



## Article

# Brown Marmorated Stink Bug (*Halyomorpha halys* Stål.) Attack Induces a Metabolic Response in Strawberry (*Fragaria × ananassa* Duch.) Fruit

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**Abstract:** The polyphagous brown marmorated stink bug (*Halyomorpha halys* Stål.) is an important pest in many countries. Recently it was noticed that it can feed on and cause damage to strawberries (*Fragaria × ananassa* Duch.). The metabolic response of strawberries to brown marmorated stink bug attacks was studied. Brown marmorated stink bugs attacked strawberry fruit which had 18% lower total sugar content compared to that of the control treatment. However, organic acid content had no significant difference among the three treatments, with the exception of shikimic acid, which had the highest content in the attacked fruit. Thirty-one phenolic compounds were identified. Results showed a strong effect on secondary metabolites due to *H. halys* attacks. *Halyomorpha halys* treatment had 27% higher total analyzed phenolic content compared to the indirect *Halyomorpha halys* treatment. The brown marmorated stink bug significantly increased total ellagic acid derivatives (33.1% to 37% higher), hydroxycinnamic acids (22.3% higher) and anthocyanins' (39% higher) contents. Fruit attacked by *Halyomorpha halys* also had higher catechin and epicatechin content than that of the control treatments. This pest had a significant influence on the plant's secondary metabolism, and this improved our understanding of how a strawberry plant reacts to the attacks of this very important pest. *H. halys*-infested fruit are not suitable for commercial production, due to the production of off-flavors.

**Keywords:** anthocyanins; ellagic acid derivatives; hydroxycinnamic acids; strawberry; total sugars; total organic acids

## 1. Introduction

The brown marmorated stink bug *Halyomorpha halys* Stål. (Hemiptera: Pentatomidae) has started to spread across Slovenia since 2017 in a similar pattern to other European countries [1]. *H. halys* originates from China, Japan, Korea and Taiwan, but has now rapidly spread to North America and Europe due to several reasons, of which the main ones are climate change and a lack of natural predators [2]. The damage is significant especially in apple, pear, peach or cherry orchards, with yield losses of up to 50% or more [3,4]. It can also cause the decrease in yield and of its quality after storage, as previously reported by Zamljen et al. [5]. Recently, farmers have also reported damage to strawberries (*Fragaria × ananassa* Duch.). *Halyomorpha halys* feeds on the fruit, causing small puncture wounds which tend to rot in storage rooms, causing significant losses to

marketable yields [6–8]. *H. halys* is recognized by white and black banding on the antennae and abdominal margins. White eggs are laid on the underside of leaves in clusters. The eggs (up to 28 per cluster) can be laid by the female every four days throughout its life span as an adult. In Europe, there are two generations of *H. halys* per year. Nymphs hatch approximately 4 to 7 days after the eggs are laid. The five nymphal stages from hatching to adulthood take from 33 to 55 days. The males are smaller than the females and have a rear ventral scoop [9]. Controlling *H. halys* populations in orchards is difficult due to several reasons, the first being the lack of appropriate plant protection substances registered [10] for *H. halys*, and the second being the great variety of host plants (over 100) that the bug feeds on [11]. A strawberry attacked by *H. halys* is darker and swollen-looking. It also has a noticeable smell which is common for a stink bug attack. The attacked fruit does not last long in storage rooms and starts to mold and rot. Strawberries attacked by *H. halys* are also not suitable for juice, since the taste and smell remain even after processing [12].

In strawberries and other plants, the natural reaction to any pests or disease attacks is the production of phenolic compounds, which act as a repellent or are toxic to certain pests and diseases [13]. Common phenolic groups in strawberries are hydroxycinnamic acids, ellagic acid derivatives, flavanols, flavonols and anthocyanins. Ellagic acid derivatives are known defensive metabolites; their tissue concentrations increase when a plant is attacked by a pest or disease [7].

Phenolics can inhibit insect oviposition, feeding, growth and development at different stages. Tannins in insects reduce growth and act as enzyme inactivators. Some phenolics can also influence insects' reproduction periods [14]. Phenolics can be directly toxic to the insect or are used as a signaling molecule which activates the synthesis of other toxic secondary metabolites [15]. There are relatively few reports in literature about pests' attacks and subsequent plants' responses in terms of metabolites.

