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A common mode of attraction of larvae and adults of insect predators to the sex pheromone of their prey (Hemiptera: Matsucoccidae)

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Abstract

The attraction of several adult predators, genera *Elatophilus*, *Hemerobius* and *Sympherobius*, to the sex pheromones of pine bast scales, *Matsucoccus* Cockerell, has already been demonstrated. Here, the hypothesis that the larvae of these predators are similarly attracted to the host prey sex pheromone is tested. The response of predators was tested in field trials using pine tree arenas baited with the sex pheromones of *M. josephi* Bodenheimer & Harpaz, *M. feytaudi* Ducasse and *M. matsumurae* Kuwana. Experiments were conducted in Israel in stands of *Pinus halepensis* infested by *M. josephi* and in Portugal in stands of *P. pinaster* infested by *M. feytaudi*, respectively. The selectivity of larvae for the three sex pheromones was tested in Petri dish arenas in the laboratory. In the field, the larval stages exhibited similar modes of attraction to those of the conspecific adults: *Elatophilus hebraicus* Pericart in Aleppo pine forest, *E. crassicornis* Reuter and *Hemerobius stigma* Stephens in the maritime pine forests. Laboratory choice tests confirmed the kairomonal selectivity of larvae. Both forest and laboratory tests demonstrated the response of a coccinellid of the genus *Rhyzobius* to the sex pheromones of *M. feytaudi* and *M. matsumurae*. A unique chemical communication system among several taxa of predators of *Matsucoccus* spp. was highlighted that may be attributed to their coevolution on a geological time scale.

Keywords: kairomone, response, sex pheromone, *Matsucoccus*, predator, *Elatophilus*, *Hemerobius stigma*, *Rhyzobius*, *Pinus*

Introduction

The members of the genus *Matsucoccus* Cockerell (Hemiptera: Matsucoccidae) feed exclusively on pines. The genus *Matsucoccus* includes the oldest known scale insect fossils: species from the lower Cretaceous, aged about 125 million years (Koteja, 1990; Foldi, 2004). Several *Matsucoccus* species are severe pests; these include *M. feytaudi* Ducasse in southeastern Europe (Covassi & Binazzi, 1992; Jactel *et al*., 1998), *M. josephi* Bodenheimer & Harpaz in the Middle East (Mendel *et al*., 1994), and *M. matsumurae* Kuwana and *M. acalyptus* Herbert in North America (McClure, 1976; Kosztarab, 1996). Currently, the sex pheromones of three *Matsucoccus* species are known, those of *M. matsumurae*, *M. feytaudi* and *M. josephi*, which are ketones that share a common ketodiene moiety (Dunkelblum *et al*., 2000). Recent

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studies showed that these sex pheromones attracted several species that are predators of these pine bast scales, and all of which are considered to be principal predators (Mendel *et al*., 2004). The *M. josephi* sex pheromone was found to have an attraction effect upon *Elatophilus hebraicus* Pericart (Hemiptera: Anthocoridae), and upon *Hemerobius stigmaterus* Fitch (Neuroptera: Hemerobiidae) (Mendel *et al*., 1995, 2004). The sex pheromones of both *M. matsumurae* and *M. feytaudi* were found to attract *E. hebraicus*, *E. crassicornis* Reuter, *E. nigricornis* Zetterstedt and *Hemerobius stigma* Stephens (Mendel *et al*., 1997, 2003, 2004). The *M. matsumurae* pheromone also attracted *Sympherobius fuscescens* Wallengern (Neuroptera: Hemerobiidae) (Mendel *et al*., 2004). These predators were also shown to be very sensitive to changes in the structure of the prey sex pheromones, especially in the diene moiety (Dunkelblum *et al*. 1996; Mendel *et al*., 2003).

The exploitation of the host sex pheromones as chemical signals for host location has been found in a number of species of parasitoids (e.g. Colazza *et al*., 1999; Hilker *et al*., 2000; Mbata *et al*., 2004). In contrast, only a few studies have demonstrated the response of predators to the sex pheromone of their prey (e.g. Boo *et al*., 1998; Millar *et al*., 2001).

