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# Diversified Cassava Agroecosystems : Chemically Mediated Searching Behaviour of Parasitoids

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# 1. SUMMARY

The aim of this study was to investigate how odours of bush bean plants planted together with cassava affect the foraging behaviour of encyrtid parasitoids of the cassava mealybug, *Phenacoccus herreni* Cox & Williams (Sternorrhyncha: Pseudococcidae). It is known that plants under herbivore attack may release specific volatiles that can be used as host location cues by parasitoids, but details for our insect species as well as the possible interference with intercropped non-host plants were unknown.

In a first step, the three encyrtid parasitoids *Aenasius vexans* Kerrich, *Apoanagyrus (Epidinocarsis) diversicornis* Howard and *Acerophagus coccois* Smith, were tested in a Y-tube olfactometer to see if they respond to cassava plant odours. It was first determined that the three female parasitoid species respond best to odours when they were 1.5 to 2.5 days old, which was the age class that was used for subsequent experiments. All three species were significantly attracted to both healthy and infested cassava plant odours, but only *A. vexans* and *A. diversicornis*, more specialised species than *A. coccois*, distinguished between the two and preferred infested over healthy leaf odours. Mealybugs spend their whole lifetime on the same plant and are always surrounded by excretion products and mould, which could release some volatiles attractive to the wasps. The role of the mealybugs themselves and their by-products in the attractiveness of infested cassava plants for *A. vexans* and *A. diversicornis* was tested, they were found not or barely attractive, while infested plants from which mealybugs and their by-products were removed remained attractive to both wasp species. Induced plant volatiles played a crucial role in the attraction of the parasitoids.

Once the attraction to induced cassava plant odours was demonstrated for *A. vexans* and *A. diversicornis*, their responses to bush bean plant odours were tested in a Y-tube olfactometer. *A. vexans* was repelled by bean odours and failed to recognise infested cassava plant in association with bean odours. In contrast, *A. diversicornis* still responded positively to infested cassava plant odours in presence of bean odours and could distinguish infested cassava leaves from healthy ones, irrespectively of the presence of beans. Given the choice between the odour of infested cassava leaves alone and the odour of bean leaves with cassava, both species preferred pure cassava.

The influence of bean plants was further tested in the field. Eight pots with a mealybug-infested cassava plant were introduced in plots with either only cassava plants, cassava intercropped with bush bean, and bush bean only. No significant difference in parasitism rate was found between the different treatments. Out of three trials only the last indicated that *A. vexans* may be affected by intercropping. In this third trial, where the bean plants were much larger than in the previous ones, the volume presented by the legumes was much higher. Parasitism by *A. vexans* was significantly lower where beans were present. Overall parasitism rate by *A. diversicornis* was lower than by *A. vexans*. Independently of the treatment, the two wasp species seemed to disperse differently inside the field. *A. diversicornis* seemed to disperse more rapidly out of the plots, while *A. vexans* had the tendency to parasitise more close to the release point.

During the study period, *A. vexans* rearing colony presented some depressions, in which sex ratio was so highly male biased, that no experiment could be done. To optimise our rearing, a sex ratio study was conducted. Host size is known to be often an important factor for the sex determination of the offspring. In choice and no-choice

tests, parasitoid sex ratios were measured on second and third instar larvae and adult mealybugs. It was shown that third instar and adult hosts produced more females than second instars. When offered the three different host stages together, *A. vexans* preferred parasitising third instars and adult stages. Fitness of the wasps, which is the relative fecundity over lifetime, is known to be correlated with wasp size. The tibia length, a commonly used measure for size, was determined for all emerging wasps and correlated with host stage. Females were in general larger than males. Third mealybug instars produced significantly larger parasitoids of both sexes. It was concluded that the third mealybug instar is best suited for rearing many females of high fecundity. The results of this study do not entirely fit the ongoing theory for the sex ratio at different host size distributions, as the sex ratio did not significantly vary between a no-choice and a choice experiment. The results also showed that koinobiont parasitoids also adjust sex ratios depending on host size, which contradict Waage's theory.

The efficiency of *A. vexans* and *A. diversicornis* as biological control agent is discussed.

## 2. ZUSAMMENFASSUNG

Ziel dieser Studie war es, den Einfluss von Maniok/Bohnen-Mischkulturen auf das Suchverhalten von Parasitoiden der Maniokschmierlaus, *Phenacoccus herreni* Cox & Williams (Sternorrhyncha:Pseudococcidae) zu untersuchen. Im Vordergrund stand dabei das Suchverhalten in Abhängigkeit von flüchtigen chemischen Stimuli. Es ist bekannt, dass befallene Pflanzen spezifische Duftstoffe freilassen können, die von Parasitoiden als Stimuli zur Wirtslokalisierung benutzt werden können. Einzelheiten zu unseren Insektenarten wie auch die möglichen Wechselwirkungen mit zwischengepflanzten Nicht-Wirtspflanzen waren unbekannt.

Im ersten Schritt wurden drei Parasitoiden der Familie Encyrtidae, *Aenasius vexans* Kerrich, *Apoanagyrus (Epidinocarsis) diversicornis* Howard and *Acerophagus coccus* Smith, in einem Wahltest im Y-Rohr Olfaktometer getestet, um ihre Reaktion auf Duftstoffe gesunder und schmierlausbefallener Maniokpflanzen zu kontrollieren. Erstens wurde festgestellt, dass die drei Arten Parasitoidenweibchen am besten auf Duftstoffen reagierten, wenn sie 1.5 bis 2.5 Tage alt waren. Diese Altersklasse wurde dann für die weiteren Experimente benutzt. Alle drei Parasitoidenarten wurden signifikant von den Duftstoffen sowohl von gesunden wie auch befallenen Maniokpflanzen angelockt, aber nur *A. vexans* und *A. diversicornis*, spezialisiertere Arten als *A. coccois*, unterschieden zwischen den beiden und zogen die Duftstoffe von den befallenen jenen vor gesunden Blättern vor. Schmierläuse verbringen ihr ganzes Leben auf derselben Pflanze und werden immer von Ausscheidungs- und Häutungsprodukten umgeben, die selber Duftstoffe freilassen könnten, welche auf die Wespen attraktiv wirken. Die Ergebnisse mit *A. vexans* und *A. diversicornis* zeigen, dass diese Herbivor-verbundenen Substanzen nicht oder kaum attraktiv waren.

Dagegen blieben befallene Pflanzen, von denen die Schmierläuse und ihre Produkte entfernt worden waren, für beide Wespenarten attraktiv. Induzierte Pflanzenduftstoffe spielten somit eine entscheidende Rolle in der Attraktion der Parasitoiden.

Nachdem die Anziehung durch induzierte Maniokpflanzenduftstoffe für *A. vexans* und *A. diversicornis* einmal nachgewiesen war, wurde ihre Reaktionsverhalten auf Duftstoffe von Bohnenpflanzen in einem Y-Rohr Olfaktometer getestet. *A. vexans* wurde von dem Geruch von Bohnen abgestossen und konnte den Geruch von den befallenen Maniokpflanzen in Assoziation mit Bohnen nicht mehr erkennen. Im Gegensatz dazu reagierte *A. diversicornis* auch in Gegenwart von Bohnengeruch positiv auf den Geruch von befallenen Maniokpflanzen und konnte befallene Maniokblätter von gesunden unterscheiden, unabhängig von der Präsenz von Bohnen. In der Wahl zwischen dem Geruch von befallenen Maniok- und Bohnenblättern zogen beide Arten reinen Maniok vor.

Der Einfluss von Bohnen war im Feld weitergetestet. Acht Töpfe mit schmierlausbefallenen Maniokpflanzen wurden in die Parzellen mit entweder nur Maniok, Maniok mit zwischengepflanzten Bohnen, und nur Bohnen eingeführt. Es wurde kein signifikanter Unterschied im Parasitismus zwischen den Behandlungen gefunden. Von den drei Wiederholungen deutete nur die letzte darauf hin, dass *A. vexans* von Zwischenpflanzungen beeinflusst werden kann. In dieser dritten Wiederholung, bei der die Bohnenpflanzen viel grösser waren als in den vorherigen Tests, war auch das Bohnenvolumen viel grösser. Der Parisitismus von *A. vexans* blieb in Gegenwart von Bohnenpflanzen signifikant niedriger. Generell war der Parasitismus von *A. diversicornis* tiefer als von *A. vexans*. Unabhängig von der Behandlung schienen sich beide Wespenarten im Feld unterschiedlich zu verhalten. *A. diversicornis* schien sich schneller über die Parzellen hinauszubreiten, während *A. vexans* die Tendenz, hatte näher am Freilassungspunkt zu parasitieren.

Während der Laufdauer der Studie zeigten sich in der Zucht von *A. vexans* Depressionen, in denen so mehr Männchen produziert wurden als Weibchen, so dass keine Experimente gemacht werden konnten. Um die Zucht zu optimieren, wurden die Mechanismen untersucht, welche das Geschlechtsverhältnis des Parasitoiden beeinflussen könnte. Es ist bekannt, dass die Grösse des Wirtes oft ein wichtiger Faktor für die Geschlechtsdeterminierung der Nachkommen ist. In Experimenten mit und ohne Wahl wurde das Geschlechtsverhältnis des Parasitoiden in Abhängigkeit der Wirtsgrösse untersucht. In steigender Grösse wurden Maniokschmierläuse des zweiten und dritten Larvenstadiums sowie Adulte gemessen. Wirte im dritten Larvenstadium wie auch Adulte produzierten mehr weibliche Parasitoiden als das zweite Larvenstadium. Wenn die drei verschiedenen Schmierlausstadien zusammen angeboten wurden, parasitierte *A. vexans* bevorzugt das dritte Larvenstadium sowie Adulte. Wespenfitness, welche als die relative Fekundität im ganzen Leben definiert ist, ist erwiesenermassen mit der Wespengrösse korreliert. Die Tibienlänge, ein übliches Mass für die Insektengrösse, wurde für alle ausschlüpfenden Wespen festgestellt und mit dem Wirtsstadium korreliert. Weibchen waren generell grösser als Männchen. Das dritte Wirtslarvenstadium produzierte von beiden Geschlechtern signifikant grössere Parasitoiden. Daraus wird geschlossen, dass das dritte Larvenstadium für die Zucht von vielen Weibchen hoher Fekundität am besten geeignet ist. Die Resultate in dieser Studie entsprachen der gegenwärtigen Theorie über die Verteilung des Geschlechtsverhältnisses bei verschiedenen Wirtsgrössen nicht; das Geschlechtsverhältnis war in beiden Experimenten mit und ohne Wahl nicht signifikant

verschieden. Die Resultate zeigten auch, dass das Geschlechtsverhältnis von koinobiotischen Parasitoiden auch von der Grösse des Wirtes abhängig sein kann, was der Theorie von Waage widerspricht.

Es wird diskutiert, wie gut *A. vexans* und *A. diversicornis* als biologische Bekämpfungsmittel geeignet sind.

## 3. GENERAL INTRODUCTION

Diversified agroecosystems offer much promise for a more sustainable agriculture. Intercropping is a common practice in the tropics used by small farmers to minimise production risk of crop failure from pest and disease epidemic (Elzen et al., 1983; Ezulike & Igwatu, 1993; Moreno, 1979; Picard & Rabaud, 1914; Pinchinat et al., 1976). Beside soil improvement and protection against erosion, intercropping is in general presumed to enhance pest control by reducing herbivore load (Gold et al., 1989). Cassava, *Manihot esculenta* Crantz is a low-grade subsistence tropical root crop, and 30 to 40 % is grown in association with other crops (Cock, 1985). Cassava originates from Latin America and was introduced into Africa and Asia in the 16th century by the Portuguese and in the 20th century, cultures were started in Australia (Silvestre & Arraudeau, 1983). Nowadays, cassava is one of the most important carbohydrate source for 300-400 million people in the tropics (Bellotti & Schoonhoven, 1978; Silvestre & Arraudeau, 1983). It is mostly grown by small farmers (Cock, 1985; Horton, 1988; Sanders & Lynam, 1981). In the Americas, 75 % of cassava growers operate on land of 20 ha or less (Horton, 1988), in the three primary producing countries, Brazil, Paraguay and Colombia, 40-50 % of cassava culture area is on farms of 10 ha and less (Cock, 1985). Though cassava was reported to grow free of arthropods, investigations showed that mite and insect pests do limit cassava production (Bellotti & Schoonhoven, 1978). Mealybugs (Sternorrhyncha: Pseudococcidae) especially are among the most damaging pests of this crop in South America and Africa (Vargas & Bellotti, 1984).

In the 1970s, two important mealybug outbreaks occurred in the world. The first one, *Phenacoccus manihoti* Matile-Ferrero (Sternorrhyncha: Pseudococcidae) was accidentally introduced into Africa and became a serious pest in most of the cassava growing regions (Herren & Neuenschwander, 1991; Matile-Ferrero, 1977). An encyrtid parasitoid, *Apoanagyrus (Epidinocarsis) lopezi* De Santis, was successfully released in the 1980s and established. It maintains now the mealybug population at an acceptable low-density in most regions (Herren & Neuenschwander, 1991), except for some 5 % of the fields, where the parasitoid is not effective in controlling the mealybug (Neuenschwander et al., 1990). Alternative control agents have been investigated such as two strains of the coccinellid predator *Hyperaspis notata* Mulsant (Stäubli Dreyer et al., 1997a; 1997b; 997c). This showed the importance of the diversification on the level of natural enemies of pest species to reach an optimal integration of the antagonists into a certain agroecosystem, which is an important basis for successful pest management (Dorn et al., 1992).

The second mealybug outbreak appeared as a problem rather suddenly in South America. *P. herreni* was first found in Northeast Brazil and then reported from Colombia, Venezuela and Guyana (Bellotti, 1983; CIAT, 1987; 1988; 1990; 1984). To control this mealybug, the three encyrtid parasitoids *Aenasius vexans* Kerrich, *Apoanagyrus (Epidinocarsis) diversicornis* Howard and *Acerophagus coccois* Smith, are being studied at CIAT (Centro International de Agricultura Tropical) in Cali, Colombia. They have been released in 1994 and in 1995 in Brazil, in the states of Bahia and Pernambuco (Smith & Bellotti, 1996); J. Bento, personal communication). The behaviour of these wasps is hardly known, neither is the influence of intercropping on their foraging ability to find their hosts. Natural antagonists do not only interfere with herbivores but also with the plant communities in agrosystems. More knowledge on this relationship between third and first trophic levels may help to judge both the strengths and the limitations of each antagonist in a certain cropping system (Dorn, 1996). Hymenopterous parasitoids have been shown to respond to volatiles of their host's food plants (review in Turlings et al., 1995), but in a mixed culture, the volatiles released by different plant species may interfere with the attractiveness of the actual host plant (Price et al., 1980; Vinson, 1981) and alter orientation ability of natural enemies.

Except for *A. diversicornis*, which was a candidate for release in Africa against *P. manihoti*, the foraging and host searching behaviour of these parasitoids is almost unknown. Parasitoid host location can be divided in several hierarchical steps beginning with habitat location (Hassell & Southwood, 1978; Vinson, 1976), in which plant volatiles may play a very important role (Nordlund et al., 1988; Tumlinson et al., 1992). The preference of *A. lopezi* for *P. manihoti*-infested cassava leaf odours over healthy ones (Nadel & Alphen van, 1986) indicates that volatiles emitted by the infested plant are important in the host location. Hymenopteran wasps have been shown to respond to their host's food plant odours even without the actual presence of their host (e.g. Schuster & Starks, 1974; Takabayashi et al., 1991; Vinson, 1981). Once the wasps reach the host habitat, they need other cues to find the actual hosts. Stimuli derived from herbivores should be the most reliable sources of information, but herbivores are small components of a whole ecosystem, and if they produce any volatiles, it will be in minute quantities (Vet & Dicke, 1992). In contrast, plants represent a larger biomass, their stimuli are in much larger quantities but less reliable for the host presence (Vet & Dicke, 1992). Volatiles are continuously released by plants and can be affected by internal and/or external factors (see review in Visser, 1986). Herbivore attack is one of these external factors that can provoke a change in the chemical composition of plants (Alphen van et al., 1990; Ayanru & Sharma, 1986; Calatayud et al., 1994; Evans, 1941). In cassava, cyanide and rutin contents as well as mineral status change when the plants are under attack of the mealybug, *P. manihoti* (Ayanru & Sharma, 1986; Calatayud et al., 1994). The increasing emission of volatiles after herbivore attack has actually been demonstrated for several plant species such as maize (McCall et al., 1993; McCall et al., 1994; Turlings et al., 1990), lima bean (Dicke et al., 1990), brussel sprouts (Mattiacci et al., 1994) or apples (Boevé et al., 1996). For parasitoids attacking feeding herbivore stages, these volatiles are reliable cues for their host presence and are relatively easy to detect (Vet & Dicke, 1992). However, volatiles released by herbivores or their by-products have also been reported to be attractive to their natural enemies (see reviews in Ramachandran et al., 1991; Rutledge, 1996; Tumlinson et al., 1992).

