Identification of a Male-Produced Aggregation Pheromone for *Monochamus scutellatus scutellatus* and an Attractant for the Congener *Monochamus notatus* (Coleoptera: Cerambycidae)

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ABSTRACT We report identification and field testing of 2-(undecyloxy)-ethanol (monochamol) as a sex-specific, aggregation pheromone component produced by males of *Monochamus scutellatus scutellatus* (Say) (Coleoptera: Cerambycidae), a longhorned beetle native to North America. A congener, *Monochamus notatus* (Drury), which uses the same hosts as *M. s. scutellatus*, also was attracted to this compound in field trials, suggesting it may be a pheromone component for this species as well. Panel traps were deployed along transects at each of five field sites in May 2010 to test attraction of native beetle species to a suite of cerambycid pheromone components, including monochamol, 3-hydroxyhexan-2-one, (2*R**,3*R**)- and (2*R**, 3*S**)-2,3-hexanediol, racemic (*E*/*Z*)-fuscumol, and (*E*/*Z*)-fuscumol acetate. In total, 209 adult *M. s. scutellatus* (136 females, 73 males) and 20 *M. notatus* (16 females, four males) were captured, of which 86 and 70%, respectively, were captured in traps baited with monochamol (means significantly different). Analysis of headspace volatiles from adult *M. s. scutellatus* by coupled gas chromatography-mass spectrometry and gas chromatography-electroantennogram detection confirmed that monochamol was produced only by males. Monochamol was not found in headspace extracts from adult *M. notatus*. This study provides further evidence that monochamol is a pheromone component common to several species in the genus *Monochamus*. The pheromone component should prove useful for monitoring native species for management purposes or conservation efforts, and for quarantine monitoring for exotic species.

KEY WORDS 2-(undecyloxy)-ethanol, aggregation pheromone, semiochemical

Species in the genus *Monochamus* (Coleoptera: Cerambycidae), commonly known as pine sawyers, are distributed throughout most of North America (Linsley and Chemsak 1984). Their wood-boring larvae provide important ecosystem services by initiating breakdown of woody tissues (Linsley 1959). Larvae can cause economic damage to sawlogs and facilitate introduction of wood rotting fungi that degrade lumber quality (Wilson 1962). *Monochamus* species native to North America pose a significant threat as exotic invasive species on other continents because they vector the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle, that can cause substantial tree mortality in coniferous forests (Linit 1988).

The first volatile aggregation pheromone of a *Monochamus* species was recently identified as 2-(undecyloxy)-ethanol (henceforth monochamol), for the European *Monochamus galloprovincialis* (Oliver) (Pajares et al. 2010). The pheromone is produced by mature males and is attractive to both sexes. The same compound has since been identified as a male-produced aggregation pheromone for the congeneric Japanese pine sawyer, *Monochamus alternatus* Hope, an important vector of pine wood nematode in Asia (Teale et al. 2011).

We report here the capture of two *Monochamus* species, *Monochamus scutellatus scutellatus* (Say) and *Monochamus notatus* (Drury) (Coleoptera: Cerambycidae), during field testing of a library of cerambycid pheromones that included monochamol in New York state. Larvae of both species develop in a diversity of coniferous hosts, and adults feed on the foliage and bark of twigs (Wilson 1962, Raske 1972). Adult male *M. s. scutellatus* locate larval hosts, aggressively defend them from other males, and mate with subsequently arriving females (Hughes and Hughes 1985). In the northeastern United States, adult *M. s. scutellatus* begin emerging in late May to early June (Rose 1957), whereas adult *M. notatus* begin to emerge in late June and continue to emerge through mid-August (Hodgdon 1957).

