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Using a kairomone-based attracting system to enhance biological control of mealybugs *(Hemiptera: Pseudococcidae)* by *Anagyrus* sp. near *pseudococci* *(Hymenoptera: Encyrtidae)* in Sicilian vineyards

Abstract - The "potato trap" technique was applied for the first time in Italian (Sicily) vineyards in an attempt to assess: a) the impact of the kairomonal activity of the vine mealybug sex pheromone (S)-(+)-lavandulyl senecioate (LS) on the parasitism of mealybugs by the encyrtid *Anagyrus* sp. near *pseudococci*, b) the influence of two commonly used insecticides on the parasitization activity of *A.* sp. near *pseudococci* towards mealybugs, and c) the efficiency of the release of *A.* sp. near *pseudococci* in enhancing parasitism rates of mealybugs. The number of captured *A.* sp. near *pseudococci* females in LS baited traps was significantly higher than that in unbaited traps. The minimal number of days for the first parasitoid emergence in LS baited traps was almost 3 days earlier, compared to unbaited control, suggesting a faster host detection by the encyrtid when LS is applied. These findings resulted in a significant increase in parasitism of mealybugs by *A.* sp. near *pseudococci* in LS baited traps relative to unbaited traps suggesting that the LS is used by the encyrtid as kairomone to ensure greater potential for host searching activity. Insecticide treatments significantly affected parasitization activity of *A.* sp. near *pseudococci* on mealybugs when compared to an untreated control with parasitoid release. The buprofezin, chlorpyriphos-methyl and untreated control with no parasitoid release treatments had statistically similar numbers of emerged parasitoids from exposed mealybugs. The obtained results provide evidence that, in the absence of conventional insecticides applications, the use of the LS could be a promising tool to improve and strengthen biological control of mealybugs by *A.* sp. near *pseudococci* within Sicilian vineyard conditions.

Riassunto - *Utilizzo di un sistema attrattivo a base di cairomone per incrementare il controllo biologico di Pseudococcini (Hemiptera: Pseudococcidae) da parte di Anagyrus sp. near pseudococci (Hymenoptera: Encyrtidae) in vigneti siciliani*
Gli autori riportano i risultati di una sperimentazione condotta in un vigneto siciliano che ha previsto l'impiego di una tecnica di nuova concezione denominata "potato trap" e mirata a valutare: a) l'impatto dell'attività cairomonica del feromone sessuale di sintesi della cocciniglia farinosa della vite *Planococcus ficus* ((S)-(+)-lavandulyl senecioate) nei confronti dell'endoparassitoide *A.* sp. near *pseudococci,* b) l'influenza

di due principi attivi, comunemente impiegati negli interventi di lotta alla cocciniglia, sull'attività di parassitizzazione del medesimo parassitoide e c) l'incidenza dei lanci inoculativi in campo di *A*. sp. near *pseudococci* sul tasso di parassitizzazione di *P. ficus*. Il numero di parassitoidi attratti all'interno delle trappole innescate con il feromone di sintesi è risultato significativamente maggiore di quello presente nelle trappole di controllo e gli sfarfallamenti della nuova generazione dell'encirtide nelle trappole innescate con il feromone sono avvenuti in un numero di giorni significativamente inferiore rispetto a quello ottenuto nelle trappole testimoni. Tali risultati suggeriscono come il parassitoide riesca a individuare più rapidamente gli ospiti da parassitizzare utilizzando quindi, il feromone sessuale di sintesi della cocciniglia come cairomone. L'attività di parassitizzazione di *A*. sp. near *pseudococci* è risultata inoltre negativamente influenzata dai trattamenti insetticidi effettuati con buprofezin e chlorpyrifos-methyl prima dei rilasci dei parassitoidi in campo. Dai dati ottenuti emerge quindi come, l'immissione in campo di erogatori del feromone sessuale di sintesi di *P. ficus* può influire positivamente sull'azione dell'antagonista *A*. sp. near *pseudococci* e rappresentare un valido ausilio nei programmi di controllo integrato della cocciniglia farinosa della vite.

Key words: Mealybugs, parasitoid release, kairomonal response, pesticide application, Integrated Pest Management.

