Attraction of the Bark Beetle Parasitoid *Roptrocerus xylophagorum* (Hymenoptera: Pteromalidae) to Host-Associated Olfactory Cues

BRIAN T. SULLIVAN,[1] EVA M. PETTERSSON,[2] KATJA C. SELTMANN, AND C. WAYNE BERISFORD[3]

Environ. Entomol. 29(6): 1136-1151 (2000)

ABSTRACT Studies were conducted to identify host location cues used by *Roptrocerus xylophagorum* (Ratzeburg), a larval/pupal parasitoid of bark beetles. In Y-tube olfactometer bioassays, female *xylophagorum* were attracted to infested bark (i.e., phloem, cambium, and outer corky bark tissues) removed from bolts of loblolly pine, *Pinus taeda* L., colonized by the late instar larvae and pupae of the bark beetle *Ips grandicollis* Eichhoff (Coleoptera: Scolytidae). In contrast, bark taken from recently cut, uninfested bolts interrupted attraction to infested bark when these were presented together. Larval and pupal hosts isolated from infested bark were not attractive to parasitoids, whereas frass removed from the larval mines in infested bark was highly attractive. Bark from which hosts or both hosts and host frass were removed remained highly attractive. Bark sandwiches (fresh bark with the exposed surface pressed to glass microscope slides) infested with either third-instar or adult female *I. grandicollis* were attractive to female parasitoids, whereas bark sandwiches with only mechanical damage to the phloem tissue were unattractive. A steam distillate of bark infested with host larvae was attractive to female *R. xylophagorum*, whereas a distillate of fresh pine resin was not attractive. Volatiles from the experimental baits were collected on Porapak Q and analyzed by coupled gas chromatography-mass spectrometry. Several compounds were identified that distinguished baits with biological activity. These data show the importance of the complete host/plant complex for attraction of *R. xylophagorum* to its host's habitat and suggest a possible role for particular odors from uninfested host plant tissue in directing foraging parasitoids away from locations with few or no hosts.

KEY WORDS *Roptrocerus xylophagorum*, *Ips grandicollis*, Scolytidae, parasitoid host location, tritrophic interactions, semiochemicals

NUMEROUS STUDIES OF parasitoid-host interactions have demonstrated the role of semiochemicals in mediating host and host habitat location by foraging female parasitoids (Lewis et al. 1976; Vinson 1981, 1984; Tumlinson et al. 1992; Godfray 1994; Vet et al. 1990). These semiochemical cues may arise from the host itself (Rice 1969, Mitchell and Man 1970, Stemlicht 1973), its products (Weseloh 1977, Cloutier and Bauduin 1990, Ngi-Song and Oversholt 1997), its food (Martin et al. 1990, Ngi-Song et al. 1996, Takacs et al. 1997, DeMoraes and Mescher 1999), or organisms found in close association with the host (Madden 1968, Greany et al. 1977, Dicke 1988, Thibout et al. 1993).

Roptrocerus xylophagorum (Ratzeburg) is a larval/pupal idiobiont parasitoid of bark beetles (Coleoptera: Scolytidae) with an apparently holarctic distribution (Mills 1983, Samson 1984, Espelie et al. 1996). Its host range encompasses well over a dozen economically important bark beetle species in Europe and North America including the beetles *Ips typographus* (L.) and *Tomicus piniperda* (L.), and the southern pine beetle, *Dendroctonus frontalis* Zimmermann, the Douglas-fir beetle, *D. pseudotsugae* Hopkins, and the western pine beetle, *D. brevicomis* LeConte (Bushing 1965, Mills 1983). This parasitoid was established in Australia in the early 1980s as a potential biological control agent for the introduced *I. grandicollis* (Berisford and Dahlsten 1989). *R. xylophagorum* is among the most abundant parasitoid species found in association with bark beetle infestations (Berisford et al. 1971, Moser 1971, Stephen and Dahlsten 1976, Goyer and Finger 1980, Ohmart and Voigt 1982, Langor 1991, Gara et al. 1995).

Roptrocerus xylophagorum females parasitize hosts concealed in bark by entering the galleries excavated by adult bark beetles in the phloem tissue and drilling through the gallery walls with their ovipositors (Dix and Franklin 1981, Samson 1984). This parasitoid apparently uses semiochemical cues to locate susceptible hosts. Female *R. xylophagorum* in the field are strongly attracted to host-infested bolts and bark as well as distillates of host-infested bark; males show no such attraction (Sullivan 1997, Sullivan et al. 1997). In addition, there is evidence that semiochemicals play a role in the location of, and discrimination among, particular host species or host life stages by this para-

[1] Southern Research Station, USDA Forest Service, 2500 Shreveport Highway, Pineville, LA 71360.
[2] Department of Chemical Ecology, Göteborg University, Box 461, SE-405 30 Göteborg, Sweden.
[3] Department of Entomology, University of Georgia, Athens, GA 30602.

0046-225X/00/1138-1151$02.00/0

sitoid. *R. xylophagorum* arrive on bark beetle colonized trees and bolts in greatest numbers when susceptible host life stages (i.e., late instar larvae and pupae) are present (Berisford and Franklin 1969, Camors and Payne 1973, Stephen and Dahlsten 1976, Dixon and Payne 1979, Dix and Franklin 1981, Ohmart and Voigt 1982). In choice experiments, *R. xylophagorum* were attracted significantly more to bolts infested with the same bark beetle species on which they had been reared than to bolts infested with an alternative, acceptable host species (Kudon and Berisford 1980).

The purpose of this study was to identify the biological sources of the host and host habitat location cues of *R. xylophagorum* and make an initial assessment of variables that might affect the production of cues and thus the efficiency of host finding by this parasitoid. This study was undertaken as part of a larger effort to characterize and synthesize the host attractants for the parasitoids of the southern pine beetle, *Dendroctonus frontalis* Zimmermann (Sullivan et al. 1997).

Materials and Methods

Insect Cultures. Adult *R. xylophagorum* were obtained from a laboratory colony maintained at the University of Georgia on 1. *grandicollis*-infested bolts of loblolly pine, *Pinus taeda* L. (Sullivan et al. 1999). Adult parasitoids were collected daily as they emerged from host-infested bolts held inside a saran-screen cage. These newly emerged parasitoids were housed in mixed-sex groups in cotton-stoppered, 250 ml Erlenmeyer flasks packed loosely with Kimwipe (Kimberly-Clark, Roswell, GA) and provisioned with honey, water, and a commercial diet (Eliminade, Entopath, Easton, PA.). Within 1 d after parasitoid collection, the flasks were placed into a cold-temperature incubator at 8 ± 1°C and aphotoperiod of 14:10 (L:D) h. Flasks were removed from the incubator daily and allowed to warm to room temperature for ≈30 min to permit parasitoids to feed, take water, and mate.

One day before bioassays, 4- to 6-d-old, presumably mated female parasitoids were exposed to their host's microhabitat in a Plexiglas chamber (30 by 30 by 30 cm) enclosing pieces of bark infested with *I. grandicollis* larvae and pupae. Only those parasitoids that antennated larval frass or galleries and were arrested on the bark for >20 s were used in tests. This preconditioning was performed to improve overall parasitoid response rates to host-associated cues (Parra et al. 1996, Röse et al. 1998). After exposure to the host/plant complex, parasitoids were transferred to culture flasks prepared as above and maintained at 26 ± 1°C and a photoperiod of 14:10 (L:D) h until bioassay.

Olfactometer Design and Methodology. The anemotaxis of walking *R. xylophagorum* in response to various odor sources was tested in a Y-tube olfactometer similar in design and operation to that of Steinberg et al. (1992). Each arm of a Pyrex glass Y-tube (4 mm i.d.; stem 40 mm; arms 50 mm at a 135° angle to the stem) was attached with Teflon tubing to a sealed glass odor source chamber (internal volume 30 ml) supplied with charcoal-filtered and humidified air (≈50–70% BH). Airflow through each arm of the Y-tube was maintained at 4 cm/s (30 ml/min) by the positive pressure of an electric pump. Parasitoids were prevented from passing out of either arm of the Y-tube by a screen barrier made of a disk of white cotton cloth (120 ct) held in place over the openings of each Y-tube arm by a Teflon O-ring. During testing, the bioassay room was 26 ± 1°C and the only light source was a 15-W, red-filtered incandescent light bulb centered over the stem of the Y-tube. Although *R. xylophagorum* is a diurnal insect (Dix and Franklin 1981), use of the red-filtered light reduced the confounding effects of insect orientation to visual stimuli during bioassay trials. Trials were generally run between the sixth and twelfth hour of photophase.

Individual parasitoids released into the olfactometer stem were recorded as choosing a given arm of the Y-tube when they spent >15 s beyond a line positioned 3 cm from the Y-intersection. Parasitoids that failed to choose an arm in 5 min were recorded as nonresponders. The assignment of odor sources to each arm of the olfactometer was reversed after every trial to eliminate directional bias. Parasitoids were not reused, and Y-tubes were replaced with clean ones after each trial, Bark extract and resin extract baits were replaced after each trial but all other baits (i.e., bark, frass, and host insects) were exchanged with fresh material after the completion of each experimental block (two replicates of each bait combination within a given experiment). Between test days, Y-tubes and all other olfactometer components potentially exposed to bait volatiles were washed with detergent, rinsed with ethanol, and dried in an oven at 120°C for at least 1 h.

