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Specificity of *Anagyrus* sp. nov. nr. *sinope* and *Leptomastix dactylopii* for six mealybug species

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Abstract. In order to understand better non-target effect and potential uses, the host specificity of two parasitoid species (*Anagyrus* sp. nov. nr. *sinope* Noyes & Menezes and *Leptomastix dactylopii* Howard) (both Hymenoptera: Encyrtidae) for six mealybug species [*Ferrisia virgata* (Cockerell), *Phenacoccus madeirensis* Green, *Phenacoccus solani* Ferris, *Planococcus citri* (Risso),*Pseudococcus longispinus* (Targioni-Tozzetti) and *Pseudococcus viburni* (Signoret)] (all Hemiptera: Pseudococcidae) was studied through behavioral observations and laboratory rearing. The selected mealybug species represent major subfamilies and tribes of Pseudococcidae. Except for *F. virgata*, all mealybug species induced examinations by *Anagyrus* sp. nov. nr. *sinope* and *L. dactylopii*. *Anagyrus* sp. nov. nr. *sinope* was specific to *P. madeirensis*, which was the only mealybug species selected for oviposition and suitable for complete development of the parasitoid. No encapsulation of *Anagyrus* sp. nov. nr. *sinope* in *P. madeirensis* was observed. *Leptomastix dactylopii* accepted multiple species for oviposition, with the ranking of species preference as *P. citri* > *P. viburni* > *P. longispinus* > *P. solani* > *P. madeirensis*. Only *P. citri*, *P. longispinus* and *P. viburni* supported the development of *L. dactylopii*. Parasitoids developing in *P. longispinus* and *P. viburni* suffered from high encapsulation rates, while no encapsulation was observed when developing in *P. citri*. The results of this study suggest that *Anagyrus* sp. nov. nr. *sinope* is highly host specific. *Leptomastix dactylopii*, on the other hand, has a wider host range. The use of *Anagyrus* sp. nov. nr. *sinope* in a mealybug biological control program is limited to *P. madeirensis* and *L. dactylopii* to *P. citri*. The results presented in this study also lead us to question the accuracy of the reported host range of *L. dactylopii*, which include all six mealybug species tested.

Key words: encapsulation, Encyrtidae, Hemiptera, host specificity, Hymenoptera, non-target effect, oviposition behavior, Pseudococcidae

Introduction

Scale insects and mealybugs (Hemiptera: Coccoidea) are some of the most damaging pests of greenhouse ornamental production (Oetting et al., 2002). Some common pestiferous mealybug species include the striped mealybug, *Ferrisia virgata* (Cockerell); the Madeira mealybug, *Phenacoccus madeirensis* Green; the solanum mealybug, *Phenacoccus solani* Ferris; the citrus mealybug, *Planococcus citri* (Risso); the long-tailed mealybug, *Pseudococcus longispinus* (Targioni-Tozzetti); the obscure mealybug, *Pseudococcus viburni* (Signoret); and the root mealybug, *Rhizoecus* spp. (all Hemiptera: Pseudococcidae). Management of mealybug populations in greenhouses has relied heavily on chemical control tactics (Townsend et al., 2001). However, chemical management is not always effective, sufficient, or feasible in some situations. For more sensitive areas, such as organic productions and facilities with high human traffic or those housing live arthropods (e.g., butterfly exhibits), biological control is an alternative to chemical control of mealybug pests. Some of the commercially available mealybug natural enemies are the parasitoids *Anagyrus pseudococci* (Girault), *Leptomastidea abnormis* (Girault) and *Leptomastix dactylopii* Howard (all Hymenoptera: Encyrtidae), and the predator *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) (Chong and Oetting, 2002).

Leptomastix dactylopii originated from Brazil (Compere, 1939; cited by Bartlett, 1978) and have been used in biological control programs against *P. citri* around the world (Noyes and Hayat, 1994). The combinations of *L. dactylopii* and other parasitoids (e.g., *L. abnormis*) and predators (e.g., *C. montrouzieri*) are most effective against *P. citri* in greenhouses (Copland et al., 1985; Chong and Oetting, 2002). This is a solitary endoparasitoid attacking more than 20 mealybug species, which include all the previously mentioned pestiferous mealybug species except *Rhizoecus* sp. (Noyes and Hayat, 1994). Although *L. dactylopii* have been reared on other mealybug species in the laboratory, field observations have suggested that *L. dactylopii* may be effective only against *P. citri* (Blumberg and Van Driesche, 2001). The inefficiency of *L. dactylopii* against mealybug species other than *P. citri* may be due to physiological incompatibility (through high level of encapsulation, see Blumberg and Van Driesche, 2001). The host specificity of *L. dactylopii* should be studied in order to understand better the compatibility and potential of the parasitoid as biological control agent of mealybug species common in greenhouse ornamental productions.

Anagyrus sp. nov. nr. *sinope* Noyes & Menezes (Hymenoptera: Encyrtidae) has shown potential as a biological control agent of *P. madeirensis* in greenhouse ornamental and vegetable productions or in field crops (reported as *Anagyrus loecki* Noyes & Menezes in Chong, 2005). *Anagyrus* sp. nov. nr. *sinope* could possibly be used in other countries where *P. madeirensis* threatens the livelihood and economy of local farmers. Before *Anagyrus* sp. nov. nr. *sinope* is introduced into new areas, ensuring that the introduction will have minimal impact on the local mealybug biodiversity is important. On the other hand, by exploring the host range of *Anagyrus* sp. nov. nr. *sinope*, we could identify other target mealybug species or those that can be used as alternative hosts in the mass rearing of the parasitoids.

