Species Richness and Phenology of Cerambycid Beetles in Urban Forest Fragments of Northern Delaware

K. HANDLEY, J. HOUGH-GOLDSTEIN, L. M. HANKS, J. G. MILLAR, AND V. D’AMICO

ABSTRACT

Cerambycid beetles are abundant and diverse in forests, but much about their host relationships and adult behavior remains unknown. Generic blends of synthetic pheromones were used as lures in traps, to assess the species richness, and phenology of cerambycids in forest fragments in northern Delaware. More than 15,000 cerambycid beetles of 69 species were trapped over 2 yr. Activity periods were similar to those found in previous studies, but many species were active 1–3 wk earlier in 2012 than in 2013, probably owing to warmer spring temperatures that year. In 2012, the blends were tested with and without ethanol, a host plant volatile produced by stressed trees. Of cerambycid species trapped in sufficient numbers for statistical analysis, ethanol synergized pheromone trap catches for seven species, but had no effect on attraction to pheromone for six species. One species was attracted only by ethanol. The generic pheromone blend, especially when combined with ethanol, was an effective tool for assessing the species richness and adult phenology of many cerambycid species, including nocturnal, crepuscular, and cryptic species that are otherwise difficult to find.

KEY WORDS

Cerambycidae, attractant, phenology, forest fragmentation

Cerambycid beetles can be serious pests of forest trees and wood products (Speight 1989, Solomon 1995). Increasing numbers of these species have been intercepted at ports of entry or have invaded new regions of the world, in some cases causing serious ecological and economic damage (Mack et al. 2000, Aukema et al. 2010). Native wood-borers, however, serve important ecological roles in forests as decomposers contributing to nutrient cycling, and as prey for woodpeckers and other vertebrates (Grove 2002). In Europe, where much of the original forest has been cleared or intensively managed for centuries, some cerambycid species associated with mature trees and dead wood have gone extinct and others are threatened or endangered (Speight 1989). This situation has inspired research to assess the biodiversity and ecological needs of these species to develop conservation strategies (Widerberg et al. 2012, Lachat et al. 2013, Bouget et al. 2014). In North America, the Cerambycidae have only recently been studied from a conservation standpoint (Hammond 1997, Langor et al. 2008, Newell and King 2009, Gustafsson et al. 2012). Although North American forests have not been managed and influenced by people for as long as those in Europe, almost half of the forests in the contiguous United States are now considered highly fragmented, especially in the heavily urbanized east coast region (SOC-CEC 2005). This fragmentation may have had negative effects on biodiversity and ecosystem function (Fahrig 2003, Bregman et al. 2014).

Beetles in the family Cerambycidae are among the most abundant and diverse of the wood-boring beetles (Solomon 1995). Although there is an extensive literature on taxonomy of the adults and on natural history of the most damaging pest species, less is known about host relationships and the behavior of adult cerambycids (Hanks 1999, Langor et al. 2008). A fairly new tool for studying cerambycid populations is trapping with blends of volatile sex or aggregation pheromones that act as general attractants for multiple species (Imrei et al. 2013, Sweeney et al. 2014). For example, Hanks and Millar (2013) and Hanks et al. (2014) used blends of synthesized cerambycid aggregation pheromones to assess species richness and seasonal phenology of cerambycids in Pennsylvania and in east-central Illinois, respectively. The advantage of using traps baited with pheromone blends over other sampling methods is that large numbers of many species can be caught at one time, including nocturnal, crepuscular, and cryptic species that otherwise are rarely observed. A limitation of the method is that pheromones remain unknown for many cerambycid species, and so these species are not attracted by currently known pheromone blends. Moreover, species whose pheromones are represented in synthetic blends may vary in how strongly they respond (Hanks et al. 2014). Thus, the number and species of beetles that are collected by pheromone-baited traps is limited by the pheromones used.
traps may not accurately represent relative or absolute population densities in nature.

