Oxidative stress mitigation in horticultural crops using foliar applications of Ilex paraguariensis extract: a dose-dependent study

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Abstract

Abiotic stress has been shown to induce the formation of reactive oxygen species (ROS) in plant cells. When the level of ROS surpasses the capacity of the endogenous defence mechanism, oxidative stress status is reached, leading to plant damage and a drop in crop productivity. Under oxidative stress conditions, ROS can react with polyunsaturated fatty acids to form oxidized derivatives called phytoprostanes (PhytoPs) and phytofurans (PhytoFs), which are recognized as biomarkers of oxidative damage advance. Modern agriculture proposes the use of biostimulants as a sustainable strategy to alleviate the negative effects of oxidative stress on plants. This work evaluates the dose effect of natural antioxidant extract to mitigate the oxidative-stress deleterious effects in melon and sweet pepper exposed to thermal stress. The plants were sprayed with *Ilex paraguariensis* (IP) aqueous extract in three different concentrations before exposure to abiotic stress. PhytoP and PhytoF levels were determined in the leaves of melon and pepper plants. IP1 and IP2 were effective against oxidative stress in both plants, with IP1 being the most protective one. IP1 decreased the levels of PhytoPs and PhytoFs by roughly 44 % in both melon plants and pepper plants. The yield, with IP1, increased by 57 and 39 % in stressed melon and pepper plants, respectively. IP3 foliar application in melon plants induced a pro-oxidant effect rather than the expected mitigating action. However, in sweet pepper plants, IP3 decreased the oxidative stress progress and increased the fruit yield.

Keywords THERMAL STRESS; MELON CROP; SWEET PEPPER CROP; BIOSTIMULANTS; ANTIOXIDANTS; PHYTOPROSTANES**;** PHYTOFURANS; FRUIT YIELD

Abbreviations

ROS: reactive oxygen species

PhytoPs: Phytoprostanes

PhytoFs: Phytofurans

IP: *Ilex paraguariensis*

PUFAs: polyunsaturated fatty acids

ALA: α-Linolenic acid

DAT: Days after transplanting

1 INTRODUCTION

Nowadays, climate change significantly affects the growth of crops, reducing their productivity. Abiotic stress caused by environmental conditions, including extreme temperatures, high salinity, and hydric restrictions, among others, increases the levels of reactive oxygen species (ROS) in plant cells (Zandalinas, Fritschi & Mittler, 2021). Plants have developed acclimatization mechanisms that give them tolerance to environmental stress conditions. However, when ROS excess cannot be handled by the endogenous defence mechanism, the oxidative stress status is reached. This condition causes cellular damage that leads, in the final stage, to plant death (Raza et al., 2019). Under oxidative stress conditions, ROS can react with polyunsaturated fatty acids (PUFAs), cell membrane constituents, to form oxidized derivatives (Ahmed et al., 2020). In higher plants, most of the oxidized PUFAs are derived from the chemical transformation catalyzed by prooxidant enzymes as lipoxidase and cyclooxygenase. In the last two decades, it has been shown that the non-enzymatic oxidation of PUFAs, such as α-Linolenic acid (ALA), leads to the formation of prostaglandin/jasmonate-like compounds called phytoprostanes (PhytoPs). Besides, furanic structures, named phytofurans (PhytoFs), can also be produced under high oxygen tension. PhytoPs and PhytoFs are two different series of compounds derived from the early stages of the lipid oxidation process. The occurrence of these metabolites in biological systems is increasingly gaining evidence, and they are considered biomarkers of the oxidative stress condition. In a previous study, we determined the PhytoP and PhytoF levels in the leaves of melon plants exposed to thermal stress (Yonny et al., 2016). This was the first study that proposed PhytoFs as oxidative stress biomarkers in stressed plants. Since then, other researchers have published similar results, as Pinciroli et al. (2018) who detected PhytoPs and PhytoFs in rice grains from plants exposed to stressful environmental conditions. These metabolites were also found in marine microalgae exposed to hydrogen peroxide (Vigor et al., 2020).

Modern agriculture needs to focus on strategies to mitigate the consequences of climate change to ensure food availability for the continuously growing global population. The wide variety of environmental stressors has intensified the search for strategies that contribute to sustainable agriculture from different angles. In this sense, biostimulants are proposed to reach high productivity when the plants are exposed to unfavourable environmental conditions; therefore, these management practices will be in good agreement with global demands for environment-friendly crops (Zulfiqar et al., 2020). Biostimulants are defined as organic and inorganic substances, natural (plant-derived) or synthetic compounds that improve plant productivity as a consequence of the novel or emergent properties of their constituents (Mrid et al., 2021). Biostimulants can function as antioxidants or elicitors for their endogenous biosynthesis. For example, proline, gallic acid and α-tocopherol have been used to mitigate saline stress effects in pepper (Abdelaal et al., 2020), rice (Ozfidan-Konakci, Yildiztugay & Kucukoduk, 2015) and onion plants (Semida et al., 2016). It has been reported that exogenous applications on bean plants of liquorice root (Rady et al., 2019) and seaweeds (Deolu-Ajayi et al., 2022) extracts stimulated the growth of seedlings and improved seed vigour, respectively. However, there are still many

unanswered questions related to biostimulant treatments, such as the number of applications, application rate (dosage), timing (in the life cycle of the crop), the dose-effect, moment of application, effect durability, among others (Teklić et al., 2021). Therefore, organized, and methodical experiments are necessary to give valuable information to establish adequate crop management practices.

The main aim of this work was to evaluate the effect of different doses of *Ilex paraguariensis* (IP) aqueous extract as a biostimulant to mitigate the oxidative stress on melon (*Cucumis melo* L.) and pepper (*Capsicum annuum* L.) crops exposed to high temperatures during their growth. PhytoP and PhytoF profiles were determined in the leaves of these crops to monitor the oxidative stress advance.