In our previous studies only adult predators, males and females, and the conspecific males of *Matsucoccus* were caught (e.g. Mendel *et al*., 1997, 2003, 2004). In our more recent studies the sticky or funnel traps were replaced with direct observation of arenas in which dispensers were placed on the stem outer bark. In these arenas, the occurrence of predator larvae near the pheromone source was observed for first time, and in the light of this observation the objective of the present study was to test the hypotheses that the larvae of the principal predators of *Matsucoccus* are also attracted to the sex pheromones of their prey; and that the patterns of kairomonal attraction of the predator larvae would match those of the conspecific adults.

Materials and methods

Field tests

Field observations were conducted in two areas: (i) in Portugal, in two *M. feytaudi*-infested maritime pine *Pinus pinaster* Ait. stands at Sintra and Península de Setúbal; and (ii) in Israel, in a *M. josephi*-infested Aleppo pine, *P. halepensis* Mill., stand at Eshta'ol in the Judean Hills. In each study area the sex pheromones of *M. josephi* (*2E, 6E, 8E*)-5,7-dimethyl-2,6,8-decatrien-4-one, *M. feytaudi* (*8E, 10E*)-3,7,9-trimethyl-dodecadien-6-one and *M. matsumurae* (*2E, 4E*)-4,6,10,12-tetramethyl-2,4-tridecadien-7-one, in the form of racemic mixtures were tested in pine tree arenas in doses of 50, 220 and 600 μg, respectively, per arena. The differences in the doses corresponded to the differing volatility of the three sex pheromones (Dunkelblum *et al*., 1996). The *M. josephi* pheromone was synthesized in the Volcani Center, Israel, that of *M. feytaudi* by Jacques Einhorn and Martine Lettere of the Unité de Phytopharmacie et des Médiateurs Chimiques, INRA, Versailles, France, and the *M. matsumurae* pheromone by Kenji Mori of the Department of Chemistry, Science University of Tokyo, Japan. The pheromones, in hexane solutions, were impregnated into American grey rubber dispensers (West Co., Pennsylvania, USA). The tree arena was a smoothed surface on the outer bark, about 1 dm^2 in area, mounted approximately 1.50 m above the ground, with a rubber septum impregnated with one of the pheromones placed in the centre. The lure was placed in the tree arena immediately before the observation. Two types of controls were used: (i) cleaned tree arenas without a pheromone lure; and (ii) tree arenas with a similar dispenser baited with 50 μg of a non-host pheromone, that of the female *Planococcus citri* Risso (Hemiptera: Pseudococcidae).

In Portugal, field observations were conducted at each site and on each monitoring date. Twenty-six pine trees were chosen, about 6–10 m apart, and were randomly assigned to one of five treatments: arena baited with *M. feytaudi*, *M. josephi*, *M. matsumurae* or *P. citri* sex pheromone and a cleaned arena, $n = 12$, 3, 3, 4 and 4, respectively. The small numbers of dispensers available dictated the smaller numbers of replicates used for *M. josephi*, *M. matsumurae* and *P. citri*. Field observations at Sintra were conducted on three sampling dates, May, June and September 2003, and those in two plots at Península de Setúbal on another four sampling dates, March, April, May and June, 2004. Observations were carried out between 1000 h and 1600 h.

In Israel, field observations were conducted only once, in July 2003, with the five treatments mentioned above, and $n = 10$, for each treatment.

Observations on each tree were repeated five times at intervals of about 10–15 min; the presence of the predators in the arena was observed each time, and all the individuals found there were counted and collected with an aspirator. The total number of individuals per tree arena was recorded and used as sampling unit.

