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IDENTIFICATION OF THE FEMALE-PRODUCED SEX PHEROMONE OF THE SCARAB BEETLE, *Hoplia equina*

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Abstract—*Hoplia equina* LeConte (Coleoptera: Scarabaeidae: Melolonthinae) is a beetle pest of cranberry beds in Massachusetts. Larvae feed on the roots of the cranberry plant, reducing yield as well as vine density. The female sex pheromone was identified as 2-tetradecanone. There were eight compounds found in the airborne volatiles collected from females that elicited antennal responses from males. Of the eight compounds tested (nonanal, decanal, dodecanal, 2-dodecanone, 2-tridecanone, 2-tetradecanone, 2-pentadecanone, and 2-hexadecanone), 2-tetradecanone was the only one that attracted male beetles in the field. Combining any of the other seven antennally active compounds with 2-tetradecanone did not increase male capture.

Key Words—Ketone, 2-tetradecanone, cranberries, electroantennogram.

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INTRODUCTION

Five species of scarab beetles have been recorded as pests in cranberry beds in Massachusetts. They include the cranberry white grub, *Phyllophaga anxia* (LeConte), the cranberry root grub, *Lichnanthe vulpina* (Hentz), the oriental beetle, *Anomala orientalis* (Waterhouse), the Japanese beetle *Popillia japonica* Newman, and *Hoplia equina* LeConte, a small (6- to 8-mm long), native, melolonthine species for which there is no common name. Larvae of the cranberry white grub and the cranberry root grub have a long history as cranberry root feeding pests in Massachusetts (Franklin, 1950). Japanese beetle grubs are regarded as minor pests of cranberry beds, almost always being found in weedy areas of the beds (Dunn and Averill, 1996), although an authenticated damaging population has been noted (P.S.R. and D.C.W., personal observations). *Hoplia* grubs had never been observed as cranberry root pests until an employee of Ocean Spray Cranberries, Inc., discovered them causing feeding damage in a cranberry bed in 1990. The adults reared from those larvae were originally identified as *Hoplia modesta* Haldeman, but were correctly identified as *H. equina* in 2001 by P.S.R. Every beetle examined by P.S.R. (unpublished observations) from a number of bogs from collections made over several years was found to be *H. equina*.

The last revision of the genus *Hoplia* indicates 12 species in America north of Mexico (Hardy, 1977). Hardy (1977) reported the range of *H. equina* as from Maryland and the eastern portion of Pennsylvania through Delaware and New Jersey to Connecticut, Massachusetts, and New Hampshire. The oriental beetle and *H. equina* (*modesta*) were first reported as new and important cranberry pests by Dunn and Averill (1996). Surveys of Massachusetts cranberry beds in 1994 and 1995 indicated that in those sampled beds where grub species were present, *H. equina* was found in 23% (5/22) in 1994 and in 30% (11/33) in 1995. Aside from the summer flood and total bog renovation, there are no recommended methods of control for any cranberry feeding scarab beetle larvae. Dunn and Averill (1997) recorded behavior indicating the presence of a female-produced sex pheromone in the mating system of *H. equina* and also demonstrated that this species is diurnal and has a 2-year life cycle.

METHODS AND MATERIALS

Pheromone Collections. Third-instar of *H. equina* were collected by digging from an infested cranberry bog in Rochester, Massachusetts, in late April 1999. Recovered larvae were housed individually in ~30-ml plastic cups in a 3:1 mix of greenhouse sand and screened peat moss raised to about 12% moisture in a controlled environment room at 25°C during the 16-hr photophase and 20°C during the 8-hr scotophase. After pupation and adult emergence, individuals were separated

by sex and about 100 females were placed into an all-glass collection vessel. During photophase, pump-drawn air was filtered through charcoal, bubbled through distilled water, passed over and among the females, and finally through a glass tube filled with adsorbent Super Q polymer material (Alltech, Deerfield, IL). Captured volatiles were eluted from the Super Q using about 2 ml of dichloromethane and then condensed under a nitrogen stream to a volume of about 20 μl.