2 Materials and Methods

2.1 Reagents and solvents

Water, methanol (MeOH), acetonitrile (ACN), and chloroform were LC-MS grade and provided from Fisher Scientific hexane (Chromasolv, for HPLC, ≥99.7 %), formic acid (FA), ammonia and potassium hydroxide (Fluka, for mass spectrometry 98 %) were provided by Sigma-Aldrich. Solid phase extraction cartridges (SPE) Oasis MAX with mixed polymer phase were purchased (3 mL, 60 mg) from Waters. Milli-Q ultrapure water was used to prepare all the aqueous solutions. The PhytoP analytical standards used were: 9-L_1-PhytoP, *ent*-9-L_1-PhytoP, *ent*-16-*epi*-16-F_{1t}-PhytoP, *ent*-9-*epi*-9-F_{1t}-PhytoP, *ent*-9-F_{1t}-PhytoP, *ent*-16-F_{1t}-PhytoP, *ent*-16-B_1-PhytoP, 16-B_1-PhytoP and 16(*RS*)-16-A_1-PhytoP. The PhytoF analytical standards used were: *ent*-16(*RS*)-9-*epi*-ST-Δ^{14}-10-PhytoF, *ent*-9(*RS*)-12-*epi*-ST-Δ^{10}-13-PhytoF and *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF. Analytical standards of PhytoPs and all PhytoFs, as well as the internal standard (IS: d_4-10-F_{4t}-NeuroP), were synthesized according to procedures previously described (Cuyamendous et al., 2015).

2.2 Preparation of biostimulant solution

The extracts were prepared by a decoction of IP-dried leaves, according to Yonny et al. (2016). Different masses of IP leaves were weighted: 10, 20, and 40 g; and 1 L of water was added to constitute IP1, IP2, and IP3 extracts, respectively. The identification and quantification of the major constituents of IP extract were carried out by HPLC-DAD in the same experiment, according to our previous report (Yonny et al. 2018). Chlorogenic (CL) and caffeic (CA) acids were the major phenolic compounds present in the IP extracts. IP1, IP2, and IP3 extracts contain 2.8 ± 0.2, 4.8 ± 0.4, and 6.9 ± 0.4 g CL L^{-1}; and 0.15 ± 0.01, 0.27 ± 0.02, and 0.39 ± 0.02 g CA L^{-1}, respectively. IP extracts were applied to the melon and pepper plant leaves in the field by spraying 25 plants per litre (40 mL/plant) with a hand sprayer.

2.3 Plant samples, crop management and treatments

The field assay was performed in the Experimental Station from I.N.T.A. Santiago del Estero "Francisco Cantos" located at 28º03'S; 64º15'W, Santiago del Estero, Argentina. Seeds of hybrids "Nun de miel"

and CLX-479 were used for melon and pepper plants, respectively. These seeds were provided by Rijkzwaan (Buenos Aires, Argentina), and they were sown in a vivarium. When melon or pepper plants had two true leaves, they were transplanted in rows. In the case of the melon crop, these rows were spaced by 1.60 m and 0.70 m between plants, representing a plant density of 8,800 plants ha^{-1}; and in the corresponding assay with pepper plants, the rows were spaced by 1.00 m and 0.40 m between plants, representing a plant density of 25,000 plants ha^{-1}. The soil characterization resulted in 0.06 % N, 64 ppm of P, 94 mg L^{-1} K, and 86 mg L^{-1} Ca, pH 7.3, and the electric conductivity (EC) was 1.20 dSm^{-1}. The irrigation system used was localized, complementary to rainfall, and one-tape irrigation was placed in each plant row. Droppers were placed every 20 cm on the tape irrigation, and each dropper yielded a rate of 0.8 L h^{-1}. Preventive phytosanitary control was carried out. Both assays (melon and pepper) were constituted of 48 plants each. Each plant group (melon or pepper) was divided into two groups: control and stressed plants. Then, control, as well as the stressed plant of each crop, were divided into four subgroups, three of them with antioxidant applications and one with water. The experimental design was completely randomized, with six plants for each treatment (subgroup). Stressed plants for both crops were covered with plastic of 30 μm thickness 35 days after transplantation (DAT) for five days. The control plants were kept uncovered during the whole experiment. Melon plants covered with plastic (stressed plants) were exposed to maximum temperatures between 38 and 44°C (Figure 1). The maximum environmental temperature range registered was 30−35°C during the mentioned five days (control ones). Concerning pepper plants, both the covered and uncovered groups were protected in turn using a shade structure because these plants are more sensitive to high temperatures. Thus, the covered pepper plants (stressed plants) were exposed to maximum temperatures between 39 and 45°C (see Figure 1), and the maximum environmental temperature range registered for control plants was 31−36°C. The foliar spray of the antioxidant solution was performed before plant exposure to stress conditions at 27 and 34 DAT. IP1, IP2, and IP3 extracts were sprayed on both control and stressed plants. The corresponding subgroups without antioxidant applications were sprayed with distilled water instead. Leaves from melon and pepper plants were collected at 40 DAT. The leaves of the different plants were put in 48 separate bags, 6 bags (six plants) for each treatment. Fresh leaves of both melon and pepper plants were lyophilized at 5 μTorr for 72 h, at −80°C. The dried samples were stored at −20°C until analysis. PhytoP and PhytoF extraction of plant tissues was carried out as in Yonny et al. 2016. (Yonny et al., 2016)

2.4 Total fruit yield

The harvest of melon and pepper fruits started at 90 DAT and 60 DAT, respectively. Fruits were harvested from six plants per treatment, and they were graded into marketable (quality commercial) and non-marketable (with disease symptoms or deformed) categories. The total weights of marketable fruits were registered per plant, and the unmarketable fruits were discarded.

155 *2.5 Statistical analyses*

156 Analysis of variance was used to determine significant differences among data using the
157 software INFOSTAT (Di Rienzo et al., 2009). Fisher LSD-test was used to compare means when the
158 effects were found to be significant ($p < 0.05$).

159

160 **3 Results**

161

162 *3.1 PhytoP and PhytoF profiles in melon and sweet pepper leaves*

163 Different PhytoPs and PhytoFs were determined in the leaves of melon and pepper plants
164 exposed to high temperatures. To the best of our knowledge, this is the first time that these metabolites
165 were evaluated in the leaves of sweet pepper plants grown under thermal stress. In both horticultural
166 crops, eight PhytoPs and two PhytoFs were quantified, and their chemical structures are shown in Figure
167 2. B_1- and L_1-PhytoP stereoisomers, could not be quantified individually due to the peak coelution. Thus,
168 PP5 and PP6 were quantified together (B_1-stereoisomers), as well as PP7 and PP8 (L_1-PhytoP
169 stereoisomers). The individual concentrations of PhytoPs and PhytoFs ranged from 1.9-50 μg g^{-1} and
170 24-70 μg g^{-1}, and 0.16-3.5 μg g^{-1} and 1.5-4.5 μg g^{-1} in melon and pepper plants, respectively.

