Research Article

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Application of sulfur-based products reduces *Halyomorpha halys* infestation and damage in pome fruit orchards

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Abstract

BACKGROUND: The brown marmorated stink bug, *Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae) is an invasive pest that causes economic damage on crops, decreasing fruit yield and quality. Conventional insecticides are frequently used to reduce infestations, but these are often with a limited residual effect, besides being costly and detrimental to nontarget organisms and the environment. In integrated pest management, novel strategies against *H. halys* are proposed, such as the use of alternative substances with an effect on insect behaviour and mobility. As one of the oldest multi-site fungicides applied against fungal pathogens and as an insecticide and acaricide to control scales and mites, sulfur is proposed here to reduce *H. halys* infestation in fruit orchards.

RESULTS: Field experiments were performed to evaluate the effect of repeated wettable sulfur applications on *H. halys* in apple and pear orchards. Sulfur-induced plant phytotoxicity effects and quanti-qualitative parameters on apple fruits were also recorded. *Halyomorpha halys* infestation was significantly reduced in sulfur-treated compared to untreated pears and apples. Furthermore, sulfur sprays reduced fruit damage caused by *H. halys*. Besides, sulfur-mediated phytotoxicity such as symptoms on leaves and fruit drop were not observed. Fruit quality was not influenced by sulfur treatments.

CONCLUSIONS: Wettable sulfur seems to be a promising formulation given the low phytotoxicity, considering the technical aspects for an effective use of sulfur-based products to counteract *H. halys* in pome fruit orchards.

Keywords: brown marmorated stink bug; fruit damage; integrated pest management; phytotoxicity; repellence; wettable sulfur

1 INTRODUCTION

The brown marmorated stink bug, *Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae) is a polyphagous pest native to East Asia[1,2] and an invasive species in several European countries,[2–5] North America (Canada and USA) and South America.[6] The establishment of breeding populations of *H. halys* in the United States and Europe suggests the occurrence of multiple introductions in the newly invaded areas.[7–10] In Asia, *H. halys* is an occasional pest in fruit orchards and often disperses among many host plants during the season.[1] *Halyomorpha halys* was reported for the first time in Europe (Liechtenstein and Switzerland) in 2004,[11] whereas in Italian orchards infestations have been observed since 2012.[12]

In territories where this species is invasive, *H. halys* population dynamics are influenced by habitat composition that in turn affects crop colonization and damage.[13,14] Damage related to *H. halys* infestations concerns major pome and stone fruits (e.g. apple, pear, peach, cherry),[3,15,16] kiwifruit,[13,17,18] but also nut crops,[19–22] row crops, and ornamentals.[1,2,23] Between spring and autumn, *H. halys* can feed on plant tissue such as flowers, leaves, trunks and twigs,[24,25] but it needs to feed on fruit structures to complete its development.[2] Early damage to developing fruits causes fruit deformation and the endocarp fails to expand, also causing early drop and fruit abortion.[26–28] In the late season, other damage includes blotchy symptoms, discoloration, and necrosis on tissues and internal corky areas below the feeding spot.[2,29] The feeding activity of *H. halys* also was associated with increased fruit damage by pathogens that can decrease yield and fruit quality.[16,23] These symptoms can strongly limit fruit marketability.

Conventional insecticides belonging to chemical classes such as pyrethroids (IRAC 3 A), neonicotinoids, butenolides (IRAC 4 A and D) and diacylhydrazines (IRAC 18) are used in Europe to reduce brown marmorated stink bug infestations. Synthetic compounds

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typically used for *H. halys* control are considered detrimental to nontarget organisms and the environment, and are characterized by a limited residual effects on this pest.[30,31] Furthermore, organic growers have few options, mostly pyrethrins (IRAC 3 A), to effectively counteract *H. halys* infestation and damage. In the integrated pest management (IPM) framework, novel strategies against *H. halys* need to be considered, such as the use of alternative substances repelling the insect and deterring its feeding, aiming also to reduce synthetic pesticide applications. These substances, able to manipulate insect behaviour and mobility, are not always effective in managing this pest.[32,33] Insect repellents are still rarely used in IPM strategies owing to practical issues such as the high volatility of most molecules, the degradation process in the environment and the limited residual effects in the field, thus influencing the time and number of applications, and in some cases the presence of residues.[34]

Recently, observations in the Veneto region (northeastern Italy) showed a reduction of *H. halys* infestations in orchards sprayed with sulfur-based pesticides, used against pathogens and other pests. Sulfur is one of the oldest and the most used pesticides worldwide,[35] with first formulations developed in 1802 against grape pathogens[36,37] now broadly applied in agricultural practices as a multisite fungicide and to avoid resistance to other fungicides.[38] Sulfur is admitted for organic agriculture, considered cheaper than other compounds[39] and is often mixable with other active ingredients. In Italy, for instance, sulfur is often applied as insecticide and acaricide to control scales and mites,[39] but also as fungicide against pathogens of grapevine, peach, apple and pear.[38,40] Sulfur-based products can cause direct mortality to pests such as mites and scale insects,[41–43] also influencing insect development and oviposition behaviour.[44–46] As an essential macronutrient for plant growth[47] and being involved in plant physiology development, regulation and abiotic stress resistance, sulfur can play a role in defence mechanisms against pests and pathogens, for example by producing secondary metabolites.[48] In orchard management, sulfur application needs to be planned carefully by considering possible detrimental effects like plant phytotoxicity (i.e. tissue burning on plant organs) and the reduction of fruit production and quality, especially related to high air temperature and droplet size application.[49]

This research aims to determine the effect of a sulfur-based product on *H. halys* infestation in apple and pear orchards. Experiments were performed to observe the effect on *H. halys* nymphs and adults of repeated sulfur applications. Sulfur-induced phytotoxicity effects were also evaluated on leaves and fruits of sprayed orchards. Additionally, quantitative and qualitative parameters on apple fruit production were recorded comparing different treatments.

