of black walnut (*Juglans nigra* L.) in the eastern United States. The disease is caused by the interaction of the aggressive bark beetle *Pityophthorus juglandis* Blackman and the canker-forming fungus, *Geosmithia morbida* M. Kolarik, E. Freeland, C. Utley & Tisserat, carried by the beetle. Other insects also colonize TCD-symptomatic trees and may also carry pathogens. A trap tree survey was conducted in Indiana and Missouri to characterize the assemblage of ambrosia beetles, bark beetles, and other weevils attracted to the main stems and crowns of stressed black walnut. More than 100 trees were girdled and treated with glyphosate (Riverdale Razor Pro, Burr Ridge, Illinois) at 27 locations. Nearly 17,000 insects were collected from logs harvested from girdled walnut trees. These insects represented 15 ambrosia beetle, four bark beetle, and seven other weevil species. The most abundant species included *Xyleborinus saxeseni* Ratzburg, *Xylosandrus crassiusculus* Motschulsky, *Xylosandrus germanus* Blandford, *Xyleborus affinis* Eichhoff, and *Stenomimus pallidus* Boheman. These species differed in their association with the stems or crowns of stressed trees. Multiple species of insects were collected from individual trees and likely colonized tissues near each other. At least three of the abundant species found (*S. pallidus*, *X. crassiusculus*, and *X. germanus*) are known to carry propagules of canker-causing fungi of black walnut. In summary, a large number of ambrosia beetles, bark beetles, and other weevils are attracted to stressed walnut trees in Indiana and Missouri. Several of these species have the potential to introduce walnut canker pathogens during colonization.

KEY WORDS scolytine, platypodine, *Geosmithia morbida*, *Juglans nigra*, thousand cankers disease

Black walnut (*Juglans nigra* L.) is native to the eastern United States and is a commonly planted ornamental tree throughout the western United States. Black walnut is highly valued for its dense grain and dark color, and it is one of the most valuable timber species per board foot in the eastern United States (Buehlmann et al. 2010, Espinoza et al. 2011). Moreover, walnut nutmeats and shells are sold as food and used in abrasives, cosmetics, and the oil industry (Reid et al. 2009). Walnut trees are especially important to states in the Midwest, including Indiana and Missouri, because they are abundant in forests, and sales of black walnut wood, nutmeats, and shells contribute greatly to local economies.

Thousand cankers disease (TCD) is caused by the interaction of the aggressive walnut twig beetle (*Pityophthorus juglandis* Blackman) and the fungal canker pathogen, *Geosmithia morbida* M. Kolarik, E. Freeland, C. Utley & Tisserat that is vectored by the beetle (Tisserat et al. 2009). This disease threatens the health of eastern black walnut throughout its native range. TCD-affected trees can succumb to the disease within a few years after infection when thousands of gallery-associated cankers caused by *G. morbida* coalesce and block the transport of photosynthates. The progression of TCD symptoms may be dependent on biotic stress after attack and infection (Griffin 2014). Although a disease with symptoms similar to TCD was reported in Utah during the 1990s, it was not until 2001 that *P. juglandis* was first associated with declining black walnuts in New Mexico (Tisserat et al. 2011), and the disease was not formally described until 2009 (Tisserat et al. 2009). The beetle and the fungus have spread beyond their native range in the southwestern United States, and, as of 2015, these are now found together in nine western states, seven eastern states, and Italy (Montecchio and Faccoli 2013). In Indiana, *G. morbida* has also been recovered from a weevil, *Stenomimus pallidus* Boheman (Juzwik et al. 2015), and walnut twig beetles have been collected from aerial flight traps at a mill in a separate location. However, it is not known if TCD is present on Indiana walnut trees, or the extent to which *S. pallidus* is capable of transmitting the pathogen to black walnut.

P. juglandis is one of the thousands of tree-infesting bark and ambrosia beetle species from the former family Scolytidae that, despite some controversy, was reduced to a subfamily within Curculionidae (Lawrence and Newton 1995, Bright 2014). Bark

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beetles within the subfamily Scolytinae consume and live in bark tissues, while ambrosia beetles within the same subfamily consume fungi and live in wood (Wood 1982, Solomon 1995). Beetles within the subfamily Platypodinae (Curculionidae) are also ambrosia beetles. These beetles and many other weevils attack woody plant tissues, especially stressed tissues. Non-scolytine and non-platypodine weevils cause damage to all tree tissues, including buds, shoots, stems, roots, bark, and wood but rarely are serious pests (Solomon 1995). A number of scolytine bark beetles and scolytine and platypodine ambrosia beetles are serious pests of trees. In some instances, damage by scolytine and platypodine beetles can directly cause dieback and death of their hosts (Six and Wingfield 2011, Ploetz et al. 2013). While in other cases, these beetles indirectly affect tree health by vectoring pathogenic fungi, especially ambrosia beetles introduced to new and naïve host trees (Nevill and Alexander 1992, Webber 2000, Jacobi et al. 2013, Kendra et al. 2013, Ploetz et al. 2013). Bark and ambrosia beetles also hasten the death of declining trees that are already weakened by other insects, fungi, or severe weather events (McPherson et al. 2005, Worrall et al. 2008).

