

Kairomonal response of the parasitoid *Anagyrus spec. nov. near pseudococci* to the sex pheromone of the vine mealybug

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Abstract

The occurrence of a kairomonal response of the parasitoid *Anagyrus spec. nov. near pseudococci* (Hymenoptera: Encyrtidae) to (+)-(1*R*,3*R*)-*cis*-2,2-dimethyl-3-isopropenyl-cyclobutanemethanol acetate (PcA, namely, planococetyl acetate) and (S)-(+)-lavandulyl senecioate (LS), the respective female sex pheromones of its hosts, the citrus mealybug, *Planococcus citri* (Risso) and the vine mealybug, *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae) was investigated. Attraction to the pheromones was tested by employing pheromone traps in field trials and by static air olfactometer bioassays in the laboratory. Female wasps showed a significant response to LS, in both field and olfactometer experiments. No significant response was registered to the sex pheromone of *P. citri*. Despite the similarity between the structures of LS and its analogue (S)-(+)-lavandulyl isovalerate (LI), no significant response to the latter compound was observed. It seems that differences between the structures of the carboxylate moiety of the respective molecules (LS and LI) markedly affect the kairomonal attractiveness to the parasitoid. The kairomonal response of *Anagyrus spec. nov. near pseudococci* was neither influenced by the host habitat nor by the host species on which it developed. This suggested innate behaviour of *Anagyrus spec. nov. near pseudococci*, possibly derived from evolutionary relationships between the parasitoid and *P. ficus*. The practical implications of the results are discussed.

Introduction

Parasitic wasps have been shown to be attracted by their host pheromones, and kairomonal responses to sex, aggregation, epideictic, and alarm pheromones have been demonstrated (see review in Powell, 1999). Sex pheromones serve as chemical cues in host location by egg parasitoids, *Trichogramma* spp., *Telenomus* spp., and *Ooencyrtus pityocampae* Mercet (Powell, 1999; Bruni et al., 2000; Reddy et al., 2002; Fiaboe et al., 2003) and larval/pupal parasitoids, for example, *Apanteles ruficrus* (Haliday), *Microplitis rufiventris* Kok., *Eupelmus vuillei* (CRW) (Powell, 1999). Sex pheromones of aphids are utilized as kairomones by their parasitoids, for example, *Diaeretiella rapae*

(McIntosh), *Praon* spp., *Aphidius* spp. (Powell, 1999; Birkett & Pickett, 2003; Powell & Pickett, 2003). The parasitoids of armoured scales *Aphytis* spp. and *Encarsia perniciosus* (Tower), which attack the California red scale *Aonidiella aurantii* (Maskell) and the San José scale *Diaspidiotus* (= *Quadraspidiotus*) *perniciosus* (Comstock), respectively, also respond to their host sex pheromone (Dunkelblum, 1999; Powell, 1999).

During a study in vineyards in southern Portugal in 2004, females of *Anagyrus pseudococci* s.l. (i.e., in the broad sense, see Triapitsyn et al., 2007) (Hymenoptera: Encyrtidae) were captured in traps baited with the sex pheromone of the vine mealybug, *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae) (JC Franco, EB Silva & Z Mendel, unpubl.). Attraction of *A. pseudococci* to similar lures has also been observed by Millar et al. (2002) in vineyards in southern California.

Anagyrus pseudococci is a solitary koinobiont endoparasitoid of mealybugs (Noyes & Hayat, 1994; Islam &

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Copland, 1997), and it has been used in classical biological control programmes and augmentative releases to control the citrus mealybug, *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) (Noyes & Hayat, 1994). Although approximately 30 mealybug species belonging to 11 genera have been recorded as hosts of *A. pseudococci* (Noyes, 2003), its most common hosts are *P. ficus* and *P. citri* (Noyes & Hayat, 1994; Daane et al., 2006). Based on the colouration of the first funicular segment (F1) of the female antenna, it has been suggested that there are two geographical races of *A. pseudococci* (Rosen & Rössler, 1966; Noyes & Hayat, 1994). Recently, Triapitsyn et al. (2007) showed, on the basis of morphological and reproductive compatibility studies and molecular data, that the taxon formerly known as *A. pseudococci* s.l. in fact comprises two sibling species, that is, *A. pseudococci* (Girault) and *Anagyrus* spec. nov. near *pseudococci*. Except for the colouration of F1 of the female antenna, these two species are morphologically indistinguishable.

Planococcus citri and *P. ficus* are the most economically important species of the genus and therefore have been subjected to many more studies than any of their congeners. *Planococcus ficus* is a major pest in many grapevine-growing regions. It is native to the Mediterranean Basin and has spread to other grapevine-growing areas, such as South Africa and California (Ben-Dov, 1994; Walton & Pringle, 2004; Daane et al., 2006). *Planococcus citri* is believed to be an Afrotropical species. It occurs in large densities on perennial crops in the tropical and subtropical zones, and on indoor ornamentals worldwide (Ben-Dov, 1994; Franco et al., 2004b). Both mealybug species are biparental. The female sex pheromone of *P. citri* is (+)-(1*R*,3*R*)-*cis*-2,2-dimethyl-3-isopropenyl-cyclobutanemethanol acetate (PcA, namely, planococcyll acetate) (Figure 1A) (Bierl-Leonhardt et al., 1981) and that of *P. ficus* is (S)-(+)-lavandulyl senecioate (LS) (Figure 1B) (Hinkens et al., 2001). Studies of Israeli populations of *P. ficus* showed that mealybugs reared in the laboratory under high densities also produced and responded to (S)-(+)-lavandulyl isovalerate (LI) (Figure 1C) (Zada et al., 2003). In both laboratory and field bioassays, feral males responded only to LS, whereas laboratory-reared males responded to both LS and LI (Zada et al., 2003). However, it was recently discovered that, in some locations, feral males are attracted to LI (Z Mendel, unpubl.).

