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Behavioural study on host plants shared by the predator *Dicyphus errans* and the prey *Tuta absoluta*

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Abstract

In the Mediterranean basin, tomato has been attacked seriously by the exotic tomato borer, *Tuta absoluta* (*Lepidoptera*: *Gelechiidae*), reported also on other *Solanaceae*. Some generalist predators, belonging to the tribe *Dicyphini* (*Hemiptera*: *Miridae*), have demonstrated a good potential in controlling this pest. Among them, the Palaearctic *Dicyphus errans* occurs naturally in organic tomato in NW Italy, preys upon several pests and lives omnivorously on several plants. This predator seems to share various host plants with the tomato borer, characteristic that could be exploited by means of proper habitat modifications to enhance its predation during all the year. Therefore, host plant selection of both predator and prey was investigated in multichoice assays with 10 plant species (aubergine, common bean, broad bean, courgette, datura, European black nightshade, herb-Robert, pepper, potato, tomato). Development and emergence rate of both predator and prey was assessed on tomato and European black nightshade, which is abundant in agro-ecosystems in NW Italy. Furthermore, behavioural responses of *D. errans* reared on tomato or European black nightshade were tested in olfactometer. Both predator and prey females oviposited on all tested plant species. However, significantly higher numbers of *D. errans* nymphs emerged on herb-Robert, whereas *T. absoluta* larvae were found almost exclusively on species of the genus *Solanum*. Despite the numerous eggs, no larvae could develop on courgette. No significant differences were found in developmental time on tomato and European black nightshade for both predator and prey. In olfactometer, *D. errans* did not show any significant preference between tomato and European black nightshade, independently of the rearing plant. In a perspective of conservation biological control, herb-Robert and courgette seem to be suitable plants to use as companion plants and in consociation with tomato to favour the predator and hamper the prey, respectively.

Keywords: multi-choice assays, olfactometer assays, tomato, European black nightshade, companion plants

INTRODUCTION

In the Mediterranean area, the exotic tomato borer *Tuta absoluta* (*Lepidoptera*: *Gelechiidae*), is responsible for severe yield losses on tomato [*Solanum lycopersicum* (*Solanaceae*)] (Tropea Garzia et al., 2012). Native to South America, from its introduction in 2006, this multivoltine pest has rapidly invaded Europe being able to develop and spread very quickly in suitable agro-ecological conditions (Desneux et al., 2010). Nevertheless, the pest can survive also in adverse climatic conditions (e.g., in winter time in Belgium), revealing its capacity to overwinter even at low temperature (Van Damme et al., 2015). Besides tomato, which is its preferred host plant, *T. absoluta* is reported on other genera and species of *Solanaceae* family, both cropped and wild, such as potato, aubergine, European black nightshade, datura and *Nicotiana glauca* (Tropea Garzia et al., 2012). For these characteristics, and for its resistance to a wide range of insecticides, the management of *T. absoluta* needs an ecologically based holistic approach (Ponti et al., 2015). Focusing on biological control, among the arthropod species recorded attacking the tomato borer (more than 70 species), only a few indigenous natural enemies have promising potential to be included in effective and environmentally friendly pest management strategies (Zappalà et al., 2013). Among them, generalist predators, such as *Dicyphus errans*, *Macrolophus pygmaeus* and *Nesidiocoris tenuis* (*Hemiptera*: *Miridae*), proved to prey actively on eggs and first larval instars of *T. absoluta* (Urbaneja et al., 2009; Mollá et al., 2011, 2014; Ingegno et al., 2013). Therefore, they have been successfully used in augmentative biological control programmes (Calvo et al., 2012; De Backer et al., 2014).

Dicyphus errans occurs naturally in IPM and organic tomato in NW Italy (Ingegno et al., 2009), preying upon several pests (e.g., whiteflies, spider mites, thrips). It lives omnivorously on many host plants (over 150 species), especially on glandular hairy plants, including both crop plants (e.g., tomato, aubergine, potato, courgette, pot marigold) and noncrop plants (e.g., European black nightshade, *Geranium* spp., hedge nettle and common nettle) (Voigt, 2005; Voigt et al., 2007; Ingegno et al., 2008). Some plants are hosts shared by the indigenous predator and the exotic pest, and consequently predation could be active over the whole year. These aspects could be exploited to enhance the presence and abundance of this generalist predator in complex agro-ecosystems by means of proper habitat modifications. Therefore, the research was aimed at studying the behaviour of *D. errans* and *T. absoluta* on different cultivated and wild plants. In particular, host preference of both predator and prey were checked on 10 plant species (aubergine, common bean, broad bean, courgette, datura, European black nightshade, herb-Robert, pepper, potato, tomato) through multi-choice assays. Furthermore, since European black nightshade is widespread and abundant in the tomato agro-ecosystem in NW Italy, development and survival of *D. errans* and *T. absoluta* on these two hosts were investigated. At the same, to evaluate any possible effect of host plant on the progeny, behavioural responses of *D. errans* adults reared on tomato or on European black nightshade were assessed on uninfested and infested with *T. absoluta* plants in olfactometer assays.