Trees stressed by drought, flooding, wounding, air pollution, herbivory, and pathogen attack emit increased levels of volatile organic compounds (VOCs; Kimmerer and Kozlowski 1982, Kelsey 2001, Holopainen 2011, Kelsey et al. 2014). Among the many VOCs produced by trees, ethanol and monoterpenes, such as α-pinene, are highly attractive to many secondary wood and bark-boring beetles (Dunn and Potter 1991, Miller and Rabaglia 2009, Ranger et al. 2010). A number of studies have effectively used girdling of trees or herbicide treatments to mimic natural tree stress and attract xylophagous and phleophagous insects (Dunn et al. 1986, Dunn and Lorio 1992, McCullough et al. 2009, Dodds et al. 2012).

In this study, we characterize the assemblage of ambrosia beetles, bark beetles, and other weevils attracted to black walnut trees stressed by girdling and herbicides in Indiana and Missouri. In addition, we describe the extent to which abundant beetles are associated with the main stems and branches of affected trees. Host use and distribution data can be used to interpret the potential of different species to facilitate decline of already stressed trees. We refer to scolytine and platypodine beetles as bark and ambrosia beetles and all other curculionids as weevils, as differences in the habits and microhabitats of these insects may determine their potential to facilitate decline.

Materials and Methods

Stressed Trap Trees and Their Locations. Two parallel chain-saw cuts, ∼13–18 cm apart, were made around each trap tree at ∼1 m above the soil line to artificially stress the tree. The depths of these cuts extended as far as 1 cm into the sapwood. Glyphosphate (Riverdale Razor Pro, Burr Ridge, Illinois) was mixed to a final concentration of 240 g/liter active ingredient and sprayed into cuts to the point of run-off immediately after girdling. The amount of herbicide applied to each tree depended on the tree circumference, with larger trees receiving more herbicide.

Tree girdling was performed in central Missouri during the fourth week of May 2011, in the eastern region of Missouri during the first week of June 2011, and in the western region of Missouri during the second week of June 2011. In Indiana, girdling was carried out successively from south to north. Girdling was performed at the 12 most southern locations in Indiana during the first week of June 2011 and at the three most northern locations during the second week of June 2011. Walnut leaves had emerged and were expanding at the time of girdling at all locations in Indiana and central Missouri. Leaves had fully expanded in eastern and western Missouri at the time of girdling.

Trap trees in the study were located in plantations and natural stands in urban and rural areas. Four trap trees were created at each of 12 locations in Missouri that were distributed among eight counties. Four locations were actively managed plantations and one was an abandoned plantation within a natural forest stand. All other Missouri locations consisted of natural stands in either rural or urban areas. Stem diameters (1.2 m in height) of Missouri trap trees ranged from 12 to 37 cm. Trap trees at Missouri locations were codominant and intermediate trees in stands except for one location with open-grown walnut trees. In Indiana, four trap trees were created at each of 13 locations and two trap trees at each of two additional locations. Indiana trap trees were distributed among 15 counties. Eight locations were managed plantations and two locations were unmanaged. Four other Indiana locations were part of naturally generated forest stands, and one was an orchard planting in an urban setting. Diameters of Indiana trap trees ranged from 15 to 29 cm. Indiana trees were codominant trees on 12 sites, open grown at one site, and edge trees at two sites. The physiological maturity of trap trees was not assessed. All trap trees appeared healthy prior to girdling and herbicide treatment. TCD, which requires the walnut twig beetle and the fungal pathogen, was not known to be present in either Missouri or Indiana.

Rearing Beetles From Main Stem and Crown Portions of Trap Trees. After 14 wk, trap trees were felled at 25 sites between mid-August and mid-September 2011, and portions from the lower main stem and from the crown were harvested so insects could be collected from them. Trap trees at two central Missouri sites were harvested 15 wk after girdling. For each tree, two logs (each 30 cm long) were harvested from the lower main stem. One log was removed ∼1.2 m from the base of the tree and the other ∼2.6 m above the base. Four logs were also removed from the tree crown. Each log was 30 cm long and all diameters were greater than 3 cm.

The cut ends of smaller diameter logs were dipped into and sealed with Gulf paraffin wax (Royal Oak Sales, Roswell, Georgia), and the wax was brushed onto the cut and split ends of those logs that were too large to be dipped. Log diameter with bark attached was measured before being placed in an insect emergence bucket. An emergence bucket consisted of a 20-cm diameter plastic funnel glued to the cut bottom of a ventilated, 19-liter bucket that was suspended from a wooden rack (Mayfield and Hanula 2012). Logs were attached to the inside of bucket lids by 7.6-cm wood screws. Specifically, screws were drilled through small wood pieces on top of the bucket lid and then into logs. The wood pieces were used to brace the lids and support the weight of the log hanging from each bucket lid. The collection cup (473 ml) was a plastic food storage container (Rubbermaid Twist n' Seal, Atlanta, Georgia) with a hole drilled in the center of the lid. The lid of the collection cup was threaded onto the narrow stem of the funnel and an LED light placed under the collection cup. The stem of the funnel had a grooved ridge that permitted the screwing of the collection cup lid onto the stem.

For Indiana trap trees, the logs were small enough in diameter that main stem portions of single trees could be combined in a single emergence bucket. Likewise, canopy logs were also combined in a single bucket. If the main stem logs from a tree were too large to combine in a bucket, they were placed in separate buckets. Logs from Missouri trap trees were treated in a similar manner as Indiana logs, except the main-stem portions were quite large and had to be quartered lengthwise to fit into emergence buckets. In such cases, the pieces from a single log were equally divided and placed in emergence buckets. Emergence buckets were placed in indoor rooms with temperatures of ~25°C.

