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EVIDENCE FOR ALLELOCHEMICAL ATTRACTION OF THE COFFEE BERRY BORER, *Hypothenemus hampei*, BY COFFEE BERRIES

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Abstract—Petri dish choice tests conducted on the coffee berry borer (CBB), *Hypothenemus hampei*, showed that females were able to discriminate between coffee berries at different ripening stages. A Y-shaped glass olfactometer was used to demonstrate that coffee berries emitted volatile chemicals that elicited upwind movement by female CBB. Olfactometer tests with three different solvent extracts of berries showed that at least some of the attractive chemical(s) released by the coffee berries could be extracted with acetone.

Key Words—Coleoptera, Scolytidae, *Hypothenemus hampei*, host selection, kairomones, olfaction, *Coffea* sp.

INTRODUCTION

The coffee berry borer (CBB), *Hypothenemus hampei* Ferrari (Coleoptera: Scolytidae) feeds and develops exclusively in the berries of coffee, especially of the species *Coffea canephora* Pierre. Since the species was first described in 1867 in Uganda, *H. hampei* has spread to most of the world's major coffee-producing areas. The last significant coffee-growing countries not yet infested are Costa Rica, Papua New Guinea, and Hawaii. The CBB is responsible for significant

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crop losses with infestation levels up to 60–80% per crop being reported (Schmitz and Crisinel, 1957; Le Pelley, 1968; Reid and Mansingh, 1985). In New Caledonia, CBB is the major pest of coffee. Control of this pest in New Caledonia can be difficult because it has developed resistance to endosulfan (Brun et al., 1989). In the two major coffee-growing areas of the territory, 94% and 100% of the surveyed CBB populations had resistant individuals (Brun et al., 1990). The spread of the resistant populations is of concern, and new methods to monitor this pest need to be developed.

Surprisingly, little is known about its biology considering the economic significance of this pest. Most of the work on this species has been concerned with its developmental cycle (Bartra et al., 1981; for a review see Borbon-Martinez, 1989). At about 12 days after eclosion, gravid females leave the berry and disperse to colonize and oviposit in new berries. Recently, evidence for parthenogenic reproduction has been reported (Munoz, 1989). Females are able to colonize berries throughout the year, when the harvest is incomplete. Some berries remain on the plant (Baker, 1984). Most of these berries dry on the plant, but some green and red berries can develop.

In contrast with other Scolytidae, little is known about the chemical ecology of CBB, particularly the factors that influence host colonization. Corbett (1933) and Morallo-Rejesus and Baldos (1980) reported that red, ripe berries are preferentially attacked over less ripe berries also present in the field. Morallo-Rejesus and Baldos (1980) dismissed the importance of visual cues in this preferential attack, arguing that insects are usually blind to red. They concluded that other factors, such as odors of ripe berries, may aid the insect in host choice. As a basis for developing a monitoring system for CBB based on host location, we were interested in determining how CBB females recognize a suitable host; i.e., are olfactory or visual cues involved in colonization by CBB females or is colonization of the host plant a result of random search by females. The present study describes laboratory tests determining whether volatile chemicals emitted from different stages of coffee berries are involved in host location by CBB females.

METHODS AND MATERIALS

Insects. Infested berries were collected in the field (Sarramea Valley, New Caledonia) and stored in small black-painted plastic containers in the laboratory, under ambient conditions (26 ± 2°C and relative humidity 80–85%). The positive phototactic response exhibited by female CBB leaving berries was used for collection of CBB females. A transparent plastic tube was connected to the black container so that females that had come out of the berries would walk into the tube. Females were used in experiments within 1 hr after they had left the berry.

Age (after emergence from the pupae) of adult females used for the experiments was approximately 12 days.

Host Plants. Berries were collected at Sarramea 3 hr before each test, from two clones of *C. canephora* var. *robusta* Linden: the primarily produced clone (HB) and a pollinator clone (H 865).

Extracts. Immediately after they were collected, sets of 35 berries were soaked in 70 ml of either hexane, acetone, or ethanol. The berries were left at −18°C for 24 hr in the solvent. Extracts were used without any further purification or condensation.