For the first time ever, we noted and analyzed a metabolic response of strawberries when attacked by *Halyomorpha halys*. *Halyomorpha halys*' damage to strawberries had previously not been analyzed and reported in a detailed manner. In addition to individual sugars and organic acids, we also studied the response of 31 individual phenolic compounds, explaining how a strawberry fruit reacts to *Halyomorpha halys* attacks on a metabolic scale. This study gives a great insight to how the quality of the fruit changes in regard to *Halyomorpha halys* damage. The results of this study improve our understanding of the significance of controlling the brown marmorated stink bug population in orchards and other plant production fields to reduce the damage to crops and agriculture.

## 2. Materials and Methods

### 2.1. Plant Material and Growing Conditions

Strawberries in our experiment were grown according to the integrated production guidelines of the Ministry of Agriculture, Forestry and Food at the experimental station of the Agricultural Institute of Slovenia, located in Brdo pri Lukovici (latitude, 46°10' N; longitude, 14°41' E). The field trial was carried out on silty loam soil, rich in potassium and nitrogen and low in phosphorus, equipped with a drip irrigation system. Frigo strawberry plants (*Fragaria* × *ananassa* Duch.) cv. Clery were planted in an open field on 15 August, 2019, on slightly raised beds covered with black polyethylene, then covered in a non-heated plastic greenhouse on 3 April, 2020. Plants were planted in double rows, with 0.25 × 0.25 m spacing between the plants and 1.3 m spacing between the rows or raised beds. For the experiment, we selected uniform strawberry plants that had 3 stems forming a crown. The strawberries in this experiment were grown in a very controlled manner (use of insect nets and pesticides if needed) so that no diseases or pests (other than *H. halys*) could affect the results.

An experiment was conducted to evaluate the effect of *Halyomorpha halys* (Stål) on strawberry fruit. Three treatments were carried out: (i) control treatment (healthy plants without pests); (ii) *H. halys* treatment, in which two flower stems per plant bearing ripening fruit were covered with a soft nylon plastic insect bag measuring L30 × W10 cm and net

measuring 104 × 94 mesh/square inch (BugDorm, Taichung City, Taiwan), infested with two second stage *H. halys* nymphs, which were caught by pheromone traps, as previously reported by Short et al. [16] and sealed with strings; (iii) indirect *Halyomorpha halys* treatment, in which healthy uninfested fruit were collected from the same plant infested with *H. halys*. The *H. halys* nymphs were examined once a week and all dead specimens were replaced with healthy ones to ensure constant infestation (This was conducted for four weeks). Each treatment was carried out on ten (10) plants; a total of 20 plants were included (10 plants for control treatment, 10 plants with *H. halys* treatment and indirect *H. halys* treatment). Fruit samples for biochemical analyses were collected at the fifth harvest on June 5. Each treatment was represented with five repetitions, each repetition consisting of five fruits, to reduce any difference in ripeness among fruit from the same treatment. Ripe fruits were picked early in the morning when temperatures did not exceed 15 °C, labeled, immediately snap-frozen in liquid nitrogen, stored in plastic bags, transferred to the laboratory in liquid nitrogen and stored at −20 °C for further analyses. For all metabolite extractions, fresh samples were first thawed at room temperature and blended in a blender while still cold to form a paste. This paste was then used for the extraction of the individual metabolites.

## 2.2. Extraction of Sugars and Organic Acids

One gram of fresh samples were extracted with 5 mL of bidistilled water for the extraction of sugars and organic acids. Samples were then shaken for 30 min and filtered through a 25 µm cellulose filter (Chromafil A–25/25; Macherey-Nagel, Düren, Germany). Thermo Finnigan Surveyor HPLC system (Thermo Scientific, San Jose, CA, USA) was used for analyses. The column, settings and detection parameters were based on Zamljen et al. [17] and Weber et al. [18]. Relevant standards were used to calculate all data and all data were expressed in g/kg FW (fresh weight).

