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Adaptation of Indigenous Larval Parasitoids to *Tuta absoluta* in Italy

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Abstract

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) is a serious threat to tomato crops in South America. In Europe, after its first detection in Spain in 2006, it rapidly spread through the Mediterranean basin, reaching Italy two years later. The aim of our work was to find indigenous effective biological control agents and to evaluate their potential role in the control of larval populations of *T. absoluta* (tomato borer) in controlled conditions. Nine species of larval parasitoids emerged from field-collected tomato leaves infested by *T. absoluta*. The most abundant, *Necremnus* near *artynes* (Walker) and *N.* near *tidius* (Walker) (Hymenoptera: Eulophidae), were tested in laboratory parasitism trials. Furthermore, since the species *N. artynes* and *N. tidius* are each reported in literature as an ectoparasitoid of *Cosmopterix pulchrimella* Chambers (Lepidoptera: Cosmopterigidae) on upright pellitory plants, olfactometer bioassays were performed to assess the response of our parasitoids to the odors of tomato and pellitory leaves infested by *T. absoluta* and *C. pulchrimella*, respectively, compared with healthy ones. Both *Necremnus* species showed good adaptation to the invasive pest, and we observed a high larval mortality of *T. absoluta* due to host feeding and parasitism. Even olfactory responses highlighted a preference of both wasps for tomato plants infested by the exotic pest. These preliminary results demonstrated a high suitability of these indigenous natural enemies for controlling the tomato borer. Further investigations are therefore needed to confirm their role as potential biological agents in commercial tomato plantations.

Keywords

biological control, tomato borer, native natural enemy, exotic invasive pest, *Necremnus* spp.

Native to Central America, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (tomato borer) has been a pest of tomato crops in South American countries since 1970, and is distributed in Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Paraguay, Peru, Uruguay and Venezuela (EPPO 2005). In 2006, it was accidentally introduced into Spain, and in the past few years has spread rapidly through many countries bordering the Mediterranean Sea, including Italy where it was first reported in 2008 (Urbaneja et al. 2009, Viggiani et al. 2009, CABI 2011). The species was added to the European and Mediterranean Plant Protection Organization (EPPO) A1 and A2 action lists for regulation as quarantine pests in 2004 and 2009, respectively, and is now considered one of the major pests on tomato crops. In fact, while its main host is tomato, infestation of potato, eggplant, pepper, tobacco, and common bean is also reported. Moreover, various solanaceous species such as *Solanum nigrum* L., *S. elaeagnifolium* Cav., *S. puberulum* Nutt, *Datura stramonium* L., *D. ferox* L. and *Nicotiana glauca* Graham are reported as wild hosts (EPPO 2005, EPPO 2009, CABI 2011).

Tuta absoluta is a multivoltine species; females lay eggs on epigeal parts of their host plants and a single female can lay up to 260 eggs during its lifetime (EPPO 2005). Through their feeding activity within the mesophyll of the leaves, the larvae produce large mines, thus affecting the plant's photosynthetic capacity; furthermore, they burrow into stalks, apical buds, and fruits. The pest affects tomatoes destined for the fresh market as well as for processing, with larvae causing losses in its area of origin of up to 80–100% (Desneux et al. 2010). Pupation may take place in the soil, on the leaf surface or within the mines, depending on environmental conditions (EPPO 2005).

The tomato borer is a very challenging pest to control. Chemical approaches are difficult because of the mine-feeding behavior of larvae, in addition to the resistance developed to many conventional insecticides and the side effects for useful organisms in integrated pest management (IPM) programs (Siqueira et al. 2000, Lietti et al. 2005, Cabello et al. 2009, IRAC 2011). So, as an alternative approach, biological control has been actively pursued. Several natural enemies occurring in its native area have been reported as fully documented by Desneux et al. (2010), and the efficacy of entomopathogenic fungi, bacteria and nematodes have also been evaluated for the implementation of biological control strategies (Batalla-Carrera et al. 2010, González-Cabrera et al. 2010, Pires et al. 2010, Hernández-Fernández et al. 2011). Natural enemies have been investigated in many South American countries with the aim of using them in biological control programs; in particular, research has been carried out on egg parasitoids such as *Trichogramma pretiosum* (Riley), *T. nerudai* (Pintureau & Gerding), *T. exiguum* Pinto & Platner and *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae) (Pratissoli and Parra 2000, Zucchi and Querino 2000, Faria et al. 2008, Desneux et al. 2010, Virgala and Botto 2010), and larval parasitoids such as *Apanteles gelechiidivoris* Marsh, *Pseudapanteles dignus* (Muesebeck), *Bracon* spp. (Hymenoptera: Braconidae), *Dineulophus phthorimaeae* (de Santis) (Hymenoptera: Eulophidae), and *Diadegma* spp. (Hymenoptera: Ichneumonidae) (Colomo et al. 2002, Marchiori et al. 2004, Miranda et al. 2005, Bajonero et al. 2008, Sánchez et al. 2009, Luna et al. 2010). However, none of these beneficial organisms seem to have so far been decisive in controlling *T. absoluta* and the research is still ongoing.