Here we present results of host preference and suitability studies of *L. dactylopii* and *Anagyrus* sp. nov. nr. *sinope* against six mealybug species (*F. virgata*, *P. madeirensis*, *P. solani*, *P. citri*, *P. longispinus*, and *P. viburni*) commonly encountered in ornamental productions of the southeastern US. The selected mealybug species also represent major clades within the family Pseudococcidae. We determined the preference for the six mealybug species through an observational study on the foraging and oviposition behaviors of the two parasitoid species. We also investigated the effects of different mealybug species on the fitness parameters (emergence, encapsulation, developmental time, progeny production, sex ratio, and body size) of the two parasitoid species.

Materials and methods

Sources of insects and maintenance of colonies

Phenacoccus madeirensis and *P. citri* were established using colonies maintained on coleus (*Solenostemon scutellarioides* Thonn.) in the Ornamental Entomology Greenhouses at the University of Georgia, Griffin Campus, Griffin, GA. Initial colonies of *P. longispinus* and *P. viburni* were collected from a local nursery. A colony of *F. virgata* was established with mealybugs collected from the Cecil B. Day Butterfly Center at the Callaway Garden, Pine Mountain, GA. The *P. solani* colony originated from several individuals collected from potatoes (*Solanum tuberosum* L.) purchased at a local grocery store. All mealybug colonies were established in 2004 and maintained on sprouted russet potatoes kept in an environmental chamber maintained at 25 °C and 14L:10D photoperiod. Only pre-reproductive

adult female mealybugs of similar body length (2.5–3.5 mm) were selected for the experiments.

Anagyrus sp. nov. nr. *sinope* colony was established in May 2002 with individuals collected from a greenhouse colony of *A. loecki* at the Griffin Campus. The *A. loecki* colony was established with parasitoids obtained from University of Florida, Mid-Florida Education and Research Station, Apopka, FL, in September 2000. The University of Florida colony was established with parasitoids reared from papaya mealybug, *Paracoccus marginatus* Williams & Granada de Willink collected in the field around Orlando in 1999 (L. S. Osborne, personal communication). We believe that *Anagyrus* sp. nov. nr. *sinope* was a result of local contamination because the species was not detected in the original *A. loecki* colony in Apopka (L.S. Osborne, personal communication). The *Anagyrus* sp. nov. nr. *sinope* colony was maintained in the laboratory with *P. madeirensis* reared on sprouted russet potatoes at 25–27 °C and 14L:10D photoperiod. Each week, the colony was examined, and mummies were collected and isolated in individual gelatin capsules. The mummies were incubated in an environmental chamber at 25 °C and L14:D10 photoperiod until adult emergence. The parasitoids were collected within 24 h of emergence and released into a plastic vial. We provided streaks of diluted honey as food. To ensure mating and a full complement of eggs, the parasitoids were held at 25 °C and L14:D10 photoperiod for 48 h. No hosts were provided during the holding period; thus, the parasitoids were inexperienced when used in the experiments.

A laboratory colony of *L. dactylopii* was established with adult parasitoids collected from Cecil B. Day Butterfly Center. The parasitoids were the remnants of a previous biological control program against *P. citri* in the live butterfly exhibit. The *L. dactylopii* colony was maintained with *P. citri* reared on sprouted russet potatoes under laboratory conditions. The parasitoids used in this study were collected and conditioned with the same procedure as described for *Anagyrus* sp. nov. nr. *sinope*.

Voucher specimens of the parasitoids and mealybugs were deposited at the Georgia Museum of Natural History, University of Georgia, Athens, GA. Additional parasitoid specimens were deposited at the Natural History Museum, London.

Oviposition behavior and host species preference

The experiments were performed between January and May of 2005. The foraging behavior of the two parasitoid species was observed on

a coleus leaf disc placed abaxial side up in a Petri dish (35 mm in diameter). Mealybugs were transferred to the leaf disc and allowed to settle 16 h before the start of the experiments. Three types of experiments were conducted: no-choice, two-choice, and all-choice tests. In the no-choice test, a single parasitoid was provided with 12 mealybugs of the same species. In the two-choice test, two mealybug species, with six individuals per species, were transferred to the leaf disc. The parasitoid was allowed to choose among the six mealybugs species (two individuals per species) in the all-choice test. In all the tests, a total of 12 mealybugs were provided for each parasitoid on a leaf disc. To facilitate the identification and tracking of the mealybugs during the behavioral observations, we marked the positions of the mealybugs on a separate sheet of paper before the experiment.

Chong (2005) and de Jong and van Alphen (1989) studied the oviposition behaviors of *Anagyrus* sp. nov. nr. *sinope* and *L. dactylopii*, respectively. In brief, the oviposition behavior of the two parasitoid species consists of stereotypical sequence of behavioral events: searching, encounter, antennal examination, ovipositor probing, and oviposition. The parasitoids search for hosts by rapidly drumming the leaf surface with antennae. Once the parasitoids encounter a host, they will repeatedly touch the host body with antennae; this behavior is called antennal examination. If the host is deemed acceptable for oviposition, the parasitoids will turn around and attempt to insert the ovipositor in a backward thrusting movement. If the host is not acceptable, the parasitoids will resume searching. Once the ovipositor is inserted, *L. dactylopii* will spend about 3 s to deposit one egg (de Long and van Alphen, 1989), while *Anagyrus* sp. nov. nr. *sinope* can spend on average between 6 and 15 min to deposit 1–6 eggs per mealybug in a single oviposition (Chong, 2005).