## 2.3. Extraction of Phenolics

Three grams of fresh fruit samples and 10 mL of 80% methanol and 3% formic acid were used for the extraction of phenolic compounds. The samples were placed in an ultrasonic bath (0 °C) for 1 h. After the ultrasonic bath, the samples were filtered through a 25 µm polyamide filter (Chromafil AO-45/25, Macherey-Nagel, Düren, Germany). Identification of individual phenols was performed by tandem mass spectrometry (MS/MS; LTQ XL; Thermo Scientific, Waltham, MA, USA) with heated electrospray ionization in negative ion mode. The settings were the same as previously reported by Medic et al. [19]. Quantification of individual phenols was performed using a UHPLC system (Vanquish; Thermo Scientific, Waltham, MA, USA). The UHPLC system settings and column were the same as previously reported by Weber et al. [18]. Chromatographic information (*m/z*) for the identification of each individual phenolic are presented in Supplemental Tables S1–S3. The identification was based on references [18,20–27]. In Supplemental Figures S1–S3 we also present the chromatographic information for each wavelength. All ellagic acid derivatives were expressed as ellagic acid equivalents; procyanidin dimer 2 and procyanidin trimer were expressed as procyanidin B1 equivalents; all glucoside of kaempferol were expressed as kaempferol-3-glucoside equivalents; *p*-coumaroylhexosides and *p*-coumaroylhexose were expressed as *p*-cumaric acid equivalents; all caffeic acid derivatives were expressed as caffeic acid equivalents; all cinnamic acid derivatives and ferulic acid derivatives were expressed as equivalents of cinnamic acid and ferulic acid, respectively. Pelargonidin-3-malonylglucoside and pelargonidin-3-rutinoside were expressed as pelargonidin-3-glucoside. The total analyzed ellagic acid derivatives, flavanols, hydroxycinnamic acids and anthocyanins were a summarization of individual phenolics in each appropriate group. All data were expressed in mg/kg FW.

## 2.4. Chemicals

The following standards were used to determine the chemical compounds: apigenin 7-glucoside, kaempferol-3-glucoside, procyanidin B1, quercetin-3-glucoside, quercetin-3-rhamnoside, ferulic acid, *p*-coumaric acid from Fluka Chemie GmbH (Buchs, Switzerland), (+)-catechin from Roth (Karlsruhe, Germany), 4-caffeoylquinic acid, chlorogenic acid (trans-5-caffeoylquinic acid), neochlorogenic acid (3-caffeoylquinic acid), quercetin-3-galactoside, quercetin-3-rhamnoside, caffeic acid, galic acid, (–)-epicatechin from Sigma–Aldrich Chemie GmbH (Steinheim, Germany), myricetin-3-rhamnoside, quercetin-3-arabinofuranoside, quercetin-3-arabinopyranoside, quercetin-3-xyloside from Apin Chemicals (Abingdon, UK).

For sugars, standards for glucose, sucrose and fructose were used from Sigma–Aldrich Chemie GmbH (Steinheim, Germany) and for organic acids, standards of citric acid, malic acid, shikimic acid and fumaric acid were used from Sigma–Aldrich Chemie GmbH (Steinheim, Germany).