In a diversified agroecosystem, parasitoid insects which use plant volatiles to locate their hosts have to face a mixture of different plant species odours. It has been reported that herbivorous insects are confused or repelled by the non-host plant odours, which leads to a decreasing pest abundance (Cromartie, 1981), although increasing pest attacks have also been reported with diversification (Risch et al., 1983; Russell, 1989). In fact, the impact of intercropping on herbivores depends on their host range, specialist herbivores should be more influenced by diversification than polyphagous ones (Root, 1973). Like herbivores, natural enemies should react differently to mixed cropping systems, dependent if they are specialists or generalists, negative effects are more likely to appear with specialists (Sheehan, 1986). Specialised parasitoids responding to their host's food plant odours may be impeded to find the target plants because of the foreign odours, but almost no investigation is reported in the literature. Monteith (1960) is the only one who tested a parasitoid species in an olfactometer with a non-host's food plant. He found that *Drino bohemica* Mesn. (Dipt.:Tachinidae) was attracted in an olfactometer to the food tree, *Picea glauca* (Moench) Voss of its host, the sawfly *Diprion hercynia* Htg., but when the plant was in presence of another tree species, which is not attacked by its host, the parasitoid preferred the food-tree alone over the association of both tree species. The non-food tree was not repellent to the tachinid, but when the amount of the non-food tree was increased, the tachinid females could not distinguish between the host food tree alone and the association of both tree species. This was attributed to an increased masking effect. Though, the masking of the odours of infested *P. glauca* by the odours of the other tree species was not tested, which would have given complementary results

In this study, I tried to find out if traditional intercropping systems used by small farmers in South America can have an impact on parasitoid foraging behaviour. As a model I chose the cassava/bean intercropping and investigated the three encyrtid parasitoids *A. vexans, A. diversicornis* and *A. coccois.* First, their response to cassava plant odours was tested in a Y-tube olfactometer, then the source of attractants for the two best responding species, *A. vexans* and *A. diversicornis*, was determined. Their responses to plant odours were compared to their responses to odours emitted by the mealybugs and their by-products. To determine the olfactory influence of bush bean plants, we tested the response of *A. vexans* and *A. diversicornis* to the legume plant odours in the olfactometer and in a field study parasitisation rate and dispersal were compared for plots in which only cassava was grown and plots that included bean plants.

# 4. CHEMICALLY-MEDIATED ATTRACTION OF THE THREE PARASITOID SPECIES TO MEALYBUG-INFESTED CASSAVA LEAVES

## 4.1. INTRODUCTION

Natural enemies of *Phenacoccus herreni* Cox & Williams have been systematically collected for the control of the mealybug in South America. Laboratory colonies of three encyrtid parasitoids *Aenasius vexans* Kerrich, *Apoanagyrus (Epidinocarsis) diversicornis* Howard (asexual strain) and *Acerophagus coccois* Smith were established at CIAT (Centro International de Agricultura Tropical), in Cali, Colombia (CIAT, 1990; 1983; 1982). 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 & Caudle, 1938; Thorpe & Jones, 1937). 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., 1995; Turlings et al., 1990; 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 & Alphen, 1987). Changes in chemicals produced by the cassava plant due to *P. manihoti* infestation (Calatayud et al., 1994) could result in the emission of volatiles and explain why *A. lopezi* and *A. diversicornis* (sexual strain) are attracted by *P. manihoti*-infested cassava plants (Alphen van et al., 1990; Nadel & Alphen, 1987). The feeding behaviour *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 Hofstee et al. (1993) showed no response by this parasitoid to the odour of *P. herreni*-infested cassava plants (var. Odungbo). A better understanding of the interactions between cassava plants, mealybugs, and parasitoids requires more behavioural as well as chemical studies.

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

## 4.2. MATERIALS AND METHODS

### *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.

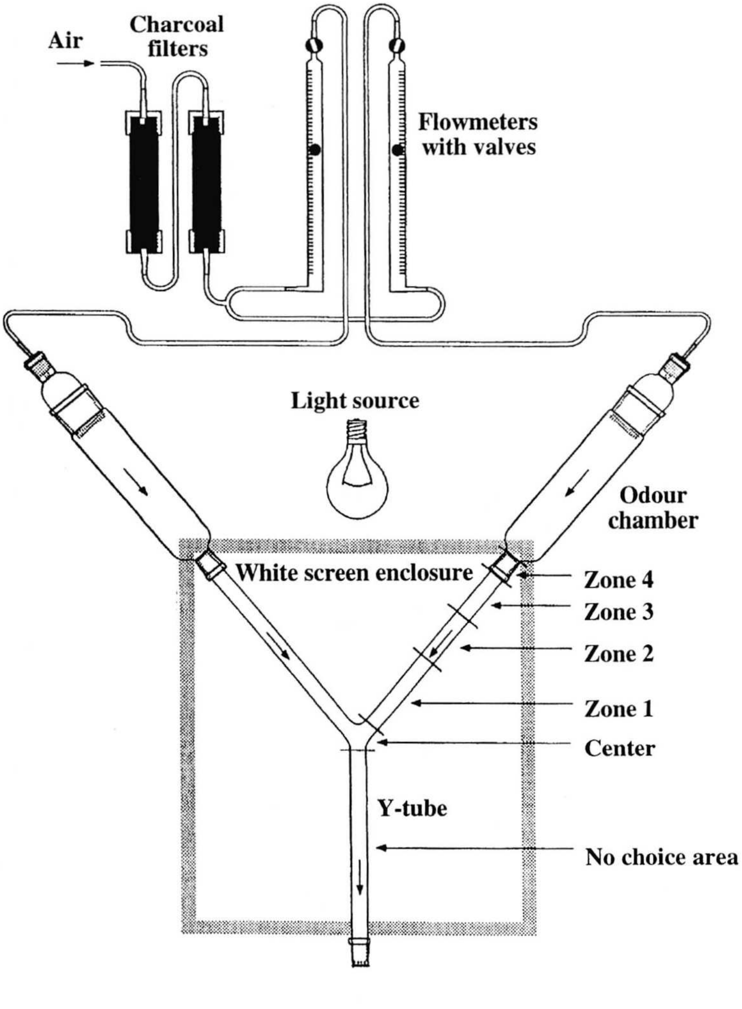
## *The Olfactometer*

A Y-tube olfactometer similar to the one first described by Sabelis & van de Baan (1983) was used in our experiments (Fig. 4.1). Two arms of a glass Y-shaped tube were connected to glass chambers (6.5 cm diam.) in which odour 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 odour sources. When a female reached the center of the Y-tube, where the three arms met, she could choose one of the offered odours.

## *Odour 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

**Figure 4.1.** Diagram of the olfactometer set-up. Drawing by Urs Lengwiler.



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 odour 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 odour chambers. The infested leaves that were selected carried honeydew and sooty mould, 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 females 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 odours (Steinberg et al., 1992; Turlings et al., 1993; Vet et al., 1995). The parasitoids were then captured in a gelatine capsule and kept there for 10 to more than 60 minutes. Before each Y-tube test, the gelatine capsule was opened and inserted at the base of the Y-tube. Females were introduced and were observed individually in the olfactometer and used only once. The odour sources were reversed each time three wasps had been tested.

### *Evaluation of Choices*

A stopwatch was started when the insect left the gelatine capsule. The female was allowed 5 minutes to walk up the no-choice-area (Fig. 4.1) to reach the centre of the olfactometer, which is the area where the three arms meet. If a female did not reach this centre 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 centre, or after they had reached the end of one of the arms. Each arm, was divided into four zones (Fig. 4.1), which measured 8, 6, 6, and 3 cm, respectively.

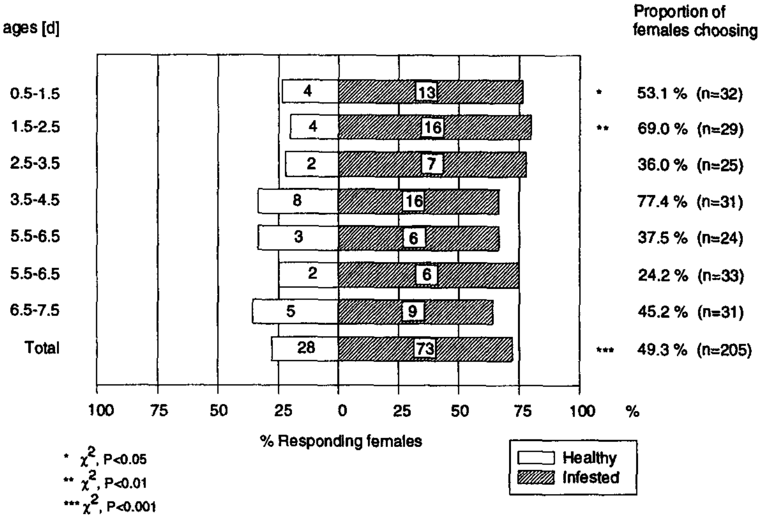
A female had to enter at least zone 2 (Fig. 4.1) to be considered to have made a choice. A few females switched arms after reaching zone 2. In those cases, females were considered to choose the arm which they entered the furthest. For statistical analyses, a chi-square test was applied, using the total number of females that made a choice for a particular odour ($\alpha$=0.05) .

## 4.3. RESULTS

### *The Effect of Wasp Age*

It has been shown that the responsiveness to odours may change when parasitoids get older (e.g. Steinberg et al., 1992; Thorpe & Caudle, 1938). 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 odours of infested and healthy cassava leaves.

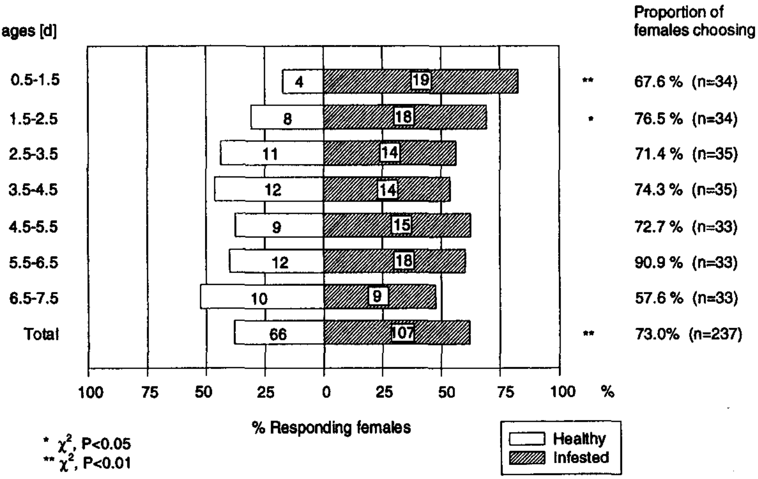
**Figure 4.2.** Age dependency of response. Choices by *A. vexans* females of different age classes between the odours of mealybug-infested and healthy cassava leaves in a Y-tube olfactometer. In each bar the actual number wasps that made a particular choice is given, while the x-axis indicates the percentages 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 odours, as well as the total number of females that were tested per age class.



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 odour source changed in two of the three species (Fig. 4.1-3). In *A. vexans* and *A. diversicornis*, the preference for the odour of infested leaves.

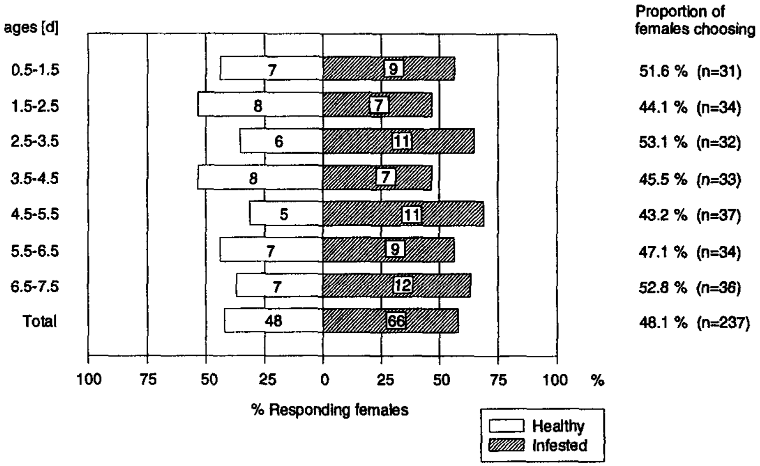
**Figure 4.3.** Age dependency of response. Choices by *A. diversicornis* females of different age classes between the odours of mealybug-infested and healthy cassava leaves in a Y-tube olfactometer. In each bar the actual number wasps that made a particular choice is given, while the x-axis indicates 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 odours, as well as the total number of females that were tested per age class.



over odour of healthy leaves was age dependent and significant for young females only. Of the younger (1.5-2.5d old) *A. vexans* females, 80% preferred infested cassava leaf odours ($\chi^2$ = 7.2, P<0.01). The youngest *A. diversicornis* (0.5-1.5d) showed the clearest preference (82.6%) for the odour of infested leaves over the odour 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 odours of infested leaves (69.2%, $\chi^2$ =3.85, P<0.05). The older wasps showed no significant preference. All age classes of *A. coccois* did not differentiate between infested and healthy plant odours. Like *A. diversicornis*, *A. coccois* walked a lot in the olfactometer, often switching between arms (26.3% of the choosing females).

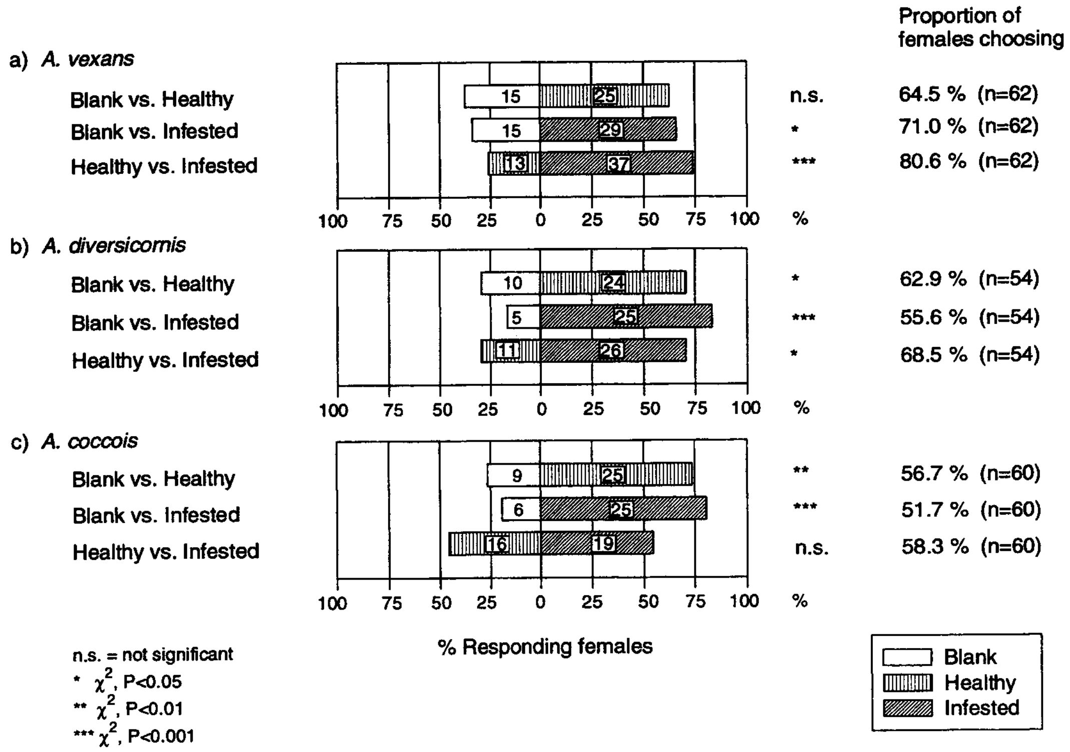
### *The Role of Plant Odours*

**Figure 4.4.** Age dependency of response. Choices by *A. coccois* females of different age classes between the odours 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 odours, as well as the total number of females that were tested per age class.



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 previous experiments, only females that were 1.5 to 2.5 days old were used. On a given day three different pairs of odour 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 odour chamber with just a piece of wet cotton wool. For each pair of odour sources, 4 to 6 insects per day were

**Figure 4.5.** Responses of the three parasitoid species. (A) *A. vexans*, (B) *A. diversicornis* and (C) *A. coccois*, in a Y-tube olfactometer. The wasps were offered choices between the odours 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 odours, as well as the total number of females that were tested per choice.



individually tested in the Y-tube. Occasionally, another series of 6 insects per odour source was tested the same day.

