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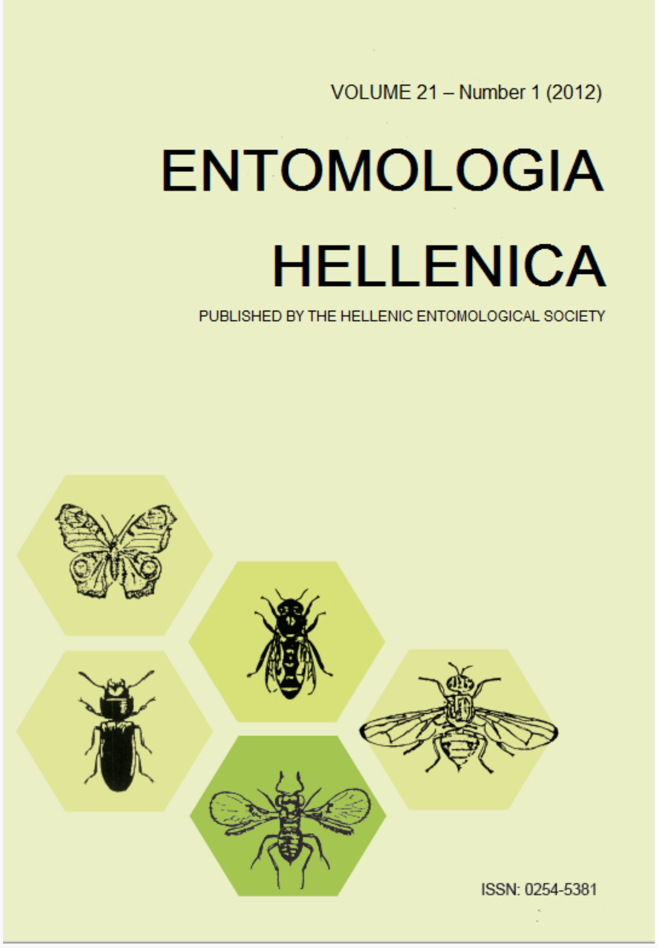

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Selection of *Macrolophus melanotoma* between its main non-crop host plant (*Dittrichia viscosa*) and eggplant, pepper and tomato, in choice experiments

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ABSTRACT

The host plant selection of the predator *Macrolophus melanotoma* (Costa) (=*M. caliginosus* Wagner) (Hemiptera: Miridae) was studied between: i) its major non-crop host plant, *Dittrichia viscosa* L. (W. Greuter) (Asteraceae) and the solanaceous plants eggplant, pepper and tomato, and ii) between eggplant, pepper and tomato, in choice experiments. The experiments were performed in cages where two potted plants without prey were placed. In each replicate, an adult predator was released in the centre of the cage or on the solanaceous plant. The position of the released predator in each cage was recorded at intervals of 20min, 1, 2, and 24h after its introduction. The results showed that *M. melanotoma* had a high selection preference rate for its origin host plant (*D. viscosa*) irrespectively of the solanaceous plant that was present in the cage. The experiments investigating the relative preference of *M. melanotoma* when given access only to solanaceous plants revealed that it preferred eggplant at higher rates than pepper and tomato. These data indicate that *M. melanotoma* originating from *D. viscosa* patches might have a very low potential to colonize pepper or tomato crops in the open field. However, its potential to colonize eggplant crops should be further evaluated.

KEY WORDS: conservation biological control, eggplant, Integrated Pest Management, Miridae, non-crop plants, pepper, tomato, weed.

Introduction

The insect predator *Macrolophus melanotoma* (Costa) (=*M. caliginosus* Wagner) (Hemiptera: Miridae) had been considered to be a major natural enemy of insect pests on tomato crops (i.e. Albajes and Alomar 1999). However, evidence based on crossings and molecular analyses has shown that *Macrolophus* specimens recorded on tomato in areas of Greece, Spain and Portugal always belonged to *Macrolophus pygmaeus* (Rambur) (Perdikis et al. 2003, Martinez-Cascales et al. 2006).