MATERIALS AND METHODS

Colonies of *D. errans* were started from individuals collected on European black nightshade [*Solanum nigrum* (*Solanaceae*)] in Piedmont (NW Italy). They were reared on tobacco [*Nicotiana tabacum* (*Solanaceae*)] for multi-choice assays, and on European black nightshade or tomato plants for olfactometer assays, inside insect cubic cages (47.5 cm edge) (MegaView, Talchung, Taiwan) in climatic chambers at 24±1°C, 55±5% RH, with a L 16:D 8 photoperiod. Individuals were fed with eggs of *Ephestia kuehniella* (*Lepidoptera*: *Pyralidae*) (Bioplanet s.c.a., Cesena, Italy) and dehydrated and decapsuled cysts of *Artemia salina* (*Anostraca*: *Artemiidae*) (La Mangrovia, Ostuni, Italy). Colonies of *T. absoluta* were started from individuals provided by a commercial producer (Bioplanet s.c.a., Cesena, Italy) and reared on tomato plants in net cages (150 W × 150 L × 110 H cm, mesh 0.23 × 0.23 mm) in an experimental heated greenhouse at 27±3°C and 55±23% RH. Ten plant species were selected among hosts and non-hosts for *D. errans* and *T. absoluta*, and used in laboratory assays: tomato 'Marmande', potato 'Villastellone', aubergine 'Bellezza nera', pepper 'Quadrato d'Asti', datura, European black nightshade, courgette 'Nero di Milano', herb-Robert, common bean 'Borlotto lingua di fuoco', broad bean 'Aguadulce Supersimonia'. Seeds were sown in plastic containers (Ø 14 cm), daily watered and fertilized. All the plants were cultivated in the heated greenhouse, at 27±3°C and 55±23% RH, and used when they reached approximately 25-40 cm height with a similar leaf area.

To assess the preference of *D. errans* and *T. absoluta* towards the selected 10 plant species, multi-choice assays were carried out in net cages (150 W × 150 L × 110 H cm, mesh 0.23×0.23 mm) in the heated greenhouses at 27±3°C and 55±23% RH. For *D. errans*, two potted plants of each species were randomly placed inside the net cage, and 40 1-week-old females and 20 males were released (i.e., 2 females and 1 male per plant). After 72 h, the plants were removed and inspected to count the adults; then, they were singly isolated in Plexiglas cylinders (Ø 12 cm, height 50 cm), and checked for nymph emergence every 48 h until no nymphs were seen for a week. For *T. absoluta*, three potted plants of each species were placed inside the net cage, in which 120 adults were released (i.e., 4 adults plant-1). After 48 h, the plants were inspected to count the eggs; then, they were singly isolated in Plexiglas cylinders (Ø 12 cm, height 50 cm), and checked for larval emergence after a week. For both predator and pest, five repetitions were done.

The survival rate and development time of *D. errans* and *T. absoluta* on tomato and on European black nightshade were assessed in climatic chambers at 24±1°C, 65±5% RH, and L 16:D 8. For *D. errans*, 20 newly emerged nymphs (<1-day-old) were placed individually on leaf disks (Ø 25 mm) of each plant species in single cells in 12-well tissue culture plate (Falcon, NJ, USA) on wet Gypsum plaster to maintain humidity, together with *A. salina* cysts (about 0.02 g) and closed with Parafilm®, and observed until adulthood or death. For *T. absoluta*, 15 eggs of the tomato borer were transferred on a potted plant of each plant species placed inside a Plexiglas cage (20×20×30 cm). All the emerged adults were counted. Six repetitions were done.

To evaluate any possible effects of host plant on progeny, behavioural responses of females and males of *D. errans* reared on tomato or European black nightshade were tested in olfactometer assays. The following six comparison experiments were performed: 1) uninfested tomato vs. infested tomato; 2) infested tomato vs. infested European black nightshade; 3) infested tomato vs. uninfested European black nightshade; 4) uninfested tomato vs. infested European black nightshade; 5) uninfested tomato vs. uninfested European black nightshade; 6) uninfested European black nightshade vs. infested European black nightshade. The assays were carried out in a vertical Y-shaped Pyrex tube following the procedure described for *M. pygmaeus* (Ingegno et al., 2011) and already used for *D. errans* (Ingegno et al., 2013). The odour sources consisted of one entire potted plant. Plants were infested by leaving them in the mass rearing of *T. absoluta* for 48 h. The infested plants were kept separately from uninfested plants. For each test, an adult was evaluated only once to prevent any behaviour conditioning by experience. Thirty responses were recorded for each pair of odour sources (overall 720 records). Adults that did not choose a side arm within 20 min were considered as "no choice" and were not counted in the subsequent data analysis. Olfactometer was cleaned, and position was switched as described by Ingegno et al. (2013). The olfactometer assays were conducted at 24±2°C, 50±10% RH, and 150±10 lux.