2 MATERIALS AND METHODS

The experiments on sulfur effect on *H. halys* were conducted in four organic apple orchards and a pear orchard in northeastern Italy. All orchards were trained with the Spindel trellis system. Experiments were conducted from May to October during two growing seasons, in 2020 and 2021. In all the experiments, the sulfur-based fungicide used was Microthiol Disperss® (UPL Openag™ – distributed by UPL Italia s.r.l., Cesena, italy), which contains 80% sulfur as active ingredient (a.i.). Microthiol Disperss is a wettable fungicide registered for apple and pear against powdery mildew, scab, blister mite, red spider mite, silver mite and two-spotted mite. This sulfur-based product is admitted in organic farming and has small particle size, minimal dust and instantaneous dispersion that makes it easy to dissolve in water. Microthiol Disperss applications were performed at the field rate of 3 kg ha^{-1} (300 g hL^{-1} in 100 L water) on apple in spring and autumn, and 2 kg ha^{-1} (200 g hL^{-1} in 100 L water) in summer with temperature >28 °C, on both apple and pear. In all of the experiments described below, sulfur treatments were applied with an axial fan air blast sprayer commonly used for treatments in orchards and in no-wind conditions, thus respecting the standard requirements for orchard sprays. These conditions were maintained in each treated plot, where the axial fan air blast sprayer uniformly applied the product to all plants within the treatment area, where rows were sprayed on both sides.

2.1 Experimental design

A first experiment (orchard A) was performed during the growing season in 2020. Orchard A was located in Bevilacqua, Verona province, northern Italy (45° 12′ 5.93″ N, 11° 24′ 1.90″ E), on 1.30 ha apple cultivation (cv. Golden Delicious, grafted on M9). This orchard was planted in 2004 and had a plant spacing of 2.0 m × 4.5 m (1110 trees ha^{-1}). Two treatments were compared for this experiment: untreated plots (CTRL) and plots treated with sulfur (S). Plots were arranged in a randomized block design and four replicates (replicate size: four rows of 18 trees) were included for each treatment. Microthiol Disperss applications were performed at the 3 kg ha^{-1} field rate according to seasonal fungicide spraying against fungal pathogens (e.g. apple scab and powdery mildew). Three sprays were performed on 6, 14 and 26 July, with a working pressure of 16 bar, spray volume 1300 L ha^{-1} and 6 km h^{-1} driving speed.

The second experiment (orchard B) was performed in both growing seasons, in 2020 and 2021. The orchard was in Bevilacqua, Verona province, northern Italy (45° 12′ 9.88″ N, 11° 24′ 2.89″ E), on 1.56 ha apple plants (cv. Golden Orange, grafted on M9). This orchard was planted in 2014 and had a plant spacing of 1.3 m × 4.0 m (1923 trees ha^{-1}). Two treatments were compared for this experiment: untreated plots (CTRL) and plots treated with sulfur (S). Plots were arranged in a randomized block design and eight replicates (replicate size: four rows of 15 trees) were included for each treatment and year. In 2020, sprays were performed on 6, 14 and 26 July, whereas in 2021 there were seven applications, on 10, 16, 21, 28 June, and 5, 17, 26 July. For all sprays, the working pressure was 15 bar, with spray volume 1000 L ha^{-1} and driving speed 6 km h^{-1}.

The experiment in orchard C was performed during the growing season in 2021, and was in Bosco di Zevio, Verona province, northern Italy (45° 20′ 51.34″ N, 11° 9′ 14.68″ E), on 5.34 ha cultivated apple trees (cv. Granny Smith, grafted on M9). This orchard was planted in 2000 and had a plant spacing of 1.5 m × 4.5 m (1481 trees ha^{-1}). Three treatments were compared in this experiment: untreated plants (CTRL); sulfur-treated plants at ≤20 mm apple diameter (T-20); sulfur-treated plants at ≥20 mm apple diameter to harvest (T-harvest). Plots were arranged in a randomized block design and six replicates (replicate size: four rows of 15 trees) were included for each treatment. Sulfur applications started on 19 May and ended on 28 September. Sulfur-treated apple plants in the T-20 plots were sprayed four times every 7–8 days, from 19 May to 8 June, whereas those in the T-harvest plots were sprayed six times every 10–12 days, from 8 June to 28 September. For all sprays, the working pressure was 13–14 bar, with spray volume 500 L ha^{-1} and driving speed 6.1 km h^{-1}.

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The experiment in orchard D was performed during the 2021 growing season. This orchard was in Legnago, Verona province, northern Italy (45° 11′ 7.30″ N, 11° 20′ 29.80″ E) and consisted of 1.45 ha cultivated apple plants (cv. Granny Smith, grafted on M9). This orchard was planted in 1991 and had a plant spacing of 2.0 m × 5.0 m (1000 trees ha^{-1}). Two treatments were compared: untreated plants (CTRL) and plants treated with sulfur (S). Plots were arranged in a randomized block design and eight replicates (replicate size: four rows of 15 trees) were included for each treatment. Sulfur applications started on 17 June and ended on 1 October. Sprays were performed 11 times, applied every 10–12 days. For all treatments, the working pressure was 18–19 bar, with spray volume 1100 L ha^{-1} and driving speed 5.5 km h^{-1}.

The field experiment on pear was performed in orchard E during the 2021 growing season. The orchard was in Terrazzo, Verona province, northern Italy (45° 9′ 28.68″ N, 11° 23′ 45.04″ E) and consisted of 2.51 ha with different pear cultivars: White William, Conference and Abate Fétel. The orchard was planted between 2000 and 2010, and mean plant spacing was 1.5 m × 4.0 m (1670 trees ha^{-1}). Two treatments were compared: untreated plants (CTRL) and plants treated with sulfur (S). Plots (five in total) were arranged in a randomized block design, and eight replicates per cultivar (replicate size: four rows of 15 trees) were included for each treatment. Sulfur sprays started on 10 June to finish on 30 July, with a total of six applications performed every 10–12 days. For all treatments, the working pressure was 14–15 bar, with spray volume 600 L ha^{-1} and driving speed 6.0 km h^{-1}.

