Short communication

Likely Aggregation-Sex Pheromones of the Invasive Beetle Callidiellum villosulum, and the Related Asian Species Allotreus asiaticus, Semanotus bifasciatus, and Xylotrechus buqueti (Coleoptera: Cerambycidae)

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Abstract

During field trials of the two known cerambycid beetle pheromone components 3-hydroxyhexan-2-one and 1-(1H-pyrrol-2-yl)-1,2-propanedione (henceforth “pyrrole”) in Guangxi and Anhui provinces in China, four species in the subfamily Cerambycinae were attracted to lures containing one of the two components, or the blend of the two. Thus, the invasive species Callidiellum villosulum (Fairmaire) (tribe Callidiini) and a second species, Xylotrechus buqueti (Castelnau & Gory) (tribe Clytini), were specifically attracted to the blend of 3-hydroxyhexan-2-one and the pyrrole. In contrast, Allotreus asiaticus (Schwarzer) (tribe Phoracanthini) and Semanotus bifasciatus Motschulsky (tribe Callidiini) were specifically attracted to the pyrrole as a single component. In most cases, both males and females were attracted, indicating that the compounds are likely to be aggregation-sex pheromones. The results indicate that the two compounds are conserved as pheromone components among species within at least three tribes within the subfamily Cerambycinae. For practical purposes, the attractants could find immediate use in surveillance programs aimed at detecting incursions of these species into new areas of the world, including the United States.

Key words: 3-hydroxyhexan-2-one, 1-(1H-pyrrol-2-yl)-1,2-propanedione, aggregation-sex pheromone, attractant

The Cerambycidae constitute a large and diverse family of beetles, with ~35,000 described species worldwide (Svácha and Lawrence 2014). In their native habitats, their wood-boring larvae perform valuable ecosystem services by initiating the degradation and recycling of woody plant material. However, when introduced into new geographic regions, usually by global commerce (Haack et al. 2010), they can become severe pests of woody plants, particularly if they vector plant pathogens as well as causing direct damage. For example, the Asian longhorned borer Anoplophora glabripennis (Motschulsky) has invaded both North America and Europe, where it represents a major threat to hardwood forests throughout both continents (Meng et al. 2015).

In the United States, two Asian cerambycid species in the genus Callidiellum (subfamily Cerambycinae; tribe Callidiini) have been intercepted entering the United States numerous times. Thus, the first, Callidiellum rufipenne (Motschulsky), successfully invaded the United States in the late 1990s (Maier and Lemmon 2000) and is now established in the northeastern United States. It also has invaded many countries in Europe (Cocquempot and Lindelow 2010, Van Meer and Cocquempot 2013). The second species, Callidiellum villosulum (Fairmaire), has been intercepted numerous times from wooden materials entering the United States from China, but is not yet known to be established (Cocquempot and Mifsud 2013). The host range of C. villosulum includes species in the Cupressaceae, such as two exotic species which are commonly grown as ornamentals in the United States, Chinese fir (Cunninghamia lanceolata [Lamb.] Hook) and Japanese cedar (Cryptomeria japonica [L. f.] D. Don) (Cocquempot and Mifsud...
Materials and Methods
Sources of Compounds
1-(1H-Pyrrole-2-yl)-1,2-propanediol (henceforth “pyrrole”) was synthesized following the methods of Zou et al. (2016), and racemic 3-hydroxyhexan-2-one (henceforth “ketol”) was purchased from Bedoukian Research (Danbury, CT).

Study Sites
Field bioassays were conducted in two areas of China: 1) just north of Nanning, Guangxi Autonomous Region (22.949 N, 108.361 E, elevation ~150 m), in a forest dominated by Chinese fir (C. lanceolata); and 2) ~100 km north of Hefei, Anhui Province (33.996 N, 116.774 E, elevation ~130 m) in a forest dominated by oriental arborvitae (Platycladus orientalis [L. Franco; Cupressaceae]).