The availability of the two mealybug sex pheromones has presented new opportunities for mealybug management through improved monitoring techniques and control tactics, such as mass trapping, mating disruption, and lure-and-kill (Franco et al., 2001, 2004a,b; Millar et al., 2002, 2005; Walton et al., 2004, 2006; Zada et al., 2004). In this respect, the kairomonal properties of sex pheromones

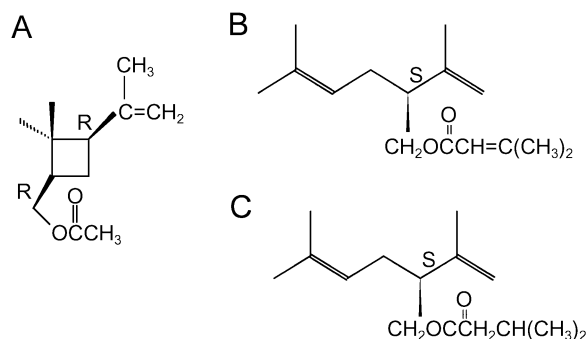


Figure 1 Chemical structure of (A) (+)-(1*R*,3*R*)-*cis*-2,2-dimethyl-3-isopropenyl-cyclobutanemethanol acetate, (B) (S)-(+)-lavandulyl senecioate, and (C) (S)-(+)-lavandulyl isovalerate.

are worthy of study for their possible detrimental impact on populations of natural enemies and potential application in mealybug pest management (e.g., Powell & Pickett, 2003).

In the present study, we used two complementary approaches, which involved pheromone traps in field trials and olfactometer bioassays in the laboratory, to study the behavioural response of *A. pseudococci* to the sex pheromones of *P. ficus* and *P. citri*. The following questions were addressed: (i) Is the female parasitoid attracted by the sex pheromones of *P. ficus* and *P. citri*? (ii) Does the plant host habitat affect the attraction of the parasitoid to these sex pheromones? and (iii) Does the mealybug species from which the parasitoid female emerges affect her response to the host sex pheromone?

Materials and methods

The parasitoid

The parasitoids used in the olfactometer experiments originated from specimens that had emerged from sweet orange fruits infested with *P. citri*. They were collected in citrus orchards, in October 2004, in Silves (37°12'12.56"N, 8°18'21.81"W), in the Algarve region, Portugal, and were reared for ca. 48 generations in *P. citri* before the experiments. During this period, specimens collected from the field were added at least twice, in order to refresh the rearing. In the present study, the laboratory-tested individuals and those trapped in orchards and vineyards matched the description of *Anagyrus* spec. nov. near *pseudococci* (sensu Triapitsyn et al., 2007).

Chemicals

LS and LI, as well as PcA, were synthesized in the chemical unit of the Department of Entomology, at the Volcani Center (Agricultural Research Organization, Bet Dagan,

Israel), according to Zada et al. (2003, 2004). All dispensers were loaded with the pheromones in hexane solution. The PcA was chiral, with an isomeric purity of 95% with respect to the (+)- α -pinene (Aldrich, Milwaukee, WI, USA) that was used as the starting material, whereas the chemical purity was 95%. Both LS and LI were racemic and their chemical purity was 94 and 97%, respectively. The unnatural stereoisomers of PcA and LS are benign, neither inhibitors nor synergists, as with most scale insect pheromones (Dunkelblum, 1999; Millar et al., 2002; Zada et al., 2004); up to now, an antagonistic effect has only been observed in the case of the pink hibiscus mealybug pheromone (Zhang et al., 2006).

Outdoor study of the attraction of *Anagyrus spec. nov. near pseudococci* to pheromone traps

The experiments were carried out in the Algarve region, in 2004–2006, in typical *P. ficus* habitats, that is, vineyards (*Vitis vinifera* L.) and fig orchards (*Ficus carica* L.), and *P. citri* habitats, that is, sweet orange orchards [*Citrus sinensis* (L.) Osbeck], from which *P. ficus* was absent. American grey rubber septa (The West Co., Lititz, PA, USA) were loaded with 50 μ g of the chemicals tested (unless otherwise indicated). The pheromone doses were selected according to previous experience (Franco et al., 2001; Zada et al., 2003, 2004; Branco et al., 2006). We used unbaited traps as controls. The lures were installed in traps consisting of (15 \times 15 cm) sticky plates of white alveolar polypropylene. All traps were suspended in the tree canopy or on the tutor wire that supported the vines, at a height of 1.0–1.5 m, with the sticky surface facing south-east (preliminary data suggested this trap location as optimal for male mealybug capture with sticky plate traps; JC Franco, unpubl.). The traps were distributed approximately 20 m apart in a completely randomized design with respect to position, with 10 replicates. Although estimates of the attraction range of mealybug pheromone traps suggest longer distances than 20 m (e.g., Millar et al., 2002; Branco et al., 2006), our experience showed that this trap spacing was adequate to minimize interference between traps. The data from individual traps corresponded to the total captures obtained during the exposure period. No saturation of traps with mealybug males was observed.