In each experimental orchard (A–E), the same fungicides (i.e. calcium polysulfide and copper) were applied during growing seasons to control the most important apple and pear diseases. A pyrethrins-based insecticides was applied against *H. halys* infestation only in orchard B on 10 May 2020, without affecting data collection for the present study. The late frost events occurring in March and April 2021 caused loss of pear production of 100% and >50% of apple fruits.[50]

2.2 *Halyomorpha halys* infestation

Halyomorpha halys abundance was periodically quantified in apple and pear orchards. Orchards A and B were sampled every 7 days in July 2020, and every 7–10 days in 2021 (only orchard B), from early June to the end of July. Orchard C was sampled every 7–10 days from mid-May to the end of September 2021. In orchard D, samplings were conducted every 7–10 days from mid-June to mid-October, and in pear orchard E from early June to the end of July 2021. The abundance of *H. halys* nymphs and adults in each orchard and experimental plot was evaluated by shaking apple or pear branches to collect insects that fell on a 1 × 1 m white beating sheet placed under the tree canopy. Beating samplings were performed on four plants located in the inner part of the two central rows of each replicate (per treatment: 16 plants in orchard A, 32 in B, 24 in C, 32 in D and 32 in E). Every plant was shaken once in each sampling date. The counts of all brown marmorated stink bug individuals found on the sheet from the four plants were merged, differentiating adults from nymphs. In all orchards, *H. halys* infestation level among plots also was assessed before starting sulfur applications.

2.3 Fruit damage caused by *Halyomorpha halys*

The percentage of damage caused by *H. halys* feeding on apples and pears was assessed at harvest time for each treatment by observing the fruiting structures. Fruits were classified into four categories[51]: healthy fruits with no damage caused by brown marmorated stink bug (D0); fruits with ≤10% of their surface damaged, thus representing a low feeding activity of *H. halys* that makes the fruit still acceptable on the fresh market (D1); fruits that presented ≤40% surface damaged, making these fruits not marketable (D2); fruits with ≤100% of the fruit surface damaged and deformed (D3). Orchard B was sampled on 25 August 2021 and 25 fruits per replicate were analyzed (200 apples per treatment), collecting fruits from three or four plants located in the inner part of the replicate. In orchard C, 100 fruits per replicate (from three or four plants located in the inner part) were collected on 22 October (400 apples per treatment), and in orchard D the 100 fruits per replicate (from three or four plants located in the inner part) were collected on 28 September (800 apples per treatment). Fruit damage was not estimated in the apple orchard A and pear orchard E surveyed in 2021 owing to the spring frost event, which caused the complete loss of fruiting structures on these crops.

2.4 Sulfur-induced phytotoxicity

In the sulfur product label, a risk of phytotoxicity is reported for Golden Delicious apple and for William pear. No information was available for Granny Smith and the other pear cultivars. Thus, we evaluated the phytotoxic effect of sulfur on Granny Smith in apple orchard C, where we assessed the effects induced by wettable sulfur treatments on leaves and fruits. Observations on phytotoxicity on fruits and leaves were made from fruit set until reaching swelling fruitlet of 20 mm in diameter, considered the most sensitive phenological stage in pome fruits.[52–55] This aspect also emerged from preliminary observations conducted on different apple cultivars in previous years. Phytotoxicity was assessed by collecting 100 leaves per treatment and replicate (600 leaves in total) on the whole leaf lamina following EPPO/OEPP standards, so that leaf phytotoxicity assessments were rated on a 0–5 scale as described by Holb *et al*.[49] Measures on leaves were recorded as: 0 = no damage; 1 = leaf 60–80% of normal size and no leaf necrosis; 2 = leaf <60% of normal size and no leaf necrosis; 3 = leaf <60% of normal size and with brown margins (<3% leaf necrosis); 4 = leaf <60% of normal size and 3–6% leaf necrosis; 5 = bumpy small leaf and >6% leaf necrosis.

Fruit phytotoxicity was evaluated as number of fruits dropped following each sulfur treatment (T-20) compared to untreated plots (CRTL). Fruit drop was calculated as the percentage of dropped fruits on the total number of apples per plant, on four plants per treatment and replicate – thus counting fruits on 24 plants per treatment. A band of white nonwoven tissue (TNT; 7.0 m × 1.20 m) was placed under the plants in orchard C to count the number of dropped fruits. The number of apple fruits still present on plants was counted at harvest (28 September). Leaf and fruit observations were carried out six times from mid-May to mid-July, on 19 and 26 May, 8 and 17 June, 8 and 21 July, respectively. Phytotoxicity was not evaluated on pear cultivars as a consequence of the lack of fruit following spring frost.

We also evaluate the effect of sulfur on apple Granny Smith fruit quality. Specifically, fruit not showing external *H. halys* damage were selected in orchards C and D. On 28 September and 22 October, these fruits were placed in a refrigerator at 3–4 °C and transported to the laboratory of DAFNAE, University of Padua. Apples were then visually inspected to asses any defect in colour (e.g. russeting) and also the following parameters were evaluated: weight (g), firmness (kg cm^{-2}) and sugar content (°Brix; only for fruits from orchard D). A skin portion was removed from two sides of the same fruit, to test fruit firmness on the skinless sections using a handheld penetrometer (FT 30; Wagner Instruments, Greenwich, CT, USA). The sugar content of each fruit was assessed by placing a drop of fruit juice on a handheld optical brix refractometer (RHB-18ATC; Lumen Optical Instrument Co., Ltd, Fuzhou, China). In these assessments, 40 fruits per treatment (10 per replicate, in four replicates) were collected in orchard C, and 40 fruits (10 per each replicate) in orchard D. All fruits were collected from four plants located in the inner part of the replicate.

2.5 Data analysis

Data on the abundance of *H. halys* observed in each experiment were analyzed with separated generalized linear mixed repeated measures models with a lognormal error distribution and an identity link function, using the GLIMMIX procedure of SAS (v9.4).[56] The same analysis was performed for the phytotoxicity data on leaves and fruits (orchard C). The number of nymphs and adults found in apple and pear orchards was considered as dependent variable in two separate models. In these models, treatment (orchards A, B, and E: CTRL *versus* S; orchard C: CTRL *versus* T-20 *versus* T-harvest) and sampling date were considered as categorical independent variables. For orchard E, the three pear cultivars were considered as a random term in the models.[57] Degrees of freedom (df) were projected using the Kenward and Roger method. Data on nymphs and adults were checked for normality assumption. The SLICE option of the LSMEANS statement was used to test treatment effect variation during observation periods. Untransformed data were used, and model assumptions were evaluated by inspecting diagnostic plots of model residuals.