*A. vexans* females were significantly attracted to infested cassava leaves compared to healthy ones or a blank (Fig. 4.5a). Healthy leaf odours 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 odour 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 odours over healthy ones ($\chi^2 = 6.08$, $P<0.025$).

Only 51.7 to 58.3% of *A. coccois* females made a choice, but these were significantly attracted by healthy and infested plant odours 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 odour source ($\chi^2 = 0.26$, $P>0.05$) (Fig. 4.5c).

## 4.4. DISCUSSION

The preference of female wasps to plant odours in the olfactometer was age dependent for *A. vexans* and *A. diversicornis*. The younger age classes of both these species significantly preferred the odour 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 odours 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. coccois*, 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 odour of infested leaves. *A. coccois* was also attracted to both healthy and infested leaves, but did not distinguish between these two odour sources. These differences in response of the three encyrtid parasitoids suggest that they may employ different foraging strategies. *A. vexans* and *A. diversicornis* recognised odours that are specifically associated with mealybug infestation. *A. coccois*, on the other hand, appeared to respond only to general cassava plant odours. It remains unknown if *A. vexans* and *A. diversicornis* are attracted to odours emanating directly from the mealybugs or if the infested plants emit the attractive odours.

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 behaviour was also observed in the olfactometer. *A. vexans* was clearly attracted to the infested cassava plants, but not to the odours 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 odours. This fast moving species did not readily distinguish between the odours of infested and healthy leaves.

The reported host preference of these parasitoids may explain their behaviour in the olfactometer to some extent. *A. vexans* prefers *P. herreni* over a related species, *Phenacoccus gossypii* (=*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. manihoti* and to parasitise both species at similar rates, but with a higher survival rate in *P. herreni.* An asexual strain from Venezuela has been shown to prefer *P. herreni* over *P. gossypii* (=*madeirensis*) (Driesche van et al., 1987). *A. coccois* seems to be the most polyphagous of the three. It parasitises 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 parasitises *P. herreni* and *P. madeirensis* more or less equally

(CIAT, 1990). As a generalist, *A. coccois* may be more responsive to general plant odours, while the more specialised wasps, *A. vexans* and *A. diversicornis*, may have adapted to exploit odours 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 odours 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 *P. manihoti*-infested cassava plants release odours that are attractive to the parasitoid *A. lopezi*. The odours probably came 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. manihoti*-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 systemically 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.

## 4.5. CONCLUSION

The three encyrtid parasitoids were attracted to the odours of cassava plants, and were most responsive and selective when they were 1.5 to 2.5 days old. *A. vexans* and *A. diversicornis* were strongly attracted to infested leaves and preferred these over healthy ones. *A. coccois*, which has the broadest known host range of the three, was significantly attracted to either healthy or infested leaves but did not distinguish between the two. This suggests that *A. coccois* may be responsive only to general plant odours, while *A. vexans* and *A. diversicornis* respond more specifically to odours associated with mealybug infestation.

# 5. THE ROLE OF MEALYBUG-INDUCED CASSAVA PLANT VOLATILES IN THE ATTRACTION OF THE ENCYRTID PARASITOIDS *AENASIUS VEXANS* AND *APOANAGYRUS DIVERSICORNIS*.

## 5.1. INTRODUCTION

The larvae of the pseudococcid, *Phenacoccus herreni*, remain within the ovisac for a short time after emergence and then migrate to the apical part of the young growing shoots of cassava plant. When the mealybug population increases, they will disperse to all plant parts (Bellotti et al., 1984). After they find a feeding site they mostly remain there if not disturbed (Bellotti et al., 1984). Because *P. herreni* females spend their whole lifetime on the same plant, they are always surrounded by their excretion products such as exuviae, faeces, defence substances, honeydew and the sooty mould that forms on it. Such systems are common for many sternorrhynchan insects that produce honeydew and spend a large part of their life on the same plant, such as aphids, scale insects and mealybugs, and to a lower extent for whiteflies which fly away as adults. Among the by-products, honeydew has received much interest for its possible importance for parasitoids and predators (Vianen van, 1989). Its attraction has been reported for the general predator, *Chrysopa carnea* Stephen (Duelli, 1980; Emden van & Hagen, 1976; Hagen et al., 1976). Effects of honeydew or other sternorrhynchan by-products on the foraging and searching behaviour of some parasitoids have often been tested, but they are mostly found to be arrestants or to stimulate searching (e.g. Bouchard & Cloutier, 1984; Roermund van & Lenteren van, 1995). Some have reported long-range attraction to parasitoids of whiteflies and aphids. The aphelinid parasitoid *Encarsia formosa* Gahan, showed an oriented flight toward infested host's plants as well as toward its host, the whitefly *Trialeurodes* *vaporariorum* Westwood in a flight tunnel (Guerrieri, 1997), similarly the parasitoids *Aphidius uzbekistanicus* Luzhetski and *A. ervi* Haliday, responded to their aphid hosts and to the plants in a Y-tube olfactometer (Powell & Zhi-Li, 1983). Honeydew was found attractive for *Aphidius rhopalosiphi* and *A. nigripes,* two aphid parasitoids (Bouchard & Cloutier, 1985; Wickremasinghe & Emden van, 1992).

Parasitoids that are in search of herbivores as hosts for their offspring often first respond to general host food-plant odours (Elzen et al., 1983; Picard & Rabaud, 1914; Udayagiri & Jones, 1993; Vinson, 1981) and attraction to plant odours even occurs if the host is not present (e.g. Baaren van & Nénon, 1996; Schuster & Starks, 1974; Takabayashi et al., 1985; Wickremasinghe & Emden van, 1992; Mattiacci et al., 1994). Wasps may be attracted to the "wrong" plants as Powell & Zhang Zhi-Li (1983) showed for *Aphidius uzbekistanicus* Luzhetski (Hym.:Aphidiidae), which is attracted to bean leaves, but their known hosts are restricted to Gramineae. Similarly, Takabayashi et al. (1991) found that *Apanteles kariyuai* Watanabe (Hym.:Braconidae), a parasitoid of a noctuid pest of maize, is attracted to odours from different plant species that are not their host's food. Parasitoids that make these "mistakes" are expected to correct their orientation once they perceive cues that more reliably are associated with hosts (Vinson, 1981). Plants under herbivore attack may provide reliable long range cues in the form of volatiles that are released in response to the attack (Boevé et al., 1996; Dicke, 1994; Turlings et al., 1995; Vet & Dicke, 1992) and volatiles from the hosts may be used rather at close range or even only after direct contact (e.g. Mattiacci & Dicke, 1995).

Cassava plants infested with the mealybug, *P. manihoti,* have been shown attractive to the encyrtid parasitoid, *A. lopezi,* which could also distinguish them from healthy plants, but they were not attracted by healthy ones, neither by mealybugs nor their by-products (Nadel & Alphen, 1987). On the other hand, van Baaren & Nénon (1996) found contradictory results with the same organisms, and reported that *A. lopezi* was attracted to unparasitised mealybugs. In chapter 4, it was shown that the encyrtid parasitoids, *Aenasius vexans* and *Apoanagyrus diversicornis* could distinguish between infested and healthy cassava plants and prefer the first ones. The few works that have reported an attraction of parasitoids of sucking herbivores in feeding stages to host cues (Bouchard & Cloutier, 1985; Guerrieri, 1997; Powell & Zhi-Li, 1983; Wickremasinghe & Emden van, 1992) and the one of van Baaren & Nénon (1996) suggest that in plant-host complex cassava/ *P. herreni*, the attraction observed in chapter 4 for the two encyrtid parasitoids may not be only due to mealybug-induced plant volatiles. Although cues that come directly from the host are not likely to play an important role in foraging behaviour of parasitoids due to their minute quantity (Vet & Dicke, 1992), it is not possible to exclude an influence of mealybugs and their by-products in the attraction of the parasitoids, especially honeydew which may cover a large surface of the plants. That is why an olfactometer study with *A. vexans* and *A. diversicornis* was conducted in which the actual role of the plant as opposed to factors more directly associated with the mealybug presence was determined.

## 5.2. MATERIALS AND METHODS

### *Plants, Insects and Olfactometer*

The description of the organisms and material is given in detail in chapter 4. The same evaluation of choices as described in chapter 4 was used and a chi-square analysis for the statistic.

### *Role of Plant Derived Odours*

To determine if the attractiveness of infested plants was due to odours emitted by the plant or to mealybugs and their by-products, the two were tested separately. Two cassava leaves infested with mealybugs and carrying exuviae, as well as honeydew and sooty mould were carefully cleaned. First the mealybugs were removed with a fine paintbrush and then all the products left on the leaves were carefully wiped off with a wet cotton wool and distilled water, without wounding the leaves. These leaves, free of their pest and by-products, were assumed to release only plant-produced odours. The petiole of each washed leaf ("Damaged") was wrapped in wet sterilised cotton wool to avoid any odour emanating from the cut petiole. They were then placed in the olfactometer and tested against other potential odour sources. Sterilised cotton wool was preferred over the normal one used in chapter 4 because the latter was observed to be somewhat attractive to the wasps.

### *Role of Mealybug Derived Odours*

To determine if mealybugs and/or their by-products contribute to the attraction of the wasps, the mealybugs and by-products that were removed as described above were also used as an odour source. The pseudococcids and their exuviae were placed on a small wet cotton wool pad and the remaining by-products were collected on another small piece of cotton wool. Like the damaged cassava leaves, this mealybug complex ("Mealybugs") was offered in the olfactometer against other potential odour sources.

### *The Other Odour Sources*

To determine their respective roles in attracting the parasitoids, the above odour sources were tested against one of the following : Two infested leaves ("Infested"), which carried mealybugs as well as honeydew and sooty mould, but no other arthropod species. Healthy leaves ("Healthy"), which were absolutely free of arthropod pests and had never carried any. As a control ("Blank"), sterilised wet cotton wool was used as an odour source.

### *Experimental Procedure*

Newly emerged parasitoids were isolated at noon and placed in Plexiglas® cages with honey and water for 1.5 day without any contact with hosts or cassava plants. 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 insects were left in the experimental room, an air-conditioned chamber (28-30° C), for one to two hours before the start of an experiment. They were given an oviposition experience by placing each female in a Petri dish (diam. 15 cm) with an infested cassava leaf. Several females at a time were observed until they had parasitized or at least stung a mealybug. The parasitoids were then captured in a gelatine capsule and kept there for 20-60 minutes. For each olfactometer test, the gelatine capsule was opened and inserted at the base of the Y-tube. Females were observed individually in the olfactometer and used only once. The position of the odour sources was reversed each time three wasps had been tested, and after six wasps a new combination of odour sources was placed in the olfactometer.

On a given day four different pairs of odour sources were tested. In the first experiment the following odour sources were tested against damaged washed leaves ("Damaged") : "Blank", "Infested", "Healthy" and "Mealybugs". In the second experiment mealybugs and their products ("Mealybugs") were tested against "Blank", "Infested" and "Healthy". A combination of healthy leaves and mealybugs and their by-products ("Healthy+Mealybugs") were tested in the olfactometer against infested leaves to confirm the presence of induced volatiles.

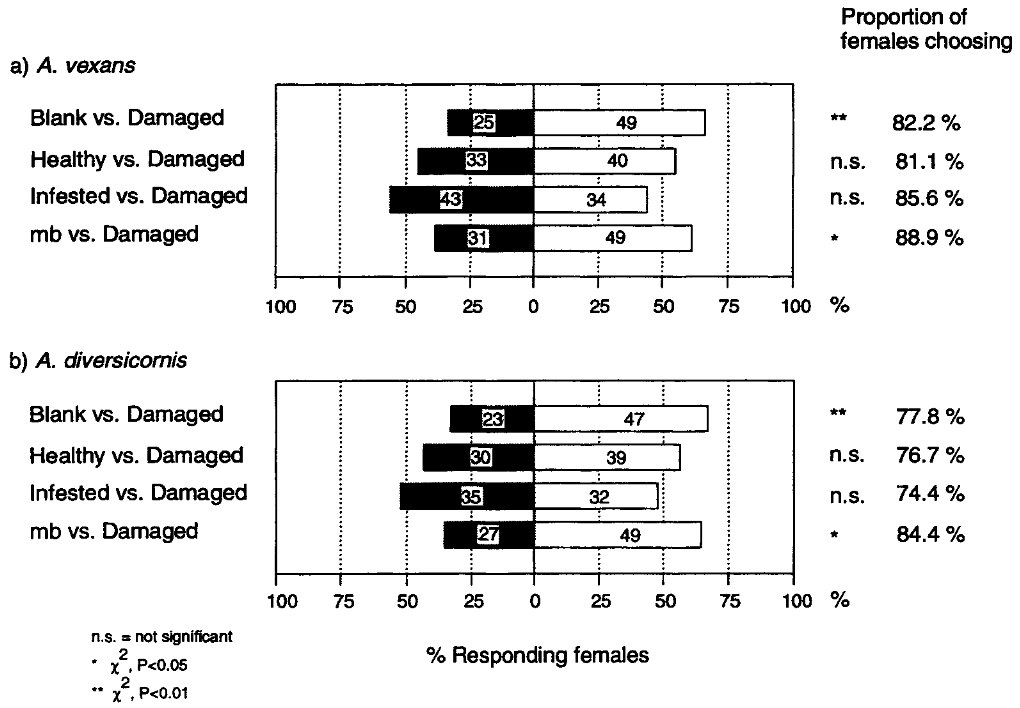
## 5.3. RESULTS

In chapter 4, it was reported that 64.5 % of *A. vexans* females preferred healthy plant odour over "Blank", but this was not statistically significant. We repeated this experiment with ninety *A. vexans* females. This time they showed a statistically significant attraction for healthy leaf odours (67.2 %, $\chi^2$=6.89, P<0.01). This discrepancy is attributed to the use of unsterilised cotton wool in the first experiment, which is attractive to *A. vexans* and may have affected its responses.

### *Role of Plant Derived Odours*

*A. vexans* females made more choices (81.1 % to 88.9 %) than *A. diversicornis* (74.4 % to 84.4. %), but both showed a similar pattern of preferences (Fig. 5.1). Damaged leaves were preferred by both species over pure air ("Blank") ($\chi^2$=7.78, P<0.01 and $\chi^2$=8.22, P<0.005 respectively for *A. vexans* and *A. diversicornis*) and

**Figure 5.1.** Responses of (A) *A. vexans* and (B) *A. diversicornis* in a Y-tube olfactometer to damaged cassava plant odours ("Damaged"). mb = mealybug and by-products. In each bar the actual number of 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 odours, as well as the total number of females that were tested per choice. n=90.



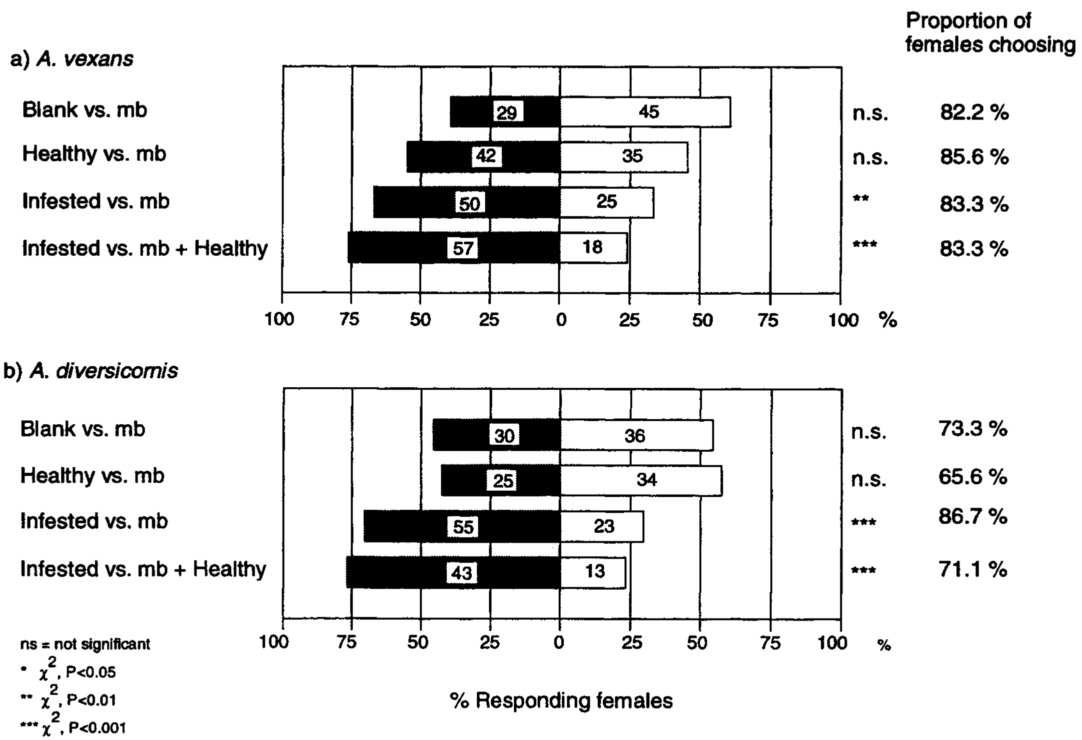
over mealybugs and their by-products ($\chi^2$=4.05, P<0.05 and $\chi^2$=6.36, P<0.025). This indicates that odours of plant origin were more important than odours that are more directly associated with mealybugs. Both wasp species could not distinguish damaged leaves from infested ones ($\chi^2$=1.05, P>0.25 and $\chi^2$=0.134, P>0.50), which confirms the release of induced volatiles by cassava leaves. They also did not distinguish between damaged leaves and healthy ones ($\chi^2$=0.67, P>0.25 and $\chi^2$=1.17, P>0.25), while they do distinguish infested from healthy leaves (Chap. 4). This may indicate that damaged cassava leaves release a lesser quantity and/or quality of attractive substances than infested leaves.