Indigenous predators and parasitoids with the ability to control this exotic leafminer have also been investigated throughout the Mediterranean area. Larval parasitoids such as *Necremnus* spp. and *Stenomesius* spp. (Hymenoptera: Eulophidae) (Arnó and Gabarra 2010, Gabarra and Arnó 2010, Rizzo et al. 2011, Zappalà et al. 2011a), and predators such as *Macrolophus pygmaeus* (Rambur), *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae), *Nabis pseudoferus* (Remane) (Hemiptera: Nabidae) (Urbaneja et al. 2009, Mollá et al. 2010, Fois et al. 2011, Zappalà et al. 2011b), have been reported in Spain and more recently in Italy. Additionally, the shift of other indigenous entomophagous species, especially braconids and ichneumonids, onto the exotic pest has been documented by recent literature (Loni et al. 2011, Rizzo et al. 2011, Zappalà et al. 2011a), revealing a gradual adaptation of these generalist parasitoids to the new host. The egg parasitoid *Trichogramma achaeae* Nagaraja & Nagarkatti, which is however reported in the list of the hymenopteran species alien to Europe (Rasplus et al. 2010), was also tested in inundative biological control programs, and has become commercially available following its promising potential (Cabello et al. 2009, Desneux et al. 2010).

Due to the huge potential of *T. absoluta* to expand its distribution range, a multidisciplinary approach exploiting natural enemies in several countries is gaining favor. With the aim of searching for indigenous parasitoids able to adapt to the new host, and which are suitable for mass-rearing and augmentation biological control programs, field surveys were firstly carried out in horticultural areas of Italy. The species that most frequently emerged from infested tomato, and that proved to be fit for mass-rearing, were therefore tested in the laboratory to evaluate their effectiveness in controlling *T. absoluta*; to contribute further to the implementation of effective and environmentally friendly control strategies, their ability to recognize and choose new host larvae was also assessed.

Materials and Methods

Field Collection and Rearing of Parasitoids. In the two-year period 2009–2010, surveys were carried out in 24 IPM-protected tomato fields located in four Italian horticultural areas: Liguria (four fields), Campania (six fields), Sardinia (five fields), and Sicily (nine fields), where *T. absoluta* was established on tomato plants. In the surveyed sites, samples of 100 infested leaves were randomly collected from tomato crop once or twice in the mid-growing season, placed in sealed plastic bags, and transferred to the laboratory. All the leaves were carefully checked using a microscope for the presence of tomato borer larvae and pupae, in order to exclude any other insect infestation. The leaves were then placed in cages in climatic chambers at 24 ± 1°C, 60 ± 5% RH, 16:8 h (L:D) photoperiod to detect the emergence of possible larval parasitoids. Each cage consisted of a cardboard box (40 × 60 × 30 cm) with a tight-fitting lid and two clear jars extending from two holes in its side. When the adults emerged, they were attracted to the light entering through the jars where they then accumulated and were easily collected with the aid of an insect aspirator. The emerged adult parasitoids were killed with ethyl acetate and stored in 70% ethanol in glass test tubes (60 mm high × 8 mm diameter) until identification. The parasitoids belonging to the

Eulophidae family were then identified at the DIVAPRA (Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, University of Torino, Italy) (identifications by co-author P. N.), while all other parasitoid species were sent to the respective specialists. Voucher specimens were deposited at the DIVAPRA.

The most abundant species of the emerged parasitoids were selected on the basis of their suitability for mass-rearing, as tested at the Bioplanet laboratories (Bioplanet s.c.a., Cesena, Italy). Therefore, two species of the genus *Necremnus* that had proved to be fit for mass-rearing on *T. absoluta* on tomato plants at the Bioplanet laboratories, were tested at the DIVAPRA laboratories to assess their effectiveness in controlling the pest, and to study their behavior and host preference under laboratory conditions. Before using them in the experiments, the parasitoid adults from Bioplanet were sexed, fed every 48 h with drops of honey on cardboard, and kept in individual glass tubes (120 mm high × 18 mm diameter), in a climatic chamber at 24 ± 1°C, 60 ± 5% RH, 16:8 h (L:D) photoperiod.

Insect Colonies and Host Plant Management. Colonies of *T. absoluta* were established starting from an initial culture collected in a tomato commercial plantation in Liguria (NW Italy). A continuous mass-rearing of all development stages was maintained on tomato plants in an open-sided greenhouse, in cages (150 × 150 × 110 cm) that had a stainless steel frame structure supporting an insect-proof net (mesh 0.23 × 0.23). Plants of *Lycopersicon esculentum* Mill. Montecarlo F1 variety were used for both mass-rearing and laboratory trials. In particular, plants used in the parasitism trials were approximately 25 cm high from the soil surface, in pots filled with a mixture of soil and covered with a layer of white sand in order to easily detect possible individuals dropped off during trials.