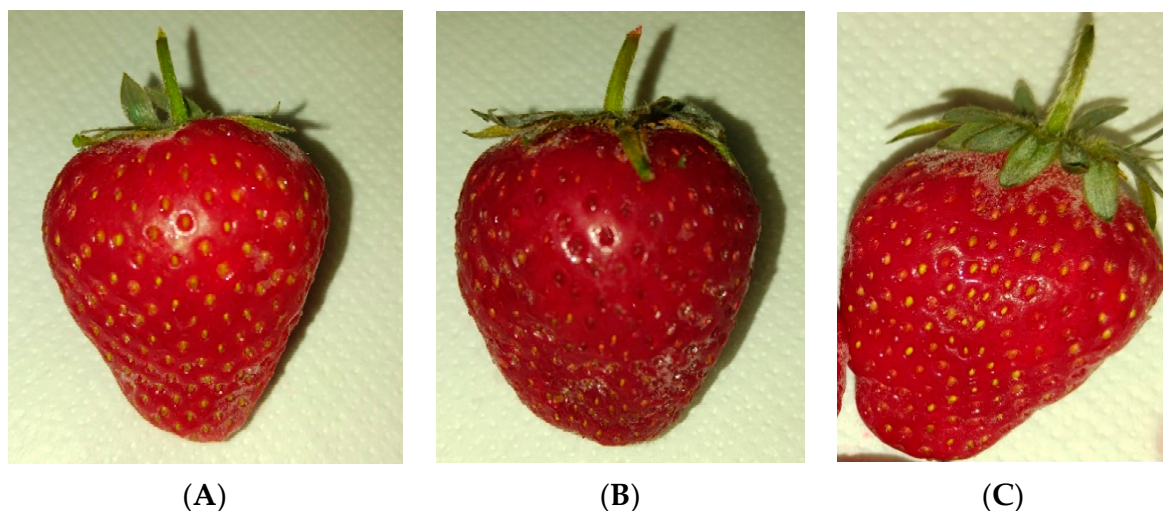
## 2.5. Statistical Analysis

Data were statistically processed using R program (Team, 2008). For all data, mean and standard error were calculated. Significant treatment effects were found using analysis of variance (ANOVA) and the LSD test. The significant level was  $\alpha \leq 0.05$ . Where differences were observed, lowercase letters (a, b), were added to distinguish between which treatment they were present for or not.

## 3. Results and Discussion

### 3.1. Visual Appearance of Strawberry

The color of the *H. halys*-infested strawberry was dark red, and the tissue was swollen-looking (Figure 1). The fruit was also characterized by a distinct odor, caused by the stink bug and associated with two defense chemicals, namely trans-2-octenal and trans-2-decenal aldehydes [28]. Strawberries with this scent cannot be sold to consumers.



**Figure 1.** Control strawberry (A); *H. halys*-infested strawberry (B); and indirectly *H. halys*-infested strawberry (C).

### 3.2. Sugars

Three individual sugars were determined in strawberry fruit (Table 1). The content of all three individual sugars (sucrose, glucose and fructose) was highest in the control treatment, with glucose and fructose being the most abundant in all three treatments. *Halyomorpha halys* treatment was characterized by the lowest content of all three sugars. The control treatment had 41.1% higher sucrose content, 14.6% higher glucose content and 10.5% higher fructose content compared to the *Halyomorpha halys*-infested strawberry.



Consequently, the control treatment had 14.3% and 18% higher total sugar content compared to the indirectly *Halyomorpha halys*-infested fruit and the *Halyomorpha halys* treatment, respectively. Strawberries of the control treatment had similar sugar content, as previously reported by Milivojević et al. [29]. Our results were similar to those of Wiman et al. [6], Zhou et al. [12] and Schumm et al. [2] which reported decreased sugar content following *Halyomorpha halys* attacks on blueberries and tart cherries. A possible reason for the decrease in sugars can be ascribed to preference in sugar consumption of *Halyomorpha halys*, since it mostly feeds on fruit juices and sugars that are present in them. The second reason for the lower sugar content may also be related to higher phenolic content, since they are synthesized as defense compounds. Glucose is used as the main substrate for the synthesis of phenolics, which would explain the lower sugar content and higher phenolic content, as previously reported by War et al. [14] and Kaur et al. [15].

**Table 1.** Individual and total sugar content (g/kg FW, mean  $\pm$  SE) in strawberry fruit.

	Control		<i>Halyomorpha halys</i>		Indirect <i>Halyomorpha halys</i>	
Sucrose	10.20 $\pm$ 0.41	a *	6.01 $\pm$ 0.62	b	6.62 $\pm$ 0.44	b
Glucose	21.82 $\pm$ 0.40	a	18.63 $\pm$ 0.83	b	19.61 $\pm$ 0.42	b
Fructose	21.91 $\pm$ 0.45	a	19.62 $\pm$ 0.76	b	20.02 $\pm$ 0.35	ab
Total sugars	53.93 $\pm$ 0.52	a	44.26 $\pm$ 0.56	b	46.25 $\pm$ 0.44	b

\* a,b lower case letters denote statistically significant differences ( $\alpha < 0.05$ ) among different treatments.

### 3.3. Organic Acids

Four organic acids were quantified in strawberries (Table 2). No significant differences were detected for citric, malic and fumaric acid content. The *Halyomorpha halys* treatment positively affected the shikimic acid content compared to the indirect *Halyomorpha halys* treatment. No significant differences were determined in total organic acid content in all three treatments. Similarly, decreased organic acid content was previously determined in *Colletotrichum nymphaeae*-infected strawberries by Weber et al. [13]. No reports on the influence of *Halyomorpha halys* on organic acid content have been reported in past studies. Certain organic acids such as shikimic acid have an important role in phenolics synthesis [30]. The significant increase in shikimic acid in fruit attacked by *Halyomorpha halys* could be linked to the increased synthesis of phenolic compounds.