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Earlier studies have documented that both sexes of *M. s. scutellatus* are attracted by host plant volatiles such as the monoterpenes α-pinene, β-pinene, myrcene, limonene, camphene, and 3-carene, and attraction is synergized by ethanol (Chénier and Philogène 1988, Phillips et al. 1988, Allison et al. 2001, de Groot and Nott 2004, Miller 2006). Here, we show that adult *M. s. scutellatus* and *M. notatus* of both sexes are attracted by monochamol, and we confirm *M. s. scutellatus* males produce the compound as a sex-specific pheromone component.

Materials and Methods

Our study sites were five forested stands along a north-to-south gradient in New York state, as follows: 1) a mature mixed northern hardwood stand adjacent to a managed conifer stand in the SUNY-ESF James F. Dubuar Memorial Forest in the northwestern Adirondack Park near Wanakena, NY (St. Lawrence County; 44° 09′45″ N, 74° 54′30″ W); 2) a contiguous stand of mature northern hardwoods in the 8,260-ha Frank E. Jadwin State Forest (Lewis County; 44° 04′35″ N, 75°22′53″ W); 3) SUNY-ESF's Lafayette Road Field Station, a 22-ha mixed forest with a broad variety of tree species, including a planted arboretum, at the southern edge of the City of Syracuse (Onondaga County; 42° 59′29″ N, 76° 07′55″ W); 4) SUNY-ESF's Heiberg Memorial Forest, a 1,600-ha forest with a mosaic of mixed hardwood and planted conifer stands, ≈25 km south of Syracuse, NY (Cortland County; 42° 46′06″ N, 76° 04′19″ W); and 5) a contiguous 26,300-ha mixed forest near Salamanca, NY at the northern edge of Allegany State Park (Cattaraugus County; 42° 05′27″ N, 78°51′04″ W).

Beetles were trapped with black cross-vane panel traps (Panel Trap model, AlphaScents, Portland, OR) coated with Fluon PTFE (Northern Products, Woonsocket, RI) to improve trap efficiency (Graham et al. 2010). Traps were hung from L-shaped frames of PVC pipe that were mounted on a 1-m steel reinforcing bar driven partway into the ground. Trap basins contained a killing agent (30% propylene glycol).

Trap lures were polyethylene sachets (Bagettes model 14770, 5.1 by 7.6 cm, Cousin Corp., Largo, FL) loaded with synthetic pheromone diluted in 1 ml of 95% ethanol (an efficient carrier for these compounds that does not attract cerambycid beetles at these doses; e.g., Hanks et al. 2007). The six compounds tested included important pheromone components for cerambycid species in the subfamilies Lamiinae, Cerambycinae, and Spondylidinae (Hanks et al. 2007, Silk et al. 2007, Mitchell et al. 2011, Teale et al. 2011) and included monochamol (25 mg per lure), racemic 3-hydroxyhexan-2-one (50 mg per lure), (2*R**,3*R**)-2,3-hexanediol (50 mg per lure), (2*R**,3*S**)-2,3-hexanediol (50 mg per lure), racemic (*E*/*Z*)-fuscumol (100 mg per lure), and racemic (*E*/*Z*)-fuscumol acetate (100 mg per lure). Compounds were synthesized as described in the following references: monochamol (Pajares et al. 2010), racemic 3-hydroxyhexan-2-one (Imrei, Z., Millar, J. G. et al. unpublished data), (2*R**,3*R**)-2,3-hexanediol and (2*R**,3*S**)-2,3-hexanediol (Lacey et al. 2004), (*E*/*Z*)-fuscumol and (*E*/*Z*)-fuscumol acetate (Mitchell et al. 2011). Treatments were randomly assigned to eight traps, including two controls: one trap baited with 1 ml of 95% ethanol (solvent control), and one trap without a lure (blank control). Traps were deployed 10 m apart in linear transects at each of the five study sites (40 traps total).

The experiment was set up during the week of 18 May 2010 and checked weekly for ≈3 mo. Lures were replaced every third week at which time treatments were rotated within transects. Taxonomy of captured beetles follows Lingafelter (2007), and beetles were sexed by relative antennal length (Linsley and Chemsak 1984). Voucher specimens are deposited in the SUNY-ESF Insect Museum in Syracuse, NY.