Our project objectives were to assess the species richness and phenology of cerambycids and associated beetle species in forest fragments in northern Delaware, using traps baited with a blend of cerambycid pheromones designed to attract a broad range of species. The synthetic pheromone blend used in this study consisted of a mixture of racemic 3-hydroxyhexan-2-one, racemic $\text{syn}$-2,3-hexanediol, racemic $\text{(E)}$-6,10-dimethylundeca-5,9-dien-2-ol (“fuscumol”) and its corresponding acetate ester (“fuscumol acetate”), 2-((undecyloxy)ethanol (“monochamol”), and racemic 2-methylbutanol. These chemicals are known pheromone components or attractants for many species of cerambycids, especially those in the subfamilies Cerambycinae and Lamiinae (Hanks and Millar 2013, Hanks et al. 2013). In 2013, two additional components were added to the pheromone blend: citral (a $\sim$1:1 isomeric mixture of neral and geranial) and racemic 3,5-dimethyldodecanolic acid (“prionic acid”). Citral is part of the male-produced pheromone of the cerambycine species Megacyllene caryae (Gahan) (Lacey et al. 2008, Mitchell et al. 2012), and does not attract other species, to our knowledge. Prionic acid is a female-produced sex pheromone of Prionus californicus Motschulsky, and also attracts males of congeneric species (Barbour et al. 2011).

In 2012, we assessed the influence of ethanol on attraction to the pheromone blend, comparing catches by traps baited with the pheromone blend (in isopropanol) with and without ethanol, traps with ethanol alone, and traps with isopropanol alone. Ethanol is a component of host plant volatiles produced by stressed trees (Kimmerer and Kozlowski 1982, Gara et al. 1993, Kelsey 1994, Kelsey and Joseph 2003, Kelsey et al. 2014), and has long been used as a general attractant and pheromone synergist for cerambycids, bark beetles (Curculionidae: Scolytinae), other wood-boring beetles, and their associates (Oliver and Mannion 2001, Allison et al. 2004, Miller 2006, Ranger et al. 2010). Ethanol is known to enhance attraction to pheromones for some cerambycid species (Hanks et al. 2012, Hanks and Millar 2013), suggesting that the insects respond to volatiles from potentially suitable larval hosts in addition to pheromones from potential mates (Ginzel and Hanks 2005).

In 2013, single traps at each site were used, baited with the pheromone blend and ethanol. Data from both years were used to assess species richness and phenology. Site-specific differences in cerambycid communities, including species richness and other community-level metrics, which may vary depending on plant communities and other variables, will be explored and presented elsewhere (preliminary analyses in Handley, 2014).

### Materials and Methods

Study sites were part of the Forest Fragments in Managed Ecosystems project, a multifaceted project that aims to develop strategies for managing fragmented urban and suburban forests to maximize ecosystem services (D’Amico et al. 2010). These sites, located in the vicinity of Newark, DE, have been partially characterized by soil, tree and understory species and density, and litter-dwelling arthropods (Rega 2012). They range in area from 2 to 16 ha, with varying levels of isolation and connectivity, and in age from 32 to >100 yr since last disturbance (Rega 2012). The dominant tree species varied from site to site, but were primarily hardwoods (Handley 2014).

Beetles were captured with black cross-vane panel traps (corrugated plastic, 1.2 m in height by 0.5 m in width, Alpha Scents Inc., West Lim, OR) that were coated with Fhion (Insect-a-Slip, Bioquip Products, Rancho Dominguez, CA), and hung from 2-m tall frames constructed of polyvinyl chloride pipe. Undiluted ethylene glycol was used as a preservative and killing agent in the collecting cup of each trap. Traps were deployed continuously from 23 April to 26 September in both 2012 and 2013.

