


Combined effect of timing, position and management on *Halyomorpha halys* feeding injury assessment on apple cultivars

Livia Zapponi¹  | Serena Giorgia Chiesa²  | Gianfranco Anfora^{3,4} | Loris Chini² | Luca Gallimbeni² | Claudio Ioriatti³ | Valerio Mazzoni³  | Alberto Saddi³ | Gino Angeli² 

¹National Research Council of Italy, Institute of BioEconomy, San Michele all'Adige, Trento, Italy

²Fondazione Edmund Mach, Technology Transfer Centre, San Michele all'Adige, Trento, Italy

³Fondazione Edmund Mach, Research and Innovation Centre, San Michele all'Adige, Trento, Italy

⁴Center for Agriculture, Food and Environment (C3A), University of Trento, San Michele all'Adige, Trento, Italy

Correspondence

Livia Zapponi, National Research Council of Italy, Institute of BioEconomy, 38098 San Michele all'Adige, Trento, Italy.
Email: livia.zapponi@cnr.it

Abstract

Halyomorpha halys is an invasive stink bug, native to east Asia, responsible for extensive economic loss in numerous crops. Since several factors can influence the severity of the recorded injury level, we performed field and semi-field experiments in commercial apple orchards to monitor their interaction. To establish how position (both in the orchard and on the canopy), management and cultivar interact in determining pre-harvest injury levels, more than 100,000 apples, distributed over 106 orchard blocks, were examined in the field. Apples located higher in the canopy, on edge rows and in orchards with organic management had a higher number of external injuries and such factors were more relevant when occurring in combination, and on mid and late-season cultivars. Exclusion cages were used to assess if the injury severity changed with the apple growth stage and with the life stage of *H. halys*, exposing Red delicious apples to adults and nymphs for 48 hours, from May to July. Early-season injuries did not evolve in distinct depressions nor in wide areas with necrotic tissue. Later in season, the injury severity was higher and similar for adults and older nymphs (fourth and fifth instars), with the development of depressions externally and necrotic tissue internally. Thus, even a low number of individuals could cause severe damage, over a short exposure period, when it happened near full maturation. The combined results offer new insights to support the development of informed and sustainable control strategies.

KEYWORDS

brown marmorated stink bug, damage, economic loss, invasive species, Pentatomidae, pest

1 | INTRODUCTION

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive species native to east Asia that in a few decades has spread to North and South America, Europe and western Asia (Leskey & Nielsen, 2018). Since its initial introduction in the mid-Atlantic region of USA, it was evident that

the feeding damage of *H. halys* was far more critical than what was recorded before 2010 by native stink bugs (Joseph et al., 2015), as its extensive feeding damage can cause injuries that make crops unmarketable (Peiffer & Felton, 2014).

Like most Pentatomidae, *H. halys* is a phytophagous species that feeds on a broad variety of plants. Trophic resources include developing seeds, fruits, and growing shoots, which adults

and nymphs pierce with their mandibular and maxillary stylets (McPherson et al., 2018). Experimental observations on soybean seeds showed that the feeding activity disrupts the cells of the endosperm, through the mechanical action of the stylets and the salivary digestive enzymes, resulting in detectable spots, outside and inside the seed (Depieri & Panizzi, 2011). Another distinct sign of *H. halys* feeding activity is the stylet sheath, composed of lipoproteins that solidify, creating a passage through which digestive enzymes reach the plant tissues (Bundy et al., 2000). The marks resulting from the feeding activity vary according to the attacked crop: light-yellow discoloured marks (often with distinguishable punctures) on the outside and spongy tissue inside on bell peppers and tomatoes (Zobel et al., 2016); necrosis of internal tissues on blueberries (Wiman et al., 2015); discoloured areas and depressions on apples; gummosis and necrosis on peaches (Acebes-Doria et al., 2016); malformations and necrotic areas on hazelnuts (Hedstrom et al., 2014); green and white spots on kiwifruit (Chen et al., 2020); collapsed kernels on sweet corn (Cissel et al., 2015) and flat pods on soy (Owens et al., 2013). Feeding has also been documented to be associated with secondary effects, such as volume loss (Wiman et al., 2015; Zapponi et al., 2022), fruit abscission (Nielsen & Hamilton, 2009; Zapponi et al., 2022), rot development (Bosco et al., 2018), lack of kernel formation (Hedstrom et al., 2014) and alteration of the content of sugars, organic acids and phenols (Zamljen et al., 2021). *Halyomorpha halys* has also been reported as a putative phytoplasma vector in Korea (Jung, 2023) and as a vector of a phytoplasma infection of the empress tree *Paulownia tomentosa* (Thunb.), the *Paulownia* witches'-broom (Hiruki, 1999), which can compromise the quality of the wood (Hiruki, 1997). Besides, increased fungal contamination (*brown rot* caused by *Monilinia* spp.) was also associated with increased levels of *H. halys* density and duration of infestation (Moore et al., 2019). However, the analysis of the content of *H. halys* watery saliva with a proteomic approach did not reveal the presence of yeast nor bacterial proteins (Peiffer & Felton, 2014), supporting that the incidence of pathogens in the environment and on the insect maybe responsible of the observed infestations (Moore et al., 2019).