Insect Collection and Identification. For a 3-mo period after tree felling, ambrosia beetles, bark beetles, and other weevils emerged from logs and were captured in collection cups. Insects were collected from cups at least twice per week, placed singly into 1.5-ml microcentrifuge tubes, and stored at −20°C for later fungal isolation. Tubes with insects from Indiana were overnight shipped with cold packs to a permitted lab at the University of Missouri. Insect collection ceased after 2 wk passed with no beetles emerging from the logs. For each tree, insects collected from the main stem portions were pooled as a single sample. Similarly, all insects collected from the four canopy logs were pooled.

All bark and ambrosia beetles were identified to species at the University of Missouri (Wood 1982, Rabaglia et al. 2006). Robert Rabaglia (U.S. Forest Service, Arlington, Virginia) confirmed the identities of less common bark and ambrosia beetle species, and E. Richard Hoebeke (Georgia Museum of Natural History, Athens, Georgia) identified specimens of all other weevil species. Voucher specimens were placed in the Enns Entomology Museum at the University of Missouri and in the Georgia Museum of Natural History.

For analysis, we combined the number of each insect that emerged from the stem and branch samples of trap trees in Indiana and Missouri because trends observed for species in each state were similar. The density at which each species emerged was calculated by dividing the number of individuals collected from the main stem and crown logs by the surface area of logs with bark-on. The non-parametric Wilcoxon signed ranks test was used to compare the densities of insects that emerged from the main stem and crown logs. All statistical analyses were performed using SAS 9.3 (SAS Institute 2011; SAS Institute, Cary, NC).

We compared the number of tree crowns colonized by each of the five most abundant insect species to the number of tree stems colonized. Data were analyzed using a generalized linear mixed model (PROC GLIMMIX). In the model, the fixed effect was tree section and the response variable was the number of tree stems or crowns attacked at each location. The function used during the procedure was a log-link and the distribution was Poisson. A least square means test was used to make comparisons of differences between treatments.

Results

Insect Diversity. Insects were collected from walnut trap trees at all locations in Indiana and Missouri (Fig. 1). Their abundance varied greatly among locations, ranging between 2,472 individuals collected at a

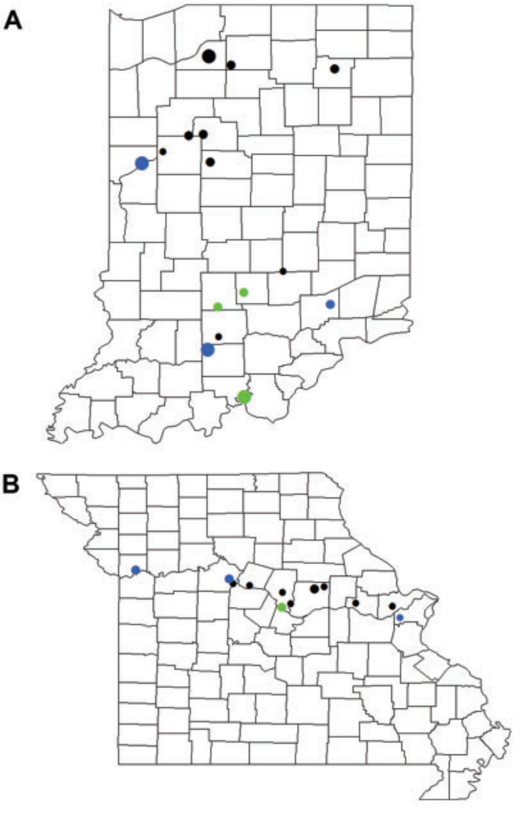

Fig. 1. Distribution of ambrosia beetles, bark beetles, and other weevils collected in Indiana (A) and Missouri (B). Small, medium, and large dots represent 1–4, 5–8, and ≥9 species, respectively, detected from all trees at a location. Black, blue, and green dots represent 1–500, 501–1,000, and 1,001–2,500 insects, respectively, trapped at each location. Indiana trees were girdled successively from south to north during the first 2 wk of June 2011. Missouri trees were girdled first in central Missouri and then eastern and western Missouri during the last week of May and first 2 wk of June 2011.

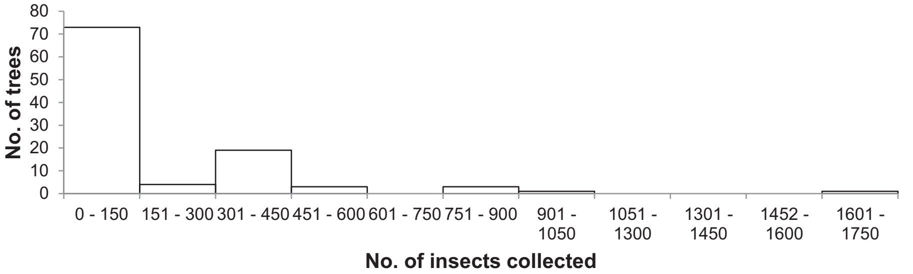

Fig. 2. Distribution of the number of ambrosia beetles, bark beetles, and other weevils collected per tree from all stressed walnut trees in Indiana and Missouri during 2011.