Bioassay Experiments. *R. xylophagorum* females were subjected to 20 binary choice tests (Table 1). These tests were grouped into eight blocked experiments consisting of up to four different such tests performed in equal numbers of trials on the same days using the same pool of test subjects. This was done to eliminate the potentially complicating effects of between-day variation in parasitoid responsiveness when comparing the results of component tests in an experiment. On each day of trials, the tests within an experiment group were performed consecutively in blocks of two to four replicates each, and the order of the tests was randomized.

Infested and Uninfested Bark. In experiments 1-3, parasitoids were assayed for their response to bark (i.e., phloem, cambium, and outer corky bark tissues) excised either from an uninfested pine bolt or from a bolt infested with susceptible hosts. The uninfested bark was excised from a bolt cut from a healthy loblolly pine felled in the previous 7 d and stored at -4°C before use. The infested bark was removed from a loblolly pine log colonized 2-3 wk earlier in the laboratory by *I. grandicollis* adults (Sullivan et al. 1999) and contained predominantly pupae and second and third instars. Bark for either treatment was excised <30 min before the experiment and had its origins in bolts (80 ± 20 cm by 15 ± 5 cm diameter) from at least

Table 1. Summarized details of individual treatment pairings in Y-tube olfactometer choice bioassays of female *R. xylophagorum*

Test	Parasitoids tested (n)	Arm 1	Arm 2
1.1	36	Cleanair	Excised bark (21 cm") from an uninfested pine bolt
1.2	36	Excised bark (21 cm^2) from a pine bolt infested 2-3 wk with *I. grandicollis* (late larval and pupal brood present)	Clean air
1.3	36	Excised bark (21 cm^2) from a pine bolt infested 2-3 wk with *I. grandicollis* (late larval and pupal brood present)	Excised bark (21 cm") from a" uninfested pine bolt
2	48	Excised bark (7 cm") from a pine bolt infested 2-3 wk with *I. grandicollis* (late larval and pupal brood present)	Excised bark (7 cm^2) from a pine bolt infested 2-3 wk with *I. grandicollis* (late larval and pupal brood present) and excised bark (7 cm") from a" uninfested loblolly pine bolt
3	42	Excised bark (7 cm^2) from a pine bolt infested 2-3 wk with *I. grandicollis* (late larval and pupal brood present)	Excised bark (14 cm^2) from a pine bolt infested 2-3 wk with I. *grandicollis* (late larval and pupal brood present)
4.1	32	Larval and pupal brood removed from bark (21 cm") from a pine bolt infested 2-3 wk with *I. grandicollis*	Clean air
4.2	32	Clean air	Bark (21 cm") from a pine bolt infested 2-3 wk with *I. grandicollis*, with larval and pupal brood removed
4.3	32	Larval and pupal brood removed from bark (21 cm^2) from a pine bolt infested 2-3 wk with *I. grandicollis*	Bark (21 cm^2) from a pine bolt infested with 2-3 wk *I. grandicollis*, with larval and pupal brood removed
5.1	30	Frass removed from larval mines in bark (21 cm^2) from a pine bolt infested 2-3 wk with *I. grandicollis*	Clean air
5.2	30	Clean air	Bark (21 cm^2) from a pine bolt infested 2-3 wk with *I. grandicollis*, with brood and frass removed
5.3	30	Frass removed from larval mines in bark (21 cm^2) from a pine bolt infested 2-3 wk with *I. grandicollis*	Bark (21 cm^2) from a pine bolt infested 2-3 wk with *I. grandicollis*, with brood and frass removed
6.1	32	Bark sandwich (21 cm") artificially infested 3 d previously with 10 I. *grandicollis* third-instar larvae	Clean air
6.2	32	Bark sandwich (21 cm") artificially infested 3 d previously with 10 *I. grandicollis* third-instar larvae	Excised bark (21cm^2) from a pine bolt infested 2-3 wk with *I. grandicollis* (late larval and pupal brood present)
7.1	30	Bark sandwich (21 cm") artificially infested 3 d previously with six *I. grandicollis* adult females (insects removed immediately before bioassay)	Clean air
7.2	30	Bark sandich (21 cm") artificially infested 3 d previously with six *I. gradicollis* adult females (insects removed immediately before bioassay)	Bark sandwich (21 cm^2) artificially infested 3 d previously with 10 *I. grandicollis* third-instar larvae (insects removed immediately before biossay)
7.3	30	Bark sandwich (21 cm^2) receiving mechanical damage 3 d previously and immediately before test	Clean air
7.4	30	Bark sandwich (21 cm^2) receiving mechanical damage 3 d previously and immediately before test	Bark sandwich (21 cm^2) artificially infested 3 d previously with 10 I. *grandicollis* third-instar larvae (insects removed immediately before bioassay)
8.1	34	Steam distillate of loblolly pine bark infested with larval and pupal *I. grandicollis*, diluted 1:50 (by volume) in isopropyl myristatc (10 μl) and applied to Whatman #1 filter paper	Neat isopropyl myristate (10 μl) applied to Whatman #1 filter paper
8.2	34	Neat isopropyl myristate (10 μl) applied to Whatman #1 filter paper	Steam distillate of fresh loblolly pine resin, diluted 1:50 (by volume) in isopropyl myristate (10 μl) and applied to Whatman #1 filter paper
8.3	34	Steam distillate of loblolly pine bark infested with larval and pupal *I. grandicollis*, diluted 1:50 (by volume) in isopropyl *myristate* (10 μl) and applied to Whatman #1 filter paper	Steam distillate of fresh loblolly pine resin, diluted 1:50 (by volume) in isopropyl myristate (10 μl) and applied to Whatman # 1 filter paper

Tests designated with the same integer composed a single experimental block. Tests within a single experimental block wore performed in equal numbers of replicates on the same days "sing the same pool of parasitoids. The order in which the tests were performed on a given day was chosen at random.

three different trees cut from the same stand. Parasitoids were presented a choice among uninfested bark (surface area, 21 cm") versus clean air (experiment 1.1), infested bark (21 cm^2) versus clean air (experiment 1.2), uninfested bark (21 cm^2) versus infested bark (21 cm^2) (experiment 1.3), infested bark (7 cm^2) versus uninfested bark (7 cm"), and infested bark (7 cm^2) presented together (experiment 2), and infested bark (7 cm^2) versus a doubled quantity of infested bark (14 cm^2) (experiment 3).

In experiment 4, female parasitoids were assayed for their response to either infested bark (as used in experiments I-3) with susceptible host stages removed or the live, excised hosts from this bark. The mean ± SD numbers and life stages of hosts isolated from each 21-cm^2 bark piece used per trial were 0.19 ± 0.39 first-instar larvae, 2.44 ± 2.42 second-instar larvae, 8.00 ± 4.42 third-instar larvae, and 1.25 ± 1.25 pupae (11.88 ± 5.00 total larvae and pupae). Bark was excised and hosts dissected <30 min before assay. Before the trials, the isolated hosts were mechanically cleaned with a camels-hair brush to remove all visible frass and plant material. Because of the inherent difficulty of locating early-stage larvae mining deeply in the bark, we were not successful in removing all hosts from the infested bark sections before the bioassays. However, additional, intensive dissection/examination of the bark following the bioassays showed that we had removed >90% of the hosts present within the tissue before testing. Parasitoids were presented a choice among isolated hosts versus clean air (experiment 4.1), the bark from which these hosts were removed versus clean air (experiment 4.2), and isolated hosts versus their bark of origin (experiment 4.3).

Isolated Frass and Frass-Free Bark. In experiment 5, female parasitoids were assayed for their response to either infested bark (as used in experiments I-3) dissected to remove host brood and host frass or the host larval frass collected from this bark. Clean, nylon-bristled brushes were used to remove frass from the larval galleries of the bark pieces. The mean ± SD fresh weight of frass used per trial was 104 ± 64 mg. Host brood and any adult frass present in the bark pieces were removed and discarded. Further dissection/examination of the tested bark following the bioassays indicated that >95% of host brood and host frass (by weight) were removed before testing. Parasitoids were presented a choice among host larval frass versus clean air (experiment 5.1), the bark from which this frass was removed versus clean air (experiment 5.2), and larval frass versus its bark of origin (experiment 5.3).

Bark Sandwiches with Adult or Larval Hosts. In experiments 6 and 7, female parasitoids were assayed for their response to individual 3 by 7-cm pieces of fresh bark damaged by 10 third-instar *I. grandicollis*, by six adult female *I. grandicollis*, or by artificial manipulation Bark pieces were infested by cutting evenly spaced niches into the phloem tissue (≈1 by 3 mm) and placing a single insect in each niche. The bark pieces receiving the artificial damage treatment were given the same number of niches (i.e., 10) as the larvae-infested pieces as well as ≈15 diagonal scalpel cuts (2 cm long) in the phloem tissue, but no insects were placed into the niches. After these preparations of the bark pieces, a clean, aseptic glass microscope slide (5 by 7 cm) was sealed to the surface of the exposed phloem with binder clips. All bark manipulations were carried out with sterile instruments in a clean environment. The sealed bark sandwiches were then incubated for 3 d at ≈28°C and 80–90% RH. Immediately before the trials for experiment 7, all live or dead insects were removed from the infested bark sandwiches by dissection, and the mechanical damage-only sandwiches were likewise dissected to inflict a similar amount of damage as occurred in dissecting the larvae-infested pieces. Female adult I. grandicollis, rather than males, were chosen for these tests because females do not produce pheromones (Smith et al. 1993), and females will mine readily in bark in the absence of males. Some females laid eggs in the bark sandwiches, but none hatched before the bioassays. The mean ± SD numbers of living insects present in the sandwiches at the end of the 3-d incubation were as follows: larvae-infested sandwiches, 7.31 ± 2.03 larvae, and 1.58 ± 1.94 pupae; adult-infested sandwiches, 5.07 ± 2.46 adults (some adults abandoned the bark sandwiches during the 3-d incubation). Parasitoids were presented a choice among a larvae-damaged bark sandwich versus clean air (experiment 6.1), a larvae-damaged bark sandwich versus a 3 by 7-cm piece of larvae/pupae-infested bark from a bolt colonized 2-3 wk earlier by 1. *grandicollis* (experiment 6.2), an adult-damaged bark sandwich versus clean air (experiment 7.1), an adult-damaged bark sandwich versus a larvae-damaged bark sandwich (experiment 7.2), an artificially damaged bark sandwich versus clean air (experiment 7.3), and an artificially damaged bark sandwich versus a larvae-damaged bark sandwich (experiment 7.4).

