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Life stage-dependent susceptibility of *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) to two pesticides commonly used in citrus orchards

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HIGHLIGHTS

- Lethal and sublethal effects of two pesticides were studied on *Aphytis melinus*.
- Significant differences in parasitoid instars susceptibility were recorded.
- Pesticide risk assessment and choice should include life-stage susceptibility.
- The natural origin of the mineral oil is not guarantee of non-target safety.
- Careful integration of *A. melinus* and the tested insecticides is recommended.

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ABSTRACT

The assessment of pesticides compatibility with natural enemies is recommended before including agrochemicals in integrated and organic pest management schemes. The lethal and sublethal effects of a mineral oil and a juvenile hormone mimic (pyriproxyfen), on adults and larvae of *Aphytis melinus*, a key ectoparasitoid of armored scale insect pests of citrus, such as *Aonidiella aurantii*, were evaluated. Mineral oil caused very high mortality on the adults, while a lower acute toxicity was recorded on young instars. No significant effects on their reproduction capacity and on the sex-ratio of the progeny were observed. Pyriproxyfen had neither lethal nor sublethal effects (in terms of survived female fertility) on *A. melinus* adults. However, parasitoid larvae exposed to this insecticide suffered strong acute toxicity and fertility reduction (progeny number and proportion of female progeny). When adults were offered the choice to parasitize treated and untreated scales they significantly preferred the control ones, and when they were exposed to only treated scaled the parasitism rate was significantly lower only with mineral oil-treated hosts. The significant differences in the susceptibility of the two parasitoid instars highlight the importance of including this aspect in pesticide risk assessment procedures and in the choice of the pesticide and of the treatment timing in the field. Overall, the results indicate potential for integrating *A. melinus*, both naturally present and artificially released, and these insecticides only by appropriate timing of insecticide spraying and parasitoid releases.

1. Introduction

The California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae), is an invasive pest of citrus and despite its common name it is believed to have Asiatic origin (Compere, 1961). Nowadays, this species is considered one of the most important pests of citrus in the Mediterranean basin and in several other citrus growing areas worldwide (Jacas et al., 2010; Zappalà, 2010). The ectoparasitoid *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) is a key natural enemy of *A. aurantii* (Compere, 1961; Siscaro et al., 1999; Sorribas and Garcia-Marí, 2011) and it is mass reared for inoculative and augmentative releases in citrus

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orchards (Moreno and Luck, 1992; Zappalà, 2010). The effectiveness of *Aphytis* spp. in the scale control could depend on many agro-ecological factors, such as the fitness of the released insects (Vasquez and Morse, 2012), their field dispersal capacity (Zappalà et al., 2012), the availability of the susceptible host instars and their size (Luck and Podoler, 1985), the interaction with other natural enemies (Heimpel et al., 1997a; Borer et al., 2003; Vanaclocha et al., 2013a), and the presence in the tree canopy of insecticide residues (Suma et al., 2009; Garcerá et al., 2013; Vanaclocha et al., 2013b).

Although various environmentally friendly pest control tools, such as habitat management, cultural practices, mating disruption, and biological control, are currently adopted and implemented in citrus crops worldwide (Lim et al., 2006; Vacas et al., 2012; Aguilar-Fenollosa and Jacas, 2013; Chueca et al., 2013), chemical treatments are frequently applied in citrus groves (Rill et al., 2008; Garcerá et al., 2014; Monzò et al., 2014). The use of selective pesticides is therefore crucial for maintaining natural enemy populations and their ecosystem services (Prabhaker et al., 2007; Suma et al., 2009). Thus, the correct evaluation of potential side effects of pesticides on non-target organisms, such as the natural enemies employed in citrus IPM and organic packages, is particularly crucial (Garcerá et al., 2013; Planes et al., 2013).

Pesticides could have both lethal (i.e. acute toxicity) and sublethal (i.e. influence on various behavioral and physiological traits) effects on the exposed natural enemies (Desneux et al., 2007; Biondi et al., 2012a). A comprehensive evaluation of undesired effects of agrochemicals should be therefore based not only on short-term acute toxicity tests, but also on the assessment of physiological and behavioral sublethal effects. These should include the potential long-term effects on natural enemy population dynamics and their ecosystem services (He et al., 2012; Biondi et al., 2013; Saber and Abedi, 2013; Bengochea et al., 2014). Moreover, most of the tests used to determine the side effects of pesticides on natural enemies are performed on only a single developmental stage, primarily the adults, which are considered to be the most exposed life stage. Immature developmental stages, however, may also be affected by pesticides, even though they are usually concealed within the host (Schneider et al., 2004). In this framework, studying the potential effects of pesticides on different developmental stages of the natural enemies could be relevant, also considering their variable susceptibility which has been often highlighted, particularly when testing Insect Growth Regulator (IGR) compounds (Ishaaya and Horowitz, 1995; Hoddle et al., 2001; Schneider et al., 2004).

