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Interference of ants (Hymenoptera: Formicidae) with biological control of the vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae)

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ABSTRACT

Anagyrus sp. and *Coccidoxenoides perminutus* are well known parasitoids used for the biological control of the vine mealybug *Planococcus ficus*, a key pest in vineyards. In South Africa, three ant species, *Anoplolepis steingroeveri*, *Crematogaster peringueyi* and *Linepithema humile* form a trophobiotic relationship with the vine mealybug in vineyards and promote the latter's infestations to unacceptable levels. In a manipulative laboratory experiment, ants and parasitoids were allowed to forage on vine mealybug-infested butternuts and numbers were recorded for a 1 min period at 10 min intervals for 2 h. Parasitoid mortality and the number of parasitized vine mealybug females were then recorded in the presence and absence of the three ant species. Data were analyzed using a repeated measures generalized linear model (GEEs). The mean number of ants and parasitoids on the mealybug-infested butternuts differed significantly between ant species and time intervals ($p < 0.0001$ in all cases). *Crematogaster peringueyi* and *L. humile* caused significantly higher mortality of both parasitoids than *A. steingroeveri* during the 24-h exposure period ($p < 0.0001$). *Coccidoxenoides perminutus* parasitized significantly more vine mealybugs than *Anagyrus* sp. for all treatments ($p < 0.0001$). Ants should therefore be controlled prior to release of parasitoids to suppress populations of ant-tended Hemiptera in vineyards.

1. Introduction

Finding new ways of understanding animal behavior is fundamental to the biological control of arthropod pest species. The trophobiotic relationship between ants and their adopted Hemiptera (Gibernau and Dejean, 2001; Jiggins et al., 1993; Hölldobler and Wilson, 1990; Buckley, 1987; Pierce and Mead, 1981; Adenuga, 1975; Bradley, 1973; Bartlett, 1961; Steyn, 1954) needs further investigation to understand various levels of aggression exhibited by ants towards natural enemies of their attended Hemiptera. Field behavioral studies of parasitoids are difficult to carry out due to the complexity of these interactions in the field. Behavior of ants and parasitoids can be broken down into simple components (e.g. ant recruitment and parasitoid oviposition) that can be manipulated under controlled conditions to predict field response that can be considered in planning and implementing a pest management programme.

Although many studies have abundantly documented the detrimental impacts of ants on biological control (Bartlett, 1961; Buckley, 1987; Itioka and Inoue, 1996; Martinez-Ferrer et al., 2003), the individual impact of each ant species on individual parasitoids has received limited experimental attention. In agricultural and natural ecosystems, ants obtain carbohydrate-rich honeydew from Hemiptera such as mealybugs, scale insects, aphids, among others, while they render protection, sanitation and sometimes transport services to sedentary Hemiptera (Buckley, 1987; Lach, 2003). Carbohydrates serve as a metabolic fuel for most insects and therefore behavioral dominance can be associated with relative availability of and demand for sugar (Grover et al., 2007). Arboreal ants exhibit greater activity and aggression where carbohydrate resources are limited compared to epigeic ants probably due to their numerical superiority in canopies and greater dependency on hemipteran honeydew. Similarly, high levels of activity and aggression exhibited by invasive ants may well be linked to their reliance on carbohydrate-rich honeydew (Way, 1963; Markin, 1970).

The Argentine ant *Linepithema humile* (Mayr) was found to be disruptive to the black scale *Saissetia oleae* Olivier, parasitoid *Coccophagus scutellaris* (Dalman) in California (Horton, 1918). In South Africa *Metaphycus helvolus* (Compere), a parasitoid of black scale was found to be effective in the absence of *L. humile* (Flanders, 1943; Compere, 1940). In California, Daane et al. (2007) found that *L. humile* promoted populations of obscure mealybug *Pseudococcus viburni* (Signoret) while lowering populations of its parasitoids *Pseudaphycus flavidulus* and *Leptomastix epona*. They also noted increased densities of the Argentine ant-tended grape mealybug *Pseudococcus maritimus* (Erhorn) accompanied by a serious reduction in its parasitoid populations.

The cocktail ant *Crematogaster peringueyi* Emery is disruptive to natural enemies of soft brown scale *Coccus hesperidum* L., and vine

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mealybugs *Planococcus ficus* (Signoret). This ant species provides protection to its hemipteran hosts by constructing carton shelters over the mealybugs (Kriegler and Whitehead, 1962). The common pugnacious ant, *Anoplolepis custodiens* (Smith), incidentally disturbed the parasitoids of California red scale *Aonidiella aurantii* (Maskell), while tending soft brown scale in citrus orchards in South Africa (Samways and Tate, 1984; Steyn, 1954).

Buckley and Gullan (1991) concluded that the incidence of coccid parasitization was correlated with the relative inoffensiveness of the attendant ant species in a field study in Australia. These authors measured low parasitism rates (at least 15%) of coccids in the presence of *Tapinoma* and *Iridomyrmex* spp. and <10% in the presence of the more aggressive *Oecophylla* and *Solenopsis* species. In California, *L. humile* reduced parasitism and host mutilation of the California red scale by the parasitoids *Comperiella bifasciata* (Howard) (59.1%) and *Aphytis melinus* De Bach (79.5%) in a laboratory trial, even if there were no honeydew-excreting soft scales (Martinez-Ferrer et al., 2003). Itioka and Inoue (1996), in a comparative field investigation, found a 94% decrease of the mealybug *Pseudococcus citriculus* Green by natural enemies in the absence of the attendant ant *Lasius niger* (L.). Previous (unpublished) studies revealed that more than one ant species and parasitoid species were sampled from the same sites and therefore parasitism rates could not reflect the individual impact of each ant species on mealybug biological control. *Anagyrus* sp. and *Coccidoxenoides perminutus* (Timberlake) (Hymenoptera: Encyrtidae) are widely distributed primary parasitoids of *Planococcus* spp. and *Pseudococcus* spp. (Davies et al., 2004; Triapitsyn et al., 2007), that have been used for classical and augmentative biological control of the vine mealybug and citrus mealybug *Planococcus citri* (Risso) in some localities (Daane et al., 2004; Walton and Pringle, 2005).