Distillates of Infested Bark or Fresh Resin. In experiment 8, female parasitoids were assayed for their response to either a steam distillate of bark infested *with I. grandicollis* larvae or a steam distillate of fresh pine resin. Fresh resin was collected from healthy loblolly pines by removing bark disks from tree trunks with a cork borer and inserting screw-top vials into the openings. After ~24 h, the vials were removed, capped, and stored at approximately -30°C before distillation. The full, uncapped vials were placed into a 1,000-ml Erlenmeyer flask with 500 ml de-ionized water and distilled according to Sullivan et al. (1997). Tested baits consisted of these distillates diluted 1:50 in isopropyl myristate, an inert, nonvolatile oil. Blanks consisted of neat isopropyl myristate, and baits and blanks were presented in the odor sample chambers as a 10-μl aliquot applied to a 2 by Z-cm filter paper. Parasitoids were presented a choice among the distillate of host-infested bark versus a blank (experiment 8.1), the resin distillate versus a blank (experiment 8.2), and the distillate of host-infested bark versus the resin distillate (experiment 8.3)

Collection and Analysis of Volatiles. Bark baits (as used in experiments 1, 6, and 7) were placed in the olfactometer odor source chambers, and an electric vacuum pump drew charcoal-filtered air for ≈90 min at 30 ml/min through the chambers and into a column (PTFE tubing; 4 cm by 3 mm i.d.) packed with Porapak Q (0.1 g; 50-80 mesh; Waters Associates, Milford, MA). Twelve different pieces of bark (originating from two trees, six pieces derived from each) of each treatment were sampled, and aerations were performed at 26 ± 1°C. Within 1 h after completion of the aerations, columns were extracted with 1.2 ml redistilled pentane, and the extracts were stored at approximately −80°C in glass vials with Teflon lined tops. Additionally, the distillates bioassayed in experiment 8 were diluted 1/ 1,000 in redistilled pentane and both these and the Porapak extracts were spiked with internal standards (100 μg undecane and 20 μg ethyl caprate). Samples of the above mentioned extracts and dilutions (1 μl) were analyzed on a Hewlett-Packard GCD 1800A coupled gas chromatograph-mass spectrometer (GC-MS) fitted with a Hewlett-Packard Innowax column (60 m by 0.25 mm i.d., 0.50 μm film thickness). Compounds were identified by their mass spectra and by retention time matches when identified standards were available. Compounds identified by mass spectra alone are noted in Table 2. Response curves were calculated for identified compounds for which pure standards were available, and the volatile compoundsin the samples were quantified by their ion abundances relative to those of the internal standards. Compounds for which no pure standard was available were quantified using the standard curve of a structurally similar compound.

Statistical Analysis. The null hypothesis that the parasitoids showed no preference for either olfactometer arm (i.e., a hypothesized response proportion of 50:50) was tested either with a G-test for goodness-of-fit (when >25 insects responded) or by calculating the cumulative probability of the observed proportions plus "all worse" outcomes using a binomial probability table (Sokal and Rohlf 1995). Quantities of volatiles present in the Porapak extracts were compared among bark treatments with a one-way analysis ofvariance (ANOVA) on ranks ($\alpha = 0.05$) followed by a Tukey test for all-pairwise comparisons (SPSS 1997).

Results

Bioassay Experiments. Female *R. xylophagorum* were attracted to bark removed from bolts infested with *I. grandicollis* larvae and pupae, and strongly preferred the odors of infested bark over those of uninfested bark (Fig. 1, experiment 1.2, 1.3). Parasitoids were not attracted to the odors of fresh, uninfested bark (Fig. 1, experiment 1.1). Parasitoids strongly preferred the odors of host-infested bark presented alone over these odors presented in combination with the odors of fresh bark (Fig. 1, experiment 2). Nearly twice as many parasitoids chose the olfactometer arm baited with a doubled surface area of host-infested bark, however this was not a significant preference (Fig. 1, experiment 3). Although more parasitoids selected the olfactometer arm baited with isolated hosts over the blank arm, the numbers of insects responding to the arm baited with isolated hosts was too low to indicate a significant level of attraction (Fig. 2, experiment 4.1). Infested bark remained highly attractive to parasitoids after nearly all hosts had been removed, and parasitoids strongly preferred the odors of this material over odors of the isolated hosts (Fig. 2, experiment 4.2, 4.3). Both host larval frass and the bark fragments from which this frass had been removed were highly attractive, and parasitoids exhibited no preference for either of these materials (Fig. 2, experiment 5.1-5.3).

Bark sandwiches infested 3 d with third-instar *I. grandicollis* larvae were attractive to *R. xylophagorum* females, and, with 23 of 32 insects responding, this parasitoid did not show a significant preference between these larvae-infested bark sandwiches and bark excised from *I. grandicollis*-colonized bolts (Fig. 3, experiment 6.1, 6.2). Bark sandwiches infested 3 d with female, adult *I. grandicollis* were likewise attractive, and with 27 of 30 insects responding, there was no significant preference observed for bark sandwiches infested with either adults or larvae (Fig. 3, experiment 7.1, 7.2). Odors from bark sandwiches receiving simulated host damage had no discernible activity (Fig. 3, experiment 7.3, 7.4).

Roptrocerus xylophagorum females were attracted to the steam distillate of bark infested with larvae and pupae of *I. grandicollis* (Fig. 4, experiment 8.1). In contrast, the distillate of fresh pine resin was not significantly attractive (Fig. 4, experiment 8.2, 8.3).

Analysis of Volatiles. Forty-eight compounds were isolated and quantified in the aerations of bark baits, and 31 of these compounds quantitatively distinguished the bait treatments paired in the Y-tube olfactometer bioassays (Table 2). Aerations of bark from bolts infested with larval- and pupal-stage *I. grandicollis* brood had significantly higher quantities of 21 compounds than aerations of bark from uninfested pine bolts. These included three hydrocarbon monoterpenes (a-fenchene, p-cymene, and α-*p*-dimethylstyrene) , I2 oxygenated monoterpenes (fenchone, linalool, camphor, isopinocamphone, pinocarvone, terpinen-4-01, myrtenal, *trans*-pinocarveol, α-terpineol, bomeol, myrtenol, and *p*-cymen-8-ol), two "green leaf volatiles" (Visser et al. 1979, Bemays and Chapman 1994) (I-hexanol and 3-hexen-1-ol), two nonterpene aromatics (styrene and benzaldehyde), and two unknown compounds. With the exception of 3-hexen-1-ol and one unidentified compound, all of these compounds were also present in higher concentrations in the steam distillate of infested bark than the steam distillate of fresh resin, Ten compounds were present in significantly higher quantities in the aerations of the bark sandwiches damaged by third-instar *I. grandicollis* larvae than sandwiches damaged mechanically. These included four oxygenated monoterpenes (pinocamphone, camphor, isopinocamphone, and borneol), one hydrocarbon sesquiterpene (α-cedrene), two nonterpene aromatics (styrene and benzaldehyde) ,

Table 2. Volatile compounds identified from bait treatments tested in Y-olfactometer bioassays of *R. xylophagorum*