Macrolophus melanotoma occurs at high numbers on the non-cultivated plant *Dittrichia viscosa* L. (W. Greuter) (Asteraceae) that is very common in the Mediterranean region (Alomar et al. 1994, Lykouressis et al. 2000, Perdikis et al. 2003, Martinez-Cascales et al. 2006). It was present throughout the year on *D. viscosa* with the highest numbers occurring in June and July, when more than 15 individuals per stem, on average, were recorded (Perdikis et

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al. 2007). Laboratory life table studies showed that *M. melanotoma* survival, reproduction and intrinsic rate of increase were highly favoured when fed on *D. viscosa* leaves with aphid prey than when leaves without prey were available, showing its predatory habits (Perdikis et al. 2007).

Although *M. melanotoma* might not colonize tomato, the fact that *M. melanotoma* develops high populations on *D. viscosa* which is a very widespread non-crop plant whereas it can prey on aphids, indicates that it would be worthwhile to study whether *M. melanotoma* might occur on other solanaceous crops such as eggplant and pepper, which are commonly colonized by *Macrolophus*. The natural colonization of tomato crops by *M. pygmaeus* originating from non-crop host plants has been shown to substantially contribute to regulation of whitefly, aphid and Lepidopteran pest populations (Albajes and Alomar 1999, Lykouressis et al. 1999-2000, Arnó et al. 2009).

The factors involved in host plant selection behaviour can be related to visual cues as was demonstrated for *Lygus hesperus* Knight (Hemiptera: Miridae) (Blackmer and Cañas 2005). Volatile cues are also major determinants of host plant selection (Visser 1986, Blackmer et al. 2004). The predators' host plant selection decisions may be mediated by volatiles emitted due to the presence of prey (Dicke 1999). Additionally, the selection decision among different host plant species may positively correlate with the offspring survival on those plants (Lundgren et al. 2008).

This paper aimed to evaluate host plant selection of the predatory species *M. melanotoma*. More specifically, the preference of this species was studied when given a choice between its major non-crop host plant, *D. viscosa,* and the solanaceous plants, eggplant, pepper and tomato, as well as between the previously mentioned solanaceous plants, in 2-choice experiments. The experiments were performed in cages with whole plants without prey.

Materials and Methods

Rearing and experimental conditions

M. melanotoma rearing was initiated from adults and nymphs collected from *D. viscosa* in the area of Kyparissia, in western Peloponnese. The rearing was kept on potted *D. viscosa* plants from that area, infested with the aphid *Capitophorus inulae* (Passerini). This aphid is common on *D. viscosa* plants and is a suitable prey for the development and reproduction of *M. melanotoma* (Perdikis et al. 2007).

The rearing was kept in wood-framed cages (80 cm length x 80 cm width x 70 cm height) in a glasshouse maintained at 22.5 ± 2.5°C (mean ± S.D.) under natural lighting. The insects used in the experiments were reared, during the spring, for 2-4 generations in the aforementioned conditions.

The choice of *M. melanotoma* between its origin host plant (i.e. *D. viscosa*) and the solanaceous plants eggplant (cv. Bonica), pepper (cv. Vidi) and tomato (cv. Arletta) was studied in 2-choice experiments. The experiments were performed in cages 30cm high and 35cm in diameter. They were made of transparent PVC sheet 0.4mm thick and bore two rectangular openings of 20x15 cm each. These openings along with the top opening of the cage were covered with fine muslin.

In each cage two young potted plants of approximately equal size (15 cm high, with 6-8 leaves) without prey were introduced. The selection rates were recorded when the following pairs of plants were simultaneously present in the cage: *D. viscosa* and eggplant, *D. viscosa* and pepper, *D. viscosa* and tomato, eggplant and pepper, eggplant and tomato, pepper and tomato.

