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Behavioural response of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) to cues from infested and non-infested olives

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Abstract: *Diachasmimorpha longicaudata* is a solitary larval-pupal endoparasitoid of several fruit flies that has been introduced to many countries as biocontrol agent of tephritid pests. The species has been reared in Italy under quarantine conditions to test its reliability in biological control programmes against fruit flies. Laboratory bioassays aimed at evaluating the behavioural response of *D. longicaudata* toward *Bactrocera oleae* infested or non infested olives have been carried out in the last two years. Data obtained through two different behavioural experiments demonstrated that the parasitoid can perceive olfactory and visual cues coming from fruits, especially from infested ones. In bioassays performed in arenas, naïve and experienced females were able to discriminate infested and non infested fruits showing also a noticeable ability in recognizing different fruit size by visual stimuli. Experiments conducted in Y-tube olfactometer revealed a remarkable response of naïve females to infested olives while experienced females preferred the pure air, showing a probable bias by the factitious host. Further research has to be planned in order to study other possible stimuli coming from the insect pest as well as from olive leaves and fruits. Moreover, additional bioassays should be performed on non-frugivorous tephritid flies before field releasing attempts, in order to avoid possible negative side effects.

Key words: *Bactrocera oleae*, biological control, Y-tube olfactometer, lab bioassays

Introduction

Diachasmimorpha longicaudata is a solitary larval-pupal endoparasitoid of several fruit flies (Diptera: Tephritidae). The species is native to the Indo-Australian region, developing on larvae of fruit flies of the genus *Bactrocera*. It has been introduced to many other areas such as Pacific, Caribbean, Neotropical, Nearctic, Afrotropical regions (Wharton, 1989; Ovruski et al., 2000). It has been also introduced in California and evaluated as a potential parasitoid for the olive fly biocontrol (Sime et al., 2006).

Field successful releases of natural enemies rely on a thorough knowledge of the biology and behavior of both biocontrol agents and targeted insect pests (van Alphen and Jervis, 1996). In this paper we report preliminary behavioral observation on *D. longicaudata* in order to investigate the species as candidate for the olive fly biocontrol in Italy. Behavioral observations in two-choice experiments conducted in arena and Y-tube olfactometer have been carried out aimed at evaluating the response of *D. longicaudata* females towards infested and non-infested olives or differently sized fruits.

Material and methods

The exotic parasitoid *D. longicaudata* has been reared at the Department of Agrifood Production and Environmental Sciences (University of Florence, Italy), under quarantine conditions. Bioassays were performed in laboratory at constant conditions (temperature 25 ± 5 °C; RH 40 ± 5%). Tested olives belonged to olive oil variety Mignolo and olive table variety Ascolana. Wasps were observed in two different experiments, one set using as arena a glass Petri dish (ø 16 cm) and the other using a Y-tube olfactometer to discriminate the relative importance of visual and olfactory stimuli.

In the first experiment two olives (one infested and one non-infested olive) were placed directly on a glass Petri dish, at equal distance from the point where the parasitoid was released (10 cm from the two olives). Only mated, 7-10 days old, *D. longicaudata* females were used for the bioassays; each wasp was tested singly, and only once. Experienced or naïve females (without prior oviposition experience) were tested. Behavioral parameters recorded were: the number of not responding females; the number of females who discriminated infested and non-infested fruit or differently sized fruit. Concerning the Y-tube olfactometer, the first choice of the wasps on the treated or untreated arm was considered. All data were analyzed with the chi-square goodness of fit test, using 95% as confidence level.

Results and discussion

Data concerning the behavioural response of *D. longicaudata*, proved that both experienced females and naïve ones were interested in olives since a statistically significant number of them moved towards the fruits. As a matter of fact, 41 naïve females out of 65 and 45 experienced females out of 65, responded to the olives while the remaining stayed on the arena ($\chi^2 = 4.45$; $p = 0.035$ and $\chi^2 = 9.62$; $p = 0.003$, respectively).

As highlighted in Table 1, the number of naïve and experienced wasps who discriminated the infested olives compared to the females who preferred the non-infested fruits was highly significant.

Table 1. *Diachasmimorpha longicaudata* discrimination ability and behavioral responses (antennal drumming and ovipositor probing) of naïve and experienced females in two-choice bioassays with infested and uninfested olives.

	Female status	Olive		$\chi 2$	p
		Non-infested	Infested		
Discrimination ability	Naïve	9	32	11.9	< 0.001
	Experienced	10	35	12.8	< 0.001
Antennal drumming	Naïve	5	45	30.4	< 0.001
	Experienced	13	45	16.6	< 0.001
Ovipositor probing	Naïve	5	47	32.3	< 0.001
	Experienced	4	81	68.0	< 0.001

During the bioassays, naïve wasps performed 45 antennal drummings and 47 ovipositor probings on the infested fruits and only 5 antennation events were recorded on the non-infested ones. Experienced females performed 45 antennation and 81 ovipositor probings on the infested fruit and 13 antennations and 4 ovipositor probings in the non-infested fruit. Also these latter behavioural parameters were highly significant.