Mindful of this context, we aimed at evaluating the compatibility of a narrow range mineral oil and a juvenile hormone mimic with *A. melinus* when applied on adults and young instars of the parasitoid. Laboratory bioassays were conducted to assess the acute toxicity and the sublethal effects on reproduction on adults and young instars, as well as *A. melinus* parasitization activity on treated hosts.

2. Material and methods

2.1. Insects

A parthenogenetic strain of the oleander scale, *Aspidiotus nerii* Bouché (Hemiptera: Diaspididae), was reared on organically grown squashes, *Cucurbita maxima* Duch. var. Butternut. Infested squashes were used for the parasitoid rearing, following *A. lingnanensis* rearing methodology as described by DeBach and White (1960), and modified by Raciti et al. (2003). All the insects used in this study were reared and kindly provided by the Sicilian Regional Insectary (*Biofabbrica Insetti Utili*, Ramacca, Catania, Italy). In particular *A. nerii*-infested and *A. nerii*-infested and parasitized squashes were promptly transferred to the laboratory, when needed for the toxicological bioassays.

2.2. Pesticides

We evaluated under laboratory conditions the effects of two agrochemicals. The first was the narrow range mineral oil Biolid E® (Emulsifiable Concentrate 80% a.s., Sipcam, Italy), generally used as adjuvant and/or alone to control mites and scale pests when at the first instars on many different crops under conventional, integrated and organic pest management programs (Biondi et al., 2012a). The other was pyriproxyfen, a juvenile hormone mimic [Admiral 10EC® (Emulsifiable Concentrate 10.86% a.s.), Sumitomo Chemical, Japan], which inhibits metamorphosis and embryogenesis in several insects (Ishaaya and Horowitz, 1995). Both chemicals were applied at their maximum label dose: 2 L hL^{-1} and 75 mL hL^{-1} respectively. Untreated controls were sprayed with tap water only. All the bioassays were conducted at 23 ± 1 °C, 60 ± 10% R.H. and a photoperiod of 16:8 (*L*:*D*).

2.3. Acute toxicity on A. melinus adults

The acute toxicity was assessed exposing newly emerged (24–48 h) adults to freshly dried pesticide residues on glass plates. Uniform deposits (1.3–1.8 mg/cm^2) of pesticide solution or of water were obtained using a Potter Precision Spray Tower (Burkard Manufacturing Co. Ltd.) to spray 6 glass plates (9 × 9 cm). Subsequently, the plates were left into a chemical hood for about 2 h to complete drying and then assembled to put together a cube (Suma et al., 2009). To avoid fumigation effect, each set of experimental arenas was provided with a electric pump (Air fizz 100, Ferplast Spa, Italy) ensuring a constant 36 mL × min^{-1} air-flow (Suma et al., 2009). To mimic the field scenario where parasitoid adults use external nutrients (Beltrá et al., 2013), a solution of pure honey and water (1:1) was provided in the experimental arena through a cotton ball imbibed of honey solution. Ten parasitoids (five females and five males) were introduced in the arena and left in contact with the pesticide residues for 24 h and then their survival was recorded. Adults were considered dead if they did not react after being touched by a fine paint brush. Ten replicates per each treatment were carried out.

2.4. Acute toxicity on A. melinus larvae

Bioassays on *A. melinus* juveniles were conducted using squashes infested with 1000 ± 100 oleander scale third instar nymphs, i.e. the parasitoid preferred host instar (Rosen and DeBach, 1979). Infested squashes were exposed to 10 females and 10 males of the parasitoid for 48 h in ventilated plastic boxes (35 × 25 × 45 cm) ($l \times w \times h$), provided with honey droplets. Two similar squashes infested with 500 third instars *A. nerii* each, were simultaneously exposed to *A. melinus* adults per each replicate and treatment. In order to expose *A. melinus* third instars to the tested insecticides or to the control solution, only one squash was sprayed until run-off [using a 2 L aerosol hand sprayer (Matabi®, Antzuola, Guipuzcoa, Spain)] 5 d after oviposition initiation (Raciti et al., 2003). While, the other infested squash was moved to a different box kept under the same environmental conditions. This second set of squashes provided a specific control data on parasitoid emergence per each sprayed squash. The number and the sex-ratio of the emerged progeny from all the squashes were scored daily for one week after the juvenile development completed, i.e. 12 d at these environmental conditions (Raciti et al., 2003). The experiment was replicated ten times. The percentage of young instars mortality was estimated as: