Host specificity and daytime activity of parasitoids of the Latin American cassava mealybug, *Phenacoccus herreni* (Sternorrhyncha: Pseudococcidae)

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The two encyrtid endo-parasitoids, *Aenasius vexans* Kerrich (Hymenoptera: Encyrtidae) and *Acerophagus coccois* Smith (Hymenoptera: Encyrtidae), are important natural antagonists of the cassava mealybug, *Phenacoccus herreni* Cox & Williams (Sternorrhyncha: Pseudococcidae), in Latin America. The cassava mealybug is a major Latin American pest of cassava (*Manihot esculenta* Crantz). Host specificity of the two parasitoids was determined in seven mealybug species. Results demonstrated that in the cassava agroecosystem, *A. vexans* is a specialist for *P. herreni*, while *A. coccois* is a generalist on the first and second trophic level. Daytime activity of the two parasitoid species was studied to estimate a release time for the biological control agents. During daytime, the two species differed in both their activity and their activity pattern. Both species increased their walking activity over the observed time period compared to standing. The host handling activity decreased for *A. coccois* over the course of the experiment, while it remained similar for *A. vexans*. In general, *A. vexans* was less active than *A. coccois*. For efficient field application we suggest to release *A. vexans* and *A. coccois* in the period of increasing activity, thus in the late morning.

Keywords: *Aenasius vexans*, *Acerophagus coccois*, Encyrtidae, *Phenacoccus herreni*, biological control, specialist, generalist, activity, host specificity, effectiveness.

INTRODUCTION

Cassava, *Manihot esculenta* Crantz (Euphorbiaceae) is a long-season staple crop, grown throughout the tropics for its starchy roots (FAO 1997). The cassava mealybug, *Phenacoccus herreni* Cox & Williams, 1981 (Sternorrhyncha: Pseudococcidae) is a major pest of cassava in Latin America. Recent epidemic outbreaks caused root yield losses of up to 80% (Bellotti et al. 1999). Its control relies primarily on the mass-release of natural antagonists, since no resistant cassava germplasm is available for this pest and farmers themselves avoid expenditures for plant protection due to the low economic return of this subsistence crop (Bellotti et al. 1999). *Aenasius vexans* Kerrich, 1967 (Hymenoptera: Encyrtidae) and *Acerophagus coccois* Smith, 1880 (Hymenoptera: Encyrtidae) are endo-parasitoids of mealybugs.

The two parasitoid species appear to have different degrees of host specialisation, with *A. coccois* being more generalist than *A. vexans* (Bertschy et al. 1997). Both parasitoid species are currently being used as biological control agents of the cassava mealybug (Bellotti et al. 1999; Bento et al. 1999). Generally, for biological control purposes, natural antagonists with a narrow host spectrum (specialists)

are assumed to be more effective than those with a broad host spectrum (generalists) (Futuyma & Moreno 1988; Sheehan 1986), and safer to non-target organisms in the ecosystem (McEvoy 1996; Secord & Kareiva 1996). We define as a specialist a parasitoid that is specialised at the herbivore level and at the plant, whereas a generalist is specialised neither at the herbivore nor the plant level (Vet & Dicke 1992).

For biological control, once natural antagonists are released, successful and reliable location of host habitat and hosts in the field becomes important. Effectiveness of biological control depends, among other factors, on the capability of natural antagonists to locate the pest insect in the area of control. Activity is a prerequisite of natural antagonists to locate the host habitat and the host, and it plays an essential role in the life cycle and in the resources-searching process of insect parasitoids (Bell 1990; Quicke 1997). Activity patterns of parasitoids tend to be species specific (Walter 1988) and can even differ between the sexes of the same species (Dyer & Landis 1997). For biological control purposes it would be of advantage to release natural antagonists in the period of their increasing activity, when they are assumed to be motivated to search for hosts. *A. vexans* and *A. coccois* are day active (pers. obs.), however their period of increased activity during daytime is not known.