This study tests the interference of three ant species on the biological control of the vine mealybug *P. ficus* using the parasitoids *Anagyrus* sp. near *pseudococci* (as identified by Triapitsyn et al., 2007) and *C. perminutus*. This investigation used the presence and absence of these ant species to quantify their individual impact on the parasitoids under controlled laboratory conditions. For the purposes of this study, *Anagyrus* sp. near *pseudococci* shall be referred to as *Anagyrus* sp. from this point onwards.

2. Materials and methods

2.1. Insect colonies

2.1.1. Vine mealybug colonies

Colonies of vine mealybugs were maintained on mature fruit of butternut squash *Cucurbita moschata* (L.) in the laboratory at 27 ± 1 °C with a 12:12 (L:D) hour photoperiod and 65 ± 5% RH. Butternut squash were obtained fresh from the local grocery store and were more or less of equal size. Before use in experiments, butternuts were washed in 5% bleach solution (to prevent fungal growth), triple rinsed, air dried and then inoculated with vine mealybug crawlers after 1 h. After the first molt, mealybugs were thinned to approximately 100 individuals that were allowed to develop up to a desired stage before use in experiments as hosts to parasitoids. For *Anagyrus* sp., 3rd instar to preovipositing female vine mealybugs were used while for *C. perminutus*, 2nd instar mealybugs were used (Islam and Copland, 1997; Joyce et al., 2001).

2.1.2. Ant colonies

A single average ant nest (at least 500 workers) for all three ant species, consisting of workers, queens and immatures, was collected from commercial vineyards. For epigeic ants, a spade was used to collect a portion of the nest and maintained in soil from the original nest. A small size carton nest was collected for the arboreal *C. peringueyi*. Nests of all three species were placed into five liter square plastic containers. The set up was designed to mimic a natural situation as far as possible, where ants are regarded as pests and as such ants were collected from vineyards where their infestation levels were above the action threshold. *Anoplolepis steingroeveri* and *C. peringueyi* were collected from Ashton (−33.85°S, 20.08°E, 186 m) in the Breede River Valley (BRV) while *L. humile* were collected from Simondium (−33.83°S, 18.83°E, 175.2 m) in the Stellenbosch area, South Africa. The three ant species were maintained in plastic containers (18 × 18 × 16 cm) in the laboratory containing soil or material from the original nests. Each ant nest was connected to a clear Perspex container (25 × 25 × 20 cm) with clear plastic tubing (20 cm long and 6 mm in diameter). A butternut infested with about 100 mealybugs was placed into each Perspex container and ants were allowed to forage freely on this butternut for honeydew until 48 h prior to the experiment. All ant colonies were kept at 27 ± 0.5 °C, 65 ± 5% RH and a 12:12 (L:D) photoperiod.

2.1.3. Parasitoid colonies

2.1.3.1. Anagyrus sp.. Field collected vine mealybugs were incubated individually in gelatin capsules at room temperature. Incubated mealybugs were monitored daily, and emerged *Anagyrus* sp. were selected for experimental use through stereo microscope identification. Only *Anagyrus* sp. were selected, based on antennal coloration according to Triapitsyn et al. (2007). The parasitoids were placed in a cage (66 × 66 × 37 cm) containing butternuts infested with vine mealybugs. Parasitoids were offered a 50:50 honey:water solution and kept at 27 °C, 65 ± 5% RH with a 12:12 (L:D) photoperiod. After seven days, parasitized mealybugs were moved into another cage for parasitoid emergence. *Anagyrus* sp. colonies were maintained in the laboratory on 3rd instar to adult vine mealybugs feeding on butternut squash (Islam et al., 1997). Testing was done when a total of 40 newly emerged female parasitoids (total per trial) were available and each individual was used only once. Newly emerged parasitoids were allowed to feed and mate before use in trials. One male parasitoid was given access to five newly emerged females for 24 h (Tingle and Copland 1988, 1989). Only mated two-day old females were used in the experiment. After every three generations, *Anagyrus* sp. from the field were added to laboratory colonies to prevent inbreeding of the laboratory colony.

2.1.3.2. Coccidoxenoides perminutus. Coccidoxenoides perminutus were obtained from DuRoi Integrated Pest Management (Letsitele, South Africa) as mature pupae. Newly emerged individuals were allowed to feed for 24 h after which they were used in the experiments. No mating was necessary as *C. perminutus* are thelytokous (Davies et al., 2004). Both parasitoid species were deprived of hosts 24 h prior to the experiment.

2.2. Quantitative observations

Ants foraged on a butternut infested with 100 vine mealybugs in each of the six experimental cages of 21.5 × 21.5 × 16 cm (one for each ant species per parasitoid species). An ant-free cage was included as a control for each parasitoid species. Each experimental cage had a hole (6 mm in diameter) in the side which was plugged with cotton wool. This hole was only unplugged during introduction of parasitoids into the cages. A single tube aspirator was used to collect parasitoids from their rearing cages and release the parasitoids into the experimental cage through the side hole by gently blowing through the tube. The ants were allowed to forage for 3 h before 20 two-day old fertilized *Anagyrus* sp. females and 20 one-day old *C. perminutus* were introduced. Observations were made 10 min after the release of parasitoids for each treatment