In 2004–2005, data of *Anagyrus spec. nov. near pseudococci* captures were obtained from field experiments that involved monitoring of *P. ficus* with pheromone traps (JC Franco, EB Silva & Z Mendel, unpubl.). Trial 1 (2004) was carried out in a fig orchard (cultivar Lampa branca and cultivar Lampa preta) in Belmonte (Tavira), and in a vineyard that contained several varieties. The attraction of feral *P. ficus* males to LS and LI was tested to study the response

of the local *P. ficus* populations to both compounds in two different hosts, that is, grapevine and fig, and the captures of *Anagyrus spec. nov. near pseudococci* were also determined. Zada et al. (2003) suggested that the production of LI in laboratory *P. ficus* culture might be attributed to selective pressures induced by potato sprouts that favour genotypes that produce both LS and LI. Therefore, we tested if Portuguese feral populations of *P. ficus* associated with different host plants could show differing responses to LI. The traps were left in the field for approximately 3 weeks, from 30 June to 23 July 2004. By mistake, no control traps were installed in the vineyard experiment.

In Trial 2 (2005), we tested the effect of the pheromone trap colour on captures of *P. ficus* males. The trial was conducted in two vineyards (cultivar Cardinal) located in Tavira and Silves. The captures of *Anagyrus spec. nov. near pseudococci* in red, white, yellow, and blue traps, baited with LS or unbaited, were compared. The traps were left in the field for approximately 3 weeks, from 30 June to 20 July 2005.

A third set of experiments, Trial 3, was conducted in a sweet orange orchard (cultivar Newhall), located in Silves. Citrus species in Portugal are not colonized by *P. ficus*; therefore, it was assumed that the *Anagyrus spec. nov. near pseudococci* population found in this citrus orchard had had no previous contact with *P. ficus*. The experiment was conducted from 12–26 September 2006 with traps baited with 200 μ g of LS and PcA and with unbaited traps. The objective of the experiment was to confirm the kairomonal response of the parasitoid to LS, in a habitat free of *P. ficus*. The captured individuals of the parasitoid were identified under 10 \times magnification.

Indoor study of kairomonal response of females of *Anagyrus spec. nov. near pseudococci* in a static-air olfactometer

The *Anagyrus spec. nov. near pseudococci* females used in the bioassays were reared on *P. citri* and *P. ficus* colonies that had been reared on potato sprouts under controlled conditions: 24.0 \pm 0.5 $^{\circ}$ C, 52.0 \pm 0.5% r.h., and L14:D10 photoperiod. The cultures were maintained at the facilities of the Plant Protection Department, Faculty of Agronomy, Technical University of Lisbon, Portugal.

The responses of parasitoid females to LS and PcA were studied in a static-air olfactometer (Vet, 1983; van Alphen & Jervis, 1996). The device consists of a row of three chambers connected to each other by a single corridor. Each female was released into the middle chamber and its subsequent choice of one of the outer chambers containing the test odour was recorded.

Two series of choice tests were carried out with parasitoid females aged less than 1 day. The females were fed with honey and allowed to mate as they emerged, together with

males, in rearing containers, before they were used in each experiment. Mating was assumed to have occurred, based on preliminary observations carried out under the same conditions.

In the first series of tests, we used wasps that had been reared on *P. citri* without previous contact with LS, or on *P. ficus*, without previous contact with PcA. The former wasps were allowed to choose between LS and a control (Trial 4), and the latter between PcA and a control (Trial 5). In the second series of tests (Trial 6), experienced wasps reared on *P. citri* mealybugs, that had had previous contact with PcA, were allowed to choose between PcA and a control. The main objective was to test the hypothesis that there was an innate response of the parasitoid in relation to both LS (Trial 4) and PcA (Trial 5). Because olfactometer tests are laborious, the second series of tests (Trial 6) examined only the response to PcA of parasitoids reared on *P. citri*, because PcA was the only compound to which no kairomonal response was suggested in the first series of tests. Therefore, in this trial, we tested the hypothesis of parasitoid learning.

Every observation was repeated 30 times, with a different wasp each time. American grey rubber septa loaded with 200 µg of pheromone were used as the odour source in one of the two external chambers of the olfactometer. The olfactometer was washed with water and detergent and then cleaned with hexane between replications. To avoid possible visual or other extraneous effects in the response of the parasitoid, we switched the position of the pheromone dispenser between the two opposite olfactometer chambers before each replication. The experiments were conducted in the laboratory at 22.0 ± 0.5 °C and $49.0 \pm 0.5\%$ r.h.

Statistical analysis

The field trial results are presented as means \pm SEM. We compared the effects of treatments on parasitoid or mealybug male capture by using one-way analysis of variance (ANOVA), followed by the Student–Newman–Keuls post hoc test. In Trial 1, an independent sample t-test was employed to compare captures of parasitoid females. As the assumption of equality of variances was not guaranteed (Levene's test), a logarithmic transformation [$\ln(x + 0.5)$] of the data was used, except for the data relating to mealybug males in Trial 3, where a square root transformation $\sqrt{x + 0.5}$ gave better results. In the olfactometer bioassays, the observed frequencies were tested according to a binomial distribution, considering as the null hypothesis that wasps would choose randomly between treatment and control ($p = q = 0.5$, $n = 30$). All analyses were performed with SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA).