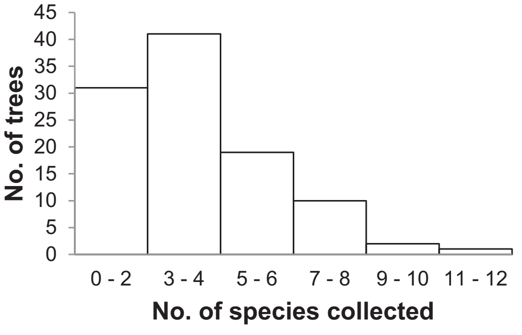

Fig. 3. Distribution of the number of ambrosia beetles, bark beetles, and other weevil species collected per tree from all stressed walnut trees in Indiana and Missouri during 2011.

location in Boone County, Missouri, to 15 individuals collected at a location in Callaway Co., Missouri. The richness of species collected also varied greatly among locations. A minimum of two species were detected at each location, and between 6 and 12 species were detected at more than 50% of study locations.

The numbers of insects collected and the taxonomic diversity also varied greatly among trap trees in the study. One tree in Orange Co., Indiana, yielded ∼1,600 individuals, but most trees produced 150 or fewer individuals (Fig. 2). Eleven species were detected in one trap tree located in Monroe Co., Indiana, but three or fewer species were detected from the majority of other trap trees (Fig. 3).

The collection of insects from Missouri and Indiana included ambrosia beetles, bark beetles, and other weevils. Richness and abundance of these taxa detected in Indiana were greater than that in Missouri. The more than 9,000 specimens from Indiana included 15 species of ambrosia beetles, three species of bark beetles, and four species of other weevils (Table 1), while the 7,295 insect specimens from Missouri included seven species of ambrosia beetles, two species of bark beetles, and six species of other weevils (Table 2).

Eight ambrosia beetle species were detected in trees only in Indiana. These included *Ambrosiophilus atratus* Eichhoff, *Anisandrus sayi* Hopkins, *Cyclorhipidion bodoanum* Wood, *Dryoxylon onoharaense* Murayama, *Euplatypus compositus* Say, *Euwallaceae validus* Eichhoff, *Monarthrum fasciatum* Say, and *Xyleborus celsus* Eichhoff. In addition, the bark beetle species, *Hylocurus rudis* Leconte and *Pityophthorus lautus* Eichhoff, and the other weevil species, *Sitophilus zeamais* Motschulsky, were also detected only in Indiana. No ambrosia beetle species was detected uniquely in Missouri; however, one bark beetle, *Hypothenemus interstitialis* Hopkins, and several other weevil species,

Table 1. The number and relative abundance of ambrosia beetles, bark beetles, and other weevils collected in Indiana during 2011

Taxa	Status[a]	Total no. insects collected	Percent relative abundance	No. of locations with taxon
Ambrosia beetles				
Platypodinae				
Euplatypus compositus (Say)	Native	15	<1	1
Scolytinae				
Xylosandrus crassiusculus (Motschulsky)	Exotic	4,168	43	14
Xyleborinus saxeseni (Ratzburg)	Exotic	3,282	34	13
Xylosandrus germanus (Blandford)	Exotic	791	8	12
Xyleborus affinis (Eichhoff)	Native	277	3	8
Ambrosiodmus rubricollis (Eichhoff)	Exotic	199	2	6
Monarthrum mali (Fitch)	Native	142	1	7
Euwallacea validus (Eichhoff)	Exotic	63	1	5
Dryoxylon onoharaense (Murayama)	Exotic	61	1	2
Ambrosiophilus atratus (Eichhoff)	Native	47	<1	6
Xyleborus ferrugineus (Boheman)	Native	22	<1	7
Xyleborus celsus Eichhoff	Native	14	<1	4
Monarthrum fasciatum (Say)	Native	2	<1	1
Anisandrus sayi Hopkins	Native	1	<1	1
Cyclorhipidion bodoanum Wood	Exotic	1	<1	1
Bark beetles				
Scolytinae				
Pityophthorus lautus Eichhoff	Native	19	<1	1
Hylocurus rudis Leconte	Native	10	<1	2
Hypothenemus eruditus Westwood	Native	14	<1	2
Other weevils				
Baridinae				
Plocamus hispidulus LeConte	Unknown	1	<1	1
Cossoninae				
Stenomimus pallidus (Boheman)	Native	435	5	12
Himatium errans LeConte	Native	10	<1	6
Dryophthorinae				
Sitophilus zeamais Motschulsky[b]	Unknown	9	<1	4
Total		9,583		

Insects collected from 56 walnut trap trees at 15 locations in Indiana.

[a] Status as exotic or native to eastern United States (Wood 1982, Rabaglia et al. 2006) or status unknown.

[b] *S. zeamais* breeds in corn kernels.

