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Field-grown soybean induces jasmonates and defensive compounds in response to thrips feeding and solar UV-B radiation

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ABSTRACT

Solar UV-B radiation has been reported to enhance constitutive and inducible plant defenses against herbivore insects in many species. However, the induction of plant defenses depends on the phytohormone profile induced by the specific herbivore feeding guild. No study has shown the impact of soybean leaf chemical defenses induced by thrips herbivory in combination with solar UV-B radiation on thrips performance. To uncover plant responses to herbivory in crop conditions, we proposed the hypothesis that solar UV-B radiation will increase constitutive and inducible defenses and phytohormones related with defenses in field-grown soybean, therefore affecting thrips performance. In this study two soybean cultivars (cv.) were grown in field conditions under attenuated or solar UV-B radiation and damaged by 6 days of herbivory of *Caliothrips phaseoli*. Our field experiments showed similar survivorship levels of thrips that fed on foliage grown under either attenuated or solar UV-B radiation, while survivorship of thrips that fed on cv. Williams was lower than those that fed on cv Charata. Cv Williams produced different flavonols and higher trypsin protease inhibitor (TPI) activity levels and more genistin than cv Charata. The increment of jasmonic acid (JA)-regulated defenses against insects in foliage of cv Williams was explained by the induction of JA and JA-Ile after herbivory and solar UV-B exposure. Independently of the UV-B environment herbivory induced salicylic acid (SA) and 12-oxo-phytodienoic acid (*cis*-OPDA) in both cvs. To our knowledge no study before has showed a complete profile of defensive hormones and defensive compounds induced by thrips feeding and solar UV-B.

1. Introduction

Thrips (*Caliothrips phaseoli*) are occasional pest in Argentina and with increasing importance in soybean crops, especially during dry summers. Many thrips species cause direct damage to commercial crops through feeding or by acting as vectors of economically important plant viruses (Diaz-Montano et al., 2011; Riley et al., 2011). Since these insects cannot be deterred by transgenic BT (*Bacillus thurigiensis*) crops and they are hard to control because of their rapid adaptation to pesticides, the development of pest management strategies to control thrips is an issue of increasing urgency (Mouden et al., 2017). A way to reduce the use of pesticide applications is identifying plant defenses, and then enhancing them by genetic improvement (Birkett and Pickett, 2014). Resistance plant traits against insect consumption can be constitutive and inducible by insect damage, and they are modulated by defensive phytohormones (Schoonhoven et al., 2005).

Plants perceive insect damage and alter the production of phytohormones of the jasmonate type such as jasmonic acid (JA), jasmonoyl-L-isoleucine (JA-Ile) and 12-oxo-phytodienoic acid (*cis*-OPDA), and also salicylic acid (SA), abscisic acid (ABA), and ethylene (ET) to up-regulate defenses against herbivores (Schuman and Baldwin, 2016). Some studies showed that thrips feeding activities induce the synthesis of JA and JA-responsive genes of plants grown under artificial light (De Vos et al., 2005; Abe et al., 2008; Escobar-Bravo et al., 2017). For example, in *Arabidopsis thaliana* almost 70% of genes induced by *Frankliniella occidentalis* damage were related to JA pathway (De Vos et al., 2005). Similar response was found in *Brassica rapa* and *Solanun lycopersicum* (tomato), where *F. occidentalis* feeding increased JA concentrations in

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plant tissues (Li et al., 2002; Abe et al., 2009). In soybean, thrips damage induced JA-mediated defenses, and application of MeJA reduced thrips population (Selig et al., 2016). One of the typical defenses regulated by JA is proteinase inhibitors (PI) that inhibit the digestive proteases of herbivores, decreasing their performance (Zavala et al., 2008). *Solanum tuberosum* (potato) overexpressing cysteine PIs deterred *F. occidentalis* attack (Outchkourov et al., 2004). In addition, it has been suggested that some polyphenolic compounds, such as tannins, may deter *Odontothrips loti* in some lines of *Medicago sativa* (alfalfa) (Wang et al., 2014).