### *Role of Mealybug Derived Odours*

Here, too, *A. vexans* made in general more choices (82.2 % to 85.6 %) than *A. diversicornis* (65.6 % to 86.7 %) (Fig. 5.2), while both showed similar preferences.

Given the choice between the mealybug system and pure air, 60.8 % of *A. vexans* females walked up to the arm leading to the mealybugs and their by-products, which just misses the level of significance at P<0.05 ($\chi^2$=3.459, 0.1<P>0.05). In the case of *A. diversicornis* only 54.5 % of the responding females chose this odour over pure air ($\chi^2$ =0.545, P>0.5). Odours from infested leaves were significantly more attractive to both *A. vexans* and *A. diversicornis* than the mealybugs and their by-products ($\chi^2$=8.33, P<0.005 and $\chi^2$=13.12, P<0.001 respectively), which confirms that odours of plant origin are dominant in the attractiveness and more important than odours of mealybug origin. The wasps also preferred infested leaves over the combination of healthy leaves and a removed mealybug complex

**Figure 5.2.** Responses of (A) *A. vexans* and (B) *A. diversicornis* in a Y-tube olfactometer to mealybugs and their by-products (mb). In each bar the actual number of 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 odours, as well as the total number of females that were tested per choice. n=90.



("Healthy+Mealybugs") ($\chi^2$=20.28, P<<0.001 and $\chi^2$=16.07, P<<0.001 respectively). This indicates emission of an attractive odour by infested leaves which is released neither by healthy leaves nor by mealybug and their by-products. The wasps did not distinguish between healthy leaves and the mealybugs and their by-products, though healthy leaves were shown to be attractive to both *A. vexans* and *A. diversicornis* over clean air (Chap. 4).

## 5.4. DISCUSSION

Both encyrtid parasitoids, *A. vexans* and *A. diversicornis,* responded in a similar way to odours from the cassava-mealybug complex. In chapter 4 it was shown that they both are attracted more to infested cassava plant odours than to healthy ones. Here we show that their attraction is due to a large degree to plant volatiles and that the mealybugs themselves and their by-products were not significantly attractive to either wasp.

For host habitat finding as well as for host finding, attractant stimuli elicit orientation to areas containing hosts, and arrestant stimuli elicit a reduction in the distance to the host (Waage, 1978). Once arrested in one level of foraging, parasitoids may respond to further attractant and arrestant stimuli which tend to lead them to smaller units of host distribution (Waage, 1978). The responses by *A. vexans* and *A. diversicornis* to healthy cassava leaves shown in chapter 4 confirms that general plant odours may be the first cues used by the parasitoids to lead them to the food plants of their hosts (Vinson, 1976). Subsequently, wasps are expected to use more specific cues to determine actual host presence. Due to the large biomass presented by plants, their cues are more readily perceived at long distance than cues emanating directly by herbivores themselves or their by-products (Vet & Dicke, 1992). Feeding by herbivorous insects can induce the emission of plant volatiles (Boevé et al., 1996; Dicke, 1994; Turlings et al., 1995), which are well detectable at long distance by parasitoids and are reliable cues of host presence (Vet & Dicke, 1992). Infested cassava plants seem to release such induced volatiles that distinguish them from healthy plants. Damaged leaves were as attractive to both *A. vexans* and *A. diversicornis* as infested leaves still carrying all mealybug factors. This confirms that the major attractive odours do come from the plant itself. Du et al. (1996) also found a similar result with the aphid parasitoid, *Aphidius ervi*, in which the wasps did not distinguish between infested plants with their hosts and the infested plants from which the hosts had been removed. In our experiments, the amount of volatiles released by the damaged cassava leaves may have been somewhat reduced because of the absence of feeding insects and water soluble attractive substances may have been removed while washing the leaves. Table 5.1 gives a synoptic view on the relative importance of individual constituents in the attractiveness to the parasitoids. The difference in the volatile quantity between healthy (3) and damaged cassava leaves (2), like between infested (1) and damaged leaves (2) may not have been high enough for the wasps to perceive.

Similar subtle differences between mealybug complex and healthy plants could explain some of the results in the second experiment. Mealybug derived factors certainly do play a minor role in the attraction of wasps to infested cassava plants. For *A. vexans*, a marginal attraction to the mealybug complex was found, but not for *A. diversicornis*. However for both wasps it is not statistically significant. Healthy plants were somewhat attractive (Chap. 4), but when healthy plants were tested next to the mealybug factors the wasps did not make a distinction (Table 5.1.). It is possible, that with the removal of the mealybug and by-products some plant substances were removed as well and were part of the mealybug complex odour source, as shown by Read et al. (1970), who found that aphids tested 24 h after removing from their food plants were not attractive anymore to their parasitoid, the braconid *Diaretiella rapae*. Such a contamination may have been enough to confuse the wasps in the choice "Mealybugs vs. Healthy", but not enough to significantly attract the parasitoids in the control experiment "Mealybugs vs. Blank". On the other hand, when the mealybug complex was combined with healthy leaves and tested against infested ones, the wasps clearly preferred the infested leaf odours.

**Table 5.1.** Interpretation of the relative importance of the various odour sources in the attraction of the parasitoids *A. vexans* and *A. diversicornis*. The sign > means significantly higher attractiveness (P>0.05).

| 1 "Infested" | 2 "Damaged" | 3 "Healthy" | 4 "mb" | 5 "Blank" |
|---|---|---|---|---|
| 1 | > | 3 | | |
| | 2 | | > 4 | |
| | | 3 | | > 5 |

The role of cues that come directly from the host may be more important at close range (Vet & Dicke, 1992). Very often natural enemies are arrested by such products and only react to the chemicals after close-range or direct contact (e.g. Hagvar & Hofsvang, 1991; Islam & Jahan, 1993; Ohara et al., 1996; Takabayashi et al., 1985). However, there are also examples of volatile substances emitted from insect honeydew that attract the natural enemies of their producers over longer distances. The green lacewing , *Chrysopa carnea* respond to honeydew scent, in which a breakdown product of tryptophan is the main attractant (Duelli, 1980; Emden van & Hagen, 1976; Hagen et al., 1976). Green lacewing adults feed on honeydew (Hagen et al., 1976), which therefore does not only represent a cue for the presence of prey for its offspring, but also a food source. Some aphid parasitoids are also attracted to the odour of their host and honeydew in an olfactometer, though their response was stronger in presence of the plant (Wickremasinghe & Emden van, 1992). In other cases where honeydew or host odours are reported attractive to parasitoids, the foraging behaviour of these wasps seems to be different. The aphidiid parasitoid *Aphidius nigripes* is strongly attracted to their host or their honeydew, but does not respond to plant odours, which was thought by the authors to indicate that *A. nigripes* may use other than olfactory means to locate the habitat of its host, such as visual cues (Bouchard & Cloutier, 1985). *Encarsia formosa*, parasitoid of the whitefly *Trialeurodes vaporariorum* Westwood, showed oriented flights in a flight tunnel to both whitefly-infested plants and whitefly larvae, but visual stimuli were important as well in the decision made by the wasps (Guerrieri, 1997).

Beside the quality blend of an odour, the quantity of attractive substances that is available needs to be considered. Induced volatiles released by infested plants are highly concentrated and are relatively reliable cues for host presence (Vet & Dicke, 1992). In the tritrophic system involving *A. vexans* and *A. diversicornis*, foraging parasitoids seem to rely predominantly on cues coming from the cassava plant after attack by *P. herreni*. Once the wasps are on an infested plant, they may use the mealybug factors for their orientation to the host. Relatively low concentrations of odours released by the host and its by-products could be sufficient to influence the short range searching behaviour of the parasitoids.

## 5.5. CONCLUSION

*A. vexans* and *A. diversicornis* responded significantly to *P. herreni*-induced cassava odours. Attraction to infested cassava plants is most likely mediated by volatile emissions that are induced in the plant by mealybug infestation. Volatiles directly derived from the mealybugs or their by-products were not significantly attractive to either antagonist species in our bioassay.

# 6. THE INFLUENCE OF BUSH BEAN PLANT ODOURS ON THE FORAGING BEHAVIOUR OF *AENASIUS VEXANS* AND *APOANAGYRUS DIVERSICORNIS*

## 6.1. INTRODUCTION

Intercropping is the growing of different crops together in same area and time. Due to economical problems and a high degree of mechanisation,, mixed cultures are not very common in the industrial countries but still very frequent in tropical America, where small farms are numerous (Pinchinat et al., 1976). The mixture of two or several genetically different plants in a plot (=polyculture) has been often, but not always, shown to bring advantages to biological and integrated pest management. The causes are not completely understood. Root (1973) proposed two hypotheses to explain the advantages and limitations of intercropping: The "enemies hypothesis" which predicts that natural enemies are more numerous in a diversified system due to presence of alternate prey, nectar sources or suitable microhabitats. The "resource concentration hypothesis" predicts that mono- or oligophagous herbivores will more easily find and stay in a monoculture.

In most cases polycultures result in a higher herbivore mortality and a decrease in the number of herbivore species (Risch et al., 1983; Russell, 1989). Still some polycultures do not appear to bring these advantages (Risch et al., 1983; Russell, 1989). Russell (1989) identified about 150 polyculture studies and found that in 53 % of them a decrease of the number of herbivore species is reported and in 18 % the number had even increased.

Root's enemies hypothesis (1973) has been tested often and/or criticised (e.g. Russell, 1989; Sheehan, 1986; Vet & Dicke, 1992). In a particular ecosystem, polycultures may increase the number of enemy species, but the influence of intercropping on a given species may be different. A higher diversity of enemies does not mean that the problematic pest will be kept at a low level, especially if the more effective enemy does not respond positively to the mixed cropping system. The interactions between enemies and the host plants of their prey may be an important factor that determines if a given enemy affects a herbivore population. For control strategies relying on useful interactions within the agroecosystem, the spatial aspects and underlying mechanisms which influence and direct dislocation of insects, become a key issue (Dorn, 1993).

Plants under herbivore attack are known to be induced to release chemicals which may be attractive to some natural enemies (reviews in Rutledge, 1996; Turlings et al., 1995). In a polyculture a mixture of odour blends is released by different plant species. The insect species that respond to herbivore-induced plant odours may be confused in a polyculture due to the interference of associated plants. Chemicals coming from a plant different from the ones on which their hosts feed may mask the attractive odours or even repel the parasitoids (Price et al., 1980), and decrease their orientation abilities (Singh-Rathore, 1995).

Cassava is commonly grown on small farms in Latin America (CIAT, 1976) and frequently intercropped (CIAT, 1975). The parasitoids *A. vexans* and *A. diversicornis* were shown in previous chapters to be attracted to healthy as well as to infested cassava plant odours and to distinguish between both of them, which was not the case for the generalist *Acerophagus coccois.* According to Sheehan (1986), it could be expected that specialist parasitoids respond negatively to a polyculture. Specialist enemies may use more specific chemical cues than generalists (Vinson, 1976), also suggested in chapter 4. This indicates that specialists may respond more strongly to masking of attractants, and consequently, they may colonise simple agroecosystems more readily than diverse systems (Sheehan, 1986). Here we report on the effect of odours from bush bean, *Phaseolus vulgaris,* (var. Pijao) on the attractiveness of cassava plants to two encyrtids in a Y-tube olfactometer. In addition, parasitism rates for these parasitoids were determined in a field experiment in mono- and diculture (bean-cassava).

## 6.2. MATERIALS AND METHODS

### *Laboratory Experiment*

### *Olfactometer*

In chapter 4 the material and experimental procedure for Y-tube olfactometer experiments were described in detail. Cassava plants and insects were kept under the same conditions as described there.

### *Beans*

Every day 12 bean seeds (var. Pijao) were planted in a plastic tray (50x40x20cm) and kept in a greenhouse at an average temperature of 32° C during the day and 23°C at night. Three weeks later the plants were used in experiments, when they carried at least two well developed trifoliums. As odour source for the olfactometer experiments, two trifoliums were cut from a plant, and their petiole was inserted in a 2 ml-glass vial. Care was taken that the leaves were not further damaged.

### *Odour Sources*

Five different pairs of odours were offered to *A. vexans* and *A. diversicornis* in the olfactometer. The experiment "Infested vs. Blank" was repeated here as control experiment : two infested cassava leaves (see chap. 4) were offered against two 2 ml-glass vials filled with water. As a blank, these glass vials filled with water replaced the cotton wool used in previous experiments (chap. 4 and 5). For the choices including "Bean", two bean leaves (trifolium) were cut and their petiole was placed into the vials with water, to avoid odour emissions from the cut end of the petiole and to prevent dessication of the leaves. "Infested" is related to two infested cassava leaves which were cut when they carried mealybugs and honeydew, while "Healthy" corresponds to two leaves from a healthy cassava plant, on which no insect pest had ever occurred. For the mixed odour sources, two leaves of each plant were placed into the odour chambers. The five different choices were tested the same day with both parasitoid species. On the same day six 1.5-2.5 day old wasps were tested per odour combination. Ninety females were tested for each combination. The experimental procedure and the evaluation of choices were identical to the ones described in chapter 4.

### *Field work*

### *Plants*

Cassava stakes (var. CMC40) came from multiplication plots at CIAT. Stakes of 20 cm length were directly planted into the field or, when used for infestation, into pots one week later. The potted plants were left in the screened compartment described in chapter 4 for 3-4 weeks, until they reached the desired developmental state (approximately 30 cm high, more than 10 leaves) to be infested with mealybug ovisacs. Pijao bush beans came from the CIAT bean-seed department. They were planted directly into the field 3 weeks after the cassava plants.

### *Infestation*

Because *A. vexans* and *A. diversicornis* prefer third instar and adult mealybugs (Herrera & Bellotti, 1986); chapter 7), the infestation of cassava plants was achieved in two steps so that they carried late second and third instar larvae by the time the plants were brought into the field and the optimal stages were available for the bulk of the five days period of exposure. Two infestations were done at one week interval. Each time four mealybug ovisacs were deposited on three leaves. One and a half week after the last infestation, all mealybugs which did not belong to the preferred age classes were removed. From the remaining mealybugs, 300 were counted and the superfluous insects were removed.

### *Parasitoids*

One day before the parasitoid release, newly emerged *A. vexans* or *A. diversicornis* were collected from the rearing colony and kept all day in Plexiglas® cages with honey and water. Males and females were kept together to assure copulation, but no plant was present.

### *Field Experimental Design*

The experiments were conducted at CIAT-Palmira, Colombia (965m a.s.l.;.3° 30' north lat./76° 21' west long.), from March 1996 to May 1997. Table 6.1. gives the meteorological details.

**Table 6.1.** : Temperatures and precipitation that occurred in the experimental field during the exposure time to parasitoids.