Data on damaged fruit categories (D0–D3; orchards B, C and D) were analyzed by considering the percentage of fruit belonging to each category and run separately with a logit regression model using the GENMOD procedure of SAS (v9.4).[56] Category rates were considered as dependent variables in separate models, and the effect was tested with a χ^2 test ($\alpha = 0.05$). Differences among treatments were evaluated with a χ^2 test ($\alpha = 0.05$) on least-square means.

For the evaluation of phytotoxicity on leaves and fruits, we ran the analysis on CTRL *versus* T- 20 treatments. The effect of all independent variables and their interactions were tested with an *F*-test ($\alpha = 0.05$). Differences among treatments were evaluated with a Bonferroni test ($\alpha = 0.05$) on least-square means. Data on fruit weight, firmness and sugar content (orchards C and D) were analyzed with a generalized linear model using the GLM procedure of SAS (v9.4).[56] In this model, the treatment was considered as categorical independent variable, and the effect was tested with a Tukey's honestly significant difference test ($\alpha = 0.05$) on least-square means. Untransformed data were used, and model assumptions were evaluated by inspecting diagnostic plots of model residuals.

3 RESULTS

3.1 *Halyomorpha halys* infestation

Halyomorpha halys population was continuously observed throughout the two growing seasons, in all experimental apple and pear orchards. In apple orchard A, the abundance of *H. halys* before starting sulfur applications did not differ among experimental plots ($F_{1,24} = 3.51$, $P = 0.073$). The infestation of nymphs was significantly influenced by the sulfur treatments and sampling date (Table 1). A lower level of nymph infestation was observed in sulfur-treated plots if compared to the untreated ones ($t = 5.51$, df = 20, $P < 0.001$; data not shown). The abundance of adults over the season was significantly influenced by the sulfur treatments, sampling date and the interaction between the two effects (Table 1). After beginning of the experiment, a lower number of adults was observed in sulfur-treated plots if compared to the untreated ones ($t = 3.07$, df = 24, $P = 0.005$), with treated plots having a reduction of ≈80% in adult infestation (Fig. 1).

In apple orchard B, the abundance of adult individuals did not differ among experimental plots in either year (2020: $F_{1,22} = 0.01$, $P = 0.999$; 2021: $F_{1,76} = 0.61$, $P = 0.437$) and nymphs appeared only later in the season (data not shown). The infestation of *H. halys* nymphs was significantly influenced by sulfur treatments in 2021 only, whereas this effect was not observed in 2020 (Table 1). The presence of *H. halys* nymphs did not show variations depending on the sampling date in either year (Table 1). In 2021, a lower number of nymphs was observed in July in sulfur-treated plots if compared to the untreated ones ($t = 2.07$, df = 55, $P = 0.043$). The number of *H. halys* adults was significantly affected by sulfur treatments in both years, with differences among sampling dates (Table 1). The interaction 'sampling date*-treatment' showed significant differences in *H. halys* adult infestation in 2020, but this was not observed in 2021 (Table 1). The number of *H. halys* adults was lower in sulfur-treated plots if compared to control ones (2020: $t = 5.82$, df = 22, $P < 0.001$; 2021: $t = 3.16$, df = 76, $P = 0.002$), and this was observed in 2020 on sampling dates following wettable sulfur applications (Fig. 2), where there was a notable reduction in infestation exceeding 80% in sulfur-treated plots compared to untreated ones.

In apple orchard C, *H. halys* population density did not differ among treatments in the first sampling date, before starting with sulfur applications (nymphs: $F_{2,150} = 0.01$, $P = 0.999$; adults: $F_{2,150} = 0.01$, $P = 0.996$). During the growing season, the number of *H. halys* nymphs was significantly influenced by sulfur treatments, sampling date and the interaction between these two factors (Table 1; data not shown). Nymph density was lower in T-harvest plots than in control ones ($t = 4.50$, df = 150, $P < 0.001$) and T-20 ($t = 2.05$, df = 150, $P = 0.042$), and in T-20 it was lower than in the control ($t = 2.45$, df = 150, $P = 0.015$). On most of the sampling dates, the interaction among factors highlighted a lower nymph infestation when sulfur treatments were made continuously during the summer (T-harvest) when compared to the limited spring period T-20 and untreated plots (Fig. 3). The abundance of brown marmorated stink bug adults was influenced by sulfur treatments, sampling date and the interaction between these two factors (Table 1). Adult density was significantly reduced in sulfur-treated plots, T-20 ($t = 5.79$, df = 150, $P < 0.001$) and T-harvest ($t = 7.69$, df = 150, $P < 0.001$), when compared to nontreated plots. *Halyomorpha halys* population increased in T-20 plots following the last sulfur spraying date (Fig. 3). Considering adult numbers, the reduction in population during sulfur application period was ≈85% in T-20 plots and ≈68% in T-harvest.

In apple orchard D, *H. halys* population density did not differ among plots before sulfur applications (nymph: $F_{1,105} = 0.01$, $P = 0.999$; adult: $F_{1,105} = 0.25$, $P = 0.619$). The abundance of *H. halys* nymphs was not influenced by sulfur treatments, sampling date or 'sampling date*treatment' (Table 1). *Halyomorpha halys* adults differed in numbers according to sulfur applications and sampling date, but this was not observed when considering their interaction (Table 1). The abundance of *H. halys* adults was significantly lower in sulfur-treated plots than in control ones ($t = 4.60$, df = 105, $P < 0.001$).