Solar ultraviolet (UV)-B radiation (λ 280–315 nm) has well-documented effects in increasing plant resistance to herbivorous insects under field conditions (Ballaré et al., 2011). One of the main responses of plants to solar UV-B radiation is increasing leaf flavonoids and other phenolic compounds (Jenkins, 2017), which are negatively associated to lepidopteran larval performance and stink bugs (*Nezara viridula*) colonization and feeding preference in soybean (Piubelli et al., 2005; Zavala et al., 2015; Dillon et al., 2017). Solar UV-B radiation also enhances plant jasmonate-dependent defenses, such as trypsin protease inhibitors (TPI) induction in response to either insect damage or MeJA applications, as demonstrated in *Nicotiana* sp., tomato and soybean plants (Stratmann et al., 2000; Izaguirre et al., 2007; Demkura et al., 2010; Dillon et al., 2017, 2018). Although thrips (*C. phaseoli*) preferred to feed on soybean foliage grown under attenuated solar UV-B radiation and it was demonstrated that these insects perceive UV-B photons (Mazza et al., 1999, 2002), no study has shown the impact of soybean leaf chemical defenses induced by thrips herbivory in combination with solar UV-B radiation on thrips performance.

Since performing field experiments are essential to uncover plant responses to herbivory in crop conditions, we proposed the hypothesis that solar UV-B radiation will increase constitutive and inducible defenses and phytohormones related with defenses in field-grown soybean, therefore affecting thrips performance. We expected that thrips damage induce jasmonates and JA-regulated defenses against herbivores that can be synergistically increased by solar UV-B radiation, and decrease thrips survivorship. To examine the role of soybean defenses induced by either herbivory or solar UV-B on thrips performance, soybean crop was grown under ambient or attenuated solar UV-B radiation generated by plastic filters. At R1 stage of the crop (Fehr et al., 1971) collected thrips were allowed to feed on soybean for 6 days and survivorship analysis was performed. In addition, plant responses to solar UV-B radiation and herbivory were determined. While solar UV-B increased flavonol glycosides and genistin production, thrips feeding induced TPI activity and an unknown phenolic compound. Moreover, thrips damage induced jasmonates and *cis*-OPDA and in a minor extent SA. Although solar UVB radiation did not affect thrips survival, insects that fed on the soybean cultivar with lower levels of genistein derivatives and JA-Ile, and higher levels of constitutive SA had higher survivorship than those that fed on the more defended cultivar (Williams).

2. Material & methods

2.1. Experimental

2.1.1. Plant material

The experiments were carried out in the experimental fields of the University of Buenos Aires (34°35′S, 58°29′W), Buenos Aires, Argentina during the summer of 2014–2015 and 2015–2016. Two soybean (*Glycine max* L. Merril, Leguminosae) cultivars with flavonoid content previously characterized in response to differences solar UV-B radiation levels (Dillon et al., 2017; Zavala et al., 2015), Williams (maturity group: III) and Charata (maturity group: VII) were grown in four plots with an inter-row spacing of 20 cm, and a spacing between plants within each row of 15 cm. The plots were watered as needed and weeds were controlled manually. Although in this study we used two soybean cultivars with differences in maturity group, the contrasting levels of total phenolic compounds between cultivars allowed us to test the proposed hypothesis (Mazza et al., 2010)

2.1.2. UV-B treatment

Plants were allowed to emerge and grow in the field under 1.8 × 1.4 m aluminum frames covered with either clear polyester films (Mylar-D, Du-Pont, Wilmington, DE; 0.1 mm thick), which virtually cut off all UV radiation below 310 nm (UV-B- treatment), or “Stretch” films (Bemis Co. Minneapolis; 0.025 mm thick), which had very high transmittance (more than 80%) over the whole UV waveband (UV-B + treatment; Izaguirre et al., 2007). Peak UV-B (λ 305 nm) irradiance for clear-sky conditions during midday in the summer in Buenos Aires, fluctuates around 3.5 μW $cm^{-2} nm^{-1}$ while the peak photosynthetic photon flux density (PPFD) is around 2200 μmol $m^{-2} s^{-1}$ (Ballaré et al., 1996). Both filters have high and similar transmittance to PAR and UV-A (more than 80%) and generate similar conditions of temperature and humidity in the canopy and soil (Ballaré et al., 1996; Mazza et al., 2002; Izaguirre et al., 2007; Zavala et al., 2015). For all measurements, only plants from the center of the plot were used (where solar UV-B radiation is less than 5% of ambient levels in the UV-B- treatment; Zavala et al., 2001). The filters were raised periodically to maintain them approximately 10 cm above the upper leaf layer. On each individual plot, the filters were changed twice during the course of the growing season because the plastics tended to deteriorate and accumulate dust.