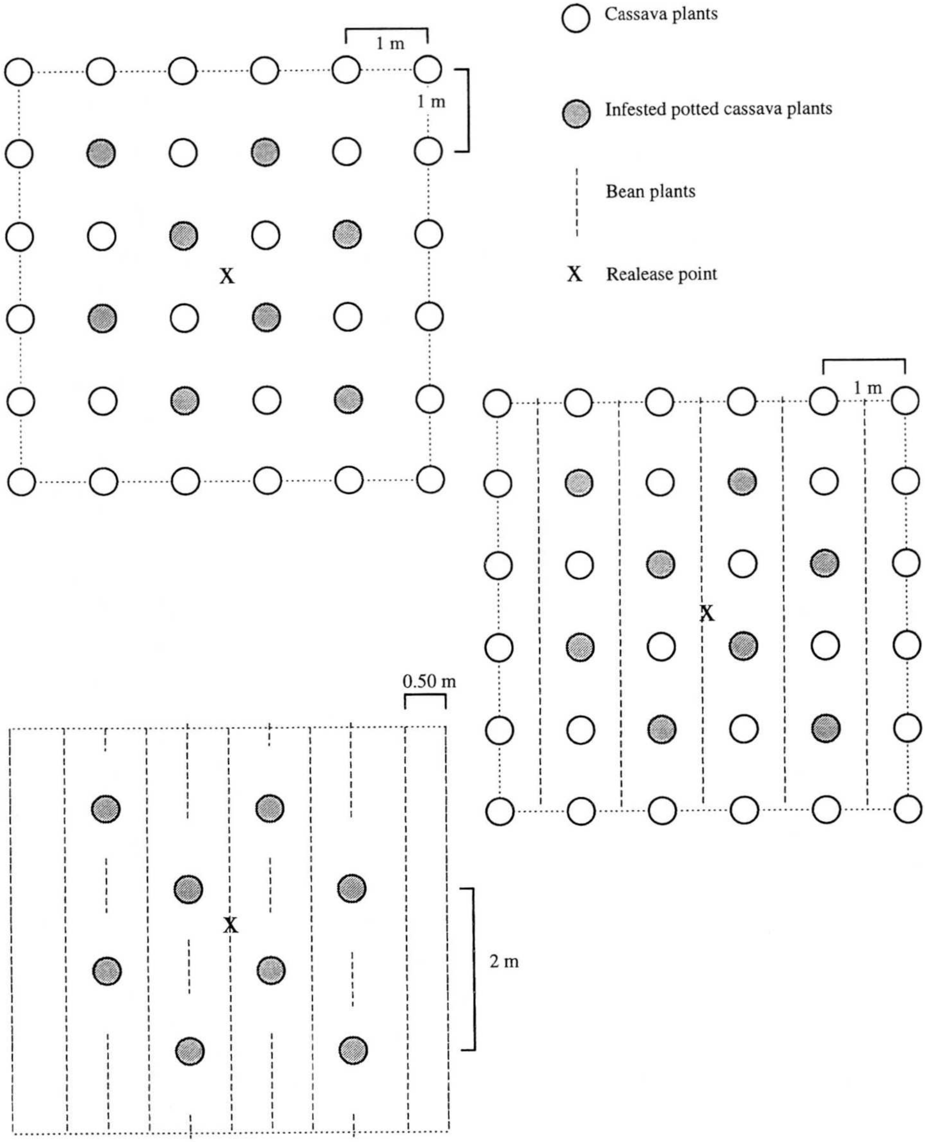
| Species | Duplicate | Date | Days with rain | Rain range (mm) | Temp. range (C°) |
|---|---|---|---|---|---|
| *A. vexans* | I | 8.3.-12.3.96 | 4 | 0-33.9 | 18.0-29.4 |
| | II | 13.12.-17.3.96 | 0 | 0 | 17.9-31.0 |
| | III | 23.5-27.5.97 | 2 | 0-15.8 | 17.5-31.8 |
| A. diversicornis | I | 28.6-2.7-96 | 5 | 0.2-20.7 | 18.9-28.0 |
| | II | 15.11.-19.11.96 | 1 | 0-0.5 | 15.9-29.5 |
| | III | 25.4.-29.4.97 | 2 | 0-2.5 | 17.2-31.3 |

Three different treatments (crop mixtures) were arranged in a block of 12 plots of 5mx5m each (Fig. 6.1). Between them a row of cassava or bush beans (var. Pijao) was planted. Bush bean rows were planted around the plots in which no cassava was planted. The rows between the plots were meant to separate each plot without free spaces between the plots, which may have influenced wind movements and created border effects. Figure 6.1 shows the different treatments : cassava pure stand, cassava intercropped with bush beans (±50 %) and bush beans alone. Each cassava plot contained 6 rows of 6 cassava plants with a distance of 1 m between rows and between each plant inside the rows. In the four central rows, two plants were left out of each row, leaving a distance of 2 m between two cassava plants (Fig. 6.1). In the mixed culture plots, the same design was used as in cassava pure stand and rows of beans were sewn 10 cm from each others between the cassava rows. In the last treatment, bean pure stand, 11 rows of beans were sewn. The two outer rows were sewn without interruption, while in each second row in the middle, the sewing was interrupted twice for 2 m each (Fig. 6.1). In the open spots in each plot, infested potted cassava plants were placed on an afternoon (2-4 p.m.) and left in the field for parasitisation for five days. Either 20 females and 10 males of *A. vexans* or 20 females of *A. diversicornis*, which is parthenogenetic, were released the next morning (8 a.m.) in the middle of each plot. The two parasitoid species were never released at the same time, but the experiment plots for the different species were contiguous. *A. vexans* seemed to be more aggressive and remained longer in the area. Except for the first trials *A. diversicornis* was first released and a few weeks later the experiment with *A. vexans* was started (Tab. 6.1).

### *Evaluation*

After five days of exposure, the potted cassava plants were brought back into a greenhouse. The mealybugs that remained on each plant were counted and used as

**Figure 6.1.** Description of the three treatments for field trials.



basis for the calculation of parasitism rates. New ovisacs were removed because they often contained eggs of the syrphid predator *Ocyptamus sp.* The larvae of this fly feed on mealybug eggs and when their egg provision is over, they will feed on the nymph and adult mealybugs. The greenhouse was maintained closed, so no other parasitoids or predators could come in. Some two weeks later, the mummies (parasitized mealybugs) were individually collected in gelatine capsules and counted. Emerging wasps were identified and sexed. For statistical analyses, percentages were transformed into arcsine and a one-way ANOVA analysis was used.

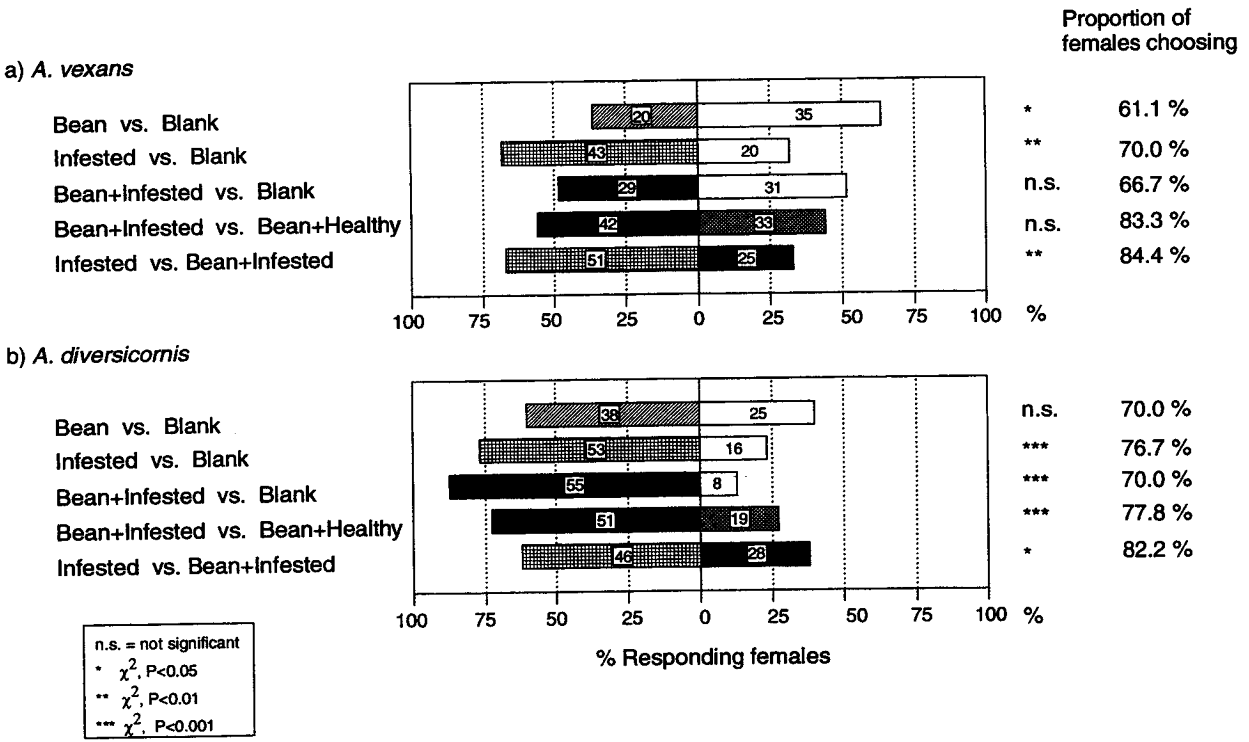
## 6.3. RESULTS

### *Laboratory Experiments*

*A. vexans* showed a stronger response to odour pairs with a higher quantity of cassava leaves (83.3 % and 84.4 %). The responsiveness was lower in odour pairs with a lower quantity of cassava leaves (70 % in “Infested vs. Blank”) and the ones that included bean leaves (61.1 % and 66.7 %) (Fig. 6.2a). *A. diversicornis* responded somewhat less than *A. vexans* in the odour pairs with two cassava leaves on both sides, but their responsiveness was higher for *A. diversicornis* in the other odour pairs (Fig. 6.2b).

*A. vexans* females significantly preferred the “Blank” (63.6 %) over bean leaf odours ($\chi^2 = 4.09$, $P<0.05$) (Fig. 6.2a), which may explain the low overall response obtained in this choice (61.1 %). This species was significantly attracted to infested cassava leaves over clean air (“Blank”)(Fig. 6.2a) as was already shown in chapter 4. Bean leaf odours apparently confused the wasps, which were not attracted to cassava

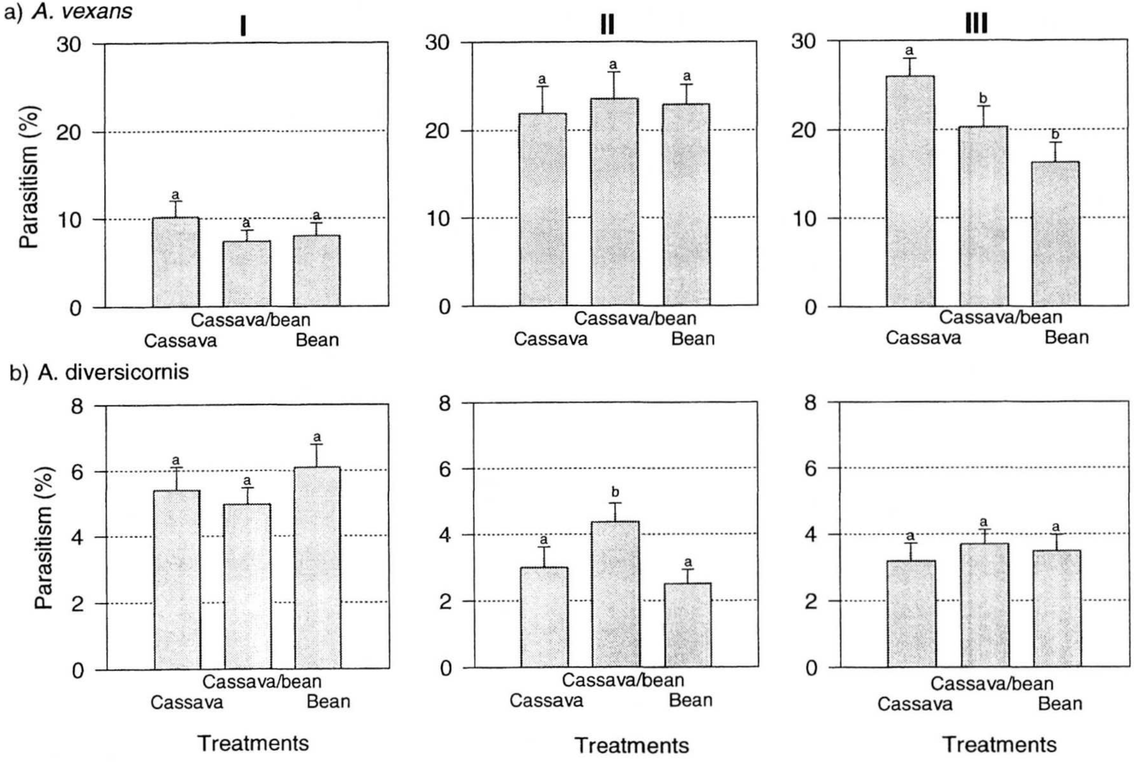
**Figure 6.2.** Responses of (A) *A. vexans* and (B) *A. diversicornis* in a Y-tube olfactometer to bean leaf odours offered alone or in combination with cassava leaves. In each bar the actual number of wasps that made a particular choice is given, while the x-axis indicates the percentages of choosing 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 odours, as well as the total number of females that were tested per choice.



leaf odours when they were mixed with bean leaf odours. When the wasps were offered a combination of infested cassava and bean leaves vs. clean air ("Bean+Infested vs. Blank), only 48.3 % of the responding females walked up to "Bean+Infested" side ($\chi^2 = 0.066$, $P>0.75$). They were not capable to distinguish between infested and healthy cassava leaves either (see chapter 4) when bean leaf odours were present on both sides ("Bean+Infested vs. Bean+Healthy") (Fig. 6.2a); Only 56 % of the choices were made for infested cassava with bean leaf odours, which is not significantly different ($\chi^2 = 1.08$, $P>0.25$) from the other choice. Of the responding *A. vexans* females, 67.1 % preferred infested cassava leaves alone over infested leaves associated with bean leaves ("Infested vs. Bean+Infested") ($\chi^2 = 8.89$, $P<0.005$).

Unlike for *A. vexans*, bean odours did not seem to affect *A. diversicornis* (Fig. 6.2b). No statistical difference was found between the proportion of females choosing either odours of bean leaves (60.3 %) or clean air ("Blank") ($\chi^2 = 2.68$, $P>0.10$). The presence of bean leaves did not interfere with the attractiveness of cassava leaves. Of the responding females, 87.3 % preferred "Bean+Infested" over "Blank" ($\chi^2 = 35.06$, $P<<0.001$), 72.9 % preferred infested over healthy cassava leaves, even when bean leaves were present ($\chi^2 = 14.628$, $P<<0.001$). However, like *A. vexans*, *A. diversicornis* females preferred infested cassava leaves alone over a combination of cassava and bean leaves ($\chi^2 = 4.37$, $P<0.05$).

**Figure 6.3.** Parasitism rate (± S.E.) on potted infested cassava plants in the field by (A) *A. vexans* and (B) *A. diversicornis* for the three different treatments : cassava = cassava pure stand, cassava/bean = mixed cropping with ±50 % bean, bean = high proportion of bean.



## *Field Work*

### *Parasitism in the Different Cropping Systems*

For most replications about 60 % of the mealybugs were recovered. Parasitism rate for *A. vexans* was higher than for *A. diversicornis*. *A. vexans* reached 20-25 % in the two last trials, while *A. diversicornis* did not exceed 6 % (Fig. 6.3 a and b).

For *A. vexans*, the second and third replicates were significantly different from the first one in a two-way ANOVA test, due in the much lower parasitism rate in the first trial. Using a one-way ANOVA test, no effects of bean plants on the parasitism rate were found in the first two field trials. For the first trial parasitism rates were 10.2 % ± SE 1.8 for cassava pure stand, 7.5 % ± 1.2 for plots containing bean and cassava and 8.0 % ± 1.4 for plots with almost only beans, in the second repetition the parasitism rates were 21.9 % ±3.1, 23.5 % ±3.0 and 22.9 ±2.2, respectively. The differences were not statistically significant. In the third trial the bean plants were higher (50-60 cm) than in the first two trials (20-30 cm) and almost reached the height of cassava plants (approx. 70 cm). In this trial, significantly lower parasitism was found for plots that included bean plants (20.3 % ± SE 2.4 and 16.3 % ± 2.2, respectively for mixed plots and the plots with almost only beans proportion) than for cassava plots (26.0 % ± 2.0) (Fig. 6.3a).