Laboratory tests

Preference trials were conducted in the laboratory, in glass Petri dish arenas, 22 cm in diameter and 2.5 cm deep, with a sheet of paper at the bottom. The preference behaviour of the larvae of *E. hebraicus*, *H. stigma* and *Rhyzobius* sp. (Coleoptera: Coccinellidae) was examined. The larvae of the two first species were reared in laboratory conditions, fed on ovisacs and on *M. josephi* females obtained from laboratory mass rearing of the scale. The larvae of *Rhyzobius* sp. were collected in a maritime pine forest, while they were devouring ovisacs of *M. feytaudi*. They were collected from band traps wrapped around the tree trunk at breast height, a device used to sample females of *M. feytaudi* (Jactel *et al*., 1996). In the laboratory test arenas the attraction of larvae to the pheromones was monitored with the aid of baits comprising 5-mm-diameter filter papers impregnated with 2 μg of the sex pheromone of *M. josephi*, *M. feytaudi* or *M. matsumurae*. Two series of choice tests were performed. In the first series larval preference was tested in separate two-choice tests in which the choice was between pheromone bait and a non-bait material. The second series was applied only to the larvae of *E. hebraicus* and *H. stigma*; it comprised multi-choice tests in which all three *Matsucoccus* pheromones, and controls were exposed in the same arena. Crude pheromone (from about ten females) of *Nipaecoccus viridis* (Newstead) (Hemiptera: Pseudococcidae) and a non-baited material served as controls. In each trial ten larvae were tested simultaneously for both *E. hebraicus* and *H. stigma*, and five larvae for *Rhyzobius* sp. Those of *Rhyzobius* sp. and *H. stigma* were from second and third instars, whereas those of *E. hebraicus* were L3–L5 larvae. The larvae were positioned in the centre of the arena, equally distanced from all baits. Each observation was conducted for 5 min in the case of *H. stigma* and *Rhyzobius* sp. and 30 min for *E. hebraicus*. The number of

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Table 1. Predators captured ($\bar{x} \pm SE$, $n = 10$) per tree arena according to the type of lure in an Aleppo pine stand in the Judean Hills, Israel, in July 2003.

Species	Pheromone				
	Matsucoccus feytaudi	*M. matsumurae*	*M. josephi*	*Planococcus citri*	Clean arena
Elatophilus hebraicus adults $F_{3,36} = 28.86$, $P < 0.0001$	6.0 ± 1.56^{b}	7.2 ± 1.57^{b}	29.0 ± 6.19^{c}	0.1 ± 0.1^{a}	0^{a}
E. hebraicus nymphs $\chi^2_3 = 9.34$*, $P = 0.025$	1.10 ± 0.50^{ab}	2.50 ± 0.97^{b}	2.20 ± 0.93^{b}	0^{a}	0^{a}

*Kruskal-Wallis test.
Mean values within each species/stage followed by the same letter are not significantly different ($P < 0.05$).

larval encounters, i.e. physical contacts with bait, was recorded in each observation; it was also noted whether the larvae remained in contact with the bait or tended to leave it. Four to six replicate observations were conducted for each species/larval stage. A new set of larvae was used in each successive trial. After each bioassay the Petri dish was washed and dried with a hair dryer, and the paper sheet in the bottom was replaced. Experiments were conducted at room temperature and under normal illumination. To avoid possible visual or other extraneous effects, the positions of the baits were changed for each bioassay by random allocation.

Statistical analyses

To determine their responses to the sex pheromone compounds in the field trials, the mean numbers of captured insects per tree, grouped according to species and stage, were subjected to the Kruskal-Wallis test, followed by the Mann-Whitney test to compare treatments, as the assumption of a normal distribution could not be guaranteed. The mean numbers of encounters of the multiple-choice tests in the laboratory were subjected to one-way ANOVA, type III, followed by a Tukey HSD post-hoc test. To determine pheromone selection in the laboratory two-choice tests a paired t-test was performed. All analyses were conducted with SPSS 13.0 for Windows.

Results

Field tests

In all field trials both larval and adult predators were observed congregating in sex pheromone-baited tree arenas, usually within 2–5 min after the lures had been set up.

In Israel, both adults and larvae of *E. hebraicus* congregated in arenas baited with each of the three tested *Matsucoccus* pheromones. In the case of larvae, results were significant for the arenas baited with *M. josephi* and *M. matsumurae* pheromones. The greatest number of adults was found in the arena with the *M. josephi* pheromone. In the case of larvae, their numbers near *M. josephi* and *M. matsumurae* pheromone baits were similar (table 1).