2.1.3. Thrips manipulative treatments

During February of summer 2015–2016, adults of thrips (*C. phaseoli*) were collected using Eppendorf tubes in groups of 15 individuals from soybean plants in a greenhouse of School of Agronomy, University of Buenos Aires. At the moment to start field experiments, while cv. Williams was in R1, cv. Charata was on V7 stage (Fehr et al., 1971). To induce soybean defenses and to determine thrips survivorship, 15 adult thrips were placed in the central leaflet of the youngest fully expanded leaf of soybean plants and bagged with transparent tulle. Similarly, control leaflets were covered with an empty tulle bag. Soybean leaflets from different plants and plots were considered as replicate (n = 6; 2 or 1 replicates per plot). Thrips were allowed to feed on leaves for 6 days. At midday, after six days of feeding, alive adult thrips that were on each leaflet or tulle bag were counted, and damaged and control leaves from the four different plots were collected and frozen in liquid nitrogen for phytohormone analysis (JA, JA-Ile; OPDA, SA and ABA) TPI activity, and phenolic compounds determination.

Ethylene emission determination was performed 24 h after thrips started to feed on soybean from damaged and control leaves. The unit of replication for statistical analyses was the individual plot (n = 4), where one leaf of each cultivar and treatment per plot was collected for analysis. Leaves were cut and the petioles covered with a matrix of water-saturated cotton to prevent desiccation and then placed in 660 mL hermetic glass pots.

2.1.4. TPI determination

To determine TPI (trypsin protease inhibitor) activity, 0.150 g of fresh leaf material were ground in a mortar and transferred to an Eppendorf tube with 0.525 mL of extraction buffer. Samples were vortexed for 5 min and centrifuged at 11,000*g* for 15 min to obtain the supernatant, which is used for TPI activity determination. The extraction buffer was made by adding to 1 L of buffer 0.1 M Tris-HCl (pH 7.6): 50 g polyvinylpyrrolidone (PVPP), 2 g phenylthiourea, 5 g diethyldithiocarbamate, 18.6 g ethylene diamine tetraacetic acid. TPI activity was determined using, bovine trypsin (Sigma) and *N*α-benzoyl-*D,L*-arginine-*p*-nitroanilide (D-L-BA*p*NA) as substrate. The reaction was performed at 37 °C in a microplate reader (Biotec ELx808; Vermont, USA) and TPI activity measured at λ 410 nm. TPI activity was normalized to soluble protein content determined by Bradford (1976), and using Bovine Serum Albumin (BSA) as standard.

2.1.5. Phenolic compounds quantification and identification

To determine individual phenolic compounds, collected leaves were ground with liquid nitrogen and 0.1 g was combined with 900 µL of 80% methanol aqueous solution in Eppendorf tubes of 2 mL. Samples were vortexed for 1 min and left at room temperature for 1 h. Afterwards samples were sonicated for 1 min and left at room temperature 2 h and then centrifuged at 11,000*g* for 5 min. After taking a clear supernatant, 400 µL of chloroform were added and after hand-shaking to homogenize, 200 µL of H_20 were added to reach an emulsion. Afterwards the extracts were centrifuged for 5 min at 11,000*g* and the aqueous phase (free of pigments) was taken for high-pressure liquid chromatography (HPLC) analysis. Chromatography analysis was performed on an HPLC Agilent 1100 A series equipped with a UV detector (Agilent Technologies, Inc., Wilmington, DE, USA), using an Eclipse XDB C18 reversed phase HPLC column (5 µm, 4.6 × 150 mm, Agilent Technologies). The mobile phase consisted of 0.1% aqueous acetic acid (solvent A) and 0.1% acetic acid in acetonitrile (solvent B). Solvent B was increased from 15% (at 0 min following injection) to 36% over 30 min. The solvent flow rate was 1 mL min^{-1}. The wavelength of the UV detector was set at λ 254, 270, and 360 nm. Identification of flavonoids was performed based on comparison of retention time and UV spectra with previously reported data, and quantification was done as rutin (flavonols), genistin (isoflavonoids) or gallic acid (unknown phenolic compound) equivalents (Dillon et al., 2017). Analytical and HPLC degree solvents used for determination of leaf phenolic compounds were purchased from Sintorgan (Argentina). True standards of rutin and genistin used in HPLC analysis were purchased from Sigma-Aldrich.