Compound	Class	Mean ± SE quantity (μg) volatiles collected from 21 cm² pieces of loblolly pine bark during a 90-min aeration										Steam distillates (μg/μl)	
		Bark sandwich damaged by:						Bark excised from bolts:					
		I. grandicollis larvae		*I. grandicollis* adults		Artificial manipulation		Uninfested		Infested 2-3 wk by *I. grandicollis*		Fresh pine resin	Larvae-infested bark
Tricyclene	H M	0.30	0.13ab[a]	0.075	0.012a	0.48	0.34ab	0.67	0.08bc	1.2	0.2c	3.6	2.1
α-Pinene	H M	36.	12.0a	14.	2.0a	22.	5.0a	12x10'	12.0b	18×10[1]	34.0b	45x 10[1]	31x10'
a-Fenchene	H M	0.056	0.046a	0.003	0.002a	0.020	0.013a	0.006	0.006a	1.6	0.4b	0.050	2.1
Camphene	H M	0.97	0.46ab	0.19	0.04a	1.4	1.1ab	1.7	0.2bc	4.2	0.7c	9.6	9.2
Hexanal[b]	G L V	2.3	0.8b	2.8	0.8b	0.97	0.36b		NDa	0.50	0.24ab	ND	0.47
β-Pinene	HM	15.	6.ab	2.7	1.3a	6.1	3.9ab	23.	7.0ab	43.	21.0b	57.	15×10[1]
Myrcene	H M	2.8	1.1ab	0.57	0.16a	3.9	3.0ab	4.7	0.4b	6.4	2.0b	18.	25.
α-Phellandrene	H M	0.009	0.005ab	0.092	<0.001a	0.010	0.006ab	0.012	0.003ab	0.030	0.012b	0.068	0.39
α-Terpinene	H M	0.015	0.010ab	0.001	0.001a	0.012	0.008abc	0.018	0.003bc	0.10	0.05c	0.089	0.82
Limonene	H M	1.5	0.9ab	0.21	0.05a	1.3	0.9ab	1.4	0.1bc	6.4	1.4c	5.8	33.
β-Phellandrene[b]	H M	0.63	0.24ab	0.15	0.05a	1.2	0.9ab	0.81	0.16b	1.6	0.6b	2.4	8.1
γ-Terpinene	H M	0.023	0.015a	0.006	0.001a	0.027	0.017ab	0.045	0.003b	0.17	0.08b	0.15	1.1
Styrene[b]	NTA	0.061	0.016c	0.023	0.005bc	<0.001ab			NDa	0.52	0.19c	ND	0.015
p-Cymene	H M	0.12	0.05b	0.023	0.005a	0.12	0.07ab	0.040	0.008ab	1.3	0.3c	0.048	1.8
Unknown #1	—	0.096	0.044b	0.026	0.004b	NDa			NDa	0.11	0.03b	ND	ND
Terpinolene	HM	0.23	0.16a	0.033	0.008a	0.20	0.13ab	0.28	0.04bc	1.6	0.8c	1.3	7.9
1-Hexanol[b]	G L V	1.1	0.4b	1.0	0.3b	0.35	O.11b		NDa	0.42	0.17b	ND	0.048
Unknown #2	—	0.055	0.010c	0.018	0.004bc	0.011	0.007ab		NDa	0.088	0.019c	ND	1.6
3-Hexen-1-ol[b]	G L V	0.89	0.23b	0.84	0.16b	0.44	0.12b	0.006	0.004a	0.30	0.09b	ND	ND
Fenchone	O M	0.031	0.013a	0.008	0.002a	0.019	0.011a	0.016	0.011a	1.5	0.4b	ND	1.1
α-p-Dimethylstyrene[b]	H M	0.038	0.012bc	0.012	0.003ab	0.037	0.020b	0.003	0.002a	0.19	0.06c	0.010	2.3
Copaene[b]	HS	0.037	0.021	0.044	0.023	0.028	0.011	0.094	0.037	0.079	0.035	ND	0.42
Linalool	O M	0.037	0.007b	0.046	0.005b	0.14	0.05b		NDa	0.12	0.03b	0.27	0.95
Benzaldehyde[b]	NTA	0.46	0.20c	0.12	0.08bc	0.005	0.001ab		NDa	0.34	0.16C	ND	0.31
Pinocamphone	O M	0.39	0.12c	0.22	0.10bc	0.13	0.11ab	0.002	0.001a	0.57	0.27ab	ND	2.1
Camphor	O M	1.2	0.1cd	0.67	0.16bc	0.098	0.032a	0.16	0.05ab	5.9	1.2d	0.056	6.3
Isopinocamphone	O M	0.14	0.05bc	0.057	0.031ab	0.044	0.035a	0.009	0.004a	1.3	0.3c	ND	5.4
Fenchyl Alcohol	O M	0.037	0.025	0.011	0.003	0.014	0.010	0.042	0.019	0.12	0.04	ND	3.4
Bergamotene[b]	HS	0.40	0.09	0.59	0.12	0.21	0.05	0.55	0.11	0.46	0.14	0.062	2.3
Bornyl acetate	OM	0.030	0.008	0.013	0.004	0.068	0.045	0.072	0.025	0.44	0.16	0.76	1.6
α-Cedrene[b]	H S	0.042	0.005b	0.044	0.006b	0.014	0.003a	0.031	0.005ab	0.027	0.007ab	ND	0.088
Pinocarvone[b]	O M	0.019	0.004bc	0.013	0.004ab	0.005	0.002ab	0.004	0.004a	0.16	0.03c	ND	0.62
Terpinen-4-ol	O M	0.023	0.012a	0.005	0.002a	0.011	0.007a	0.003	0.003a	0.94	0.72b	0.056	21.
Caryophyllene	H S	4.3	2.5	5.7	3.2	3.7	1.5	13.	5.	10.	5.	ND	68.
Unknown #3		0.27	0.04b	0.21	0.02b	0.008	0.001a	0.004	0.003a	0.012	0.005a	0.096	0.091
Unknown #4	—	0.023	0.005	0.038	0.010	0.011	0.002	0.026	0.006	0.019	0.007	ND	0.64
Myrtenal	OM	0.031	0.007bc	0.016	0.003b	0.008	0.002ab		NDa	.013	0.03c	ND	0.76
trans-Pinocarveol	O M	0.069	0.025b	0.034	0.015ab	0.031	0.024ab	0.003	0.003a	0.099	0.037b	ND	1.8
4-Allylanisole	P P	2.8	0.6ab	1.5	0.3a	2.5	1.6a	2.1	0.4ab	4.9	1.4b	24.	40.
α-Terpineol	O M	0.062	0.045a	0.012	0.009a	0.019	0.014a		NDa	0.21	0.09b	0.096	33.
α-Humulene	H S	0.68	0.41	1.1	0.6	0.55	0.22	2.0	0.8	1.6	0.7	ND	16.

(Continued)

Table 2. (Continued)

Compound	Class	Mean ± SE quantity (μg) volatiles collected from 21 cm² pieces of loblolly pine bark during a 90-min aeration									
		Bark sandwich damaged by:				Bark excised from bolts:			Steam distillates (μg/μl)	
		I. grandicollis larvae	*I. grandicollis* adults	Artificial mamillation		Uninfested		Infested 2–3 wk by *I. grandicollis*	Fresh pine resin	Larvae-infested bark
Borneol	UM	0.13	0.02c	0.11	0.03bc	0.032	0.012ab	0.013	0.058a	0.49	0.12c	0.21	2.9
Unknown #5	—	0.067	0.019	0.12	0.03	0.035	0.008	0.077	0.017	0.054	0.016	ND	0.65
Unknown #6	—	0.028	0.009a	0.053	0.014ab	0.027	0.008a	0.075	0.010b	0.067	0.016ab	ND	0.40
Carvone	OM	0.013	0.005	0.009	0.004	0.001	0.001		ND	0.027	0.013	ND	0.31
Myrtenol	OM	0.013	0.003bc	0.005	0.002abc	0.002	0.001ab		NDa	0.042	0.009c	0.004	1.1
p-Cymen-8-ol	OM	0.025	0.007bc	0.012	0.005bc	0.009	0.005ab		NDa	0.047	0.008c	ND	0.58
Methyleugenol	PP	0.009	0.003b	0.005	0.001b	0.007	0.004ab		NDa	0.002	0.001ab	1.2	0.77

[a] Within rows, means denoted with the same letter were not significantly different with $\alpha = 0.05$ (Tukey test on ranks).
[b] Compounds identified by mass spectral matches only; identification not confirmed by retention time match because standard was not available.
[c] HM, hydrocarbon monoterpene. OM, oxygenated monoterpene. HS, hydrocarbon sesquiterpene. GLV, green leaf volatile. NTA, non-terpenoid aromatic. PP, phenylpropanoid.

and three compounds of uncertain chemical classification. Aerations of the bark sandwiches damaged by female adult I. *grandicollis* had significantly lower quantities of a single hydrocarbon monoterpene (*p*-cymene) than aerations of bark sandwiches damaged by larvae. Aerations of bark sandwiches damaged by third-instar *I. grandicollis* differed significantly from aerations of bark fragments from bolts infested with larval and pupal-stage *I. grandicollis*. Relative to the latter, the former had significantly lower quantities of eight hydrocarbon monoterpenes (tricyclene, α-pinene, α-fenchene, camphene, α-terpinene, limonene, p-cymene, and terpinolene) and three oxygenated monoterpenes (fenchone, terpinen-4-ol, and a-terpineol) and had significantly higher quantities of a single unknown compound.

Discussion

Our data show that the cues that attract *R. xylophagorum* to its host's habitat arise from the interaction between the host insect and the tissues of its host plant, whereas odors from isolated hosts or uninfested tree tissue apparently play a limited and secondary role in host habitat location. Attraction of parasitoids to pieces of fresh, excised bark was stimulated after 3 d of feeding by host larvae, whereas such barkincubated for the same period and receiving only artificial damage showed no evidence of being attractive (experiments 6.1, 7.3, 7.4). Hosts isolated from highly attractive, infested bark were not appreciably attractive when presented alone, and parasitoids unfailingly chose the olfactometer arm baited with bark from which >90% of the hosts had been removed over the olfactometer arm baited with the hosts themselves (experiment 4). These findings reflect previous observations that isolated host larvae and pupae will neither arrest nor induce oviposition behaviors in female R. *xylophagorum* that come in direct physical contact with them (Samson 1984; B.T.S., unpublished data), Host location cues that originate directly from the bodies of host insects are probably quite rare in nature, likely because of strong natural selection against hosts producing such cues (Vet et al. 1991, Tumlinson et al. 1992, Vet and Dicke 1992, Godfray 1994). Conversely, infested bark remained highly attractive despite the removal of hosts (experiment 4.2), and host-depleted bark did not differ significantly from bark with hosts left in place (experiment 1.2) in the proportion of parasitoids responding to the bark-baited olfactometer arm ($P = 0.1$, χ^2 test). Similar findings occurred in field studies in which female A. *xylophagorum* were attracted to bolts cut from pines infested by larval *D. frontalis* despite the removal of all bark and hosts, and this parasitoid responded in similar numbers both to this host-free xylem tissue and to the infested bark from which it had been separated (Sullivan 1997).