Several factors can influence the severity of the resulting damage. In terms of the development stage at which stink bug feeding occurs, Cissel et al. (2015) observed that while the highest yield loss in corn occurred with early-stage infestation, the most dramatic quality reductions were associated with later-stages attacks. Moreover, the damage distribution can also be affected by a significant edge effect, with higher densities of both adults and nymphs at field edges (Venugopal et al., 2015), with consequent higher feeding levels (Joseph et al., 2014; Venugopal et al., 2014). Besides, Quinn et al. (2019) observed that both *H. halys* adults and nymphs were more abundant in the mid- and upper canopy and preliminary observations by Joseph et al. (2014) suggested that the proportion of injured apples increased from the lower to the upper canopy.

In order to optimize feeding damage assessment, supporting the development of sustainable management strategies, we designed

two complementary experiments. The specific objectives of this study were to: (1) determine how position (both in the orchard and on the canopy), management and cultivar interact in determining pre-harvest injury levels and (2) assess if the injury severity changes with the fruit growth stage and with the life stage of *H. halys*.

2 | MATERIALS AND METHODS

2.1 | Study sites

All the surveys were conducted in commercial apple orchards located in northern Italy (Figure S1a), in the Trentino province (centroid: 46°6'13" N, 11°4'22" E), where a substantial part of national apple production takes place. Two independent field trials were carried out: on the field damage before harvest (2020) and on the effect of timing on the recorded feeding damage (2021).

2.2 | Evaluation of field damage before harvest

To evaluate the influence of cultivar, canopy height, plant distance to the edge and management (IPM/organic), we selected 106 orchard blocks, distributed in the most productive areas of the region (Figure S1b). The management of *H. halys* in IPM orchards relied predominantly on specific treatments with acetamiprid (e.g. Epik SL, Kestrel and Gazelle), on average 2 treatments in 2020, but also benefited from the collateral effect of other synthetic insecticides potentially used throughout the season to control other pests (e.g. codling moth and Mediterranean fruit fly). Conversely, organic orchards relied on a single active ingredient, the natural pyrethrum (e.g. Pyganic and Asset Five), whose residual action is significantly lower than that achievable with synthetic insecticides, thus insect-proof netting was widely used.

We focused on five cultivars that are grown in the area, which have different ripening times: from late August to mid-September ('Golden Delicious', $n=24$ and 'Red Delicious', $n=24$), mid-late September ('Granny Smith', $n=16$) and late September to late October ('Fuji', $n=26$ and 'Pink Lady', $n=16$).

The surveys were performed from the first of September to the end of October, in the week preceding the harvest of each cultivar. For each orchard block, we selected two survey points: 'edge', the external row of the orchard, within 5 m from semi-natural vegetation, and 'core', a row located beyond 15–20 m from the edge, as in Venugopal et al. (2015). In each survey point, we evaluated 501 apples (1002 apples per block), distributed over 8–10 plants. On each plant, we identified three areas, according to the height from the ground: low (<1 m), medium (1–1.8 m) and high (>1.8 m), so that the 501 apples were equally distributed in the three zones (i.e. 167 apples per area). External feeding injury from *H. halys* on apple surface was assessed by counting the number of discoloured spots and depressions, which are the marks evaluated by inspectors to establish the economic injury level (Bergh

et al., 2019; Joseph et al., 2015). For the analyses, the total number of external injuries recorded in each zone was summed, to treat each apple as a replicate.

2.3 | Feeding damage in inclusion cages

A 'Red Delicious' orchard was selected because of its high sensitivity to stink bug damage (Aubry et al., 2016), which makes fruit injury assessment easier, compared to other available cultivars.

Halyomorpha halys individuals were collected in the area and reared in plastic cages (BugDorm-4M3030) at Fondazione Edmund Mach, on green beans (*Phaseolus vulgaris* L.), tomato (*Solanum lycopersicum* L.), carrots (*Dacus carota* L.) and peanuts (*Arachis hypogaea* L.). They were kept in controlled conditions ($25 \pm 3^\circ\text{C}$, $60\% \pm 10\%$ RH and 16:8hL:D), before they were placed in the field cages. These were built following Joseph et al. (2015), using insect rearing bags (BugDorm, size 40×25 cm, 104×94 mesh/square inch), in which we inserted semi-rigid, green plastic mesh (13×13 mm openings) and a bamboo dowel (8 cm) placed perpendicularly, to prevent the collapse of the cage (Figure S1c). To avoid the occurrence of insect injury before the assessment, we position 180 field cages right after fruit set (10 May 2020). Treatments were randomly assigned and consisted of fruit never exposed to *H. halys* (control), fruit exposed to three adults for 48 h in May, June or July and fruit exposed to three nymphs (testing three nymphal instars separately, third to fifth) for 48 h, in July. Nymphs were included only in the last period of exposure since they were not present in the orchards earlier in the season (Zapponi et al., 2023). The plants were treated with etofenprox and flonicamid during pre-bloom and sulfoxaflor in post-bloom; no other insecticides were applied afterwards. To reduce the possible confusion of the effect of 'cork spot' (a physiological disorder associated with calcium deficiency), with feeding damage, a calcium chloride foliar fertilizer was applied four times between July and August. The injury evaluation was performed on the same day of harvest. External feeding injury was assessed as the number of discoloured spots and depressions, as in the previous experiment. Internal injury was recorded as the area of necrotic tissue, slicing each apple to the core in each quadrant to expose the area underneath the external injuries (Joseph et al., 2014). Each internal injury was photographed (Canon 80D with Canon EF 100mm f/2.8L Macro IS USM and Manfrotto tripod 055XDB) and measured with ImageJ software version 1.53 (Schneider et al., 2012).