Electrophysiological Recordings. A Hewlett Packard 5890 Series II plus gas chromatograph equipped with a polar DB-WAXetr capillary column (60 m $\times$ 0.25 mm ID, 0.25-mm film thickness, J&W Scientific, Folsom, CA) in the splitless mode was used for coupled gas chromatographic–electroantennographic detection (GC-EAD) analyses (Zhang et al., 1997). The oven temperature was programmed at 80°C for 2 min, then raised 10°C/min to 250°C, and held for 15 min. Injector and flame ionization detector (FID) temperatures were 260°C. Nitrogen was the carrier gas with a flow rate of 2.6 ml/min and inlet pressure was set at 276 kPa. An antenna plucked from a beetle was placed tip and scape between two gold wire electrodes immersed in saline-filled wells in a small acrylic holding station so that the three lamellae of the antennal club were spread. The gold wire output recording electrodes were connected by a short coaxial cable to a high-impedance 1:300 amplifier with automatic baseline drift compensation. The temperature of the antennae on the acrylic station was maintained at about 5°C by flushing 0°C water from a benchtop refrigerated circulator (RTE-100, NESLAB Instruments, Inc., Portsmouth, NH) through the insulated layer of the modified condenser containing the acrylic holding station mounted on top of the GC. An HP dual channel ChemServer/ChemStation A/D interface, 35900E, was used for the EAD recordings.

GC–mass spectrometry (GC-MS) was carried out with a Hewlett Packard 6890 gas chromatograph coupled to an HP 5973 Mass Selective Detector using the same 60-m DB-WAXetr or 60-m DB-5 capillary column and conditions as above, but with helium as the carrier gas.

Chemicals. All synthetic aldehydes, ketones, and the butylated hydroxytoluene (BHT) were purchased from Lancaster Synthesis, Inc. (Windham, NH), or Aldrich (Milwaukee, WI). Purities were $>$98% based on GC analysis. The 2-tetradecanone was synthesized from the corresponding alcohol by PCC (pyridinium chlorochromate) oxidation (Corey and Suggs, 1975).

Preparation and Field Evaluation of Synthetic Lures. EAD active components were tested on rubber septa in the field in 1999, 2001, and 2002. Lures were formulated by dissolving the neat compounds in hexane, dispensing appropriate amounts into 5-mm rubber stopper septa (Thomas Scientific, Swedesboro, NJ), and allowing the hexane to evaporate in a fume hood. Lures were deployed in the field in lab-constructed cross-vane traps. The bottom of the trap was hung about 50 cm from the ground. Traps were placed at about 20-m intervals. Trap placement was randomized at initial deployment.

TABLE 1. RELATIVE PERCENTAGES OF THE EAD-ACTIVE COMPOUNDS FOUND IN THE AIRBORNE VOLATILES COLLECTED FROM FEMALES OF *Hoplia equina*, AS DETERMINED BY GAS CHROMATOGRAPHY

Compound	Percentage
Nonanal	27.7
Decanal	21.4
Dodecanal	10.0
2-Dodecanone	2.1
2-Tridecanone	1.7
2-Tetradecanone	24.0
2-Pentadecanone	4.0
2-Hexadecanone	9.1

In 1999, the nine treatments included each of the five ketones tested alone at 300-μg/septa, a blend of the five ketones formulated in the ratio determined from the captured volatiles (Table 1) tested at 100 μg/septa and 300 μg/septa, 2-tetradecanone and 2-hexadecanone combined in the exact amounts found in the 300-μg mix, and a solvent control. One replicate of the nine treatments was deployed at each of four Massachusetts cranberry bogs during June and July. Traps were checked and emptied two to three times each week and rerandomized each time they were checked.

In 2001, the seven treatments included 2-tetradecanone at 300 μg/septum, the 300 μg/septum mix of the five ketones as above, the ketone mix + nonanal, the ketone mix + decanal, the ketone mix + dodecanal, the ketone mixture + all three aldehydes, and a solvent control. The aldehydes were added to the 300 μg of five ketone mixtures according to the percentages in Table 1. BHT was added (0.5%) to these lure mixes to slow trimerization of aldehydes. Six replicates of the treatments were tested during June and July at a cranberry bog that was infested with *H. equina*. Trap catch was recorded nine times and traps were rerandomized twice.

In 2002, three treatments were deployed including 2-tetradecanone alone at both 300 μg/septa and 176 μg/septa, and the mix of the five ketones together at 300 μg/septa. Four replicates of the three treatments were tested during June and July at an infested Massachusetts bog. Traps were checked three times and rerandomized each time.

