

ROLE OF HOST PLANT VOLATILES IN MATE LOCATION FOR THREE SPECIES OF LONGHORNED BEETLES

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Abstract—Stressed woody plants represent an ephemeral and unpredictable resource for larvae of some species of longhorned beetles (Coleoptera: Cerambycidae) because prime subcortical tissues are rapidly degraded by a guild of xylophagous competitors. Selection favors efficient mechanisms of host and mate location to expedite colonization of hosts by larvae. Based on previous research, we hypothesize that mate location in some species of the subfamily Cerambycinae involves three sequential behavioral stages: (1) both sexes are attracted to larval hosts by plant volatiles; (2) males attract females over shorter distances with pheromones; and (3) males recognize females by contact pheromones in their epicuticular wax layer. We already have evidence of second-stage and third-stage behaviors in three species in this subfamily whose xylophagous larvae feed in hardwood trees: *Xylotrechus colonus*, *Megacyllene caryae*, and *Neoclytus mucronatus mucronatus*. In this report, we evaluate the first behavioral stage of mate location behavior (i.e., independent response of both sexes to host plant volatiles) for the same three species. Supporting our hypothesis, both males and females responded to volatiles emanating from hickory logs in Y-tube olfactometer bioassays.

Key Words—Host location, mating behavior, pheromone, Cerambycidae, *Xylotrechus*, *Megacyllene*, *Neoclytus*.

INTRODUCTION

A better understanding of host and mate location is critical for developing management strategies of longhorned beetles (Coleoptera: Cerambycidae), among the most important pests of woody plants in natural and managed systems world wide

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(Hanks, 1999). Disparate studies of many species in this family have suggested that the sexes are brought together by a mutual attraction to the larval host rather than by long-range pheromones (reviewed by Hanks, 1999). Recent research on several species in the subfamily Cerambycinae, however, has revealed that males produce pheromones that operate over short or moderate distances (reviewed by Lacey et al., 2004). The pheromones are comprised of single or multiple compounds based on a common structural motif: straight chains of six, eight, or ten carbons with hydroxyl or carbonyl groups at C₂ and C₃.

We have documented the first aggregation pheromone in a longhorned beetle, that of the cerambycine species *Neoclytus acuminatus acuminatus* (F.) (Lacey et al., 2004), and the structure of the single active component conforms to this structural motif. Nevertheless, both sexes of *N. a. acuminatus* also are attracted in the field by volatiles emanating from larval hosts, dying hardwoods (Lacey et al., 2004). Adults of other cerambycine species also respond to plant volatiles (e.g., Hanks et al., 1996; Fetzkoether et al., 2000), and males use their antennae to recognize females by contact chemoreception of cuticular hydrocarbons (Ginzel and Hanks, 2003; Ginzel et al., 2003). We, therefore, hypothesize that mate location and recognition in cerambycine species involves three sequential behavioral stages: (1) both sexes are independently attracted to larval hosts by plant volatiles; (2) males attract females over shorter distances with pheromones; and (3) males recognize females by contact pheromones.

In this paper, we evaluate the first stage of mate location in three species of cerambycines by testing the hypothesis that adult males and females are independently attracted to volatiles produced by larval hosts. We have evidence of second-stage behaviors in two species: male *Xylotrechus colonus* F. and *Megacyllene caryae* (Gahan) produce compounds consistent with the pheromone structural motif, and we are evaluating their activity (E. S. Lacey, J. G. Millar, L. M. Hanks, unpublished data). We predict that males of the third species, *Neoclytus mucronatus mucronatus* (F.), also produce pheromones because it is a congener of *N. a. acuminatus* and the males display a “perching” behavior that is associated with release of pheromones in that species (Lacey et al., 2004). We have documented the third-stage behaviors for all three species: males use their antennae to recognize females by contact chemoreception (Ginzel and Hanks, 2003; Ginzel et al., 2003).

All three study species are native to eastern North America and their larvae commonly feed in stressed or moribund hickories (Linsley, 1964). Both sexes of all three species congregate on freshly cut logs of hickory in the area of the study (Ginzel et al., 2003; unpub. data). This behavior could be cued by host plant volatiles or entirely by long-range pheromones. Larvae of *X. colonus* develop in many species of hardwoods, and the crepuscular adults are active from May to September (Linsley, 1964; Ginzel et al., 2003). Larval hosts of *M. caryae* include several species of hardwoods, but especially hickory, and adults are diurnal, aposematically-colored wasp mimics that are active from April to June

(Linsley, 1964). Larvae of *N. m. mucronatus* develop in hickories, and the adults are crepuscular and nocturnal and active in June and July (Linsley, 1964).