Field Bioassays
Beetles were captured with panel traps (black corrugated plastic, 1.2 x 0.3 m; AlphaScents, Portland, OR) that were coated with a 50% aqueous dilution of the fluoropolymer Fluon to render traps more slippery and improve trap efficiency (see Graham et al. 2010). Traps were hung from trees, or attached to wooden posts, ~1 m above the ground. At the site in Guangxi, basins of traps were partially filled with ethylene glycol to kill and preserve captured insects, whereas at the site in Anhui basins were left dry so that beetles could be captured alive for collection of headspace volatiles. Pheromone lures consisted of resealable polyethylene sachets (5.1 by 7.6 cm; Fisher Scientific, Pittsburgh, PA) which were loaded with 50 mg of racemic ketol, 25 mg of pyrrole, or the blend of both, dissolved in 1 ml isopropanol. Control lures contained 1 ml of neat isopropanol.

At both sites, trap treatments were as follows: 1) ketol, 2) pyrrole, 3) ketol + pyrrole, 4) control. Traps were positioned ~50 m apart in six linear transects, which in turn were ~50–65 m apart at the site in Guangxi, and the experiment ran from 12 March to 24 April 2016. Traps were deployed ~20 m apart in five transects ~50 m apart at the site in Anhui, and the experiment ran from 23 March to 23 April 2016. Traps were serviced at intervals of 1 wk (Guangxi) or 6–10 d (Huaibei); lures were replenished every second week, and treatments were rotated one position down transects at each count to minimize positional effects.

Results
A total of 1,448 cerambycid beetles were caught in traps at the two field sites (Table 1), with 1,361 beetles at the Guangxi site and 87 beetles at the site in Anhui. The species represented three tribes within the subfamily Cerambycinae. The two most numerous species were the target species C. villosulum (caught at both sites), and Allotropa asiatica (Schwarzer) (caught only at the Guangxi site). These two species differed in their responses to the experimental treatments (Fig. 1). That is, adults of C. villosulum were not attracted to either compound alone, but were strongly attracted to the blend of the ketol and pyrrole (Q3,180 = 122.5, P < 0.0001). Conversely, adults of A. asiatica were significantly attracted by the pyrrole alone, and attraction was not influenced by the ketol, nor were they attracted by the ketol alone (Friedman’s Q3,20 = 60.5, P < 0.0001). The sex ratios (% female) of trapped beetles were 66% for C. villosulum and 61% for A. asiatica.

Traps also caught another five species of cerambycines in smaller numbers (Table 1), including 8 adults (6 females, 2 males) of Semanotus bifasciatus Motschulsky, 2 females of Semanotus sinoaustus (Gressitt), 11 females of Ceresium sinicum White, 9 females of Xylotrechus buqueti (Castelnau & Gory), and 2 beetles of a cerambycine species that could not be identified. Semanotus bifasciatus and X. buqueti were trapped on multiple dates, across trap transects, and only in baited traps, resulting in statistically significant treatment effects, despite their low numbers. Seven of the eight S. bifasciatus were caught in traps baited with the

Table 1. Numbers of beetles of seven cerambycid species that were caught by panel traps during a field experiment conducted in Guangxi Autonomous Region and Anhui Province, China, during March to April 2016

<table>
<thead>
<tr>
<th>Tribe/species</th>
<th>Guangxi</th>
<th>Anhui</th>
</tr>
</thead>
<tbody>
<tr>
<td>Callidini</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Callidium villosulum (Fairmaire)</td>
<td>418</td>
<td>79</td>
</tr>
<tr>
<td>Semanotus bifasciatus Motschulsky</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Semanotus sinoaustus (Gressitt)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Callidiopini</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceresium sinicum White</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Clytini</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylotrechus buqueti (Castelnau &amp; Gory)</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Phoracanthini</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allotropa asiatica (Schwarzer)</td>
<td>919</td>
<td>0</td>
</tr>
<tr>
<td>Unknown species 1</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1,361</td>
<td>87</td>
</tr>
</tbody>
</table>
pyrrole, and that treatment mean (1.17 ± 0.31) was significantly greater than the mean for the ketol + pyrrole blend (0.17 ± 0.17), and those for the ketol and control treatments (both 0; Q_3,24 = 13.0, P = 0.0045). Similarly, all 9 female X. buqueti were caught in traps baited with the ketol + pyrrole blend, and the mean for that treatment (1.29 ± 0.18), was significantly greater than the zero means for the remaining treatments (Q_3,28 = 25.2, P < 0.0001). Catches of the remaining species were spread across treatments, with no indications of specific attraction (Friedman’s Q, P > 0.05).