Moreover, for olfactometer bioassays, colonies of *Cosmopterix pulchrimella* Chambers (Lepidoptera: Cosmopterigidae), reported as a natural host of the related *Necremnus* species (Bernardo and Viggiani 2002, The Natural History Museum 2011), were also established, starting from an initial culture collected on upright pellitory [*Parietaria officinalis* L. (Urticaceae)] in

Piedmont (NW Italy), and maintained in the cages described above. Upright pellitory plants were collected in wastelands and cuttings were taken in order to obtain new plants for *C. pulchrimella* rearing.

All the plants were cultivated in plastic pots (diameter 20 cm), watered daily and fertilized, and kept in an open-sided greenhouse at 27 ± 3°C, 55 ± 23% RH, 16:8 h (L:D) photoperiod.

Parasitism Trials. Before testing the selected parasitoids for their effectiveness in controlling tomato borer, laboratory trials were performed to assess if the parasitoid females showed any preference for different instar larvae. Potted tomato plants were infested with 20 larvae of *T. absoluta* of different instars, and encaged in Plexiglas cylinders (40 cm high × 18 cm diameter). Newly molted larvae (i.e., five for each of the four instars) were chosen and randomly distributed over the plant. Single five-day-old females ready to oviposit, after being mated and fed with honey, were introduced and maintained on the infested tomato plants for 48 h. Following this, the females were removed and the larvae were observed under a stereomicroscope to determine if they were dead or alive. Five replicates were performed for each parasitoid species.

To evaluate the effectiveness of the selected parasitoids as biocontrol agents, tomato leaves with *T. absoluta* larvae no older than 24 h were collected from mass-rearing cages, and the portion of the leaf which contained the larva inside the mine was cut off after checking that the mine was not empty using the stereomicroscope. This leaf portion was then fixed onto a leaf of healthy potted tomato plant with the aid of a drop of silicone. On each tomato plant consisting of four leaves, 20 leaf sections were fixed homogeneously, so as to obtain a density of five larvae per leaf to ensure a consistent infestation while preventing plant collapse. The potted plant was placed separately inside a Plexiglas cage (20 × 20 × 30 cm) with two sides and a lid of fine gauze (30/10 net) and a mesh sleeve inserted in the middle of a side (diameter 11 cm) to allow access to the plant.

Larvae were left for 48 h to allow them to transfer from the leaf section to the plants and establish new galleries. One female parasitoid was then released into each cage and removed five days later. Five-day-old females ready to oviposit were used, after being mated and fed with honey inside the rearing glass tubes. Ten replicates were performed for each parasitoid species and 10 plants were also set up as control.

Cages were checked for parasitoid and moth emergence every day, and all the emerged individuals were aspirated off the cage through the sleeve and counted; the parasitoids were then sexed and sex ratio was provided. Collection continued for two weeks after initial emergence. After 30 days, each experimental cage was dismantled and all the leaves observed under a stereomicroscope.

All the trials were performed in a climatic chamber at 24 ± 1°C, 60 ± 5% RH, 16:8 h (L:D) photoperiod.

Moreover, single females of each parasitoid species were offered tomato leaves with five first-instar larvae, placed on a wet filter paper inside a Petri dish (diameter 10 cm), in order to obtain preliminary data on their behavior in relation to parasitism and host feeding. So, females, mated, fed with honey, and not experienced with a host were used for the experiments. Their behavioral sequence (e.g., host location and acceptance, oviposition, host-feeding) on larvae of *T. absoluta* was observed using a steromicroscope for 30 min, and 10 replications were carried out for each parasitoid species.

Olfactometer Bioassays. In the olfactometer bioassays, five day-old females of the selected parasitoid species were used to assess their olfactory responses to the odors of tomato plants uninfested and infested by *T. absoluta*, and to the odors of *P. officinalis* uninfested and infested by *C. pulchrimella* as an alternative host. In particular, three comparisons were carried out for each parasitoid species: a) upright pellitory leaves infested by *C. pulchrimella* compared to tomato leaves infested by *T. absoluta*; b) healthy upright pellitory leaves compared to healthy tomato leaves; and c) healthy tomato leaves compared to tomato leaves infested by *T. absoluta*. Each odor source consisted of five tomato leaflets or pellitory leaves, uninfested or infested with a density of three larvae per leaf, for a total of 15 larvae. Before trials, insects were kept at 15°C without any host or plant in a glass tube for 18 h with a humid cotton cap and microdrops of honey. The bioassays were carried out in a Y-shaped Pyrex tube (internal diameter 1.2 cm) formed by an entry arm and two side arms, each 10 cm long (70° angle), and positioned horizontally. Each side arm was connected to a round modified beaker (250 mL volume capacity, diameter 9 cm) as an odor-source container. Airflow was provided by an air pump (Air 275R, Sera, Germany), then filtered in an activated CO_2 filter, regulated with a flow meter at 2.5 L min^{-1} (EK-2NRK, Comer, Italy) and humidified in a 1-L water bubbler half-filled with deionized water. After the flow was established, a single parasitoid female was introduced into the entry arm. Each female was observed until she had moved at least 2 cm up one of the side arms or until 10 min had elapsed. Females that did not choose a side arm within 10 min were considered as "no choice" and were not counted in the subsequent data analysis. For each test, a female was evaluated only once to prevent any behavior conditioned by experience. The odor sources chosen by females that responded were recorded. Thirty responses were recorded for each pair of odor sources. After testing five females, odor sources were switched between the left-hand and right-hand side arms to minimize any spatial effect on choices. The Y-tube and cameras were cleaned with mild soap and alcohol ($70\%_v$) and sterilized in an autoclave at 120°C for 20 min. The olfactory bioassays were conducted at 24 ± 2°C, 50 ± 10% RH and 150 ± 10 lux.