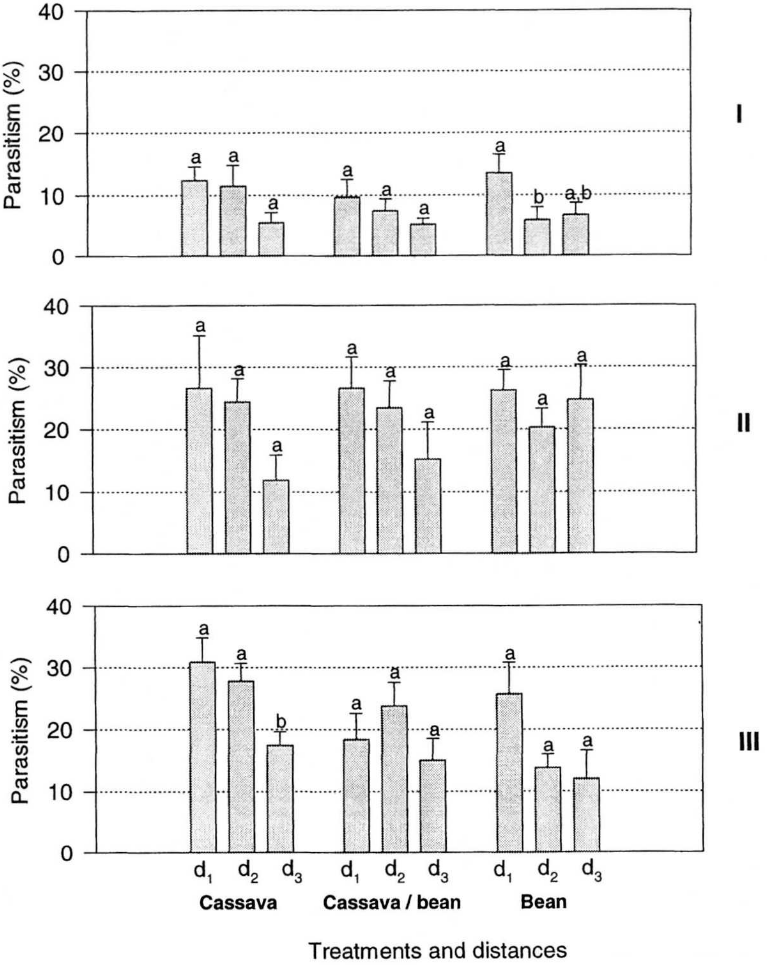
Two observations on parasitism by wild populations of *A. vexans* brought further indications that this wasp species forages better in cassava plots. Prior to the last trial an infested potted cassava plant was left in each treatment for five days to know if parasitoids were locally present. About the same number of *A. vexans* emerged from parasitised mealybugs in the cassava (15) and mixed plots (13), but no *A. vexans* emerged from the mealybugs in the bean plots. Moreover, during the third replicate with *A. diversicornis*, the parasitism due to wild *A. vexans* was particularly high (453 offspring). In the plots with a high proportion of bean, *A. vexans* parasitised half as many mealybugs (77 = 1.43 %) as in cassava (178 = 3.3 %) or in mixed plots (198 = 3.9 %), which is statistically significant (P=0.012).

Overall parasitism by *A. diversicornis* was very low (Fig. 6.3b). The first replicate is significantly different from second and third ones in the two-way ANOVA test, it showed a higher parasitism rate. Each replicate was analysed separately in a one-way ANOVA test. In the first trial *A. diversicornis* parasitised 5.4 % ± SE 0.7 in cassava pure stand, 5.0 % ± 0.5 in the cassava/bean plots and 6.1 % ± 0.7 in bean plots, in the third trial this was 3.2 % ± 0.5, 3.7 % ±0.4 and 3.5 % ± 0.5 respectively (Fig. 6.4). The differences are not significant. In the second trial, the parasitism rate for cassava/bean plots was significantly higher than in the two other treatments, which may be an artefact of poor mealybug recovery. In this trial several plants dried out and only 40 % were recovered from the cassava plots and the ones with a high proportion of bean and 50 % from the cassava/bean plots, but these differences were not statistically significant.

### *Parasitism as a Function of the Distance from the Release Site*

To test whether intercropping with beans influences the dispersal of parasitoids and the resulting pattern of parasitisation within a plot, the potted infested cassava plants were classified according to their distance from the release point (Fig. 6.1). The two closest infested plants were at a distance of 0.7 m (d1) from release point, four plants at 1.6 m (d2), and the two furthest plants at 2.1 m (d3). Fig. 6.4 and 6.5. reflect

**Figure 6.4.** Parasitism rate (± S.E.) on potted infested cassava plants in the field by *A. vexans* related to treatments (cassava - cassava/bean - bean) and distances from release point. Cassava = cassava pure stand, cassava/bean = mixed cropping with ±50 % bean, bean = high proportion of bean. $d_1$ = 0.7 m, $d_2$ = 1.6 m, $d_3$ = 2.1 m.



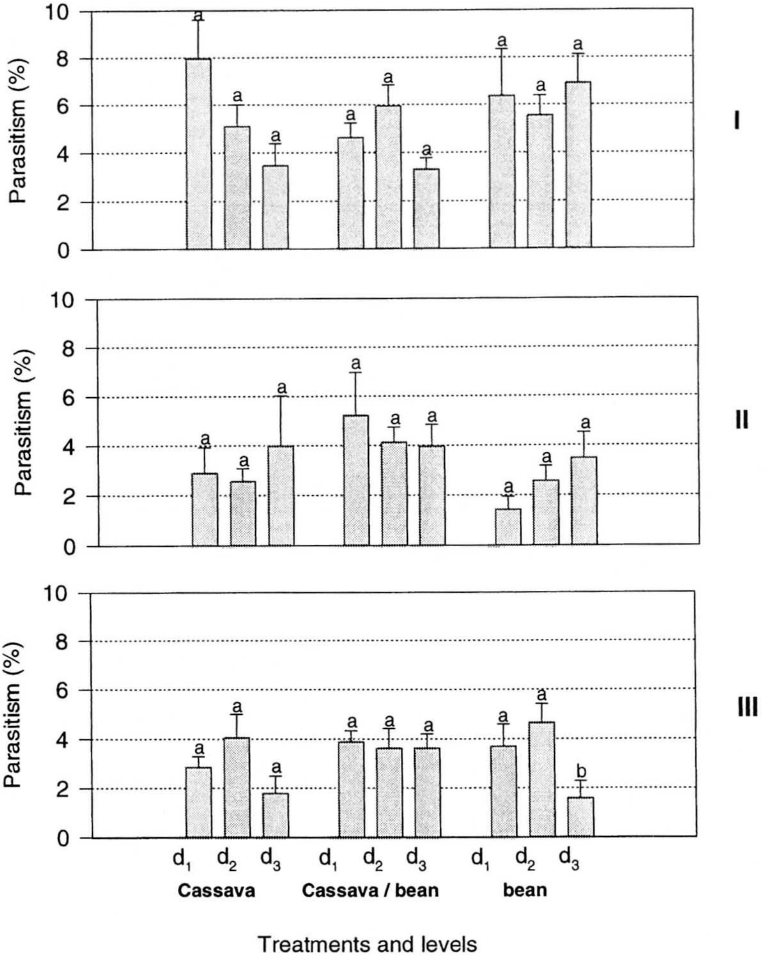
the findings.

For *A. vexans*, the spatial pattern of parasitisation in the first and second trials was not significantly different between treatments in a two-way ANOVA test, so the data were pooled and a one-way analysis was made for each replicate. In the first trial parasitism rates were higher for the nearest plants (11.8 % ± SE 1.5) than for the two other distances (8.3 % ± 1.5 and 5.9 % ± 0.9, respectively for distances d2 and d3). As mentioned above, in the second trial the parasitism rate was higher than in the first one, but showed in tendency a similar pattern (26.5 % ± 3.3, 22.8 % ± 2.1 and 19.0 % ± 3.4 respectively for d1, d2 and d3), but the differences were not statistically significant (P=0.135).

In the third trial, there is a significant difference between treatments and between distances, but without interaction. In cassava plots and those containing a high proportion of bean, females parasitised more on the plants nearest to the release point. The parasitism rate at the most remote distance was in tendency always the lowest one. This was significant only in the pure cassava stand. In general, treatments did not appear to influence this measure of dispersal by *A. vexans* females.

The low parasitism rate by *A. diversicornis* renders a statistical analysis of the spatial patterns difficult (Fig. 6.4). For the first trial, a two-way ANOVA test showed no difference between treatments and the data were pooled. Females tended to parasitise more on the nearest plants, but the differences between distances were not statistically significant (P=0.252). In the second replicate, treatments were statistically different, but without interaction for distances. The difference found for the intercropped plots was probably due to the higher parasitism rate observed in this treatment. As mentioned before this apparently higher parasitism rate is probably an

**Figure 6.5.** Parasitism rate (± S.E.) on potted infested cassava plants in the field by *A. diversicornis* related to treatments (cassava - cassava/bean - bean) and distances from release point. Cassava = cassava pure stand, cassava/bean = mixed cropping with ±50 % bean, bean = high proportion of bean. $d_1$ = 0.7 m, $d_2$ = 1.6 m, $d_3$ = 2.1 m.



artefact. In the third trial, there was no statistically significant differences between treatments and the data for the different plots pooled. Parasitism was higher on the plants close to the point of release (distances d1 and d2, 3.5 % ± SE 0.4 and 4.1 % ± 4.8, respectively) than on the most remote plants (distance d3, 2.3 %. ±0.4). Like for *A. vexans*, the different treatments did not affect *A. diversicornis* in its dispersal, but the latter dispersed more evenly over the plots.

## 6.4. DISCUSSION

The results for *A. vexans* in the olfactometer indicate that parasitoid foraging behaviour can be negatively influenced by non-target plant odours. This parasitoid species showed a significantly reduced attraction in the presence of bean plant odours. The legume even exerted some repellence on the wasps, resulting in a preference for clean air over bean plant odours. *A. vexans* was incapable to recognise infested cassava plant odours when they were mixed with bean odours.

For herbivorous insects there is evidence that non-host plants can disrupt their orientation (Guerin, 1987; Litsinger & Moody, 1976; Thiery & Visser, 1986). For example, the Colorado potato beetle which is attracted to the odours of its cultivated host plant, *Solanum tuberosum* L., does not respond to them any more when potatoes are mixed with wild tomatoes or cabbage (Thiery & Visser, 1986). Masking effects for natural enemies were considered to be less important than for herbivores because they are more often polyphagous and find their hosts on taxonomically diverse plants (Guerin, 1987). This would not be true for specialised parasitoids. If a parasitoid uses odours of plants attacked by its herbivorous host as cues in its foraging behaviour, it has been speculated that the presence of other plants may disrupt its orientation (Price et al., 1980; Sheehan, 1986). In the few studies reported on olfactory disruption for natural enemies, masking attraction of entomophagous insects has been suggested (Cromartie, 1981; Monteith, 1960; Shahjahan & Streams, 1973). Only Monteith (1960) showed a negative effect of non-host food plant odours for *Drino bohemica* Mesn., a tachinid parasitoid of the larch sawfly, *Pristiphora erichsonii* (Htg.). In an olfactometer, it preferred the odours of the coniferous tree *Picea glauca* alone over the association of the latter with another tree species, *Ledum groenlandicum* and did not make a choice when the quantity of the non-host plant was increased. The author attributed the result to a masking effect of the non-host plant. However, a part of Monteith's results (1960) are similar to the one of *A. diversicornis*, which preferred the infested cassava leaf odours alone over the association cassava/bean. In my olfactometer experiment, *A. diversicornis* clearly showed that bean plant odours did not influence its ability to recognise infested cassava plant odours.

In the field study, the influence of bean plants on the foraging behaviour of *A. vexans*, was only apparent in the third trial. In this trial, the wasps parasitised significantly fewer mealybugs in the two plots containing bean plants. One difference with the other trials was that in this last one, the legume plants were much higher. As the olfactometer experiments suggest that bean odours do confuse the wasps, it is possible that higher concentration of odours emitted by the relatively large bean plants did affect the foraging behaviour of *A. vexans* in the field. The actual bean plant biomass probably did not act as a physical barrier that prevented the wasps from searching efficiently, because dispersal of the parasitoids was similar for each plot type. The parasitism rates of the wild *A. vexans* in the field were low for plots with mainly beans. These observations suggest that only a few *A. vexans* wasps may have entered plots with beans if they had been released outside the experiment plots.

If we compare the number of *A. diversicornis* that emerged during the three successive experiments in which *A. vexans* was released (0, 11 and 29) with *A. vexans* that emerged in experiments where *A. diversicornis* was released (0, 69 and 453), the discrepancy is obvious. Just prior to the second trial, cassava was planted alongside our plots giving the parasitoids the opportunity to migrate and establish themselves in this field. One week before the beginning of the final experiment with *A. diversicornis*, the bordering field was sprayed with an organophosphate insecticide (Sistemin®= Dimethoate). This may have had a repellent effect on *A. vexans*, and provoked a massive migration into my experimental plots. The highest natural rate of parasitisation was found in the plots adjacent to the cassava field (about 90 parasitoids emergence as opposed to 30-40). Repellence to pesticides has already been reported for parasitoids. *Aphidius spp.* (Hym.: Aphidiinae) for example showed an increased dispersing and leaving behaviour in the presence of the pyrethroid deltamethrin (Longley & Jepson, 1996a; Longley & Jepson, 1996 b). Also *A. vexans* was observed in the olfactometer to be repelled by a pyrethroid insecticide(Cupex®, containing 0.07 % tetramethrin and 0.01 % deltamethrin), which was used against Dipteran insects (personal observation). Though, increasing natural parasitism may not have influenced the results of the last trial with *A. vexans*, as dispersal from release point is similar in cassava plots to the other trials, the wasps parasitised significantly more on plants near the release point. On the other hand the parasitism was not higher in the plots adjacent to the cassava field.

Parasitism by *A. diversicornis* was much lower than by *A. vexans*, especially in the second and third replications (less than 4 % against more than 20 %, respectively)

(Fig. 6.3 and 6.4). *A. diversicornis* was observed to spend more time parasitising a host (ca. 5 min.) than *A. vexans* (ca. 30 sec.). It also tends to deposit more than one eggs per host (Driesche van et al., 1986), which may further reduce its efficiency. On the other hand, *A. diversicornis* has been reported to be a bad competitor against *A. vexans* and *A. coccois* on *P. herreni* (J. Castillo, personal communication) or against *A. lopezi* in *Phenacoccus manihoti* (Pijls et al., 1995). The decreasing parasitism rate by *A. diversicornis* in the trials may be partly due to the increasing competition with *A. vexans.*

Dispersion within the plots could not be measured very accurately because of the low numbers, but it seemed that *A. diversicornis* spread out more after release, and it is likely that they leave the plots more readily. *A. vexans* seemed to parasitise more on plants at the shortest distance from their release point. Mass releases of the wasps against *P. herreni* in Brazil, at a totally different scale also showed that *A. vexans* did not disperse and was only recaptured at release sites, while *A. diversicornis* and *A. coccois* spread over hundreds of kilometres (J. Bento, personal communication).

## 6.5. CONCLUSION

In the olfactometer, the response of *A. vexans* to cassava odours was negatively affected by the presence of bean odours. In the field, this was only reflected in the trial with large bean plants, which may have interfered with the wasp's foraging. The bean plants in the first two trials may have been too small to confuse *A. vexans,* but when bean plants were twice as high, the wasps showed lower parasitism in plots with bean as compared to pure cassava stand. Natural parasitisation by the wasps suggested that the results may have been more pronounced if the wasps had been released outside the plots. *A. diversicornis* was not disturbed by the presence of bean plants, neither in the olfactometer nor in the field. This species seemed to disperse more rapidly away from the release point which may be an advantage in inoculative biological control, in which the wasps could establish rapidly in vast areas, but its parasitism rate was very low. *A. vexans* showed a higher parasitism rate in the field, but does not seem to disperse well. Its efficiency as a control agent may be negatively affected by mixed-cropping.

# 7. SECONDARY SEX RATIO AFFECTED BY HOST STAGE IN *AENASIUS VEXANS*

## 7.1. INTRODUCTION

Successful augmentative releases of parasitoids against pest require that large numbers of healthy females are reared. Similarly the success of several of the experiments for this project depended to a large part on the rearing of parasitoid colonies. For the Y-tube experiments as well as for field work, a high number of parasitoid females were needed. On several occasions the sex ratio of the *A. vexans* colony was so extremely male biased that no experiment could be done for several weeks. In order to alleviate this problem, which was disrupting the experimental time table, an experiment was started to optimise the sex ratio of the colony.

Sex ratios of Hymenoptera are known to be affected by environmental factors (Flanders, 1965). Most of them show an arrhenotokous parthenogenesis, which means that haploid eggs (unfertilised) develop into males and diploid ones into females (Bouletreau, 1976). The sex determination mechanism allows ovipositing females to precisely determine the sex ratio of their offspring. Different biotic and abiotic factors can influence the sex allocation, like temperature (Kfir & Luck, 1979; Laraichi, 1978; Löhr et al., 1989; Rechav, 1978), photoperiod (Bouletreau, 1976; Rechav, 1978) and host density (Green, 1982; Hu et al., 1986; Kolodny-Hirsch, 1988; Legner, 1967; Rotary & Gerling, 1973). But also the size of the host is a very important factor, which has attracted much attention from numerous biologists. Charnov's model (Charnov et al., 1981), which predicts that larger hosts are used for the production of female parasitoids has been frequently confirmed (e.g. Nechols & Kikuchi, 1985; Rechav, 1978; Sandlan, 1979). Larvae developing in larger hosts benefit of a larger food supply and show increased sizes (Charnov et al., *1981*; Sandlan, *1979*). Both sexes gain by being larger, but females gain more than males in fitness (Charnov et al., 1981). One of the main consequences is that they lay more eggs over their lifetime (Charnov et al., 1981; Dijken & Alphen, 1991; Sandlan, 1979; Srivastava & Singh, 1995).

