









# Brown marmorated stink bug (*Halyomorpha halys*) feeding damage determines early drop in olive crops

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## Abstract

The brown marmorated stink bug (BMSB), *Halyomorpha halys* Stål, is an invasive species and a polyphagous pest. BMSB feeding activity was suspected to be responsible for olive damage. To evaluate the effect of feeding damage from adults and nymphs of BMSB, 30 rearing sleeves were positioned in an olive grove, at an early stage of drupe development. The individuals were kept in the rearing sleeves for 48 h, and the number of olives in each sleeve was checked weekly, visually assessing signs of damage and measuring their volume. After the 48-hr exposure, the number of early dropped olives was significantly higher for rearing sleeves containing BMSB adults and nymphs compared with control, with visible signs of damage. The volume of olives still attached was significantly lower for rearing sleeves with adults. These results provide key evidence on BMSB damage in developing olives. If the numbers of BMSB keep increasing in Mediterranean regions (where most of the olive production occurs), actions should be taken to prevent economic losses.

## KEYWORDS

economic loss, feeding damage, Hemiptera, invasive species, olive pest, Pentatomidae

## 1 | INTRODUCTION

The brown marmorated stink bug (BMSB), *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), is an invasive species, nowadays worldwide listed among the key agricultural pests. Native of China, Japan and Korea, BMSB is a very polyphagous stink bug with an increasing list of recorded host plants (Hamilton et al., 2018). When it becomes established, it represents a major threat for several commercial crops such as apple (Bergh et al., 2019), hazelnuts (Hedstrom et al., 2014), kiwi (Chen et al., 2020), pears and peaches (Nielsen & Hamilton, 2009).

Brown marmorated stink bug was first recorded in Italy in 2012, and since then it rapidly spread reaching high densities in the north of the country (Maistrello et al., 2018; Malek et al., 2019). In this area, olive trees (*Olea europaea* L.) produce modest quantities of high-quality extra virgin olive oil for both local market and export.

Previous observations suggested that the damage of BMSB feeding activity on olive can be severe (D'Ascenzo et al., 2020; Damos et al., 2019) and that it may be associated with early drop (Minuto, 2021). Thus, the objective of this study was to examine the effect of BMSB feeding activity on developing olives by assessing

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the severity of the damage with the time and if it varied between adults and nymphs.

## 2 | MATERIALS AND METHODS

### 2.1 | Experimental assessment of olive damage

In an IPM olive grove of the 'Casaliva' cultivar located near Cavedine, Italy (46.0142677 N, 10.9622535 E), rearing sleeves (BugDorm 40 × 20 cm, nylon netting 104 × 94 mesh) were used to evaluate BMSB feeding (Figure S1, Supplementary Material). We considered three treatments: (1) Exposure of olives to adults for 48 h; (2) to nymphs for 48 h and (3) as a control, rearing sleeves with no individuals. Thirty rearing sleeves were distributed on ten trees, 3 per plant, placed on singles branches randomly selected (with at least 10 fruits), to have a sleeve per treatment on each tree (10 per treatment).

Brown marmorated stink bug individuals were collected at the beginning of July 2021 using attractive traps placed along a hedgerow at the Fondazione Edmund Mach campus, San Michele, Italy (46.1862381 N, 11.1339682 E). They were reared on green beans (*Phaseolus vulgaris* L.), tomato (*Solanum lycopersicum* L.), carrots (*Dacus carota* L.) and peanuts (*Arachis hypogaea* L.), in plastic cages (BugDorm-4M3030). They were kept in controlled conditions (25 ± 3°C and 60% ± 10% RH) with 16:8 h L:D photoperiod, before they were placed in the rearing sleeves.