2.4 | Statistical analysis

All analyses were performed with R version 4.2.2 (R Core Team, 2021) and ggplot2 (Wickham, 2016), using RStudio (Posit team, 2023). Since data were not normally distributed, differences in the number and severity of internal and external injuries among treatments were analysed with Mann–Whitney test and with Kruskal–Wallis test, followed by Dunn's post hoc test with Bonferroni correction.

To analyse the influence of several factors (i.e. position in the field, position in the canopy, management and cultivar) on the number of external injuries (which showed a skewed data distribution with exact zeros), a Tweedie generalized linear mixed model (GLMM) with a log link function was applied using the package glmmTMB (Brooks et al., 2017). To account for potential similarities among orchard blocks, orchard ID was used as a random factor. The GLMMs were built to account for all possible interactions among the involved variables. Model assumptions were verified following Zuur and Ieno (2016) and with package DHARMA (Hartig, 2021). Akaike information criterion (AIC) was used for model ranking, with package MuMIn (Bartoń, 2023).

For the feeding damage in exclusion cages, the number of external injuries was analysed with negative binomial generalized linear model (GLM) while for internal injury extent (continuous data with exact zeros) we used a Tweedie GLM. In both cases, the recorded injury was model as a function of exposure time and *H. halys* life stage. Following Dunn and Smyth (2018), for the Tweedie GLM parametrization and execution we used the following packages respectively: tweedie (Dunn & Smyth, 2008) and statmod (Giner & Smyth, 2016). For the selected models, marginal effects were plotted with ggeffects (Lüdtke, 2018) to visualize the predicted values.

3 | RESULTS

3.1 | Apple injury field evaluation

Of the 106,212 evaluated apples, 8.08% (1688) showed signs of damage (Figure S2). In terms of cultivar (Figure 1a), the number of injuries was significantly higher for Fuji and Granny Smith (Kruskal–Wallis: $H = 31.529$, $p < 0.001$). Significant differences were also detected in terms of position in the orchard (Figure 1b), with damage significantly higher at the edge than in the core (Mann–Whitney: $W = 63,921$, $p < 0.001$). For canopy height (Figure 1c), the injury level was higher on the top (Kruskal–Wallis: $H = 26.99$, $p < 0.001$) compared to medium and low. For what concerns management (Figure 1d), the recorded damage was significantly higher in organic than in IPM orchards (Mann–Whitney: $W = 14,790$, $p < 0.001$).

According to model selection (Table 1), the best model involved the interaction between cultivar and management: number of injuries (response variable) \sim cultivar \times management + canopy height + position in the orchard (Table 2). The model confirmed that apples located higher in the canopy, on edge rows and in orchards with organic management had a higher number of external injuries. However, the interaction between organic management and cultivar highlighted that such factors were more relevant when occurring in combination, and on Fuji and Granny Smith cultivars (Figure 2). Post-hoc comparisons showed that the number of external injuries was significantly higher for organic Fuji apples compared to both IPM and organic Golden Delicious and Red Delicious and only IPM for Granny Smith, Pink Lady and Fuji

