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## SHORT COMMUNICATION

### Survival Rates of Planidial Larvae of the Parasitoid Fly *Ormia ochracea* (Diptera: Tachinidae)

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#### Introduction

The tachinid fly, *Ormia ochracea* Bigot 1889, is a major parasitoid of field crickets in North America. The fly ranges from Florida to California, and it has also been introduced to Hawaii. Across this range, *O. ochracea* uses at least 6 different *Gryllus* species as hosts for its larvae (Cade, 1975; Walker, 1986; Walker and Wineriter, 1991; Zuk *et al.*, 1993; Wagner, 1996; Hedrick and Kortet, 2006). *O. ochracea* finds its hosts by eavesdropping on male mating songs (Cade, 1975). The fly deposits up to 3 planidial larvae (Adamo *et al.*, 1995a) directly on the male and these larvae burrow into the cricket to continue development. After 7–10 days, the larvae emerge from the host and kill it during this process (Adamo *et al.*, 1995b). Interestingly, the fly also deposits approximately 6 planidial larvae (Adamo *et al.*, 1995a) on the ground around the male cricket. These larvae wave their heads while standing on the ground (Cade, 1975) and snap forward with their anterior end when touched (*pers. obs.*), suggesting that they wait for a host to pass by to cling onto rather than actively searching for a host (Allen *et al.*, 1999). Possible hosts targeted by these larvae could be the singing male, passing males, females approaching the singing male for mating (Martin and Wagner, 2010), or even juvenile crickets (Vincent and Bertram, 2009a). In contrast to the planidia deposited directly on the cricket, the potential of incidental infestations by the surrounding larvae is poorly understood. We measured the survival of planidial larvae of *O. ochracea* in a dry and a damp environment to estimate how long the larvae survive and pose a potential threat to passing crickets.

#### Methods

We collected gravid *O. ochracea* females from Rancho Sierra Vista in the Santa Monica Mountain National Recreation Area near Camarillo, CA, using broadcasts of *G. lineaticeps* songs (for details see Wagner and Basolo, 2007) and transported them to the University of Nebraska. Flies were housed individually in plastic containers (13 × 17 × 8 cm) and supplied every second day with apple sauce (Best Choice™) and cotton (Padco™) soaked in saturated sugar solution. Larvae were obtained by killing the adult female fly by removing its head and then dissecting its abdomen (for detailed description see Vincent and Bertram, 2009b). We sacrificed one fly per experimental day and transferred a portion of its larvae onto a single ‘wet’ filter paper (Whatman International Ltd.™) and a portion onto a single ‘dry’ filter paper. We transferred between 3 to 10 larvae per fly on each of the two filter papers. Treatment groups did not differ by more than one larva on any of the experimental days. We used a total of 5 flies and measured survival of 36 larvae in each treatment group. For the ‘damp’ treatment, we used a pump spray bottle (Bottle crew™) to spray distilled water on the filter papers. Each of these papers was sprayed with five pumps of water at an approximate distance of 30 cm. For the ‘dry’ treatment, we used untreated filter papers. Each filter paper was placed on the bottom of a petri dish (Fisher Scientific™, 10 cm diameter) and was covered with a lid after the transfer of the larvae. Except for the periodic observations, petri dishes were kept in the dark and covered with the lid throughout the experiment. Daily average humidity levels range between approximately 65% and 85% near the collection site during peak fly activity (mid-July to mid-September; *pers. obs.*) (URL: <<http://www.wunderground.com/cgi-bin/findweather/getForecast?query=camarillo>>; accessed on 06/23/11), suggesting that our ‘damp’ treatment simulates more natural conditions and that the ‘dry’ treatment simulates more adverse conditions. We determined larval survival rates in each treatment group by checking larval activity with a dissecting microscope (American Optical

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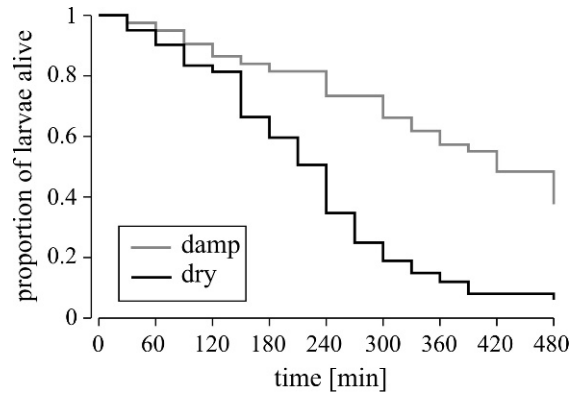


Fig. 1. Survival functions of larvae of *Ormia ochracea* on 'dry' (black line, bottom) and 'damp' filter paper (grey line, top). The trials of each treatment started with 36 living larvae that were obtained from 5 flies. For each treatment, we calculated for each fly the proportion of living larvae after every 30-minute time interval. Shown are the average proportions of the living larvae over time.

Corporation™) every 30 minutes for a total of eight hours. Larvae were scored as 'alive' if they displayed behavior such as head waving, moving across the filter paper, or body movement in response to a gentle touch with a blunt probe. They were scored as 'dead' if none of these behaviors was observed. We determined the survival rates of larvae of one fly per day. Observations took place between 1400 and 2200 hr at ambient temperatures between 21 and 22°C. We compared survival rates between treatments using the statistical software 'R' version 2–12 (R Foundation for statistical Computing, Vienna, Austria).

#### Results and Discussion

Larvae on the damp filter paper survived significantly longer than those placed on dry filter paper (Cox proportional hazards frailty model: d.f. = 1,  $\chi^2 = 13.2$ ,  $P < 0.001$ ; Fig. 1). After 8 hours of observation, 37.6% of the larvae on the damp paper were still alive, whereas only 6% on the dry filter paper were still alive.

We did not measure the ability of the larvae to infest crickets but used their activity as a proxy to estimate how long they may pose a threat to their hosts. It is possible that the risk that living larvae pose to a passing cricket declines over time. In this case, we would expect the head waving movement of the larvae, which indicates the search for a passing host, to be substantially reduced if they lost their infectious potential. It took approximately 1 hr for the planidial larvae to change their activity state from standing up and waving the head to being unresponsive to physical touch (pers. obs.). Considering that up to ~38% of the larvae were still alive after 8 hours (damp treatment), a substantial fraction of larvae may pose a threat to passing crickets for 7 or more hours after they are deposited. Some larvae may even represent a risk throughout the entire nightly cycle of reproductive activity by the cricket host. Thus, depositing extra larvae in the vicinity of a singing cricket increases the chance of infesting more than just the focal cricket.

The tachinid fly, *Homotrixa alleni* Barraclough 1996, parasitizes calling males of the katydid *Sciarasaga quadrata* (Allen, 1995). In the lab, at a relative humidity between 56–80% and a temperature of  $20 \pm 1^\circ\text{C}$ , more than 50% of the planidial larvae died within one hour of deposition, and all of the larvae had died within two hours (Allen, 1999). These times are substantially shorter than the times we found for *O. ochracea* larvae for both the 'dry' and 'damp' treatment (50% of larvae were still alive after 3.5 h and 6.5 h, respectively; Fig. 1). Interestingly, the encounter rate between male and female *S. quadrata* is very low, and females have never been found to be parasitized (Allen, 1995; Allen et al., 1999). The latter may be explained in part by the short survival of the *H. alleni* larvae. In contrast to *S. quadrata*, 6% of female *G. lineaticeps* are parasitized (Martin and Wagner, 2010). The greater survival of *O. ochracea* larvae might be an adaptation to infect silent females that are attracted to males after larval deposition.

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