The sex ratio of a koinobiont parasitoid, which lays eggs in still growing hosts, is not usually size dependent (Waage, 1982), as it is unlikely for the parasitoid to determine the eventual host size at the time of oviposition (Godfray, 1994). However, some studies (see review in King, 1989) report size-dependant sex ratio for koinobiotic parasitoids. After parasitisation by *A. vexans*, *P. herreni* still lives 10 days and can mould during this time (P. Calatayud, personal communication). Although *A. vexans* is a koinobiotic parasitoid, we suspected that host stage (size) at oviposition may play a role in sex allocation. To test this hypothesis, also with the objective to determine the best strategy for mass-rearing of *A. vexans*, we conducted a series of tests to measure host stage preference and host stage dependent sex allocation in no-choice and choice experiments.

## 7.2. MATERIALS AND METHODS

The experimental organisms, plants and insects, were reared and kept under the same conditions as described in chapter 4.

### *Hosts*

Each stage of the mealybugs was chosen at a mid-development average size. Second instar larvae of the mealybug were chosen when they were big enough to be recognised as females, which remain white, while males turn rosy before they start spinning their cocoon. Adult females were used after they had moulded but before they started producing ovisacs. Only large females were used.

### *Wasps*

Five days before the experiments, mummies (parasitised mealybugs), which can be distinguished from their dark grey colour and hard consistence, were collected from the colony. Each one was kept individually in a gelatine capsule and stored at about 28° C. The largest wasps of the emerging batch were used for the experiments.

### *Preparation of the Experimental Plants*

Leaves from a living plant were enclosed in Petri dishes of 15 cm diameter. The dishes were fixed to a thick iron wire planted into the soil to support the Petri dish so that the leaves would remain in their natural position. In order to reduce the condensation due to plant respiration, two holes of 10 cm each were cut in the upper and lower sides and sealed with nylon screen. Two holes of 1 cm diameter were drilled on opposite sides of the vertical wall of the Petri dish. One was used to introduce plant petiole. Cotton wool was wrapped around the petiole to prevent damaging it and to plug the hole, the aperture above it was sealed with Parafilm®. The second hole was used for the introduction and capture of the parasitoids. The dishes were wrapped with Parafilm® to seal all holes and the space between the two dish halves. No insects could escape or enter. Each plant carried 3 Petri dishes.

### *No-choice experiment*

First instar mealybugs are much smaller than *A. vexans* adults and are seldom parasitised. If so, no offspring will emerge (CIAT, 1987-1991). This is why only later stages were used for the experiments. Fifty mealybugs of the same stage were carefully transferred with a small paint brush from an infested cassava plant onto a leaf enclosed in a Petri dish. The mealybugs were lifted with the brush at their abdomen moving toward the mouth to prevent breaking their stiletto, which were often inserted in the plant. The insects were placed on the upper side of the leaf, from where most of them moved to the lower side. 19 females were tested for second larva instars and adults and 17 for third instars.

### *Choice Experiment*

Twenty mealybugs of each stage were transferred to the same leaf as described above. The following day, after they had been exposed to a parasitoid, the mealybugs were separated by stages and transferred into three other Petri dishes on a plant. The experiment was achieved with 21 *A. vexans* females.

### *Parasitisation*

The first morning of an experiment, newly emerged *A. vexans* females (1-6 hour old) were individually introduced into a Petri dish containing a mealybug-infested leaf. A few females were introduced alone and unmated to confirm the arrhenotokous parthenogeny. The others were accompanied by a male to allow them to copulate. Each morning at about 10 a.m. the parasitoid couples were transferred into a new dish with fresh mealybugs of the same stage or stage combination. The sixth morning the wasps were discarded.

The plants with the parasitised mealybugs remained two weeks in a glasshouse at an average temperature of 32° C (max. 40° C) during the day and 23° C (min. 19° C) at night and an average daytime humidity of 40 % and 74 % at night. After this period, mummies were removed and individually kept in gelatine capsules. When the parasitoids emerged, their sex was recorded.

### *Parasitoid Size*

In the choice experiment, after sexing the emerging adults, the length of the hind tibias was measured for each wasp and related to the mealybug stages they came from.

### *Statistic*

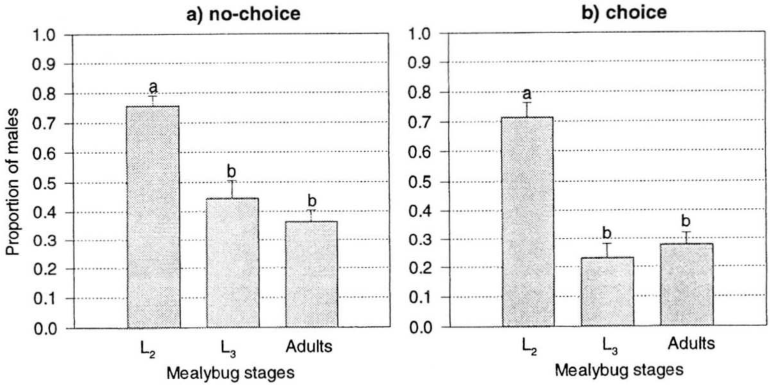
Percentage data for sex ratio and preference experiments were transformed to their arcsine to approach a nearly normal distribution, then a one-way ANOVA analysis was performed. The lengths of the tibia were not normal distributed, even after a log transformation. Kruskal-Wallis one-way ANOVA on ranks was used.

## 7.3. RESULTS

### *Sex Ratio*

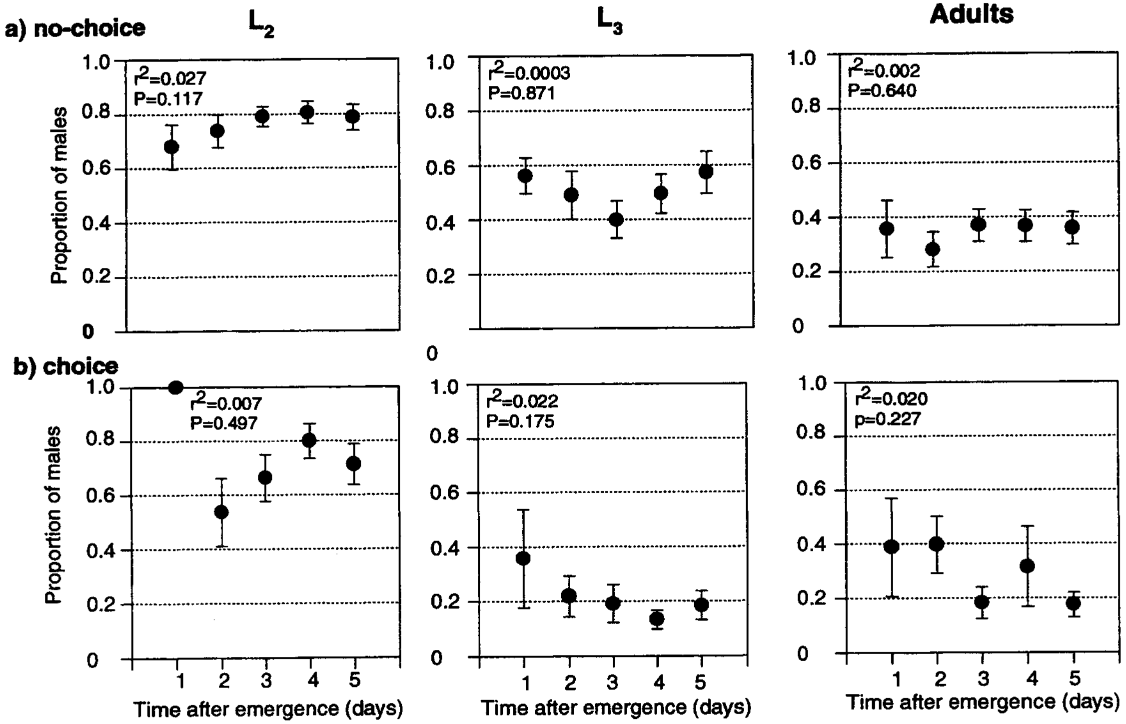
Mealybugs of the second larval instar yielded fewer *A. vexans* females than third instars and adults in both no-choice and choice experiments (Fig. 7.1a and b). The average male ratios (± SE) for second instar host larvae were 0.76 (± 0.03) and 0.71 (± 0.05), respectively for the two experiments. For third instars the male ratios were 0.45 (± 0.06) and 0.23 (± 0.05) respectively and 0.36 (± 0.04) and 0.28 (± 0.04) for mealybug adults. The sex ratio of the emergence should be regarded as secondary sex ratio. The differential mortality before mummification may have contributed to differences in sex ratio among the different host stages. However, *A. vexans* mortality after the mummy stage for all host stages is similar (about 5 % and 8 %, respectively for no-choice and choice experiments).

**Figure 7.1.** Proportion (± S.E.) of *A. vexans* males emerged from second, third and fourth mealybug stages. a) *A. vexans* females were offered 50 hosts of only one stage (n=19, 17, 19, respectively for second, third and fourth stages). b) 20 mealybugs of each stage were offered to *A. vexans* females (n=21). Different letters are statistically different (P<0.05).



The male ratio did not change much with time when the parasitoids did not have a choice between different mealybug stages. In third instars and adult stages, the proportion of males produced per day did not vary, but in second instars it slightly increased with time, but not significantly (Fig. 7.2a). In the choice experiment, the

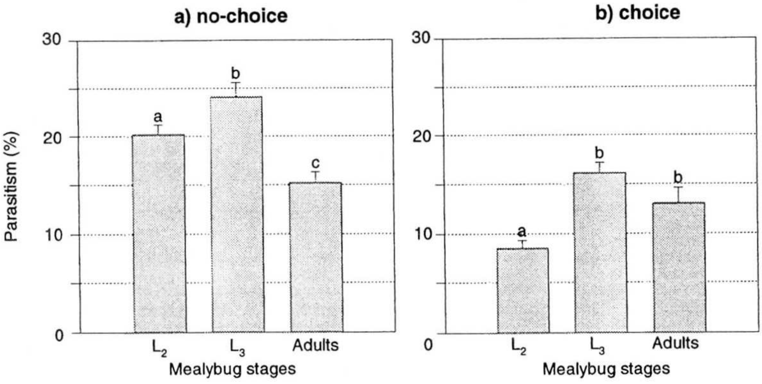
**Figure 7.2.** Proportion (± S.E.) of males produced per day and per host stage by *A. vexans* females during five days. a) *A. vexans* females were offered 50 hosts of only one stage (n=19, 17, 19, respectively for second, third and fourth stages). b) 20 mealybugs of each stage were offered to *A. vexans* females (n=21). Different letters are statistically different (P<0.05).



male ratio decreased slightly over time for third and fourth stages but increased for second instars (Fig 7.2b), but these changes were not statistically significant. The increase of male ratio for second instar hosts appeared stronger in the choice (from 0.53 to 0.83) than no choice experiments (from 0.66 to 0.80). The male ratio for the first day is not included in choice experiment because only three females successfully parasitised four mealybugs in total. They all produced males. In third instars and adults only 7 and 6 *A. vexans* females respectively had progeny, so that the first day ratios should be taken with care.

### *Preference*

**Figure 7.3.** Parasitism rate (± S.E.) in each host stage. a) *A. vexans* females were offered 50 hosts of only one stage (n=19, 17, 19, respectively for second, third and fourth stages). b) 20 mealybugs of each stage were offered to *A. vexans* females (n=21). Different letters are statistically different (P<0.05).

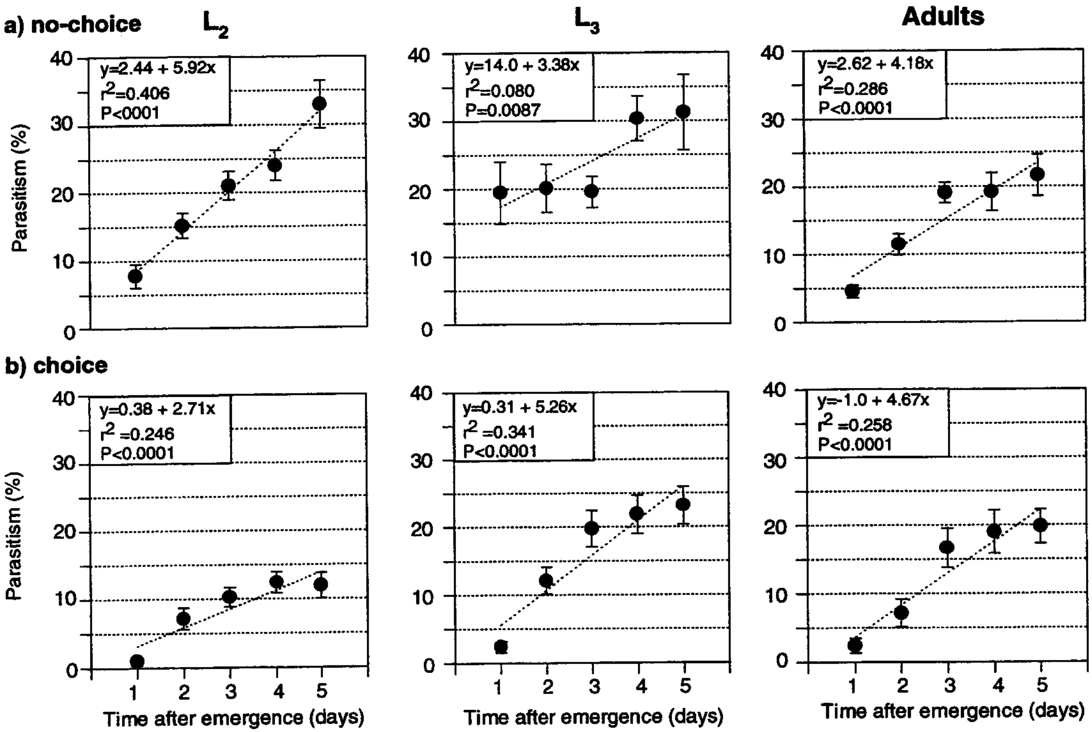


In the choice experiment, *A. vexans* females parasitised significantly more third instars and adults (16.1 % ± SE 5.0 and 13.0 % ± 7.4, respectively) than second instars (8.5 % ± SE 3.9) (Fig. 7.3b). The preference for third mealybug instars was confirmed in the no-choice experiment. The emergence from third instars was significantly more numerous than for second instars and adults (24.1 % ± SE 1.5, 20.2 % ± 1.0, and 15.2 % ± 1.1, respectively) (Fig. 7.3a). When the encyrtids had a choice, they significantly preferred adults over second mealybug instars, but not in the no-choice experiment, in which *A. vexans* showed a significant higher emergence in second instars than in adults (Fig. 7.3a and b).

The curves for the daily parasitism appear to be different in choice and no-choice experiments (Fig. 7.4). When only one mealybug stage was present, females started parasitising on the first day after emergence (7.8 %, 19.4 % and 4.5 % in second and third instars and in adults, respectively). Females laying in the preferred third mealybug instars continued parasitising a similar number of mealybugs during the following two days (about 20 %) and increased the production the fourth (30.4 %) and fifth days (31.2 %). In contrast, for the second mealybug instar, the wasps started with only a few eggs the first day and almost doubled their production the second day (15.2 %). The parasitism rate kept increasing the following days more or less linearly (Fig. 7.4). Similarly, in the mealybug adults, *A. vexans* doubled their production the second day (11.5 %), but then they stabilised their production to about 20 % for the last three days.

