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CHEMICALLY MEDIATED HOST FINDING BY *Biosteres (Opius) longicaudatus*, A PARASITOID OF TEPHRITID FRUIT FLY LARVAE

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Abstract—Host finding by the fruit fly parasitoid *Biosteres (Opius) longicaudatus* Ashmead was found to involve attraction to fermentation products emanating from rotting fruit, a probable site for location of host larvae. Bioassays conducted in the greenhouse with all saturated 1-, 2-, and 3-carbon primary alcohols, aldehydes, and organic acids indicated that acetaldehyde was the most active agent followed by ethanol and acetic acid. Rotting fruit was attractive irrespective of the presence of host larvae, and attraction was attributed to fungal fermentation products rather than to kairomones from host larvae.

Key Words—Host finding, *Biosteres (Opius) longicaudatus* Ashmead, ethanol, acetaldehyde, fungi, fermentation, Tephritidae, parasitoid.

INTRODUCTION

Appreciable attention has been directed recently to elucidation of the physical and chemical stimuli used in host finding by parasitic wasps, and a number of kairomones have been chemically identified (Vinson, 1975, 1976). In addition to cases involving kairomones, wherein the parasitoids respond to compounds produced by the host insects themselves, there are other instances in which parasitoids are attracted to compounds associated with their hosts but not produced by them. For example, Spradbery (1970) found that certain

parasitoids of siricid woodwasps are attracted to odors produced by fungal symbionts of their hosts, and Vinson (1975) reported that some parasitoids are attracted to odors liberated from wounded plant tissues. Although these types of host indicators cannot be regarded as true kairomones, they function similarly. There appears to be no recognized term available to describe such compounds as they do not comply with any of the definitions given by Nordlund and Lewis (1976) for various types of allelochemics.

The object of the present study was to investigate the mechanisms of host finding used by the braconid *Biosteres* (*Opius*) *longicaudatus* Ashmead. This solitary endoparasitoid attacks larvae of a variety of Tephritidae (Greany et al., 1976), normally encountering them in decomposing fruit. We obtained evidence indicating that host finding involves attraction to specific fermentation products emanating from rotting fruit. These compounds were produced not by host larvae, but by fungi growing in the fruit.

METHODS

Bioassay Techniques

Cultures of *B. longicaudatus* were maintained using larvae of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), as the laboratory host (Greany et al., 1976). Bioassays were conducted in screen cages in a greenhouse maintained at $28 \pm 4°C$ and ca. 50% relative humidity. The cages were $36 \times 36 \times 46$ cm and were constructed of aluminum frame with fiberglass window screen covering all surfaces other than the end that contained a sleeve. Candidate attractants and solvent controls (100 μl ea.) were applied to 4.3 cm-diam pieces of filter paper enclosed in evaporators. The evaporators were 20-dr snap-cap plastic vials modified by replacing the bottom with nylon netting (ca. 1-mm^2 openings) and with a 7-mm-diam orifice in the cap. Two evaporators (treatment and control) were suspended in each cage 10 cm from the top and 15 cm from either end (16 cm apart), with the netted end abutted against the upwind side of the cage. Air was drawn through the cages at a rate of 1.5 m/sec by the greenhouse exhaust fan.

Bioassays were performed with 100 5–6-day-old *B. longicaudatus* females, and at least 1 hr was allowed for acclimation prior to bioassay. The parasitoids were used not more than twice each day, with at least 4 hr between bioassays. All tests were conducted between 9:00 AM and 4:00 PM; no time-of-day effects were noted during this period. The peak number of parasitoids observed inside and on the surface of the vials within 5 min after application of material to the evaporators was recorded for both the test and control units.

Due to the fact that a simple cage was used as an olfactometer, not

all test insects were exposed to the odorants; i.e., some were located on the top and sides of the cage out of the air stream of either the treatment or control evaporator. We therefore tried to increase the proportion of the test population that was attracted by using other types of olfactometers, including Y tubes. None of these other olfactometers allowed the parasitoids to fly freely, and they proved less satisfactory than the screen cages because orientation to the odor source apparently occurred only after initiation of flight. Thus, a fairly large number of parasitoids (100) was exposed so as to obtain observable differences in capture rates between treatment and control units. Tests conducted in the laboratory failed to elicit desired responses, whereas these activities were readily displayed in the greenhouse, perhaps due to the high intensity light that prevailed there.

Microbiological

Cultures of microorganisms were obtained from rotting peaches and were isolated on acidified potato-dextrose-agar. Colonies that developed after 7 days at 22–23°C were identified by the methods of Alexopoulas (1962) and Barnett and Hunter (1972).