2.1.6. Ethylene measurements

Collected damaged and undamaged leaves were incubated in hermetic glasses for 5 h at room temperature (25 °C) and white light (approx. PPFD 700 µmol m^{-2} s^{-1}). Ethylene was measured with a gas chromatograph (Hewlett Packard 4890, Agilent) equipped with a Porapak N 80/100 column of 2 m length and a flame ionizing detector. Temperatures of the injector, column, and detector were 110 °C, 90 °C, and 250 °C, respectively. Flow rate of the carrier N_2 gas was 0.37 mL s^{-1}. The detector response was standardized by injecting known amounts of standard ethylene gas (Sigma–Aldrich, St. Louis, MO, USA) by serial dilutions. Retention time was 1.8 min. Amount of ethylene produced was quantified by the peak area of the standard and expressed as nmoles g^{-1} fresh weight (FW) h^{-1} for leaves.

2.1.7. Quantification of jasmonic acid, JA-Ile, cis-OPDA, salicylic acid, and abscisic acid measurements

JA, JA-Ile, *cis*-OPDA, SA, and ABA content in soybean leaves was analyzed by LC–MS/MS as described by Vadassery et al. (2012). Briefly, dry finely ground plant material was weight (25 mg) and extracted with 1.5 mL of methanol containing 60 ng D_6-JA (HPC Standards GmbH, Cunnersdorf, Germany), 60 ng of D_4-SA (Santa Cruz Biotechnology, Santa Cruz, U.S.A.), 60 ng of D_6-ABA (Toronto Research Chemicals, Toronto, ON. CA), and 12 ng of JA-[$^{13}C_6$]Ile conjugate (synthesized as in Kramell et al., 1988) as internal standards. The homogenate was mixed for 30 min and centrifuged at 11,000*g* for 20 min at 4 °C. After the supernatant was collected, the homogenate was re-extracted with 500 mL of methanol, mixed, and centrifuged, and supernatants were pooled. The combined extracts was evaporated in a SpeedVac at 30 °C and redissolved in 500 µL of methanol. Chromatography was performed on an Agilent 1200 HPLC system (Agilent Technologies). Separation was achieved on a Zorbax Eclipse XDB-C18 column (4.6 mm × 50 mm, 1.8 µm; Agilent Technologies). Formic acid (0.05%) in water and acetonitrile were employed as mobile phases A and B, respectively. An API 5000 tandem mass spectrometer (Applied Biosystems) equipped with a Turbospray ion source was operated in the negative ionization mode with multiple reaction monitoring (MRM). Phytohormones were quantified relative to the signal of their corresponding internal standard. For quantification of 12-oxophytodienoic acid (*cis*-OPDA), D6-JA was used as the internal standard applying an experimentally determined response factor of 0.5.

2.1.8. Statistical analyses

Statistical analyses were carried out using INFOSTAT software (Student Version 1.1). Phenolic compounds and phytohormone concentrations, and TPI activity were analyzed using a three-way ANOVA with UV-B, herbivory, and genotype as factors. When the interaction of three principal effects was significant Tukey Test was performed. Contrast test between undamaged vs damaged leaf was performed for each cultivar/UV-B treatment. Thrips survivorship was analyzed with a two way ANOVA with genotype and UV-B radiation as factors.

3. Results

3.1. Thrips survivorship

While adult thrips had higher survivorship after feeding on foliage of cv. Charata for 6 d than those that fed on cv. Williams ($p = 0.003$), no differences in survivorship were found between solar UV-B treatments (Fig. 1).

3.2. Soybean defenses induced by thrips herbivory and solar UV-B radiation

With the exception of kaempferol triglycoside 3 (KT3), flavonols glycosides differed between cultivars (Table 1). Although flavonols glycosides were induced by solar UV-B radiation, herbivory did not change flavonol glycosides concentrations in leaves of both cultivars (Fig. 2). Solar UV-B mediated increase of flavonols glycosides was higher for quercetin derivatives (QT1 + QT2, QT3, QD1 + QD2) than for kaempferol and isorhamnetin derivatives (KT1, KT2 + IT1, KT3) in soybean leaves (Table 1).