Female *R. xylophagorum* did not discriminate between host frass and the surrounding, host-damaged plant tissue in which this frass had been deposited, hence our data do not indicate one of these as the

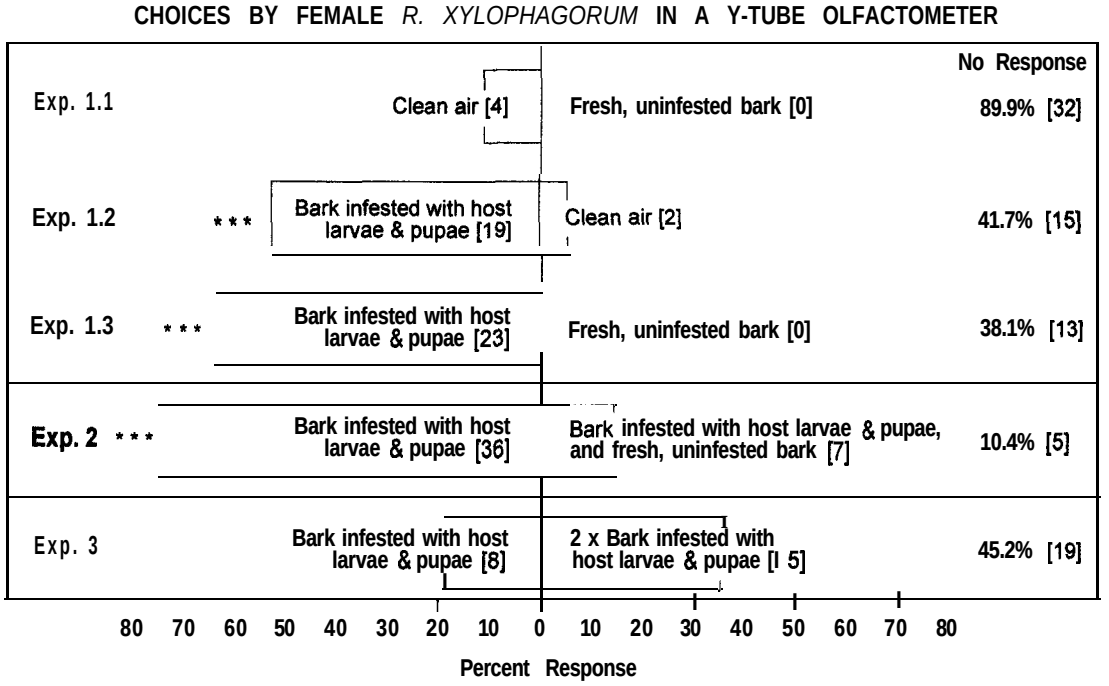

Fig. 1. Responses of individual walking *R. xylophagorum* females in a Y-tube olfactometer to pieces of bark excised from loblolly pine bolts either uninfested or infested 2-3 wk earlier by *I. grandicollis* adults (brood predominantly in the late larval and pupal stages of development). Raw numbers of insects responding/not responding are shown in brackets. Bars denoted by asterisks indicate a significant preference for that treatment (***, $P < 0.001$).

exclusive origin of the attractants. Because both the plant tissue and the frass were in physical contact for hours or days before the tests, some diffusion of attractants from one to the other probably occurred, potentially lessening observable differences in attractiveness. Nonetheless, because bark beetle frass largely consists of masticated, undigested plant tissue (Hopf 1937, Reid 1958, Gouger 1971; B.T.S., unpublished data), our data are consistent with the hypothesis that the attractants arise from host-damaged or host-exposed plant tissue rather than from host excreta.

Females of numerous parasitoid species are attracted to odors from the uninfested food plant of their hosts (Elzen et al. 1983, 1984; Martin et al. 1990; Whitman and Eller 1990; Tumlinson et al. 1992), and hence such volatiles appear to play a significant role in guiding some parasitoids to habitats where hosts are likely to be found. Our data suggest that *R. xylophagorum* do not rely upon this particular host location strategy because they were not attracted to odors of the bark tissue or resin of uninfested *P. taeda*. Bark (or, more precisely, the phloem and cambium layers) is the only tissue fed upon by hosts of *R. xylophagorum*. Although mechanically damaged, this tissue was not attractive to *R. xylophagorum* in the absence of infestation by hosts. Similarly, female parasitoids were not attracted to volatile components of the host plant's constitutive defensive system (i.e., loblolly pine resin distilled as turpentine), Constitutive resin is exuded by pines in response to any mechanical damage to vascular tissues including that inflicted by bark beetle attack (Berryman 1972, Lewinsohn et al. 1991). Hence, resin production and release appear to be a generalized defensive response, and resin odors likely provide no specific cues that might indicate the cause of a tree's injury. This reasoning is supported by our volatiles analyses of the olfactometer baits. None of the major monoterpene components of pine resin (e.g., α-pinene, β-pinene, myrcene) were found in association with any of the host-infested bark treatments in concentrations that differed significantly from those of the corresponding uninfested treatments. This suggests that these compounds were not used by parasitoids to detect host presence. Our conclusions are supported by field studies in which turpentine failed to attract *R.* xylophugorum to baited traps (Dixon and Payne 1980), and peak parasitoid arrival on infested trees was found not to coincide with maximum exudation of resin odors (Sullivan 1997). In addition, the major chemical constituents of the turpentine (including α-pinene, β-pinene, limonene, myrcene, and camphene) failed to elicit electroantennogram responses with *R. xylophagorum* (Pettersson et al. 2000), hence *R. xylophagorum* may lack receptors for these odors.

However, our results suggest that odors of the uninfested food plant, though unattractive, may nonetheless play a role in parasitoid host finding. Parasitoids were less attracted to pieces of host-infested bark

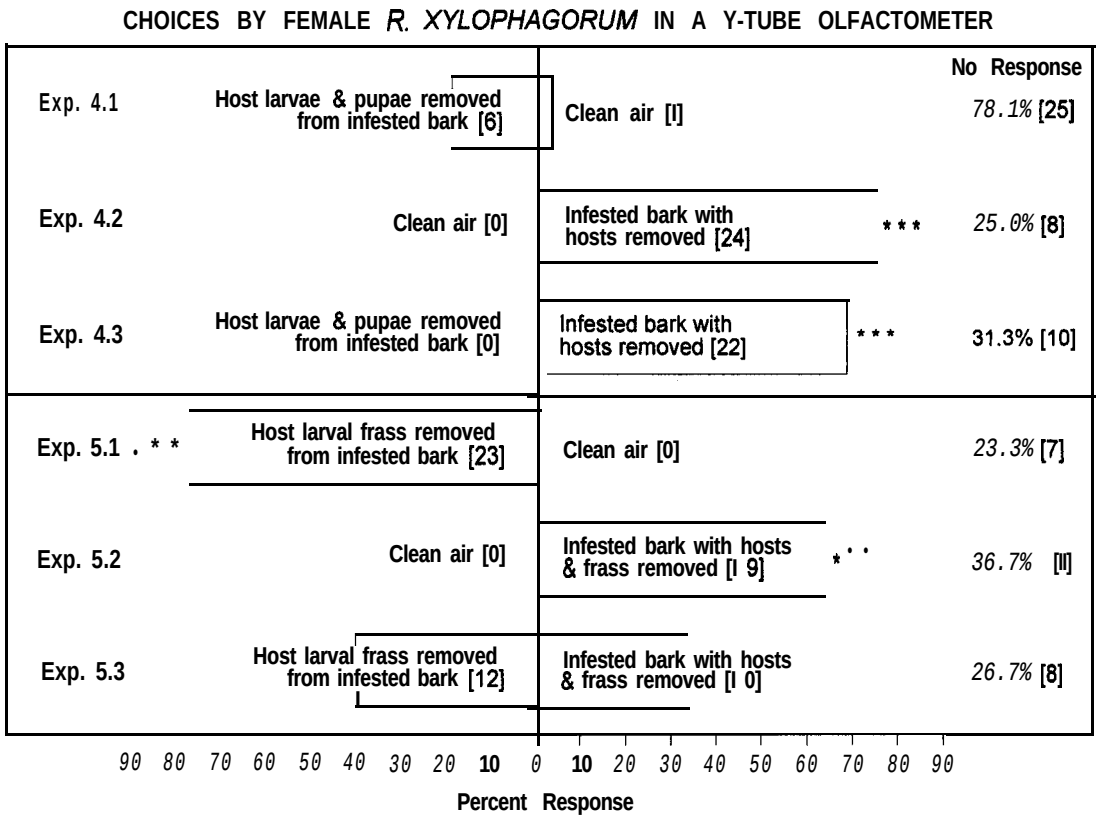

Fig. 2. **Responses of individual walking *R. xylophagorum* females in a Y-tube olfactometer to *I. grandicollis* larvae and pupae, larval frass, or the loblolly pine bark from which these were removed. Raw numbers of insects responding/not responding are shown in brackets. Bars denoted by asterisks indicate a significant preference for that treatment (***, $P < 0.001$).**

when these were presented alongside identically sized pieces of fresh, uninfested bark (experiment 2), hence the odors of fresh bark appeared to be acting as attractant antagonists. This apparent antagonism was not caused merely by an inverse dose-response to odors present in similar quantities in both fresh and infested bark, because doubling the surface area of infested bark presented in one arm of the olfactometer resulted in higher numbers of insects responding to that arm (experiment 3). Thus, our findings show that volatiles from the host's uninfested food plant can to some extent mask odors associated with the host/plant complex. The importance of these masking odors to parasitoid survival and reproduction is unclear. Because the antagonistic odors appear to be associated with tissues lacking hosts, it is possible that *R. xylophagorum* might use them to assess host patch profitability and avoid investing search time in beetle galleries associated with few or no susceptible hosts (Godfray 1994, Geervliet et al. 1998). The response may also represent an avoidance of toxic plant exudates associated specifically with newly excavated beetle galleries or other freshly damaged pine tissue. Comparison of the volatile blends associated with both the infested and uninfested bark did not suggest any obvious candidates as the compounds responsible for the attractant antagonism; no volatiles were found associated in significantly higher quantities with uninfested than infested bark. It is possible that we failed to detect the antagonists during our analyses. Alternatively, parasitoid attraction/inhibition may be influenced to a greater extent by the ratios of components in the odor blend than the absolute concentrations of the individual components. Hence the fresh bark-associated odors may have reduced parasitoid attraction to infested bark by altering the relative proportions among odor blend components perceived by the responding insects (e.g., increasing ratio of sesquiterpenes to oxygenated monoterpenes) rather than increasing the absolute concentration of specific antagonistic compounds.