INTRODUCTION

In several grape-growing areas around the world, mealybugs (Hemiptera : Pseudococcidae) are considered serious insect pests on grapevine (Sforza *et al*., 2003; Walton *et al*., 2004; Daane *et al*., 2006; Buonocore *et al*., 2008; Franco *et al*., 2008b; Lo *et al*., 2009). Mealybugs produce honeydew which supports the development of the black sooty mold fungi. Some mealybug species, including the vine mealybug (VM) *Planococcus ficus* (Signoret) and the citrus mealybug (CM) *P. citri* (Risso), are efficient vectors of grapevine viral diseases (Cabaleiro & Segura 1997; Cid *et al*., 2007; Mahfoudhi *et al*., 2009; Tsai *et al*., 2010). These threats by mealybugs has incited researchers to develop management tools needed to cope with this group of pests in vineyards. The VM was found to be the most abundant mealybug species in several grapevine cultivated areas throughout the world, such as in Italy (Dalla Montá *et al*., 2001), in South Africa (Walton *et al*., 2009), in California (Daane *et al*., 2006) and in Argentina (Trjapitzin & Trjapitzin, 1999). The wasp *Anagyrus pseudococci* s.l. (Girault) (Hymenoptera: Encyrtidae) is a common parasitoid of both VM (Trjapitzin & Trjapitzin, 1999; Dalla Montá *et al*., 2001; Gutierrez *et al*., 2008; Franco *et al*., 2009) and CM (Franco *et al*., 2009). Therefore, its use in an IPM strategy could provide a promising solution for mealybug problem in vineyards. For instance, in California, biological control by mass release of this parasitoid contributed to a decrease in VM populations (Daane *et al*., 2006, 2008). Since the identification and synthesis of the sex pheromone of the VM by Hinkens *et al*. (2001) pheromone-based monitoring systems have been developed and applied as alternative tools in decision making for the control of VM (Millar *et al*., 2002; Walton *et al*., 2004;

Zada *et al*., 2008). More recently, the VM sex pheromone (S)-(+)-lavandulyl senecioate (LS) proved to attract the females of the VM parasitoid *A*. sp. near *pseudococci* sensu Triapitsyn *et al*. (2007) in field and under laboratory conditions (Franco *et al*., 2008b). This kairomonal response is an innate behavior trait (Franco *et al*., 2008b) and LS was shown to increase parasitism rates of mealybugs by *A*. sp. near *pseudococci* in Mediterranean citrus orchards (Franco *et al*., 2008a, unpublished data).

Insecticide are still the most common control tactic used against mealybug pests (Franco *et al*., 2009), with new compound being integrated in mealybug management strategies. For example neonicotinoid insecticides such as imidacloprid are applied through drip irrigation system and contributed in limiting mealybug outbreaks on grapevine (Daane *et al*., 2006; Mansour *et al*., 2010b), and recently tetramic acid insecticide spirotetramat also proved as a successful compound for VM control (Brück *et al*., 2009; Mansour *et al*., 2010a). However, in some cases, chemical treatments may negatively impact mealybug natural enemies occurring in vineyards (Walton & Pringle, 1999; Mgocheki & Addison, 2009a). In light of this and respecting Integrated Pest Management aims, insecticides applications should be environmentally safe and sufficiently compatibles with the behavior and movements of the mealybug pest's auxiliary fauna.

In Sicily, the VM and the CM have been recorded in vineyards and it was proven that they can cause serious damages on grapevine (Buonocore *et al*., 2008). Hence, implementation of control programs is needed for sustainable mealybug pest management in Sicilian grape-growing areas.

In the present study, we evaluated, as a new approach to enhance the biological control of mealybugs in Southern Italian vineyards, the capability of the combination "release of the parasitoid *A*. sp. near *pseudococci* + the use of the LS as a kairomone-attractive source" in increasing parasitism rates of these pests by the wasp, in the presence and in the absence of insecticide applications for mealybugs.

MATERIALS AND METHODS

Study site and climatic data

The study was performed in a drip irrigated vineyard located in Sicily Southern Coast (Acate, Ragusa Province) (37° 00' 03'' N; 14° 24' 38'' E), which was divided into four separate 0.5 ha plots. Each plot consisted of grapevines cultivar Frappato, installed in straight lines with a distance of plantation of 1.00 x 2.20 m. The climatic data of the study site are indicated in Table 1.

Table 1 - Climatic conditions in the experiment site (January-September 2010).