A single adult predator (1-2 weeks old) was introduced into each cage. Each predator was considered a replicate. In all tests, the predator was smoothly released: 1) at the bottom surface of the cage between the two plants or 2) on the upper leaves of the solanaceous plant (5 replicates in each case, i.e. 10 replicates in total). In the experiments that only solanaceous plants were tested, 5 adult predators were released separately on each of the two plants, in addition to the 5 adult predators released between the two plants in the cage (i.e. 15 replicates in total).

The release of the predator on its origin host plant, *D. viscosa,* could increase the time required for a response to occur (i.e. landing on the other plant) since in previous experiments it was shown that *M. melanotoma* did not depart from *D. viscosa* to colonize tomato. For this reason, it was preferred to release the predator between the *D. viscosa* and the solanaceous plant or only on the solanaceous plant in the cage. However, as a control treatment, the above procedure was followed when a single plant of *D. viscosa* was available in the cage. In that case, 5 adult predators were released at the bottom of the cage and 5 on the top leaves of the plant (i.e. 10 replicates in total). In each experiment, females and males were used at almost equal frequencies.

The experiments were initiated between 14:00–16:00 h since the closely associated predator *M. pygmaeus* was found more active during the afternoon (Perdikis et al. 2004). The position of the predator was recorded on each of the two plants but also elsewhere in the cage. The first record was taken after a period of 20min from the predator's introduction in the cage. During this period the cage was kept at room temperature (22-23°C). Then, the cage was transferred into a growth cabinet set at 25±1°C with relative humidity (RH) 65±5% and 16:8h L:D. The position of the predator in the cage was further recorded at intervals of 1, 2 and 24h after its release into the cage.

Preliminary experiments showed that when only its rearing host plant was available in the cage, then the predator was able to have selected it much earlier than 24h and subsequently, 24h-experimental period was considered as long enough to show the host plant selection choice decision of the predator.

The respective frequencies were calculated and pooled in respect to the released site (between the two plants or on the plant) as both concerned releases elsewhere than *D. viscosa*. The data were compared using chi-square statistics for observed and the expected frequencies. The null hypothesis was that the two plants used in each treatment would be selected at equal rate by the released predator.

Results

M. melanotoma showed a high selection rate for its origin host plant (*D. viscosa*) being recorded at a percentage of 90% on this plant as early as 1h after its introduction in cages with *D. viscosa* only (χ^2 = 4.9, df=1, $P < 0.05$) whereas, 100% of the predators were recorded on *D. viscosa* after a period of 24h (χ^2 = 8.1, df=1, $P < 0.01$) (Fig. 1a).

When *M. melanotoma* was given the chance to select between its origin host plant (*D. viscosa*) and eggplant, 1h after its release had already selected *D. viscosa* at a much higher rate than eggplant (60 vs 10%, respectively) (Fig. 1b). At the later records it was always recorded at a much higher rate on *D. viscosa* than eggplant (Fig. 1b). At the end of the experiment, *M. melanotoma* was recorded at 80% of the cases on *D. viscosa* in comparison to 20% on eggplant.

In the case that pepper was used as an alternative host plant, *M. melanotoma* showed a clear preference for its origin host plant (*D. viscosa*), that was shown as early as the first record (80 vs 0%, χ^2 = 6.1, df=1, $P < 0.05$), whereas at the later records this rate was increased reaching to 100% after 2h from the predator's release in the cage (Fig. 1c).

FIG. 1. Selection rates (% of records on the plants or the cage walls) of *Macrolophus melanotoma* in cages a single plant of *Dittrichia viscosa* was placed alone (a) or together with a plant of eggplant (b), pepper (c) or tomato (d). Records on the position of the released adult predator were taken 20min, 1, 2 and 24h after its introduction in the cage. Only records of the predator on the plants (not elsewhere in the cage) are shown. Significant differences are indicated by asterisks (chi-square test, *: P < 0.05; **: P < 0.01).