Pheromone emitters were clear low-density polyethylene press-seal sachets (5.1 by 7.6 cm, 0.05-mm wall thickness, Cousin Corp., Largo, FL) that were suspended at trap centers. In 2012, traps were baited with a blend of synthetic pheromones similar to that used in earlier studies (Hanks et al. 2012, Hanks and Millar 2013), which was formulated to contain 25 mg of each isomer per 1 ml of solvent carrier (91% isopropanol) per lure: racemic 3-hydroxyhexan-2-one (50 mg/lure), monochamol (25 mg), racemic (3R)-fuscumol, and (E)-fuscumol acetate (50 mg each), all from Bedoukian Research (Danbury, CT), syn-2,3-hexanediol (i.e., a racemic blend of (2R,3R)-hexanediol and (2S,3S)-hexanediol; 50 mg, synthesized as described by Lacey et al. 2004), and racemic 2-methylbutan-2-ol (50 mg; Sigma-Aldrich, St. Louis, MO). In 2013, citral (50 mg; Aldrich Chemical Co.) and racemic prionic acid (1 mg; synthesized as described by Rodstein et al. 2000) were added to the pheromone blend. Milligram quantities of prionic acid were used because males of $\text{Prionus}$ species are attracted by very small quantities of pheromone (Rodstein et al. 2011). Emitters were replaced every 2 wk. Ethanol lures were 10.2 by 15.3 cm sachets (Cousin Corp.) containing 100 ml of 100% ethanol (Pharma Products Inc., Brookfield, CT) that were clipped to trap centers. Ethanol lures lasted for an entire season. Release rates of the individual components averaged $\sim$1 mg/d in laboratory studies over a 2-wk period, except for monochamol, which had a release rate of $\sim$0.01 mg/d (L.M.H., unpublished data). These release rates are relatively low, but have been shown to be effective in numerous trials conducted over the past 5 yr (Hanks et al. 2012, Hanks and Millar 2013). There was no evidence that interactions among the components influenced the rates at which they were released.

In 2012, four traps were placed at each of 12 study sites (Fig. 1), with traps $\sim$25 m apart in a square configuration. Four treatments were assigned randomly to traps within sites: 1) pheromone blend, 2) pheromone blend + ethanol, 3) ethanol alone, and 4) solvent control (1 ml isopropanol). In 2013, single
traps baited with the pheromone blend (including citral and prionic acid) and ethanol were set up in each of 24 forest fragments, with six larger fragments having two traps that were positioned 100–180 m apart (Fig. 1). During the second year, traps were used only to determine phenology (all counts summed each week) and to assess site-specific differences in cerambycid communities (results to be presented elsewhere; Handley, 2014).

Insects were collected once per week in both years. All cerambycids were identified to species (Yanega 1996, Lingafelter 2007), and taxonomy follows Bezark (2014). Voucher specimens of cerambycid species have been deposited in the University of Delaware Insect Reference Collection.

For cerambycid species represented by more than 20 specimens in a year, seasonal phenology was characterized graphically. For 2012, weekly trap catches from all four traps at each site were pooled to determine phenology. For cerambycid species represented by >50 specimens in 2012, treatment effects on the mean numbers caught over the entire field season were compared using a two-way analysis of variance (ANOVA) blocked by trap site, followed by Tukey’s test for separation of means. Data were checked for homoscedasticity using Levene’s test, and square root-transformed as needed. Means and SEs in figures were calculated from untransformed data. Analyses were conducted using R (R Core Team, 2012, Vienna, Austria).

Results

In total, 15,370 cerambycid beetles of 69 species in seven subfamilies were trapped over both years, including 7 species that have not been reported previously from Delaware (Table 1). The only species known to be exotic is Phymatodes testaceus (L.), which is native to Europe (Swift and Ray 2010). Thirty species were represented by 1–5 specimens; 9 species by 6–20 specimens, and 30 species were caught in numbers greater than 20 individuals (Table 1). The most common species were all in the subfamily Cerambycinae and included Xylotrechus colonus (F.), which accounted for 30% of the total trap catch; Phymatodes amoenus (Say), 17% of the total; and Megacyllene caryae (Gahan), 12% of the total, almost all in the second year. The most common species in the Lamiinae was Graphisurus fasciatus (DeGeer), which made up 8% of the total (Table 1). Over both years, 78% of the captured beetles were cerambycines and 15% were lamaines; the remaining 7% of specimens were distributed among the five remaining subfamilies. Both sexes responded for all species except Prionus laticollis (Drury), which was represented only by males (consistent with attraction by the female-produced sex pheromone).
Table 1. Taxonomy of cerambycid beetles, and numbers captured by semiochemical-baited cross-vane panel traps in Delaware during 2012 and 2013