Statistical Analyses. Significance of mean values of the data was analyzed by one-way analysis of variance (ANOVA), after ascertaining the homogeneity of variance (Levene's test). In the olfactory bioassays, responses of parasitoid females were analyzed by a Chi-square test. The null hypothesis was that parasitoid females had a 50:50 distribution across the two odor sources. All analyses were performed using the software SPSS version 17.0 (SPSS, Chicago, IL, USA).

Results

Nine species of indigenous parasitoids emerged from tomato leaves infested by *T. absoluta* collected in Arma di Taggia (43°50'54" N - 07°50'27" E), Ceriale (44°06'0" N - 08°13'60" E), Pula (39°00'36" N - 09°00'6" E), Portopalo (36°40'24" N - 15°05'20" E), Sampieri (36°46'60" N - 14°41'60" E), namely: *Necremnus* near *artynes* (Walker), *N.* near *tidius* (Walker), *Neochrysocharis* *formosa* (Westwood), *Pnigalio* (=*Ratzeburgiola*) *cristatus* (Ratzeburg), *Pnigalio* sp. *soemius* complex (Hymenoptera: Eulophidae), *Diadegma ledicola* Horstmann (Hymenoptera: Ichneumonidae), *Bracon osculator* (Nees), *B. hebetor* Say, and *Agathis* sp. (Hymenoptera: Braconidae) (Table 1). The presence of these parasitoids varied in relation to the surveyed area; however the most abundant species were *N.* near *artynes* coming from Sardinia and Sicily, and *N.* near *tidius* and *D. ledicola* from Liguria (Table 1).

In preliminary multiplication trials performed at the Bioplanet, *D. ledicola* was found to be not suitable for wasp offspring and was subsequently discarded, whereas both *Necremnus* species proved to be fit for mass-rearing on *T. absoluta* on tomato plants (data not shown). Therefore, populations of *N.* near *artynes* and *N.* near *tidius*, obtained from tomato leaves collected in Pula and Arma di Taggia respectively, and mass-reared at the Bioplanet, were used for parasitism and olfactory bioassays.

Parasitism Trials. In the experiments, when different instar larvae were simultaneously offered, both *N.* near *artynes* and *N.* near *tidius* females were shown to prefer and set on larvae of the first two instars, which were well-accepted as hosts, both for feeding and oviposition ($F = 87.55$; $df = 3$; $P < 0.001$ for *N.* near *artynes*; $F = 195.85$; $df = 3$; $P < 0.001$ for *N.* near *tidius*). In fact, high percentages of larvae of the first- and of the second-instar were killed by the wasps, whereas no larvae of the third- and fourth-instar were attacked (Table 2).

Therefore, the effectiveness of *N.* near *artynes* and *N.* near *tidius* as biocontrol agents was evaluated on larvae of the first two instars on potted tomato plants in cages. In these experiments, a high mortality of *T. absoluta* larvae was recorded when they were exposed to the parasitoids, with statistically significant differences between control and treated plants. The average number of adults of the emerged moths (± SE) was 2.6 ± 0.9 and 1.2 ± 0.5 in the presence of *N.* near *artynes* and *N.* near *tidius*, respectively, in contrast to 17.2 ± 0.6 in the control ($F = 180.28$; $df = 2$; $P < 0.001$).

Females of both *Necremnus* species were able to parasitize host larvae, and the average offspring (± SE) obtained was 2.7 ± 1.1 and 1.5 ± 0.4 with a sex ratio of 1:3.5 and 1:1♀:♂) for

N. near *artynes* and *N.* near *tidius*, respectively. The developmental time from the introduction of the wasps in the cage to the emergence of the progeny was shorter for *N.* near *artynes* at 10.2±0.1 days and longer for *N.* near *tidius* at 14.3±0.2 days, while at least three weeks were needed for the adult moths to emerge. In the leaves observed with the stereomicroscope, 64.8% and 78.6% of the larvae exposed to *N.* near *artynes* and *N.* near *tidius*, respectively, were found dead inside the mines. Considering the death rate in control plants (14.0%), the previous mortalities can be ascribed to host-feeding or to failed larval development of the parasitoid.