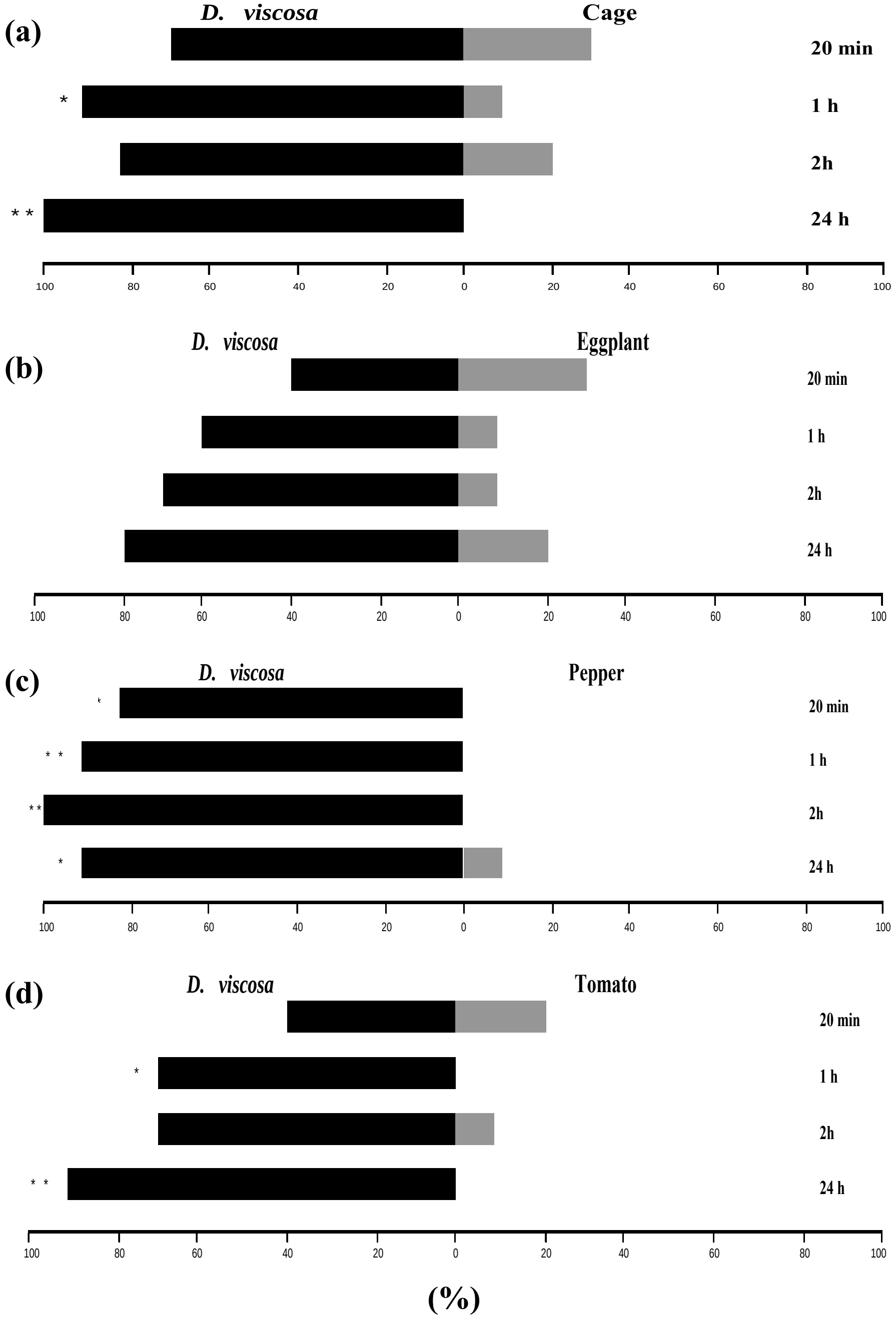