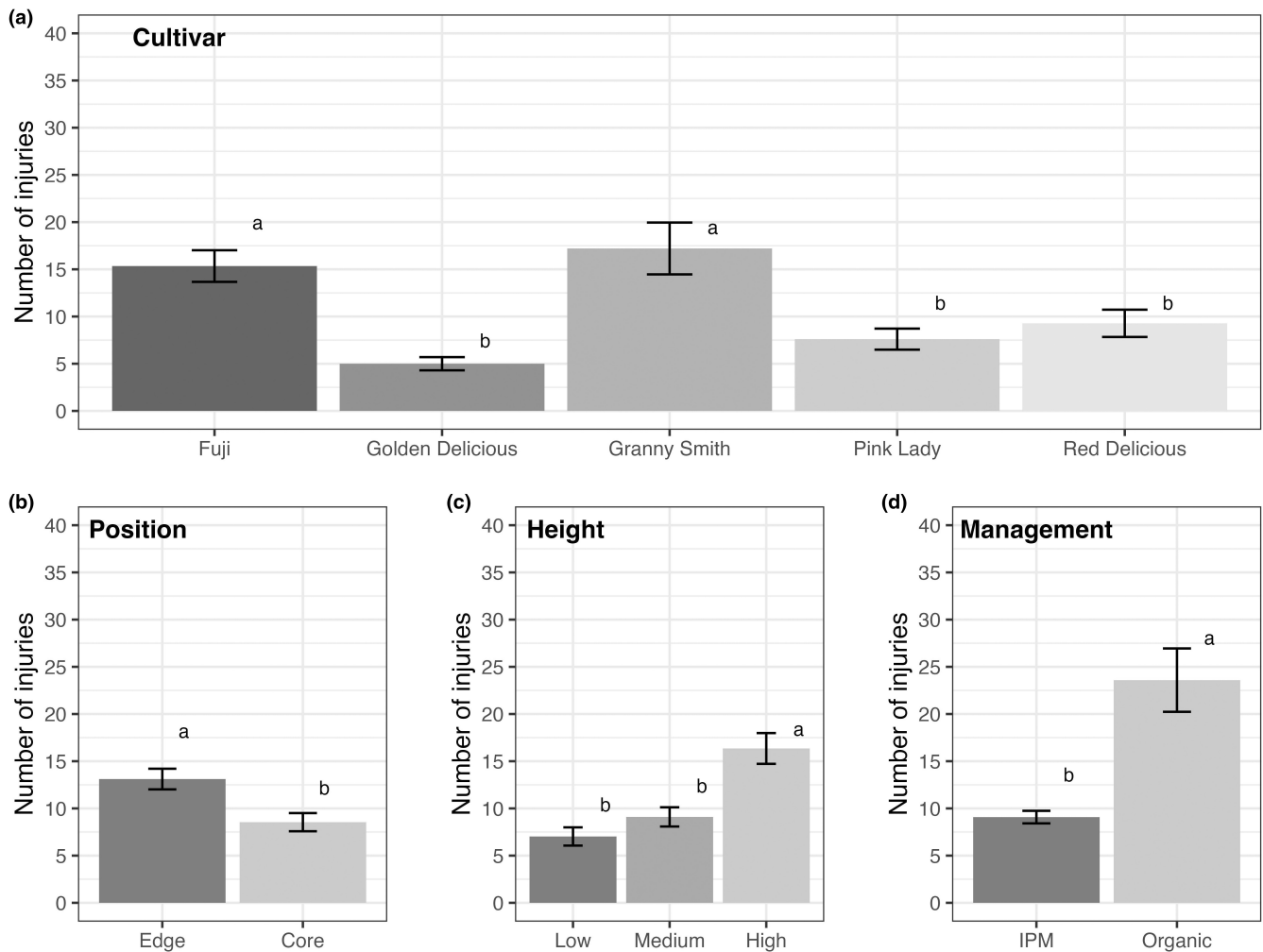


FIGURE 1 Total number of recorded injuries per orchard (mean ± SE) for the examined apples according to (a) cultivar, (b) position in the field, (c) height on the tree and (d) management. Letters indicate significant differences among the categories (Mann–Whitney and Kruskal–Wallis tests, $p < 0.005$).

TABLE 1 Akaike Information Criterion (AIC), corrected AIC (AICc) and relative likelihood of the model (weight) for the Tweedie GLMM models on the data on field damage before harvest.

Model	df	AIC	AICc	Weight
Numb. injuries ~ Cultivar × Management + Height + Position	16	3660.7	3661.6	0.726
Numb. injuries ~ Cultivar × Position × Management + Height	25	3661.5	3663.6	0.268
Numb. injuries ~ Cultivar + Management + Height + Position	12	3671.3	3671.8	0.004
Numb. injuries ~ Cultivar × Position + Height + Management	16	3673.9	3674.8	0.001
Numb. injuries ~ Cultivar × Height + Management + Position	20	3675.1	3676.5	0.000
Numb. injuries ~ Cultivar × Management × Height + Position	34	3675.8	3679.7	0.000
Numb. injuries ~ Cultivar × Position × Height + Management	34	3694.0	3697.9	0.000
Numb. injuries ~ Cultivar × Position × Height × Management	63	3697.6	3711.4	0.000

(Table S1). Moreover, there was also a significant three-way interaction between cultivar, management and position (edge > core) for Fuji IPM and organic, Golden Delicious IPM, Granny Smith IPM and organic, Pink Lady IPM and Red Delicious IPM and organic (Table S2).

3.2 | Apple injury in exclusion cages

A total of 360 apples, which developed inside the 180 exclusion cages, were evaluated (Figure S2 and Table S3). Control apples did not show any signs of internal/external injury, confirming that the

TABLE 2 Estimated regression parameters, standard errors, z-values and p-values for the best Tweedie GLMM model on the number of injuries recorded in the field pre-harvest.

	Estimate	SE	z Value	p-Value
Intercept	1.868	0.205	9.131	<0.001
Cultivar: Golden Delicious	-0.764	0.260	-2.942	0.003
Cultivar: Granny Smith	-0.247	0.278	-0.888	0.375
Cultivar: Pink Lady	-0.627	0.299	-2.093	0.036
Cultivar: Red Delicious	-0.216	0.241	-0.898	0.369
Management: Organic	2.236	0.512	4.372	<0.001
Height: Medium	0.267	0.110	2.429	0.015
Height: High	0.861	0.101	8.512	<0.001
Position: Core	-0.489	0.082	-5.940	<0.001
Cultivar: Golden Delicious * Management: Organic	-0.737	0.467	-1.579	0.114
Cultivar: Granny Smith * Management: Organic	-0.383	0.882	-0.435	0.664
Cultivar: Pink Lady * Management: Organic	-1.859	0.934	-1.992	0.046
Cultivar: Red Delicious * Management: Organic	-2.092	0.563	-3.715	<0.001