Chemical

Crude extracts were prepared from rotting peaches using methanol, which preliminary studies showed to be superior to less polar solvents for extraction of the attractant. The peach pits were removed and the tissue was homogenized in a blender. The homogenate was centrifuged and the supernatant held aside. The precipitate was rehomogenized in reagent-grade methanol with 25 ml/10 g tissue, and this homogenate was also centrifuged. The supernatants from both steps were combined and distilled at atmospheric pressure using an 8-cm Vigreux column. Three fractions were collected (64–66°C, 67–90°C, plus pot residue), and the activity of each was monitored by bioassay.

Gas–liquid chromatographic (GLC) analyses of the test materials were conducted using a Packard Model 804® equipped with a flame ionization detector and 2 m × 2.3 mm (ID) stainless-steel columns operated with an He carrier gas flow rate of 20 cc/min. All active distillation fractions were analyzed by GLC on Porapak Q, 80/100 mesh (Waters Associates). This column was operated either isothermally at 170°C or was programmed from 140 to 220°C at 8°/min.

Identification of the major peak in active distillation fractions was facilitated by comparing the GLC retention times of known standards with

that of the major peak, and by comparing the retention times of 3,5-dinitrobenzoate (3,5-DNB) derivatives of standard alcohols with the retention time of the derivatized unknown. Derivatives were prepared from the corresponding benzoyl chlorides according to the procedure of Shriner et al. (1956). Benzoate derivatives were analyzed by GLC on 5% OV-101 on 80/100 mesh Chromsorb G-HP; the column was operated isothermally at 200°C.

RESULTS AND DISCUSSION

In preliminary studies of host finding by *B. longicaudatus*, it was noted that the parasitoid females, but not males, were attracted to peaches that had begun to decompose either due to mechanical injury or because of infestation by Caribbean fruit fly larvae. Further studies showed that fresh, uninfested peaches were not attractive. As the presence of host larvae apparently was not necessary to produce activity, all subsequent extractions were made using rotting but uninfested fruit. These findings agree with those of Nishida and Napompeth (1974), who found that *B. longicaudatus*, *Opius oophilus* Fullaway, and *O. incisi* Silvestri were attracted to uninfested fruits as readily as to infested ones; however, they did not state whether the fruit was rotting. Nishida (1956) also demonstrated that *O. fietcheri* Silvestri was attracted to host plant tissue rather than to host larvae. Thorpe and Caudle (1938) described other instances in which parasitoids were attracted to the food plants of their hosts.

The fungus obtained from rotting fruit was identified as *Monolinia fructicola* (Wint.), known to cause brown rot in plums, apples, pears and peaches (Dunegan 1953). To establish the role of this fungus in attraction, fresh peaches were disinfected with 1% NaOCl, rinsed with sterile water, inoculated with pure colonies of *M. fructicola*, and allowed to incubate. After moderate decomposition, an extract was prepared and found to attract a mean ($\pm$SE) of $7.7 \pm 2.0\%$ of the females vs. $3.1 \pm 0.8\%$ for the control units (100 μl of each solution applied; 10 replicates; significantly different at the 1% level by χ^2 analysis). This extract was found to contain 0.13% ethanol by GLC analysis. Next, pure cultures of *M. fructicola* were incubated in potato-dextrose broth, which was then centrifuged and the supernatant bioassayed. This solution was active also, attracting a mean of $8.0 \pm 1.0\%$ of the females vs. $3.1 \pm 1.0\%$ for the controls (10 replicates, significantly different at the 1% level by χ^2 analysis). These tests suggested not only that it was the fungus that produced the attractant(s), but also that it could do so even without being incubated on fruit. A similar test was conducted using a pure culture of *Penicillium digitatum* Sacc. grown on disinfected grapefruit. This extract (0.37% ethanol by GLC) was active also ($8.2 \pm 1.0\%$ vs. $1.8 \pm$

0.5% attraction to treatment and control units, respectively), indicating that attraction was probably due to a common fermentation product(s).

Following demonstration of the role of fungi in attraction, chemical fractionation studies were performed to isolate and identify the attractant(s). Distillation fractions collected from 64 to 66°C and from 67 to 90°C were both active, but the higher boiling fraction was more active. Gas chromatography of both fractions showed a major peak with a retention time slightly greater than that of methanol, but identical to that of ethanol. Coinjection of ethanol with active fractions resulted in addition to the major peak rather than appearance of a new peak, suggesting that the peak corresponded to ethanol. Furthermore, derivatization of an active distillation fraction to the 3,5-DNB yielded a product with a retention time identical to that of a 3,5-DNB derivative of ethanol, corroborating identification of the peak as ethanol. Gas chromatography showed that the lower boiling fraction of most crude extracts contained about 0.2–0.4% ethanol, presumably carried over by codistillation with methanol, whereas the higher boiling fraction contained about 0.7–0.95% ethanol.