Table 1. Output of repeated measures ANOVA run on *Halyomorpha halys* infestation level for the experimental orchards A, B, C, D and E, in the growing seasons 2020 and 2021, separately for nymphs and adults

Orchard	Year	*H. halys* stage	Effect or interaction	*F*	df	*P*
Orchard A	2020	Nymphs	Treatment	30.31	1, 20	<0.001
			Sampling date	4.81	4, 20	0.007
			Sampling date*treatment	2.78	4, 20	0.055
		Adults	Treatment	9.44	1, 24	0.005
			Sampling date	6.43	4, 24	0.001
			Sampling date*treatment	4.81	4, 24	0.005
Orchard B	2020	Nymphs	Treatment	0.54	1, 26	0.469
			Sampling date	2.02	4, 26	0.121
			Sampling date*treatment	0.31	4, 26	0.866
		Adults	Treatment	33.83	1, 22	<0.001
			Sampling date	3.15	4, 22	0.034
			Sampling date*treatment	3.69	4, 22	0.019
Orchard B	2021	Nymphs	Treatment	4.28	1, 55	0.043
			Sampling date	1.54	8, 55	0.165
			Sampling date*treatment	0.52	8, 55	0.835
		Adults	Treatment	9.98	1, 76	0.002
			Sampling date	5.94	8, 76	<0.001
			Sampling date*treatment	0.30	8, 76	0.963
Orchard C	2021	Nymphs	Treatment	10.14	2, 150	<0.001
			Sampling date	15.07	14, 75	<0.001
			Sampling date*treatment	1.93	28, 150	0.006
		Adults	Treatment	32.11	2, 150	<0.001
			Sampling date	2.20	14, 75	<0.001
			Sampling date*treatment	2.45	28, 150	<0.001
Orchard D	2021	Nymphs	Treatment	0.46	1, 105	0.501
			Sampling date	1.09	14, 105	0.378
			Sampling date*treatment	1.05	14, 105	0.411
		Adults	Treatment	21.12	1, 105	<0.001
			Sampling date	3.42	14, 105	<0.001
			Sampling date*treatment	0.83	14, 105	0.637
Orchard E	2021	Nymphs	Treatment	3.70	1, 264	0.055
			Sampling date	25.63	6, 264	<0.001
			Sampling date*treatment	3.00	6, 264	0.007
		Adults	Treatment	27.22	1, 257	<0.001
			Sampling date	31.57	6, 257	<0.001
			Sampling date*treatment	2.77	6, 257	0.013

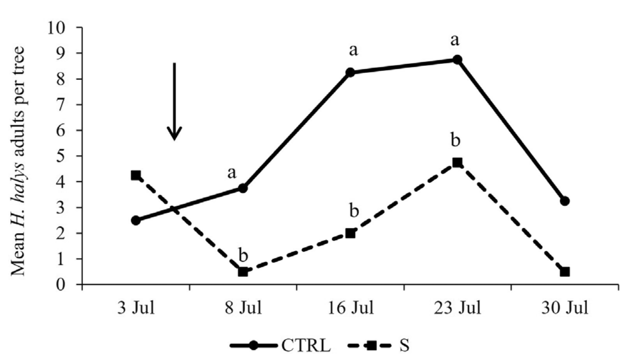

Figure 1. Number of *Halyomorpha halys* adults sampled with beating sampling in apple orchard A in 2020. CTRL, untreated plots; S, sulfur-treated plots; arrow indicates when treatments started. Different letters indicate differences at Bonferroni test ($\alpha = 0.05$).

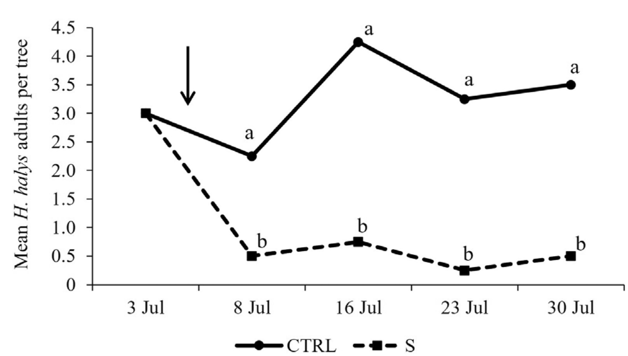

Figure 2. Number of *Halyomorpha halys* adults sampled with beating sampling in apple orchard B in 2020. CTRL, untreated plots; S, sulfur-treated plots; arrow indicates when treatments started. Different letters indicate differences at Bonferroni test ($\alpha = 0.05$).

In pear orchard E, *H. halys* population density did not differ among plots before sulfur applications (for both nymphs and adults, $F_{1,257} = 0.01$, $P = 0.999$). The number of nymphs was not influenced by sulfur applications, but differences were observed when we considered sampling date and the interaction 'sampling date*treatment' (Table 1). At the beginning and end of their activity period in the field, nymph infestation was lower in sulfur-treated plots than in control plots (Fig. 4). The number of adults was influenced by the treatments as well, and by sampling date and the interaction between these two factors (Table 1). From mid-June until late-July 2021, a lower infestation was observed in sulfur-treated plots than in control ones ($t = 5.22$, df = 257, $P < 0.001$; Fig. 4).

3.2 Fruit damage caused by *Halyomorpha halys*

In orchards B and D, the percentage of undamaged apples at harvest differed among treatments (B: $\chi^2 = 50.47$, df = 1, $P < 0.001$; D: $\chi^2 = 132.69$, df = 1, $P < 0.001$) and was higher in S than in CTRL (Fig. 5). Differences among treatments on fruits classified as D1 in both orchards were observed in the two orchards (B: $\chi^2 = 39.58$, df = 1, $P < 0.001$; D: $\chi^2 = 9.54$, df = 1, $P = 0.002$). The number of slightly damaged apple fruits (D1) was higher in sulfur-treated plots than in control ones in orchard D, but the opposite trend was observed in orchard B (Fig. 5). Sulfur treatment influenced the amount of fruit classified ad D2 for both orchards (B: $\chi^2 = 125.13$, df = 1, $P < 0.001$; D: $\chi^2 = 111.62$, df = 1, $P < 0.001$), with a lower number of D2 fruits in S plots than in CTRL ones (Fig. 5). Heavily damaged apples (D3) were affected by the treatment in orchard D only ($\chi^2 = 11.13$, df = 1, $P < 0.001$), with an overall reduction of fruits classified as D3 in the sulfur-treated plots (Fig. 5). In orchard B, D3 apples were not observed.

In orchard C, the percentage of undamaged apples differed among treatments ($\chi^2 = 9.88$, df = 2, $P = 0.007$) and was higher in T-harvest plots than in CTRL ones, yet intermediate in the T-20 plots (Fig. 6). Differences among treatments on fruits classified as D1 also were observed ($\chi^2 = 8.90$, df = 2, $P = 0.012$), with CTRL plots showing more D1 apples than in T-harvest, and intermediate levels in T-20 plots. Sulfur treatment influenced the amount of fruit in the D2 category ($\chi^2 = 11.13$, df = 2, $P < 0.001$), and a low number of D2 fruits was observed in T-harvest plots in comparison to T-20 and CTRL ones (Fig. 6). In this orchard, D3 fruits were not observed.