Fragments of fresh bark manually infested 3 d earlier with *I. grandicollis* larvae became attractive to *R. xylophagorum* females (experiment 6.1). In the side-by-side choice test, nearly one-third of responding parasitoids chose the olfactometer arm baited with these larvae-infested bark sandwiches over the opposite arm baited with bark from naturally infested bolts bearing larval brood in similar mean densities (experiment 6.2, $P = 0.105$). Thus, our data show that the

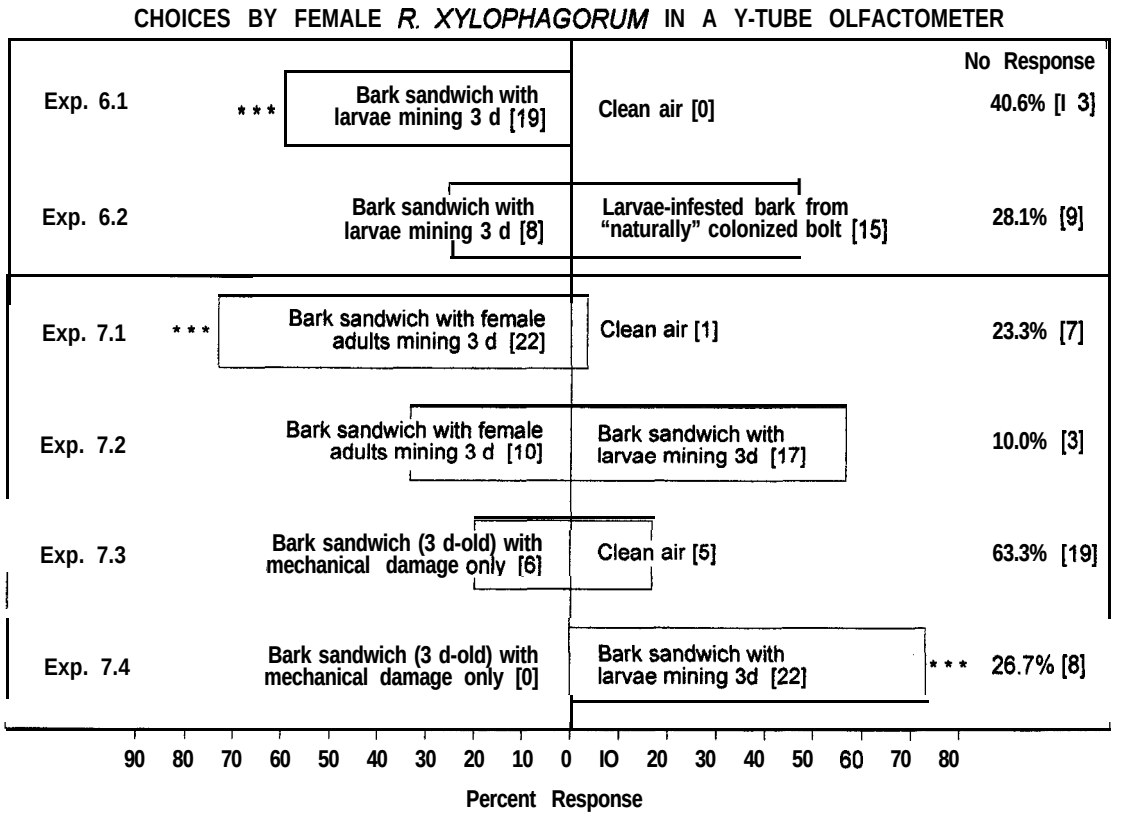

Fig. 3. **Responses of individual walking R. *xylophagorum* females in a Y-tube olfactometer to bark excised from an *I. grandicollis*-infested bolt or bark sandwiches damaged by either feeding *I. grandicollis* larvae, feeding female adults, or artificial manipulation. Raw numbers of insects responding/not responding are shown in parentheses. Bars denoted by asterisks indicate a significant preference for that treatment** (***, $P < 0.001$).

parasitoid host location cues are generated largely within the host-damaged bark tissue without any essential contribution from the xylem tissue. Host larvae in the bark sandwiches were feeding in phloem that had been detached from other living tissue of the tree bole, hence the normal mobilization of defensive com-

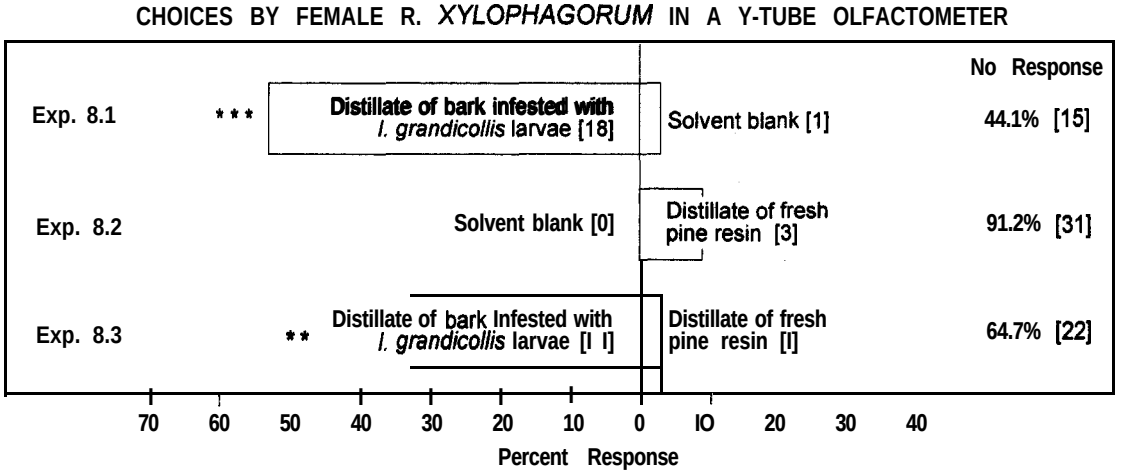

Fig. 4. **Responses of individual walking *R. xylophagorum* females in a Y-tube olfactometer to diluted steam distillates of either bark infested with I. *grandicollis* larvae or fresh loblolly pine resin. Raw numbers of insects responding/not responding** are shown in parentheses. Bars denoted by asterisks indicate a significant preference for that treatment (**, $P < 0.01$; ***, $P < 0.001$).

pounds such as terpenoids from the sapwood into the damaged phloem tissue was prevented (Berryman 1972, Lewinsohn et al. 1991). Accordingly, substantially lower levels of hydrocarbon monoterpenes such as a-pinene and limonene were present in the larvae-infested bark sandwiches compared with the bark from naturally infested bolts. In addition, these data indicate that the production of attractants within pine tissues can occur much faster than the typical 2-3 wk required for host brood to develop into susceptible instars following attack initiation on a tree or bolt. Hence, in nature the typically extended period of interaction between beetle and tree tissue before parasitoid arrival is probably not necessary for successful host location by this parasitoid. This suggests that certain relatively slow biological processes initiated by bark beetle attack (e.g., colonization and sporulation by specific bark beetle associated fungi, or gradual physical and chemical degradation within the tree's tissues) may not play a particularly important role in either generating host-habitat cues for *R. xylophagorum* or governing the timing of this species' arrival on bark beetle attacked trees (Dahlsten and Berisford 1995, Sullivan 1997).

Female *R. xylophagorum* were attracted to excised, fresh bark damaged 3 d by adult female *I. grandicollis* (experiment 7.1). Although there were no susceptible host stages present in this bark, numbers of attracted parasitoids were not significantly different from those attracted by similar bark infested with third-instar larvae (experiment 7.2), the preferred host stage for oviposition by *R. xylophagorum (Samson* 1984). Therefore, our data show that the cues to which *R. xylophagorum* responded in these choice bioassays were not especially host-stage specific and suggest that host seeking parasitoids may lack highly reliable cues for distinguishing host life stages at a distance.

This apparent inability of foraging female *R. xylophagorum* to consistently discriminate between odors associated with susceptible and nonsusceptible host life stages was unexpected. *R. xylophagorum are* trapped in much greater numbers landing on bark beetle infested trees when late larval instars are abundant than when adults are initiating galleries (Berisford and Franklin 1969, Stephen and Dahlsten 1976, Dixon and Payne 1979, Dix and Franklin 1981), suggesting that foraging *R. xylophagorum* in nature can effectively discriminate among host stages during host habitat location. In addition, *R. xylophagorum* do not respond to aggregation pheromones associated specifically with attacking adult bark beetles (Dixon and Payne 1980), in contrast to parasitoids and predators that attack bark beetle adults (Payne 1989).

