CHEMICALLY-MEDIATED ATTRACTION OF THREE PARASITOID SPECIES TO MEALYBUG-INFESTED CASSAVA LEAVES

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ABSTRACT

We investigated whether cassava plants that are infested by the cassava mealybug, Phenacoccus herreni (Pseudococcidae, Sternorrhyncha), emit attractants for the encyrtid parasitoids Aenasius vexans Kerrich, Apoanagyrus (Epidinocarsis) diversicornis Howard, and Acerophagus cocois Smith. Bioassays with a Y-tube olfactometer showed for all three species that female wasps were most responsive and selective when they were 1.5 to 2.5 days old. Females of these age groups were used to test their ability to distinguish between the odor of plants with and without mealybugs. The wasps were offered choices between infested cassava leaves vs. healthy ones, infested leaves vs. clean air, and healthy leaves vs. clean air. A. vexans and A. diversicornis were strongly attracted to infested leaves and preferred these over healthy ones. In contrast, A. cocois was significantly attracted to either healthy or infested leaves, and did not distinguish between the two. The results suggest that A. cocois, which has the broadest known host range of the three, may be responsive only to general plant odors, while A. vexans and A. diversicornis respond more specifically to odors associated with mealybug infestation.

Key Words: Aenasius vexans, Apoanagyrus (Epidinocarsis) diversicornis, Acerophagus cocois, cassava (Manihot esculenta), host location, semiochemicals

RESUMEN

Se investigó si las plantas de yuca que son infestadas por el piojo harinoso, Phenacoccus herreni (STERNORRHYNCHA: Pseudococcidae), emiten sustancias atractivas para los parasitoides Encyrtidae Aenasius vexans, Apoanagyrus (Epidinocarsis) diversicornis y Acerophagus cocois. Ensayos con un tubo olfactómetro en Y mostraron que las tres especies tienden a responder y seleccionar más frecuentemente cuando tienen de 1.5 a 2.5 días de edad. Las hembras de esta edad fueron usadas para determinar su capacidad de distinguir entre el olor de plantas con y sin piojos. Se ofreció a las hembras olores de yuca infestadas o limpias, hojas infestadas o aire puro y hojas limpias o aire puro. A. vexans y A. diversicornis fueron atraídas fuertemente por las hojas infestadas y presentaron preferencia por estas hojas contra las hojas limpias. En contraste, A. cocois fue atraído de manera significativa por hojas limpias u hojas infestadas contra aire, y no pudo distinguir entre ambos olores. Los resultados sugieren que A. cocois, que tiene el más alto rango de huéspedes de los tres, puede responder sólo a los olores generales de las plantas, mientras A. vexans y A. diversicornis responden más específicamente a los olores asociados con la presencia de los piojos harinosos.
Cassava mealybugs are among the most damaging pests of cassava in South America and Africa (Vargas & Bellotti, 1984). The two most important species are *Phenacoccus herreni* Cox & Williams and *P. manihoti* Matile-Ferrero (Sternorrhyncha: Pseudococcidae), which both originate from South America (Cox & Williams, 1981; Bellotti et al., 1984). *P. herreni* appeared as a problem rather suddenly in Northeast Brazil in the mid-1970s and was then reported from Colombia, Venezuela and Guyana (CIAT, 1984; 1987; 1988; 1990); it can cause root yield losses up to 80% (Bellotti et al., 1984; Bellotti, 1983). In Africa, the closely related *P. manihoti* became a serious pest in most of the cassava growing regions after its accidental introduction in the 1970s (Matile-Ferrero, 1977; Herren & Neuenschwander, 1991). For both pest species biological control programs have been developed. The encyrtid parasitoid *Apoanagyrus* (Epidinocarsis) *lopezi* (De Santis) was successfully released in African in the 1980s. It established and now maintains the mealybug population at an acceptable low-density in most regions (Herren & Neuenschwander, 1991; CIAT, 1992). For the 5% of the African cassava fields where the parasitoid has not been effective in controlling the mealybug (Neuenschwander et al., 1991), alternative control agents were investigated such as two strains of the coccinellid predator *Hyperaspis notata* Mul-sant (Stäubli Dreyer et al., 1997a; 1997b; 1997c).

