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Fruit Damage by *Dicyphus cerastii* and *Nesidiocoris tenuis* (Hemiptera: Miridae) on Tomato †

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Abstract: Zoophytophagous dicyphine mirids can produce economically important damage on tomato. We evaluated the influence of fruit ripeness and presence of prey and water, on fruit damage caused by nymphs and adults of two species (*Dicyphus cerastii* and *Nesidiocoris tenuis*) in 24h. For both species combined, fruit ripeness was the most important factor and unripe fruit suffered more damage. The presence of prey only reduced damage on unripe fruits. Factor importance on fruit damage varied between species. For *D. cerastii* fruit ripeness was the most important factor whereas for *N. tenuis* it was mirid age. Overall, *N. tenuis* females produced more damage than *D. cerastii*.

Keywords: fruit injury; biological control; mirid; dicyphini; protected crops

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1. Introduction

Zoophytophagous mirid species (Hemiptera: Heteroptera: Miridae) are important biological control agents in several crops. Dicyphine (Miridae: Bryocorinae: Dicyphini) species, such as *Nesidiocoris tenuis* (Reuter), and several species of the genera *Macrolophus* Fieber and *Dicyphus* Fieber, are used worldwide against whiteflies, aphids, and lepidopteran eggs and larvae [1] in several vegetable crops, in conservation or augmentative biological control strategies. However, some of these species are known to damage stems, leaves, flowers, and fruits on the crops they are used to protect [2–4]. These omnivorous mirid species can obtain water and nutrients, such as carbohydrates, from plant tissues. This strategy allows them to withstand periods of lower prey abundance. On the other hand, it can also decrease predation, and the damage can be of economic importance, such as necrotic rings in stems and petioles of leaf, as well as flower or fruit abortion, and punctures in the fruits. Damage greatly varies with host plant species and mirid species [3].

Dicyphus cerastii Wagner is a palearctic mirid, report to the Mediterranean Basin, naturally found in Portuguese greenhouses, whereas *Nesidiocoris tenuis* is largely released to control whiteflies and South American tomato moth in Mediterranean greenhouses [1,4–6]. The increased demand for products without pesticide residues, combined with the need to control pests, highlights the urgency for sustainable alternatives that reduce the negative effects on the consumer and the environment's health. The use of predatory mirids in pest control has been very successful in protected tomato crop [e.g. 6–8]. However, it is necessary to understand the damage these species can cause in the crop, to help decision making in choosing the species to be used as a biological control agent. While damage caused by *N. tenuis* has been studied [e.g. 2,9–11], little is known about those induced by *D. cerastii. Dicyphus cerastii* has been observed producing chlorotic punctures on leaves [4] but also necrotic damage on tomato stems and leaf petioles, as well as feeding punctures in fruits (our personal observation). However, the influence of these damage on plant development and possible economic impacts has never been tested or compared with other mirid species.

The aim of this study was to assess the effect of factors like presence of prey, water, tomato ripeness, mirid age and species on tomato fruit damage (both unripe and ripe) made by these two Dicyphini species, *Dicyphus cerastii* and *Nesidiocoris tenuis*, nymphs and adult females.

2. Materials and methods

2.1. Rearing of mirid predators

Stock colonies of both species (*D. cerastii* and *N. tenuis*) are maintained at the Instituto Superior de Agronomia, Lisbon, Portugal, on tobacco plants (*Nicotiana tabacum* L.). The rearing populations came from different geographical sites in Portugal, and are frequently refreshed with wild individuals, mostly from the Oeste region (for rearing details, see [5]). Young adult females (between 1 and 7 days old) were obtained from the regular collection of large nymphs from breeding cages that were transferred to separate cages, where they could reach adulthood. For nymph experiments, 4th/5th instar nymphs were collected from immature rearing cages.

2.2. Fruit damage bioassay

Tomato fruits (Fig. 1, cv. Suntasty) were obtained from an organic commercial tomato greenhouse. For each species three adult females or three nymphs were placed in plastic cups (8 cm high and 6 cm diameter) with one tomato fruit approximately 4–5 cm long. The lid of the cups had a hole (3 cm diameter) covered with fabric to allow ventilation. Four treatments were considered (15 replications): a) fruit only (N); b) fruit with water (W) (supplied through an Eppendorf vial with moist cotton wool); c) fruit with water and alternative food (FW) (*Ephestia kuehniella* eggs and *Artemia* sp. cysts Entofood®, Koppert Biological Systems, Berkel en Rodenrijs, The Netherlands), supplied on a sticky paper strip (2 x 0.8 cm); d) fruit with alternative food (F) (Entofood®) but no water. The experiments were carried on both unripe (mature green) and fully ripe (red) fruits. Insects were allowed to feed for 24 h after which punctures (injuries resulting from fruit feeding) were counted under a stereomicroscope with a magnification of 50x. Injury was considered as a puncture surrounded by small whitish or yellowish halo (Fig. 1) [3,12]. Replicates in which death occurred or nymphs molted into adults were discarded.