Months	**Jan.**	**Feb.**	**March**	**April**	**May**	**June**	**July**	**August**	**Sept.**
MT (°C)	11.1	11.6	12.4	15.7	18.7	22	24.9	24.8	21.9
TR (mm)	127	56.7	63.5	14	2.8	2.4	0	0	154.2

MT: Mean temperature; TR: total rainfall

Pheromone

The vine mealybug sex pheromone (S)-(+)-lavandulyl senecioate (LS) was synthesized in the Chemical Unit of the Department of Entomology, at the Volcani Center (ARO, Bet Dagan, Israel), according to Zada *et al*. (2003). The dispensers were loaded with 400 μg of the pheromones as hexane solution.

Insecticides

Two commonly used insecticides for mealybug control in Italian vineyards were applied: buprofezin (Applaud, Sipcam), an insect growth regulator applied at the dose 1 L/ha, and the organophosphate chlorpyriphos-methyl (Alisé 75 WG, Sipcam) mixed with mineral oil (Eko oil spray, Kollant s.r.l.) applied at the doses 0.7 L/ha + 10 L/ha, respectively.

Mealybugs

The *P. citri* colonies used in the study originated from individuals collected in citrus orchard and reared on sprouted potatoes (*Solanum tuberosum* L., var. "Spunta") within plastic boxes (17.5 x 11.5 x 5 cm) with net covered openings for ventilation. They were kept in a rearing room at 26±1 °C, 50-65 % RH, and 16L:8D photoperiod.

Potato traps

As an experimental device for field trials, we used "potato traps", consisted of a plastic cylindrical container (11 cm diameter, 13 cm height) with circular openings (3 cm diameter), to allow the access of the parasitoids, within which was placed one sprouted potato infested with a colony of 100-150 third instar nymphs or adult females of the CM (Franco *et al*., 2008a).

Parasitoids

The parasitoids *A*. sp. near *pseudococci* were provided by Bio-Bee Biological Systems Ltd. Company (Israel), packaged in small boxes containing 500 mummified mealybugs mixed in sawdust.

Trials

All trials were carried out on June 11th (T), corresponding to the first day of potato traps' exposure in the experiment vineyard. Four plots (2 insecticide-treated plots with parasitoid release, one untreated plot with parasitoid release and one untreated plot with no parasitoid release), in each one of which 4 traps (2 with LS bait and 2 with no LS bait) were placed (Table 2). Insecticide treatments were applied at T early in the morning on vines of plots 1 (buprofezin) and 2 (chlorpyriphos-methyl + mineral oil). Four hours after insecticide applications, potato traps were suspended from drip irrigation pipes in the same level of the vine canopy, at about 1m above the ground. To avoid the access of mealybug predators and/or ants, the upper area of the traps was covered by a non-drying spreadable glue (Zapicol Paint, Zapi Industrie Chimiche S. p. A., Italy). Afterwards, in the center of each of the plots 1, 2 and 3, about 1.000 parasitoids *A*. sp. near *pseudococci* were released. After one week of exposure, the potato traps were collected from

Table 2 - Details on the experimental plots. In each plot (each week) four potato traps were exposed, two of the traps were baited dispenser impregnated the vine mealybug sex pheromone.

Plot	Insecticide treatment	Parasitoid release
1	buprofezin	yes
2	chlorpyriphos-methyl	yes
3	no	yes
4	no	no

the vineyard, each covered by a self-sealing and flexible laboratory film (Parafilm® M) and transported to the laboratory. The collected traps were replaced by new ones for the second week of exposure. Traps' exposure was repeated for 4 consecutive weeks, from June 11th (T) to July 2nd (T + 21 days). Traps were arranged in a randomized block design according to position/plot. In every new week of exposure, all traps were shifted along each plot by one position. In the laboratory, number of *A*. sp. near *pseudococci* females present within each trap/each week-exposure was counted. Subsequently, the sprouted potatoes were placed in new cylindrical transparency containers and kept to allow the emergence of *A*. sp. near *pseudococci* from the parasitized mealybugs.