Females of both *Necremnus* species showed the same behavior when they were introduced into the arena with tomato leaves infested by first-instar larvae of *T. absoluta*. First, the females walked and searched at random on the leaves until they located the mine. Then, they explored the mine inserting the ovipositor repeatedly at different points; they were guided by the host larva's movement until they could sting to inject venom and paralyze the larva. The ovipositing females laid one or more eggs per larva at different points of the mine, whenever reinserting the ovipositor. The location of eggs was related to the size and shape of the mine. Since the larva became paralyzed many hours after being injected by venom from the parasitoid female, the eggs were generally laid far from the host to be safe from its movement. After oviposition, the parasitoid females did not touch nor brush the point of insertion with the tip of the gaster. The females showed a more aggressive behavior when they killed the larva for host-feeding. In fact, they pierced the body of the larva with their ovipositor repeatedly, causing an irreversible paralysis followed by death. Drilling was accomplished by inserting the ovipositor, and swinging the valves up and down against each other. Once the ovipositor was completely withdrawn, females began to suck the haemolymph exuded at the puncture. This behavior lasted for up to 2–3 min.

Olfactometer Bioassays. Since the species *Necremnus artynes* (Walker) and *N. tidius* (Walker) are reported in literature as ectoparasitoids of *C. pulchrimella* on upright pellitory (Bernardo and Viggiani 2002, The Natural History Museum 2011), the olfactometer bioassays were performed to testing the attractiveness both of tomato and of pellitory plants for the wasp females.

Almost all the females tested in olfactometer bioassays responded by making a choice within the fixed time. In the experiment with *N.* near *artynes*, females proved to be more attracted by infested tomato compared to infested upright pellitory plants (χ^2 = 19.20; df = 1; P < 0.001), and by healthy tomato compared to healthy upright pellitory plants (χ^2 = 4.80; df = 1; P = 0.028). Significant differences in responses of *N.* near *artynes* females were also found when comparing infested with healthy tomato plants (χ^2 = 10.80; df = 1; P = 0.001) (Figure 1). In the experiment with *N.* near *tidius*, no significant preference of females was detected between healthy tomato and healthy upright pellitory plants (χ^2 = 0.53; df = 1; P = 0.465), and between infested tomato and infested upright pellitory plants (χ^2 = 3.33; df = 1; P 0.068). By contrast, results for infested tomato plants showed them to be largely attractive in comparison with healthy plants (χ^2 = 8.53; df = 1; P = 0.003) (Figure 2).

Discussion

Tuta absoluta is considered one of the major pests on tomato and other solanaceous crops, both in the field and under protected conditions (EPPO 2005). Because of the ongoing spread of this exotic moth throughout Europe and the lack of totally satisfactory effective management options, most control attempts have moved towards a biological control approach. Several natural enemies have been reported, in particular Eulophidae, Braconidae and Trichogrammatidae as parasitoids, and Miridae, Nabidae and Pentatomidae as predators (Desneux et al. 2010). Nevertheless, some species that we obtained from field-collected samples are recorded here for the first time as larval parasitoids of *T. absoluta*. In fact, although species of the genera *Agathis*, *Bracon* (Hymenoptera: Braconidae), and *Diadegma* (Hymenoptera: Ichneumonidae) are already known as larval parasitoids of the tomato borer in South America (Colomo et al. 2002, Marchiori et al. 2004, Miranda et al. 2005), *B. hebetor* and *D. ledicola* have not been previously reported, while *A. fuscipennis* (Zetterstedt) and *B. osculator* along with *D. pulchripes* (Kokujev) have been recently observed in

Tuscany (Loni et al. 2011) and Sicily (Zappalà et al. 2011a). However, these species are larval parasitoids of many Lepidoptera species (Milonas 2005).

At the same time, several species belonging to the Eulophidae family have been reported among larval parasitoids of *T. absoluta* in South America, and now also in the Mediterranean area (Desneux et al. 2010). These data are not surprising because this family includes several parasitoids of leafminers and gall-making larvae, often able to adapt to exotic hosts. Although records of *Neochrysocharis formosa*, *Pnigalio cristatus* and a species of the *P. soemius* complex have also been recently reported (Luna et al., 2011; Zappalà et al. 2011a), parasitoids of the genus *Necremnus* emerged from larvae of tomato borer in our studies as well as in others (Mollá et al. 2008, 2010; Gabarra and Arnó 2010, Fois et al. 2011, Rizzo et al 2011, Zappalà et al. 2011a). Thus, when considering their suitability for mass-rearing as assessed at the Bioplanet, these species appear to be promising native biological control agents. In contrast, *D. ledicola* proved not to be fit for mass-rearing in spite of its abundance on *T. absoluta* in one of the surveyed tomato fields.