Table 2. The number and relative abundance of ambrosia beetles, bark beetles, and other weevils collected in Missouri during 2011

Taxa	Status[a]	Total no. insects collected	Percent relative abundance	No. of locations with taxon
Ambrosia beetles				
Scolytinae				
Xyleborinus saxeseni (Ratzburg)	Exotic	6,055	83	12
Xyleborus affinis (Eichhoff)	Native	431	6	9
Xylosandrus germanus (Blandford)	Exotic	402	6	6
Xylosandrus crassiusculus (Motschulsky)	Exotic	240	3	11
Monarthrum mali (Fitch)	Native	40	<1	5
Xyleborus ferrugineus (Boheman)	Native	15	<1	4
Ambrosiodmus rubricollis (Eichhoff)	Exotic	2	<1	1
Bark beetles				
Scolytinae				
Hypothenemus interstitialis Hopkins	Native	3	<1	2
Hypothenemus eruditus Westwood	Native	2	<1	1
Other weevils				
Baridinae				
Plocamus hispidulus LeConte	Unknown	2	<1	2
Cossoninae				
Himatium errans LeConte	Native	91	1	8
Stenomimus pallidus (Boheman)	Native	9	<1	2
Caulophilus dubius (Horn)	Unknown	5	<1	3
Curculioninae				
Tychius picirostris (F.)[b]	Exotic	1	<1	1
Dryophthorinae				
Dryophthorus americanus (Bedel)	Native	6	<1	3
Total		7,295	100	

Insects collected from 48 walnut trap trees at 12 locations in Missouri

[a] Status as exotic or native to eastern United States (Wood 1982, Anderson and Howden 1994, Rabaglia et al. 2006) or status unknown.

[b] *Tychius picirostris* breeds in clover.

Table 3. Detection of the five most abundant taxa from main stem and crown logs taken from stressed black walnut trees in Indiana and Missouri during 2011

Taxa	No. site detections	Overall percentage trees with >1 insect collected		Average no. trees (± SE) with collected beetles	
		Main stem	Crown sample	Main stem	Crown
Xyleborinus saxeseni	25	74	58	3.1 ± 0.2	2.4 ± 0.3
Xylosandrus crassiusculus	24	48	21	1.9 ± 0.2	0.9 ± 0.2
Xylosandrus germanus	18	38	7	2.2 ± 0.4	0.4 ± 0.2
Xyleborus affinis	17	30	6	1.8 ± 0.3	0.4 ± 0.1
Stenomimus pallidus	14	24	13	1.8 ± 0.4	0.9 ± 0.3

Based on insects emerged from logs collected from 104 trees at 27 locations in Indiana and Missouri with four trees per location.

Table 4. Emergence densities of the five most abundant taxa collected from main stem and crown pieces taken from stressed black walnut trees in Indiana and Missouri during 2011

Taxa	No. trees attacked	Average no. (±SE) collected beetles/m^2	
		Main stem	Crown
Xyleborinus saxeseni	85	265 ± 57	114 ± 25
Xylosandrus crassiusculus	61	122 ± 48	8 ± 3
Xylosandrus germanus	45	42 ± 12	3 ± 2
Xyleborus affinis	35	27 ± 9	1 ± 0
Stenomimus pallidus	31	10 ± 4	2 ± 1

Averages based on number of insects emerged from 104 trees at 27 locations in Indiana and Missouri with four trees per location.

including *Caulophilus dubuis* Horn, *Tychius picirostris* F., and *Dyrophthorus americanus* Bedel, were detected only in this state.

Ambrosia beetles dominated the insect collections from both states. In Indiana, *Xylosandrus crassiusculus* Motschulsky was the most commonly collected species, followed by *Xyleborinus saxeseni* Ratzburg, *Xylosandrus germanus* Blandford, and *Xyleborus affinis* Eichhoff. In Missouri, *X. saxeseni* was collected most commonly, followed by *X. affinis*, *X germanus*, and *X. crassiusculus.* In both Indiana and Missouri, *X. saxeseni* and *X. crassiusculus* were detected at more than 90% of trap tree locations, while *X. germanus* and *X. affinis* were detected at more than 50% of trap tree locations sporadically over the sampled regions of each state. *X. saxeseni* or *X. crassiusculus* was the dominant species at almost all trap tree locations.

Weevils not in the scolytine or platypodine subfamilies made up the next most commonly collected group of insects. Of the seven weevil species, *S. pallidus* Boheman and *Himatium errans* LeConte weevils were collected most frequently and were the most abundant in Indiana and Missouri, respectively. *S. pallidus* weevils were collected at 80% of Indiana locations and at 17% of Missouri locations. *H. errans* weevils were collected at 50% of Missouri locations and at 40% of Indiana locations. Scolytine bark beetles were collected infrequently in both states, including *P. lautus* beetles, which were collected at one location in Indiana and not collected in Missouri.

Host Selection. The five most abundant species were collected from trees of all sizes. Individuals were collected from trees with diameters ranging between 12 and 37 cm for *X. saxeseni*, 12 and 37 cm for *X. crassiusculus*, 15 and 32 cm for *X. germanus*, 13 and 29 cm for *X. affinis,* and 15 and 29 cm for *S. pallidus* beetles. Beetles of the rarely collected, *P. lautus* were collected from the lower main stems of two trees with diameters between 25 and 27 cm.