With regard to the ability to discriminate the size of infested fruits, 31 naïve out of 56 females responded while 25 wasps remained in the arena without choosing one of the two olives (χ^2 = 0.64; p = 0.423 and (χ^2 = 1.14). About the experienced females, 32 out of 56 responded while 24 did not (χ^2 = 1.14; p = 0.29).

Naïve wasps' discrimination between large (n = 18) and small infested olives (n = 13) was not significant (χ^2 = 0.52; p = 0.47). On the other hand, experienced females preferred larger infested olives (n = 26) respect to small infested olives (n = 6) in a highly significant way (χ^2 = 11.3; p < 0.001).

No remarkable differences were observed regarding antennal drummings and ovipositor probings on differently sized olives for either wasps.

However, females, after getting in contact with the olive fruits, performed a total of 3 antennal drumming on large olives and 8 on small ones (χ^2 = 1.45; p = 0.23). Experienced females displayed only 3 antennal drummings on large olives and behaved similarly on the small ones (n = 4).

No significant differences were found in the number of ovipositor probings performed by naive (χ^2 = 0.27; p = 0.61) and experienced (χ^2 = 0.1; p = 0.75) females on the small and large olives.

In the Y-tube olfactometer experiments either naïve and experienced females changed their response when exposed to olfactory cues from infested olives. Naïve wasps moved more frequently toward the olfactometer arm carrying odours from the infested olives (untreated arm 37.5% *vs* treated arm 62.5%, (χ^2 = 5.76, p = 0.02) while experienced female moved to the infested olive arm less frequently (untreated arm 37.5% *vs* treated arm 62.5%, (χ^2 = 6.41, p = 0.01) (Figure 1).

Behavioural observations on the response of *D. longicaudata* to non-infested and infested olives show interest of the species towards stimuli from infested olives. Naïve females displayed a noticeable response in discriminating infested fruits in bioassays performed in arena. The two-choice tests with differently sized infested olives, proved that this parasitoid female did not rely solely on visual stimuli, being not innately attracted by larger fruits. These findings diverge from those obtained in previous researches (Segura et al., 2007), nevertheless, we observed that the preference toward larger olives infested by *B. oleae*, is more remarkable in experienced females, despite they had already been in contact and parasitized *C. capitata* larvae.

Although the opposite behaviour of naïve and experienced female towards infested olives, olfactory cues are clearly involved in the response to the potential host fruit, as supported by the Y-tube olfactometer experiments. These achievements demonstrated that females' choice exposed to olives is based on several cues as reported for other host fruits such as papaya, guava, mango, orange and other fruits (Jang et al., 2000). However, as already observed in the wasp *Psyttalia concolor* (Giunti et al., 2016), also olfactory stimuli are relevant in the host finding.