The genus *Necremnus* includes solitary and gregarious ectoparasitoids of larvae of coleopteran, lepidopteran and dipteran species (Coudron et al. 2000, Bernardo and Viggiani 2002, Dosdall et al. 2007), but most species have been poorly investigated so far and very little information, not always reliable, is available in the literature on their life history, behavior, and above all their hosts. For example, *N. tidius* is a solitary ectoparasitoid of coleopteran larvae with a Holarctic distribution (Gibson et al. 2005); Diptera and Lepidoptera have also been listed among its hosts but the non-coleopteran host association probably resulted from incorrect identification of the parasitoid (Dosdall et al. 2007). This misidentification would seem to be further confirmed by the different behavior of females after oviposition. In fact, unlike what has been observed for females of *N. tidius* (Dosdall et al. 2007), in our experiments females did not touch and brush the mine after oviposition. Additionally, some characters of *N. artynes*, described in the keys of Boucek (1958), Graham (1959) and Askew (1968), did not match completely those of our individuals. Hence, we chose to name the species emerged from *T. absoluta* and here studied as *N.* near *tidius* and *N.* near *artynes* to avoid any misidentification while awaiting their systematic clarification.

Despite their specific identification requiring further investigation, the results obtained from laboratory experiments demonstrated that both *Necremnus* species effectively recognized and parasitized *T. absoluta* in our caged experiments. Therefore, these parasitoids are able not only to accept this new host, as also observed in other studies (Gabarra and Arnó 2010, Mollá et al. 2010), but can also reduce significantly infestations of the tomato borer under laboratory conditions. Parasitoid females exhibited a preference for larvae of the first two instars, on which they could oviposit or feed. Moreover, females of both species proved to be able to recognize and choose tomato leaves infested by *T. absoluta* larvae in the olfactometer bioassays. In particular, females of *N.* near *artynes* showed a very strong response to tomato leaves, both uninfested and infested, which could explain the frequent record of this native parasitoid on the exotic pest. It now remains to be assessed what stimuli form the trigger for behavioral responses of these parasitoids in order to obtain a better understanding of their behavior.

In our experiments the impact of the parasitoids was calculated as all dead hosts resulting from the presence of the parasitoids, not only hosts utilized for parasitoid reproduction. Females of both *Necremnus* species were observed to kill *T. absoluta* larvae and feed on their haemolymph; they probably need to feed on the host for maintenance and/or egg production, as do other synovigenic parasitic wasps (Giron et al. 2004). Destructive host-feeders can be considered to be better biological control agents even if host-feeding must not be used as the sole selection criterion (Jervis et al. 1996). However, in our experiments the impact of host-feeding by both *Necremnus* species could be overestimated. In fact, the females were kept in contact with the same larvae in the cages for five days, and consequently the probability that they could feed on the larvae in the mines, where they had previously laid eggs, was increased. When new larvae were offered, on *C. pulchrimella* approx 20% and 80% of host larvae were killed by females of *N.* near *tidius* for feeding and oviposition respectively, whereas host-stinging behavior was very rarely observed (P.

N., unpublished data), unlike other eulophid parasitoids of leafminers such as *Pnigalio soemius* (Walker) (Bernardo et al. 2006). Therefore, the high larval mortality of *T. absoluta* could be due partly to host-feeding and partly to the failure of parasitoid larval development on this new host under laboratory conditions, and needs to be further investigated.

Since conditions in the open field are much more varied than those in the laboratory and, among other factors, temperature is observed to play an important role in the development of arthropods, further investigations are needed to assess the behavior of both *Necremnus* species at different thermal conditions. Moreover, our preliminary findings about the host location and acceptance suggest that both *Necremnus* species have several behavioral traits that positively influence their performance as biological control agents. Hence, studies should be continued to investigate the suitability of the selected host for the parasitoid immature development with the aim of improving the efficiency of mass production and implementing augmentation of biological control programs.

The ability of *Necremnus* species to find and parasitize *T. absoluta* larvae makes them potential candidates for mass production and biological control, adding to the list another example of the adaptation of an indigenous parasitoid to an exotic pest and highlighting the importance of a rich and variegated biodiversity in finding new associations with harmful pests, as already reported in Nicoli and Burgio (1997). In the light of these satisfactory results, additional studies on both *Necremnus* species will be required to clarify their systematic position, detect their primary hosts, and to evaluate them in biological control and IPM programs in commercial tomato plantations.

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Figure captions

Fig. 1. Responses of *N.* near *artynes* (no. of responding females in bars) in a Y-tube olfactometer and, when present, number of non-responding individuals (NS) to the odors of infested (δ), or uninfested (π) tomato plants with *T. absoluta* and of infested (α), or uninfested (β) upright pellitory plants with *C. pulchrimella* for each compared pair. Numbers in bars represent individuals that moved toward the volatiles. χ^2 statistics (*P<0.10; **P<0.05; ***P<0.01; df=1) tested the hypothesis that the distribution of side-arm choices deviated from a null model where odor sources were chosen with equal frequency.