Ambrosia beetles, bark beetles, and other weevil species were collected from tree stems more frequently than tree crowns, with individuals collected from 92% of stem samples in comparison to only 72% of the paired crown samples. The five most abundant species were collected from stems more frequently than crowns (Table 3). However, differences in collection frequency between the main stem and crown samples were significant only in the case of *X. crassiusculus* ($F_{1,23} = 8.10$, $P < 0.01$), *X. germanus* ($F_{1,17} = 18.10$, $P < 0.01$), and *X. affinis* ($F_{1,16} = 13.56$, $P < 0.01$), and neither *X. saxeseni* ($F_{1,24} = 2.10$, $P = 0.16$) nor *S. pallidus* ($F_{1,13} = 3.66$, $P = 0.08$). Emergence densities of all of these species were 2–27 times greater from the main stem samples in comparison to the crown samples (Table 4). There were significant differences in the

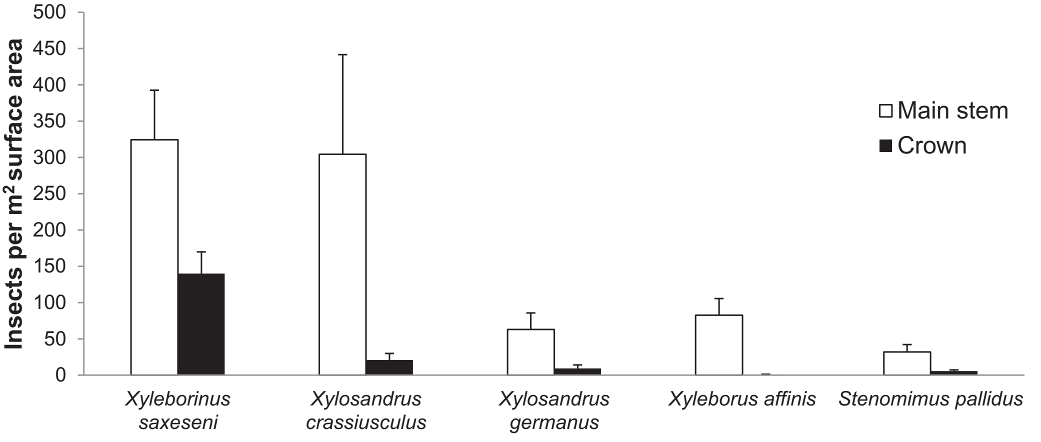

Fig. 4. Densities of the five most abundant taxa (mean ± SE) collected from attacked main stems and crowns of stressed black walnut trees in Indiana and Missouri during 2011. Densities of insects were significantly higher in the main stems than in the crowns for all taxa ($P < 0.05$). Error bars show standard error.

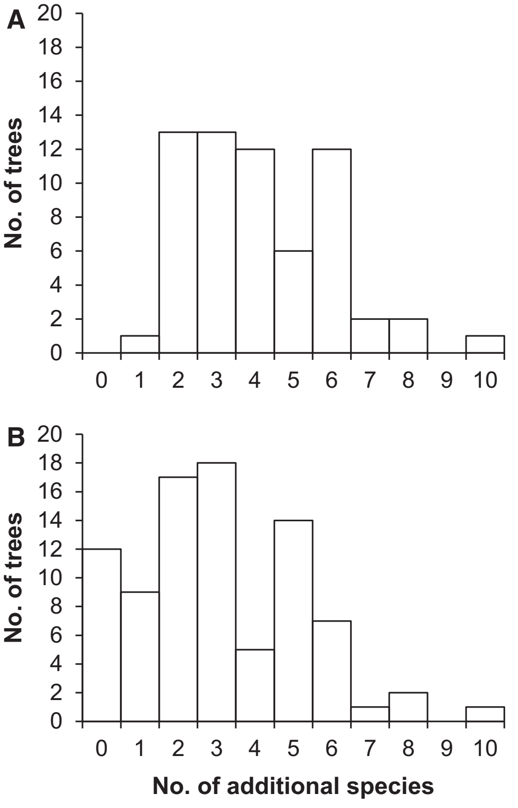

Fig. 5. Number of additional taxa collected per tree from stressed black walnut trees that had been attacked by (A) *X. crassiusculus* and (B) *X. saxeseni* during 2011.

emergence densities between the main stem and the crown samples for *X. saxeseni* ($N = 86$, $S = 1043$, $P < 0.001$), *X. crassiusculus* ($N = 61$, $S = 646$, $P < 0.001$), *X. germanus* ($N = 46$, $S = 409$, $P < 0.001$), and *X. affinis* ($N = 35$, $S = 284$, $P < 0.001$) beetles, as well as *S. pallidus* ($N = 31$, $S = 152$, $P < 0.002$) weevils (Fig. 4).

Two to six additional species were always collected from trees that were attacked by *X. crassiusculus* (Fig. 5), *X. affinis*, or *S. pallidus*. In comparison, 15 and 9% of trees were attacked solely by *X. saxeseni* (Fig. 5) and *X. germanus* beetles, respectively.

Discussion

Assemblages of ambrosia beetles, bark beetles, and other weevils varied in density and species richness among black walnut trap tree locations in Indiana and Missouri. Similar variations among assemblages were reported in other surveys of hardwood species in the midwestern and eastern United States (Oliver and Mannion 2001, Coyle et al. 2005). Although the reason for such a variation is unclear, previous studies suggest that tree density, type, age, size, and forest management practices may influence beetle populations (Harmon et al. 1986, Fettig et al. 2007, Reed and Muzika 2010). In addition, differences in tree phenology among sites and tree placement within sites may have influenced the attractiveness of individual trees and, in turn, affected beetle abundance and richness (Peltonen and Heliövaara 1999, Oliver and Mannion 2001).