171

172 *3.2 Monitoring of ALA oxidized metabolites in thermal-stressed and non-stressed melon plants under*
173 *IP treatment.*

174 Foliar applications of three levels of IP and distilled water were done in both thermal-stressed
175 plants and control ones. Total PhytoP and PhytoF concentrations in control and thermal-stressed samples
176 were statistically different (Figure 3), being 1.5 times higher in stressed plants than in control. Thus,
177 PhytoPs and PhytoFs confirm their role as oxidative stress biomarkers. The PhytoP and PhytoF profiles,
178 which show the variations in the individual concentrations of these oxidative biomarkers in thermal-
179 stressed melon plants for the treatments evaluated, are presented in supplemental material S1.

180 The result of IP application differed in stressed and non-stressed plants (Fig. 3). The IP1 dose
181 was the most effective against oxidative stress since the PhytoP and PhytoF levels in thermal-stressed
182 plants were statistically lower, 42.4 and 47.2 %, respectively, than the corresponding ones in the stressed
183 plants sprayed with water. It should be highlighted that there was no statistical difference in the total
184 concentrations of PhytoPs and PhytoFs between stressed samples sprayed with IP1 and non-stressed
185 samples sprayed with water. Besides, the total PhytoP levels in non-stressed samples sprayed with IP1
186 were statistically lower than in non-stressed samples sprayed with water (31.9 %). The IP1 extract had
187 not only a mitigation effect on oxidative damage but also a biostimulant effect. By contrast, IP2 spraying
188 on thermal-stressed plants decreased PhytoP and PhytoF levels by 26.9 and 30.1 %, respectively,
189 compared to stressed plants sprayed with water, but PhytoP and PhytoF concentrations were similar in
190 non-stressed samples sprayed with IP2 or water. In respect to IP3 dose effect, PhytoP and PhytoF levels
191 in thermally stressed plants were also lower than in stressed plants sprayed with water, 10.9 and 16.8 %,

respectively. However, in non-stressed plants sprayed with IP3, the PhytoP and PhytoF levels were statistically higher than the corresponding ones sprayed with water, 22.6 and 24.1 %, respectively. Moreover, there was no statistical difference in the total PhytoP and PhytoF levels between stressed and non-stressed melon plants sprayed with IP3 and the corresponding PhytoP and PhytoF contents in thermal-stressed plants. Finally, the total PhytoP levels in thermal-stressed samples sprayed with IP3 were similar to the corresponding ones in stressed samples sprayed with water.

3.3 Monitoring of ALA oxidized metabolites in stressed and non-stressed pepper plants under IP treatments.

Different IP doses were sprayed in pepper plants before thermal stress exposure in both control and stressed plants to attenuate their negative effects (Fig. 4). PhytoP and PhytoF concentration levels of stressed and control samples were statistically different, being 53.5 % on average higher the former than the latter. The PhytoP and PhytoF profile that show the variations in the individual concentrations of these oxidative biomarkers in thermal-stressed pepper plants for the treatments evaluated is presented in the supplemental material S2.

PhytoP and PhytoF levels in thermal-stressed pepper plants sprayed with IP1 were statistically lower than in stressed plants sprayed with water, being 38.5 and 48.2 %, respectively. PhytoP and PhytoF levels of IP1 treatment were also similar to those of non-stressed plants sprayed with water. This fact demonstrates the oxidative stress mitigation effect (Fig. 4). Moreover, as like in melon plants, the total PhytoP levels in non-stressed samples sprayed with IP1 are statistically lower (20.2 %) than corresponding non-stressed samples sprayed with water. Thus, both effects, oxidative stress mitigation and biostimulant, were also observed in this crop. With respect to IP2 and IP3 treatments, their effectiveness in mitigating oxidative stress decreased but was observed with both doses. Specifically, the PhytoP and PhytoF levels in thermal-stressed pepper plants sprayed with IP2 were statistically lower, 31.4 and 35.2 %, respectively, than those in stressed plants without treatment. For the IP3 dose, it should be noted that in this crop, the oxidative stress mitigating effect remained as the PhytoP and PhytoF levels in thermal-stressed pepper plants sprayed were statistically lower, 21.4 and 25.4 %, respectively, than in stressed plants sprayed with water. Concerning non-stressed pepper plants, similar results in PhytoP and PhytoF levels were found among IP2, IP3 and water sprayings.

3.4 Evaluation of IP treatment effect in melon and pepper yields from thermal-stressed and non-stressed plants

The fruit yields as the total weights of marketable fruits were determined in melon and pepper plants exposed to high temperatures in the presence of different IP doses (or distilled water) compared to the corresponding controls (non-stressed plants). It should be noted that this is the first report of productivity parameters in thermal-stressed melon and pepper evaluated under IP biostimulant effect (Fig. 5 and 6).

In terms of melon (Fig. 5), the mean yield in thermally stressed plants sprayed with water was statistically lower (37 %) than the corresponding value in non-stressed plants. There was no statistical difference between the yields of stressed plants sprayed with IP1 and non-stressed plants (sprayed with water). The former was only 1.2 % lower than the latter but 57 % higher than the thermal-stressed plants without IP. Thus, the mitigation of the negative effect of the thermal (and oxidative) stress of this productivity parameter was also observed. Furthermore, the yields of non-stressed plants sprayed with IP1 were slightly higher (10 %) than those of non-stressed plants without IP extract. This finding gives another probe of the biostimulant effect of IP extract at the first dose evaluated. Considering IP2 and IP3 foliar applications, the results showed that there were statistical differences between the yields of stressed plants sprayed with these doses and the corresponding ones in and non-stressed plants (sprayed with water), being 18 and 43 % lower the former than the latter, respectively. The spraying of non-stressed samples with IP2 decreased the fruit yield by 5 % in relation to the non-stressed plants sprayed with water, while with IP3, this decrease was 38 %. Finally, IP2 spraying of thermally stressed samples increased fruit yield with 29 % compared to water-sprayed stressed plants. In contrast, IP3 decreased by 10 % compared to water-sprayed stressed plants. Therefore, IP3 spraying was slightly harmful to the melon crop, rather than beneficial.

