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Interaction between ants and the Mediterranean fruit fly: New insights for biological control

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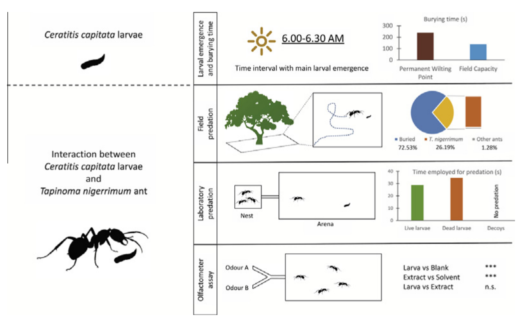
HIGHLIGHTS

- We investigated the predatory relationship between *C. capitata* and *T. nigerrimum*.
- Furthermore we investigated some bio-ethological aspects of *C. capitata* larva.
- About 27% of *C. capitata* larvae were preyed on by ants on field.
- The movement of medfly larvae, as well as olfactory cues, affected prey location.
- Mature larvae of *C. capitata* leave drop from fruit mainly in the early morning.

GRAPHICAL ABSTRACT



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ABSTRACT

During recent years, the positive role of ants as biological control agents in agro-ecosystems has gained growing interest. We investigated the predatory relationship between *Ceratitis capitata* (Wiedemann) and the ant *Tapinoma nigerrimum* (Nylander), commonly widespread in the Mediterranean area. Additionally we investigated some bio-ethological aspects of *C. capitata* larvae that could be relevant timing factors for predation. Field observations highlighted that *C. capitata* larvae were preyed on by ants, and laboratory assays suggested that movement of medfly larvae, as well as olfactory cues, affected prey location by *T. nigerrimum*. Further observations on the circadian activity of *C. capitata* suggest that mature larvae leave the fruit to pupate in the soil mainly in the early morning, and they can bury faster in moistened soil. These ecological aspects are discussed in the context of sustainable agriculture.

1. Introduction

Insect trophic interactions occurring within terrestrial ecosystems constitute a complex network (Weisser and Siemann, 2004; Dyer et al., 2014), so the study of these interactions can provide novel insights and new approaches for the application of biological control strategies and Integrated Pest Management (IPM) programs (Schoonhoven et al., 2005; Smagghe et al., 2012). In most terrestrial habitats, ants are dominant organisms that may establish a complex network of interactions, at every trophic level, with virtually every component of their foodweb (Hölldobler and Wilson, 1990, 2009; Ness et al., 2010).

Although ants have high species diversity and represent a large fraction of biomass in several ecosystems, their impact and ecological role have been neglected for several decades (Hölldobler and Wilson, 1990). This trend has been reversed in recent years, and

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ants have received a great deal of attention related to many ecological issues, from their use as an ecological indicator to their impact in urban and agro-ecosystems (Kaspari and Majer, 2010; Philpott et al., 2010; Fisher et al., 2014). Because of their relationships with many sap-sucking hemipterans, ants are often considered to be problematic in agriculture, mainly because they can disturb beneficial insects (Beattie, 1985; Rosumek et al., 2009; Piñol et al., 2012). However, there is increasing evidence that ants have a positive impact in agro-ecosystems, in particular on trophic and guild interactions in biological control (Rosumek et al., 2009; Benckiser, 2010; Choate and Drummond, 2011). Indeed, several species are both sugary-liquid collectors and fierce predators (Stadler and Dixon, 2008; Cerdá and Dejean, 2011), frequently exhibiting strong territorial defense, so their mere presence on plants may be a deterrent for many potential herbivores and seedeaters.

In Italy, to date, there has been no extensive investigation on ants as potential control agents of pest insects in agricultural systems, although Italian ant fauna is quite rich and may represent an important component in agro-ecosystems (Castracani and Mori, 2006; Castracani et al., 2010). The spread of invasive insect pests could represent a threat to agricultural production, as with the introduction of *Unaspis yanonensis* (Kuwana) and *Chrysomphalus aonidum* (L.) (Hemiptera: Diaspididae) in southern Italy, where citrus is one of the most economically important crops (Campolo et al., 2013, 2014). On the other hand, well-known key pests could continue to represent a severe issue in agriculture, like the Mediterranean fruit fly or medfly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). This fly is able to feed on a large variety of fruit of different plant families, and represents a serious threat to most citrus varieties, peaches, nectarines, persimmons, apricots, prickly pears, and many others. *C. capitata* lays eggs inside susceptible fruit and, after hatching, larvae feed on the fruit flesh. Third instar larvae leave the fruit to pupate in soil, and adults complete the life cycle in the canopy. In southern Italy, *C. capitata* is able to complete 6–7 generations per year because of the continuous availability of susceptible fruit (Tremblay, 2003). The development of the medfly involves different habitats (fruit, soil, and canopy) so these variables must be taken into account in biological control. Augmentative release of a large number of parasitoids (i.e. the braconid wasp *Diachasmimorpha tryoni* (Cameron) may be a viable control method against immature stages of *C. capitata* (Wong et al., 1991; Medina et al., 2014). Medfly immature stages are also controlled by a large number of ground-dwelling predators (i.e. ants, carabid beetles and arachnids; Urbaneja et al., 2006). Other approaches, such as foliage baiting, the use of baited traps or the sterile insect technique, are also used to reduce the population level and to attempt medfly eradication all over the world (Hendrichs et al., 2002; Navarro-Llopis et al., 2013).