Evaluation procedure

The number of days for the first parasitoid emergence was noted to assess the ability of *A*. sp. near *pseudococci* females in locating the trap. Besides, the total number of emerged *A*. sp. near *pseudococci* in each trap was counted. Means (of four week-exposure) of the obtained data were computed and comparisons were made between data corresponding to:

a) Traps with LS bait and traps with no LS bait in order to assess the impact of the application of the LS on parasitism rates of mealybugs by *A*. sp. near *pseudococci*.
b) Traps in treated (chlorpyriphos-methyl or buprofezin) and traps in untreated plots in order to estimate the influence of insecticide treatments on the movement of the released *A*. sp. near *pseudococci* and hence on its capability to parasitize mealybugs.
c) Traps in plots with parasitoid release and traps in plots with no parasitoid release (all traps with LS baits) to evaluate the efficiency of the release of *A*. sp. near *pseudococci* in increasing overall parasitism of mealybugs by the wasp.

Statistical analyses

The results are presented as means of separate-week (4 weeks) collected data from each 2 similar (baited with LS and/or with no LS bait) traps/plot ± SE. To standardize the data distribution and to stabilize the variance, a logarithmic transformation [log (x + 1)] was applied. The transformed data were subjected to one-way analysis of variance ANOVA. Means were separated using the LSD-test at a level of significance $P = 0.05$. All analyses were performed using STATISTICA 6.0 (StatSoft Inc., 2003).

RESULTS

Across the whole check period, no parasitoid species other than *A.* sp. near *pseudococci* was recorded to emerge from mealybug colonies on sprouted potatoes, regardless of the plot aspect (chemically treated or chemically untreated, with parasitoid release or with no parasitoid release) and the applied modality (traps baited with LS or traps with no LS bait).

Both the number of captured parasitoids and the number of emerged parasitoids in LS baited traps were significantly higher ($F_{1,22}$=7.385, P=0.013 and $F_{1,30}$=9.59, P=0.0042, respectively) than that found in unbaited traps (Table 3). Additionally, the number of days for first parasitoid emergence in LS baited traps was significantly ($F_{1,22}$=7.132; P=0.016) lower than in unbaited traps, with ca. 3 days of difference between the two modalities. On the other hand, a higher significant ($F_{3,12}$=5.18, P=0.015) number of emerged parasitoids was obtained in traps corresponding to untreated plot, compared to that recovered from the traps collected in the two treated (chlorpyriphos-methyl or buprofezin) plots (Table 4). Besides, no significant differences ($F_{3,12}$=5.18, P=0.015) were revealed between number of emerged parasitoids in LS baited traps of chlorpyrihos-methyl treatment, compared to that recorded in LS baited traps of buprofezin treatment. However, a significant impact of the release of *A.* sp. near *pseudococci* on the parasitism of mealybugs was found, since the number of emerged parasitoids in LS baited traps of the untreated plot with parasitoid release was significantly higher ($F_{3,12}$=5.18, P=0.015)

Table 3 - Overall effect of the vine mealybug sex pheromone (LS) bait on occurrence and emergence of Anagyrus *sp. near* pseudococci *(mean ± SE).*

Traps	Females per trap	Days for the first parasitoid emergence	Number of emerged parasitoids per trap
Traps baited with pheromone	0.75 ± 0.25 a*	16.04 ± 0.65 a	19.78 ± 4.29 a
Traps with no pheromone bait	0.13 ± 0.07 b	18.85 ± 0.98 b	5.31 ± 1.99 b

* Means followed by the same letter within each column are not significantly different (P < 0.05; LSD test).

Table 4 **-** Overall effect of insecticide treatments and parasitoid release on the performance of the parasitization activity of *Anagyrus* sp. near *pseudococci* on mealybugs (mean ± SE)

Treatment	Number of emerged A. sp. near pseudococci / trap
Chlorpyriphos application + parasitoid release	15.5 ± 7.92 a*
Buprofezin application + parasitoid release	19.62 ± 4.64 a
Untreated + parasitoid release	40 ± 7.62 b
Untreated with no parasitoid release	4 ± 2.12 a

* Means followed by the same letter are not significantly different (P < 0.05; LSD test).

than that in LS baited traps collected from the untreated plot with no parasitoid release (difference averaged 36 emerged parasitoids) (Table 4). The first parasitoid emergence from traps belonging to plots with parasitoid release was recorded on June 27th, namely at T + 16 days, from one LS baited trap of the untreated plot. Furthermore, the number of emerged parasitoids recorded in traps of the untreated plot with no parasitoid release did not differ significantly ($F_{3,12}$=5.18, P=0.015) from number of emerged parasitoids found in traps of the two insecticide-treated plots (Table 4).