Choice Bioassay. These bioassays were conducted in plastic Petri dishes (9 cm ID) under ambient laboratory conditions. Natural light was provided through a window and was used to elicit motion. Maximum female flight activity occurs at the beginning of the afternoon (Morallo-Rejesus and Baldos, 1980; Baker, 1984), and therefore the tests were conducted during this period. The Petri dishes were placed at the same distance from the light source. Four different categories of berries were tested: two green, one red, one brown. The two different types of green berries were distinguished by endosperm characteristics: mature green berries have vitrified endosperm, whereas immature berries do not. Red berries are ripe, and brown berries have dried on the trees.

For each test, two different categories of berries were placed on a filter paper, 6 cm apart and perpendicular to the natural light source. The position of each type of berry was reversed after each test and the filter paper renewed. A single female was placed in the middle of a Petri dish, equidistant from the berries. After 3 hr, the test was stopped and berries were checked for infestation. As females do not lay eggs until two days after entry into berries, we used signs of boring activity into berries as a measure of successful colonization rather than the presence of eggs. Females were tested once and each comparison was replicated at least 139 times.

Olfactory Tests. These were conducted in a Y-shaped glass olfactometer (0.5 cm ID for each part of the Y, 40° angle between the arms, length of the main part 5 cm, length of each arm 4 cm) connected to two vertical open containers (2 cm ID) constructed of PVC. The berries, or dental cotton impregnated with 3 ml of extract [i.e., 1.5 berry equivalents (BE)], were placed into one of the PVC tubes and the control (solvent only) placed in the other. The airstream passed through the sources, sucked by a pump providing an airflow of about 1.8 m/sec in the main part of the Y tube. The light conditions were as described previously. Tests were conducted as 26 ± 2°C and 80 ± 10% relative humidity.

The solvent was allowed to evaporate from wicks before the wicks were tested. Tests were run for 20 min following introduction of stimuli and of a single insect. After 20 min, the arm in which the female was located was recorded. About 30% of the tested females did not move into either arm; data on these females were not included in the statistical analysis. A female released at the extremity of the main arm and reaching the far end of the odorous arm was scored as a positive response. After five replications of each combination of stimuli, the tubes were cleaned in solvents and the positions of the sources reversed. The same tests were then replicated another five times. Chi-squared tests, assuming an equal distribution of females in both arms, were used for statistical analysis of the data.

RESULTS AND DISCUSSION

In the Petri dish experiments, females exhibited distinct preferences for different phenological stages of the coffee berry (Table 1). The proportion of females that did not attack berries was relatively constant for all choice tests and was always less than 10% of the tested insects. These females remained hidden and did not appear to react to the light. Females showed a preference for red berries over mature green berries ($\chi^2 = 31.7$; $df = 1$), for red berries over dry berries ($\chi^2 = 32.8$; $df = 1$), and for dry over immature green berries ($\chi^2 = 18.1$; $df = 1$). In a comparison of the two types of green berries, females showed a preference for mature over immature berries ($\chi^2 = 7.7$; $df = 1$). Field observations (Giordanengo, 1992) indicate that CBB females only oviposit in red berries or in green berries with at least a vitrified endosperm. Nevertheless, they can bore into and inhabit less ripe berries. It has been suggested that feeding upon immature green berries permits adult females to survive until ripe

TABLE 1. CHOICE TESTS IN PETRI DISHES AND LEVEL OF BERRY INFESTATION WHEN SUBMITTED TO CBB FEMALES AT DIFFERENT STAGES OF BERRY MATURATION[a]

Females infesting green immature berries	Females infesting green mature berries	Females infesting fresh red berries (tested within 4 hr of collection)	Females infesting dry berries	Females infesting red berries collected 24 hr ago	Unresponsive females	χ^2, $df = 1$
48	78				13	7.7
	34	101			9	31.7
		101	33		10	32.8
42			92		10	18.1
		88		50	6	10.3