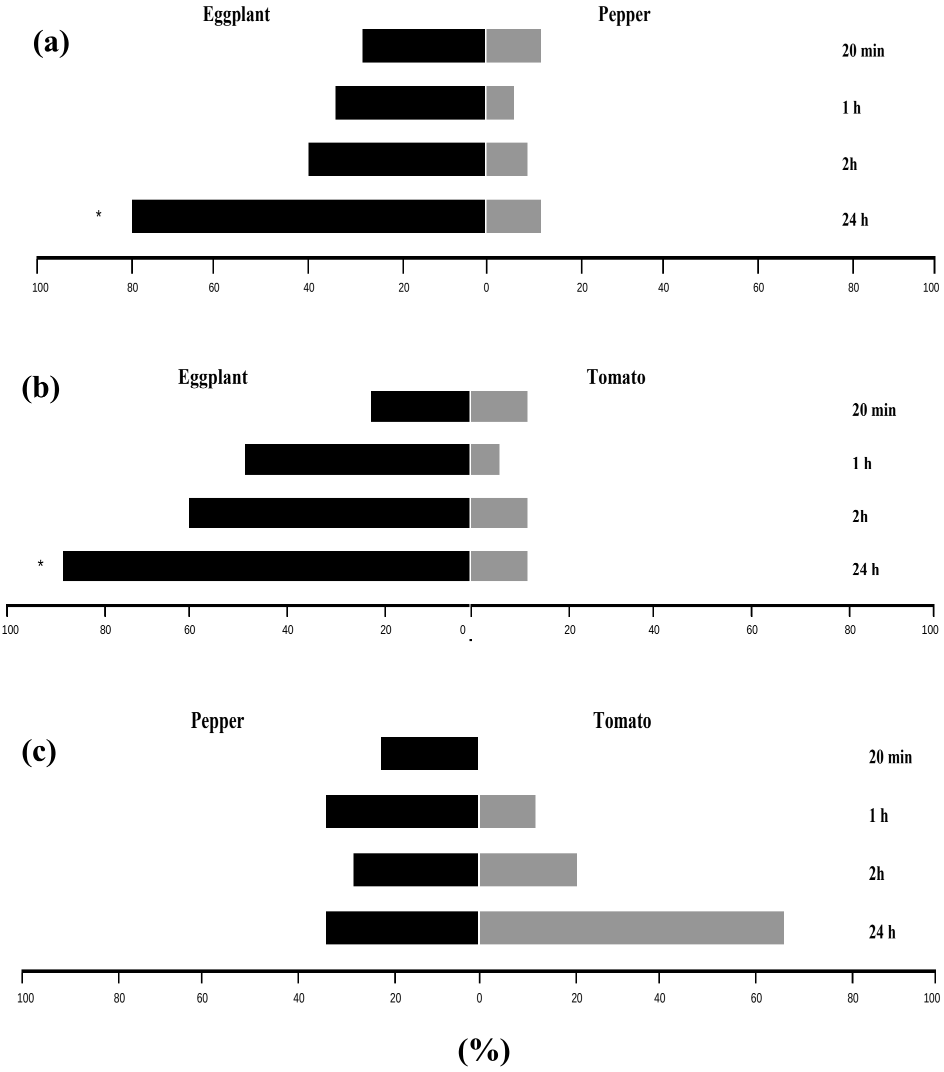