In the choice experiment, very few mealybugs of all stages were parasitised on the first day (Fig. 7.4a); about half of the females did not lay any eggs (11.1 % in no-choice experiment). The production increased with time for all three stages. Only for

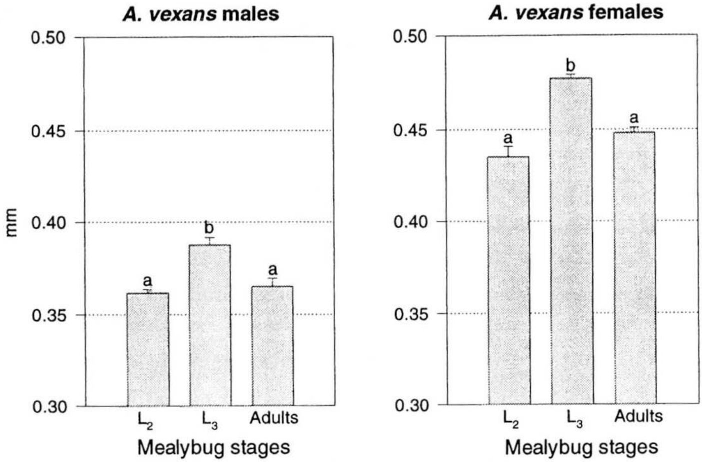
**Figure 7.4.** Parasitism rate (± S.E.) in each host stage per day. a) *A. vexans* females were offered 50 hosts of only one stage (n=19, 17, 19, respectively for second, third and fourth stages). b) 20 mealybugs of each stage were offered to *A. vexans* females (n=21). Different letters are statistically different (P<0.05).



second mealybug instars, *A. vexans* maintained a low parasitism rate, it did not exceed 12.4 % (Fig. 7.4a). The preference pattern was more or less the same for all days. For each day between 40 % and 50 % of all the mummies came from third instar hosts.

For second host instars, *A. vexans* increased the production in the no-choice experiment from 7.8 % to 32.9 % over five days, while it did not pass 12.4 % in the choice-experiment (Fig. 7.4b). For adult stages the parasitism rate curves for no-choice and choice-experiments are similar. In the no-choice experiment it goes from 4.5 % to 21.6 % and from 2.4 % to 19.8 % in the choice-experiment.

**Figure 7.5.** Tibia lengths (mm) of the *A. vexans* insects that emerged from different host stages. a) Tibia length of the emerging males b) Tibia length of the emerging females. Different letters are statistically different. (n=21). Different letters are statistically different.



### *Wasp Size*

*A. vexans* females were significantly larger than males (P<0.0001). Female as well as male parasitoids emerging from hosts of third instars were significantly larger than the ones from second instars and adults (Fig. 7.5). The latter were not significantly different from each other.

## 7.4. DISCUSSION

The size of emerging wasps from *P. herreni* was highly correlated with the host size at the time of parasitisation. Third instar larvae of the mealybug produced larger parasitoids than adults and second instars, which is similar to what Cadée & van Alphen (1997) found for the citrus mealybug, *Planococcus citri* (Hom. : Pseudococcidae) and its encyrtid parasitoid, *Leptomastidea abnormis*. They found that parasitoids that emerged from egg-producing mealybugs were lighter than the preovipositing ones. The authors suggest that the mealybugs use resources for egg production, which are then not available to the parasitoid larvae. The size of adult parasitoids is usually correlated with their fitness; larger females lay more eggs over their lifetime (Charnov et al., 1981; Dijken & Alphen, 1991; Sandlan, 1979; Srivastava & Singh, 1995). Third instar larvae of mealybugs are therefore likely to be most suited for *A. vexans*.

The sex ratio I found for the three different host stages is in accordance with the suitability of the third instar host larvae. Third instars and adult mealybugs produced more female parasitoids, while second instars produced more males. This host dependant sex ratio for a koinobiont parasitoid goes against Waage's theory (1982), that suggests a size-dependant sex ratio only for idiobiont parasitoids, their hosts do not develop after parasitisation, but confirms Charnov's model (Charnov et al., 1981), which postulates that larger hosts should produce more females. Charnov et al. (1981) also predict in their model that sex ratio depends on host size distribution. The size of a host is not perceived as the same absolute mass by the parasitoids. A host of a certain size may be considered relatively small in some populations or relatively large in others. If a population has a higher proportion of small insects, parasitoids would use more small hosts to produce females, so that insects that are relatively big in a small insect population produce more females than insects of the same size in a population of large insects. In the choice experiment the female production for larger mealybug stages slightly decreased over time while it increased for second instars, though not statistically significantly (Fig. 7.2b). This may indicate that the females learned the host distribution over time, as predicted by Charnov's model (Charnov et al., 1981). However, when wasps were exposed to one stage only separately, the sex ratio did not change over time (Fig. 7.2). A decrease in male production for second instar hosts was expected, but it actually showed a slight increase over time. This suggests that the wasps may have an innate ability to recognise second instar hosts as unsuitable for female (Charnov, 1979). For all three stages sex ratio did not significantly change over time.

Charnov et al. (1981) offered weevil larvae of a certain size to females of the pteromalid *Lariophagus distinguendus*. When these larvae were in company of larger hosts, the sex ratio increased, it decreased when the reference larvae were offered in presence of smaller ones. This is contrary to my findings. In general the sex ratio was more female biased for choice than no-choice experiments. Second mealybug instars offered alone (no-choice experiment) produced 75.8 % males, in presence of larger hosts (choice experiment) the sex ratio dropped to 71.4 %. Similarly, adult mealybugs yielded 36.3 % males in the no-choice experiment against 22.9 % in the choice experiment, where smaller hosts were also present. This slightly higher female production in the choice experiment could be explained by the role of the spermathecal gland in sex allocation (Flanders, 1939). Its function can be influenced by external factors(Flanders, 1965) and changes the proportion of sex ratio. Parasitoids lay male eggs in less preferred hosts, but when parasitisation happens shortly after egg deposition into a preferred host, they may fertilise eggs that would have remained unfertilised in other cases (Flanders, 1965). Thus, the encounters with larger hosts in the choice experiment may have resulted in a higher female production in second instar hosts. Because we did the choice and no-choice experiments with different generations of wasps a few months apart, any differences that we observed may have been caused by differences other than the factors I looked for. A true comparison is therefore difficult.

No-choice and choice experiments showed some other apparent differences. In general *A. vexans* parasitised more mealybugs when the three host stages were offered separately. This may indicate that when the parasitoids only encounter same sized-hosts they accept them more rapidly. Nechols & Kikuchi (1985) also found different preferences in host stages in choice and no-choice experiments for the encyrtid *Anagyrus indicus* and its host, the spherical mealybug. In all cases, *A. vexans* preferred third instar mealybugs which confirms its suitability for *A. vexans*. In the no-choice experiment fourth instar was least parasitised but was statistically not less preferred than third instar in the choice-experiment. However, the parasitism rates in both experiments were very close. Also the increase over time of parasitisation is very similar for both experiments, both curves for the daily parasitism look very alike (Fig. 7.4). Adult mealybugs have an aggressive defence behaviour, which may have reduced its parasitisation. In contrast, second instars, which almost do not defend themselves against the wasps, were more than twice as much parasitised in the no-choice than in the choice experiments. In the no-choice experiment the parasitisation increased fast over time and reached a similarly high level to the one for third instars, while it remained low in the choice-experiment (Fig. 7.4). The egg load of the wasps may have forced the females to parasitise at any rate in the only mealybug instar available, but was prevented doing so in adults, because of their good defence ability.

Hamilton (1967) introduced the theory of local mate competition, which suggests that a female biased sex ratio occurs in cases where related males compete for mates, given that one male can copulate with more than one female. Thus the sex ratio should also depend on the number of parasitoid females present (Hamilton, 1967). The female biased sex ratio found for third instars and adults may be explained with the local mate competition theory (Hamilton, 1967). *A. vexans*'s mating behaviour is not described in the literature. This species is solitary but parasitises a gregarious insect, since nymphs and female adults of *P. herreni* stay in groups most of the time. One parasitoid female will attack several hosts within a group, therefore, mating between brothers and sisters is expected. In another encyrtid parasitoid, *Apoanagyrus lopezi*, which parasitises the closely related gregarious host, *Phenacoccus manihoti*, local mate competition did not play any role in the female biased sex ratio; its sex ratio did not change in response to local mate competition (Dijken et al., 1989). In the field work with *P. herreni* and *A. vexans* described in chapter 6, the sex ratios found for second (0.29) and third (0.31) trials (sex ratio for the first field test was not recorded) appear very similar to the one calculated for the choice experiment (0.31) or the one for third instars and adults together (0.41) in the no-choice experiment. Twenty females and ten males were released in the field in each plot and the sex ratios were similar to the ones in sex ratio experiments. Although the conditions in the sex ratio experiments were completely different from the field tests, the sex ratio remained more or less unchanged, this could suggests that the female density does not influence much the sex ratio and that the local mate competition did not have a strong influence in the results found.

My results are based on the emergence, which may not correspond to the real allocation at oviposition. Differential mortality during larval development may have influenced the emergence, giving a wrong preference and sex ratio image. The mortality from mummy stage to emergence was, however, found to be low and did not significantly differ between the instars, nor between both experiments (about 6 % and 8.5 %, respectively for no-choice and choice experiments), which indicates that the preference calculated out of the emergence was not influenced by differential mortality at that stage. Still the sex ratio may have been influenced by a sexual differential mortality at an earlier preimaginal stage. Following Sandlan's example (1979), we compared the mortality of the progeny of virgin and mated females. Male offspring of virgin females showed a mortality for third instars (11.4 %) and adults (14.6 %) that was four times as high as for second instars (3 %) (the difference between second instars and adults was significant). Although these results should be taken with care because of the low sample size (n=5 for second instars and adults, n=7 for third instars), they may suggest that males have a higher mortality in larger hosts, which may have influenced the emergent sex ratio.

Differential mortality for parasitoid males and females in case of superparasitism may also occur (King, 1987). In my experiments superparasitism is unlikely to have played a significant role. In previous studies at CIAT (1992), five *A. vexans* couples were offered different mealybug nymphs densities during 24 hours. At the density that we used for the no-choice experiment (250 mealybugs for five *A. vexans* couples), superparasitism reached an average of only 1.3 % (CIAT, 1992). Such a low superparasitism should not have influenced the results of my experiments. On the other hand, encapsulation may also be the cause of differential mortality. Encapsulation ability is often better for later stages (Benrey & Denno, 1997; Blumberg, 1997; Brodeur & Vet, 1995), but for *P. herreni*; adult mealybugs are less successful at encapsulating the parasitoid *A. diversicornis* than second instar mealybugs (Driesche van et al., 1986). Though, the difference (15 % over 10 %) (Driesche van et al., 1986) is not very high and is unlikely to have a significant effect on the results.

## 7.5. CONCLUSION

Sex ratios in the laboratory experiments were similar to the ones observed in the field. The sex ratio was in accordance with preference of the wasps. They produced more females in their preferred host stages, namely third instar and adult mealybugs, and more males in the smaller second instar larvae of the host. However, the preference for adult stages changes for no-choice and choice experiments. The parasitism rate remained constant but was lower than the two other stages in no-choice experiment, while it was not significantly different from third instar in choice experiment, probably due to the least preference for second instars. The apparent constant parasitism rate is probably due to the good defence ability of adult hosts. Third mealybug instars seem to be the best suited stage for mass rearing of *A. vexans*.

Female and male parasitoids emerging from this stage were larger, what is usually correlated with a higher performance and in females it results in a higher lifetime fecundity (Sandlan, 1979). The results of this study do not entirely fit Charnov's model (Charnov et al., 1981); the sex ratio at different host size distributions did not significantly vary between the no-choice and choice experiments, but the host size dependence was in accordance with the model (Charnov et al., 1981), which predicted that larger hosts produce more females. *A. vexans* produced significantly more males in second instar (smaller) hosts, which contradicts Waage's theory (Waage, 1982), which says that koinobiont insects do not adjust sex ratios depending on host size.

# 8. GENERAL DISCUSSION

The main goal of this study was to determine the impact of intercropping on the foraging behaviour of parasitoids of the cassava mealybug, *Phenacoccus herreni.* This tropical plant is partly cultivated in mixed cropping (Cock, 1985) which means that parasitoids of cassava pests very often have to search for their host in diversified ecosystems.

The three encyrtid parasitoids, *Aenasius vexans*, *Apoanagyrus diversicornis* (asexual strain) and *Acerophagus coccois*, are attracted to cassava plant odours, which indicates that these wasps use olfactory cues to find their host habitat. Though with some differences, only *A. vexans* and *A. diversicornis* could distinguish between odours from infested cassava plants and odours from healthy plants. The fact that *A. coccois* does not make this distinction could be because it is a generalist and as such may use different foraging strategies than more specialised solitary wasps. Herbivore-induced volatiles released by infested plants have been shown to be attractive to natural enemies (Dicke, 1994; Turlings et al., 1995). This was also found in this study. *A. vexans* and *A. diversicornis* (asexual strain) females were significantly attracted to infested cassava plant odours but *P. herreni* and their by-products were not significantly attractive to both wasp species (chapter 5). In similar studies, others have found that the encyrtid wasps, *Apoanagyrus lopezi* and *A. diversicornis* (sexual strain) were attracted to mealybug-infested cassava plants, but not to the mealybugs themselves nor their by-products (Alphen van et al., 1990; Nadel & Alphen, 1987). If a herbivore can avoid information conveyance, parasitoids are more likely to turn to information from plants (Vet & Dicke, 1992), which seems to be the case for parasitoids of the cassava mealybug. However, van Baaren & Nénon (1996) found that

*A. lopezi* coming from Congo was attracted to unparasitised *P. manihoti.* Unlike Nadel & van Alphen (1987) they also found an attraction to healthy cassava plant odours. These two different results with the same plant and insect species suggest variability and the interference of other factors. One of them may be the origin of the insects, which was shown by Du et al. (1996) to significantly affect a parasitoid response. They tested two strains of the aphid parasitoid *Aphidius ervi* in a wind-tunnel, one of them originated from Bulgaria responded significantly better to target odours than the other one from Britain. The plant variety could also influence the insect response in producing different volatile blends. Studies with parasitoids of *P. manihoti* in Africa did not constantly found a respond to healthy cassava plant odours (Alphen van et al., 1990; Baaren van & Nénon, 1996; Nadel & Alphen, 1987), while the ones of *P. herreni* I tested did (chapter 4). This raises the question if *P. manihoti* system is different from *P. herreni* system, or if the plant variety used in this study played an important role in the outcome.

Because volatiles emanating from plants appear to be important for both *A. vexans* and *A. diversicornis* to find their hosts, the mixture of odours emitted by other plants in a diversified system may interfere with their searching efficiency. I have shown in this study that bean plant odours interfered with the attraction of *A. vexans* to infested cassava in the olfactometer. In contrast, *A. diversicornis* was not affected by bean odours and could still distinguish the odours of infested cassava plants in presence of odours of bean. Although the difference between the two species could not be finally confirmed in the field experiments, the negative effect of bean odours on search ability of *A. vexans* can be important for the effectiveness of this parasitoids as a control agent.

In inoculative biological control, parasitoids have to search in the target crop for their hosts. If repellent factors are mixed in a culture, wasps may not enter a field, even if their hosts are present and this may be the case for *A. vexans*. In the classical biological control program in Brazil, where wasps were introduced to establish and disperse (Smith & Bellotti, 1996), the parasitoids must look for cassava fields and choose to enter them. There is a risk that *A. vexans* does not enter a cassava field intercropped with bean. It is possible that the negative effect exerted by bean odours as observed in the olfactometer may be of importance if the biomass of bean plants is relatively high. The apparently low dispersion capacity of *A. vexans* is an additional disadvantage. In this study *A. vexans* had the tendency to parasitise more in plants near the release point (chapter 6), in Brazil it was observed that the wasps did not disperse throughout the region, while the two other species did over hundreds of kilometres (Bento, personal communication). As far as its dispersal ability is concerned *A. diversicornis* seems to be more suited for inoculative biological control, it disperses well and is not affected by bean plant odours, however the parasitism rates were very low for this wasp and it seems to compete poorly with *A. vexans* and *A. coccois* (J. Castillo, personal communication).

For inundative mass releases, *A. vexans* may be a good candidate. Its poor dispersion is an advantage in this case, as the wasps would probably remain relatively long in a target field. However, the sex ratio experiment (chapter 7) showed that *A. vexans* mostly prefers parasitising larger instars, this means that adult mealybugs may still have time to produce eggs before dying, which lowers the benefits of this antagonist.

If *A. vexans* is to be used as a biological control agent, an optimal rearing colony is necessary. I showed in chapter 7 how mealybug stage influences the sex ratio and size of the parasitoid. *A. vexans* produced more females and preferred both third larval instar and adult stage hosts and more females emerged from these instars. Finally, third instars seemed to be more appropriate to a rearing colony because the emerging parasitoids were larger. Larger parasitoids are thought to have a higher fecundity over lifetime (Sandlan, 1979), which was for example shown for the encyrtid *Leptomastix dactylopii* Howard (Yang & Sadof, 1997). To be used in a mass release biological program parasitoids must be fit but also they should have an optimal host searching capacity. Before they were used in the olfactometer, wasps had the opportunity to acquire experience, during which they may learn to respond to the odours that they encounter through a process of association (Turlings et al., 1993; Vet et al., 1995). Insects that were used in the field had no experience before release and may have been less responsive to host location cues. The potential of giving parasitoids an appropriate experience before releasing them against a specific pest on a specific plant deserves further attention.

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