The foraging and oviposition behaviors of *L. dactylopii* and *Anagyrus* sp. nov. nr. *sinope* in different mealybug species compositions were observed for 30 min. Observation began when a parasitoid was released onto the coleus leaf disc. The Petri dish was not covered, thus allowing the parasitoid to determine its residence time. Observation was terminated at the end of the 30-min observational period. Observation might also be terminated before the 30-min period if the parasitoid left the Petri dish or if the parasitoid did not engage in any foraging behavior for more than 10 min. During the observation, the numbers of mealybugs encountered, examined, and probed were tallied with a behavioral observation program, the Observer® (version 4.1, Noldus Information Technology, Wageningen, the Netherlands).

The oviposition behavior of 20 different individuals of *L. dactylopii* and *Anagyrus* sp. nov. nr. *sinope* were observed in each mealybug species composition. The foraging behavior of a total of 140 individuals each of *Anagyrus* sp. nov. nr. *sinope* and *L. dactylopii* were observed.

To verify successful egg deposition and to determine the clutch sizes (the numbers of eggs deposited in each mealybug), the parasitized mealybugs were removed 3 h after the observation period, dissected in a drop of distilled water on a microscope slide, and examined at 100× magnification. Parasitoid eggs were released into the dissecting fluid when the mealybug body was ruptured. These eggs appeared oblong with a short stalk, a characteristic that made them easily distinguishable from the larger, rounded mealybug eggs.

In the no-choice test, the number and fate of the mealybugs were tallied and the proportion of mealybugs encountered, examined, probed, and successfully oviposited in by each of the two parasitoid species were determined. The null hypothesis of no species difference in the proportion of mealybugs subjected to encounter, antennal examination, ovipositor probing, and oviposition was tested with a Kruskal-Wallis test at a significant threshold of 0.05 (PROC NPAR1WAY; SAS Institute, 1999). A multiple comparison test described in Conover (1999) was performed to separate the means only when the null hypothesis was rejected.

In the two-choice test and all-choice test, the numbers of mealybug successfully parasitized were recorded and used as the criteria for determining the parasitoids preference among the mealybug species. Paired chi-square analyses were performed to determine if the number of mealybug oviposited in by *L. dactylopii* or *Anagyrus* sp. nov. nr. *sinope* was different among the pairs of mealybug species at a significance threshold level of 0.05 (PROC FREQ; SAS Institute, 1999). Based on the result of the chi-square frequency analyses, a ranking of the preference for the six mealybug species was produced for each parasitoid species.

Host species suitability

Suitability of the six mealybug species for the development and survival of *Anagyrus* sp. nov. nr. *sinope* and *L. dactylopii* was studied in no-choice and all-choice tests. The number and species composition of the mealybugs in the two tests were identical to the same tests prepared in the behavioral observation study. The mealybugs were collected from their respective colonies and transferred to a whole coleus leaf in a Petri dish (90 mm in diameter). To maintain vigor of the

coleus leaf for the length of this experiment, the leaf petiole was immersed in a cup of water through a hole drilled on the bottom of the Petri dish. The Petri dish was covered with chiffon to allow ventilation and prevent escape of the mealybugs and parasitoids.

A female *Anagyrus* sp. nov. nr. *sinope* or *L. dactylopii* was released into each Petri dish and allowed to forage for 24 h at 25 °C and 14L:10D photoperiod. After the removal of the parasitoids, the mealybugs were incubated at 25 °C until mummification. Ten days after the exposure to the parasitoids, the mealybug cohorts were examined, and mummies were collected and isolated in individual gelatin capsules. The mummies were incubated at 25 °C until adult emergence. Parasitism rate (proportion of mealybugs mummified), duration of development (period between parasitism and adult emergence), and the number and sex ratio of offspring of each parasitoid species were determined.

Hind tibial length was used as a surrogate for body length (Sagarra et al., 2001b; Chong, 2005). The adult parasitoids were killed in 70% ethanol within 24 h of emergence. Their left hind tibia were removed and mounted on microscope slides. The left hind tibial length of all adult parasitoids were measured with an ocular micrometer at 60× magnification to investigate the effect of host species on the fitness of parasitoids.

Surviving mealybugs in the no-choice and all-choice tests were dissected to determine the presence and fate of the deposited parasitoid eggs. The mealybugs were transferred to and cleared in a 1:1 solution of chloralphenol and acetic acid (Blumberg and Van Driesche, 2001). The solution removes wax and other residue on the mealybug bodies, thus facilitating detection of encapsulated parasitoid eggs or larvae. The mealybugs were dissected on a microscope slide after 24 h in the clearing solution. The number of mealybugs showing encapsulation and the number of parasitoid eggs or larvae encapsulated by the mealybugs were determined. The encapsulation rate of *Anagyrus* sp. nov. nr. *sinope* and *L. dactylopii* in each mealybug species was calculated by dividing the number of eggs or larvae encapsulated by the total number of eggs deposited.