FIG. 2. Plant selection rates (%) of *Macrolophus melanotoma* in cages where one plant of eggplant was placed together with one pepper (a) or tomato plant (b), or when a pepper together with a tomato plant (c) were placed. Records on the position of the released adult predator were taken 20min, 1, 2 and 24h after its introduction in the cage. Only records of the predator on the plants (not elsewhere in the cage) are shown. Significant differences are indicated by asterisks (chi-square test, *: P < 0.05).



The study on the choice of *M. melanotoma* for tomato when present along with *D. viscosa* showed that *M. melanotoma* was mostly recorded on *D. viscosa* (Fig. 1d). After 1h, the predator selected *D. viscosa* at a rate of 70% vs 0% for tomato (χ^2 = 5.14, df=1, P < 0.05), whereas at the end of the experimental period (24h from its release) the predator was recorded on *D. viscosa* at a rate of 90% (χ^2 = 7.11, df=1, P < 0.01).

When *M. melanotoma* was given a choice between eggplant and pepper, it showed a stronger preference for eggplant, that was significant at 24h (80 vs 13 %, χ^2 = 5.7, df=1, P < 0.05) (Fig. 2a).

In the choice tests between eggplant and tomato, *M. melanotoma* showed a strong preference for eggplant that was evident 1h after its release in the cage (47 vs 7 %) and became even stronger at the end of the experiment (87 vs 13%, χ^2= 6.67, df=1, P < 0.05) (Fig. 2b).

In the case that pepper and tomato were available in the cage, *M. melanotoma* had a higher selection rate for pepper at the first observation (20min from its introduction) (Fig. 2c). At the following records, its selection rate for tomato steadily increased. At the last record, its selection rate on tomato was higher but not significantly so compared to that for pepper.

Discussion

According to the results, *M. melanotoma* showed a high preference for its origin host plant (*D. viscosa*) irrespectively of the solanaceous plant present in the experimental arena (eggplant, pepper or tomato). Considering that the predators were released on the solanaceous plant or between the two plants in the cage, this result indicates the low potential of this predator to colonize these plant species. In a previous study, it was documented that *D. viscosa* with, and at a lower rate without prey, was a suitable diet for *M. melanotoma* population increase (Perdikis et al. 2007). These results show a positive correlation between *M. melanotoma* performance and preference for *D. viscosa* and in sequence might indicate specialization on this host plant (Via 1991). Generally, high adaptation rate of a natural enemy to a certain host plant might be associated with its low potential for colonization of nearby crops (Perrin 1975).

The results are in agreement with these of previous studies conducted in cages where mature tomato plants infested with the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) (Homoptera: Aleyrodidae) were enclosed together with *D. viscosa* plants without prey. In these experiments it was revealed that *M. melanotoma* avoided tomato (Perdikis et al. 2008). Moreover, studies on DNA analysis of *Macrolophus* specimens found on tomato crops showed that they belonged to the species *M. pygmaeus* (Perdikis et al. 2003, Martinez-Cascales et al. 2006). Therefore, if considering that *M. melanotoma* showed a very low selection rate for tomato plants, our data support these of previous studies indicating that *M. melanotoma* should be considered as a predator most likely not occurring on tomato crops. Therefore, as it has been also reported by Castañé et al. (2011), previous studies should be considered as erroneously reporting *M. melanotoma* on tomato; instead *M. pygmaeus* should have been reported.

Interestingly, *M. melanotoma* showed a much lower selection rate for tomato than *D. viscosa*, despite the fact that both plants are characterized by their dense trichomes. Likely, the volatiles or other identities of the glandular trichomes are quite different between the two plants as they belong to different families. The glandular hairs of tomato plants were thought to interfere with the low preference of another mirid *Lygocoris pabulinus* (L.) for tomato in comparison to green beans, despite the fact that this mirid originated from *S. nigrum*, a plant of the same family with tomato (Groot et al. 2003).

However, the results showed that although *M. melanotoma* had a higher selection rate for *D. viscosa* than the three solanaceous plants tested, eggplant was a more frequently selected plant in comparison to tomato or pepper. As this preference ranking is established in the absence of previous experience of the predator on any of the solanaceous plants under evaluation, it may indicate a comparatively higher innate preference rate of *M. melanotoma* for eggplant. Therefore, our results indicate that the potential of *M. melanotoma* to occur on eggplant and their possible associations have to be closely verified.