The environmental impact resulting from the wide use of insecticides has led to initiatives directed at reducing reliance on chemical control (Biondi et al., 2015; Qu et al., 2015), such as releasing biological control agents, as has happened for other key pests such as *Aonidiella aurantii* Maskell (Hemiptera: Diaspididae) (Zappalà et al., 2012) and mealybugs. Several ant species are known to prey on *C. capitata*: *Pheidole megacephala* (Fabricius), *Linepithema humile* (Mayr) and *Solenopsis geminata* (Fabricius) are well reported examples. *P. megacephala* destroys a large number of larvae in Hawaii (Pemberton and Willard, 1918; Willard, 1927) and *L. humile* has been shown to kill larvae (2.3–3.9%) and teneral adult flies (38.8%) in a guava plantation in Hawaii (Wong et al., 1984). *S. geminata* and other predacious ants predate on both pupae (73.4%) and teneral adults (31%) beneath guava trees (Wong and Wong, 1988) while in coffee and citrus plantations in Guatemala, the fire ant was able to kill 7–25% of mature medfly larvae (Eksafi and Kolbe, 1990). Other ants, such as *Monomorium subopacum* (Smith), *Tapinoma simrothi* Krausse, *Cataglyphis viatica* (Fabricius) and *Messor picturatus maura* Santschi preyed on 18.8% of third instar medfly larvae in the Argan forest in Morocco (El Keroumi et al., 2010). Predation of *C. capitata* by other arthropods in citrus orchards has also been reported (Eksafi and Kolbe, 1990; Urbaneja et al., 2006; Monzó et al., 2009), as well as the predation of other fruit fly species by ants (e.g. Van Mele et al., 2007; Fernandes et al., 2012). Hence, results of previous research suggest that arthropod predators, and more specifically ants, could influence the population dynamics of *C. capitata* in fields (Wong et al., 1984).

Tapinoma nigerrimum (Nylander) is a dominant ant species commonly widespread in the Mediterranean area (Cerdá et al., 1998). It forms large polygynous colonies with interconnected nests, and thousands of very active and aggressive workers that mainly feed on honeydew and insects. Patrolling activity is performed with a composite and over dispersed distribution pattern directed at discovering new resources for the colony, thereafter the food is collected by workers foraging using a mass recruitment strategy (Cerdá et al., 1989, 1997).

The aim of this study was to collect information on the potential of *T. nigerrimum* as a control agent of the fruit fly *C. capitata* in citrus orchards. Some aspects of predator–prey interactions involving these species were investigated both in the field and under laboratory conditions. We also inspected the role of stimuli perceived by ants for intercepting the prey. Additionally we investigated some bio-ethological aspects of medfly larvae, because the larval emergence from fruit and the time spent burying into soil could be relevant timing factors for predation.

2. Materials and methods

2.1. Experimental field

The trial was carried out in August–September 2013 in a citrus orchard under IPM program located in Gallico (province of Reggio Calabria, Italy; 38° 11′ N, 15° 38′ E) at 30 m a.s.l., where no chemical treatments had been applied for 3 years before the observations. The experimental field (2.4 ha) was planted in 2004 with Satsuma trees (cultivar Miyagawa) grafted on Troyer citranges, in a 4×5 m grid. Fruit ripens in late September and become susceptible to *C. capitata* from mid-August. The trees in the experimental field were regularly fertilized, pruned and irrigated, and the soil between the plant rows was hoed twice per year. The local vegetation was mainly represented by weeds, *Amaranthus retroflexus* L., *Portulaca oleracea* L. and *Setaria verticillata* (L.), controlled with mowing when needed. During the observations, temperature and relative humidity (R.H.) were measured by means of DS1923 data logging units (iButton™, San Jose, CA, U.S.) placed below the canopy.

2.2. Insects

C. capitata was reared at the Entomology Laboratory of AGRARIA Department (University of Reggio Calabria, Italy) from a wild population initially collected in Locri (Italy – 38° 13′ N, 16° 14′ E) on citrus fruit (cv Clementine); wild adult specimens from the same locality were introduced yearly in the medfly rearing cages. Adult flies were provided with water and a water–honey solution (1 + 1 by weight), whereas larvae were reared on a wheat bran-based diet made of sodium benzoate 9 g/L, citric acid 10, g/L, brewer's yeast 150 g/L, sucrose 300 g/L, wheat bran 450 g/L, and water 1 L. Rearing conditions were maintained at 25 ± 1 °C, 55 ± 5% R.H. with a photoperiod of 12:12 LD cycle.

On the basis of field preliminary observations and experiments (see Field predation) showing that most larvae were preyed on by