2.4. Data analysis

Classification tree methods were used to understand the relative importance of the variables used (i.e., the absence or presence of water and/or food, developmental stage of the mirid, tomato ripeness, and species) on the number of feeding punctures. Statistical analyses were performed using R software version 4.1.0 implemented in RStudio version 1.4.1106. Conditional inference trees were made using the "ctree" function (R package party, http://cran.r-project.org/web/packages/party/index.html), which bases node splitting on statistical tests, providing a p-value for the significance of splitting [13]. The importance of the variables was measured using the random forest algorithm [14] and computations in the randomForest package. The random forest algorithm combines many classification trees to produce more accurate classifications and has measures of variable importance and measures of similarity of data points as by-products of its calculations [15]. Data were analyzed together (i.e., considering counts for both species) and separately for each species. All preliminary analysis considered the modalities in two groups: (i) without food (N and W); and (ii) with food (F and FW). We grouped them into Absence (A) and Presence (P) of food and/or water, respectively, in the presented output.

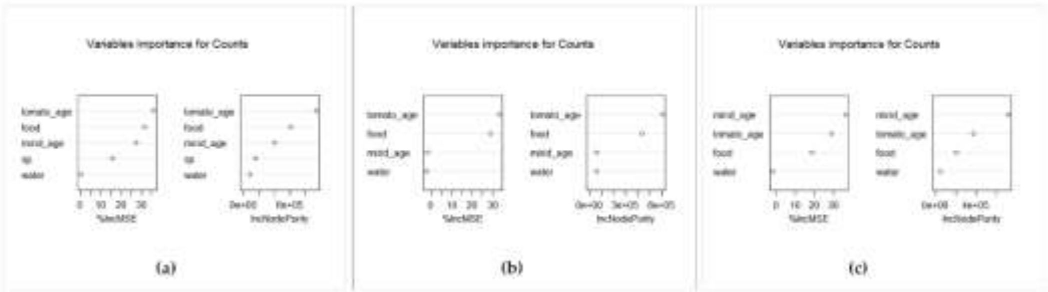
3. Results

Both species fed on tomato fruits and produced punctures that appeared as damaged epicarp/mesocarp cells. It was often possible to observe that the damaged area extended beyond the puncture point following stylet movement inside the fruit. Punctures were structurally similar but the pattern of each species was different. *Dicyphus cerastii* punctures tended to be aggregated, forming clearly visible patches in cases of highly damaged fruits. *Nesidiocoris tenuis* punctures appeared less aggregated compared to *D. cerastii*. Punctures in fruits didn't heal. Punctures on green fruits did not disappear even when fruits changed colour during maturation. Punctures produced by females and nymphs appeared similar, for both species. We observed that, occasionally, females of both species laid eggs on fruits. It is possible that an amount of the punctures may have been egg laying attempts or the result of probing.

A high variability was observed in the number of punctures inflicted in all treatments and in both species, with low numbers or even no punctures to high numbers of punctures in the same treatment, which generated great variance in the data for both species studied. When analysing the dataset considering both species or for each species separately there was no difference between the two treatments without food (N and W) or between the two treatments with food (F and FW). Considering both species, the most important variable was the tomato ripening stage (tomato_age), with unripe (green) tomato the one with the highest number of feeding punctures. Food presence (F, FW) or absence (N, W) was only significant in the case of green fruit, and species was only significant for females in the presence of food in the case of green fruits, and for females in the ripe fruits. While the most important factor for *D. cerastii* was also the tomato ripeness, for *N. tenuis* the most important factor was the individual's stage of development (i.e., whether it is a nymph or an adult) (Figs. 2 and 3).