Adult *M. s. scutellatus* and *M. notatus* were reared from infested pine logs harvested from Heiberg Forest, Tully, NY, in April 2011. Four standing dead white pines (*Pinus strobus* L.) exhibiting signs of pine sawyer larval activity (frass and galleries) were felled and infested sections were identified and cut into 1-m bolts for transport to rearing facilities at the State University of New York, College of Environmental Science and Forestry in Syracuse, NY. The ends of infested bolts were sealed with paraffin wax, and bolts were placed in rearing containers at 20–24°C with a continuous 24-h light photoperiod. In total, rearing efforts yielded 14 *M. s. scutellatus* (five females, nine males) and 67 *M. notatus* (38 females and 29 males). Beetles were collected daily, separated by sex, and single-sex cohorts were held under laboratory conditions (air temperature 20–24°C) in glass jars and provided fresh white pine twigs and foliage. Because so few *M. s. scutellatus* were reared from bolts, flight traps (described above) were baited with monochamol and α-pinene and deployed at Heiberg Forest in June 2011 to collect more live beetles. As beetles were caught, they were brought back to the laboratory and held as described above.

Nine male and nine female *M. s. scutellatus*, and 14 male and 13 female *M. notatus* were aerated to collect beetle-produced volatiles. Individual beetles were aerated separately in 500-ml Erlenmeyer flasks fitted with inlet and outlet tubes, and each insect was provided with pine foliage as food. Control aerations, with chambers containing only pine foliage, were run in parallel with aerations of beetles. Charcoal-filtered air was drawn through each chamber at 500 ml/min, and headspace volatiles were trapped on Porapak Q (200 mg, 80–100 mesh; Supelco Inc., Bellefonte PA) held between glass wool plugs in a glass tube. Trapped volatiles were eluted with dichloromethane and the resulting extracts were analyzed in splitless mode with a 6890N gas chromatograph ([GC]; Agilent, Wilmington, DE) fitted with a DB-5 column (30 m × 0.25 mm i.d., 0.25-μm film; J&W Scientific, Folsom CA), interfaced to a 5975C mass selective detector (Agilent). The GC was temperature programmed from 40°C/1 min and then 10°C/min to 280°C for 20 min. Monochamol in samples (see Results) was identified by

Table 1. Number of adult male and female beetles of two *Monochamus* species captured in flight intercept traps baited with known cerambycid pheromones at five sites in New York state

	M. s. scutellatus		*M. notatus*	
	Males	Females	Males	Females
Site				
Allegany	5	18	2	4
Heiberg	29	33	1	6
Lafayette	0	1	1	5
Wanakena	34	75	0	0
Wisner	5	9	0	1
Lure				
Monochamol	62	118	3	11
3-Hydroxyhexan-2-one	7	9	0	2
(*R**,*R**)-2,3-Hexanediol	0	1	0	0
(*R**,*S**)-2,3-Hexanediol	3	6	1	1
Fuscumol	0	0	0	0
Fuscumol acetate	1	2	0	1
Solvent control	0	0	0	0
Blank control	0	0	0	1
Totals	73	136	4	16

matching its retention time and mass spectrum to those of an authentic standard.

Extracts in which monochamol was detected were reanalyzed by coupled gas chromatography-electroantennography (GC-EAD) to confirm its function as a pheromone, using live *M. s. scutellatus* shipped to University of California–Riverside for this purpose (USDA–APHIS permit P526P-09-01886). The GC-EAD apparatus and analysis conditions were as described in Ray et al. (2012).