In the maritime pine forests in Portugal, both adults and larvae of the three species were recorded in the pheromone arenas: *E. crassicornis*, *H. stigma* and *Rhyzobius* sp. (probably a new species of the genus *Rhyzobius* Stephens; Armando Raimundo, a coccinellids taxonomist, personal communication). Nevertheless, different numbers of the various species and stages were registered on different sampling dates reflecting different phenological traits. Both adults and larvae of *E. crassicornis* were sampled in significant numbers in September 2003 and in April and March 2004; adults were also sampled in June 2003. Adults and larvae of the brown lacewing *H. stigma* were present in significant numbers in May, June and September 2003, and May and June 2004. The larvae of the ladybeetle were sampled in May 2003 and again in March, April and May 2004, whereas the adults were found only in June, in both years. Both adults and larvae of the three predatory species captured in the maritime pine forests displayed clear orientation and typical searching behaviour and attraction towards arenas baited with the pheromones of *M. feytaudi* and *M. matsumurae*, but not to those baited with that of *M. josephi* (table 2). A peak of daily activity of the predators was observed between 1000 and 1300 h.

In general, adults, both of *Elatophilus* spp. and of *H. stigma*, were observed in larger numbers than their larvae. Occasionally, depending on season, only adults or only larvae were observed in some tree arenas. This was the rule in the case of the coccinellid, which attracted larvae and adult beetles at different seasons. The coccinellid was also distinguished by attracting, on the whole, larger numbers of larvae than of adult beetles (table 2).

Preference tests with larvae in the laboratory

In two-choice tests that compared pheromone-impregnated filter paper discs with non-baited discs, significant attraction was displayed by larvae of *H. stigma* to all three pheromones, by larvae of *Rhyzobius* sp. to the pheromones of *M. matsumurae* and *M. feytaudi*, and by larvae of *E. hebraicus* to the pheromones of *M. josephi* and *M. matsumurae* (fig. 1). In the multi-choice test, where the choices were between discs impregnated with *Matsucoccus* pheromones, crude pheromone of *N. viridis* and an unbaited disc, the larvae of *H. stigma* were attracted to the pheromones of *M. matsumurae* and *M. feytaudi*, whereas their attraction to the other baits did not significantly differ from that to the unbaited paper (fig. 2). The larvae of *E. hebraicus* were attracted to the pheromone of *M. josephi*, less attracted to that of *M. matsumurae*, and not attracted to the *M. feytaudi* pheromone (fig. 2).

The observations on the behaviour of the larvae in the laboratory showed that after a contact has been established with attractive bait the larvae tend to stay very close to the pheromone-impregnated material. Such behaviour was not observed towards non-*Matsucoccus* baits.

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Table 2. Number of predators captured ($\bar{x} \pm SE$) per tree arena on each type of lure in two maritime pine stands in Sintra and Ferraria, Portugal, in June and September 2003 and spring 2004.

Species	Pheromone				
	Matsucoccus feytaudi	*M. matsumurae*	*M. josephi*	*Planococcus citri*	Clean arena
Elatophilus crassicornis adults $\chi^2_3 = 101.26^*$, $P < 0.001$	11.14 ± 1.06^b	6.86 ± 1.52^b	0.33 ± 0.16^a	0.10 ± 0.07^a	0^a
E. crassicornis larvae $\chi^2_3 = 30.09^*$, $P < 0.001$	1.06 ± 0.17^c	0.62 ± 0.28^{bc}	0.24 ± 0.15^{ab}	0^a	0^a
Hamerobius stigma adults $\chi^2_3 = 51.69^*$, $P < 0.001$	5.74 ± 1.07^b	5.55 ± 2.19^b	0^a	0^a	0^a
H. stigma larvae $\chi^2_3 = 15.49^*$, $P = 0.001$	0.48 ± 0.25^b	0.66 ± 0.33^b	0^a	0^a	0^a
Rhyzobius sp. adults $\chi^2_3 = 11.97^*$, $P = 0.007$	0.26 ± 0.08^b	0.25 ± 0.13^{ab}	0^a	0^a	0^a
Rhyzobius sp. larvae $\chi^2_3 = 70.67^*$, $P < 0.001$	3.48 ± 0.53^c	2.14 ± 0.71^{bc}	0.66 ± 0.21^b	0.04 ± 0.04^a	0^a

*Kruskal-Wallis test.
Mean values within each species/stage followed by the same letter are not significantly different ($P < 0.05$).