Statistics. Numbers of beetles caught in the field in the various treatments were analyzed using ANOVA and Fisher's LSD test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Pheromone Identification. Using the GC-EAD system, seven EAD active compounds ($t_R = 8.60$, 9.94, 12.49, 13.58, 14.72, 15.89, 16.83 min) were

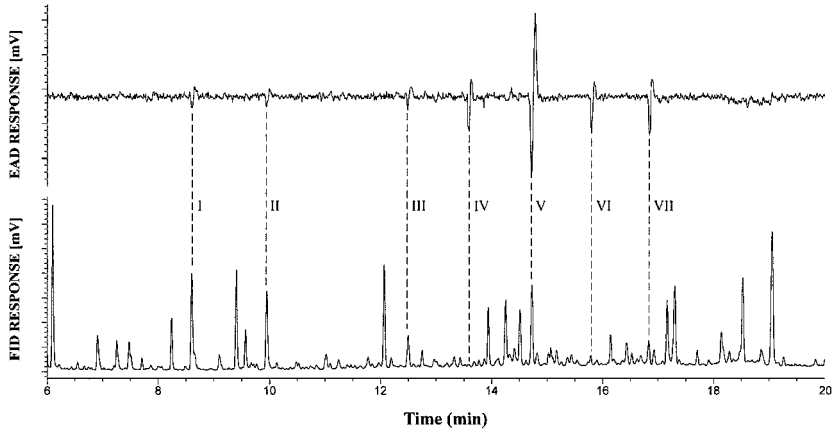

FIG. 1. Simultaneous responses of flame ionization detection (FID) and electroantennographic detection (EAD) of an adult male *Hoplia equina* antenna stimulated with female volatiles on a 60-m DB-WAXetr capillary column. I – nonanal. II – decanal, III – dodecanal and 2 – dodecanone (see text), IV – 2-tridecanone; V – 2-tetradecanone; VI – 2-pentadecanone; VII – 2-hexadecanone.

revealed using male antennae stimulated with female volatiles (Figure 1). These same volatiles did not elicit any significant response from female antennae. The EAD active compounds with peak numbers as in Figure 1 and relative ratios as in Table 1 were identified as nonanal (I), decanal (II), dodecanal (III), 2-tridecanone (IV), 2-tetradecanone (V), 2-pentadecanone (VI), and 2-hexadecanone (VII) by comparison of mass spectra and GC retention times on both polar and nonpolar capillary columns with synthetic standards. When a selected ion monitoring method (m/z 184, 198, 212, 226, and 240) was used to check the series of 2-ketones in female airborne extract, a tiny amount of 2-dodecanone was found to elute just before the third EAD active peak at $t_R = 12.35$) under the GC conditions used.

Field Evaluation of Synthetic Lures. No female beetles were found in the traps during the 3 years of field testing of the various compounds. In 1999, 2-tetradecanone at 300 μg was the only single compound to catch more males than the control (Figure 2). Septa loaded with the 300-μg blend of the five ketones attracted more beetles than did the 100-μg blend. There were no differences among captures in traps baited with 300 μg of the 2-tetradecanone alone, the 300-μg blend of all five ketones, and the blend of 2-tetradecanone and 2-hexadecanone. Traps baited with the 100-μg ketone blend did not capture significantly more male beetles than the solvent baited control traps (Figure 2).

In 2001, traps baited with 300 μg of 2-tetradecanone alone caught more beetles than the 300-μg five ketone blend (containing 176 μg of 2-tetradecanone).

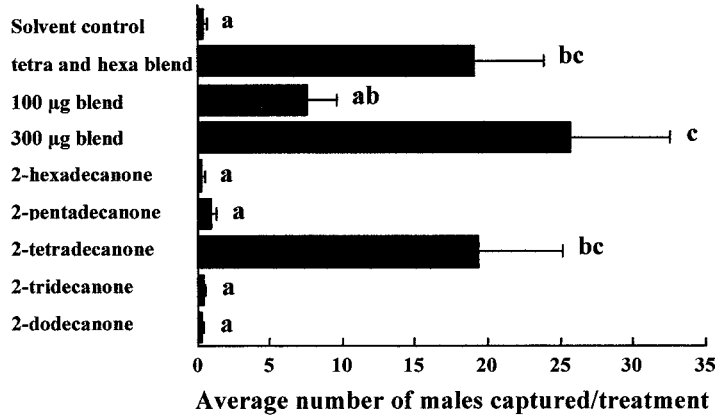

FIG. 2. Mean number of male *Hoplia equina* beetles caught/treatment in 1999. Bars with the same letters are not significantly different, $P < 0.05$, Fisher's LSD test. Total male beetles trapped = 1261.