These studies suggested that ethanol might serve as an attractant for *B. longicaudatus* females; hence bioassays were performed using aqueous solutions of ethanol. Aliquots of 100 μl of a 50% solution attracted a mean of 8.5% of the parasitoids, whereas absolute ethanol attracted 14.5% (Table 1). When a 10% solution was tested, however, the level of response was less than expected (Table 1), and no significant difference was detected between treatment and control units. The ethanol concentration of crude extracts averaged less than 1% by GLC analysis, yet they were more attractive to the parasitoids than 10% ethanol, indicating involvement of additional components in the active fractions Tests were therefore conducted on 1-, 2-, and 3-carbon saturated alcohols, aldehydes, and organic acids. Of these, acetaldehyde was found to be ca. 50 times as attractive as ethanol, with 1% acetaldehyde capturing as many parasitoids as 50% ethanol (Table 1). Acetic acid also showed some activity (Table 1). Attraction was not observed in response to 10 or 50% methanol or propanol, 0.1 or 1.0% formaldehyde or propionaldehyde, or 0.1 or 1.0% formic or propionic acid. Although attempts were not made to isolate acetaldehyde or acetic acid from the crude extracts tested in the present study, oxidation of ethanol to acetaldehyde in fruit is a well-known phenomenon (Davis, 1970), and acetaldehyde and acetic acid have previously been reported from peaches (Power and Chestnut, 1921).

Ethanol has been reported as an attractant for a number of other insects, including the scolytid beetle *Trypodendron domesticum* (L.) (Kerck, 1972). Other instances of attraction to ethanol and acetic acid are cited by Dethier (1947). Acetaldehyde was reported to be slightly attractive to the codling

TABLE 1. RESPONSE OF *Biosteres longicaudatus* FEMALES TO CERTAIN FERMENTATION PRODUCTS[a]

Concentration (%)	No. trials	No. parasitoids counted[b]				
		Total[c]			Mean[d]	
		Treatment		Control	Treatment	Control
Ethanol						
10	9	24	(NSD)	14	2.7±0.6 (a)	1.6±0.3 (a)
50	17	152	(**)	18	8.9±0.7 (b)	1.1±0.3 (a)
100	10	145	(**)	14	14.5±2.7 (c)	1.4±0.3 (a)
Acetaldehyde						
0.01	9	41	(*)	24	4.6±0.7 (a)	2.7±0.5 (a)
0.1	10	73	(**)	3	7.3±0.7 (b)	0.3±0.1 (b)
1.0	13	113	(**)	15	8.7±0.6 (b)	1.2±0.3 (b)
Acetic acid						
1.0	10	32	(**)	19	3.2±1.0 (a)	1.9±0.7 (a)
10	10	38	(**)	14	3.8±0.6 (a)	1.4±0.4 (a)

[a] Each evaporator contained 100 μl of an aqueous dilution of the candidate attractant (treatment unit) or distilled water (control unit).
[b] Peak number of *B. longicaudatus* females present during 5-min bioassay period; each cage provisioned with 100 females.
[c] Treatment and control totals significantly different at the 5% (*) or 1% (**) level by χ^2 analysis (NSD = not significantly different).
[d] Means followed by different letters in the same column for a given compound are significantly different at the 1% level by Duncan's new multiple range test.

moth, *Laspeyresia pomonella* (L.) (Eyer and Medler, 1940), but no other published accounts of attraction to acetaldehyde were discovered.

Field trials are needed to verify the attractiveness of ethanol, acetaldehyde, and acetic acid alone and in combination upon presentation to a wild population of *B. longicaudatus*. While additional compounds and physical cues may be used in host finding by *B. longicaudatus* females, the findings of the present study agree well with our field observations in which *B. longicaudatus* females were seen probing for hosts only on fruits which had begun to decompose. Upon inspection, these fruits generally contained nearly mature host larvae, the preferred stage. Considering the number of tephritid species attacked by *B. longicaudatus* (Greany et al. 1976), and the even wider host plant ranges of these fruit flies, it is significant that the parasitoids apparently use fermentation products as host indicators. These compounds would normally be associated with infested fruit, irrespective of the species of host or host plant.

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