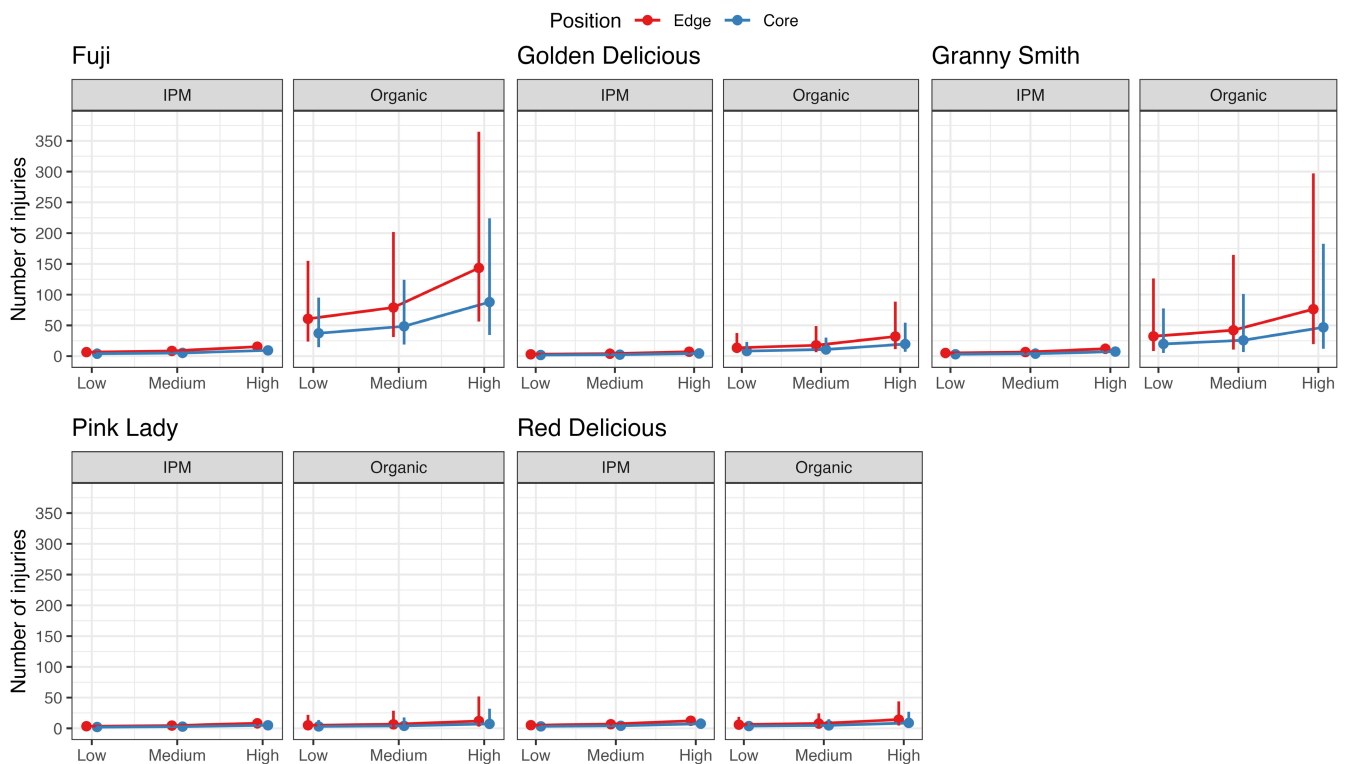


FIGURE 2 Marginal effects and adjusted predictions for the GLMM model for the number of external injuries recorded before harvest, for each cultivar.

design of the cages was appropriate to prevent insect injury, as previously proved (Bergh et al., 2019). For apples exposed to adult *H. halys* in May, the highest number of injuries was recorded in June, while the number of detectable marks decreased in the following controls (Figure 3a). Such trend, however, was not significant (Kruskal–Wallis: $H=4.013$, $p=0.135$). Internally, the mean size of the feeding injuries was higher in the last control but below 0.3mm^2 and not significantly different than in previous controls (Kruskal–Wallis:

$H=0.380$, $p=0.827$). For the apples exposed to adult *H. halys* in June (Figure 3b), we observed a significant decrease in the number of injuries detected in last control compared to the previous one (Mann–Whitney: $W=1044$, $p<0.001$), while in terms of injury size the difference was not significant (Mann–Whitney: $W=739$, $p=0.840$). When comparing the injury levels observed at the last control between the different treatments (Figure 4), the highest number of injuries (Kruskal–Wallis: $H=18.807$, $p=0.002$) and largest areas with

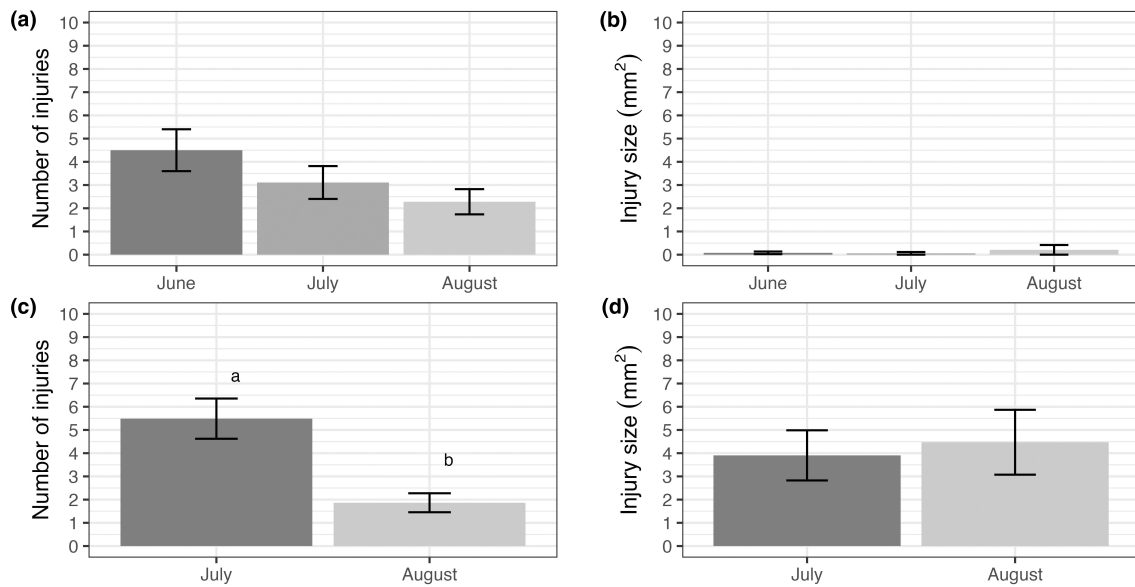


FIGURE 3 Injury evaluation for the apples inside the exclusion cages, for the monthly controls: (a) mean number of injuries (\pm SE) for apples exposed to adult stink bugs in May; (b) mean size of internal injuries (\pm SE) for apples exposed to adult stink bugs in May; (c) mean number of injuries (\pm SE) for apples exposed to adult stink bugs in June; (d) mean size of internal injuries (\pm SE) for apples exposed to adult stink bugs in June. Letters indicate significant differences (Mann-Whitney and Kruskal-Wallis tests, $p < 0.005$).