Considering the fruit yields of the pepper crop (Figure 6), the mean yield in thermally stressed plants was statistically lower (21 %) than the corresponding value in non-stressed plants. There were statistical differences between the yield of thermal-stressed plants sprayed with IP1 and non-stressed plants (sprayed with water), being the former 9.1 % higher than the latter, and 39 % higher than the yield in thermal-stressed plants without IP. Therefore, as like in melon plants, IP1 dose also had both stress mitigation and biostimulant effects in the pepper plants exposed to thermal stress during their growth. Regarding IP2, there were no statistical differences between the yield of stressed plants sprayed with this dose and non-stressed plants (sprayed with water), even the former was only 6.6 % lower than the latter and 16 % higher than the yield in thermal-stressed plants without IP. Finally, the results concerning to IP3 foliar application showed a similar behavior that IP2, because the yield of stressed plants sprayed with this dose is comparable to yield of non-stressed plants without IP, being the former 8.3 % lower than the latter and 14 % higher than the yield in thermal-stressed plants without IP. Thus, in the pepper crop, IP2 and IP3 doses also had a mitigating effect.

4 Discussion

4.1PhytoP and Phyto F levels in melon and pepper plants

The PhytoP and PhytoF levels determined in melon leaves in the present work were between 15 and 185 times higher than those previously reported (Yonny et al., 2016). This fact can be explained considering that the melon plant hybrid used in the present work was *Nun de miel*; while, *Sweet ball* and *Early spring* hybrids were those used in the experiment held in 2016, showing the dependence on the

plant genotypes. Other studies reported the same situation in other plant species. Pinciroli et al. (2018) reported, in rice grain, 0.19 µg g^{-1} of PhytoPs in 'H475-3-1-1-2' genotype and 0.004 µg g^{-1} of PhytoPs in 'H484-9-1'genotype; and 0.025 µg g^{-1} of PhytoFs in 'H458-41-1-1-1' genotype and 0.002 µg g^{-1} of PhytoFs in 'H484-9-1' genotype. Vigor et al. (2020), working with microalgae, reported extreme PhytoP values of 2.2 µg g^{-1} in *Rhodomonas salina* and 0.0008 µgg^{-1} in *Phaeodactylum tricornutum*, and extreme PhytoF values of 2.2 µgg^{-1} in *Rhodomonas salina* and 0.001 µg g^{-1} in *Phaeodactylum tricornutum*.

Regarding the PhytoP and PhytoF profiles measured in stress melon and pepper plant (Supplementary Material S1 and S2, respectively), most of the ALA oxidation products found are consistent with those described in our previous report melon leaves (Yonny et al., 2016), as well as in other plant systems, such as herb leaves (Imbusch & Muller, 2000), rice grain (Pinciroli et al., 2018) and microalgae (Vigor et al., 2020). However, compared with our previous study on melon leaves (Yonny et al., 2016), in this work, a new PhytoF molecule was found. Specifically in the present work, the *ent*-16(*RS*)-13-*epi*-ST-Δ^{14}-9-PhytoF (PF1) was detected and quantified for the first time in a plant system. In contrast, the *ent*-9(RS)-12-*epi*-ST-Δ^{10}-13-PhytoF, detected and quantified as mentioned, in our previous study, in the present report, it was detected but its level was not enough to reach the methodology limit of quantification and so, it could not be quantified. This situation is another consequence of working with different genotypes or varieties from the same crop as it was explained and discussed before.

For the quantification of PhytoPs and PhytoFs in pepper leaves, there are no previous studies, and this is the first report. The PhytoP and PhytoF profiles and concentration ranges are similar to those found in melon leaves, this an important finding of this work since a common pattern of OS biomarkers could be found in two different species and different hybrids of the same species when exposed to similar abiotic stress conditions.

Regarding to thermal stress effect in melon and pepper plant yields, the decrease percentages registered in stressful conditions, were 37 and 21 % respectively. These results can be explained by considering that at temperatures above 40 °C, these horticultural crops face heat damage and severe water loss due to excessive transpiration and evaporation, therefore, it limits physiological processes such as photosynthesis and respiration, which substantially decrease the crop yields (Mittler, Finka, & Goloubinoff, 2012). In this sense, it was reported that the horticultural crop yields decrease 40-50 % because of the thermal stress induced principally by the climate change (Saquib et al., 2022).

4.2 The biostimulant dose effect on melon plants exposed to thermal stress.

Melon plants were exposed to high temperatures as stressing factor. An antioxidant extract was sprayed on these plants in different doses to determine their effect to reduce or delay the oxidative stress condition. The IP1 dose was the most effective against thermal (and oxidative) stress in the melon plant because this dose, under thermal stress conditions, decreased PhytoP and PhytoF levels and increased the total fruit weight per plant in percentages equal to 44.8 and 57 %, respectively. The decrease in the

levels of biomarkers of oxidative stress was similar to, or even slightly higher than the value reported (37 %) in our previous work (Yonny et al., 2016). Regarding the increase in yield, the obtained value was higher than that reported by Amarasinghe et al. (2022) in melon plants exposed to high temperatures after the foliar application of brassinoesteroid extract (12.5 %).

By contrast to IP1, IP3 extract application was found to be prooxidant because when this extract dose was applied to control samples (without stressful factor), the PhytoP and PhytoF levels increase instead of decreasing compared to the plant sprayed with water (**Figure 3**). In addition to that, there were no statistical differences between the levels of PhytoPs and PhytoFs corresponding to the unstressed and stressed melon plants, both sprayed with IP3. This potential pro-oxidant effect could also be attributed to a too high concentration of phenolic compound present in the sprayed extracts (Yonny et al., 2018). These phenolic compounds have well-recognised antioxidant activity, but it has also been reported that they can have a pro-oxidant effect at high concentrations (Sakihama et al., 2002). The mentioned pro-oxidant effect was also observed in fruit yield because this productivity parameter in thermal stressed plants sprayed with IP3 decreased 43 % in respect to non-stressed plants (without IP), being higher than the obtained in thermal stressed plants sprayed with water in respect to non-stressed plants also sprayed with water (37 %).These results evidence the importance of a dose effect experiment, to establish the safe concentration for biostimulant applications that avoid the change of behaviour to an oxidative stress promoter.