Differences between treatments in mean trap capture, blocked by site and date, were tested separately for *M. s. scutellatus* and *M. notatus* by using the nonparametric Friedman's test (PROC FREQ, option CMH; SAS Institute 2001). Differences between pairs of means were tested with the REGWQ means-separation test which controls for maximum experimentwise error rates (PROC GLM; SAS Institute, 2001). We included in each analysis only replicates that had a minimum number of specimens so as to ensure sufficient replication for a robust analysis (at least six and one beetles per replicate for *M. s. scutellatus* and *M. notatus*, respectively). We also tested for deviations from a 1:1 sex ratio by using the chi-square test. Samples were lost on two separate sampling dates at the Wisner site and one date at Wanakena due to traps being damaged by bears.

Results

In total, 209 *M. s. scutellatus* and 20 *M. notatus* were captured during the experiment (Table 1). Traps baited with monochamol captured >6 times as many adult *M. s. scutellatus* as the remaining treatments (Fig. 1), which did not differ significantly from one another (Friedman's $Q_{7,87} = 53.2$, $P < 0.0001$). The sex ratio of *M. s. scutellatus* caught in the monochamol treatment was significantly female-biased (118 females, 62 males; $\chi^2 = 17.4$, $P < 0.0001$; Table 1). Even though many fewer *M. notatus* were captured during the study, the mean for the monochamol treatment was significantly greater than that for the other treatments (Friedman's $Q_{7,100} = 26.1$, $P = 0.0005$), and the sex ratio of *M. notatus* attracted to the monochamol treatment again was significantly female-biased (11 females, three males; $\chi^2 = 4.57$, $P = 0.033$; Table 1).

Five of the nine male *M. s. scutellatus* that were aerated produced detectable amounts of monochamol, whereas this compound was not detected in any headspace collections from the nine females. In GC-EAD analyses of extracts (Fig. 2), and a monochamol standard (Fig. 3), antennae of both sexes of *M. s.*

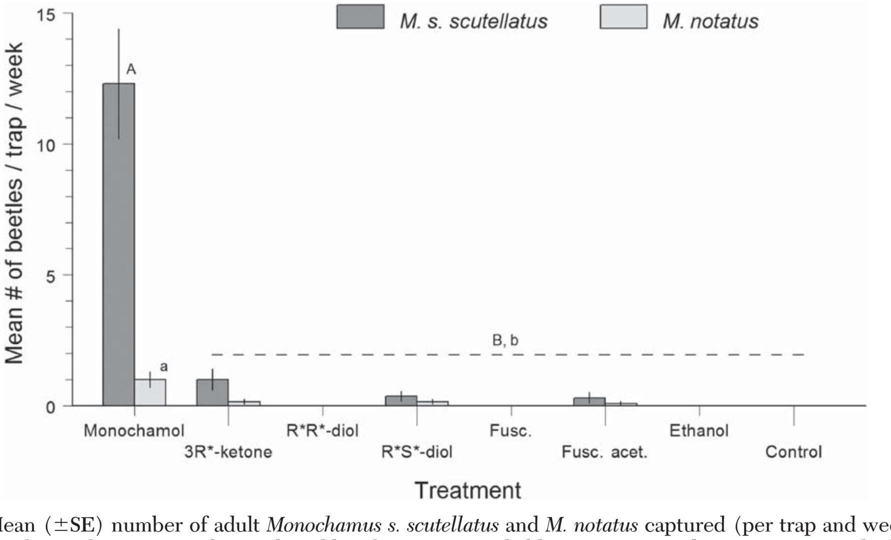

Fig. 1. Mean (±SE) number of adult *Monochamus s. scutellatus* and *M. notatus* captured (per trap and week) in traps baited with synthetic pheromones of cerambycid beetles. Compound abbreviations: 3R*-ketone, racemic 3-hydroxyhexan-2-one; R*R*-diol, (2*R**,3*R**)-2,3-hexanediol; R*S*-diol, (2*R**,3*S**)-2,3-hexanediol; Fusc., (*E*/*Z*)-fuscumol; Fusc. acet., (*E*/*Z*)-fuscumol acetate; ethanol, solvent control; and Control, unbaited trap. Means with different letters within species are significantly different (Ryan-Einot-Gabriel-Welsch Q means separation test, $P < 0.05$).