The parasitism rate, the developmental time, the number, sex ratio and hind tibial length of the offspring, and the number and rate of encapsulation of *Anagyrus* sp. nov. nr. *sinope* and *L. dactylopii* in each of the six mealybug species were analyzed for differences among the host species; for this we used a Kruskal–Wallis test (SAS Institute, 1999). Means were separated with Conover's (1999) multiple

comparison procedure when significant differences were detected among the means.

Results

Host specificity of Anagyrus sp. nov. nr. sinope

In the no-choice tests, *Anagyrus* sp. nov. nr. *sinope* encountered more *P. solani*, *P. citri*, *P. longispinus* and *P. viburni* than *P. madeirensis* and *F. virgata* (Table 1). Majority of the encountered *P. madeirensis* were subjected to antennal examination (mean = 93%). Approximately 30–50% of the encountered mealybugs of other species, except *F. virgata*, were examined. No *F. virgata* was examined by *Anagyrus* sp. nov. nr. *sinope*. The long sticky wax filaments projected from the body of *F. virgata* provided good protection to the mealybugs. The antennae of both parasitoid species become entangled with the wax filaments, causing the parasitoids to retreat rapidly and immediately leave the arena or spend the rest of the observation period in grooming. No host feeding was observed in the tests.

Anagyrus sp. nov. nr. *sinope* attempted to probe fewer than 5% of the examined *P. solani*, *P. citri*, *P. longispinus* and *P. viburni*. There was not a single egg deposited in any of these species (Table 1). On average, 83% of the examined *P. madeirensis* were probed, and 1–5 eggs were successfully deposited in 77% of these probed mealybugs. Only *P. madeirensis* was successfully parasitized by *Anagyrus* sp. nov. nr. *sinope* in the no-choice tests.

When provided with *P. madeirensis* and one of the other five mealybug species in the two-choice test, *P. madeirensis* was the only species parasitized by *Anagyrus* sp. nov. nr. *sinope* (mean proportion of hosts parasitized = 0.42). Although other mealybug species were encountered or examined during the two-choice test, no mealybugs were probed or parasitized. Since *P. madeirensis* was the only species parasitized, no ranking of preference was performed.

The specificity of *Anagyrus* sp. nov. nr. *sinope* for *P. madeirensis* was confirmed in the all-choice tests, where six mealybug species were provided simultaneously. All mealybug species were encountered by *Anagyrus* sp. nov. nr. *sinope* in the all-choice test, with the encountered rate of *F. virgata* (40%) lower than other species (60–75%). *Anagyrus* sp. nov. nr. *sinope* examined all mealybug species, except *F. virgata*. *Phenacoccus madeirensis*, *P. solani*, and *P. longispinus* were probed; however, only *P. madeirensis* was parasitized by *Anagyrus* sp. nov. nr. *sinope*. *Phenacoccus madeirensis* was thus the only preferred species.

Table 1. Proportion (mean ± SEM) of mealybugs encountered, examined, probed and oviposited in by *Anagyrus* sp. nov. nr. *sinope* and *Leptomastix dactylopii* within a 30-min observation time in the no-choice tests. Twelve individuals of each of the six mealybug species were exposed to a single parasitoid. A total of 20 parasitoids were tested on each mealybug species

Parasitoid species	Mealybug species	Prop. of hosts encountered	Prop. of encountered hosts examined	Prop. of examined hosts probed	Prop. of probed hosts oviposited in	Prop. of total available hosts oviposited in
Anagyrus sp.	*F. virgata*	0.15 ± 0.05c	0d	–	–	0b
nov. nr. *sinope*	*P. madeirensis*	0.37 ± 0.08b	0.93 ± 0.05a	0.83 ± 0.07a	0.77 ± 0.07a	0.17 ± 0.03a
	P. solani	0.75 ± 0.04a	0.46 ± 0.08b	0.03 ± 0.03b	0b	0b
	P. citri	0.67 ± 0.05a	0.39 ± 0.06bc	0.01 ± 0.01b	0b	0b
	P. longispinus	0.65 ± 0.08a	0.30 ± 0.06c	0c	–	0b
	P. viburni	0.68 ± 0.07a	0.29 ± 0.06c	0.02 ± 0.02b	0b	0b
	n	120	113	95	25	120
	χ^2	58.85	71.36	72.21	14.42	117.82
	d.f.	5	5	4	3	5
	p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
L. dactylopii	*F. virgata*	0.11 ± 0.02c	0d	–	–	0c
	P. madeirensis	0.58 ± 0.05b	0.20 ± 0.11c	0.26 ± 0.19b	0.10 ± 0.10d	0.01 ± 0.01bc
	P. solani	0.70 ± 0.06a	0.35 ± 0.11b	0.28 ± 0.10b	0.35 ± 0.15c	0.04 ± 0.03b
	P. citri	0.55 ± 0.07b	0.74 ± 0.07a	0.64 ± 0.07a	0.89 ± 0.05a	0.20 ± 0.03a
	P. longispinus	0.53 ± 0.07b	0.26 ± 0.09bc	0.50 ± 0.20ab	0.60 ± 0.24b	0.04 ± 0.02b
	P. viburni	0.57 ± 0.07b	0.68 ± 0.08a	0.66 ± 0.14a	0.69 ± 0.06b	0.16 ± 0.03a
	n	120	118	68	58	120
	χ^2	53.50	60.56	14.77	25.22	77.04
	d.f.	5	5	4	4	5
	p	<0.0001	<0.0001	0.0052	< 0.0001	<0.0001

Means followed by the same letter within each column are not significantly different.