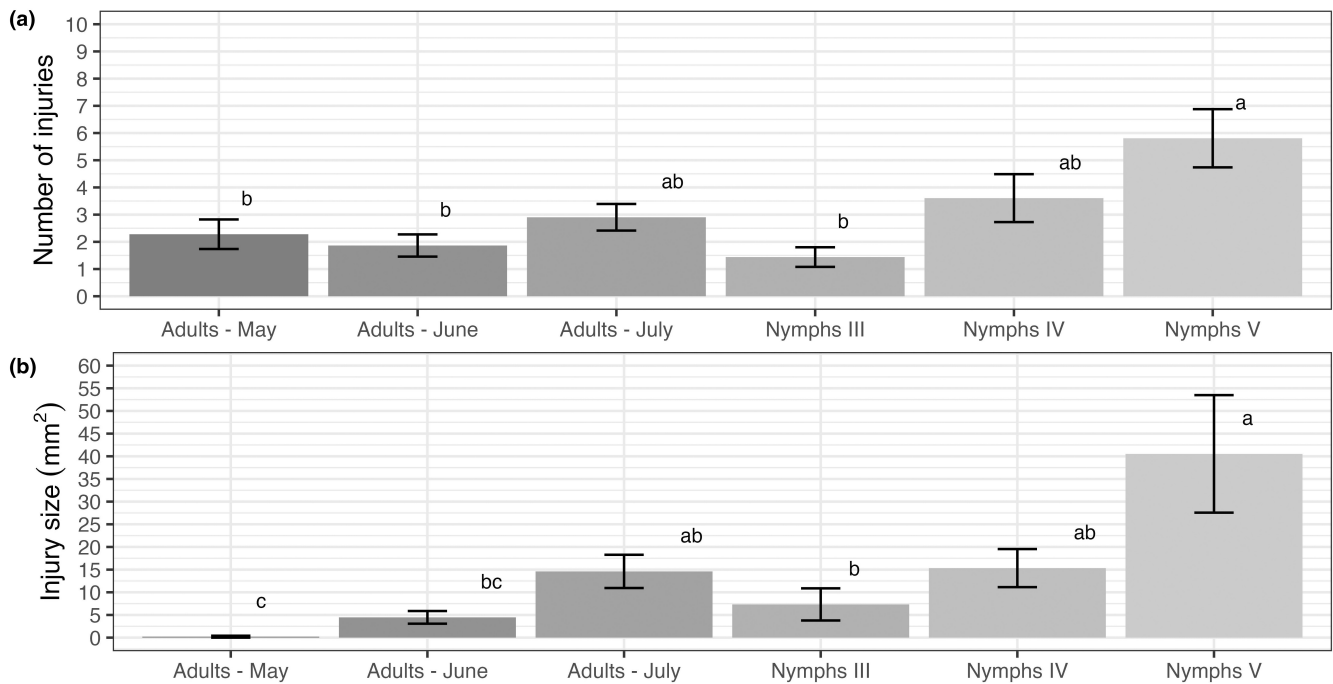


FIGURE 4 Injury evaluation for the last control performed in August: (a) mean number of injuries (\pm SE) and (b) mean size of internal injuries (\pm SE). Letters indicate significant differences among the categories (Mann-Whitney and Kruskal-Wallis tests, $p < 0.005$).

necrotic tissue (Kruskal-Wallis: $H = 28.401$, $p < 0.001$) were both recorded for fifth instars, which however were not statistically different from those observed for fourth instars and adults. The highest impact of the fifth instar, compared to other instars and adults, was detected also with the negative binomial and Tweedie GLMs (Figure 5, Table 3). Post-hoc comparisons revealed that the number

of external injuries was significantly higher for fifth instars compared to all other treatments, apart from fourth instars (Table S4). In terms of internal injuries, the affected area was significantly larger for fifth instars compared to all other tested life stages; besides, the impact of fourth instars and adults in June was significantly higher than what was observed for apples exposed to adults in May (Table S5).