**Table 2.** Individual and total organic acid content (g/kg FW, mean  $\pm$  SE) in strawberry fruit.

	Control		<i>Halyomorpha halys</i>		Indirect <i>Halyomorpha halys</i>	
Citric acid	8.15 $\pm$ 0.92	a *	6.85 $\pm$ 0.32	a	6.23 $\pm$ 0.27	a
Malic acid	3.86 $\pm$ 0.35	a	3.95 $\pm$ 0.24	a	3.23 $\pm$ 0.22	a
Shikimic acid	0.05 $\pm$ 0.01	ab	0.06 $\pm$ 0.00	a	0.04 $\pm$ 0.00	b
Fumaric acid	0.02 $\pm$ 0.00	a	0.02 $\pm$ 0.00	a	0.02 $\pm$ 0.00	a
Total organic acid	12.08 $\pm$ 1.4	a	10.88 $\pm$ 1.8	a	9.52 $\pm$ 0.7	a

\* a,b lower case letters denote statistically significant differences ( $\alpha < 0.05$ ) among different treatments.

### 3.4. Phenolics

Seven phenolics groups and thirty-one individual phenolic compounds were identified in strawberry fruit (Table 3). Fruits infested with *Halyomorpha halys* contained higher levels of total ellagic acid derivatives, hydroxycinnamic acids and anthocyanins compared to all control treatments. Bis-HHDP-hexose was the most common phenolic compound among total ellagic acid derivatives, procyanidin dimer 1 and 2 among flavanols, kaempferol-3-glucoside among flavonols, cinnamic acid hexoside among hydroxycinnamic acids and pelargonidin-3-glucoside and pelargonidin-3-rutinoside among anthocyanins. The total ellagic acid derivative content was 33.1% and 37% higher in *Halyomorpha halys* treatment compared to the control and indirect *Halyomorpha halys* treatment, respectively. The control fruit was characterized by 22.3% less hydroxycinnamic acid compared to strawberries infested with *Halyomorpha halys*. Moreover, the control fruit contained 400.12 mg/kg FW, and indirectly *Halyomorpha halys*-infested strawberries contained 357.46 mg/kg FW

fewer anthocyanins compared to the *Halyomorpha halys* treatment. The control and indirect *H. halys* strawberries had 27% and 28% fewer total analyzed phenolics compared to fruit infested by brown marmorated stinkbugs. Similar phenolic content as our control treatment was also reported by Weber et al. [31].