Addition of any of the aldehydes to the 300-μg blend of ketones, alone or in combination, resulted in a trend towards reduced trap catches (Figure 3). The data from this test raised the question of whether the increased capture in the 300-μg dose of 2-tetradecanone resulted a dose response to the smaller amount of 2-tetradecanone in the 300-μg blend of ketones. However, in 2002, there were no significant differences among the three treatments of 300 μg of 2-tetradecanone alone, 176 μg

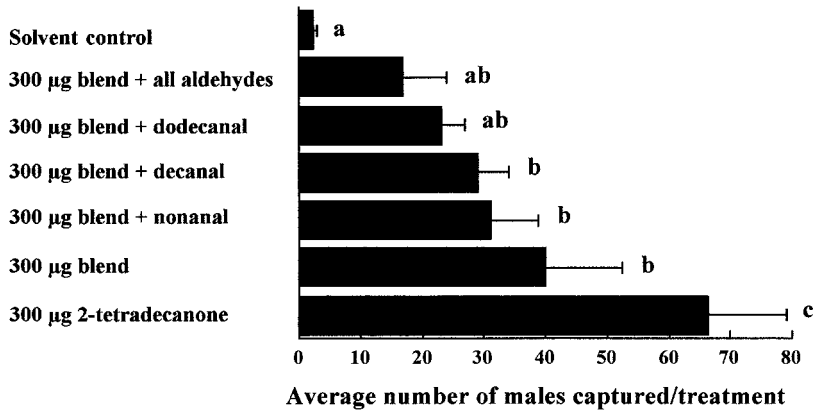

FIG. 3. Mean number of male *Hoplia equina* beetles caught/treatment in 2001. Bars with the same letters are not significantly different, $p < 0.05$, Fisher's LSD test. Total beetles trapped = 2505.

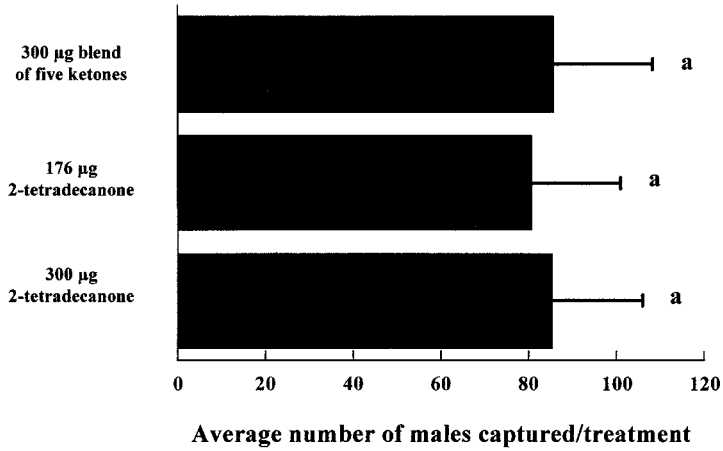

FIG. 4. Mean number of male *Hoplia equina* beetles caught/treatment in 2002. Bars with the same letters are not significantly different, $p < .05$, Fisher's LSD test. Total beetles trapped = 3020.

of 2-tetradecanone alone, and the 300-μg five ketone blend (containing 176 μg of 2-tetradecanone). Furthermore, addition of other EAD active ketone compounds to 2-tetradecanone did not result in increased male catches relative to 2-tetradecanone alone (Figure 4).

In three seasons of field testing, addition of any of the other EAD active components, either alone or in combinations, to 2-tetradecanone, did not result in increased male capture rates relative to 2-tetradecanone alone. We conclude that 2-tetradecanone is the sex pheromone of *H. equina*.

This is the second report of a ketone employed as the sex pheromone of a scarab beetle. Two similar ketones, 7-(Z)- and 7-(E)-2-tetradecanone, constitute the sex pheromone of *Anomala orientalis*, the oriental beetle (Leal, 1993; Zhang et al., 1994). Other known melolonthine sex pheromones include phenol (Henzell and Lowe, 1970), L-isoleucine and linalool (Leal et al., 1992), anisole (Leal et al., 1996a; Ward et al., 2002), (R,Z)-7,15-hexadecadien-4-olide (Leal et al., 1996b), (Z,E)-α-farnesene (Yarden et al., 1996), and a combination of L-isoleucine and L-valine (Zhang et al., 1997) (for a discussion of the sex pheromones of other scarab beetles, see Leal, 1998).

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