Ambrosia beetles were the primary insects collected from trap trees in Indiana and Missouri. Six of the 15 beetle species detected had not been previously associated with black walnut (Wood 1982, Solomon 1995). These six species, *X. affinis*, *A. atratus*, *M. fasciatum*, *Monarthrum mali* Fitch, *E. compositus*, and *D. onoharaense*, are generalists and were reported previously to infest tree species in the same family or order as black walnut, including hickory, pecan, and oak (Wood 1982, Solomon 1995).

All but four of the 15 ambrosia beetle and three bark beetle species detected in the Indiana survey had been reported in the state prior to 1980 (Deyrup 1981). Of the four remaining species, *C. bodoanum* and *X.*

crassiusculus were found throughout the Indiana survey area. *C. bodoanum* is a palearctic species that was first reported in the western United States during the 1940s and was later detected in the eastern United States during the 1990s (Wood 1982, Vandenberg et al. 2000). *X. crassiusculus* is native to Asia and first became established in the southeastern United States during the 1970s (Rabaglia et al. 2006). *A. atratus* was detected in the northern and southern regions of Indiana. This species was reported previously in the southern region of the state during Early Detection and Rapid Response surveys (EDRR) (EDRR 2014). Also of Asian origin, *A. atratus* was first detected in the eastern United States during the 1990s (Rabaglia et. al 2006). *D. onoharaense* was collected from trap trees in two southern Indiana counties, Orange and Crawford. *D. onoharaense* was previously reported at two nearby locations in southern Indiana (EDRR 2014). This species is native to Asia and was first recognized as established in the southeastern United States during the 1970s (Bright and Rabaglia 1999).

Nine common bark and ambrosia beetle species were detected in Indiana but not in Missouri. Accordingly, *A. atratus*, *A. sayi*, *C. bodoanum, M. fasciatum*, *X. celsus*, *H. rudis,* and *P. lautus* are native to the midwestern United States or are abundant exotic species in Missouri (Wood 1982, Reed and Muzika 2010). *D. onoharaense* and *E. validus* are also exotic to Missouri but are detected less frequently (D. LeDoux, personal communication). It is possible that fewer beetle species were collected from Missouri as a result of when trees were girdled and logs collected. The majority of these beetle species are known to fly between March and October in Missouri with individuals collected in aerial flight traps most of these months (Reed and Muzika 2010, EDRR 2014). Aerial flight captures are greatest in Missouri from April to mid-June and August to September. Flight periods of these beetle species are similar in Missouri and Indiana (EDRR 2014). Nevertheless, nine fewer bark and ambrosia beetle species were recovered from trap trees in Missouri.

Four ambrosia beetle species made up the majority of the collected insects. Of these species, *X. saxeseni*, *X. crassiusculus*, and *X. germanus* are exotic to the United States, while *X. affinis* is native. These species have previously been detected in bark and ambrosia beetle aerial trap surveys performed in the eastern United States and are often the dominant species (Coyle et al. 2005, Reed and Muzika 2010). *X. crassiusculus* and *X. germanus* are pests of a wide range of horticulturally important woody plant species in the northeastern United States and are thought to be more aggressive than other native and exotic species. *X. crassiusculus* and *X. germanus* are often reported as attacking trees that appear healthy but are experiencing some physiological stress (Oliver and Mannion 2001, Ranger et al. 2010). *X. germanus* beetles attack young black walnut trees in the midwestern United States, and these attacks are associated with stem dieback and tree death (Weber and McPherson 1984a). Wood (1982) also associated attack by *X. saxeseni* and *X. affinis* beetles with tree death, especially in tropical regions.

All non-scolytine and non-platypodine weevil species, except the corn-inhabiting *S. zeamais* and the clover-inhabiting *T. picirostris*, collected in this study have been documented living in or under tree bark (Blatchley and Leng 1916). The most abundant and frequently collected weevils were *S. pallidus* and *H. errans. S. pallidus* was collected from trees in 12 counties in Indiana and from 2 counties in Missouri. The species is native to and found throughout the eastern United States, and it has been found under the bark of wounded hickory trees, black walnut trees, and dead oak trees (Beutenmuller 1893, Ciegler 2010). *S. pallidus* adults, contaminated with *G. morbida* spores were recently collected at one location in southern Indiana (Juzwik et al. 2015), but the implications of this detection on the epidemiology of TCD are unclear. *H. errans* was collected from five counties in Indiana and seven counties in Missouri. This weevil has been collected previously from bark beetle galleries (Beutenmuller 1893) and from dead pine wood in bottomland and upland forests of the southeastern United States (Ulyshen and Hanula 2009).

The collection of the bark beetle, *P. lautus*, in Carroll Co., Indiana, was of interest because of its close taxonomic relationship to *P. juglandis*, the vector of *G. morbida.* This native species is distributed throughout the eastern United States and has been collected from a variety of hosts, including elm, sassafras, redbud, pines, poison ivy, and sumac (Bright 1981, Wood 1982, Atkinson et al. 1991). In Illinois, it was recovered in aerial traps within black walnut plantations (Weber and McPherson 1991).

In addition to other species in the present study, other curculionid species may attack black walnut trees. Trees were left standing for ∼3 mo and some ambrosia beetle species are known to have generation times of 60 d or less in the southeastern United States (Oliver and Mannion 2001). It would be difficult to assess if ambrosia beetles had emerged prior to placing logs in emergence buckets, as they enter and exit from the same entry hole. In addition, some ambrosia beetle, bark beetle, and other weevil species may not have completed their life cycle if they require a cold period for development or a different moisture regiment. It is also possible that some of the less abundant species in this study were attracted to the stressed walnut trees but failed to reproduce because walnut trees were poor hosts. Despite this, we collected nearly 17,000 individual bark and ambrosia beetles and other weevils from 104 trees at 27 locations. The abundance of some beetle and weevil species is likely an indicator that they are among those species most likely to be associated with stressed black walnut in the midwestern United States.