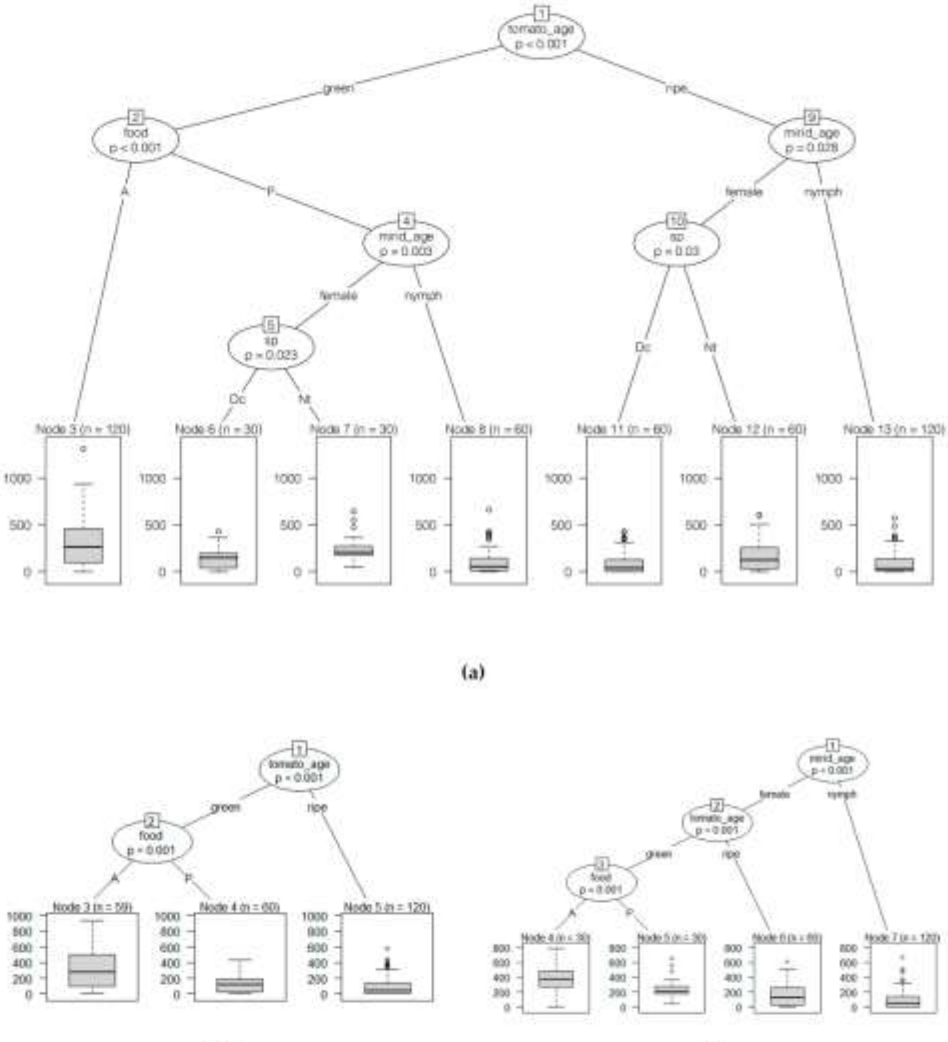

(a) (b)

Figure 1. Feeding punctures in tomato fruit: (**a**) unripe fruit; (**b**) ripe fruit.



(**a**) (**b**) (**c**)

Figure 2. Variable importance plot from Random Forest model (randomForest). The variables are ordered top-to-bottom as most-to-least important for an increase in feeding punctures (counts) on tomato fruit. (**a**) Using all dataset with data from both species, *Dicyphus cerastii* and *Nesidiocoris tenuis*; (**b**) Database containing data collected only for *D. cerastii*; (**c**) Database containing data collected only for *N. tenuis*.



(**a**)

(**b**) (**c**)

Figure 3. Classification trees from the Conditional Inference Trees (ctree) model. For each internal node, input variable and P values are provided, the boxplot of the number of feed punctures is displayed for each end node. Numbers in boxes above the variable indicate the node number. (**a**) Using all dataset with data from both species, *Dicyphus cerastii* and *Nesidiocoris tenuis*; (**b**) Database containing data collected only for *D. cerastii*; (**c**) Database containing data collected only for N. tenuis. Dc: *Dicyphus cerastii*; Nt: *Nesidiocoris tenuis*; A: absence and P: presence of food.

4. Discussion

Zoophytophagous predators such as dicyphines may resort to plant feeding in order to obtain water and nutrients. In this study, the presence of water did not influence fruit puncture level. Therefore, both mirid species and both development stages could obtain the water they needed from green or ripe tomato fruits, at least when water was not provided. Water provision has been reported as one reason for phytophagy on heteropteran predators [e.g. 16]. However, as puncture numbers did not differ when water was supplied for both *N. tenuis* and *D. cerastii*, these mirids may have looked for other resources when they fed on the fruits.

Among the nutrients obtained from phytophagy, carbohydrates may have a particular ecological function since they have been reported to influence both predation and reproduction in dicyphines. This was demonstrated for *N. tenuis* that was able to reduce the amount of prey feeding needed to establish on tomato plants [6] and increased its progeny [17], in the presence of sucrose dispensers. In another study, *N. tenuis* reduced its phytophagy when provisioned with sucrose dispensers [18].