4.3 The biostimulant dose effect on pepper plants exposed to thermal stress.

Pepper plants were exposed to high temperatures after biostimulant applications. Both, stressed and non-stressed plants were sprayed with the biostimulant doses in the assay. PhytoP and PhytoF levels were significantly higher in the leaves of stressed plants than in those of non-stressed ones. PhytoP and PhytoF levels were evaluated for the first time to monitor the oxidative stress status of the pepper plant after exposure to thermal stress. Therefore, considering the results obtained, PhytoPs and PhytoFs were also found to be biomarkers of the progression of oxidative stress in this crop. IP1 was determined as the optimum dose for this crop as well as that for melon plants; besides, PhytoP and PhytoF levels decreased in similar percentages to those observed in melon plants. Although, their profile presented variations. This situation suggests that each species responds in a specific way to the same stressing condition within a common pattern as horticultural crops. Unlike what happens in the melon crop, in pepper plants sprayed with IP2 and IP3, the evaluated concentrations of PhytoPs and PhytoFs were statistically different from stressed plants sprayed with water. Thus, it could be concluded that to pepper plants, the IP extract in the maximum dose assayed was still effective to delay the oxidative stress process generated by thermal stress. Although, the percentage decreases of PhytoP and PhytoF were lower than the corresponding ones found in melon plants. Thus, the biostimulant foliar application could be a useful crop management practice to fortify the defense mechanisms of the pepper plant, when it is cultivated in its normal season when high temperatures are expected.

The oxidative stress mitigation effects of IP1-IP3 doses were consistent with the corresponding effects on fruit yield as a productivity parameter. In this sense, the total fruit weights per plant in thermal stressed plants sprayed with IP1, IP2 and IP3 were 39, 16 and 14 % higher than the corresponding ones in thermal-stressed plants sprayed with water, respectively. Thus, the biostimulant foliar application could be a useful crop management practice to fortify the defense mechanisms of the sweet pepper plant, when it is cultivated in its normal season when high temperatures are expected. The increase in yield reached with the IP1-IP3 extracts was similar or even higher than that reported by Paradikovic et al. (2013), who registered an increase of 14 % in pepper plant yield because of foliar application with four commercial biostimulants based on a mixture of amino acids, polysaccharides, and organic acids as active compounds. The mentioned study was performed in a hot summer season, when high temperatures in a greenhouse caused physiological stress in plants.

Finally, the mentioned results showed that the IP active compounds were capable to mitigate the oxidative stress, reducing cell damage caused by thermal stress; moreover, biostimulants effects were observed in both, melon and pepper crops. It has been described that biostimulants activate plant endogenous defence mechanisms producing a faster recovery of plants when they were exposed to abiotic and biotic stresses (Cardonia et al., 2018; Yahkin et al., 2017). However, these activation mechanisms are often difficult to identify, and they are still under research (du Jardin, Xu & Geelen, , 2020).

The dose of biostimulant that is most effective to activate the plant's defense mechanisms may vary depending on the cultivated species. Further studies on the metabolic pathways activated in the presence of biostimulants for each species during their biochemical response to stress are important to understand the underlying mechanisms of action.

Conclusion

In the present work, an eco-friendly crop management practice was assayed to mitigate the oxidative stress negative effects on melon and sweet pepper plants of the exposure to high temperatures. The biochemical response of stressed plants previously treated with three different doses of IP extracts was evaluated in terms of early-stage biomarkers of oxidative stress. Moreover, the total fruit yields were also measured. The most convenient IP doses to mitigate the oxidative stress progress and increase the crop production under thermal stress conditions, were found for both, melon and pepper plants. Besides, a prooxidant effect of IP extract found in melon plants exposed to thermal stress is reported here for the first time. These effects, with the IP3 dose, not only increased PhytoP and PhytoF levels, but also decreased the yield of the melon plant. Thus, it could be highlighted that crop yields had a similar trend that the oxidative stress markers levels, which were determined before the harvesting time. This situation suggests that the evaluation of oxidative stress in advance, during plant growth, could give an excellent opportunity to decide if introduce or not introduce some agronomic practise, such as biostimulant application, to fortify the defences of the endogenous plant mechanisms defences before

the harvested. In this way, the sustainable management practice propose here will have more possibilities to be effective in order to set a defence plant stress response and anticipate the environmental changes that might affect plant growth irreversibly. These findings become important to mitigate losses in crop productivity in the context of climate change in which crops will have to grow in increasingly unfavourable conditions. Finally, to take advantage of this promising agricultural management practice in warmer years due to climate change, specific conditions should be evaluated for different crops and their hybrids under different abiotic stresses.

Author contributions

Melisa E. Yonny: Conceptualization, Methodology, Investigation, Writing-Original Draft, Formal analysis, Funding acquisition. **Maria L. Toscano Adamo**: Investigation. **Ariel Rodríguez Torresi**: Resources, Investigation. **Guillaume Reversat:** Investigation. **Binqging Zhou:** Investigation. **Camille Oger:** Writing-Review & Editing. **Jean-Marie Galano:** Writing-Review & Editing. **Thierry Durand:** Writing-Review & Editing, Funding acquisition. **Claire Vigor:** Investigation, Formal analysis, Writing-Review & Editing. **Mónica A. Nazareno:** Supervision, Writing- Review & Editing, Funding acquisition.

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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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Figure Captions

Figure 1. Evolution of the maximum temperatures during the growth cycles of melon and pepper plants with or without plastic coverture during the thermal stress period, control and thermal stressed plants, respectively.

Figure 2. Chemical structures and nomenclature of PhytoPs and PhytoFs as early oxidative stress advance biomarkers.