Herbivory induced an unknown phenolic derivative (with retention time similar to chlorogenic acid, a maxima λ_{abs} at 275 nm and MW of 430; Dillon et al., 2017) and TPI activity levels independently of the soybean cultivar or UV-B treatment ($p < 0.0001$; Fig. 2AB). While independently of solar UV-B radiation cv. Williams had higher genistin and malonyl genistin concentrations compared to cv. Charata ($p < 0.0001$), thrips damage induced malonyl genistin in cv. Charata ($p = 0.01$; Fig 2C & D). Neither daidzein and its derivatives nor other isoflavonoids were detected in any leaf sample.

3.3. Phytohormonal concentrations

While cv. Charata had higher constitutive SA levels than cv. Williams, thrips damage increased SA content in both cultivars and UV-

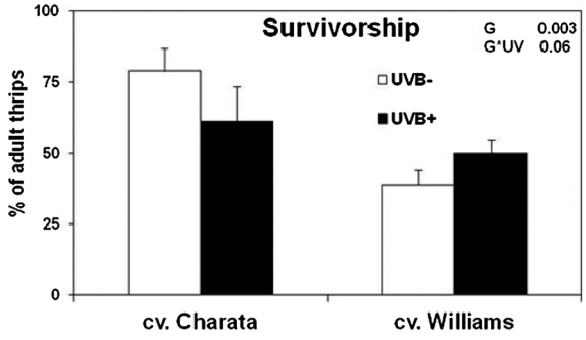

Fig. 1. Survivorship of adult thrips that fed during 6 days on two commercial soybean genotypes (cv. Williams; cv. Charata) grown under either solar or attenuated UV-B radiation (UVB+; UVB-). Significant *p* values of a two way ANOVA are shown (G: genotype; UV-B radiation and interaction terms). Values are means and error bars represent SEM (n = 6).

Table 1
Flavonoid concentration (μg/mg fresh weight) and P values of a two-way ANOVA from control or thrips damaged leaves of cv. Charata and cv. Williams grown under either solar or attenuated UV-B radiation (UVB+; UVB-). Values are means ± standard error (n = 3) or P values of a two way ANOVA. QT1 + QT2: Quercetin triglycoside 1 & 2, KT1: kaempferol triglycoside 1, KT3: kaempferol triglycoside 3, QT3: quercetin triglycoside 3, KT2 + IT1: kaempferol triglycoside 2 & isorhamnetin 1 and QD1 + QD2 quercetin diglycoside 1 & 2. QT1 + 2, KT1 and KT2 + IT1 were not detected in cv. Williams while QT3 and QD1 + QD2 were not detected in cv. Charata.

Cultivar	Flavonol glycoside	UVB- control	UVB- thrips	UVB + control	UVB + thrips	P_{UV}	$P_{herbi\text{-}vory}$	P_{UV*H}
cv. Charata	**QT1 + QT2**	1.04 ± 0.05	0.89 ± 0.07	1.66 ± 0.27	1.81 ± 0.28	**0.005**	1.0	0.5
	KT1	0.67 ± 0.06	0.62 ± 0.01	0.86 ± 0.1	1.06 ± 0.23	**0.04**	0.6	0.3
	IT1 + KT2	0.46 ± 0.04	0.54 ± 0.03	0.75 ± 0.13	0.91 ± 0.09	**0.005**	0.2	0.7
	KT3	0.16 ± 0.01	0.16 ± 0.01	0.2 ± 0.03	0.29 ± 0.05	**0.02**	0.1	0.2
cv. Williams	**QT3**	0.63 ± 0.05	0.7 ± 0.1	1.03 ± 0.09	0.91 ± 0.07	**0.005**	0.8	0.3
	KT3	0.16 ± 0.02	0.2 ± 0.04	0.24 ± 0.02	0.18 ± 0.02	0.3	0.8	0.1
	QD1 + QD2	0.76 ± 0.05	0.95 ± 0.12	1.53 ± 0.2	1.51 ± 0.02	**0.0005**	0.5	0.4

Bold values indicate different means ($P < 0.05$).