<table>
<thead>
<tr>
<th>Taxonomy</th>
<th>2012</th>
<th>2013</th>
<th>Total</th>
<th>Previous</th>
<th>PA, IL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saperdini</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td></td>
<td>PA, IL</td>
</tr>
<tr>
<td>Lepturina</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lepturina</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belleminera scalaris</td>
<td>23</td>
<td>1</td>
<td>24</td>
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<td></td>
</tr>
<tr>
<td>Brachydeptus ruderis</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>PA, IL</td>
<td></td>
</tr>
<tr>
<td>Fulgoria cecidogena</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>PA</td>
<td></td>
</tr>
<tr>
<td>Strychnele aprebieta</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>PA, IL</td>
<td></td>
</tr>
<tr>
<td>Stephanae nitera</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>PA, IL</td>
<td></td>
</tr>
<tr>
<td>Trichophora narsidiala</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>PA, IL</td>
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</tr>
<tr>
<td>Trigonarthris minnesotana</td>
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<td>1</td>
<td>1</td>
<td>PA, IL</td>
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<tr>
<td>Trigonarthris proxima</td>
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<td>Typoceros hagubris</td>
<td>11</td>
<td>4</td>
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<tr>
<td>Typoceros cebutius cebutius</td>
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<td>2</td>
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<td>Rhagadi</td>
<td>132</td>
<td>90</td>
<td>222</td>
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<td>Metacerus cecidogena</td>
<td>9</td>
<td>1</td>
<td>10</td>
<td>PA</td>
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<td>Xylocerus</td>
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<tr>
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<td>2</td>
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<td>2</td>
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<tr>
<td>Nacelma</td>
<td>59</td>
<td>12</td>
<td>71</td>
<td>PA, IL</td>
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<td>Pronius</td>
<td></td>
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<td></td>
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<tr>
<td>Pronius</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Orthomerus brownianus</td>
<td>20</td>
<td>1</td>
<td>21</td>
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<tr>
<td>Pronius laticollis</td>
<td>748</td>
<td>754</td>
<td>1502</td>
<td>PA, IL</td>
<td></td>
</tr>
<tr>
<td>Spondylophila</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aescini</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arhopalus rusticus rusticus</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td>PA, IL</td>
<td></td>
</tr>
<tr>
<td>Aescini strinatus (L.)</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>PA, IL</td>
<td></td>
</tr>
<tr>
<td>Total number of specimens:</td>
<td>6,015</td>
<td>8,455</td>
<td>15,370</td>
<td>PA, IL</td>
<td></td>
</tr>
<tr>
<td>Total number of species:</td>
<td>61</td>
<td>47</td>
<td>69</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*PA, found in Pennsylvania (Hanks and Millar 2013); IL, found in Illinois (Hanks et al. 2014).*

Adding citral and prionic acid to the pheromone blend in 2013 increased the capture of *M. caruae* from nine specimens in 2012 to >1,800 males and females in 2013, and *P. laticollis* from 6 to >700 males (Table 1). Other species that were absent or in low numbers in 2012, but caught in fairly large numbers in 2013, were the early season cerambycine species *Eudercus pini* (Olivier) and *Neoclytus caprea* (Say) (Table 1).

In 2012, seven species of cerambycids were present in the first trap collection, and this number increased rapidly to 21 species in the third collection week (mid-May, Fig. 2). More than 20 species were collected each week until mid- to late July, when species richness began to decline. A similar pattern was observed in 2013, except that increased species richness in the spring did not occur until collection week 5 (end of May), 3 wk later than in 2013 (Fig. 2).