**Table 3.** Individual and total phenolic content (mg/kg FW, mean  $\pm$  SE) in strawberry fruit.

	Control			<i>Halyomorpha halys</i>		Indirect <i>Halyomorpha halys</i>	
<b>Total elgaic acid derivatives</b>	<b>326.0 <math>\pm</math> 12.0</b>	<b>b *</b>		<b>487.8 <math>\pm</math> 26.2</b>	<b>a</b>	<b>307.6 <math>\pm</math> 21.8</b>	<b>b</b>
bis-HHDP-glucose	85.0 $\pm$ 4.7	b		132.3 $\pm$ 10.3	a	71.0 $\pm$ 3.2	b
bis-HHDP-hexose	154.0 $\pm$ 11.4	b		222.3 $\pm$ 24.8	a	152.6 $\pm$ 17.8	b
ellagic acid deoxyhexoside	11.0 $\pm$ 0.5	b		15.9 $\pm$ 0.9	a	13.2 $\pm$ 1.2	ab
<b>Flavanols</b>	<b>398.1 <math>\pm</math> 13.5</b>	<b>a</b>		<b>436.4 <math>\pm</math> 29.2</b>	<b>a</b>	<b>375.2 <math>\pm</math> 16.8</b>	<b>a</b>
procyanidin dimer 1	115.5 $\pm$ 8.6	a		149.7 $\pm$ 3.6	a	137.1 $\pm$ 10.6	a
procyanidin dimer 2	137.0 $\pm$ 7.6	a		124.8 $\pm$ 6.8	ab	104.9 $\pm$ 8.0	b
procyanidin trimer	78.5 $\pm$ 2.9	a		78.5 $\pm$ 7.4	a	79.5 $\pm$ 8.1	a
propelargonidin dimer	12.1 $\pm$ 1.4	a		10.5 $\pm$ 3.1	a	10.2 $\pm$ 2.6	a
epicatechin	38.6 $\pm$ 4.5	b		52.9 $\pm$ 12.5	a	29.7 $\pm$ 5.3	b
catechin	16.4 $\pm$ 0.8	ab		19.6 $\pm$ 0.4	a	13.5 $\pm$ 2.0	b
<b>Flavone</b>							
apigenin rhamnoside	1.10 $\pm$ 0.1	a		1.0 $\pm$ 0.0	a	0.9 $\pm$ 0.1	a
<b>Flavonols</b>	<b>21.7 <math>\pm</math> 1.2</b>	<b>a</b>		<b>20.4 <math>\pm</math> 1.4</b>	<b>ab</b>	<b>15.7 <math>\pm</math> 1.9</b>	<b>b</b>
kaempferol-3-coumaroyl glucoside	0.2 $\pm$ 0.0	a		0.1 $\pm$ 0.0	a	0.2 $\pm$ 0.0	a
quercetin-3-glucuronide	0.5 $\pm$ 0.1	a		0.3 $\pm$ 0.0	a	0.4 $\pm$ 0.1	a
kaempferol-3-glucoside	12.2 $\pm$ 0.7	a		9.4 $\pm$ 1.0	ab	7.4 $\pm$ 1.0	b
quercetin-3-malonyl glucoside	1.3 $\pm$ 0.1	b		1.9 $\pm$ 0.1	a	1.1 $\pm$ 0.1	b
kaempferol-3- glucuronide	4.7 $\pm$ 0.3	a		4.6 $\pm$ 0.2	a	4.1 $\pm$ 0.5	a
isorhamnetin-3- glucuronide	0.7 $\pm$ 0.1	a		0.2 $\pm$ 0.1	b	0.3 $\pm$ 0.1	b
kaempferol-3-acetyl glucoside	2.3 $\pm$ 0.1	b		3.5 $\pm$ 0.1	a	2.2 $\pm$ 0.1	b
<b>Hydroxycinnamic acids</b>	<b>310.1 <math>\pm</math> 3.9</b>	<b>b</b>		<b>401.7 <math>\pm</math> 5.4</b>	<b>a</b>	<b>354.1 <math>\pm</math> 11.9</b>	<b>ab</b>
p-cumaroyl hexoside 1	12.6 $\pm$ 0.3	ab		14.3 $\pm$ 0.6	a	11.4 $\pm$ 0.5	b
p-cumaroyl hexoside 2	3.4 $\pm$ 0.1	a		2.8 $\pm$ 0.4	a	1.4 $\pm$ 0.2	b
p-cumaroyl hexoside 3	3.2 $\pm$ 0.3	a		3.6 $\pm$ 0.5	a	2.5 $\pm$ 0.5	a
cinnamic acid hexoside	190.4 $\pm$ 2.3	a		182.7 $\pm$ 4.1	ab	163.1 $\pm$ 10.7	b
caffeoylhexose	0.9 $\pm$ 0.0	a		0.9 $\pm$ 0.0	a	0.6 $\pm$ 0.1	b
caffeic acid derivate	0.5 $\pm$ 0.1	b		1.3 $\pm$ 0.0	a	0.5 $\pm$ 0.1	b
ferulic acid hexose derivate	87.0 $\pm$ 2.2	b		133.1 $\pm$ 5.7	a	83.9 $\pm$ 3.0	b
p-cumaroylhexose	4.4 $\pm$ 0.1	b		5.2 $\pm$ 0.1	a	4.1 $\pm$ 1.1	b
cinnamic acid-3 acetylhexoside	4.3 $\pm$ 0.3	a		3.52 $\pm$ 0.20	a	3.5 $\pm$ 0.4	a
<b>Hydroxybenzoic acids</b>							
ellagic acid	4.2 $\pm$ 0.8	a		4.87 $\pm$ 0.46	a	2.9 $\pm$ 0.1	a
<b>Anthocyanins</b>	<b>640.8 <math>\pm</math> 18.9</b>	<b>b</b>		<b>1040.95 <math>\pm</math> 48.07</b>	<b>a</b>	<b>683.5 <math>\pm</math> 38.9</b>	<b>b</b>
Cyanidin-3-glucoside	38.8 $\pm$ 0.6	b		57.98 $\pm$ 3.79	a	36.3 $\pm$ 5.4	b
Pelargonidin-3-glucoside	413.4 $\pm$ 15.1	b		672.37 $\pm$ 34.84	a	420.7 $\pm$ 20.4	b
Pelargonidin-3-malonylglucoside	14.4 $\pm$ 0.7	b		21.23 $\pm$ 1.77	a	18.3 $\pm$ 1.1	b
Pelargonidin-3-rutinoside	174.3 $\pm$ 4.5	b		289.59 $\pm$ 11.14	a	208.4 $\pm$ 16.7	ab
<b>Total analyzed phenolics</b>	<b>1625.2 <math>\pm</math> 28.5</b>	<b>b</b>		<b>2226.64 <math>\pm</math> 58.60</b>	<b>a</b>	<b>1589.6 <math>\pm</math> 53.0</b>	<b>b</b>