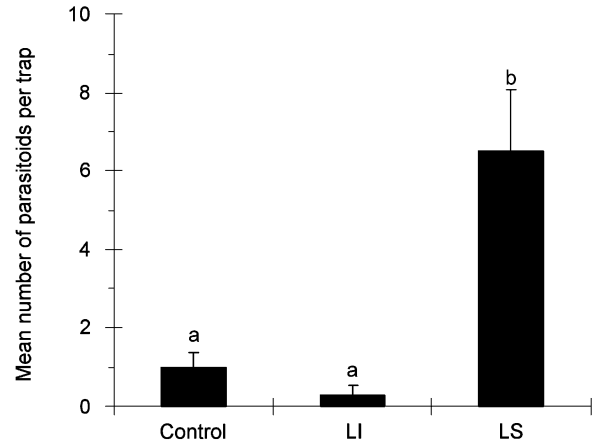


Figure 2 Comparison of the mean numbers (\pm SE) of *Anagyrus spec. nov. near pseudococci* females captured per sampling period in sticky traps baited with (S)-(+)-lavandulyl senecioate (LS) or with (S)-(+)-lavandulyl isovalerate (LI), and in control traps. The traps were suspended in a fig orchard in Belmonte (Algarve region, Portugal), from 30 June to 23 July 2004 ($n = 10$). Bars capped with the same letter are not significantly different ($P = 0.05$).

Results

Evaluation of kairomonal response of *Anagyrus spec. nov. near pseudococci* by using pheromone traps

Trial 1. The mean numbers of parasitoids captured with LS as bait were significantly higher than the numbers captured in traps baited with LI, in both the fig orchard ($F_{2,27} = 23.6$, $P < 0.001$; Figure 2) and the vineyard ($t = 11.97$, d.f. = 18, $P < 0.001$; Figure 3); they were also higher than the numbers captured in unbaited traps in the fig orchard (Figure 2). No significant difference ($P > 0.05$) was observed in the fig orchard between the numbers of wasps captured in the traps baited with LI and in unbaited traps (Figure 2).

Trial 2. Significant numbers of wasps were attracted to LS in traps of all tested colours, in both vineyards (Tavira: $F_{1,72} = 106.90$, $P < 0.001$; Silves: $F_{1,72} = 193.96$, $P < 0.001$; Figure 4). No significant interaction was found between bait and trap colour (Tavira: $F_{3,72} = 1.25$, $P = 0.30$; Silves: $F_{3,72} = 1.5$, $P = 0.223$; Figure 4).

Trial 3. The number of *Anagyrus spec. nov. near pseudococci* females captured in traps baited with LS in the citrus orchard in September 2006 was relatively high and significantly higher than the numbers captured in traps baited with PcA and in unbaited traps ($F_{2,27} = 76.2$, $P < 0.001$; Figure 5). No significant differences were found between mean capture numbers in the last two treatments

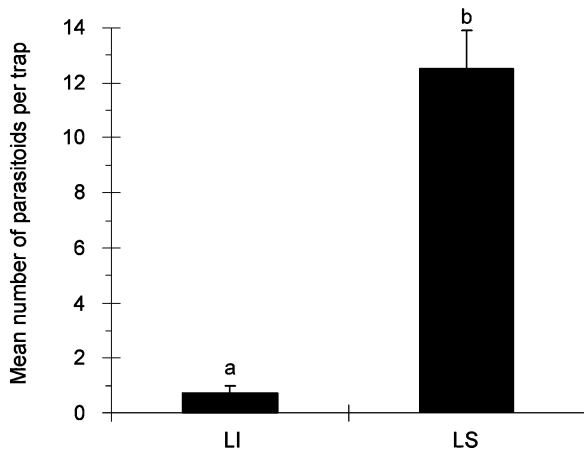


Figure 3 Mean number (+ SE) of *Anagyrus* spec. nov. near *pseudococci* females captured per sampling period in sticky traps baited with (S)-(+)-lavandulyl senecioate (LS) or with (S)-(+)-lavandulyl isovalerate (LI), suspended in a vineyard in Tavira (Algarve region, Portugal) from 30 June to 23 July 2004 ($n = 10$). Bars capped with the same letter are not significantly different ($P = 0.05$).

(Figure 5). Very few *P. citri* males were captured in traps baited with LS or in unbaited traps, with no significant differences between these treatments, whereas high and significant numbers of *P. citri* males were captured in the PcA-baited traps ($F_{2,27} = 91.0$, $P < 0.001$; Figure 5). *Anagyrus* spec. nov. near *pseudococci* males were rarely captured and, therefore, were not considered for analysis.

Indoor study of kairomonal response of females of *Anagyrus* spec. nov. near *pseudococci* in static-air olfactometer

Trial 4. The number of positive responses to LS of *Anagyrus* spec. nov. near *pseudococci* females reared on *P. citri* mealybugs was significantly higher than that expected if the parasitoid females had chosen randomly between treatment and control, according to a binomial distribution ($p = q = 0.5$, $n = 30$) (Figure 6).

Trials 5–6. The number of *Anagyrus* spec. nov. near *pseudococci* females responding to PcA did not differ significantly from the number responding to the no-bait choice. This result was obtained both with parasitoids reared on *P. ficus* (Trial 5) and with those reared on *P. citri* (Trial 6) (Figure 6).

Discussion

The results of both field and olfactometer experiments support the hypothesis that the females of *Anagyrus* spec.

nov. near *pseudococci* utilize LS, the sex pheromone of *P. ficus*, as a kairomonal cue in host selection. These findings are in accordance with the results reported by Millar et al. (2002), who suggested that *A. pseudococci* (most likely *Anagyrus* spec. nov. near *pseudococci* as well) in California vineyards was attracted to the pheromone of *P. ficus*. Our study further investigated this kairomonal attraction by conducting both field and laboratory trials. No kairomonal response was detected to PcA, the sex pheromone of *P. citri*, which confirms the findings of previous field studies (Suma et al., 2001) and electro-antennography tests (Suma et al., 2004). The number of captured *Anagyrus* spec. nov. near *pseudococci* was relatively low, but the parasitoid responses were consistent between the field and laboratory tests, suggesting that LS has kairomonal activity. Further studies are needed to

