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Fruit fly larval trail acts as a cue in the host location process of the pupal parasitoid *Coptera occidentalis*

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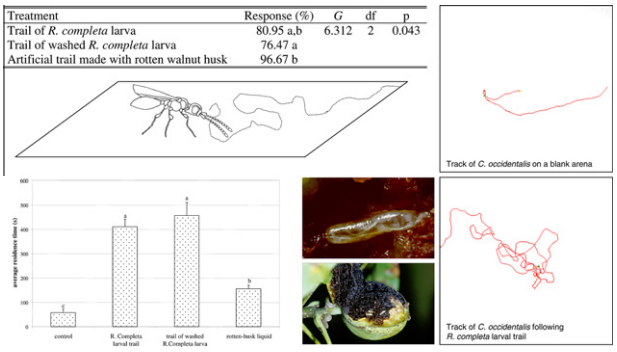
HIGHLIGHTS

- *Coptera occidentalis* females use larval trails to locate the host.
- Rearing on factitious host does not bias the host location process.
- *C. occidentalis* females also follow trails of Tephritid washed larvae.

GRAPHICAL ABSTRACT



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ABSTRACT

The pupal parasitoid *Coptera occidentalis* Muesebeck (Hymenoptera: Diapriidae) was reared in the 1970s in California for the biological control of the Walnut husk fly, *Rhagoletis completa* Cresson (Diptera: Tephritidae) and later introduced into Europe to be used as a biological control agent against fruit flies. Some behavioral aspects relating to the host location process were investigated in the laboratory in order to improve the chance of success in possible biological control programmes. The searching behavior of the female parasitoid was examined in an open arena in order to evaluate the role of larval trails or rotten-fruit trails (walnut) in the host location process. The response of *C. occidentalis* to four Tephritid hosts was evaluated: the natural host *R. completa,* the factitious host *Ceratitis capitata* (Wiedemann) and two fruit flies of economic importance, *Rhagoletis* cerasi Linnaeus and *Bactrocera oleae* (Rossi). In order to assess the response of the parasitoid, a number of behavioral parameters were considered, such as: arrestment behavior, resident time, walking distance, linear and angular speed. The larval trails of *R. completa,* as well as rotten-fruit liquid trails, were clearly detected by *C. occidentalis* females, even though the species has been reared for more than 80 generations on *C. capitata.* In addition, bioassays performed with *C. capitata* larvae showed a good behavioral response of parasitoid females to the larval trails. Finally, *R. cerasi* and *B.* oleae larval trails were barely detected in comparison to the traces left by *R. completa* and *C. capitata* larvae. The results are discussed in the light of the possible use of *C. occidentalis* in biological control programmes.

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1. Introduction

In Italy four species of fruit flies are listed as being of economic importance: *Ceratitis capitata* Wiedemann (Medfly), *Bactrocera oleae* (Rossi) (Olive fly), *Rhagoletis cerasi* Linnaeus (European cherry fruit fly) and *Rhagoletis completa* Cresson (Walnut husk fly). The control methods adopted against these pests are at present mainly chemical-based, and very often the growth of fruit fly populations is such that sprays have to be repeated several times, which is detrimental to human health and the environment, particularly to beneficial fauna in agroecosystems. Biotechnical control methods, on the other hand, are costly to manage and are only effective against low and medium population densities (Petacchi et al., 2003). Recently, a *Beauveria*-based bioinsecticide has been tested against fruit flies, with promising results (Benuzzi et al., 2007; Ladurner et al., 2008; Ortu et al., 2009).

Biological control have mainly been tested against the Olive fly. Several attempts at classical biological control were carried out at the beginning of the last century, when Silvestri, following a lengthy survey in Africa, introduced natural enemies of fruit flies into Southern Italy with a view to controlling the Olive fly (Silvestri, 1913). Many years later, further attempts were made to establish natural enemies by releasing parasitoids (Russo, 1959; Monastero, 1968; Delrio et al., 2005), but none of them acclimatized except for *Psyttalia concolor* (Szépligeti) (Hymenoptera, Braconidae), which is consistently found in milder areas of Italy, albeit with a very low parasitization rate (Raspi et al., 2007). As far as the other fruit fly pests are concerned, native parasitoids were obtained only from *R. cerasi* (Monaco, 1984) and *B oleae*. The latter species hosts several parasitoids with noticeable but very variable parasitization levels (Fimiani, 1971; Raspi et al., 2007). No data are available for the parasitoids of *C. capitata* and *R. completa*. In short, it can safely be affirmed that the parasitoid complex of fruit flies in Italy is very sparse and natural biological control is ineffective, with no noticeable reduction of the pest population density. One possible way of addressing this problem would be to consider introducing exotic parasitoids into Italy in order to increase the natural enemy complex; pupal parasitoids, amongst others, would seem to merit attention, since they parasitize the pupal stage in the soil, escaping insecticidal sprays and enabling the development of an effective combination of different IPM strategies. Although biological control measures against fruit flies have produced effective results in some countries, often they do not lead to an entirely successful reduction of the pest population, because of problems in the establishment and diffusion of parasitic wasps (Purcell, 1998). These difficulties are the result of many factors, one of which is the host location process (Purcell, 1998). As a consequence, pinpointing the stimuli involved in the host finding process appears to be essential in evaluating any possible biological agent.