To distinguish olive phenological growth stages, the international standardized Biologische, Bundesanstalt, Bundessortenamt and Chemische Industrie (BBCH) scale, described for the olive tree by Sanz-Cortés et al. (2002) was used. To prevent the olives from being attacked before the assessment, the exclusion cages were placed at the end of flowering (69 BBCH), on 01/07/21. At approximately 10% of fruit development and before full lignification of the stone (71 BBCH), adults (5 individuals, 3 females and 2 males) and nymphs (5 individuals, III–IV stage) were inserted in the rearing sleeves for 48 h (21–23/07/21).

Every week, until complete fruit development, the number of early dropped olives in each rearing sleeve were counted. Dropped olives were visually inspected under dissecting microscope (Leica Wild M10) with integrated camera (Leica IC80HD) to assess the occurrence of feeding damage (e.g., spots, depressions and feeding sheaths, Joseph et al., 2015). When stones were fully lignified and fruits reached 90% of final size, all olives still attached to the branches were collected, width and length of each olive were measured with a digital calliper (accuracy: ±0.1 mm) and their volume was estimated with the equation developed for the seed volume data set (Ganhão & Dias, 2019).

### 2.2 | Data analysis

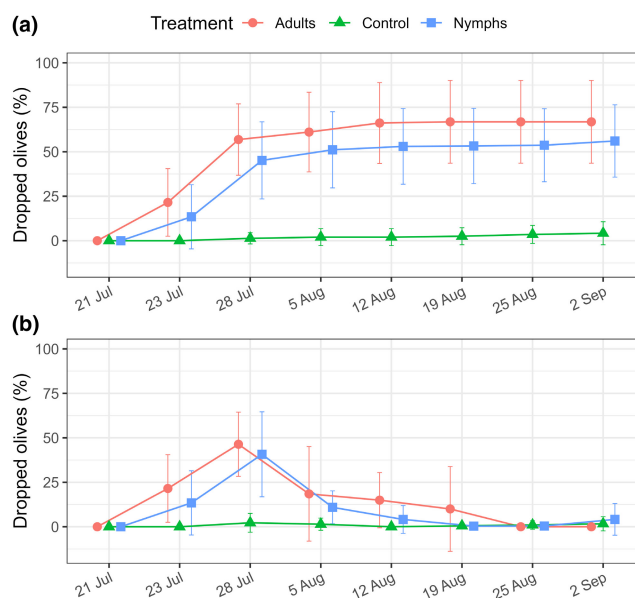
Data analysis and plotting were performed with R (R Core Team, 2018). Kruskal–Wallis test was used to compare the number

and the volume of olives for each treatment at each session, followed by Dunn's post hoc test with Bonferroni correction.

To model the observed olive early drop, a beta-binomial generalized mixed model (GLMM) with a logit link was applied (Brooks et al., 2017). Rearing sleeve ID was used as a random factor, while session number and treatment were used as explanatory variables. Model assumptions were verified following Zuur and Ieno (2016) and with package DHARMA (Hartig, 2021). Akaike information criterion (AIC) was used for model selection. Predictor effect plots (Fox & Weisberg, 2018) were used to visualize the fitted coefficients.

## 3 | RESULTS

The percentage of dropped olives in the rearing sleeves at the last session was on average (±SD) 66.83% ± 30.85% for adults, 56.06 ± 26.97 for nymphs and 4.19 ± 8.58 for the control. The number of early dropped olives was significantly higher (Kruskal–Wallis:  $H = 90.97$ ,  $df = 2$ ,  $p < 0.001$ ) for rearing sleeves containing BMSB adults and nymphs compared with control. For the running total (Figure 1a), the pattern was consistent for adults for every observation and for nymphs from the second week onwards. Analysing the sessions separately (Figure 1b), the number of dropped olives peaked 7 days from BMSB removal from the rearing sleeves, for both adults and nymphs, and kept increasing for more than 20 days. The results of the beta-binomial GLMM confirmed that the percentage of dropped olives is maximum for



**FIGURE 1** Mean number of early dropped olives (%) in rearing sleeves with different treatments (the presence of adults, nymphs and control): (a) Running total and (b) At each session. Kruskal–Wallis test and post hoc Dunn's test with Bonferroni correction, where letters indicate significant differences ( $p < 0.05$ ). Starting mean number of olives (±SD) per rearing sleeve: 17.40 ± 11.85 for adults, 20.10 ± 10.75 for nymphs and 20.10 ± 11.97 for control

adults and significant for both nymphs and adults. Since the effect of *Session* was not relevant, the simple model with only *Treatment* was selected (Table 1, Figure 2).