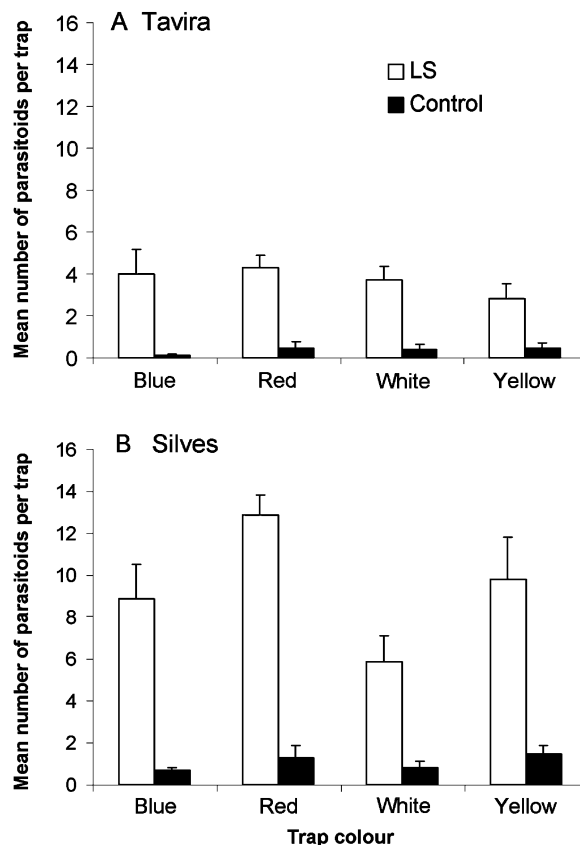


Figure 4 Mean number (+ SE) of *Anagyrus* spec. nov. near *pseudococci* females captured per sampling period in sticky traps of various colours baited with (S)-(+)-lavandulyl senecioate (LS), and in unbaited traps. The traps were activated from 30 June to 20 July 2005 in two vineyards (Tavira and Silves, in the Algarve region, Portugal) ($n = 10$). For each colour, the number of captured wasps in the pheromone-baited traps differed significantly ($P < 0.05$) from the number in the respective control traps.

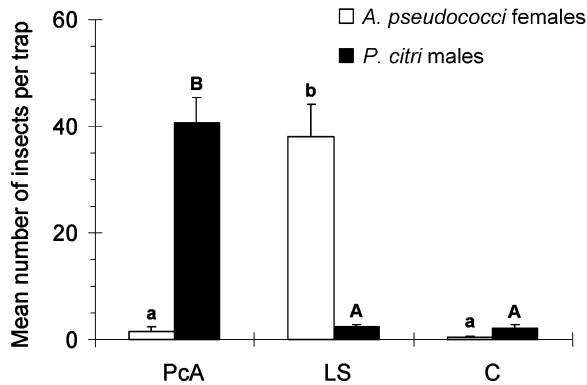


Figure 5 Mean number (+ SE) of *Anagyrus* spec. nov. near *pseudococci* females and *Planococcus citri* males captured per sampling period in sticky traps baited with (+)-(1*R*,3*R*)-*cis*-2,2-dimethyl-3-isopropenyl-cyclobutanemethanol acetate (PcA) or (S)-(+)-lavandulyl senecioate (LS), and in control traps (C). The traps were activated from 12 to 26 September 2006 in a citrus orchard in Silves (Algarve region, Portugal) ($n = 10$). Within each species, bars capped with the same letter are not significantly different ($P = 0.05$).

elucidate its eventual role in host location and to determine if it enhances parasitization.

Our present results were similar in vineyards and in fig and citrus orchards. In citrus orchards, the parasitoid showed a clear response to LS in the experiment carried out in September 2006. *Planococcus ficus* has not been reported on citrus in Portugal, where *P. citri* is the most

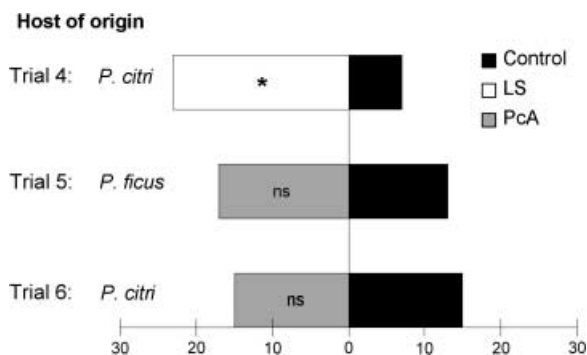


Figure 6 Number of responses of *Anagyrus* spec. nov. near *pseudococci* females in dual-choice tests in a static-air olfactometer. Parasitoids reared on *Planococcus citri* or on *Planococcus ficus* were exposed to (+)-(1*R*,3*R*)-*cis*-2,2-dimethyl-3-isopropenyl-cyclobutanemethanol acetate (PcA) or to (S)-(+)-lavandulyl senecioate (LS). An * indicates that the observed frequency responses differed significantly ($P < 0.05$) from those to be expected if the parasitoid females had chosen at random between treatments ($n = 30$).

frequent and abundant mealybug species in this habitat (Franco & Marotta, 1999). This was demonstrated in our study by the pattern of male mealybug captures obtained in Trial 3 (Figure 5). As there were no typical *P. ficus* habitats (e.g., fig plantations or vineyards) in the areas around the study plot, which was mainly surrounded by other citrus orchards, it was very unlikely that the captured parasitoids had been attracted from outside the citrus environment. Therefore, we may assume that the population of *Anagyrus* spec. nov. near *pseudococci* in these orchards had had no previous contact with *P. ficus*. Thus, the kairomonal response of the parasitoid to LS seems to be innate. This conclusion is supported by the results of the olfactometer tests, in which naïve females of *Anagyrus* spec. nov. near *pseudococci* reared on *P. citri*, and with no previous experience of *P. ficus* and its pheromone, responded to LS.