Phenacoccus madeirensis was also the only species that could support the development of *Anagyrus* sp. nov. nr. *sinope*. In the no-choice and all-choice tests of host species suitability study, 72 and 75%, respectively, of *P. madeirensis* were parasitized. No mummies were collected from other mealybug species treatments. Majority of the mummies (98%) recovered in the *P. madeirensis* treatment yielded offspring. The average (± SE) total number of offspring produced by each *Anagyrus* sp. nov. nr. *sinope* in *P. madeirensis* was 13.7 (1.6), with the proportion of males at 0.35. Both male and female *Anagyrus* sp. nov. nr. *sinope* completed development in *P. madeirensis* in 16.0 ± 0.3 days. Female *Anagyrus* nov. sp. nr. *sinope* emerged from *P. madeirensis* was bigger than males, using left hind tibial length (mean = 0.29 mm for females and 0.24 mm for males) as a surrogate for body size. No encapsulated eggs or larvae were recovered in any of the six mealybugs species tested.

Host specificity of L. dactylopii

A higher proportion (70%) of *P. solani* was encountered by *L. dactylopii* in the no-choice tests of the host preference study than other mealybug species (Table 1). Only 10% of the available *F. virgata* was encountered. No *L. dactylopii* examined any of the encountered *F. virgata* due to the sticky wax filaments of the mealybugs. Significantly more *P. citri* and *P. viburni* were examined and probed than were other mealybug species. No host feeding was observed during the experiments. Of the *P. madeirensis* probed by *L. dactylopii*, eggs were recovered from only two individuals. *Leptomastix dactylopii* deposited eggs in 89% of the probed *P. citri*, 60% of *P. longispinus*, and 69% *P. viburni*. When comparing the proportion of total available hosts which showed evidence of oviposition, *L. dactylopii* preferred to attack *P. citri* (20% parasitized), followed by *P. viburni* (16%), while fewer than 5% of *P. madeirensis*, *P. solani* and *P. longispinus* were parasitized. Only one egg was recovered from each parasitized host of the five species.

When paired with other mealybug species, no *F. virgata* were parasitized, while other species were parasitized at rates between 10 and 60%. *Phenacoccus madeirensis* (10%) was parasitized only when paired with *P. viburni* (30%). Based on the results of chi-square frequency analyses, the ranking of preference for *L. dactylopii*, from the most preferred to the least preferred, was *P. citri*, *P. viburni*, *P. longispinus*, *P. solani*, *P. madeirensis*, and *F. virgata*.

When all mealybug species were offered, *L. dactylopii* encountered 75–90% of *P. madeirensis*, *P. solani*, *P. citri*, *P. longispinus*, and

P. viburni. Like the no-choice tests, no *F. virgata* were examined in the all-choice tests. Only *P. citri* (83%) and *P. viburni* (20%) were accepted for oviposition after probing. Overall, 55% of the total available *P. citri* in the all-choice tests of host preference study were successfully parasitized, while only 5% of *P. viburni* suffered the same fate. No *F. virgata*, *P. madeirensis*, *P. solani*, and *P. longispinus* were parasitized in the all-choice tests.

After 10 days of incubation, 50 ± 9% of *P. citri*, 4 ± 3% of *P. viburni*, and 3 ± 2% of *P. longispinus* formed mummies in the no-choice tests of host suitability study. No mummies were collected from other mealybug species (Kruskal–Wallis test: n = 180, $\chi^2 = 109.28$, d.f. = 5, $p < 0.0001$). Only *P. citri* formed mummies in the all-choice tests (n = 180, $\chi^2 = 178.63$, d.f. = 5, $p < 0.0001$). The emergence rates of offspring in the no-choice tests were 67 ± 33% from *P. viburni*, 90 ± 6% from *P. citri*, and 100% from *P. longispinus*; these were not statistically different among the host species (n = 38, $\chi^2 = 2.6086$, d.f. = 2, $p = 0.2714$).

Within 24 h, each *L. dactylopii* female assigned to the no-choice tests of the host suitability study produced on average 2.8 offspring from *P. citri*, while only one offspring was produced from *P. longispinus* and *P. viburni* (n = 42, $\chi^2 = 4.2719$, d.f. = 2, $p = 0.0537$) (Figure 1). Significantly more female offspring were produced in *P. citri* (n = 42, $\chi^2 = 6.2756$, d.f. = 2, $p = 0.0434$) (Figure 1). The numbers of male offspring produced from the three mealybug species were not significantly different (n = 42, $\chi^2 = 1.8476$, d.f. = 2, $p = 0.3874$) (Figure 1). Only male offspring emerged from *P. viburni*, while the proportion of males was 57% from *P. citri* and 67% from *P. longispinus* (n = 36, $\chi^2 = 4.2719$, d.f. = 2, $p = 0.1181$).