We used a comparative approach to evaluate in a laboratory study (1) the host specificity level of the two encyrtid parasitoid species and (2) the daytime activity and activity pattern of *A. vexans* and *A. coccois* using a simple test unit to estimate a release time.

MATERIAL AND METHODS

Rearing procedures

Plants: Individually potted cassava plants (cv. CMC 40) were grown in a greenhouse at 28–35°C and 60–70 % r.h. under natural light conditions at the Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia (at 5°N, about 12L: 12D). The cassava variety CMC 40 was used throughout the rearing and experimental procedures. The plants were 5 to 6 weeks old when used in the experiments.

Mealybugs: The colony of cassava mealybug, *P. herreni*, was established from field populations originating from the Valle del Cauca region, Colombia. They were reared on potted cassava plants. Healthy plants were infested weekly with mealybug ovisacs, as described by Van Driesche et al. (1987), and these were kept as a distinct age class in a cage.

Parasitoids: *Aenasius vexans* and *Acerophagus coccois* were continuously reared at CIAT on cassava plants infested by *Phenacoccus herreni*. Both colonies were initiated with insects collected in Venezuela in 1990. Colonies were kept in a cage in a greenhouse at 28–35°C under natural light conditions. For the two bioassays the parasitoids were kept from emergence on in screen lidded glass jars (15 x 7 cm) for two days, to enable egg-maturation and mating. They were fed with honey.

Host Specificity

Mealybug species were collected in and around the cassava agro-ecosystem. They were *Phenacoccus herreni*, *Phenacoccus madeirensis* Green, 1923, *Pseudo-*

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coccus jackbeardsleyi Gimpel & Miller, *Ferrisia virgata* (Cockerell, 1893), *Planococcus citri* (Risso, 1813), *Pseudococcus* sp. near *importatus* McKenzie, 1960, and *Pseudococcus longispinus* (Targioni, 1867).

Phenacoccus herreni, P. madeirensis, P. jacksbeardsleyi and *F. virgata* were reared on cassava. For the bioassay, a leaf of a potted cassava plant was enclosed in a Petri dish (15 cm diameter) which itself was supported by the loop of an iron wire anchored in the soil of the pot so that the leaf would remain in its natural position. Two holes of 10 cm diameter were cut in each half of the Petri dish and sealed with nylon screen. A hole of 1 cm diameter on the vertical side of the Petri dish served to introduce the plant petiole.

Fifty third-instar female mealybugs were transferred from an infested cassava plant onto the upper side of a leaf enclosed in a Petri dish. The dishes were sealed with Parafilm to prevent mealybugs from escaping. After the mealybugs had settled and started to feed (24 h), one female parasitoid was introduced from a small glass vial into the Petri dish and removed after further 24 h.

Phenacoccus citri/P. sp. near *importatus* were reared on potted bean plants (cv. Rio Tibagi). For the bioassay, a trifolium of a bean plant was introduced in a Petri dish, as described for the cassava leaf. Bean plant infestation and parasitoid introduction were conducted as described for cassava.

Phenacoccus longispinus was reared on fern *Neophrolepis biserrata* (var. *furcans)*. For the bioassay, the tip of a fern leaf was placed into a Petri dish. Plant infestation and parasitoid release was conducted as described for cassava.

Plants with the parasitised mealybugs were kept in a glasshouse at 28–33°C and 62–75% r.h. under natural light conditions (at 5N, about L12:D12). As soon as parasitised mealybugs mummified, they were removed and kept individually in a gelatine capsule until parasitoid emergence. Three replicates were made each with eight female parasitoids, for a total of 24 parasitoids per mealybug species. For more details on mealybug rearing and the host specificity bioassay see Dorn et al. (2001).