The most abundant of the species caught in the first collection in 2012 (≥20 in total) were the cerambycines *P. amoenus* and *Cyrtocephus verrucosus* (Olivier) (Fig. 3). These species would have become active before or during the week of 23–30 April 2012. The following year, the first trap collection date had substantial numbers of *C. verrucosus*, but *P. amoenus* adults were not captured until 2–3 wk later (Fig. 4). Additional species found in numbers ≥20 at the first trap catch in 2013 were *M. caruae*, *E. pini*, and *N. caprea*. Of the 13 cerambycid species caught in numbers ≥20 both years (marked with an asterisk in Figs. 3
and 4), most were caught 1–3 wk earlier in 2012 than in 2013 (Figs. 3 and 4).

Cerambycine species varied greatly in the duration of their flight periods, with most individuals of many species captured during a few weeks (Figs. 3 and 4). In contrast, adults of the cerambycines Neoclytus a. acuminatus (F.) and X. colonus were active in the field for as long as 5 mo (Figs. 3 and 4). Aside from Psenoce-rus supernotatus LeConte, caught during May and early June both years, lamiine species were caught during late June through early July in 2012 and through late July in 2013 (Figs. 3 and 4). The distributions of capture dates were generally unimodal for the dominant species, with a single peak of trap capture each year (Figs. 3 and 4).

Among the most common cerambycine species in 2012 (represented by more than 50 specimens total), C. verrucosus, Neoclytus m. mucronatus (F.), P. amoe-nus, and X. colonus were most strongly attracted to the pheromone–ethanol combination, with significantly greater numbers in the combination traps than in the solvent controls, whereas the numbers caught in traps baited only with pheromone were not significantly different from controls (except for X. colonus, where numbers caught in the pheromone traps alone were higher than the control but lower than the combination treatment with ethanol; Fig. 5; $F_{3,11} = 3.59–10.2$, $P < 0.02$ for all). Other lamiine species were significantly attracted by the pheromone blend and were not influenced by ethanol, including Aegomorphus modestus (Gyllenhal in Schoenherr), Graphisurus despectus (LeConte), and G. fasciatus (Fig. 6; $F_{3,11} = 32.5–41.7$, $P < 0.0001$ for all). The lep-turine Gaurotes cyanipennis (Say) was the only ceram-bycid species that was significantly attracted to ethanol alone (Fig. 6; $F_{3,11} = 6.42$, $P = 0.002$). Neandra brunnea (F.), in the Parandrinae, was more strongly attracted to the pheromone blend than to the ethanol alone (Fig. 6; $F_{3,11} = 4.28$, $P = 0.01$); however, there were no significant differences between the isopropanol control and any other treatment, and thus results are not conclusive for this species.

Discussion

The most common cerambycid species caught in the present study, and their seasonal phenology, were similar to those reported for earlier field studies conducted in Pennsylvania (Hanks and Millar 2013) and east-central Illinois (Hanks et al. 2014; species found in the two previous studies are indicated in Table 1). Species caught in numbers >300 total in the present study were all among the 15 most numerous species collected in Pennsylvania, Illinois, or both, except for P. laticollis, which had not been targeted with the addition of prionic acid in either of the two previous studies.