The olives exposed to BMSB adults and nymphs presented external and internal feeding injury, with evident punctures and stylet sheaths (Figure 3). At 80 BBCH, the volume of the olives still attached to the trees was significantly lower (Kruskal–Wallis:  $H = 12.82$ ,  $df = 2$ ,  $p < 0.005$ ) for the rearing sleeves that contained adult BMSB compared to those exposed to nymphs and control, while there was not significant difference between nymphs and control (Figure 4).

TABLE 1 Estimated regression parameters, standard errors, z-values and p-values for the beta-binomial binomial GLMM model

	Estimate	Std. Error	z value	Pr(> z )
Intercept	-1.908	0.262	-7.271	<0.001
Treatment: Nymphs	-0.338	0.363	-0.931	0.352
Treatment: Control	-2.478	0.562	-4.407	<0.001

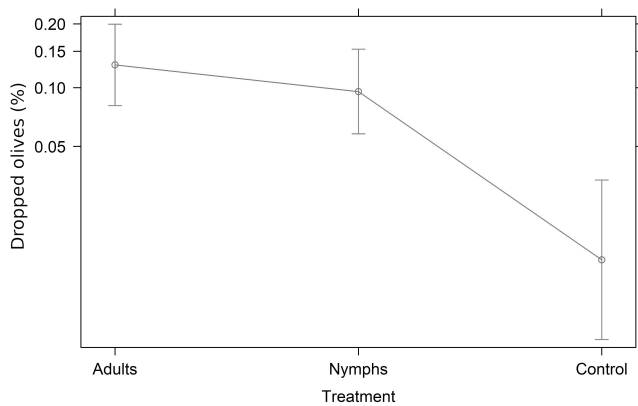


FIGURE 2 Effect plot for the beta-binomial GLMM, showing the model outcome for the different treatments

## 4 | DISCUSSION

As the list of crops attacked by BMSB still increases, new symptoms appear related to its feeding activity. Since several factors can be responsible for fruit drop (e.g., physiology, biotic and abiotic factors), this study offers key evidence of the potential role of BMSB as olive pest. Our work aimed at establishing that olives are subjected to BMSB feeding damage, which is expressed in terms of both early olive drop and reduced volume, as previously suggested (D'Ascenzo et al., 2020). Even if confining insects represents an artificial condition, it is an effective approach to correctly identify and characterize feeding damage under field conditions, a useful information to detect BMSB damage to olives in time. To our knowledge, this is the first study that has experimentally documented symptoms associated with BMSB feeding damage on olive, when the fruits are at an intermediate stage of development.