DISCUSSION AND CONCLUSIONS

During the experiment, all emerged parasitoids were *A*. sp. near *pseudococci*. The fact to did not record other emerged parasitoid species, especially in traps of plot 4 (with no release) is a strong indication that *A*. sp. near *pseudococci* is the most common parasitoid species of mealybugs in the experiment vineyard, during the trial period. According to Longo *et al*. (1991), the two indigenous wasps *A. pseudococci* and *Leptomastidea abnormis* (Girault) (Hymenoptera: Encyrtidae) were found to be the most common parasitoids of mealybugs in Southern Sicilian vineyards.

The number of captured *A*. sp. near *pseudococci* females per trap was significantly higher in LS baited traps relative to that in unbaited control, confirming previous results showed by Franco *et al*. (2008a). The shorter time that was needed for the first emergence of *A*. sp. near *pseudococci* in LS baited traps indicates that these traps were detected faster than the potato trap with no pheromone bait (Franco *et al*. 2008a). Consequently, a higher number of emerged parasitoids was collected from LS baited traps, compared to unbaited control which further supports the impact of the kairomonal effect on *A*. sp. near *pseudococci* by LS. Our findings confirmed results of previous study carried out in several Mediterranean citrus orchards showing that the enhancement of the parasitoid performance is the result of both higher number of wasp females that were attracted close to the pheromone source and a faster host detection as compared with the mealybug colonies without the pheromone (Franco *et al*. 2008a).

Results suggest that the number of emerged parasitoids in LS baited traps in plot with no chemical treatment and parasitoid release was significantly higher relative to that recorded from LS baited traps collected in the plot with no chemical treatment and no parasitoid release. Furthermore, the number of emerged parasitoids in LS baited traps in plot with parasitoid release was significantly higher relative to that recorded from LS baited traps collected from plot with no parasitoid release. Theses outcomes suggest the important role played by parasitoid release in the presence of LS baits in enhancing parasitism of mealybugs by *A*. sp. near *pseudococci*. Statistically similar numbers of emerged parasitoids were obtained from traps hung on chlorpyriphos-methyl and buprofezin-treated vines. Previous laboratory studies showed that buprofezin was not toxic to *A*. sp. near *pseudococci* (Suma & Mazzeo, 2008; Mgocheki & Addison, 2009a), different from chlorpyriphos-methyl which was proven to be highly toxic in the contact towards this wasp (Suma & Mazzeo, 2008).

Based on the obtained results, it is clearly understandable that the use of the LS could be incorporated, as a new approach, in future biological control strategies against mealybugs in Sicilian vineyards. Such proposal would be in agreement with the models explored by Kean *et al*. (2003), respecting "conservation biological control" practices, and showing the efficiency of both the enhancement of natural enemies search rate and spatial attraction of the latter to a determined site in decreasing targeted pest densities. In that context, Franco *et al*. (2009) indicated that the kairomonal response of parasitoids to the mealybug sex pheromone can be utilized to keep the released individuals in the targeted area. Furthermore, our study proved that the most commonly used insecticide treatments for mealybug control in Italian vineyards significantly affected the parasitoid capability to parasitize its mealybug hosts. Accordingly, insecticides should be selected based on positive compatibility with biological control of mealybugs by *A*. sp. near *pseudococci*. For these reasons, it would be useful to avoid insecticide application when parasitoids are released in vineyards. Franco *et al*. (2009) suggested, as a major component of a suitable future management strategy, the use of environmentally safe insecticides to control mealybug "hot spots" and stated that a low-residue short-life insecticide is the most appropriate. Our overall findings would be a basis for performing further field studies aiming to assess the impact of "parasitoid release combined with a kairomone-based attracting system" on the vineyard's mealybug population. In that context, control of tending ants should be considered in adopting accurate decisions of biological control programs against mealybugs in vineyards, since it was previously proved that tending ants increased mealybug densities by disrupting the activity of their natural enemies in vineyards (Daane *et al*., 2007; Tollerup *et al*., 2007; Mgocheki & Addison, 2009b)ACKNOWLEDGEMENTS

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