Both male and female *L. dactylopii* developing in *P. viburni* had longer duration of development than those developing in *P. citri* and *P. longispinus* (for female: n = 20, $\chi^2 = 5.2454$, d.f. = 1, $p = 0.0220$; for male: n = 26, $\chi^2 = 12.9501$, d.f. = 2, $p = 0.0015$) (Figure 2). *Leptomastix dactylopii* developing in *P. citri* emerged 17 days after parasitism; this was 2 and 4 days shorter than those in *P. longispinus* and *P. viburni*, respectively. *Leptomaxtis dactylopii* emerging from *P. citri* were also larger than those from *P. longispinus* and *P. viburni* (for female: n = 15, $\chi^2 = 2.88$, d.f. = 1, $p = 0.0897$; for male: n = 31, $\chi^2 = 6.9210$, d.f. = 2, $p = 0.0314$) (Figure 3).

In the host species suitability tests, eggs and larvae of *L. dactylopii* were encapsulated by four mealybug species, except *F. virgata* and

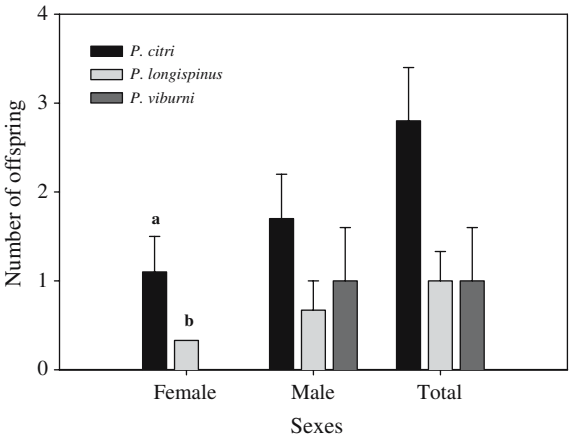

Figure 1. Number of female and male offspring (mean ± SEM) produced by each *Leptomastix dactylopii* female in three species of mealybugs. Different letters on top of the bars indicate significant difference.

P. citri (Table 2). Encapsulation rates ranged from 86 to 100% in the no-choice tests. All eggs or larvae of *L. dactylopii* were encapsulated in *P. solani*,*P. longispinus* and *P. viburni* in the all-choice tests. The number of encapsulated eggs recovered from each mealybug ranged from 0.6 to 1.8, and the number of encapsulated larvae ranged from 0.2 to 2 in the four mealybug species showing effective encapsulation.

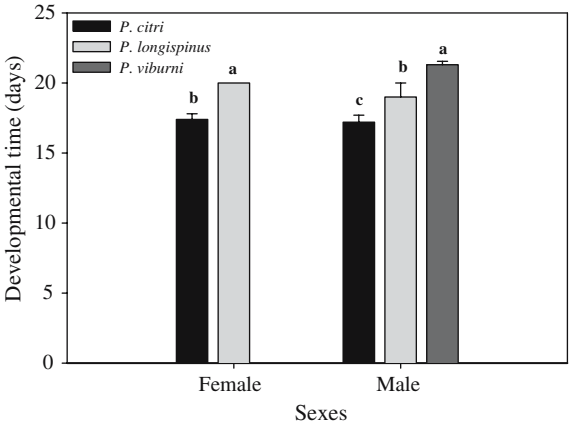

Figure 2. Developmental time (mean ± SEM) of *Leptomastix dactylopii* in three mealybug species. Different letters on top of the bars indicate significant difference.

Discussion

Host specificity of a biological control agent is one of the most important criteria in evaluating its potential uses and assessing its risks to non-target organisms (Nechols et al., 1992; van Lenteren et al., 2003; Louda et al., 2003; Briese, 2005; Sheppard et al., 2005). The host range, i.e. the set of host species that are accepted and utilized for progeny development, of a parasitoid is therefore one of the first steps in the evaluation of the parasitoid (Van Driesche and Hoddle, 1997; van Lenteren et al., 2003). The group of species that are utilized by the parasitoid in the field is the ecological host range (Onstad and McManus, 1996). Fundamental host range is the set of

Table 2. Mean (± SEM) of the number of eggs and larvae of *Leptomastix dactylopii* encapsulated and the encapsulation rates by five mealybug species. n = number of replicates with dissected mummies

Mealybug species	Number encapsulated		Encapsulation rate (%)
	Eggs	Larvae	
No-choice test			
P. madeirensis	0.6 ± 0.2b	2.0 ± 0.9a	100a
P. solani	1.6 ± 0.2a	0c	100a
P. citri	0c	0c	0c
P. longispinus	1.8 ± 0.6a	0.2b	90 ± 3b
P. viburni	1.8 ± 0.5a	0.2 ± 0.1b	86 ± 5b
n	93	93	93
χ^2	47.95	45.53	68.22
d.f.	4	4	4
p	< 0.0001	< 0.0001	< 0.0001
Choice test			
P. madeirensis	–	–	–
P. solani	0.8 ± 0.2c	0.2	100a
P. citri	0d	0	0b
P. longispinus	1.0b	0	100a
P. viburni	1.6 ± 0.3a	0.6	100a
n	77	77	77
χ^2	66.61	5.05	76.00
d.f.	3	3	3
p	< 0.0001	0.1684	< 0.0001

Means followed by the same letters within each column are not significantly different.