Fig. 2. Responses of *N.* near *tidius* (no. of responding females in bars) in a Y-tube olfactometer and, when present, number of non-responding individuals (NS) to the odors of infested (δ), or uninfested (π) tomato plants with *T. absoluta* and of infested (α), or uninfested (β) upright pellitory plants with *C. pulchrimella* for each compared pair. Numbers in bars represent individuals that moved toward the volatiles. χ^2 statistics (*P<0.10; **P<0.05; ***P<0.01; df=1) tested the hypothesis that the distribution of side-arm choices deviated from a null model where odor sources were chosen with equal frequency.

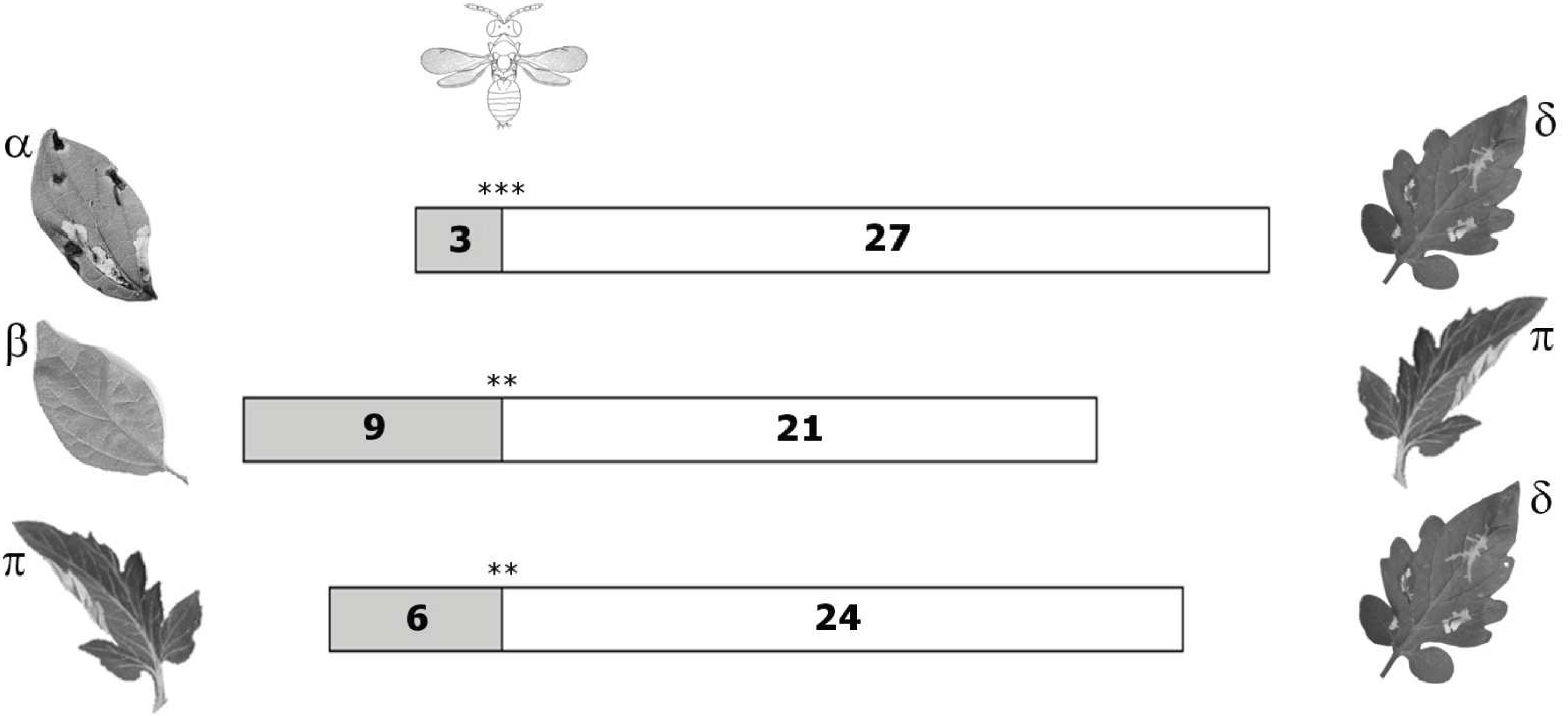
Table 1 Indigenous larval parasitoid species emerged from tomato leaves infested by *T. absoluta*, collected in Italian horticultural areas