This outcome might be related to the potentially higher suitability rate of eggplant as a food source for *M. melanotoma* than tomato or pepper. This can be anticipated if considering that, in prey absence, among these three solanaceous plants, eggplant was shown to be the most suitable for the development and reproduction of *M. pygmaeus,* which is a closely related species to *M. melanotoma* (Perdikis and Lykouressis 2000, 2004a,b). The selection process of *Orius insidiosus* (Say) (Heteroptera: Anthocoridae) has been associated with the suitability of the plant species for the predator's nymphal development in prey absence (Lundgren et al. 2008). A positive correlation was also recorded between the suitability of host plants without prey for performance and the oviposition preference of *O. insidiosus* (Coll 1996). Similarly, *Dicyphus hesperus* Knight (Hemiptera: Miridae) and *M. pygmaeus* were also recorded to prefer host plants on which nymphal survival without prey was favoured in comparison to other plants (Sanchez et al. 2004, Ingegno et al. 2011). However, plant volatiles may also be involved in the plant selection process in omnivorous predatory bugs (Kaplan 2011) and thus, additional research is required to explore this further in the case of *M. melanotoma*.

In conclusion, our results indicate the low potential of *M. melanotoma* to select tomato, eggplant and pepper, in comparison to its origin non-crop host plant *D. viscosa* as shown in choice experiments, with whole plants present. This outcome supports the results of previous studies which indicated that *M. melanotoma* did not occur on tomato crops. In addition, the potential of *M. melanotoma* to select pepper plants was shown as most likely being negligible. However, it was shown that *M. melanotoma* had a comparatively high selection rate for eggplant. Thus, it would be worthwhile to clarify the potential of *M. melanotoma* to occur, develop or reproduce on eggplant crops.

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Προτίμηση του *Macrolophus melanotoma* μεταξύ του κύριου αυτοφυούς φυτού-ξενιστή του, *Dittrichia viscosa*, και της μελιτζάνας, πιπεριάς και τομάτας, σε πειράματα επιλογής

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ΠΕΡΙΛΗΨΗ

Στη μελέτη αυτή εξετάσθηκε η προτίμηση του αρπακτικού *Macrolophus melanotoma* (Costa) (=*M. caliginosus* Wagner) (Hemiptera: Miridae) μεταξύ του *Dittrichia viscosa* L. (W. Greuter) (Asteraceae), που αποτελεί το κύριο φυτό ξενιστή του, σε σχέση με τη μελιτζάνα, πιπεριά και τομάτα, καθώς και μεταξύ της μελιτζάνας, πιπεριάς και τομάτας. Τα πειράματα πραγματοποιήθηκαν σε κλωβούς όπου το αρπακτικό είχε τη δυνατότητα να επιλέξει μεταξύ δύο φυτών διαφορετικών ειδών. Σε κάθε επανάληψη, ένα ενήλικο του αρπακτικού ελευθερωνόταν είτε επί της βάσης του κλωβού μεταξύ των δύο φυτών είτε στα κορυφαία φύλλα του φυτού μελιτζάνας, πιπεριάς ή τομάτας. Η θέση του αρπακτικού καταγραφόταν μετά από 20min, 1, 2, and 24h από την ελευθέρωσή του στον κλωβό. Σύμφωνα με τα αποτελέσματα, το *M. melanotoma* έδειξε υψηλή προτίμηση για το φυτό από το οποίο προερχόταν, δηλαδή το *D. viscosa,* σε σχέση με το φυτό μελιτζάνας, πιπεριάς ή τομάτας. Μεταξύ των τριών σολανωδών φυτών είχε σχετικά μεγαλύτερη προτίμηση για τη μελιτζάνα. Επομένως, το *M. melanotoma* φάνηκε να μην προτιμά την τομάτα και την πιπεριά, όμως θα πρέπει να μελετηθεί περαιτέρω η δυνατότητά του να αποικίζει καλλιέργειες μελιτζάνας.

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