\* a,b lower case letters denote statistically significant differences ( $\alpha < 0.05$ ) among different treatments.

A significant increase in total ellagic derivatives, hydroxycinnamic acids and anthocyanins was previously reported by Weber et al. [13] who studied *Colletotrichum nymphaeae*-infested strawberry fruit. An increase in phenolics was also reported by Young et al. [32] in pak choi (*Brassica rapa* subsp. *chinensis*) infested with flea beetles (*Altica* spp.). *Halyomorpha halys*-infested blueberry fruit contained higher phenolic levels compared to non-infested fruit, as previously reported by Zhou et al. [12] which corresponds with our results. Catechin and epicatechin levels also increased in brown marmorated stink bug-infested strawberries, which corresponds with the antimicrobial, antifungal and insect repellent functions of these compounds, as reported by Veluri et al. [33] and Ullah et al. [34].

The *Halyomorpha halys*-infested strawberries' reaction is to increase the synthesis of phenolic compounds. This is a common response, since phenolics are known for their defensive role in plants [14,15]. Phenolics can act as repellents, disturbers or even toxins for insects. With the increased synthesis of certain phenolics, plants such as strawberries try to prevent the insect from feeding on the fruit. Increasing anthocyanin synthesis in *Halyomorpha halys*-infested strawberry could be a method of repelling or confusing the

insect. Anthocyanins act as attractors for pollinators, as visual repellents for pests or for camouflage [35]. Anthocyanins also act as defensive molecules against fungal disease, as previously reported in grapes infected with *Botrytis cinerea*, reducing the infection by 95% as reported by Schaefer et al. [36]. The hazel's (*Corylus avellana* L.) caffeic acid content is higher in *Myzocallis coryli* Goetze (filbert aphid)-resistant cultivars, which demonstrates that caffeic acid is synthesized in the plant for the purpose of defense against sucking and piercing insects, as previously reported by Gantner et al. [37]. Similarly, an increase in caffeic acid content was also detected in our study.

#### 4. Conclusions

The brown marmorated stink bug (*Halyomorpha halys* Stål.) is an invasive pest in Europe, America and Asia. Because of its wide host range, it is difficult to keep it from spreading. This study has shown that it can also target strawberry plants. A natural defense mechanism of strawberry fruit is the synthesis of different metabolites, which can repel, confuse or be toxic to the attacker. Our study is a detailed report on the metabolic response of strawberries to brown marmorated stink bug (*Halyomorpha halys* Stål.) attacks. The attacked fruit had 18% lower sugar content and a higher total analyzed phenolics content compared to the control treatment. The main phenolics that increased in the attacked fruit were total ellagic acid derivatives, hydroxycinnamic acids and anthocyanins with significant increases of 33%, 22% and 39%, respectively. This pest had a significant influence on the plant's secondary metabolism, which improved our understanding of how a strawberry plant reacts to the attacks of this very fast-spreading pest. We have shown that *H. halys* significantly influenced the plant's secondary metabolism. Our study demonstrates how important it is to keep a close watch on the brown marmorated stink bug population and to follow the pest control protocols diligently and responsibly, since *H. halys*-infested fruit are not suitable for commercial production due to the production of off-flavors, which make the fruit inedible.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/horticulturae7120561/s1>, Figure S1: Chromatograph of phenolics at 530 nm wavelength, Figure S2: Chromatograph of phenolics at 350 nm wavelength, Figure S3: Chromatograph of phenolics at 280 nm wavelength, Table S1: Individual phenolics identification at 530 nm, Table S2: Individual phenolics identification at 350 nm, Table S3: Individual phenolics identification at 280 nm.

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