Discussion

The compound 3-hydroxyhexan-2-one now has been identified as a pheromone or likely pheromone component from numerous species in the subfamily Cerambycinae, including species from all continents except Antarctica (reviewed in Hanks and Millar 2016). In contrast, the pyrrole was only recently identified from headspace volatiles from male cerambycids, but it has now been found in headspace volatiles collected from males of a number of species in North and South America (J.G.M. and L.M.H., unpub. data). The results reported here, along with previous results showing that a blend of the ketol and the pyrrole were highly attractive to Callidiellum rufipenne (Zou et al. 2016), suggest that the pyrrole will prove to be another conserved pheromone motif within the subfamily Cerambycinae. Furthermore, the fact that adult females or beetles of both sexes were attracted for the four species which showed significant treatment effects (C. villosulum, A. asiaticus, S. bifasciatus, and X. buqueti), indicates that the single compounds or the blend of the two are probably acting as male-produced aggregation-sex pheromones, as occurs in all other species within the subfamily for which pheromones are known (reviewed in Hanks and Millar 2016).

The capture of large numbers of the poorly known species A. asiaticus in traps baited with the pyrrole also is noteworthy because this is the first report of any pheromone or likely pheromone from a species in the tribe Phoracanthini. This tribe includes invasive species such as the eucalyptus longhorned borers Phoracantha semipunctata (F.) and P. recurva Newman, which have caused major damage to eucalyptus plantings in countries in the Mediterranean basin, southern Africa, the southwestern United States, and Brazil (Paine et al. 2011). The fact that A. asiaticus was equally attracted to the blend of the pyrrole with 3-hydroxyhexan-2-one indicated that the latter component was not inhibitory, while the more limited data for S. bifasciatus suggested that attraction to the pyrrole was inhibited by the ketol. In contrast, both C. villosulum and X. buqueti were attracted only by the synergistic blend of the ketol and pyrrole, and not to the individual components. The critical nature of that synergism for X. buqueti explains why few of that species were captured during an earlier pheromone screening trial conducted in southern China which included traps baited with 3-hydroxyhexan-2-one (Wickham et al. 2014). Such differences among sympatric cerambycine species in the synergistic and inhibitory effects of various pheromone components may prevent deleterious interspecific attraction (e.g., Mitchell et al. 2015).

The specific attraction of small numbers of Semanotus bifasciatus to the pyrrole provides a useful lead into the likely pheromone chemistry of this species, and more broadly, the genus Semanotus. In particular, S. bifasciatus is an important pest of cupressaceous trees in Japan (Kobayashi 1985), and has been rated as having a high risk of invading the United States (Anonymous 2011). Similarly, the specific attraction of X. buqueti to the mixture of the ketol and pyrrole provides strong indications that its pheromone blend is likely to contain these two compounds.

Production of aggregation-sex pheromones by males of A. asiaticus and X. buqueti is consistent with a report by Wong (2016) that only the males of those species have pores on the pronotum, which in other cerambycines are known to be associated with pheromone glands. Those authors also reported that the sex-specific pores are present in C. sinicum, suggesting that males of that species also may produce an aggregation-sex pheromone. The lack of a significant treatment effect for this species in the present study suggests that its pheromone chemistry differs from that of the other species.

For practical purposes, attractant-based monitoring systems would be valuable tools for ongoing surveillance efforts aimed at excluding these Asian species from the United States and elsewhere. The attractant for C. villosulum could find immediate use in surveillance programs aimed at detecting incursions of this species into new areas of the world, including the United States and Europe. This species already has been intercepted numerous times in wood products originating from China and entering the United States, Japan, and most recently, Malta (Iwata et al. 2006, Cocquempot and Mifsud 2013).

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