Daytime activity

Observations were carried out in Petri dishes (5.5 x 1.5 cm) lined with moist filter paper (Whatman No 42). On the day of the bioassay, a leaf disc (2.5 cm diameter) of a cassava leaf infested by third larval instar of the cassava mealybug, the preferred host stage for *A. vexans* (Bertschy et al. 2000) and *A. coccois* (CIAT 1990), was placed inside the Petri dish. An individual female parasitoid was released into the Petri dish. The observations started two and a half hours after the onset of the photophase and ended two hours before the scotophase. Female daytime activity was measured every half hour from 8:00 to 16:30 recording the following activities: *"standing"*, *"walking"*, and *"host handling"*. *"Host handling"* was defined as antennal host examination and/or oviposition. Additionally the position of the females as on the leaf disk or in the experimental arena was recorded. The first observation was made 30 minutes after the release of the parasitoids. Only daytime activity was monitored, as in the rearing facility the two species are relatively inactive in the first hours before and after dawn (pers. obs.). Experiments were conducted outdoors in a shaded place at 28–33°C, on several experimental days for a total of 50 females observed per parasitoid species. Observations of the two species were carried out on the same day.

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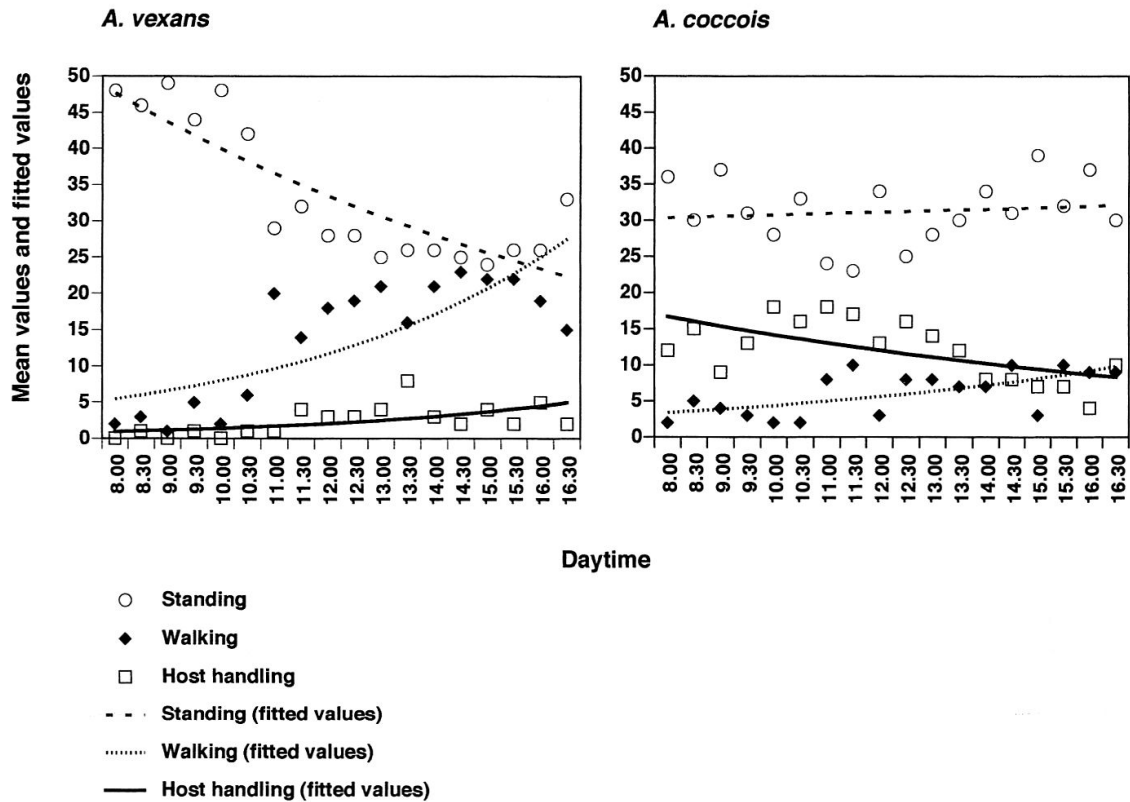
Fig. 1. Daytime activity, represented by the activities "*standing*", "*walking*" and "*host handling*" of *Aenasius vexans* and *Acerophagus coccois*. Symbols represent the actual number of females recorded for each activity over daytime. Lines represent the fitted values of the model.
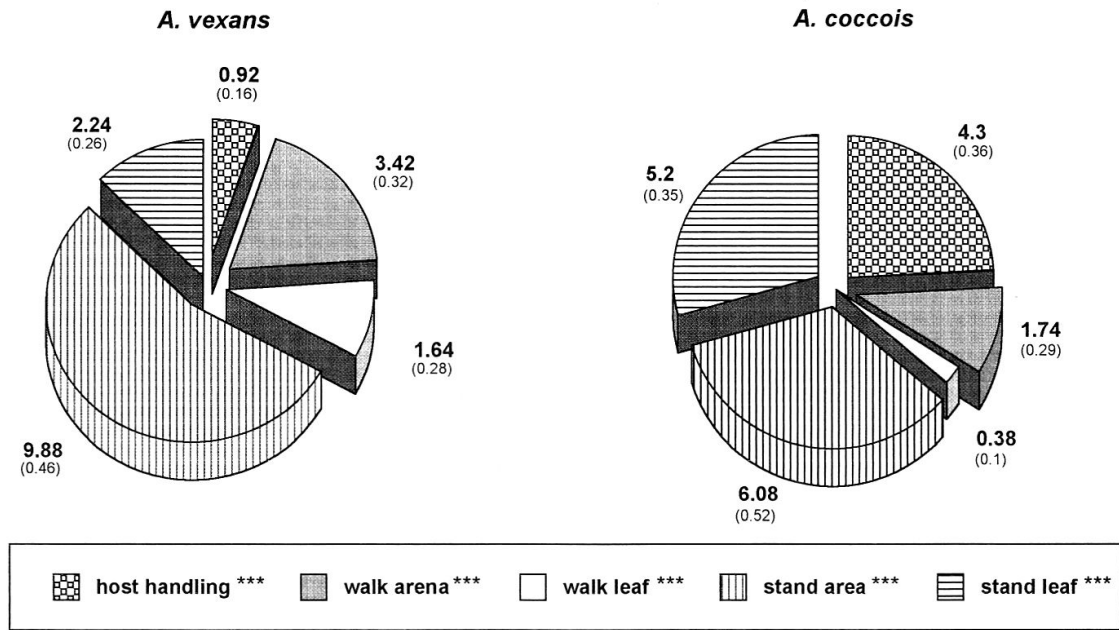
Fig. 2. Frequency of activities of *Aenasius vexans* and *Acerophagus coccois* in the course of an experimental day. Pie surfaces reflect the average frequency. Bold numbers close to slices indicate average frequency of each activity (s. e. in parenthesis). Asterisks close to activities indicate significant differences between *A. vexans* and *A. coccois* in the frequency of each activity. ***P < 0.001 (Mann-Whitney U-test).