The differential response of *Anagyrus* spec. nov. near *pseudococci* females to the sex pheromones of its two major hosts, *P. citri* and *P. ficus*, suggests that the parasitoid has a more intimate evolutionary relationship with *P. ficus* than with *P. citri*. The area of origin of *P. ficus* is believed to be the Mediterranean Basin (Cox & Ben-Dov, 1986; Ben-Dov, 1994; Islam & Copland, 2000), and it is suggested that the same is true for *Anagyrus* spec. nov. near *pseudococci*. The higher level of egg encapsulation when *A. pseudococci* oviposits on *P. citri* than when it does so on *P. ficus* indicates that this parasitoid exhibits a relatively low level of physiological adaptation to *P. citri* (Blumberg et al., 1995). This observation corroborates the hypothesis that this host-parasitoid relationship evolved more recently. It was suggested that *P. citri* might be native to South America (Compere, 1939) or the Far East (Bartlett, 1978), but recent findings concerning its principal parasitoid, *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae), indicate that it may have spread from central Africa with the slave trade (J Noyes, pers. comm.).

The finding that the kairomonal response of *Anagyrus* spec. nov. near *pseudococci* females is an innate behaviour trait is in accordance with the hypothesis of dietary specialization and use of semiochemicals by natural enemies in a tritrophic context (Vet & Dicke, 1992): specialists at the herbivore level and generalists at the plant level are expected to present an innate response to host/prey kairomones (Vet & Dicke, 1992). The innate response of *Anagyrus* spec. nov. near *pseudococci* to LS matches this expectation. In fact, *Anagyrus* spec. nov. near *pseudococci* is a specialist parasitoid of mealybugs, but its potential hosts include mealybug species that are polyphagous (Noyes, 2003; Triapitsyn et al., 2007).

We hypothesize that *P. ficus* was the primary host in the region of origin of *Anagyrus* spec. nov. near *pseudococci*.

Other mealybug species, such as *P. citri*, occurred in the host range of the parasitoid because of the expansion of the geographic range of the parasitoid and/or the mealybugs as a result of human activities. *Anagyrus* spec. nov. near *pseudococci* has been reported in several countries on different continents, including the USA, Spain, Italy, Ukraine, Turkmenistan, and Israel (Triapitsyn et al., 2007). The kairomonal response of *Anagyrus* spec. nov. near *pseudococci* is specific only with respect to LS, the sex pheromone of *P. ficus*. The wasp was not attracted to LI, despite the close similarity between the structures of these two molecules (Figure 1). It seems that differences between the structures of the carboxylate moiety of the two molecules (LS and LI) markedly affect their kairomonal attractiveness to the parasitoid, in a similar way to that observed in relation to feral males of *P. ficus* (Zada et al., 2003). However, besides the positive response in relation to LI already reported in mass-reared *P. ficus* males (Zada et al., 2003), recent findings suggest the existence, in wild populations of *P. ficus*, of male phenotypes that are also attracted to LI (Z Mendel, unpubl.).

The conservation of the innate kairomonal response of *Anagyrus* spec. nov. near *pseudococci* to the sex pheromone of *P. ficus* may be attributed to the fact that changes in responses to herbivore-derived semiochemicals are relatively conservative in both an ontogenetic and an evolutionary sense (Vet & Dicke, 1992). Pheromones are reliable stimuli to carnivores because they are intimately linked to the actual presence of an herbivore prey, as has been documented for other carnivore species. For example, the Madeira population of *Hemeroobius stigma* Stephen, a predator of the pine bast scale, *Matsucoccus feytaudi* Ducasse (Homoptera; Matsucoccidae), retains an innate kairomonal response to the sex pheromone of its prey, despite the fact that *M. feytaudi* is not present on the island (Branco et al., 2001). The possibility of carnivore species evolving from monophagous ancestors was also hypothesized to account for the innate use of semiochemicals by generalist carnivores (see review by Steidle & van Loon, 2003).

In addition to the *P. ficus* pheromone, other mealybug products, such as honeydew, are likely to be used by *Anagyrus* spec. nov. near *pseudococci* as kairomonal cues in host location. *Anagyrus pseudococci* was shown to respond to honeydew excreted by *P. citri* (Islam & Jahan, 1993).

The kairomonal response of *Anagyrus* spec. nov. near *pseudococci* to LS may impair the use of LS for pest management of *P. ficus* by mass trapping and lure-and-kill tactics. This side-effect may be avoided by using pheromone analogues that lack kairomonal activity but that still preserve the pheromonal attractiveness to the males, as was successfully accomplished for *Matsucoccus* species (Mendel et al., 2003). Optimizing the devices used in these

experiments, for example, with regard to their design and colour, can also minimize the negative impact on natural enemies. Millar et al. (2002) suggested that delta traps are better than the two-sided 'red scale' sticky card traps for trapping *P. ficus* while simultaneously excluding *A. pseudococci*. The results obtained by Walton et al. (2006) with mating disruption suggest that the treatment had no negative effect on the level of parasitization of *P. ficus* by *A. pseudococci*. According to our present results, no significant impact on the *Anagyrus* spec. nov. near *pseudococci* populations is to be expected in the case of *P. citri*, as the parasitoid showed no kairomonal response to PcA.