3.3 Sulfur-induced phytotoxicity

In orchard C, leaf symptoms associated with phytotoxicity were never observed. Besides, no differences on dropped fruits

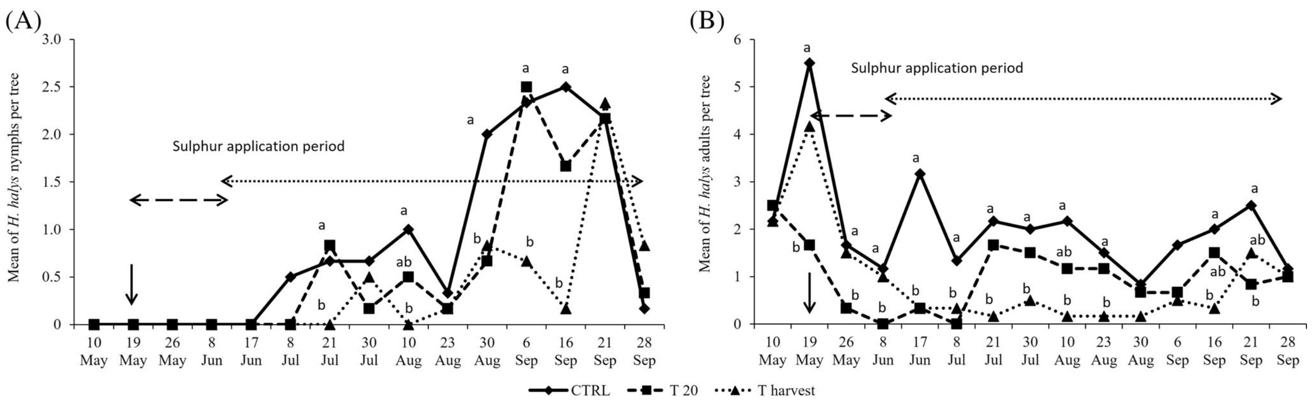

Figure 3. *Halyomorpha halys* nymphs (A) and adults (B) sampled with beating sampling in apple orchard C in 2021. CTRL, untreated plots; T-20, sulfur-treated plots with apple diameter ≤20 mm; T-harvest, sulfur-treated plots with apple diameter from 20 mm to fruit harvest. Vertical arrow indicates the start of treatments. Horizontal dashed double-headed arrow indicates the sulfur applications period in T-20 plots, whereas the horizontal dotted double-headed arrow indicates the sulfur applications period in T-harvest plots. Different letters indicate differences at Bonferroni test ($\alpha = 0.05$).

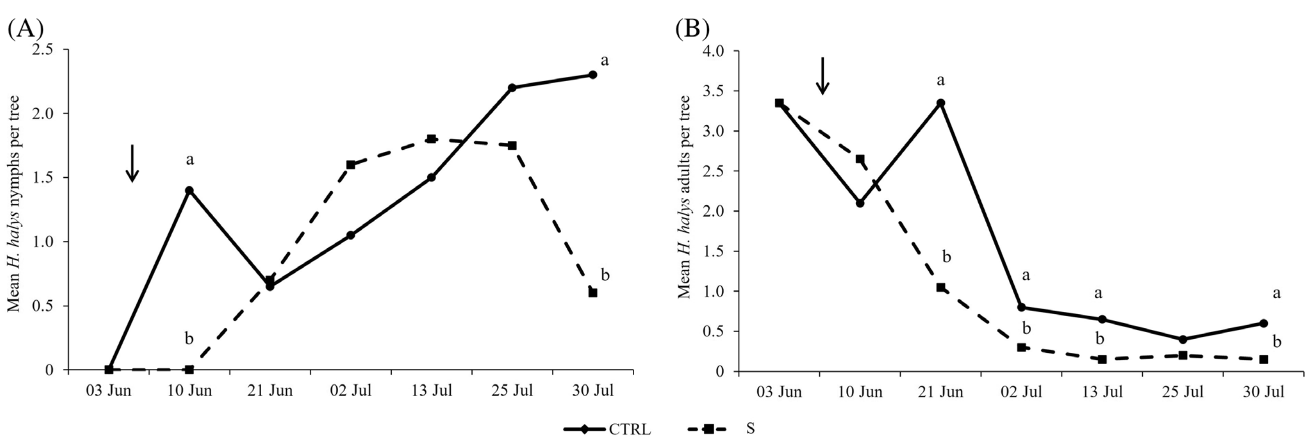

Figure 4. *Halyomorpha halys* nymphs (A) and adults (B) sampled with beating sampling in pear orchard E in 2021. CTRL, untreated plots; S, sulfur-treated plots; arrow indicates when treatments started. Different letters indicate differences at Bonferroni test ($\alpha = 0.05$).

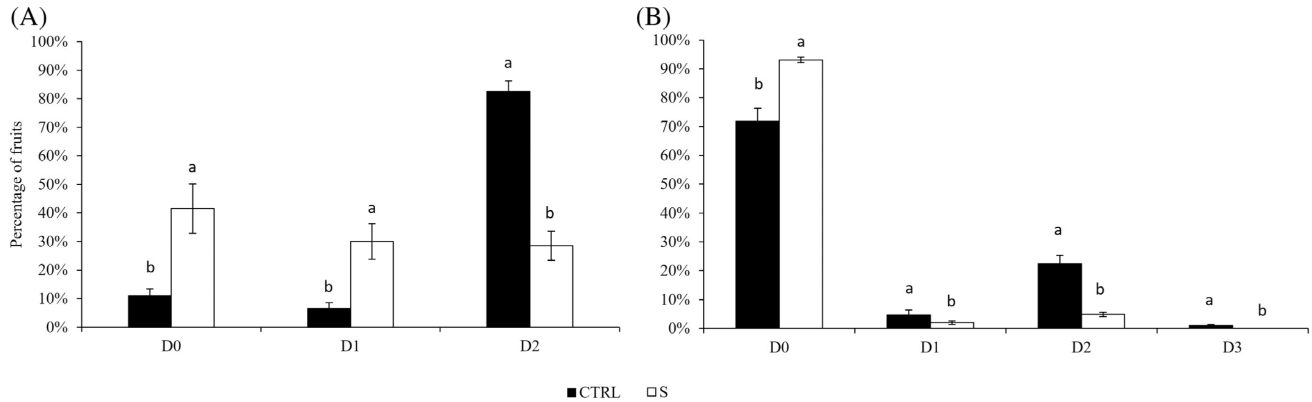

Figure 5. Percentage of healthy and damaged fruits (three classes) caused by *Halyomorpha halys*. Damage was estimated at apple harvest in orchard B (A) and orchard D (B). CTRL, untreated plots; S, sulfur-treated plots. Four classes: D0, healthy fruit; D1, damage 1 (≤10%); D2, damage 2 (≤40%); D3, damage 3 (≤100%). Error bars represent standard error of the mean. Different letters indicate differences via χ^2 test ($\alpha = 0.05$).