Many species in the present study were caught earlier in 2012 than in 2013 by ~2 wk, consistent with warmer spring weather in 2012: as of 1 May, 164 cumulative degree–days had accumulated in 2012 as compared with 101 in 2013 (base 10°C, accumulation beginning January 1, data for Newark DE from www.weather.com). Cumulative degree–day accumulation continued to be lower in 2013 than in 2012 throughout the season (Figs. 3 and 4).
Fig. 3. Total numbers of cerambycid beetles collected each week during 2012 for species represented by >20 specimens, arranged chronologically by subfamily. Bars indicate range of activity period.
The catch of *P. laticollis* males in 2013 when prionic acid was added to the lure blend is consistent with the attraction of males of that species to prionic acid in an earlier study (Agnello et al. 2011). Similarly, *M. caryae* was caught in much greater numbers in 2013 than in 2012 owing to the addition of citral to the lure blend because citral has been demonstrated to be an important part of the male-produced pheromone blend of that species.
Fig. 5. Mean (± 1 SEM) total number of beetles captured per trap (sexes combined) with respect to lures for eight species in the Cerambycinae. Means with the same letters are not significantly different (two-way ANOVA, Tukey’s test \( P > 0.05 \)).
Mean number (± 1 SEM) total beetles per trap

Fig. 6. Mean (± 1 SEM) total number of beetles captured per trap (sexes combined) with respect to lures for six species in the Lamiinae, *G. cyanipennis* in the Lepturinae, and *N. brunnea* in the Parandrinae. Means with the same letters are not significantly different (two-way ANOVA, Tukey’s test $P > 0.05$).
species (Lacey et al. 2008, Mitchell et al. 2012). Adults of very early season E. pini and N. caprea were attracted by the 3-hydroxyhexan-2-one in the pheromone blend (Graham et al. 2012, Hanks et al. 2014), and probably were not collected in 2012 because traps had not been deployed until after their flight period was over.

Temporal isolation owing to different seasonal flight periods may play a part in reproductive isolation for sympatric cerambycid species that respond to the same pheromone compounds (Hanks and Millar 2013, Hanks et al. 2014). Several species with flight periods early in the season, including M. caryae, E. pini, Euderces picipes (F), N. caprea, and P. amoenaus, were no longer active by the end of May in both years, whereas other species did not become active before the beginning of June; therefore, these two groups were temporally isolated. Nevertheless, the flight periods of many species that responded to the same components of the pheromone blend did overlap, sometimes extensively. Cross-attraction between such species may be limited by differences in daily activity periods, or by the presence or absence of minor components in their pheromone blends. Conversely, some species may eavesdrop on the pheromone communication of fellow guild members, as an efficient method of finding suitable hosts for mating and oviposition (Hanks and Millar 2014). For these species, contact pheromones present in the wax layer of females may provide an additional reproductive isolation mechanism following host location (Ginzel and Hanks 2003, Ginzel et al. 2006, Lacey et al. 2008).

Ethanol significantly enhanced attraction to the pheromone blend for several cerambycine species, as has been reported previously for attraction of N. m. mucronatus and X. colonus to a similar blend of pheromones (Hanks et al. 2012). The lamine G. fasciatus was not influenced by ethanol in the present study, but attraction to pheromones was enhanced by ethanol in the earlier study (Hanks et al. 2012). Possibly a larger sample size in our study would have shown a significant effect here. In the present study, the lepturine G. cyanipennis was attracted to the ethanol treatment but not to the pheromone blend. The lack of significant attraction of lepturines to pheromone-baited traps was not unexpected because the limited evidence to date suggests that species in that subfamily use female-produced sex pheromones with very different structures than any of the pheromones used in the blends tested in the present study (Ray et al. 2011, 2012).

Ethanol emissions have been shown to increase in trees after a stress event (Kimmerer and Kozlowski 1952, Gara et al. 1993, Kelsey 1994, Kelsey and Joseph 2003, Kelsey et al. 2014), and thus some cerambycids probably use ethanol to locate stressed host trees, even in the absence of pheromone signals.

In conclusion, the generic cerambycid pheromone blend, especially when combined with ethanol, proved to be a useful tool for assessing the presence and phenology of many cerambycid species. The effectiveness of this sampling method is highlighted by the fact that the cerambycids trapped included seven new state records for Delaware. As pheromones of more cerambycid species are identified it may be possible to further improve blends, or to use more than one blend, to produce even more potent multispecies attractants for further study of these diverse forest inhabitants.

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