Percentages of *D. errans* nymphs, and of *T. absoluta* eggs and larvae obtained per plant species in multi-choice assays were arcsine square root transformed, and checked for homogeneity of variance and normality. Then, data of *D. errans* were analysed using one-way ANOVA, and means were compared by Tukey's test, whereas data of *T. absoluta* were compared using Kruskal Wallis analysis, and means were separated by U-Mann Whitney test. Data on developmental time of *D. errans* and *T. absoluta* on tomato and European black nightshade were compared with an independent sample T test. In the olfactometer assays, responses of *D. errans* females were analysed by $\chi 2$ test with significance levels of 95%. The null hypothesis was that predatory females had 50:50 distribution across the two odour sources. Statistical analyses were performed using SPSS v21.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Dicyphus errans nymphs emerged on all tested plant species, even if in highly variable amounts. Significantly higher numbers were observed on herb-Robert than on broad bean and potato (ANOVA, DF=9, 49, F=2.441, P=0.026). Also *T. absoluta* females oviposited on all tested plant species, especially on tomato, European black nightshade, aubergine, courgette and potato. However, larvae were found almost exclusively on species of the genus *Solanum*, on which significantly higher numbers of both eggs and larvae were observed (Kruskal Wallis: eggs, DF=9, $\chi 2$=94.307, P<0.001; larvae, DF=9, $\chi 2$=115.438, P<0.001). Tomato was the most preferred species for both oviposition and larval emergence. By contrast, no larvae could develop on courgette despite the numerous eggs found.

For both predator and prey, no significant differences were found in developmental time from egg-hatching to adulthood on tomato and European black night shade (*D. errans*: T test, DF=1, 28, F=0.656, P=0.425; *T. absoluta*: T test, DF=1, 28, F=0.656, P=0.425). Development on tomato and European black nightshade took 15.29±0.34 and 14.71±0.44 d, respectively, for *D. errans*, and 18.54±0.20 and 18.50±0.21 d, respectively, for *T. absoluta*.

In olfactometer assays, no differences between the tested odour sources were found in most comparison, except for uninfested tomato vs. infested tomato where females reared on European black nightshade preferred the infested plant ($\chi2$=4.80, P=0.028) while males reared on tomato chose uninfested tomato ($\chi2$=4.80, P=0.028) (Figure 1).

DISCUSSION

Among the tested crop plant species, tomato and aubergine were the most favourite hosts, while broad bean was the least chosen for both *D. errans* and *T. absoluta*. Courgette, even if selected by the pest for oviposition, did not allow the larval development, whereas it was one of the favourite host plants of *D. errans*. This discordance may be due to either morphological characteristics or defence mechanisms in response to egg presence, impacting embryonic development (Bawin et al., 2015). A possible inhibition of embryonic development could be exploited at the within-crop level in organic farming systems, by adopting consociation of courgette and tomato to reduce pest populations.

Among the non-crop plant species, datura, even if it is reported as host for the tomato borer (Tropea Garzia et al., 2012), was unattractive neither for *D. errans*, nor for *T. absoluta*, as already observed (Bawin et al., 2015). The European black nightshade was one of the most favourite non-crop plants for both species. Therefore, it can act as reservoir of predator, but also of prey in the agro-ecosystem. By contrast, herb-Robert was the most attractive plant species for *D. errans* but not for *T. absoluta*. Consequently, it could be a potential companion plant in a perspective of habitat management, to enhance the early crop colonization by the predator and preserve the crop production from pest attacks, as reported by Lambion (2010). Further studies on practical use of this plant species as flower strips should be performed.

Olfactometer results showed some discrepancies in comparison with previous studies, in which *D. errans*, like other dicyphine species, was more attracted by infested plants than uninfested tomato plants (Ingegno et al., 2011, 2013). In this study, only in two comparisons out of 24 *D. errans* chose significantly an odour source. In particular, females preferred infested tomato to uninfested one. This preference could be due to the search of the best substrate as reproduction site. The similar response to the odour emitted by uninfested tomato and European black nightshade confirmed the results obtained in the multi-choice assays, where both plant species were equally attractive. Moreover, the behavioural responses were not affected by the plant used for rearing, suggesting the lack of influence of the natal experience.