The kairomonal response of *Anagyrus* spec. nov. near *pseudococci* could also be explored in connection with biological control tactics, by enhancing parasitization of *P. citri* as a component of integrated pest management strategies, by means of a similar approach to that used against aphid pests (Powell & Pickett, 2003). We are doing field tests in order to determine whether the parasitization efficiency of *Anagyrus* spec. nov. near *pseudococci* can be enhanced by using LS as an attractant in citrus orchards infested with *P. citri*.

In the present study, all the observed parasitoid females matched the description of *Anagyrus* spec. nov. near *pseudococci* (sensu Triapitsyn et al., 2007). Further studies are needed to confirm that these two sibling species, namely, *A. pseudococci* and *Anagyrus* spec. nov. near *pseudococci* (Triapitsyn et al., 2007), are distinguishable according to the kairomonal responses to their host sex pheromone.

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References

van Alphen JJM & Jervis MA (1996) Foraging behaviour. Insect Natural Enemies: Practical Approaches to Their Study and

- Evaluation (ed. by MA Jervis & N Kidd), pp. 1–62. Chapman & Hall, London, UK.
- Bartlett BR (1978) Pseudococcidae. Introduced Parasites and Predators of Arthropod Pests and Weeds: a World Review (ed. by CP Clausen), pp. 137–170. Agricultural Research Service, United States Department of Agriculture, Washington, DC, USA.
- Ben-Dov Y (1994) A Systematic Catalogue of the Mealybugs of the World (Insecta: Homoptera: Coccoidea: Pseudococcidae and Putoidae), with Data on Geographical Distribution, Host Plants, Biology and Economic Importance. Intercept Limited, Andover, UK.
- Bierl-Leonhardt BA, Moreno DS, Schwarz M, Fargerlund J & Plimmer JR (1981) Isolation, identification and synthesis of the sex pheromone of the citrus mealybug, *Planococcus citri* (Risso). *Tetrahedron Letters* 22: 389–392.
- Birkett MA & Pickett JA (2003) Aphid sex pheromones: from discovery to commercial production. *Phytochemistry* 62: 651–656.
- Blumberg D, Klein M & Mendel Z (1995) Response by encapsulation of four mealybug species (Homoptera: Pseudococcidae) to parasitization by *Anagyrus pseudococci*. *Phytoparasitica* 23: 157–163.
- Branco M, Franco JC, Carvalho JC & Mendel Z (2001) Occurrence of *Hemerobius stigma* in pine bast scale (*Matsucoccus* spp.) populations: opportunistic predation or obligatory association. *Bollettino di Zoologia Agraria e di Bachicoltura* 33: 397–407.
- Branco M, Jactel H, Franco JC & Mendel Z (2006) Modelling response of insect trap captures to pheromone dose. *Ecological Modelling* 197: 247–257.
- Bruni R, Sant'Ana J, Aldrich JR & Bin F (2000) Influence of host pheromone on egg parasitism by scelionid wasps: comparison of phoretic and nonphoretic parasitoids. *Journal of Insect Behavior* 13: 165–173.
- Compere H (1939) Mealybugs and their insect enemies in South America. University of California Publications in Entomology 7: 57–73.
- Cox JM & Ben-Dov Y (1986) Planococcine mealybugs of economic importance from the Mediterranean Basin and their distinction from a new African genus (Homoptera: Pseudococcidae). *Bulletin of Entomological Research* 76: 481–489.
- Daane KM, Bentley WJ, Walton VM, Malakar-Kuenen R, Millar JG et al. (2006) New controls investigated for vine mealybug. *California Agriculture* 60: 31–38.
- Dunkelblum E (1999) Scale insects. Pheromones of Non-Lepidopteran Insects Associated with Agricultural Plants (ed. by J Hardie & AK Minks), pp. 251–276. CAB International, Wallingford, UK.
- Fiaboe MK, Smith H, Borgemeister C, Chabi-Olaye A, Gounou S & Schulthess F (2003) *Sesamia calamistis* calling behavior and its role in host finding of egg parasitoids *Telenomus busseolae*, *Telenomus isis*, and *Lathromeris ovicida*. *Journal of Chemical Ecology* 29: 921–929.
- Franco JC, Gross S, Silva EB, Suma P, Russo A & Mendel Z (2004a) Is mass-trapping a feasible management tactic of the citrus mealybug in citrus orchards? *Anais do Instituto Superior de Agronomia* 49: 353–367.
- Franco JC & Marotta S (1999) A survey of mealybugs (Homoptera: Coccoidea: Pseudococcidae) in citrus groves in continental Portugal. *Entomologica* 33: 191–196.
- Franco JC, Russo A, Suma P, Silva EB, Dunkelblum E & Mendel Z (2001) Monitoring strategies for the citrus mealybug in citrus orchards. *Bollettino di Zoologia Agraria e di Bachicoltura* 33: 297–303.
- Franco JC, Suma P, Silva EB, Blumberg D & Mendel Z (2004b) Management strategies of mealybug pests of citrus in Mediterranean countries. *Phytoparasitica* 32: 507–522.
- Hinkens DM, McElfresh JS & Millar JG (2001) Identification and synthesis of the sex pheromone of the vine mealybug, *Planococcus ficus*. *Tetrahedron Letters* 42: 1619–1621.
- Islam KS & Copland MJW (1997) Host preference and progeny sex ratio in a solitary koinobiont mealybug endoparasitoid, *Anagyrus pseudococci* (Girault), in response to its host stage. *Biocontrol Science and Technology* 7: 449–456.
- Islam KS & Copland MJW (2000) Influence of egg load and oviposition time interval on the host discrimination and offspring survival of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae), a solitary endoparasitoid of citrus mealybug, *Planococcus citri* (Homoptera: Pseudococcidae). *Bulletin of Entomological Research* 90: 69–75.
- Islam KS & Jahan M (1993) Influence of honeydew of citrus mealybug (*Planococcus citri*) on searching behaviour of its parasitoid, *Anagyrus pseudococci*. *Indian Journal of Agricultural Science* 63: 743–746.
- Mendel Z, Dunkelblum E, Branco M, Franco JC, Kurosawa S & Mori K (2003) Synthesis and structure-activity relationship of diene modified analogs of *Matsucoccus* sex pheromones. *Naturwissenschaften* 90: 313–317.
- Millar JG, Daane KM, McElfresh JS, Moreira JA & Bentley WJ (2005) Chemistry and applications of mealybug sex pheromones. *Semiochemicals in Pest and Weed Control* (ed. by RJ Petroski, MR Tellez & RW Behle), pp. 11–27. American Chemical Society, Washington, DC, USA.
- Millar JG, Daane KM, McElfresh JS, Moreira JA, Malakar-Kuenen R et al. (2002) Development and optimization of methods for using sex pheromone for monitoring the mealybug *Planococcus ficus* (Homoptera: Pseudococcidae) in California vineyards. *Journal of Economic Entomology* 95: 706–714.
- Noyes JS (2003) Universal Chalcidoidea Database. Available from www.nhm.ac.uk/entomology/chalcidoids/index.html (accessed 10 October 2006)
- Noyes JS & Hayat M (1994) Oriental Mealybug Parasitoids of the Anagyrini (Hymenoptera: Encyrtidae). CAB International, Wallingford, UK.
- Powell W (1999) Parasitoid hosts. Pheromones of Non-Lepidopteran Insects Associated with Agricultural Plants (ed. by J Hardie & AK Minks), pp. 405–427. CAB International, Wallingford, UK.
- Powell W & Pickett JA (2003) Manipulation of parasitoids for aphid pest management: progress and prospects. *Pest Management Science* 59: 149–155.
- Reddy GVP, Holopainen JK & Guerrero A (2002) Olfactory