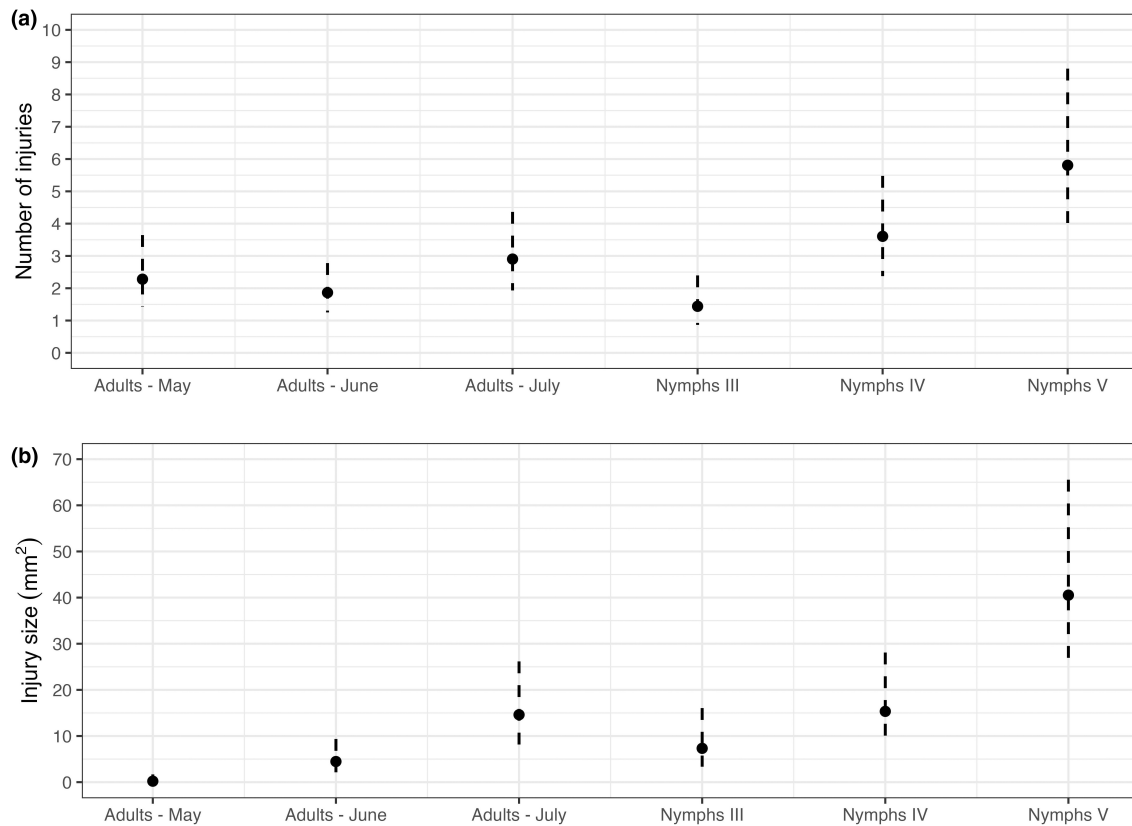


FIGURE 5 Marginal effects and adjusted predictions for the GLM models for the feeding damage in inclusion cages, recorded in August for each category: (a) number of external injuries and (b) area of necrotic tissue of each internal injury.

TABLE 3 Estimated regression parameters, standard errors, z-values and p-values for the GLM models on the number of injuries and injury size recorded at the last control (August).

	Estimate	SE	z Value	p-Value
Number of injuries				
Intercept	0.824	0.240	3.440	0.001
Adults - June	-0.201	0.314	-0.639	0.523
Adults - July	0.242	0.317	0.762	0.446
Nymphs III	-0.460	0.354	-1.300	0.194
Nymphs IV	0.459	0.321	1.430	0.153
Nymphs V	0.935	0.320	2.923	0.003
Injury size				
Intercept	-1.568	1.066	-1.471	0.143
Adults - June	3.066	1.130	2.712	0.007
Adults - July	4.250	1.106	3.841	<0.001
Nymphs III	3.559	1.138	3.127	0.002
Nymphs IV	4.298	1.110	3.874	<0.001
Nymphs V	5.270	1.094	4.819	<0.001

4 | DISCUSSION

The combination of high mobility at the landscape scale and polyphagia (being able to mix spontaneous, ornamental and cultivated

species), allow *H. halys* to quickly colonize new areas and escape management interventions (Akotsen-Mensah et al., 2020). In its invaded range, *H. halys* showed a high adaptability to exploit novel landscape elements (e.g., host plants, crops and overwintering sites), which in turn contributed to the establishment of high-density populations (Wallner et al., 2014). Its damage is often higher along field margins (Venugopal et al., 2015), as the species tends to be more abundant at the orchard borders (Rice et al., 2017). Its damaging potential is so significant that it could be recorded from the early stages of its introduction in new countries (Maistrello et al., 2017). Our results support that the actual risk in terms of pest pressure is mediated by both cultivar and management, and influenced by the timing when the feeding activity occurs. In terms of cultivar, Granny Smith (mid-season) and Fuji (late-season) showed the highest level of injury, while Pink Lady (late-season) did not. This finding is consistent with Shanovich et al. (2020) study on US cold-hardy apple cultivars (Zestar!, Honeycrisp, and Haralson), supporting that the risk of *H. halys* injury is higher for mid- and late-season cultivars, but with exceptions: Haralson for Shanovich et al. (2020), Pink Lady in our case.

When comparing the effect of management practices, we found that the number of injuries was significantly higher for organic compared to IPM orchards. This is not surprising as it has been observed in apple orchards for the codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) as well, where, despite the higher

availability of predators, infestation levels were driven by management, edge density and the presence of semi-natural habitats (Daelemans et al., 2023). Prior studies showed that the proximity to borders, both wooded (Bakken et al., 2015) and not wooded (Bergh et al., 2021), increased the probability of *H. halys* injury. In the region where the current experiments were performed, we found that both *H. halys* and its co-evolved parasitoid *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) were more abundant at the margins of perennial-crops, while generalist parasitoids and egg predation were more common in areas with abundant semi-natural habitats (Falagiarda et al., 2023). Thus, we could expect a potential damage mitigation due to biocontrol in both IPM and organic orchards, however specific experiments should be performed in the future to investigate this aspect.