According to our results, the omnivorous and widespread *D. errans* could be a key predator of the exotic tomato borer, allowing a higher probability of encountering on several plant species and a consequent more successful pest control in organic greenhouses.

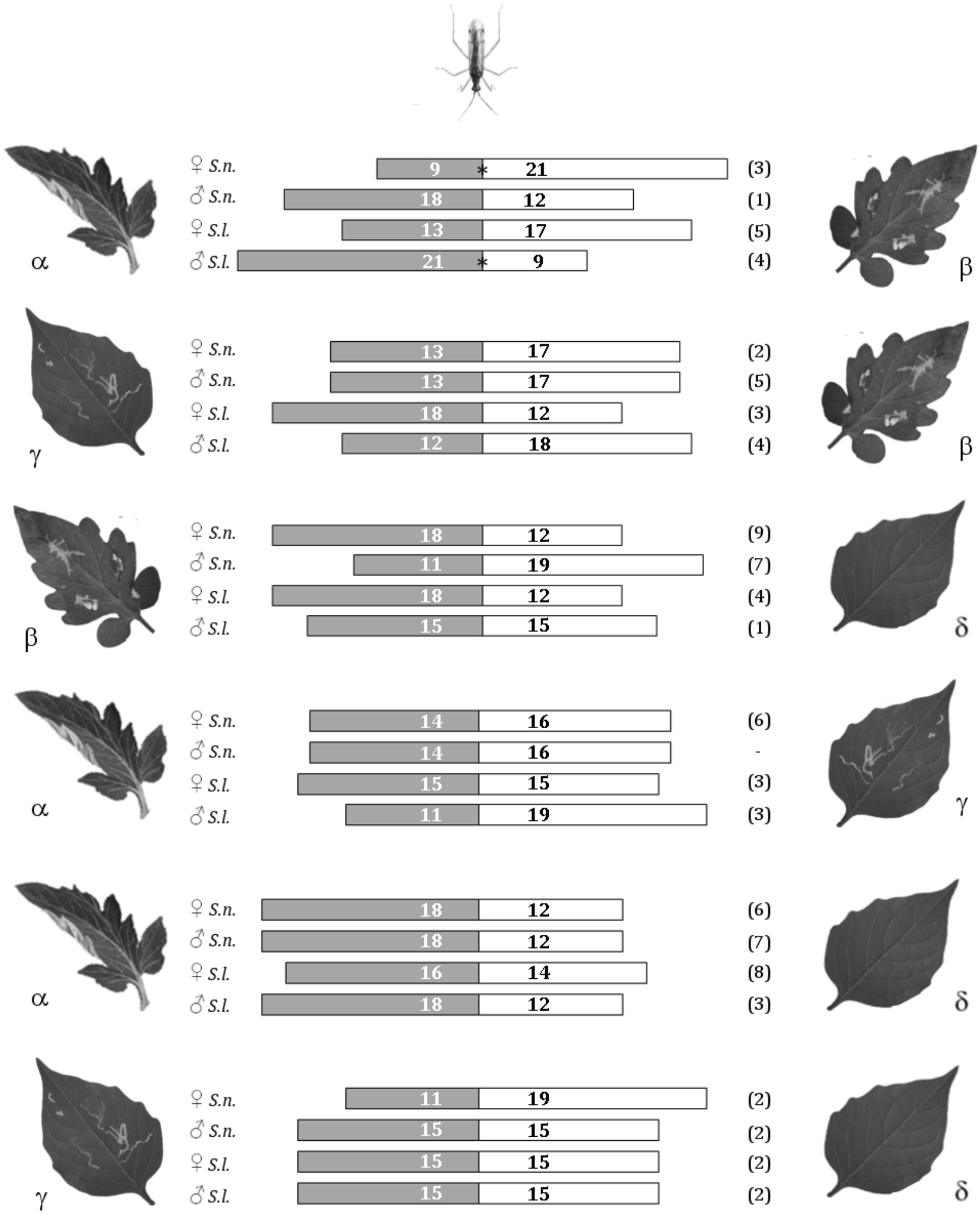

Figure 1. Responses in olfactometer assays of females and males of *Dicyphus errans* reared on tomato *Solanum lycopersicum* (*S.l.*) or European black nightshade *Solanum nigrum* (*S.n.*) to the following odour sources: uninfested tomato (α), tomato infested with *Tuta absoluta* (β), uninfested European black nightshade (δ), European black nightshade infested with *Tuta absoluta* (γ). Number inside bars represents the number of choices, while no choice is reported in brackets.

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