In our study, when considering both species combined, tomato ripeness was the most important factor, with green fruits suffering more punctures than mature ones. This difference may be due to distinct nutritional profiles between unripe and ripe fruits. Sugar concentration, among other nutrients, may be higher on ripe tomato fruits [19,20], so it is possible that mirids may get more nutritional value per feeding puncture on ripe fruits than on green ones. On green fruits, the main effect was the presence of prey, which reduced fruit damage. There were differences between species as *N. tenuis* females produced more damage than those of *D. cerastii*. On ripe fruits the most important factor was mirid age, with females producing most damage and, in these fruits, food did not significantly reduce damage. However, and once again, females of *N. tenuis* produced more damage than those of *D. cerastii*.

Considering *N. tenuis*, the most important factor was age, with females damaging more fruits than nymphs. Differently, in studies with whole plants, *N. tenuis* nymphs showed higher carbohydrate content [18], and spent more time feeding on the apical part of the plant [21], compared to adults. A similar trend was found for nymphs of the neotropical mirids *Macrolophus basicornis* (Stål), *Engytatus varians* (Distant) and *Campyloneuropsis infumatus* (Carvalho) that also produced fruit punctures whereas females didn't [12]. Even though we observed less fruit damage by *N. tenuis* nymphs than females, our results indicate that nymphs are less influenced by factors such as fruit ripeness or presence of prey, suggesting that *N. tenuis* nymphs may be less prone to change their phytophagous behaviour than adults. Following mirid age, fruit ripeness was the next important factor for *N. tenuis* with green fruit sustaining more damage. The presence of prey reduced the amount of damage on green fruits, whereas on ripe fruits it didn't produce differences. This further suggests that green fruits may be a less valuable nutritional source for *N. tenuis*.

Tomato ripeness was the most relevant factor to explain fruit punctures by *D. cerastii*. Here too, the presence of prey was important in fruit damage reduction on green fruits. Differently to *N. tenuis,* mirid age was not important in this species, which may suggest that *D. cerastii* may not be as dissimilar in phytophagy between adults and nymphs as *N. tenuis*.

Plant damage by zoophytophagous mirids has been associated with prey scarcity [9,11. However, as referred, in this study the presence of food did not affect puncture level in ripe fruits, or in green fruits with *N. tenuis* nymphs. Similarly, McGregor et al. [22] reported that the presence of food did not influence the level of fruit feeding by *Dicyphus hesperus* Knight on mature tomato fruits, and Lucas and Alomar [23] that in whole caged plants the presence of *E. kuehniella* eggs did not prevent fruit injury by *D. tamaninii*.

Plant damage by zoophytophagous mirids may be determined by a complex combination of factors besides prey abundance. Different species may have distinct preferences or behaviours that produce different types and levels of damage. Under the same conditions *M. caliginosus* (syn. *M. pygmaeus*) did not produce fruit damage whereas *D. tamaninii* did [23]. A different dicyphine, the nearctic *D. hesperus* preferred to feed on tomato leaves producing negligible damage on fruits [22]. Host plant and cultivar may also determine phytophagy, as was demonstrated for *N. tenuis* that varied its phytophagy among different tomato cultivars [24]. The health of the host plant may also shape phytophagy by dicyphines. *Macrolophus pygmaeus* was reported to increase the number and produce more evident fruit damage on tomato plants infected with Pepino mosaic virus (PepMV) [3], but the same did not occur with *N. tenuis* [25]. Defence activated plants may also be less susceptible to phytophagous behaviour. This was demonstrated for *N. tenuis* that produced less plant damage on tomato inoculated with the endophytic *Fusarium solani* K strain, a fungal isolate that confers tomato resistance to foliar and root fungal pathogens [26].

Other factors may explain differences in phytophagy such as genetic variation within species [21,27]. In fact, for the same treatments, we observed high variability in puncture numbers inflicted on fruits. This suggests that other factors, than those considered in our study, may be driving fruit feeding in both *N. tenuis* and *D. cerastii*, and genetically determined behaviours should probably be considered in future research.

In the present study, only fruits were used. In order to fully assess how these species, particularly *D. cerastii*, may affect tomato production, further research is needed in semi-field conditions and commercial greenhouses where phytophagy may be evaluated on whole plants and flowers besides fruits. Assessing plant organ preference may be crucial as fruit damage level can be lower and even negligible if other organs are preferred, as with *D. hesperus* females that produced almost no fruit damage to mature fruits when tomato leaves were available [22].

5. Conclusions

This study suggests that fruit damage by different species of zoophytophagous mirids may be determined by distinct factors such as fruit ripeness and the presence of prey. We also found that damage production was highly variable within treatments. This indicates that future selection of dicyphines for biological control will have to consider phytophagy differences in both species and individual levels. This study is also the first to report and characterize fruit damage by *D. cerastii*, which urges further information regarding damage on other plant tissues for this species, in order to evaluate its potential as biological control agent for tomato crops.

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