Discussion

It was previously demonstrated that adults of several species of predators of the pine bast scales, *Matsucoccus* spp. were attracted to the sex pheromone of the female prey (Dunkleblum *et al*., 1996; Mendel *et al*., 2003, 2004), whereas the responses of other insect predators to the sex pheromone of their prey is scarcely documented (but see Boo *et al*., 1998; Millar *et al*., 2001). The present results revealed that the larvae of two anthocorids (*E. crassicornis* and *E. hebraicus*), a hemerobiid (*H. stigma*), and a coccinellid (*Rhyzobius* sp.) were also attracted by the sex pheromone of the pine bast scales.

In both the field and the laboratory experiments, larvae tended to display similar specificity of attraction to that of the conspecific adults. In the field, adults of *E. hebraicus* preferred *M. josephi* sex pheromone and were less attracted to those of *M. matsumurae* and *M. feytaudi* in harmony with earlier observations (Dunkleblum, 1996; Mendel *et al*., 2004). In the multi-choice test larvae displayed a similar pattern (fig. 2). Nevertheless, in the field observations larvae of *E. hebraicus* seemed to be less selective than adults, as no significant differences between *M. matsumurae* and *josephi* were observed (table 1). This might be conditioned by the low numbers of larvae sampled and their patchy distribution; since larvae are flightless their attraction to the lure is conditioned to their presence on the tree trunk. Adults and larvae of *E. crassicornis*, *H. stigma* and *Rhyzobius* sp. were chiefly attracted to the *M. feytaudi* and *M. matsumurae* sex pheromones and not at all to that of *M. josephi* (table 2, fig. 2); this is consistent with previous findings on the responses of the adults of these predators (Mendel *et al*., 2003, 2004; Branco *et al*., 2005). An exception was found in the case of *H. stigma* in the laboratory two-choice test, where larvae exhibited a selection for *M. josephi* compared to control (fig. 1). The tested larvae of *H. stigma* had been reared on ovisacs and females of *M. josephi*, and this may explain their different behaviour in the present two-choice test: the previous experience and learning could have altered their infochemical detection capability (e.g. Krips *et al*., 1999; Fukushima *et al*., 2002). Nevertheless, this behaviour was not exhibited in the multi-choice test, in which larvae could choose among several pheromone sources.

A few studies have found responses of predatory larvae to prey odour, i.e. the alarm pheromone of aphids (e.g. Francis *et al*., 2004) and of phytophagous larvae to the host plant volatiles (e.g. Knight & Light, 2001; Singh & Mullick, 2002). The present study provides evidence that the larvae of predators can also respond to the sex pheromone of their prey. The responses of both adults and larvae to the pheromone source occurred within only a few minutes of the exposure of the lure in the tree arena, indicating that the response to kairomone plays an important role in prey location by these predators. The hypothesis of a response of larvae to an adult-produced aggregation signal is disregarded, as larvae of *H. stigma* and *E. crassicornis* were sometimes observed to be attracted to lures with no adults or prior to adults. Further, larvae and adults of *Rhyzobius* sp. occurred at different seasons.

In the present study it was found for the first time that a coccinellid, *Rhyzobius* sp., was also attracted to the sex pheromones of the pine bast scales. It displays some morphological features that distinguish it from all the other *Rhyzobius* spp. known in the Iberian Peninsula and the rest of Europe (A. Raimundo *et al*., unpublished). The larvae of *Rhyzobius* sp. were sampled from March to May whereas the adults were found only in June. These findings suggest that it is a univoltine species, as is *Rhyzobius chrysomeloides* Herbst, another coccinellid associated with *M. feytaudi* in Italy (Covassi *et al*., 1991). Similarly to the other two predatory species of the *M. feytaudi* guild, *Rhyzobius* sp. is also lured by the pheromones of *M. feytaudi* and *M. matsumurae* but not by that of *M. josephi*. To the best of our knowledge, this is the first evidence of a coccinellid exploiting the sex pheromone of its prey. However, other kairomonal substances, namely the alarm pheromones produced by aphids, have been associated with the attraction of ladybirds to the prey colonies (e.g. Shonouda, 1999; Francis *et al*., 2004). In the light of these results, we may hypothesize that other coccinellids that are closely associated with *Matsucoccus*, such as *R. chrysomeloides* (e.g. Toccafondi *et al*., 1991), may be lured by the sex pheromone of their prey.