B treatments ($p < 0.0001$; Fig. 3A). Although thrips herbivory induced both JA and JA-Ile ($p = 0.01$ and $p = 0.001$), JA-Ile induction was higher in leaves of cv. Williams than in cv. Charata ($p = 0.018$; Fig. 3BE). *Cis*-OPDA was also induced by herbivory in both cultivars and treatments ($p < 0.0001$; Fig. 3). Neither ABA nor ET was increased by herbivory in soybean leaves (Fig. 3).

4. Discussion

Solar UV-B radiation not only increases plant resistant to herbivory by inducing chemical defenses in soybean, but also by promoting UV-B-avoidance behavior in some phytophagous insects, as documented for the thrips *C. phaseoli* (Mazza et al., 1999, 2002; Dillon et al., 2018).

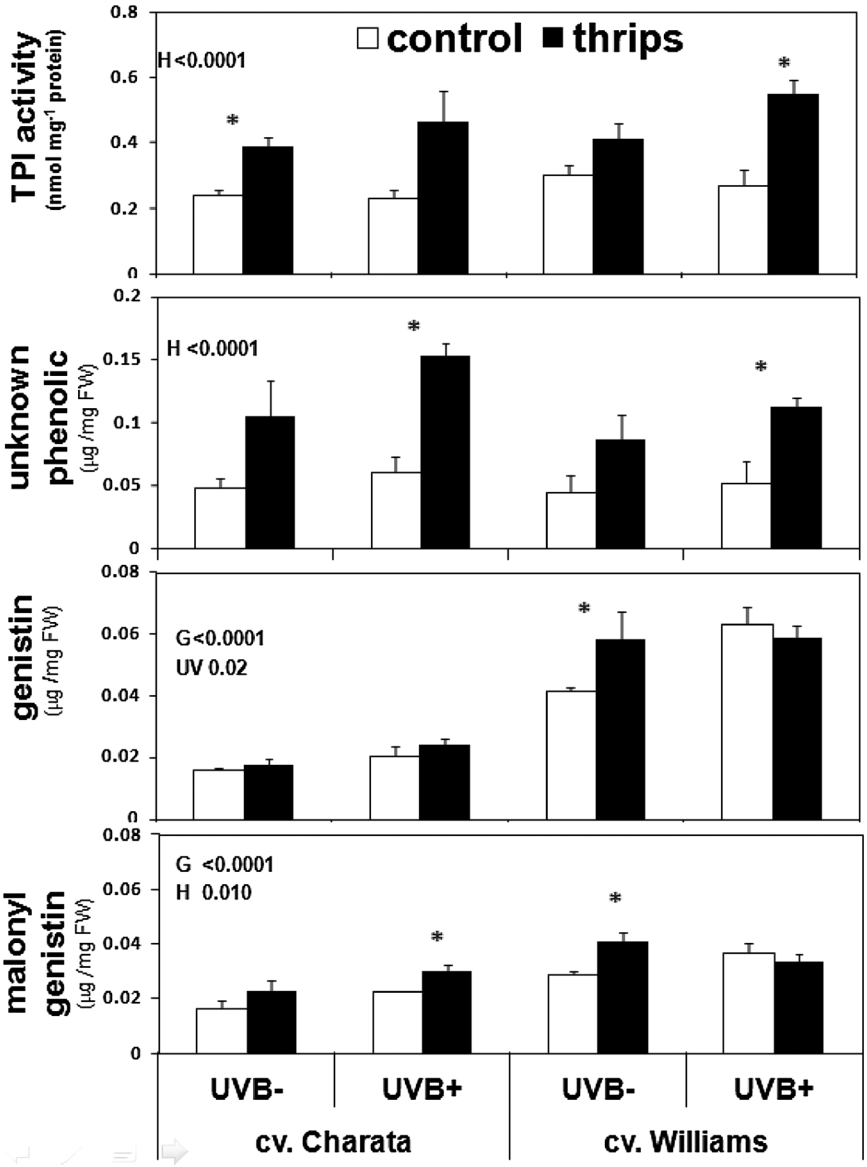

Fig. 2. Trypsin protease inhibitor (TPI) activity (A), unknown phenolic compound (B), genistin (C), and malonyl genistin (D) concentrations in undamaged leaves (from plants without thrips; white bars) and damaged leaves (black bars) after 6 days of treatment with *Caliothrips phaseoli* adults on two soybean cultivars (cv. Charata; cv. Williams) grown under either solar or attenuated UV-B radiation (UVB-; UVB+). *P* values of significant effects of three way ANOVA are