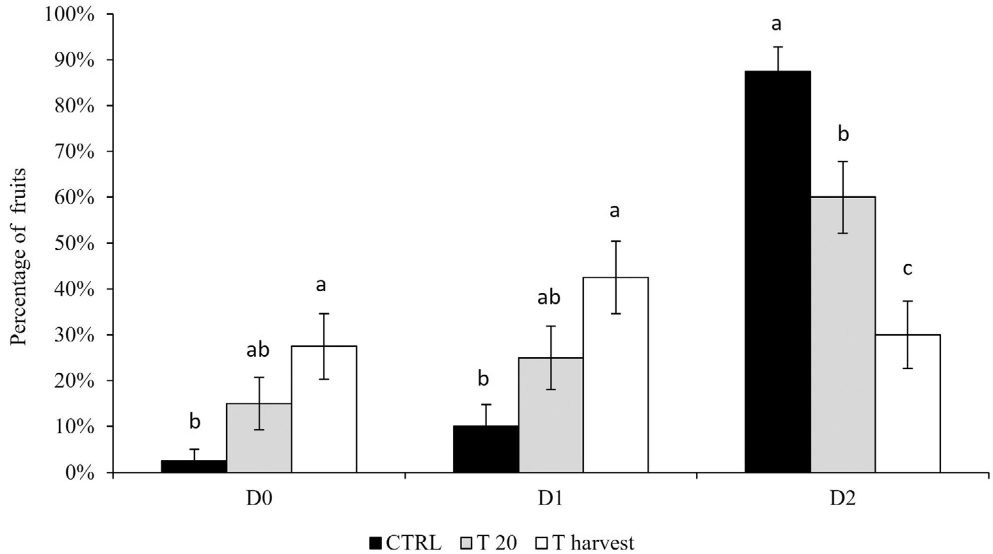

Figure 6. Percentage of healthy or damaged fruits (three classes) caused by *Halyomorpha halys* estimated at apple harvest in orchard C. CTRL, untreated; T-20, sulfur treatments with apple diameter ≤20 mm; T-harvest, sulfur treatments with apple diameter from 20 mm to harvest. Four classes: D0, healthy fruit; D1, damage 1 (≤10%); D2, damage 2 (≤40%); D3, damage 3 (≤100%). Error bars represent standard error of the mean. Different letters indicate differences at χ^2 test ($\alpha = 0.05$).

were observed among treatments (CTRL *versus* T-20; $F_{1,10} = 1.88$, P = 0.201) nor the interaction 'sampling date*treatment' ($F_{4,40} = 1.20$, P = 0.327). An effect of time was observed ($F_{4,40} = 57.55$, $P < 0.001$), with a high fruit drop noticed at the first, second and third sampling dates (i.e. 26 May, 8 and 17 June), but this was observed in both treated and control plots.

No colour defects were detected in visual inspection of apple fruits from orchards C and D. The apple weight at harvest was not influenced by sulfur applications (orchard C, $\text{weight}_{\text{CTRL}}$: 191.45 ± 40.00 g, $\text{weight}_{\text{T-20}}$: 192.77 ± 35.46 g, $\text{weight}_{\text{T-harvest}}$: 199.55 ± 37.56 g; $F_{2,9} = 0.16$, P = 0.855; orchard D, $\text{weight}_{\text{CTRL}}$: 186.99 ± 11.36 g, $\text{weight}_{\text{T-20}}$: 225.22 ± 14.98 g, $\text{weight}_{\text{T-harvest}}$: 199.55 ± 37.56 g; $F_{1,6} = 4.78$, P = 0.071). No significant differences in fruit firmness were observed in orchard D ($\text{firmness}_{\text{CTRL}}$: 7.03 ± 0.33 kg cm^{-2}, $\text{firmness}_{\text{Sulfur}}$: 6.48 ± 0.56 kg cm^{-2}; $F_{1,6} = 5.34$, P = 0.060) nor in orchard C ($\text{firmness}_{\text{CTRL}}$: 7.80 ± 0.61 kg cm^{-2}, $\text{firmness}_{\text{T-20}}$: 7.66 ± 0.72 kg cm^{-2}, $\text{firmness}_{\text{T-harvest}}$: 7.60 ± 0.62 kg cm^{-2}; $F_{2,9} = 0.46$, P = 0.646). Fruit sugar content was not influenced by sulfur treatments in orchard D ($\text{sugar}_{\text{CTRL}}$: 12.49 ± 0.21°Brix, $\text{sugar}_{\text{Sulfur}}$: 12.14 ± 0.33°Brix; $F_{1,6} = 2.29$, P = 0.181).