[a]Two different maturation stages of berries were placed at equal distances from a single female. Duration of the test was 3 hr. Green immature berry: aquous endosperm; green mature berry: vitrified endosperm; red berry: mature endosperm; dry berry: dry endosperm.

berries are available for oviposition (Waterhouse and Norris, 1989). However, we found that a gradient of preference in the choice of berries appeared to be related with the degree of maturity of coffee berries. The ability to discriminate between different types of berries could ensure with low expenditure, the choice of a suitable host to establish a new colony. In the final Petri dish experiment (Table 1), red berries tested within 4 hr of collection were preferred ($\chi^2 = 10.3$; $df = 1$) by females than were red berries tested 24 hr after collection. This decrease in attractiveness of coffee berries with time after collection suggested to us that one of the factors involved in the host colonization process may be chemical and that the chemical(s) involved is either volatile (and therefore rapidly evaporates following collection) or labile. This could be advantageous for colonization, since a volatile or labile nature would reduce the likelihood of adsorption of the chemical(s) on the foliage thereby confusing location of the berries.

To test our hypothesis that volatile chemicals and olfaction are involved and perceived by CBB females, an airflow carrying odors of red berries was tested in the Y-shaped olfactometer against a blank stimulus. This experiment showed that volatile chemicals emitted by red coffee berries do influence CBB females. Thus, significantly ($\chi^2 = 25.9$; $df = 1$) more females were found in the end part of arm baited with red berries than in the control arm (Table 2). Most of the tested females walked back and forth through the main part of the olfactometer before entering the arm and walking directly to the source. This movement may be considered as upwind progress, mediated by an anemotactic response. Further results, paralleling those of Petri dish bioassays, showed that the effluvium of red berries in the olfactometer was more attractive ($\chi^2 = 11.5$; $df = 1$) to female CBB than the effluvium of green berries (Table 2).

Due to a shortage of uninfested red berries at the time the experiments involving extracts of berries were conducted, only green berries were used for the olfactometer tests with extracts. Of the three extracts tested, only acetone elicited significant ($\chi^2 = 8.45$; $df = 1$) upwind progress of females in the olfactometer (Table 3).

While our study has shown that volatile chemicals emitted by coffee berries

TABLE 2. RESPONSES OF CBB FEMALES TO EFFLUVIA RELEASED BY DIFFERENT MATURATION STAGES OF COFFEE BERRIES TESTED IN Y-SHAPED OLFACTOMETER

5 red berries	5 green berries	Blank	Unresponsive females	χ^2, $df = 1$
43		7	9	25.9
37	13		7	11.5

TABLE 3. RESPONSES OF CBB FEMALES TO DIFFERENT EXTRACTS OF COFFEE BERRIES TESTED IN Y-SHAPED OLFACTOMETER[a]

2.5 BE, Solvant acetone	2.5 BE, Solvant hexane	2.5 BE, Solvant alcohol	Blank	Unresponsive female	χ^2, $df = 1$
53			27	16	8.45
	45		35	27	1.25
		45	35	23	1.25

[a](BE: berry equivalent).

may be involved in host colonization by female CBB, we have not tested the importance of visual cues such as color and shape in this process. It is possible that female CBB use a combination of these two sensory modalities during host colonization (Prokopy, 1986). Attraction of CBB females to light is related to flight and walking activity but can be switched by the availability of volatiles that act on host choice, as shown in the Petri dish tests. The number of females that did not react during the different tests could be explained by accidental suppression of moving activity, as suggested by Wellington (1980). In CBB, the transition from phototactic orientation to vegetative orientation appeared similar to that previously described for bark beetles (Borden et al., 1986) and for a species of Nitidulidae (Blackmer and Phelan, 1991).

The host colonization phase represents a vulnerable step for *H. hampei*, but orientation of adult females to a suitable host seems to be facilitated by kairomone-like chemicals produced by the berries. The CBB females oriented and walked to odor released by red berries over a short distance. Identification of the volatile compounds involved in host colonization by female CBB is being undertaken. Although our results did not show that a long-range orientation flight mediated by red berry odors occurs, the use of plant odor may eventually allow the development of a field trapping method for monitoring this pest.

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