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Tab. 1. Host specificity of *Aenasius vexans* and *Acerophagus coccois* on seven mealybug species from the cassava agroecosystem.

Mealybug species	Plant	A. vexans Mummies	Parasitoid emergence	A. coccois Mummies	Parasitoid emergence
Phenacoccous herreni	Cassava	yes	yes	yes	yes
Phenacoccous madeirensis	Cassava	no	no	yes	yes
Ferrisia virgata	Cassava	no	no	yes	yes
Pseudococcus jackbeardsleyi	Cassava	no	no	no	no
Planococcous citri	Bean	no	no	no	no
Pseudococcous sp. nr. *importatus*	Bean	no	no	no	no
Pseudococcus longispinus	Fern	no	no	no	no

Tab. 2. Results of the log linear model with d.f. = 11.96, deviance ratio 72.87 and P < 0.001 for the difference in daytime activity between the two parasitoid species *Aenasius vexans* and *Acerophagus coccois*.

Effect	Estimate	s. e.	T-values	P	Antilog of estimate
Constant	3.41	0.10	33.21	***	30.29
Time	0.00	0.01	0.34	ns	1.00
Activity walk	-2.24	0.29	-7.79	***	0.11
Activity host handling	-0.56	0.18	-3.04	**	0.57
Species *A. vexans*	0.49	0.14	3.65	***	1.65
Time x Activity walk	0.06	0.02	2.45	*	1.06
Time x Activity host handling	-0.04	0.02	-2.44	*	0.96
Time x Species *A. vexans*	-0.048	0.01	-3.60	***	0.95
Activity walk x Species *A. vexans*	-0.07	0.36	-0.19	ns	0.93
Activity host handling x Species *A. vexans*	-3.51	0.51	-6.87	***	0.03
Time x Activity walk x Species *A. vexans*	0.08	0.03	2.72	**	1.08
Time x Activity host handling x Species *A. vexans*	0.19	0.04	4.48	***	1.20

Note. The activity standing and the species *A. coccois* were the constant in the log linear model

Statistical analysis

Host specificity was analysed qualitatively for the two parasitoid species. The occurrence of mummies resulting from the 24 hours contact with mealybugs, and of a new parasitoid generation emerged from these mummies was assessed.

Daytime activity of the two parasitoid species was analysed with a log linear model using Genstat (Version 5; Release 4.1 for Windows NT). The factors for the model were time, activity and species. The activity parameter "*standing*" was used as a constant for the activity variable and *A. coccois* was the constant for the species variable (Crawley 1993). The difference of the frequency of the behaviours between the two parasitoid species was compared using a Mann-Whitney U-test (Zar 1996).

RESULTS

Host specificity

A. vexans accepted and completed development in only one mealybug species, *P. herreni*. In contrast, *A. coccois* accepted and developed in three of the seven mealybug species tested *P. herreni*, *P. madeirensis* and *F. virgata* (Tab. 1).

Daytime activity

The daytime activity differed significantly between *A. vexans* and *A. coccois* (P < 0.001) (Fig. 1, Tab. 2). The factor "*time*" was not significant as a main effect alone (P > 0.05). There was an interaction between time, the activity "*walking*" and the species, indicating that the "*walking*" activity increased over the observed time period compared to "*standing*" for both parasitoid species. The interaction between time and the activity "*host handling*" was negative, indicating that the "*host handling*" activity decreased over the course of the experiment in *A. coccois*, compared to "*standing*". Significant differences were apparent for the different activities observed, "*walking*" (P < 0.001) and "*host handling*" (P < 0.01) were rarer activities than "*standing*" (Fig. 2, Tab. 2). The mean activity over daytime estimated from combining "*walking*" and "*host handling*" was 32.45% for *A. vexans* and 36.32% for *A. coccois*. The two parasitoid species differed in the mean frequency of activities throughout the observational period (Fig. 2). As compared to *A. coccois*, *A. vexans* walked more often on the mealybug infested leaf (Mann-Whitney U-test, T = 3139.0, P < 0.0001), the experimental arena (Mann-Whitney U-test, T = 3091.0, P < 0.0001), and stood more often in the experimental arena (Mann-Whitney U-test, T = 3230.5, P < 0.0001). In contrast, *A. coccois* handled host (Mann-Whitney U-test, T = 1573.0, P < 0.0001) and stood on the mealybug infested leaf (Mann-Whitney U-test, T = 1723.0, P < 0.0001) more often than *A. vexans*.

DISCUSSION

Host specificity of two encyrtid mealybug parasitoid species was determined by testing plant and mealybug species occurring in and around cassava fields. Mealybug species displaying different degrees in polyphagy were chosen. *A. vexans* was able to parasitise and develop only in one mealybug species, the monophagus mealybug species *P. herreni* (Williams & Willink 1992). *A. vexans* therefore can be considered to be a specialist in the cassava agroecosystem, both at the plant and at the herbivore level (Dorn et al. 2001). In contrast, *A. coccois* showed a broader host range as it parasitised and developed in three different mealybug species, the monophagous mealybug species *P. herreni* and the two polyphagous mealybug species *P. madeirensis* and *F. virgata*. In addition, this species was also recovered from *Oracella acuta* (Lobdell) (Sternorrhyncha: Pseudococcidae) feeding on Loblolly Pine (*Pinus taeda* L.) (Clarke et al. 1990). *A. coccois* therefore is a generalist at the plant and at the herbivore level (Dorn et al. 2001). The results of this host specificity test clarified hypothesis of host range of the two parasitoid species *A. vexans* and *A. coccois* in the cassava agroecosystem (Bertschy et al. 1997; Noyes & Ren 1995).