Natural enemies of *P. herreni* have been systematically collected for the control of the mealybug in South America, and laboratory colonies of three encyrtid parasitoids were established at CIAT (Centro Internacional de Agricultura Tropical), in Cali, Colombia. These parasitoids are *Aenasius vexans* Kerrich, *Apoanagyrus* (Epidinocarsis) *diversicornis* Howard (asexual strain) and *Acerophagus coccis* Smith (CIAT, 1982; 1983; 1990). Knowledge on the biology of these insects is limited. Published information is mostly restricted to CIAT reports (1982-1992).

At the beginning of this century, studies showed that parasitic wasps use olfaction to locate hosts and that they may first be attracted to the food that their hosts feed on (Picard & Rabaud, 1914; Thorpe & J ones, 1937; Thorpe & Caudle, 1938). More recently, it was demonstrated that herbivore-damaged plants can play a key role in attracting enemies of insect herbivores (Dicke et al., 1990; Turlings et al., 1990; 1995; Vet & Dicke, 1992). For example, lima bean plants under spider mite attack release specific volatiles that are attractive to predatory mites (Dicke et al., 1990) and similar volatile compounds released by caterpillar-infested maize plants are used by parasitoids to locate caterpillars (Turlings et al., 1990).

Volatiles emitted by mealybug-infested plants are also suspected to attract natural enemies of the mealybug (Nadel & van Alphen, 1987). Changes in chemicals produced by the cassava plant due to *P. manihoti* infestation have been reported by Calatayud et al. (1994). Such changes could result in the emission of volatiles and explain why *A. lopazi* and *A. diversicornis* (sexual strain) are attracted by *P. manihoti*-infested cassava plants (Nadel & van Alphen, 1987; van Alphen et al., 1990). The feeding behavior of *P. herreni* is very similar to that of *P. manihoti* (Castillo & Bellotti, 1990), and it can be expected that they evoke similar reactions in the cassava plant. However, studies with the asexual strain of *A. diversicornis* of South America by Hof-stee et al. (1993) showed no response by this parasitoid to the odor of *P. herreni*-infested cassava plants (var. Odungbo). A better understanding of the interactions between cassava plants, mealybugs, and parasitoids requires more behavioral as well as chemical studies.

In this paper, we report on olfactometer studies with the three encyrtid parasitoids reared at CIAT, *A. vexans*, *A. diversicornis* (asexual strain), and *A. coccis*. The studies were conducted to determine whether these parasitoids are attracted to odors that may emanate from cassava plants infested by *P. herreni*. 
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**M**ATERIALS AND **M**ETHODS

**Plants**

CMC40 cassava stakes (20 cm long) were planted every week in pots and kept in a screened compartment, where they were subjected to natural weather conditions at Palmira, Colombia, though protected from rain. The plants were used in experiments when they carried 10-30 leaves (approximately 6 weeks after planting).

**Insects**

The cassava mealybug, *P. herreni* was reared at CIAT on potted cassava plants (var. CMC40). Every week 30-40 cm high plants were infested with 15 mealybug ovisacs, as described by van Driesche et al. (1987). The plants were separated in different cages based on the age of the mealybugs they carried.

The parasitoids, *A. vexans*, *A. diversicornis* and *A. coccois* were continuously reared at CIAT on mealybug-infested cassava plants (var. Mcol 1505). The colonies of *A. vexans* and *A. coccois* were initiated with insects collected in Venezuela in 1990 and the colony of *A. diversicornis* with insects from Colombia (1984). The colonies were maintained in a greenhouse at 35°C and under natural light conditions.

**The Olfactometer**

A Y-tube olfactometer similar to the one first described by Sabelis & van de Baan (1983) was used in our experiments (Fig. 1). Two arms of a glass Y-shaped tube were connected to glass chambers (6.5 cm diam.) in which odor sources could be placed. Activated charcoal filtered air at a rate of 400 ml/min was pushed into each glass chamber. To avoid visual distractions and to diffuse the light, a wooden frame covered with white cloth was placed around the Y-tube. A lamp (100 watt) was placed outside this visual barrier opposite from the entrance where the insects were introduced. As these parasitoids are attracted by light, the lamp helped to induce the insects to walk upwind in the direction of the odor sources. When a female reached the center of the Y-tube, where the three arms met, she could choose one of the offered odors.