METHODS AND MATERIALS

We collected adult beetles of all three species from felled shagbark hickories, *Carya glabra* (Mill) Sweet, or reared them from the logs. Adult *X. colonus* were collected at dusk from trees felled in May and June 1998 at Allerton Park, Piatt County, IL. Adult *M. caryae* emerged in March–April 2001 from logs of a tree felled in Athens County, OH, in Spring 2000. Adult *N. m. mucronatus* emerged in May 2003 from logs of a tree felled at Allerton Park in June 2002.

We housed beetles individually in the laboratory in cylindrical cages of aluminum window screen (9 cm diam, 12 cm tall) with clean 9-cm glass Petri dishes at top and bottom. Every 2–3 days we provided fresh 10% sucrose solution in a glass vial into which was inserted a cotton dental roll (Patterson Dental, South Edina, MN). Reared beetles could have mated with a few individuals before they were caged, but the mating history of field-captured beetles was unknown. Beetles used in bioassays had been isolated in cages for at least 24 hr and were active.

We measured the response of walking adult beetles to volatiles from logs of shagbark hickory with a glass Y-tube olfactometer (6 cm diam, main tube 26 cm long, arm length 22 cm, angle between arms 70°) positioned on a table with the arms directed toward north-facing windows. Bioassays were conducted under laboratory conditions and natural light during the normal activity periods of the species: late afternoon and evening (*X. colonus* and *N. mucronatus*) or morning (*M. caryae*). To bioassay *X. colonus*, we placed a freshly cut hickory log (~15 cm diam, 30 cm long) in a Plexiglas® box (30 × 30 × 120 cm tall) with the open bottom sealed by standing it in ~10 cm of water. Air entered the box through a hose connector at the top, and air was drawn from the box through another hose connector on the opposite side of the top that was connected with 1 cm i.d. Teflon® tubing to one arm of the Y-tube. An empty box of identical design was connected to the other arm of the Y-tube as a control. To bioassay *M. caryae* and *N. mucronatus*, we placed a hickory log (~8 cm diam, 15 cm long) in a plastic cylinder (10 cm diam, 20 cm long) sealed at one end with aluminum foil with a ~3 cm diam hole to allow air to enter and the other end connected with Teflon® tubing to one arm of the Y-tube. An identical cylinder containing moistened cotton dental rolls served as a control. Air was drawn through these systems (~1.7 l/min) with a 1 hp vacuum cleaner (Shop-vac®, Williamsport, PA) on a variable power supply, and entering air was purified with ~450 g of activated charcoal.

Odor sources were randomized between arms of the olfactometer for each bioassay to control for location effects, and the olfactometer was rinsed with acetone and air dried between trials. A beetle was released in the olfactometer at the downwind end of the main tube and responded by walking at least

TABLE 1. PERCENTAGES OF FEMALE AND MALE LONGHORN BEETLES OF THREE SPECIES THAT RESPONDED IN AN OLFACTOMETER TO VOLATILES EMANATING FROM HICKORY LOGS VERSUS A BLANK CONTROL

Species	Sex	N	# Responding	% Responding		χ^2 statistic (<i>P</i>)
				Hickory	Control	
<i>Xylotrechus colonus</i>	Female	36	30	77	23	8.53 (<i>P</i> < 0.01)
	Male	34	30	80	20	10.8 (<i>P</i> < 0.01)
<i>Megacyllene caryae</i>	Female	36	30	80	20	10.8 (<i>P</i> < 0.01)
	Male	40	30	73	27	6.53 (<i>P</i> < 0.01)
<i>Neoclytus m. mucronatus</i>	Female	25	25	80	20	9.0 (<i>P</i> < 0.01)
	Male	30	30	83	27	13.3 (<i>P</i> < 0.001)

16 cm down one of the arms. Beetles that did not enter either arm of the olfactometer within 15 min were recorded as "no response." We tested 25–40 males and females of each species and compared percentages of beetles responding to hickory volatiles with percentages responding to the control with a χ^2 goodness-of-fit test.

RESULTS AND DISCUSSION

Adult beetles of all three species showed a significant response to plant volatiles, with an average of ~80% of each sex responding to volatiles produced by hickory logs (Table 1). These findings support the hypothesis that the sexes are brought together by a mutual attraction to volatiles of the larval host. These data also lend support to our proposed three-stage behavioral sequence of mate location.

Stressed and dying host trees become available to wood borers sporadically and unpredictably when they are damaged or weakened by such environmental factors as wind, lightning strike, fire, and water deficit (reviewed by Hanks, 1999). Their quality as hosts declines rapidly, however, as subcortical tissues are degraded by xylophagous competitors, including buprestid and scolytid beetles, as well as by other cerambycid species. Thus, the quality of larval nutrition depends on the timing of colonization by larvae, and selection favors behaviors in adults that expedite that process, including mutual attraction of males and females to the larval host, and brief copulation followed immediately by oviposition. Adults of other species of the Cerambycinae that require stressed hardwood hosts show similar reproductive behaviors, presumably due to convergent selective pressures (Hanks, 1999).

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