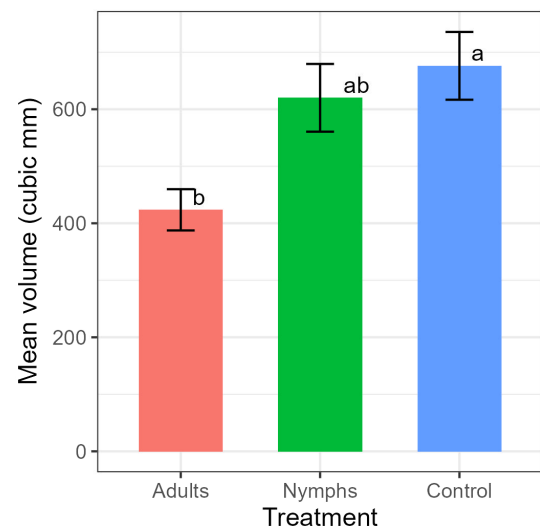
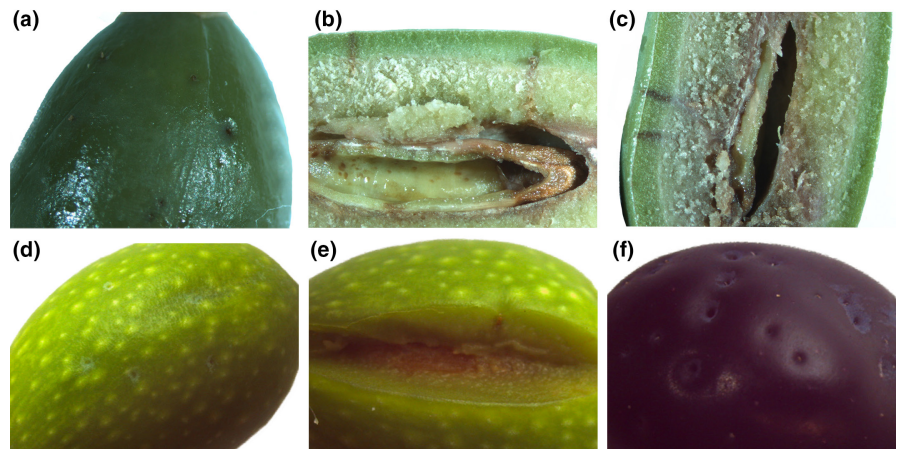


FIGURE 4 Volume of olives still attached to the branches, when the rearing sleeves were removed; letters indicate significant differences ( $p < 0.05$ ) after Dunn's test. Mean number of olives ( $\pm$ SD) per rearing sleeve:  $5.20 \pm 6.34$  for adults,  $10.30 \pm 9.17$  for nymphs and  $19.70 \pm 12.13$  for control

FIGURE 3 *Halyomorpha halys* punctures on olives, at different developmental stages. Early dropped olives, 75 BBCH: (a) Feeding puncture; (b and c) stylet sheaths. Olives removed from tree at the last session: (d) Feeding puncture, 80 BBCH; (e) stylet sheath 80 BBCH; (f) feeding puncture, 89 BBCH



The symptoms are similar for adults and nymphs (but less severe for the latter) and, as it has been observed on other crops, are evident even after a short exposure to BMSB (Cissel et al., 2015). As it has been suggested for blueberries (Wiman et al., 2015), the damage produced by stink bug feeding activity largely exceeds the area of the puncture also causing fruit necrosis that could be caused by stylet penetration (i.e., mechanical laceration), associated with direct digestive action of saliva and/or to the presence of microbes (vectored by BMSB or opportunistic) (Mitchell et al., 2018). In addition, these first results suggest that BMSB feeding damage may extend to inner tissues and be associated with olive endocarp abortion as well. Further experiments should aim at studying BMSB feeding damage over a longer timeframe to examine the physiological mechanisms responsible for both early drop and volume loss.

In conclusion, it will be important to monitor those areas where BMSB and olives occur together especially in view of a possible massive BMSB spread to Mediterranean regions. In particular, considering the olive production specificity, the potential economic loss could be worse in years of low production; therefore, informed management actions should be considered to limit its impact (Hahn et al., 2017).

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#### CONFLICT OF INTEREST

The authors declare no conflicts of interest.

#### AUTHOR CONTRIBUTIONS

Livia Zapponi, Michele Morten and Serena Chiesa conceived the research. Livia Zapponi, Michele Morten, Serena Chiesa, Giacomo Borri and Monica Sofia conducted experiments. Livia Zapponi analysed data and conducted statistical analyses. Livia Zapponi, Michele Morten and Serena Chiesa drafted the manuscript, which was reviewed by Gianfranco Anfora, Gino Angeli and Valerio Mazzoni. All authors read and approved the manuscript.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are publicly available at <https://doi.org/10.5281/zenodo.5992384>

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## SUPPORTING INFORMATION

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