**Odor Sources**

Every week ovisac-infested cassava plants were transferred into a greenhouse, where they were kept in nylon cages for three weeks before being used for the Y-tube experiments. Control (healthy) plants were transferred weekly from the screened compartment and enclosed in a nylon screen cage in the same greenhouse as the infested plants. Care was taken that no mealybugs came in contact with healthy plants. To serve as an odor source, two leaves of either infested or healthy plants were cut off and the cut ends were wrapped in wet cotton wool. The leaves were carefully placed in one of the odor chambers. The infested leaves that were selected carried honeydew and sooty mold, as well as mealybugs and exuviae.

**Experimental Procedure**

On the day of each experiment, parasitoid females were removed from their cage and kept in a glass jar (400 ml) with some honey. The jar was placed in the air-conditioned chamber (28-30°C) where the experiments would take place. The insects were left one or two hours in their new environment to become adjusted. Before each olfactometer test, female parasitoids were allowed to parasitize a mealybug on a cassava leaf. An infested cassava leaf was placed upside down in a petri dish and several fe-
males were introduced and observed until they had parasitized, or at least stung a mealybug. The parasitoids were given this experience as it may increase their responsiveness to host-related odors (Turlings et al., 1993; Vet et al., 1995, Steinberg et al., 1992). The parasitoids were then captured in a gelatin capsule and kept there for 10 to more than 60 minutes. Before each Y-tube test, the gelatin capsule was opened and inserted at the base of the Y-tube. Females were introduced and were observed indi-
vidually in the olfactometer and used only once. The odor sources were reversed each time three wasps had been tested.

Evaluation of Choices

A stopwatch was started when the insect left the gelatin capsule. The female was allowed 5 minutes to walk up the no-choice-area (Fig. 1) to reach the center of the olfactometer, which is the area where the three arms meet. If a female did not reach this center within 5 minutes, she was counted as a “no-choice”. For the other females, the observation was stopped 5 minutes after they had made it to the center, or after they had reached the end of one of the arms. Each arm, was divided into four zones (Fig. 1), which measured 8, 6, 6, and 3 cm, respectively.

A female had to enter at least zone 2 (Fig. 1) to be considered to have made a choice. A few females switched arms after reaching zone 2. In those cases, females were considered to choose the arm which they entered the furthest. For statistical analyses, a chi-square test was applied, using the total number of females that made a choice for a particular odor ($\alpha = 0.05$).

PROCEDURES AND RESULTS

The Effect of Wasp Age

It has been shown that the responsiveness to odors may change when parasitoids get older (e.g. Thorpe & Caudle, 1938; Steinberg et al., 1992). To determine the optimal age of our parasitoids for olfactometer bioassays, parasitoid females of different ages were tested. Newly emerged wasps were isolated daily at about noon and transferred to Plexiglas® cages in which they were provided honey and water. The insects remained in the cage until they had reached a certain age. Six different age classes were tested, varying from 0.5 to 6.5 days after emergence. Each wasp was given an oviposition experience, and then introduced into the olfactometer, in which they had a choice between the odors of infested and healthy cassava leaves.

Responsiveness, i.e. proportion of females that made a choice, did not decrease with increasing age of females. Overall it was high for A. diversicornis with an average of 73% and medium to low for both A. vexans and A. coccois with an average of respectively 49 and 48% of the responding females.

Preference for an odor source changed in two of the three species (Fig. 1-3). In A. vexans and A. diversicornis, the preference for the odor of infested leaves over odor of healthy leaves was age dependent and significant for young females only. Of the younger (1.5-2.5 d old) A. vexans females, 80% preferred infested cassava leaf odors ($\chi^2 = 7.2, P < 0.01$). The youngest A. diversicornis (0.5-1.5 d) showed the clearest preference (82.6%) for the odor of infested leaves over the odor of healthy leaves ($\chi^2 = 9.78, P < 0.005$), but 17.4% of the females that made a choice switched between arms before making a final decision. The 1.5 to 2.5-day-old A. diversicornis switched arms much less (3.8%), but exhibited a weaker preference for odors of infested leaves (69.2%, $\chi^2 = 3.05, P < 0.05$). The older wasps showed no significant preference. All age classes of A. coccois did not differentiate between infested and healthy plant odors. Like A. diversicornis, A. coccois walked a lot in the olfactometer, often switching between arms (26.3% of the choosing females).