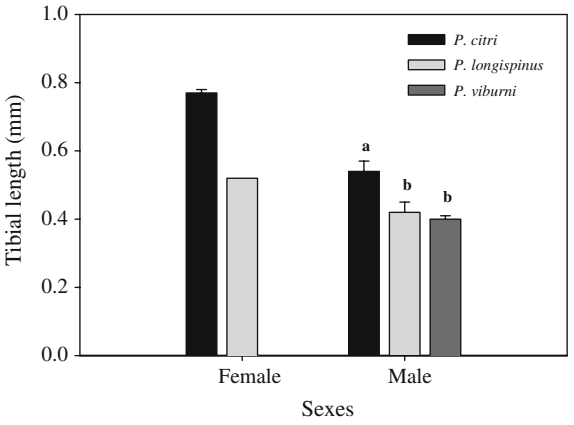

Figure 3. Left hind tibial length (mean ± SEM) of *Leptomastix dactylopii* developed in three mealybug species. Different letters on top of the bars indicate significant difference.

species that are accepted by the foraging parasitoid and can support the development under laboratory conditions (Onstad and McManus, 1996). Fundamental host range differs from the previously used physiological host range in that the host acceptance behaviors, instead of simple physiological compatibility, are taken into account (van Klinken, 2000). The selection of test species in fundamental host range studies should base on the phylogenetic relationships between the target and the non-target species (van Klinken, 2000; Kuhlman and Mason, 2003; Sheppard et al., 2005). We present here a limited fundamental host range study of two mealybug parasitoid species based on laboratory testing.

The six mealybug species selected for this study are valuable predictors of the fundamental host range of *Anagyrus* sp. nov. nr. *sinope* and *L. dactylopii* as they represent major clades within the family Pseudococcidae. Various mealybug taxonomists have proposed 3–5 subfamilies: Phenacoccinae, Pseudococcinae, Rhizoecinae, Sphaerococcinae and Trabutininae (reviewed by Ben-Dov, 1994; Downie and Gullan, 2004). However, these classifications are often unstable and unsatisfactory (Ben-Dov 1994; Downie and Gullan, 2004). We base our discussion on the higher classification of mealybug proposed by Downie and Gullan (2004), which is the most recent study and included all our test species. Downie and Gullan's (2004) phylogenetic analysis was based on molecular analyses of three nuclear genes (elongated factor 1α, ribosomal 28S and ribosomal 18S). Downie and Gullan (2004) recognized three subfamilies within Pseudococcidae:

Phenacoccinae, Psedococcidae and Rhozoecinae. We observed that the two parasitoid species tested in this study forage exclusively in the canopy, and thus we do not expect them to attack members of the Rhizoecinae, which are mostly root-feeding mealybugs (e.g., *Rhizoecus* sp.), in the field. Phenacoccinae is a paraphyletic clade and include the closely related *P. madeirensis* and *P. solani*. Along with a small number of ungrouped taxa, Downie and Gullan (2004) recognized three tribes and a distinct group within Pseudococcinae: Pseudococcini (include *P. longispinus* and *P. viburni*, but the two species are probably polyphyletic), Planococcini (*P. citri*), Trabutini (no member was included in this study), and the *Ferrisia* group (*F. virgata*).

Anagyrus sp. nov. nr. *sinope* is specific to *P. madeirensis* in this study. *Anagyrus* sp. nov. nr. *sinope* only oviposited and developed in *P. madeirensis*. *Anagyrus* sp. nov. nr. *sinope* did not oviposit in any *P. solani*, a closely related species to *P. madeirensis*. A foraging *Anagyrus* sp. nov. nr. *sinope* recognized potential hosts through antennal examination. Ovipositor probing provided further discrimination as most examined *P. solani*, *P. citri*, *P. longispinus* and *P. viburni* were not accepted for ovipositor probing. *Anagyrus* sp. nov. nr. *sinope* developed as gregarious parasitoid in *P. madeirensis*, with an average of two females and one male emerging from each parasitized *P. madeirensis* after 16 days of incubation at 25 °C. This result is similar to that reported in a life history study of *Anagyrus* sp. nov. nr. *sinope* (reported as *A. loecki*) by Chong (2005). No encapsulated eggs or larvae were recovered from any of the six mealybug species tested, suggesting that *Anagyrus* sp. nov. nr. *sinope* did not deposit eggs in the five nonpreferred species and that *Anagyrus* sp. nov. nr. *sinope* was successful in overcoming the immune system of *P. madeirensis*.

Sagarra et al. (2001a) investigated the host range of *Anagyrus kamali* Moursi in the laboratory. The selection of nine mealybug species by Sagarra et al. (2001a) was based on their commonness and economic importance in Trinidad, and represent only members from the subfamily Pseudococcinae. *Anagyrus kamali* oviposited in the target species, *Maconellicoccus hirsutus* Green [an ungrouped basal taxa according to Downie and Gullan (2004)], *P. citri* and *Planococcus halli* Ezzat & McConnel (both Planococcini). However, the parasitoid completed development in only *M. hirsutus*. A comparison of the results of this study and that of Sagarra et al. (2001a) is inappropriate because the two studies shared only one common mealybug species (*P. citri*). The fundamental host range of *A. kamali* could be understood better had Sagarra et al. (2000a) included species from other

subfamilies or genera, especially members of Phenacoccinae and other reported hosts, e.g., *F. virgata* (Noyes and Hayat, 1994), that are found in Trinidad (Ben-Dov, 1994).