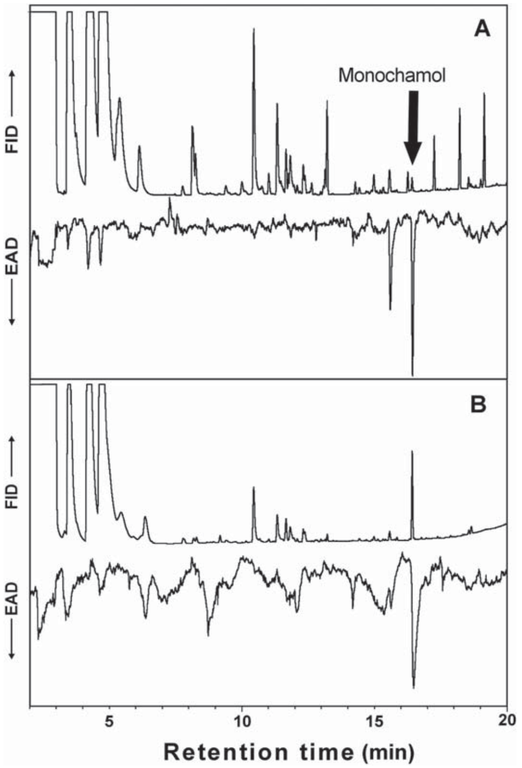

Fig. 2. Representative coupled GC-EAD analyses of aeration extracts of male *Monochamus s. scutellatus*, by using antennae from a male (A) and a female (B). Within each part, top trace shows GC chromatogram and bottom, inverted trace shows antennal response. Broad arrow indicates monochamol peak. Analyses were carried out on a DB-WAX GC column.

scutellatus responded strongly to this compound. In addition, antennae of males responded quite strongly to an earlier eluting compound (Fig. 2), tentatively identified as a sesquiterpene alcohol on the basis of its mass spectrum. However, because this compound was present in aeration extracts of both sexes and of white pine controls, it is almost certainly a pine volatile rather than being of insect origin. The identity of monochamol in the extracts was confirmed by matching its retention time and mass spectrum (Fig. 4) to that of an authentic standard of monochamol. Monochamol was not detected in headspace collections of either sex of the 27 *M. notatus.*

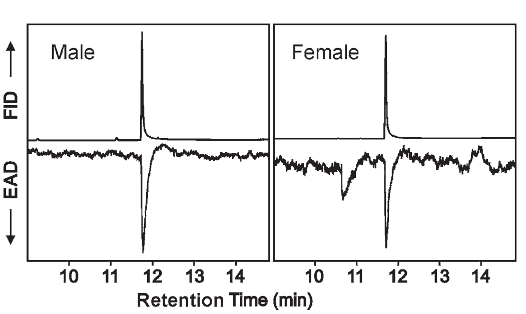

Fig. 3. Representative coupled GC-EAD analyses of an authentic standard of monochamol, by using antennae from a male (A) and a female (B) *Monochamus s. scutellatus*. Within each part, top trace shows GC chromatogram and bottom, inverted trace shows antennal response. Analyses were carried out on a DB-WAX GC column.

Discussion

A detailed review of cerambycid semiochemistry in 2004 listed fewer than 10 volatile pheromones from the >30,000 described cerambycid species (Allison et al. 2004), but since then, a rapidly accumulating body of evidence suggests that volatile pheromones are probably widespread within the family Cerambycidae. The results reported here add to that body of evidence: our field and analytical data provide strong evidence that monochamol is a male-produced pheromone component for *M. s. scutellatus.* Our field trapping data also suggested that monochamol is likely to be a pheromone component for the congeneric *M. notatus.* Taken together, our results provide support for the hypothesis that monochamol is conserved as a male-produced pheromone within the genus *Monochamus*, in parallel with recent findings that a number of other known cerambycid pheromone components, such as 3-hydroxyhexan-2-one, isomers of 2,3-hexanediols and 2,3-octanediols, fuscumol, fuscumol acetate, and 3,5-dimethyldodecanoic acid, are also highly conserved pheromone structures. There are now numerous examples of the same pheromone compound being used by congeners, by more distantly related species in the same tribe or subfamily, and even by species in different subfamilies (Hanks et al. 2007, Lacey et al. 2009, Barbour et al. 2011, Mitchell et al. 2011).