The Role of Plant Odors

In a subsequent series of experiments we more specifically determined the relative attractiveness of healthy and infested cassava leaves. Based on the results of the pre-
Fig. 2. Age dependency of response. Choices by A. vexans females of different age classes between the odors of mealybug-infested and healthy cassava leaves in a Y-tube olfactometer. In each bar the actual number wasps that made a particular choice is given, while the x-axis indicates the percentages of choosing wasps that the numbers represent. To the right of the bars is the proportion of females that made a choice for one of the two odors, as well as the total number of females that were tested per age class.

\( * \chi^2, P<0.05 \)
\( ** \chi^2, P<0.01 \)
\( *** \chi^2, P<0.001 \)
Fig. 3. Age dependency of response. Choices by A. diversicornis females of different age classes between the odors of mealybug-infested and healthy cassava leaves in a Y-tube olfactometer. In each bar the actual number wasps that made a particular choice is given, while the x-axis indicates the percentages of choosing wasps that the numbers represent. To the right of the bars is the proportion of females that made a choice for one of the two odors, as well as the total number of females that were tested per age class.
Previous experiments, only females that were 1.5 to 2.5 days old were used. On a given day three different pairs of odor sources were tested, namely “Infested vs. Healthy”, “Infested vs. Blank”, and “Healthy vs. Blank”. In the case of “Blank”, one arm introduced clean air that had passed through an odor chamber with just a piece of wet cotton wool. For each pair of odor sources, 4 to 6 insects per day were individually tested in the Y-tube. Occasionally, another series of 6 insects per odor source was tested the same day.

*A. vexans* females were significantly attracted to infested cassava leaves compared to healthy ones or a blank (Fig. 5a). Healthy leaf odors were less attractive, since only 64.5% of the females responded in the “Healthy vs. Blank” test without showing a significant preference for one of the two odor sources ($\chi^2 = 2.5$, $P > 0.05$).

*A. diversicornis* females were significantly attracted to infested and healthy cassava leaves when offered against a blank. They also showed a significant preference for infested cassava plant odors over healthy ones ($\chi^2 = 6.08$, $P < 0.025$).

Only 51.7 to 58.3% of *A. coccoides* females made a choice, but these were significantly attracted by healthy and infested plant odors when offered against a blank ($\chi^2 = 7.53$, $P < 0.01$ and $\chi^2 = 11.65$, $P < 0.001$). In the “Infested vs. Healthy” test, the choosing females very often switched sides before going up one arm, and they showed no significant preference for either odor source ($\chi^2 = 0.26$, $P > 0.05$) (Fig. 5c).

**DISCUSSION**

The preference of female wasps to plant odors in the olfactometer was age dependent for *A. vexans* and *A. diversicornis*. The younger age classes of both these species significantly preferred the odor of infested leaves, while older females showed no particular preference. The preferences exhibited by young *A. vexans* and *A. diversicornis* may have been due to the experience that the wasps received with an infested leaf just before their introduction into the olfactometer. During such an experience the females may learn to respond to the odors that they encounter through a process of association (Turlings et al., 1993; Vet et al., 1995), which may be age dependent. Some parasitoids only learn as young adults (Kester & Barbosa, 1991), which could explain why older wasps did not make a distinction in our tests. It is possible that if these older wasps had been given an experience at a younger age, they would have shown a preference as well. In the subsequent experiments only younger females were used.

For *A. coccoides*, the lack of preference of females of any age class may be due to the particular choice offered. This species obviously did not distinguish between infested and healthy cassava leaves. An alternative choice, such as between plants and a blank might have revealed a similar age dependency of the response as found for the two other species.

All three species distinguished between plant material and clean air (blank). *A. vexans* showed only a marginal attraction to healthy leaves, but was strongly attracted to infested leaves. *A. diversicornis* was attracted to healthy leaves, but preferred the odor of infested leaves. *A. coccoides* was also attracted to both healthy and infested leaves, but did not distinguish between these two odor sources. These differences in response of the three encyrtid parasitoids suggest that they may employ different foraging strategies. *A. vexans* and *A. diversicornis* recognized odors that are specifically associated with mealybug infestation. *A. coccoides*, on the other hand, appeared to respond only to general cassava plant odors. It remains unknown if *A. vexans* and *A. diversicornis* are attracted to odors emanating directly from the mealybugs or if the infested plants emit the attractive odors.