The daytime activity of *A. vexans* and *A. coccois* differed in the course of the day. For *A. vexans*, the "*walking*" activity increased and the "*standing*" activity decreased over daytime. For *A. coccois*, the "*walking*" activity increased and the "*host*

handling" activity decreased, while the "*standing*" activity remained similar over daytime. Most parasitic hymenoptera are assumed to be day active (Godfray 1994) and for many species activities were greatest in the mornings, due to cooler temperatures (Quicke 1997). However, in two *Pseudacteon* fly parasitoid species, *P. litoralis* Borgmeier showed morning and late evening activities and *P. tricuspis* Borgmeier is most active during mid-day (Pesquero et al. 1996), and in two *Trichogramma* species, *T. cacoeciae* Marchal shows high activity throughout the photophase while *T. brassicae* Bezdenko shows an earlier decrease of activity (Pompanon et al. 1994). High activity is assumed to be related with high responsiveness, thus readiness for host searching and host location. For biological control, activity and frequency of behavioural events, such as host searching, oviposition and resting, could be an important criterion for the selection of a release time of natural antagonists. The wide range of activities of natural antagonists needs a simple test unit to estimate the activity for each species under standardised conditions. The laboratory activity unit showed to be a useful test for assessing the daytime activity of *A. vexans* and *A. coccois* as results on activity are coherent with the more elaborated foraging behavioural observations (Dorn et al. 2001). They are therefore transferable to parasitoid performance in the field. Field activity estimates of natural antagonists are most reliable, however they are labour- and time intensive (Dutton & Bigler 1995). From the agricultural point of view, activity could be an important element in the selection of time of release on natural enemies in biological control and may provide important clues for elucidating the temporal occurrence of biological events in the field, such as oviposition, resting, and other behaviours. For efficient field releases of *A. vexans* and *A. coccois*, the parasitoids should be released at a time of day when they are most active and therefore responsive to search for hosts. This possibly decreases the wasp's tendency to disperse from the release point without looking for hosts (Lewis & Martin 1990) or being exposed to unfavourable weather conditions and predators.

ZUSAMMENFASSUNG

Die beiden Parasitoiden *Aenasius vexans* Kerrich (Hymenoptera: Encyrtidae) und *Acerophagus coccois* Smith (Hymenoptera: Encyrtidae) sind bedeutend für die biologische Bekämpfung der Maniokschmierlaus, *Phenacoccus herreni* Cox & Williams (Sternorrhyncha: Pseudococcidae). In Lateinamerika ist diese Schmierlausspezies der Hauptschädling der Wurzelpflanze Maniok (*Manihot esculenta* Crantz). Anhand von sieben verschiedenen Schmierlausarten wurde die Wirtsspezifität der beiden Parasitoidenarten getestet. Die Resultate zeigten, dass im Maniokökosystem *A. vexans* ein Spezialist für *P. herreni* ist. *A. coccois* ist auf der ersten sowie auf der zweiten trophischen Stufe ein Generalist mit einem relativ engen Wirtsspektrum. Die tägliche Aktivität der beiden Parasitoiden wurde untersucht, um so deren Freilassungszeit im Feld zu bestimmen. Die beiden Arten unterschieden sich sowohl in ihrer täglichen Aktivität als auch in ihrem Aktivitätsmuster. Während der Beobachtungszeit nahm die Aktivität "*Gehen*" bei beiden Arten zu. *A. coccois* steigerte seine Kontaktzeit mit dem Wirt, während sie für *A. vexans* im Tagesverlauf gleich blieb. Im Allgemeinen war *A. vexans* weniger aktiv als *A. coccois*. Der späte Vormittag wird als beste Freilassungszeit der beiden Parasitoiden vorgeschlagen, da dann ihre Aktivität erhöht ist.

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