Relatively few *M. notatus* were collected in baited traps compared with *M. s. scutellatus.* Although a larger ratio of *M. notatus* emerged from infested white pine bolts reared in the laboratory compared with *M. s. scutellatus* (≈5:1), this pattern of emergence data would have to be confirmed from more trees and at more field sites to ascertain relative densities of these species in the landscape. The emergence ratio documented between the two species from infested trees was similar to numbers of *M. notatus* versus *M. s. scutellatus* counted on 12 white and 12 red pine (*Pinus resinosa* Sol. ex Aiton) trap trees (≈6:1) in northern Ontario, Canada (Gardiner 1957). Knowing the relative ratios of the two species would help to determine whether *M. s. scutellatus* is more strongly attracted to monochamol than *M. notatus.*

Although the sex ratios of adult cerambycids found on larval hosts are often male biased (Hanks 1999), those numbers may not reflect the sex ratio of beetles that actually emerged. Brodie (2008) found female:male sex ratios of 1.7:1 when rearing *M. s. scutellatus* out of infested red pine from central New York, which was similar to our data (1.8:1) from infested white pine from Heiberg Forest in central New York. Female-to-male sex ratios in trap catches for *M. s. scutellatus*

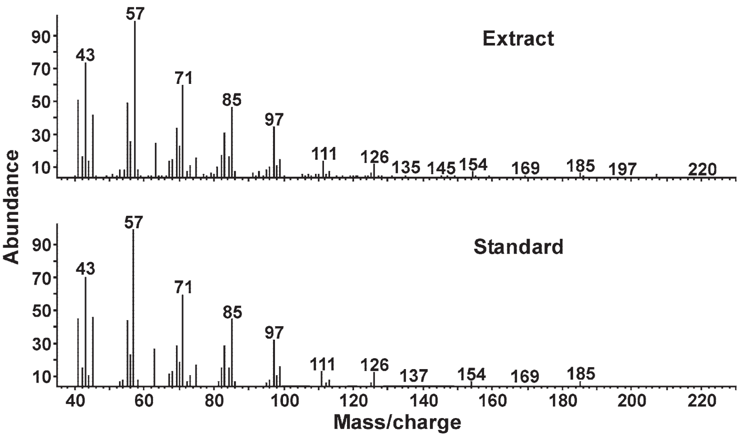

Fig. 4. Electron impact ionization (70-eV) mass spectra of monochamol in a headspace extract from male *Monochamus s. scutellatus* (top) and an authentic standard (bottom).

(≈2:1) were similar to rearing ratios. In contrast, our rearing data indicated a female:male ratio of ≈1:1 for *M. notatus* from infested bolts [as did rearing by Hodgdon (1957)], whereas trap catches were female biased (4:1), indicating females may be more strongly attracted to monochamol than males.

It is likely that attraction of both of our study species to monochamol could be significantly improved by combining monochamol with host plant compounds such as ethanol and α-pinene. In particular, these and other host plant compounds have been shown to be somewhat attractive to *Monochamus* spp. in the absence of monochamol (see Introduction). Host plant volatiles also have been shown to synergize attraction of the congeners *M. galloprovincialis* (Pajares et al. 2010) and *M. alternatus* (Teale et al. 2011) to monochamol. We currently are investigating the effects of combining host plant volatiles with monochamol for attraction of *M. s. scutellatus* and *M. notatus.*

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