Figure 3. PhytoP and PhytoF total contents in control and thermal stressed melon plants in response to different IP dose. Bars represent means ± SD (n = 6). Different letters show statistically different mean oxidative stress biomarkers levels ($p < 0.05$) according to ANOVA and LSD Fischer tests. Lowercase letters represent statistically different PhytoP contents. Capital letters represent statistically different PhytoP contents.

Figure 4. PhytoP and PhytoF total contents in control and thermal stressed pepper plants in response to different IP dose. Bars represent means ± SD (n = 6). Different letters show statistically different mean oxidative stress biomarkers levels ($p < 0.05$) according to ANOVA and LSD Fischer tests. Lowercase letters represent statistically different PhytoP contents. Capital letters represent statistically different mean PhytoP contents.

Figure 5. Effect on fruit yield of the exogenous IP application in control and thermal stressed melon plants. Bars represent means ± standard deviation (n = 6 in each crop). Different letters show statistically different mean oxidative stress biomarkers levels ($p < 0.05$) according to ANOVA and LSD Fischer tests.

Figure 6. Effect on fruit yield of the exogenous IP application in control and thermal stressed pepper plants. Bars represent means ± standard deviation (n = 6 in each crop). Different letters show statistically different mean oxidative stress biomarkers levels ($p < 0.05$) according to ANOVA and LSD Fischer tests.

Suplementary material

S1- PhytoP and PhytoF profiles in control and thermal stressed melon plants in response to different IP dose. Bars represent means ± standard deviation (n = 6). Different letters show statistically different mean oxidative stress biomarkers levels ($P<0.05$) according to ANOVA and LSD Fischer tests.

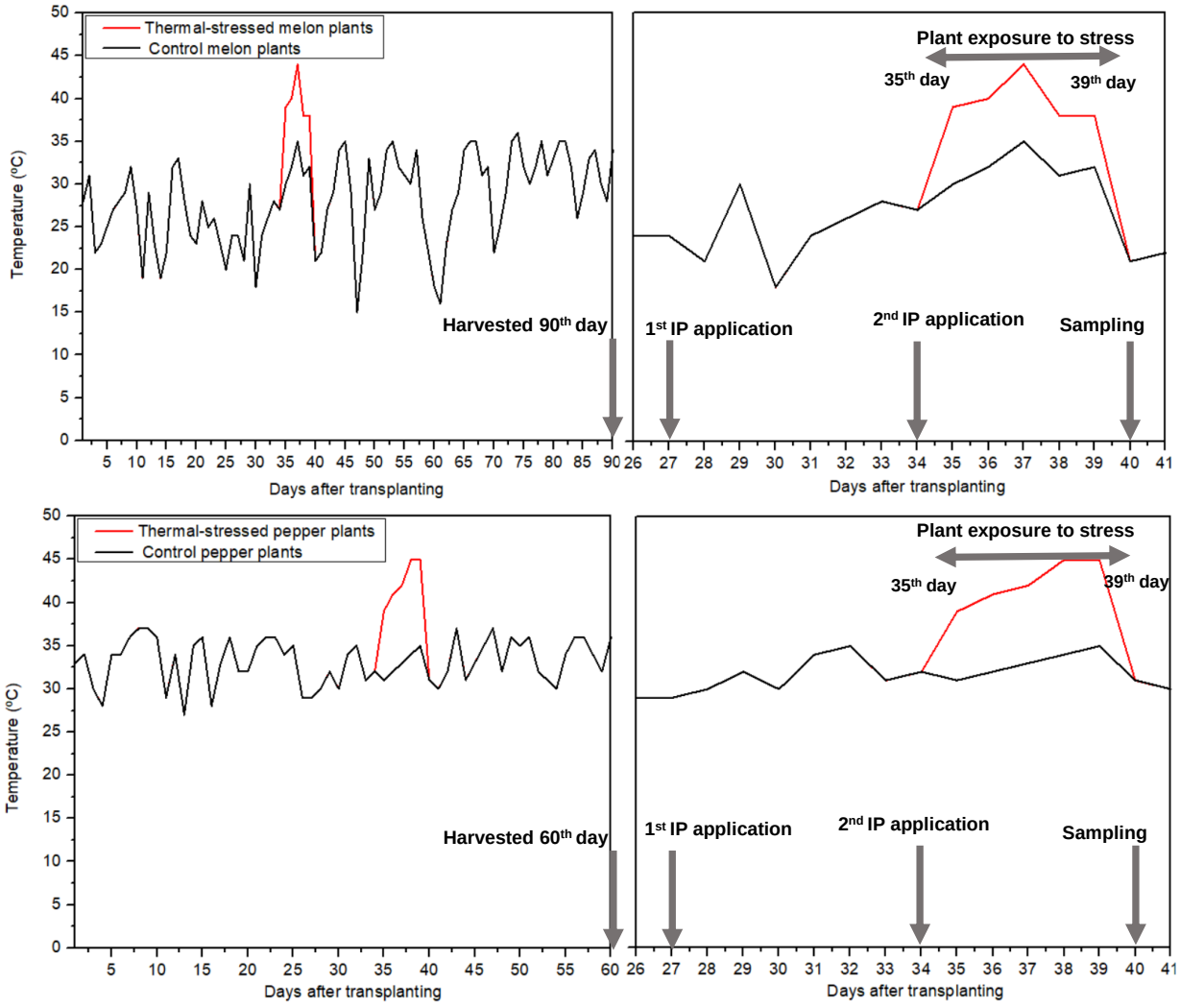
S2- PhytoP and PhytoF profiles in control and thermal stressed pepper plants in response to different IP dose. Bars represent means ± standard deviation (n = 6). Different letters show statistically different mean oxidative stress biomarkers levels ($P<0.05$) according to ANOVA and LSD Fischer tests.