Numerous compounds were identified that individually distinguished the odors associated with the bioassayed bait treatments, hence any or all of these compounds might have been used as cues by *R. xylophagorum* in discriminating among, and responding to, the odor sources. For most compounds, these differences were merely quantitative. Relatively few compounds were identified that distinguished attractive from unattractive baits with a high degree of reliability. Only six compounds were consistently found in higher quantities in association with baits derived from pine tissues infested with parasitism-susceptible host stages (artificially infested bark sandwiches, bark from infested bolts, and distillate of infested bark) than baits derived from uninfested pine tissues (mechanically damaged bark sandwiches, freshly excised bark, and distillate of fresh resin). These included three oxygenated monoterpenes (camphor, isopinocamphone, and borneol), two nonterpene aromatics (styrene and benzaldehyde), and one compound of uncertain identity. However, none of these compounds were likewise found in association with female adult *I. grandicollis*-infested bark sandwiches insignificantly higher quantities than both uninfested bark treatments (mechanically damaged bark sandwiches and freshly excised bark), although the mean quantities of these compounds in association with the adult-infested sandwiches were always higher. It is possible that our analyses failed to detect the precise cues to which the parasitoids were responding. However, it is also possible that a variety of compounds or their combinations are similarly capable of attracting *R. xylophagorum* to its host's habitat, and it is possible that many (if not all) of the specific behavioral chemicals stimulating parasitoid attraction are interchangeable with others of similar potency (Vet et al. 1990).

The oxygenated monoterpenes are a class of compounds that appear to play an important role in host-habitat Ending by *R. xylophagorum.* This parasitoid possesses olfactory receptors for at least 10 different oxygenated monoterpenes, and a synthetic blend of seven of these compounds was found to attract *R.* xylophagorum in a Y-tube olfactometer (Pettersson et al. 2000). Our analyses of the baits in the current study indicated that oxygenated monoterpenes (including nine of those identified as having biological activity in the above mentioned study) were associated with one or more host-infested baits in significantly higher quantities than uninfested baits. This suggests that oxygenated monoterpenes are fair indicators of the presence of susceptible hosts for *R. xylophagorum. Our* findings agree with studies in which several oxygenated monoterpenes were found consistently associated with trees infested with the larval and pupal stages of the southern pine beetle, *D. frontalis, an* alternate host for *R. xylophagorum* (Birgersson et al. 1992, Sullivan 1997). Oxygenated monoterpenes probably arise in bark beetle-infested pines through the spontaneous oxidation of hydrocarbon precursors exposed to the air, detoxification of monoterpenes by invading insects and microorganisms, and other processes as well (Leufven and Birgersson 1987, Leufvén et al. 1988, Hunt et al. 1989, Leufvén 1991, Smith et al. 1993). These compounds may serve as signs of the initial stages of decay of pines that have been killed recently and that are therefore more likely to be infested by bark beetles.

The results of this study demonstrate that host-foraging *R. xylophagorum* respond to a combination of attractants and attractant antagonists associated with,

respectively, the host/plant complex and the uninfested tissues of their host's food plant. This suggests that *R. xylophagorum* may use these cues in combination to guide them to concentrations of susceptible hosts and away from sites lacking host activity. Future research should focus on further elucidating the precise chemistry of these cues and the biological variables affecting their production. Such information will provide a better understanding of the host foraging strategies of bark beetle parasitoids, the potential for the regulation of host populations by these parasitoids, and the occurrence of bark beetle outbreaks.

Acknowledgments

We are grateful for technical assistance provided by Richard Garland and Mark Daluskv. Mike Hunter and other staff of the Whitehall Experimental Forest provided us with trees necessary for maintenance of the insect colonies and conducting experiments. We also thank Chris Fettig, Dan Miller, and John Nowak for helpful comments on the original manuscript. This work was supported in part by a grant from the USDA Forest Service, Forest Health Protection, project R8-*99-03.*

References Cited

Berisford, C. W., and R. T. Franklin. 1969. Attack sequence of *Zps grandicollis* (Coleoptera: Scolytidae) and some associated hymenopterous parasites. J. Ga. Entomol. Soc. *4: 93-96.*

Berisford, C. W., H. M. Kulman, R. L. Pienkowski, and H. J. Heikkenen. 1971. Factors affecting the distribution and abundance of hymenopterous parasites of *Zps* spp. bark beetles in Virginia (Coleoptera: Scolytidae). Can. Entomol. 103: 235-239.

Berisford, C. W., and D. L. Dahlsten. *1989.* Biological control of Zps *grandicollis* (Eichoff) (Coleoptera: Scolytidae) in Australia-a preliminary evaluation, pp. 81-93. *In* D. L. Kulhavy and M. C. Miller [eds.], Potential for biological control of *Dendroctonus* and Zps bark beetles. University of Texas Press, Austin, TX.

Bernays, E. A., and R. F. Chapman. 1994. Host-plant selection by phytophagous insects. Chapman & Hall, New York.

Berryman, A. A. 1972. Resistance of conifers to invasion by bark beetle-fungus associations. Bioscience 22: 598–602.

Birgersson, G., M. J. Dalusky, K. E. Espelie, and C. W. Berisford. 1992. Isolation and identification of kairomones utilized by southern pine beetle parasitoids, p. 30. *In* Proceedings: North American Forest Insect Work Conference. USDA For. Serv. Gen. Tech. Rep. PNW-GTR-*294.*

Bushing, R. W. 1965. A synoptic list of the parasites of Scolytidae (Coleoptera) in North America north of Mexico. Can. Entomol. 97: 449–492.

Camors, F. B., Jr., and T. L. Payne. 1973. Sequence of arrival of entomophagous insects to trees infested with the southern pine beetle. Environ. Entomol. 2: 267-270.

Cloutier, C., and F. Bauduin. 1999. Searching behavior of the aphid parasitoid *Aphidus nigripes* (Hymenoptera: Aphidiidae) foraging on potato plants. Environ. Entomol. 19: 222-228.

Dahlsten, D. L., and C. W. Berisford. 1995. Diversity of bark beetle natural enemies. *In* F. P. Hain, S. M. Salom. W. F. Ravlin, T. L. Payne, and K. F. Raffa [eds.], Behavior, Population Dynamics and Control of Forest Insects, Proceedings of the International Union of Forestry Research Organizations Joint Conference, February 1994, Maui, HI. Ohio State University, Agricultural Research & Development Center, Wooster, OH and U.S.D.A. Forest Service, Radnor, PA.

DeMoraes, C. M., and M. C. Mescher. 1999. Interactions in entomology: plant-parasitoid interaction in tritrophic systems. J. Entomol. Sci. 34: 31-39.

Dicke, M. 1988. Microbial allelochemicals affecting the behavior of insects, mites, nematodes, and protozoa in different trophic levels, pp. 125-163. *In* P. Barbosa and D. K. Letoumeau [eds.], Novel aspects of insect-plant interactions. Wiley, New York.

Dix, M. E., and R. T. Franklin. 1981. Observations on the behavior of the southern pine beetle parasite *Roptrocerus eccoptogastri* Ratz. (Hymenoptera Torymidae). J. Ga Entomol. Soc. 16: 239-248.

Dixon, W. N., and T. L. Payne. 1979. Sequence ofarrival and spatial distribution of entomophagous and associate insects on southern pine beetle-infested trees. Tex. Agric. Exp. Stn. Bull. MP-1432.

Dixon, W. N., and T. L. Payne. 1980. Attraction of entomophagous and associate insects of the southern pine beetle to beetle- and host tree-produced volatiles. J. Ga Entomol. Soc. 15: 378-389.

Elzen, G. W., H. J. Williams, and S. B. Vinson. 1983. Response by the parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae) to chemicals (synomones) in plants: implications for host habitat location. Environ. Entomol. 126: 1873-1877.

Elzen, G. W., H. J. Williams, and S. B. Vinson. 1984. Isolation and identification of cotton synomones mediating searching behavior by the parasitoid *Campoletis sonorensis.* J. Chem Ecol. 10: 1251-1264.

Espelie, K. E., C. W. Berisford, and D. L. Dahlsten. 1996. Use of cuticular hydrocarbons in bark beetle parasitoid taxonomy: a study of *Roptrocerus xylophagorum* (Ratzeburg) (Hymenoptera: Torymidae) from the United States, Europe and Australia. Comp. Biochem. Physiol. 113B: 193-198.

Gara, R. I., R. A. Werner, M. C. Whitmore, and E. H. Holsten. 1995. Arthropod associates of the spruce beetle *Dendroctonus rufipennis* (Kirby) (Col., Scolytidae) in spruce stands in south central and interior Alaska. J. Appl. Entomol. 119: 585-590.

Geervliet, J.B.F., S. Ariëns, M. Dicke, and L.E.M. Vet. 1998. Long-distance assessment of patch profitability through volatile infochemicals by the parasitoids *Cotesia glomerata* and *C. rubecula* (Hymenoptera: Braconidae) . Biol. Control 11: 113-121.

Godfray, H.C.J. 1994. Parasitoids. Princeton University Press, Princeton, NJ.

Gouger, R. J. 1971. Interrelations of *Ips avulsus* (Eichh.) and associated fungi. Ph.D. dissertation, University of Florida, Gainesville, FL.

Goyer, R. A., and C. K. Finger. 1980. Relative abundance and seasonal distributions of the major hymenopterous parasites of the southern pine beetle, *Dendroctonus frontalis* Zimmerman on loblolly pine. Environ. Entomol. 9: 97–100.

Greany, P. D., J. H. Tumlinson, D. L. Chambers, and G. M. Boush. 1977. Chemically mediated host finding by *Biosteres (Opius) longicaudatus,* aparasitoid of tephritid fruit fly larvae. J. Chem. Ecol. 3: 189-195.