X. crassiusculus, *X. germanus*, *X. saxeseni*, *X. affinis*, and *S. pallidus* insects were collected from trees of almost all diameters, and there was no preference for specific sizes. Except for *S. pallidus*, these insects have been reported attacking small- and large-diameter trees (Wood 1982). For example, *X. saxeseni* beetles have been collected from main stems 5–50 cm in diameter and branches as small as 2.5 cm, *X. crassiusculus* beetles have been collected from root collars of seedlings and tree stems larger than 20 cm in diameter, and *X. germanus* beetles have been collected from sapling stems 0.9 cm in diameter and beech stumps 50 cm in diameter (Wood 1982, Solomon 1995). These beetles sometimes appear to preferentially attack smaller diameter hosts, especially in nurseries and plantations (Weber and McPherson 1984a, Solomon 1995). In this study, however, trees were artificially and severely stressed by girdling, and an associated reduction in host plant defenses may explain the lack of a diameter preference by ambrosia beetles (Ranger et al. 2013).

Overall, the ambrosia beetles *X. crassiusculus*, *X. germanus*, *X. affinis,* and *X. saxeseni*, as well as the weevil *S. pallidus,* were associated with the main stems of trees more frequently than tree crowns. However, at some locations in Indiana and Missouri, *X. saxeseni* and *S. pallidus* were associated with main stems and crowns equally or crowns more often than stems. The more frequent association with stems in comparison to crowns by *X. crassiusculus*, X. *germanus,* and *X. affinis* beetles supports flight behavior studies performed previously. Abundant ambrosia beetle species in this study are reported to mostly fly within a few meters of the ground, with *X. saxeseni* beetles sometimes flying at greater heights than the other three species (Turnbow and Franklin 1980, Atkinson et al. 1988, Weber and McPherson 1991). Moreover, Weber and McPherson (1984) noted that *X. germanus* beetles preferred to attack the lower main stem of black walnut plantation trees, and Oliver and Mannion (2001) report that *X. germanus* attacks decreased with stem height. Also, similar observations were made for *X. crassiusculus* beetles (Solomon 1995, Oliver and Mannion 2001). The reasons for some beetle species preferentially attacking the main stems in comparison to the crowns are not known. Previous studies have suggested that differences in moisture within a tree may influence the distribution of colonizing beetles (Fisher et al. 1953, Francke-Grossmann 1967).

In the Indiana and Missouri surveys, multiple ambrosia beetles, bark beetles and other weevil species were collected from each black walnut tree, and it is likely that these insects created galleries in close proximity to each other. Such sharing of host material by breeding populations of beetles and weevils may be a common phenomenon. For example, ambrosia beetles of multiple species have been observed to breed near each other in chestnut, swamp bay, and avocado trees (Oliver and Mannion 2001, Kendra et al. 2011). In a host-use study in an oak–hickory forest, Reed (2010) found that between two and seven ambrosia beetle species emerged from woody debris from hickory, elm, white oak, red oak, hackberry, hornbeam, and walnut trees. Moreover, Oliver and Mannion (2001) observed *X. saxeseni* and *X. germanus* emerging from the same gallery exit holes as did *X. germanus* and *X. crassiusculus.*

The extent to which ambrosia beetles, bark beetles, and other weevils detected in the Indiana and Missouri surveys influence the rate of decline of black walnut trees affected by TCD is not known. However, these insects may increase the rate of decline by forming galleries that disrupt vascular tissue function and the movement of water or photosynthates. Alternatively, such insects could also hasten decline through introduction of fungal pathogens that cause tissue necrosis or vascular wilt. For example, *Fusarium solani* (Martius) Saccardo has been isolated from a number of ambrosia beetle species that were detected in this study (Weber and McPherson 1984b, Kasson et al. 2013). In addition, *F. solani*, which produces cankers in black walnut trees and seedlings (Carlson et al. 1993, Reed et al. 2014), has been isolated from *X. saxeseni*, *X. crassiusculus*, *X. germanus*, and *X. affinis* beetles and galleries in the Midwest (Weber and McPherson 1984b, Reed 2010, Reed et al. 2013). A separate, related study of associated fungi was conducted with a subset of the beetles and weevils from this study. The results of those findings are forthcoming.

Finally, the recent detection of *G. morbida* spores on *S. pallidus* weevils collected during this study from an Indiana plantation where no *P. juglandis* beetles have been detected suggests that this insect could at least be a casual vector of *G. morbida* (Juzwik et al. 2015). It is important to note that trees in the plantation where these beetles were recovered show no symptoms of the disease. Moreover, ambrosia beetles that emerged from the same host material as *G. morbida*-contaminated *S. pallidus* weevils did not carry *G. morbida* spores, suggesting that other wood-boring ambrosia beetles may not transmit spores of *G. morbida* (Juzwik et al. 2015). Additional research will be needed to evaluate mechanisms by which assemblages of ambrosia beetles, bark beetles, and other weevil species affect the management of declining TCD-affected black walnut.

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