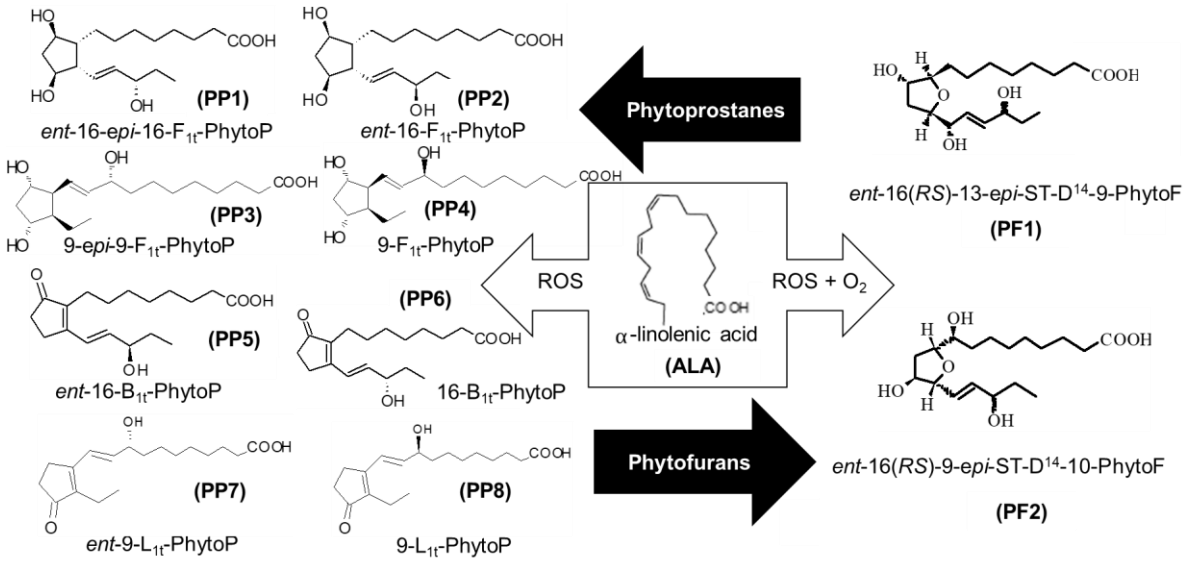
Highlights

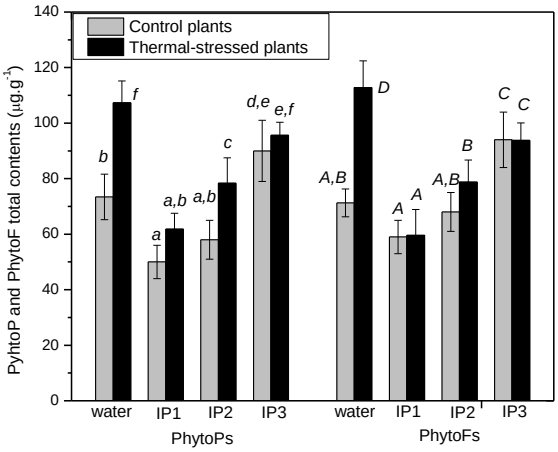
- Eco-friendly strategies to mitigate heat stress were tried in horticultural crops.
- Phytoprostanes and phytofurans were established as biomarkers in stressed plants.
- IP effect on fruit yields of stressed melon and pepper plants was dose dependent.
- IP1 extract was the most effective dose to reduce stress biomarkers for both crops.
- IP1 was the most effective dose to increase the fruit yield for both crops.
- Prooxidant effect on melon crop was found at IP3 extract dose.

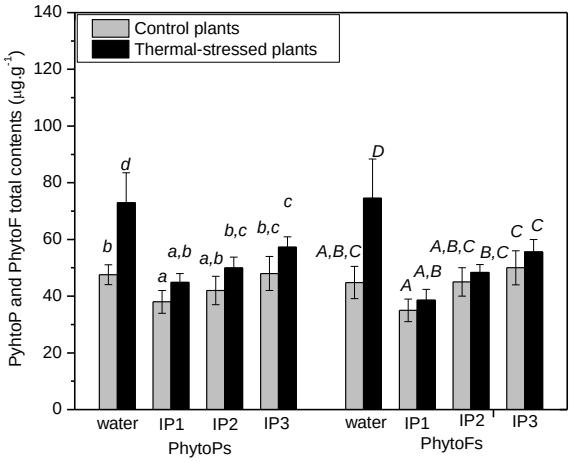
Statement

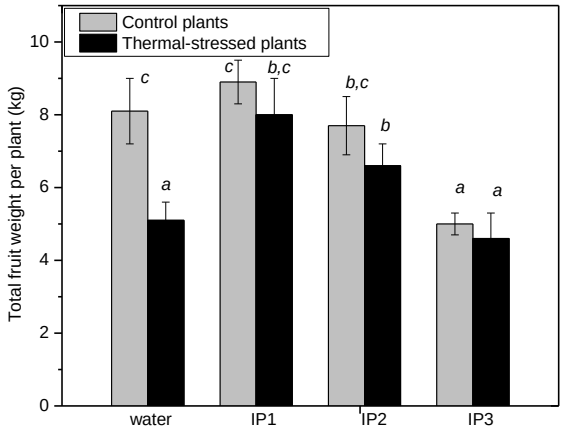
Extreme high temperatures induce abiotic stress in melon and pepper plants, increase oxidative stress biomarker levels and decrease plant yields. The biostimulant foliar application with *Ilex paraguariensis* extracts to these plants, before plant exposure to stress, attenuate their negative effects according to the dose sprayed.

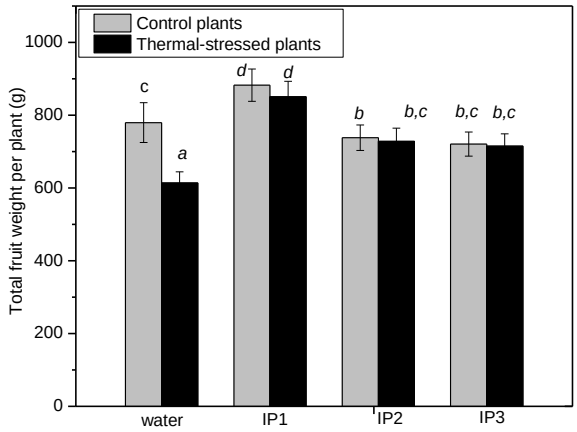

Thermal-stressed melon plants
Control melon plants
Temperature (°C)
Days after transplanting
Harvested 90th day
Plant exposure to stress
35th day
39th day
1st IP application
2nd IP application
Sampling
Thermal-stressed pepper plants
Control pepper plants
Harvested 60th day