shown (G: genotype; H: herbivory; UV-B radiation and interaction terms). Different means are represented with different letters (Tukey Test $p < 0.05$). Asterisks indicate significant differences ($p < 0.05$) between control and thrips treated leaf (one-way ANOVA). Values are means and error bars represent SEM (n = 3 or 4).

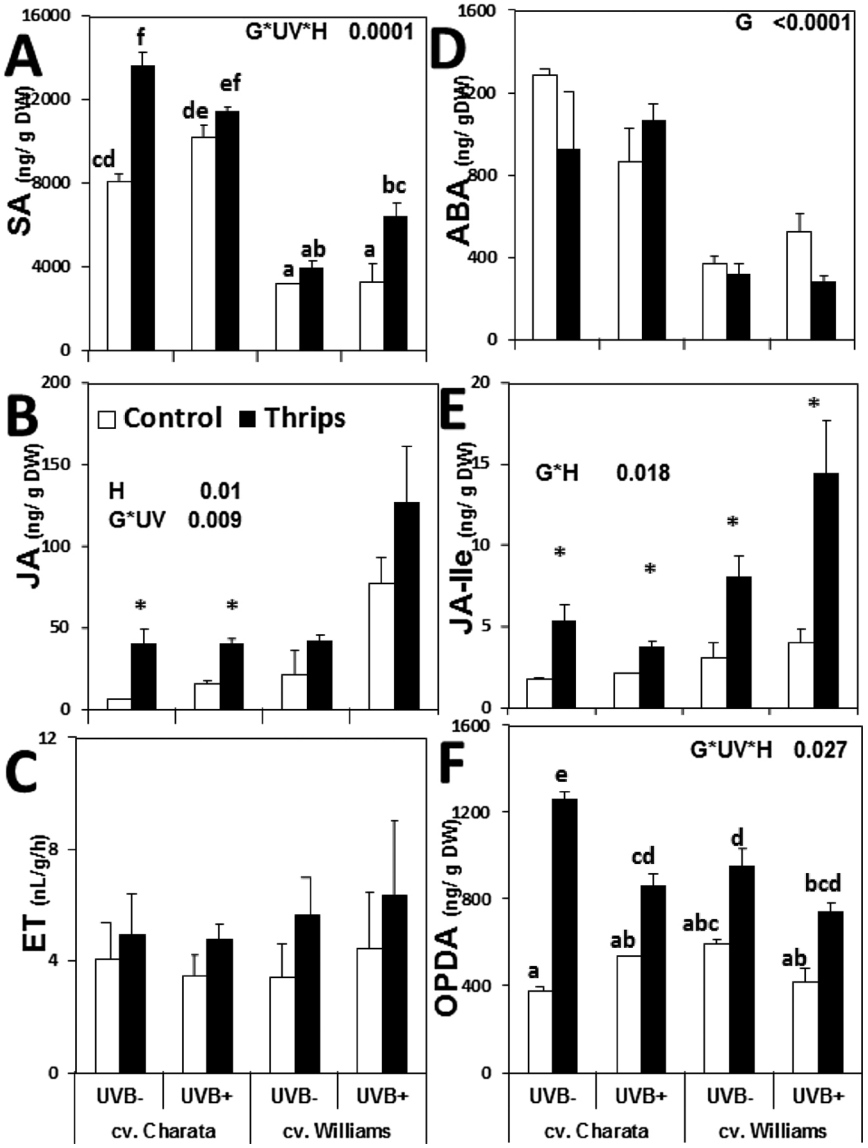

Fig. 3. Salicylic acid (SA; A), jasmonic acid (JA; B), ethylene (ET; C), abscisic acid (ABA; D), jasmonic isoleucine (JA-Ile; E), and oxo-phytodienoic acid (OPDA; F) concentrations in undamaged leaves (from plants without thrips; white bars) and damaged leaves (black bars) after 6 days of treatment with *Caliothrips phaseoli* adults on two soybean cultivars (cv. Charata; cv. Williams) grown under either solar or attenuated UV-B radiation (UVB-; UVB+). *P* values of significant effects of three way ANOVA are shown (G: genotype; H: herbivory; UV-B radiation and interaction terms). Different means are represented with different letters (Tukey Test $p < 0.05$). Asterisks indicate significant differences ($p < 0.05$) between control and thrips treated leaf (one-way ANOVA). Values are means and error bars represent SEM (n = 4).