We found a significant edge-effect, that unexpectedly was consistent for all IPM cultivars and for most organic cultivars (Fuji, Granny Smith and Red Delicious). There is a potential bias associated with the fact that the field data was collected over only 1 year, however its reliability is supported by recent studies which showed a strong consistency of both spatial and temporal *H. halys* distributions, between studied sites (Park et al., 2024), and in relation to ecosystem features (Bosco et al., 2020). The height on the canopy also significantly influenced injury levels, which could reflect *H. halys* distribution: as vertical sampling experiments indicated (Quinn et al., 2019), both adults and nymphs tend to be more abundant in the mid- and upper canopy.

The results of the semi-field experiment showed that early-season injuries presented themselves externally as punctures with only minor internal injuries and part of these marks were not recordable in the following months. This discrepancy is consistent with what was observed by Brown and Short (2010) on *Euschistus servus* (Say) (Heteroptera: Pentatomidae) feeding activity on apple: apart from early ripening cultivars, June and July injuries not always developed beyond a discoloured dot, suggesting that a key factor could be the relative fruit maturity when the feeding activity occurs. While early in the season the absolute number of punctures may be high, or highest as reported by Acebes-Doria et al. (2016), it is important to stress that they did not evolve in distinct depressions, nor did they generate wide areas with necrotic tissue. In contrast with what was observed on corn (Cissel et al., 2015), early exposure to stink bug feeding activity did not compromise apple quality at harvest.

Contrary to what was observed by Acebes-Doria et al. (2016), the injury severity from adults and older nymphs (fourth and fifth instars) were similar, causing the development of depressions externally and necrotic tissue internally, which would be recorded as economic injury at harvest. Similar results were obtained using exclusion cages on kiwifruit, where the incidence of damage from adults and nymphs (fifth instar) did not differ for the three analysed cultivars (Chen et al., 2020), on olives, with a comparable number of dropped olives following feeding activity for adults and nymphs (third and fourth instars together) (Zapponi et al., 2022), and on pistachios, with a similar amount of damage, but fewer aborted

kernels for nymphs (Stahl et al., 2020). A higher level of damage from later life stages was observed for other stink bug species as well, suggesting that it could be associated with the elevated energy requirement for developing wings and reproductive structures (Bueno et al., 2021).

Compared to previous studies (Acebes-Doria et al., 2016; Bergh et al., 2019), we tested the effect of a shorter exposure period, comparing the effect of a small and constant number of both adults and nymphs. Our results showed that even a low number of specimens in contact with the apples for over 48 h can cause significant damage, especially when the exposure happens near full maturation. Thus, regardless the reduced severity of early damage, infestation control should be implemented from the beginning of the season, to prevent the establishment of *H. halys* populations in the orchards, which could be responsible for conspicuous damage later on. Such strategy is particularly relevant for mid- and late-season cultivars.

In conclusion, the combination of two complementary approaches allowed to highlight how several factors that range from orchard management to edge proximity and canopy height, influence the recorded apple injury level, and how such level is also determined by the life stage of both apples and *H. halys* individuals. These results should be used to support the development of informed and sustainable monitoring and control strategies, which are fundamental to improve the efficacy of *H. halys* management (Hahn et al., 2017).

AUTHOR CONTRIBUTIONS

Livia Zapponi: Conceptualization; methodology; writing – original draft; formal analysis; investigation; data curation. **Serena Giorgia Chiesa:** Conceptualization; methodology; writing – review and editing; investigation. **Gianfranco Anfora:** Funding acquisition; writing – review and editing; supervision. **Loris Chini:** Data curation; writing – review and editing; investigation. **Luca Gallimbeni:** Data curation; writing – review and editing; investigation. **Claudio Ioriatti:** Funding acquisition; writing – review and editing; supervision. **Valerio Mazzoni:** Funding acquisition; writing – review and editing; supervision. **Alberto Saggi:** Data curation; writing – review and editing; investigation. **Gino Angeli:** Funding acquisition; writing – review and editing; supervision.

ACKNOWLEDGEMENTS

We thank Sara Zanoni, Matteo Soini, Michele Berti, Giacomo Borri, Teresa Delmarco, Alexis Marchesini, Claudio Panizza, Sacha Bargna and Monica Sofia for their contribution to the field surveys. We would like to thank the anonymous reviewers whose comments helped us improve a previous version of the manuscript.

FUNDING INFORMATION

This study was supported by the SWAT Project (Fondazione Edmund Mach with the contribution of the Autonomous Province of Trento).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are publicly available at <https://doi.org/10.5281/zenodo.8211211>.

ORCID

Livia Zapponi  <https://orcid.org/0000-0003-4033-9001>

Serena Giorgia Chiesa  <https://orcid.org/0000-0001-6209-3933>

Valerio Mazzoni  <https://orcid.org/0000-0003-4036-5322>

Gino Angeli  <https://orcid.org/0000-0002-4924-0481>

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How to cite this article: Zapponi, L., Chiesa, S. G., Anfora, G., Chini, L., Gallimbeni, L., Ioriatti, C., Mazzoni, V., Saggi, A., & Angeli, G. (2024). Combined effect of timing, position and management on *Halyomorpha halys* feeding injury assessment on apple cultivars. *Journal of Applied Entomology*, 00, 1–10. <https://doi.org/10.1111/jen.13319>