In the petri dish, where females were experienced by giving them the opportunity to walk over a cassava leaf and sting a mealybug, *A. vexans* walked slower, but showed a more direct orientation towards mealybugs. This slower, but directed searching be-
Fig. 4. Age dependency of response. Choices by A. cocusis females of different age classes between the odors of mealybug-infested and healthy cassava leaves in a Y-tube olfactometer. In each bar the actual number wasps that made a particular choice is given, while the x-axis indicates the percentages of choosing wasps that the numbers represent. To the right of the bars is the proportion of females that made a choice for one of the two odors, as well as the total number of females that were tested per age class.
Fig. 5. Responses of the three parasitoid species. (A) *A. vexans*, (B) *A. diversicornis* and (C) *A. coccoides* in a Y-tube olfactometer. The wasps were offered choices between the odors of clean air vs. healthy leaves, clean air vs. infested leaves, and healthy leaves vs. infested leaves. In each bar the actual number wasps that made a particular choice is given, while the x-axis indicates the percentages of choosing wasps that the numbers represent. Next to the bars the proportion is given of the females that made a choice for one of the two odors, as well as the total number of females that were tested per choice.
havior was also observed in the olfactometer. *A. vexans* was clearly attracted to the infested cassava plants, but not to the odors of healthy plants. After it found a mealybug, this solitary parasitoid needed only a few seconds to parasitize it. *A. coccois* is gregarious and took up to an hour to parasitize a host. It spent a lot of time walking rapidly around the petri dish and had fewer encounters with mealybugs. Also in the olfactometer, this species walked much faster and in more different directions than the other species, particularly when the females were given the choice between infested and healthy plant odors. This fast moving species did not readily distinguish between the odors of infested and healthy leaves.

The reported host preference of these parasitoids may explain their behavior in the olfactometer to some extent. *A. vexans* prefers *Phenacoccus herreni* over a related species, *P. gossypi* (=madeirensis) (CIAT, 1990). It has been most frequently recovered from *P. herreni* on cassava, but its host range does include other *Phenacoccus* species on different plants (Noyes & Ren, 1995). Pijls & van Alphen (1996) studied the specificity of a sexual strain of *A. diversicornis* on cassava. It appears to be specific to *P. herreni* and *P. manihotii*. An asexual strain from Venezuela has been shown to prefer *P. herreni* over *P. gossypi* (=madeirensis) (Van Driesche et al., 1987). *A. coccois* seems to be the most polyphagous of the three. It parasitizes Pseudococcidae species of different genus such as *Oracella acuta* (Homoptera: Pseudococcidae) on loblolly pine (*Pinus taeda* L.) (Clarke et al., 1990). On cassava plants, it parasitizes *P. herreni* and *P. madeirensis* more or less equally (CIAT, 1990). As a generalist, *A. coccois* may be more responsive to general plant odors, while the more specialized wasps, *A. vexans* and *A. diversicornis*, may have adapted to exploit odors that are specifically associated with the presence of mealybugs on cassava.

It remains to be determined if cassava volatiles play an important role in the specific attraction to infested plants, or if the mealybug and its by-products emit odors that are attractive. It is known that some herbivores induce reactions in plants that make them highly attractive to some parasitic wasps (Turlings et al., 1995). Nadel & van Alphen (1987) found evidence that mealybug-infested cassava plants also release odors that are attractive to the parasitoid *A. lopezi*. The odors probably come from the plant itself, as the parasitoid was not attracted by the mealybug and its by-products. Van Alphen et al. (1990) also found an attraction to *P. manihotii*-infested cassava plants by *A. diversicornis*. Unlike our results, the females were not attracted by healthy cassava plants but showed a clear attraction to uninfested leaves taken from a partially infested plant, which suggests that the infested plant emits attractants.

Little is known about the exact source and identity of parasitoid attractants. Our ongoing experiments aim to determine the exact role of the cassava plant in the foraging success of the parasitoids in order to consider and exploit this role in further control measures against the cassava mealybug.

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