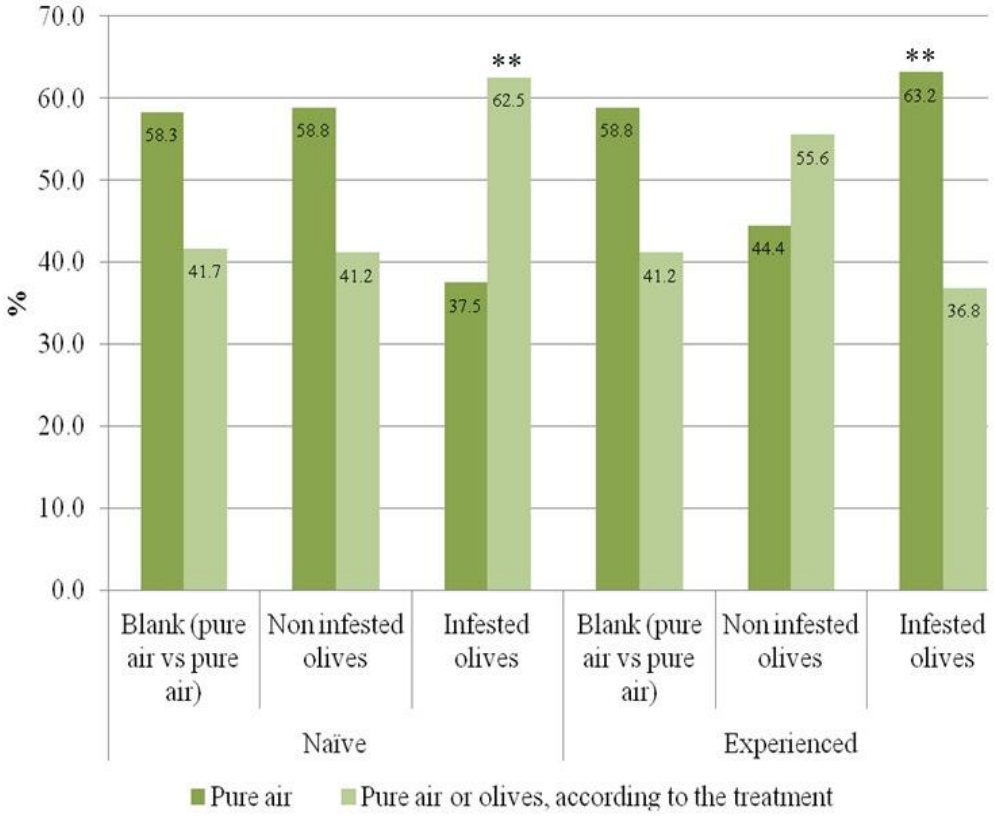

Figure 1. First choice of naïve and experienced females of *D. longicaudata* in Y-tube olfactometer bioassays to infested and non-infested olives and blank.

Behavioural bioassays of the exotic parasitoid *D. longicaudata* towards olives showed a prompt response of mated females for infested fruits. Surprisingly, naïve females displayed great interest in cues originated from infested olives, notwithstanding they had not experienced this host fruit, being reared from the factitious host, *Ceratitis capitata*. In bioassays carried out in arena, visual stimuli probably played an important role in driving wasps to the fruits, anyway, the high percentages of females that chose the infested olives depended on chemical cues produced by olive fly larvae or coming from infested fruits. Indeed, *D. longicaudata* was innately able to discriminate the infested olives, as well as experienced females, that were previously sensitized on *C. capitata* larvae. Furthermore, due to the high number of behavioral actions registered on this fruit, related to cues' perception in the host location phase, we could asses, as Segura et al. (2007) previously stated, that kairomones secreted by different species of Tephritidae are quite similar.

Concerning the future applications of *D. longicaudata* as biocontrol agent, we have to remark that the species have to be assayed for environmental risks (van Lenteren et al., 2006) before any field releasing attempts, in order to avoid possible side effects on non-frugivorous tephritid flies.

References

Giunti, G., Benelli, G., Conte, G., Mele, M., Caruso, G., Gucci, R., Flamini, G. and Canale, G. 2016. VOCs-mediated location of olive fly larvae by the Braconid parasitoid *Psyttalia concolor*: A multivariate comparison among VOC bouquets from three olive cultivars. BioMed Research International, Volume 2016, Article ID 7827615, 10 pp.

Jang, E. B., Messing, R. H., Klungness, L. M., Klungness, L. M. and Carvalho, L. A. 2000. Flight tunnel responses of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) to olfactory and visual stimuli. J. Insect Behav. 13: 525-538.

Ovruski, S. M., Aluja, M., Sivinski, J. and Wharton, R. A. 2000. Hymenopteran parasitoids on fruit-infesting tephritidae (Diptera) in Latin America and the southern United States: Diversity, distribution, taxonomic status and their use in fruit fly biological control. Integrated Pest Management Reviews 5: 81-107.

Segura, D. F., Viscarret, M. M., Carabajal Paladino, L. Z., Ovruski, S. M. and Cladera, J. L. 2007. Role of visual information and learning in habitat selection by a generalist parasitoid foraging for concealed hosts. Anim. Behav. 74: 131-142.

Segura, D. F., Viscarret, M. M., Ovruski, S. M. and Cladera, J. L. 2012. Response of the fruit fly parasitoid *Diachasmimorpha longicaudata* to host and host-habitat volatile cues. Entomol. Exp. Appl. 143: 164-176.

Sime, K. R., Daane, K. M., Nadel, H., Funk, C. S., Messing, R. H., Andrews, Jr. J. W., Johnson, M. W. and Pickett, C. H. 2006. *Diachasmimorpha longicaudata* and *D. kraussii* (Hymenoptera: Braconidae), potential parasitoids of the olive fruit fly. Biocontrol Sci. Tech. 16: 169-179.

van Alphen, J. J. M. and Jervis, M. A. 1996. Foraging behaviour. In: Insect natural enemies practical approaches to their study and evaluation (eds. Jervis, M. A. and Kidd, N.): 1-62. Chapman and Hall, London.

van Lenteren, J. C., Bale, J., Bigler, F., Hokkanen, H. M. T. and Loomans, A. J. M. 2006. Assessing risks of releasing exotic biological control agents of arthropod pests. Annu. Rev. Entomol. 51: 609-634

Wharton, R. A. 1989. Classical biological control of fruit-infesting Tephritidae. In: Fruit Flies, Their Biology, Natural Enemies and Control (eds. Robinson, A. S. and Hooper, G.): 303-313. Elsevier, Amsterdam.