- responses of *Plutella xylostella* natural enemies to host pheromone, larval frass, and green leaf cabbage volatiles. *Journal of Chemical Ecology* 28: 131–143.
- Rosen D & Rössler Y (1966) Studies on an Israeli strain of *Anagyrus pseudococci* (Girault) (Hymenoptera, Encyrtidae). I. Morphology of the adults and developmental stages. *Entomophaga* 11: 269–277.
- Steidle JLM & van Loon JJA (2003) Dietary specialization and infochemical use in carnivorous arthropods: testing a concept. *Entomologia Experimentalis et Applicata* 108: 133–148.
- Suma P, De Cristofaro A & Russo A (2004) Osservazioni sull'attività di semiochimici di sintesi di *Planococcus citri* (Risso). *Atti XIX Congresso Nazionale Italiano di Entomologia*, Catania, 10–15 Giugno 2002, pp. 541–546.
- Suma SP, Russo A, Dunkelblum E, Zada A & Mendel Z (2001) Pheromonal and kairomonal activity of *Planococcus citri* pheromone and some of its analogs. *Bollettino di Zoologia Agraria e di Bachicoltura* 33: 305–312.
- Triapitsyn SV, González D, Danel B, Vickerman DB, Noyes JS & Ernest BW (2007) Morphological, biological, and molecular comparisons among the different geographical populations of *Anagyrus pseudococci* (Hymenoptera: Encyrtidae), parasitoids of *Planococcus* spp. (Hemiptera: Pseudococcidae), with notes on *Anagyrus dactylopii*. *Biological Control* 41: 14–24.
- Vet LEM (1983) Host-habitat location through olfactory cues by *Leptopilina clavipes* (Hartig) (Hym.: Eucoilidae), a parasitoid of fungivorous *Drosophila*: the influence of conditioning. *Netherlands Journal of Zoology* 33: 225–248.
- Vet LEM & Dicke M (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Annual Review of Entomology* 37: 141–172.
- Walton VM, Daane KM, Bentley WJ, Millar JG, Larsen TE & Malakar-Kuenen R (2006) Pheromone-based mating disruption of *Planococcus ficus* (Hemiptera: Pseudococcidae) in California vineyards. *Journal of Economic Entomology* 99: 1280–1290.
- Walton VM, Daane KM & Pringle KL (2004) Monitoring *Planococcus ficus* in South African vineyards with sex pheromone-baited traps. *Crop Protection* 23: 1089–1096.
- Walton VM & Pringle KL (2004) Vine mealybug, *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae), a key pest in South African vineyards: a review. *South African Journal of Enology and Viticulture* 25: 54–62.
- Zada A, Dunkelblum E, Assael F, Harel M, Cojocar M & Mendel Z (2003) Sex pheromone of the vine mealybug, *Planococcus ficus* in Israel: occurrence of a second component in a mass-reared population. *Journal of Chemical Ecology* 29: 977–988.
- Zada A, Dunkelblum E, Harel M, Assael F, Gross S & Mendel Z (2004) Sex pheromone of the citrus mealybug *Planococcus citri*: synthesis and optimization of trap parameters. *Journal of Economic Entomology* 97: 361–368.
- Zhang A, Wang S, Vitullo J, Roda A, Mannion C & Bergh JC (2006) Olfactory discrimination among sex pheromone stereoisomers: Chirality recognition by pink hibiscus mealybug males. *Chemical Senses* 31: 621–626.

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