Coptera occidentalis Muesebeck is a solitary pupal parasitoid of Nearctic species, belonging to the genus *Rhagoletis*; according to Muesebeck (1980) it was obtained from *R. completa* in California and *R. cingulata* (Loew) in Oregon. Besides its natural hosts the species can develop in other fruit flies, such as *R. indifferens* Curran and *C. capitata*, commonly used as a factitious host in laboratory rearing (Hagen et al., 1995; Kazimírová and Vallo, 1999). *C. occidentalis* has been introduced in Slovakia as a biological control agent against *R. cerasi* (Vallo, 1990, 1996).

At the end of the 1970s, a mass rearing of *C. occidentalis* was started in California in order to release parasitoids against *R. completa*. Further releases were carried out in the 1980s in other locations with both commercial and wild walnuts (Hagen et al., 1995). The species became established over a wide area but despite assiduous and protracted efforts lasting almost 20 years, the control was inadequate. In fact, in a field trial aimed at evaluating inundative releases of *C. occidentalis* as a control method, the parasitism rate was "lower than expected". Since the puparia hosts were artificially buried in the soil, the failure was probably due, as Hagen et al. (1995) suggest, to the lack of chemical attractants (synomones and/or kairomones) needed to stimulate parasitoids to search.

In light of the fact that in Italy *C. occidentalis* was intended to be used as a biological control agent against fruit flies, it is crucial to understand how the parasitoid discovers the host pupated in the soil. We hypothesized that *C. occidentalis* females are able to detect and follow kairomonal substances released by host larvae when they crawl across the soil surface in search of suitable pupation sites. We studied the behavioral response of *C. occidentalis* females to semiochemicals left by larvae of four Tephritid species – *R. completa*, *C. capitata*, *R. cerasi* and *B. oleae* – and to the liquid produced by infested husks.

2. Materials and methods

2.1. Insect rearing

C. capitata has been reared in the laboratory on an artificial diet for over 200 generations (Cavalloro and Girolami, 1969). *R. completa, R. cerasi* and *B. oleae* larvae were obtained from their host fruits, respectively fresh walnuts, cherries and olives. Infested fruits were collected in the field, depending on seasonal availability, and kept at room temperature if used within a few days, or at 10 °C if used later (but always within 10 days).

C. occidentalis has been reared on *C. capitata* for over 80 generations (10 years), starting from a colony provided by Dr. M. Kazimírová (SAV, Bratislava, Slovakia). Adult parasitoids were given free access to water and honey; cages were kept in a climatic chamber at 25 ± 2 °C, RH 50–60%, L:D 16:8 photoperiod.

In order to plan bioassays, parasitized pupae were singly isolated in small vials that were checked on a daily basis to collect adults. Newly emerged parasitoids were mated – proved by observing the peculiar antennation during the copulatory phases (Sacchetti et al. 1999) – and fed with honey diluted in water (1:1); each mated female was kept singly in the same vial at room conditions until the bioassay. Only inexperienced females aged from one to four days were tested in bioassays.

2.2. Experimental protocol

All the bioassays consisted of two phases; in the first phase, a 3rd instar fruit fly larva was released in the center of a 20 × 20 cm sheet of paper (Copy3 Fabriano, Italy), used as an open arena. The larva was allowed to move freely on the paper for a maximum of 10 min or until it left the arena, then the larva was removed with a pair of forceps. In the second phase a female parasitoid was gently released into the center of the arena and allowed to move freely on the paper. The bioassay lasted until the parasitoid left the arena or after 10 min had elapsed. An evaluation was made of the parasitoid response to four fruit fly species: *R. completa*, *R. cerasi*, *C. capitata*, and *B. oleae*. Full grown larvae were collected after leaving the feeding substrate. Since substrate traces may adhere to the larval body, in order to ascertain the characteristics of probable kairomone compounds left by the host, a batch of bioassays was performed using larvae of *R. completa* and *C. capitata*, washed with deionized water before the test: larvae were submerged and gently agitated with a pair of forceps for 30 s.

Finally a series of bioassays was conducted to investigate the parasitoid response to infested host fruit. The first step in this case was to trace an artificial track with a thin brush soaked in the liquid