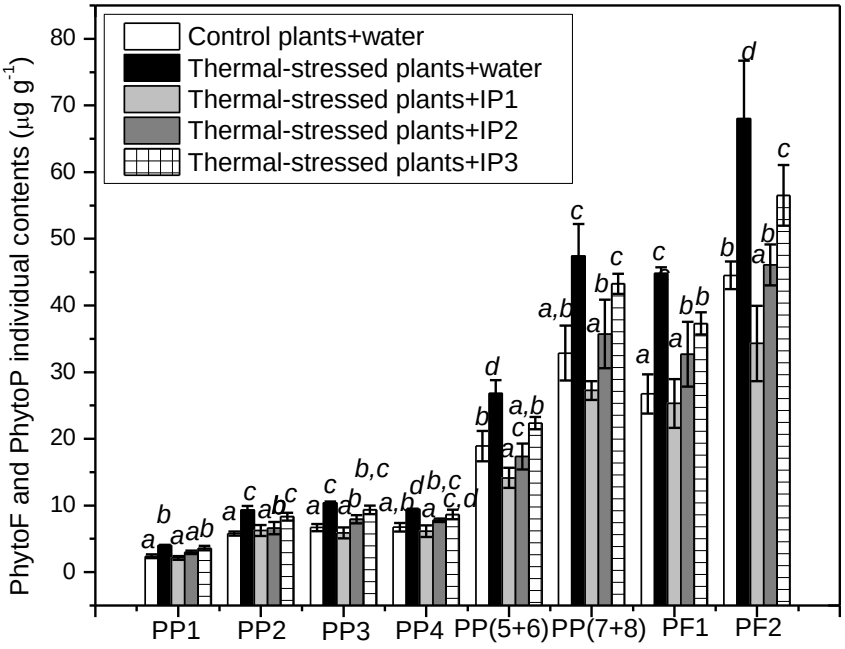

(PP1)
ent-16-epi-16-F1t-PhytoP
(PP2)
ent-16-F1t-PhytoP
Phytoprostanes
ent-16(RS)-13-epi-ST-D14-9-PhytoF
(PF1)
(PP3)
9-epi-9-F1t-PhytoP
(PP4)
9-F1t-PhytoP
ROS
ROS + O2
α-linolenic acid
(ALA)
(PP6)
(PP5)
ent-16-B1t-PhytoP
16-B1t-PhytoP
(PP7)
ent-9-L1t-PhytoP
(PP8)
9-L1t-PhytoP
Phytofurans
ent-16(RS)-9-epi-ST-D14-10-PhytoF
(PF2)

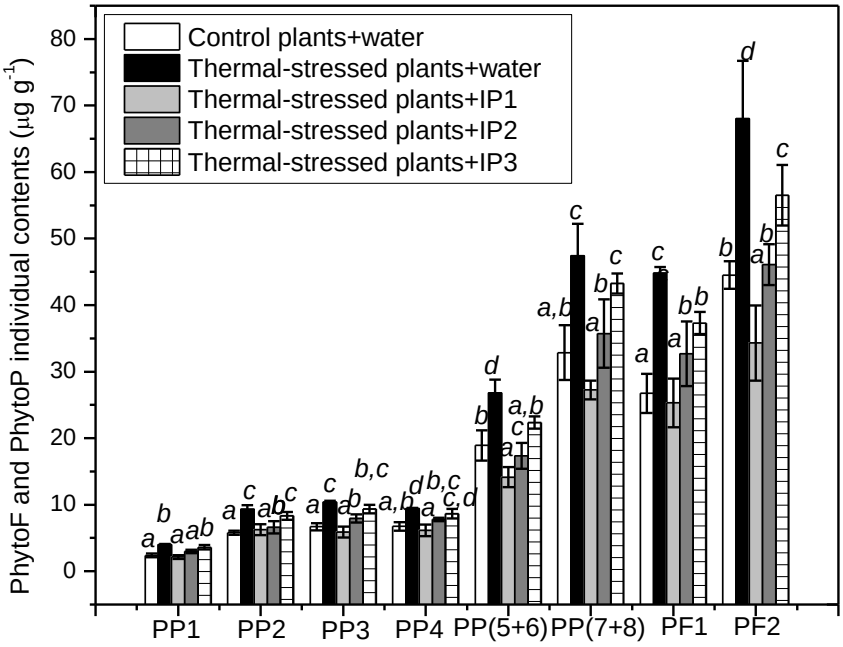

Control plants
Thermal-stressed plants
PyhtoP and PhytoF total contents (μg.g-1)
140
120
100
80
60
40
20
0
b
f
a
a,b
a,b
c
d,e
e,f
A,B
D
A
A
A,B
B
C
C
water
IP1
IP2
IP3
water
IP1
IP2
IP3
PhytoPs
PhytoFs


Control plants
Thermal-stressed plants
PyhtoP and PhytoF total contents (µg.g-1)
140
120
100
80
60
40
20
0
b
d
a
a,b
a,b
b,c
b,c
c
A,B,C
D
A
A,B
A,B,C
B,C
C
C
water
IP1
IP2
IP3
water
IP1
IP2
IP3
PhytoPs
PhytoFs


Control plants
Thermal-stressed plants
Total fruit weight per plant (kg)
0
2
4
6
8
10
c
a
c
b,c
b,c
b
a
a
water
IP1
IP2
IP3


Control plants
Thermal-stressed plants
Total fruit weight per plant (g)
0
200
400
600
800
1000
c
a
d
d
b
b,c
b,c
b,c
water
IP1
IP2
IP3


Control plants+water
Thermal-stressed plants+water
Thermal-stressed plants+IP1
Thermal-stressed plants+IP2
Thermal-stressed plants+IP3
PhytoF and PhytoP individual contents (μg g-1)
0
10
20
30
40
50
60
70
80
PP1
PP2
PP3
PP4
PP(5+6)
PP(7+8)
PF1
PF2


Control plants+water
Thermal-stressed plants+water
Thermal-stressed plants+IP1
Thermal-stressed plants+IP2
Thermal-stressed plants+IP3
PhytoF and PhytoP individual contents (μg g-1)
0
10
20
30
40
50
60
70
80
PP1
PP2
PP3
PP4
PP(5+6)
PP(7+8)
PF1
PF2