Hopf, H. S. 1937. Protein digestion of wood-boring insects. Nature (Lond.) 139: 286-287.

Hunt, D.W.A., J. H. Borden, B. S. Lindgren, and G. Gries. 1989. The role of autoxidation of α-pinene in the production of pheromones of *Dendroctonus ponderosae* (Coleoptera: Scolytidae). Can. J. For. Res. 19: 1275-1282.

Kudon, L. H., and C. W. Berisford. 1980. Influence ofhrood hosts on host preferences of bark beetle parasites. Nature (Lond.) 283: 288–290.

Langor, D. W. 1991. Arthropods and nematodes co-occuring with the *eastern* larch beetle, *Dendroctonus* simplex (Col.: Scolytidae) , in Newfoundland. Entomophaga 36: 303-313.

Leufvén, A., and G. Birgersson. 1987. Quantitative variation of different monoterpenes around galleries of *Ips typographus* (Coleoptera: Scolytidae) attacking Norway spruce. Can. J. Bot. 65: 1038–1044.

Leufvén, A., G. Bergstrom, and E. Falsen. 1988. Oxygenated monoterpenes produced by yeasts, isolated from *Ips typographus* (Coleoptera: Scolytidae) and grown in phloem medium. J. Chem. Ecol. 14: 353-362.

Leufvén, A. 1991. Role of microorganisms in spruce bark beetle-conifer interactions, pp. 467-483. *In* P. Barbosa, V. A. Krischik, and C. G. Jones [eds.], Microbial mediation of plant-herbivore interactions. Wiley, New York.

Lewinsohn, E., M. Gijzen, and R. Croteau. 1991. Defense mechanisms of conifers. Plant Physiol. 96: 44-49.

Lewis, W. J., R. L. Jones, H. R. Gross, Jr., and D. A. Nordlund 1976. The role ofkairomones and other behavioral chemicals in host finding by parasitic insects. Behav. Biol. 16: 267-289.

Madden, J. L. 1968. Behavioral responses of parasites to the symbiotic fungus associated with Sirex Noctilio. Nature (Lond.) 218: 189.

Martin, W. R., Jr., D. A. Nordlund, and W. C. Nettles, Jr. 1990. Response of parasitoid *Eucelatoria bryani* to selectedplant materialin an olfactometer. J. Chem. Ecol. 16: 499 -508.

Mills, N. J. 1983. The natural enemies of scolytids infesting conifer bark in Europe in relation to the biological control of *Dendroctonus* spp. in Canada. Biocontrol News Inf. 4: 305–328.

Mitchell, W. C., and R.F.L. Man. 1970. Response of the female southern green stink bug and its parasite *Trichopoda pennipes* to male stink bug pheromone. J. Econ. Entomol. 64: 856-859.

Moser, J. C. 1971. Relative abundance of southern pine beetle associates in east Texas. Ann. Entomol. Soc. Am. 64: 72-77.

Ngi-Song, A. J., W. A. Oversholt, P.G.N. Njagi, M. Dicke, J. N. Ayertey, and W. Lwande. 1996. Volatile infochemicals used in host and host habitat location by *Cotesia flavipes* Cameron and *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae), larval parasitoids of stemborers on Graminae. J. Chem. Ecol. 22: 307-323.

Ngi-Song A. J., and W. A. Oversholt. 1997. Host location and acceptance by *Cotesia flavipes* Cameron and C. *sesamiae* (Cameron) (Hymenoptera: Braconidae), parasitoids of African gramineous stemborers: role of frass and other host cues. Biol. Control 9: 136-142.

Ohmart, C. P., and W. G. Voigt. 1982. Temporal and spatial arrival patterns of *Ips plastographus maritimus* (Coleoptera: Scolytidae) and its insect associates on freshly felled *Pinus radiata* in California. Can. Entomol. 114: 337-348.

Parra, J.R.P., S. B. Vinson, S. M. Gomes, and F. L. Consoli. 1996. Flight response of *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) in a wind tunnel to volatiles associated with infestations of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) . Biol. Control 6: 143–150.

Payne, T. L. 1989. Olfactory basis for insect enemies of allied species, pp. 55-69. In D. L. Kulhavy and M. C. Miller [eds.], Potential for biological control of *Dendroctonus* and *Ips* bark beetles. University of Texas Press, Austin.

Pettersson, E. M., B. T. Sullivan, P. Anderson, C. W. Berisford, and G. Birgersson. 2000. Odor perception in the bark beetle parasitoid *Roptrocerus xylophagorum* (Ratzeburg) (Hymenoptera: Pteromalidae) exposed to host-associated volatiles. J. Chem. Ecol. (in press).

Reid, R. W. 1958. The behaviour of the mountain pine beetle, *Dendroctonus monticolae* Hopk., during mating, egg laying, and gallery construction. Can Entomol. 9: 505-509.

Rice, R. E. 1969. Response of some predators and parasites of *Ips confusus* (Lec.) (Coleoptera: Scolytidae) to olfactory attractants. Contrib. Boyce Thompson Inst. 24: 189–194.

Röse, U.S.R., W. J. Lewis, and J. H. Tumlinson. 1998. Specificity of systemically released cotton volatiles as attractants for specialist and generalist parasitic wasps. J. Chem. Ecol. 24: 303–319.

Samson, P. R. 1984. The biology of *Roptrocerus xylophagorum* (Hym.: Torymidae), with a note on its taxonomic status. Entomophaga 29: 287-298.

Smith, M. T., S. M. Salom, and T. L. Payne. 1993. The southern pine bark beetle guild: an historical review of the research on the semiochemical-based communication system of the five principal species. Bulletin 93-4. Virginia Agricultural Experiment Station, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry, 3rd ed. Freeman, New York.

SPSS. 1997. SigmaStat User's Guide, version 2.0, Chicago, IL.

Steinberg, S., M. Dicke, L.E.M. Vet, and R. Wanningen. 1992. Response of the braconid parasitoid *Cotesia* (=*Apanteles*) *glomerata* to volatile infochemicals: effects of bioassay set-up, parasitoid age and experience and barometric flux. Entomol. Exp. Appl. 63: 163-175.

Stephen, F. M., and D. L. Dahlsten. 1976. The arrival sequence of the arthropod complex following attack by *Dendroctonus brevicomis* (Coleoptera: Scolytidae) in ponderosa pine. Can. Entomol. 108: 283-304.

Sternlicht, M. 1973. Parasitic wasps attracted by the sex pheromone of their coccid host. Entomophaga 18: 339-342.

Sullivan, B. T. 1997. The chemical ecology of host habitat location by larval parasitoids of the southern pine beetle, *Dendroctonus frontalis* Zimmermann: Olfactory cues and their possible sources. Ph.D. dissertation, University of Georgia, Athens.

Sullivan, B. T., C. W. Berisford, and M. J. Dalusky. 1997. Field response of southern pine beetle parasitoids to some natural attractants. J. Chem. Ecol. 23: 837-856.

Sullivan, B. T., K. C. Seltmann, and C. W. Berisford. 1999. A simple continuous-rearing technique for the bark beetle parasitoid, *Roptrocerus xylophagorum* (Ratzeburg). J. Entomol. Sci. 34: 260-264.

Takacs, S., G. Gries, and R. Gries. 1997. Semiochemical-mediated location of host habitat by *Apanteles carpatus* (Say) (Hymenoptera: braconidae) a parasitoid of clothes moth larvae. J. Chem. Ecol. 23: 459-472.

Thibout, E., J. F. Guillot, and J. Auger. 1993. Microorganisms are involved in the production ofvolatile kairomones affecting the host seeking behaviour of *Diadromus pulchellus*, a parasitoid of *Acrolepiopsis assectella*. Physiol. Entomol. 18: 176-182.

Tumlinson, J. H.,T.C.J. Turlings, and W. J. Lewis. 1992. The semiochemical complexes that mediate insect parasitoid foraging. Agric. Zool. Rev. 5: 22-252.

Vet, L.E.M., W. J. Lewis, D. R. Papaj, and J. C. van Lenteren. 1990. A variable -response model for parasitoid foraging behavior. J. Insect Behav. 3: 471–490.

Vet, L.E.M., F. L. Wäckers, and M. Dicke. 1991. How to hunt for hiding hosts: the reliability-detectability problem in foraging parasitoids. Neth. J. Zool. 41: 202-213.

Vet, L.E.M., and M. Dicke. 1992. Ecology of infochemical use by natural enemies in a tritrophic context. Annu. Rev. Entomol. 37: 141-172.

Vinson, S. B. 1981. Habitat location, pp. 51-78. In D. A. Nordland, R. L. Jones, and W. J. Lewis [eds.], Semiochemicals: their role in pest control. Wiley, New York.

Vinson, S. B. 1984. Parasitoid-host relationship, pp. 205-236. *In* W. J. Bell and R. T. Carde [eds.], Chemical ecology of insects. Chapman & Hall, London.

Visser, J. H., S. Van Straten, and H. Maarse. 1979. Isolation and identification of volatiles in the foliage of potato, *Solanum tuberosum*, a host plant of the Colorado beetle, *Leptinotarsa decemlineata*. J. Chem. Ecol. 5: 13-25.

Weseloh, R. M. 1977. Behavioral responses of the parasite, *Apanteles melanoscelus*, to gypsy moth silk. Environ. Entomol. 5: 1128-1132.

Whitman, D. W., and F. J. Eller. 1990. Parasitic wasps orient to green leaf volatiles. Chemoecology 1: 69-76.

Received for publication 20 March 2000; accepted 26 July 2000.