The present observations on the behaviour of the predators were limited to the habitats of two *Matsucoccus*

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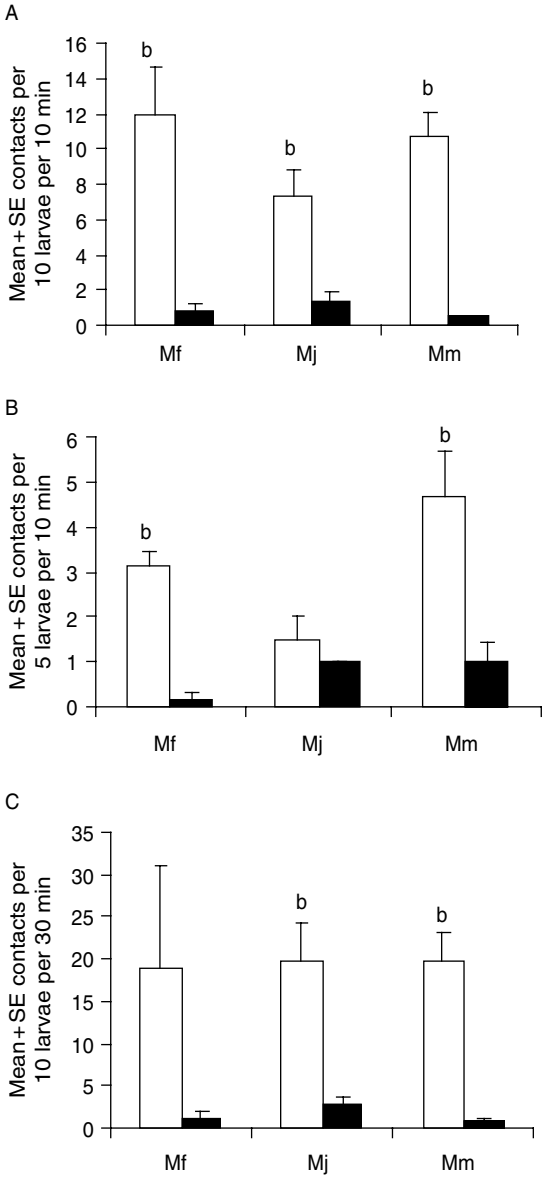

Fig. 1. Numbers of encounters ($\bar{x} + SE$) of (A) *Hemerobius stigma*, (B) *Rhyzobius* sp. and (C) *Elatophilus hebraicus* larvae with baits of *Matsucoccus feytaudi* (Mf), *M. josephi* (Mj) and *M. matsumurae* (Mm). Each pheromone-impregnated (□) filter paper disc was tested in a separate arena against an unbaited (■) disc. Mean values within each species followed by a letter are significantly different from control according to paired sample t-test ($P < 0.05$).