4 DISCUSSION

The use of synthetic insecticides in the control of *H. halys* poses some issues related to a lack of complete efficacy against this invasive pest associated with a limited residual effect that has caused the increase in sprayings by farmers.[30,31] The use of repellents or deterrent has been proposed for *H. halys* management and previous research focused mainly on kaolin clays and essential oils.[58–61]

While the effectiveness of sulfur treatments in reducing *H. halys* infestation appears to rely on repeated applications, the significance of these tactics as alternative methods in IPM for the brown marmorated stink bug should not be overlooked. This could have a particularly positive impact for organic growers, who often lack other solutions to effectively counteract pest infestation and damage. Sulfur-based compounds are used in plant protection as acaricide[39] and as fungicide against fungal pathogens such as powdery mildews.[38,40] Sulfur is admitted in organic agriculture with yearly amounts that usually range from 10 to 100 kg ha^{-1} $year^{-1}$.[39] Results reported here showed an effect of sulfur treatments on *H. halys* infestation in apple and pear orchards, where the infestation was significantly reduced by repeated sulfur applications. This was observed in all apple and pear orchards with an overall reduction in both adult and nymph numbers. However, there were some variations among years and orchards. The reduction in pest population observed where sulfur was applied throughout most of the season consistently exceeded 65% for apples and 45% for pears. It should be noted that calcium polysulfide – a fungicide containing sulfur – was equally applied on all treatments with no measurable effect on *H. halys* infestation. Thus, the effect observed here is expected to be related to the different sulfur-based treatments tested in the experiments. Following the spring frost event observed in 2021, there were either fewer or no fruit in the apple and pear orchards, respectively. This could have affected *H. halys* infestation by reducing the attractiveness and suitability of these fields to the insect owing to the low availability of food. However, we could not evaluate the impact of this phenomenon on *H. halys* because the effect of spring frost was equal across all treatments.

Furthermore, sulfur sprays reduced the fruit damage caused by the feeding activity of brown marmorated stink bug; severe damage (90–100% of deformed fruit surface or ungrown fruits) was not observed. Sulfur treatments seem to not cause *H. halys* mortality in laboratory bioassays, but may act as repellent/deterrent towards *H. halys*,[62] thus explaining the reduction of field infestation. Results on long-term treatments during different parts of the growing season revealed an overall reduction of nymph and adult density, especially where sulfur treatments were made from fruit-setting to harvest. On apple, results obtained in orchard C suggest that sulfur applications performed in the second part of the season can determine a reduction in fruit damage. On the contrary, reduced applications of sulfur over time, and early in the season, may not decrease *H. halys* infestation potential and fruit damage.

Repellence/deterrent effects induced by sulfur application can be influenced by formulation type, particle size and mode-of-action (MoA), and all of these aspects need to be considered for an effective application and degradation of this product in an orchard.[38] Sulfur was applied as wettable granular formulation with particle size ranging from 2 to 5 μm. The mixture of smaller and larger particles can increase both long- and short-term persistence and vaporization in the field,[38,63] because formulation type may affect sulfur persistence, degradation and efficacy. Formulations differ by particle size, and the most used include dusts, wettable powders, micronized sulfur and flowable liquid. Particle size and air temperature are key aspects to consider in the selection of the correct formulation – thus, the larger the particle, the lower its degradation and efficacy.[38] The MoA of sulfur-based products, which is 'unknown/uncertain' based on the IRAC classification,[64] seems to be mostly related to vapour activity that is dependent on temperature, with an optimal range of 18–22 °C.[35,38,65] Because sulfur degradation is related to temperature and particle size, timing and application frequency are key aspects to consider. Spraying at a temperature >28 °C makes sulfur less persistent on leaves and decreases its retaining efficacy over 10 days,[38,66] whereas in spring or autumn sulfur may be applied at higher concentrations owing to the low temperature.

Climatic conditions observed during the experimentation, particularly temperature and relative humidity, did not appear to favour sulfur-induced phytotoxicity. However, this issue should always be considered when applying sulfur-based pesticides in the field. Indeed, elemental sulfur application may cause toxic effects to plants as a result of its vapour form,[35] together with two volatile breakdown compounds – hydrogen sulfide and sulfur dioxide.[38] On this concern, high temperature and relative humidity increase sulfur volatilization rate and also the risk of plant phytotoxicity, which can induce a reduction in the number of fruits and an increase of colour defects such as russeting.[67–73] Indeed, sulfur particles interact with dry surfaces at temperatures >28 °C, inducing phytotoxicity following the oxidation of elemental sulfur to SO_2 and SO_3. When adsorbed in water, the oxidation product, sulfuric acid, can irreversibly damage the plants that exhibit tissue burning.[38] Sulfur treatments on apple plant canopy may also reduce photosynthesis,[70] but other studies reported a reduction of apple russeting and an increase of fruit quality following sulfur applications.[71,74] Our analysis of Granny Smith apples collected from two orchards during one season did not show differences in terms of phytotoxicity and fruit quality between sulfur-treated and untreated plots, but further investigations should be performed to confirm our results and to understand the factors that are involved in sulfur-induced phytotoxicity.

Concerning abiotic features that deal with the treated plant, sulfur efficacy against diseases decreases at <15 °C, its interaction with the plant surface being related to both the contact with plant tissue and the vapour activity. On the one hand the abiotic conditions may cause sulfur-induced phytotoxicity,[38] yet on the other high temperature and low relative humidity may reduce the population density of *H. halys* by having lethal and sublethal effects on its growth.[75–79] This may imply that a lower dose of sulfur could be applied, even though this condition needs to be evaluated case-by-case. In this study, however, we did not observe sulfur-mediated phytotoxicity such as symptoms on leaves or fruit drop, and from late May to mid-June the latter appeared to be homogeneous among treatments, and thus is ascribed to the physiological status of the plant and not to an effect of the sprays. The other fruit parameters which we investigated – weight and sugar content – were not influenced by sulfur treatments either.

5 CONCLUSION

Controlling *H. halys* requires an integrated management strategy that should incorporate various tactics, including for instance the utilization of exclusion netting,[51,80,81] the implementation of crop perimeter restructuring IPM through field border spraying[32] or the inclusion of strategies for improving biological control,[13,82,83] helping in keeping infestations of this pest below economic injury levels. Sulfur-based products are already used in agriculture as fungicide and insecticide and may be considered in brown marmorated stink bug management for their action in decreasing pest infestation and damage. Sulfur can be used for *H. halys* management in organic agriculture where limited tools are available. Sulfur applications should, however, be timed on apple and pear phenological stages, to reach a proper goal in managing *H. halys* infestations and reducing fruit damage without causing plant phytotoxicity. Wettable sulfur appears to be a promising formulation also owing to its low phytotoxicity, at least as presented in the current study, making these sulfur-based products a good tool to counteract brown marmorated stink bug in pome fruit orchards. Sulfur applications are tools that can contribute to minimize the repeated use of synthetic insecticides in line with current agricultural policies such as the EU Farm to Fork strategy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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