Species	Site	No. of emerged individuals	Date
Necremnus near *artynes* (Walker)	Portopalo (SR), Sicily	11	9 July 2009
	Pula (CA), Sardinia	27	21 July 2009 22 April 2010
Necremnus near *tidius* (Walker)	Arma di Taggia (IM), Liguria	30	7 Oct 2009
Neochrysocharis formosa (Westwood)	Ceriale (SV), Liguria	4	26 Aug 2010
Pnigalio (=*Ratzeburgiola*) *cristatus* (Ratzeburg)	Ceriale (SV), Liguria	3	26 Aug 2010 10 Oct 2010
Pnigalio sp. *soemius* complex	Ceriale (SV), Liguria	3	10 Oct 2010
Diadegma ledicola Horstmann	Arma di Taggia (IM), Liguria	25	7 Oct 2009
Agathis sp.	Sampieri (RG), Sicily	3	10 July 2009
Bracon osculator (Nees)	Ceriale (SV), Liguria	6	26 Aug 2010 10 Oct 2010
Bracon hebetor Say	Pula (CA), Sardinia	2	21 July 2009

Table 2 Mean number of *T. absoluta* larvae (±SE) killed by single females of *N.* near *artynes* and *N.* near *tidius*. Each female was simultaneously offered 20 larvae, five for each instar, for 48 h

Species	Mean no. of *T. absoluta* larvae killed by the parasitoid			
	1st-instar larvae	2nd-instar larvae	3rd-instar larvae	4th-instar larvae
N. near *artynes*	4.20±0.37 a	3.80±0.20 a	0.00±0.00 b	0.00±0.00 b
N. near *tidius*	4.80±0.20 a	4.60±0.24 a	0.00±0.00 b	0.00±0.00 b

Within the same line, data (mean±SE) followed by a different letter are significantly different (P<0.001; ANOVA)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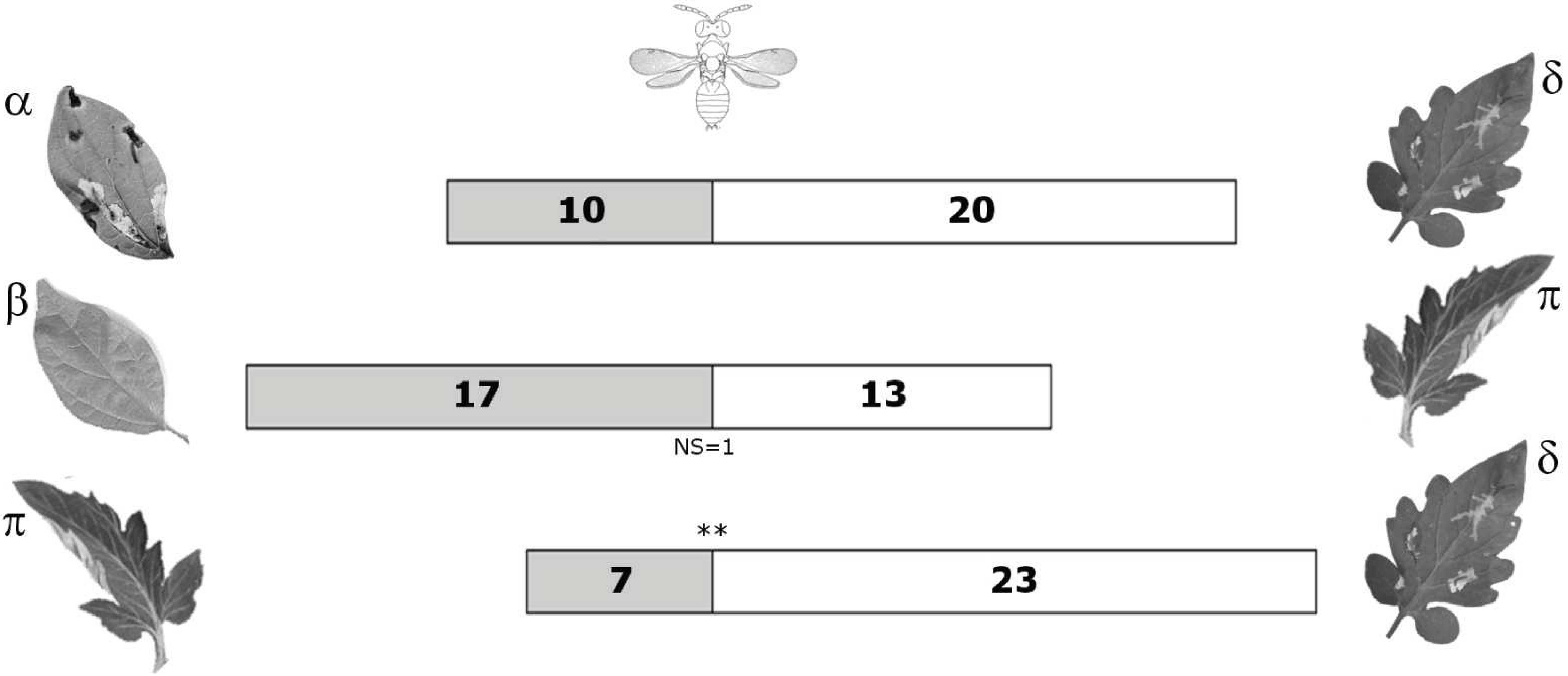
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