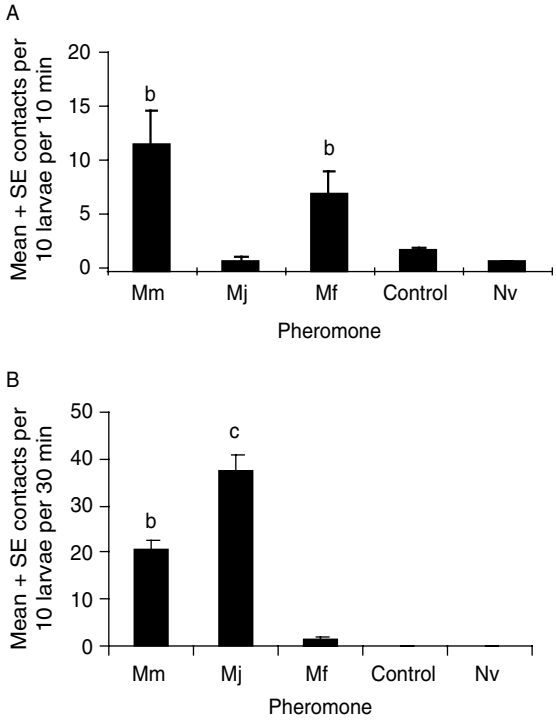

Fig. 2. Numbers of encounters ($\bar{x} \pm SE$) of the larva of (A) *Hemerobius stigma* and (B) *Elatophilus hebraicus* with filter paper discs impregnated with the host pheromones of *Matsucoccus feytaudi* (Mf), *M. josephi* (Mj) and *M. matsumurae* (Mm), the non-host sex pheromone of *Nipaecoccus viridis* (Nv), and with an non-baited disc (control). All the pheromones were tested in the same arena. $F_{4,13} = 132.7$, $P < 0.001$ and $F_{4,39} = 13.32$, $P < 0.001$ for the multi-choice tests of *E. hebraicus* and *H. stigma*, respectively. Mean values within each species followed by the same letter are not significantly different according to Tukey's HSD post-hoc test ($P < 0.05$).

spp.: *M. feytaudi* in Portugal and *M. josephi* in Israel. However, the attraction of adult *Elatophilus* spp. and adult brown lacewings in other areas in the West Palaearctic as well as in North America (Mendel *et al*., 2004) suggests that this pattern of kairomonal response of the larvae to the sex pheromones of their prey is exhibited by other species in guilds of predators of *Matsucoccus* in other areas.

As expected, the larvae, which approached the arena by walking along the stem, were outnumbered by the adults, which arrived at the arena by flight. This was not valid for *Rhyzobius* species. Adult *Rhyzobius* sp. were never captured by the funnel and delta traps that were deployed in our previous studies (e.g. Mendel *et al*., 2003), probably because of their mode of approaching the arena, i.e. by walking along the stem, in contrast to adults of *Elatophilus* spp. and *H. stigma*, which approach the bait by flight. This fact highlights the potential usefulness of the tree arena with a pheromone source as a tool for detecting and studying species that are not susceptible to other trapping methods, especially those that approach the trap by walking and not by flying, as in the case of the larvae.

The finding of such a diverse array of predatory species, from three orders, of which both the adult and larval stages exploit the sex pheromone of the prey, suggests that this phenomenon is most probably much more widespread, specifically among predators associated with pine bast scales, and that it reveals a unique chemical communication system. The field observations demonstrated that predators reacted very quickly to the presence of a pheromone source; thus this response to chemical cues is a highly efficient prey detection mechanism. In addition, the daily activity of the predators was observed to have a peak between 1000 and 1300 h which agrees with the main period of pheromone

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production and mating observed on pine scale insects (Riom & Fabre, 1977; Park *et al*., 1994). This fact supports more evidence that the attraction of these predators is efficient and that the predators may be a major regulating force on the scale populations. The populations of the pine bast scale in their native environment are kept at endemic levels, evidencing regulation mechanisms. These may be in part explained by an interaction with their native host trees (Mendel, 1998); extensive lesions in the pine tissue causing a depletion of the maritime pine bast scale populations at high densities (Riom *et al*., 1971). Even so, the result that several predator species and stages respond efficiently to the *Matsucoccus* sp. sex pheromones draws attention to the possible relevant role of predators in helping to keep populations at low levels. In Israel, outbreaks of *M. josephi* frequently occur in pine stands adjacent to cotton fields, caused by insecticide drift (Mendel *et al*., 1994).

Matsucoccus spp. are among the oldest known scale insects and they have no known parasitoids (Foldi, 2004). Thus if any pressure from natural enemies has constrained the evolution of the pine bast scales species this might have been from specialist predators. The females of several pine bast scales share a habit of locating inside the bark of tree trunks or twigs, which might be explained as a way of predator evasion. On the other hand, this habit, the low mobility of females and the fact that in their native environment pine bast scales occur in extremely low densities may have driven this broad array of predators to evolve chemically mediated mechanisms to locate their prey.

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