In the no-choice tests, *L. dactylopii* was a general parasitoid that parasitized all mealybug species tested except *F. virgata*. *Leptomastix dactylopii* preferred to parasitize *P. citri* in the choice test. The preference ranking of the six mealybug species was *P. citri* > *P. viburni* > *P. longispinus* > *P. solani* > *P. madeirensis* > *F. virgata*. *Leptomastix dactylopii* appeared to discriminate against members of Phenacoccinae (*P. madeirensis* and *P. solani*) during antennal examination (Table 1). Discrimination against *P. longispinus* was the strongest during the ovipositor-probing phase (Table 1).

Planococcus citri, *P. longispinus*, and *P. viburni* were able to support the complete development of *L. dactylopii*. *Leptomastix dactylopii* parasitized significantly more *P. citri* and produced more progeny from *P. citri* than *P. longispinus* and *P. viburni*. *Leptomastix dactylopii* developing in *P. longispinus* and *P. viburni* emerged later, smaller and had a male-biased sex ratio than those from *P. citri*. Similar to Blumberg and Van Driesche's findings (2001), the results of this study clearly demonstrated that *P. citri* was a more suitable host species than *P. longispinus* and *P. viburni* for the development of *L. dactylopii*.

The degree to which a parasitoid is encapsulated is an important parameter of host suitability (Blumberg, 1997). Unsuitability of mealybug species other than *P. citri* for the development of *L. dactylopii* was the result of high encapsulation rate of parasitoid eggs and larvae. Parasitism of *P. citri* by *L. dactylopii* did not induce any encapsulation responses, a finding previously reported by Blumberg and Van Driesche (2001). Almost all parasitoid eggs deposited in *P. longispinus* and *P. viburni* were encapsulated. Blumberg and Van Driesche (2001) reported 59 and 100% encapsulation rates by *P. longispinus* and *P. viburni*, respectively. The encapsulation rates of *L. dactylopii* by *P. madeirensis* and *P. solani* were 100% in both no-choice and all-choice tests.

A comprehensive host specificity study should include both ecological and physiological host range (Strand and Obrycki, 1996). Fundamental (or physiological) host range is often greater than the ecological host range because the later is limited by availability of the hosts, behavior of the parasitoids, and the climate (Morehead and Feener, 2000; Alleyne and Wiedenmann, 2001; Haye et al., 2005). We have not performed an exhaustive evaluation of the suitability of all mealybug species for *L. dactylopii*. Based on our results, *F. virgata*,

P. madeirensis and *P. solani*, which are listed as host species of *L. dactylopii* (Noyes and Hayat, 1994), were in fact not suitable for the development of the parasitoid. Thus, the ecological host range of *L. dactylopii* should exclude *F. virgata*, *P. madeirensis* and *P. solani*, and may be narrower than the 20 plus species as suggested by Noyes and Hayat (1994). The discrepancy indicates a need to verify the host records of *L. dactylopii* in which misidentification of mealybug species may have occurred. Practically, the use of *L. dactylopii* will only be successful in a biological control program against *P. citri* because this is the only mealybug species that allow high levels of parasitism and development of the parasitoid.

The presence of alternative hosts is important to the success of a biological control program in that such presence allows the persistence of parasitoid populations over periods of scarcity of the primary hosts (DeBach and Bartlett, 1964). The specificity of *Anagyrus* sp. nov. nr. *sinope* for *P. madeirensis* may increase the risk of local extinction of the parasitoid population when *P. madeirensis* is at low densities. A population of *L. dactylopii*, on the other hand, may persist if populations of *P. longispinus* and *P. viburni* are available during period of low *P. citri* abundance. If an *Anagyrus* sp. nov. nr. *sinope* population fails to persist, reintroduction or augmentation may be required to reestablish or increase the parasitoid's presence.

The specificity of *Anagyrus* sp. nov. nr. *sinope* for *P. madeirensis* could also hinder the use of alternative hosts for mass rearing and limit the use of this parasitoid species against other pestiferous mealybug species. Mass rearing of *L. dactylopii* should rely on *P. citri* as hosts in order to achieve high parasitoid fitness in terms of high survival rate, shorter developmental time, larger body size, and a more female-biased sex ratio. High encapsulation rates of *L. dactylopii* developing in *P. longispinus* and *P. viburni* precluded the use of the two mealybug species as alternative hosts in mass rearing. The specificity of *Anagyrus* sp. nov. nr. *sinope* maybe a positive attribute as the risk of non-target effect is reduced or non-existent. However, a more comprehensive host specificity testing is required to verify the claim of low or no non-target effect by *A.* sp. nov. nr. *sinope*. Such host specificity testing should consist of a fundamental host range study which include a wider representation from all clades within Pseudococcidae, and an ecological host range study that investigate the actual host range utilized by the parasitoid in the field.

In summary, *Anagyrus* sp. nov. nr. *sinope* is a highly host-specific parasitoid that develops in only *P. madeirensis* even when the closely

related *P. solani* is present. *Leptomastix dactylopii* is capable of attacking most mealybug clades represented in this study (except *F. virgata*) but is only able to complete development in members of Planococcini (*P. citri*) and Pseudococcini (*P. longispinus* and *P. viburni*). The mealybug species that could be effectively parasitized by *L. dactylopii* is *P. citri*. The use of *Anagyrus* sp. nov. nr. *sinope* and *L. dactylopii* against mealybug species other than their respective primary hosts should be limited, as the parasitoids may not achieve the highest efficacy and fitness.

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