Unexpectedly, our field experiments showed similar survivorship levels of thrips (*C. phaseoli*) that fed on foliage grown under either attenuated or solar UV-B radiation, while survivorship of thrips that fed on cv. Williams was lower than those that fed on cv. Charata (Fig. 1). Although the cultivars used in the experiments are from different maturity group, our previous studies and field experience working with these cultivars indicate that the differences found in our study were not affected by soybean stage of development (e.g., Mazza et al., 2000; Zavala et al., 2015; Giacometti et al., 2016; Dillon et al., 2017, 2018). Chemical analysis of foliage showed that cv. Williams produced different flavonol glycosides (QT3 and QD1 + QD2), and more genistein derivatives than cv. Charata (Table 1 and Fig. 2). The increment of the JA-regulated TPIs against insects in foliage of cv Williams was associated with the induction of JA and JA-Ile after herbivory and solar UV-B exposure (Fig. 3). In addition, herbivory induced SA and *cis*-OPDA concentrations in foliage grown under either attenuated or solar UV-B radiation (Fig. 3). To our knowledge no study before has showed a complete profile of defensive hormones induced by thrips feeding and demonstrated that herbivory not only induce JA and JA-Ile in soybean foliage, but also *cis*-OPDA and defenses, such as TPI, genistin and an unknown phenolic compound

Whereas plant responses to leaf-chewing insects and to phloem piercing–sucking feeders are well characterized (e.g., Leitner et al., 2005; Giacometti et al., 2016; Zavala et al., 2008), much less is known regarding cell-content feeders. Thrips insert their stylets into plant tissue and ingest cell content while feeding, inducing defensive plant responses (Kindt et al., 2003; De Vos et al., 2005). Previous studies have shown that thrips feeding induces the JA pathway (reviewed in Steenbergen et al., 2018). In our study, thrips damage not only induced JA, but also cis-OPDA accumulated in the foliage of field-grown soybean (Fig. 3). Similarly, necrotrophic pathogens or the cell-content feeder root-knot nematodes induced both JA/JA-Ile and *cis*-OPDA in attacked plants. *Cis*-OPDA played a key role in regulating tolerance to the root-knot nematode *Meiloidgyne hapla* in *Arabidopsis*, and adding exogenous MeJA induced resistance against this nematode (Gleason et al., 2016). Binding of *cis*-OPDA to cyclophilin 20-3 regulates cellular redox homeostasis in response to stress and induces gene expression during times of stress (Park et al., 2013). The high induction of *cis*-OPDA and in part SA may be a consequence of the oxidative stress generated by thrips damage (Fig. 4). Moreover, silencing the *cis*-OPDA reductase gene (*opr3*) in tomato demonstrated the participation of *cis*-OPDA in callose deposition as response to the necrotrophic pathogen *Botrytis cinerea* (Scalschi et al., 2015). Since *cis*-OPDA is a precursor of JA pathway, this hormone can also be involved in increasing defenses against insects.

The phytohormone jasmonic acid (JA) is considered a key player in

the defense regulatory network effective against thrips. While exogenous application of JA reduced plant susceptibility towards thrips herbivory, plants deficient in JA accumulation were more susceptible to thrips attack (Steenbergen et al., 2018). Moreover, thrips feeding induced JA pathway in *Arabidopsis* and soybean grown in pots under artificial light conditions (De Vos et al., 2005; Abe et al., 2008, 2009; Selig et al., 2016). Similarly in our study, JA and its active form JA-Ile increased in foliage of field-grown soybean after thrips feeding together with TPI, genistin and an unknown phenolic compound (Figs. 2 and 3). Overexpressing multidomain PIs in potato improved resistance to the thrips *F. occidentalis* (Outchkourov et al., 2004), suggesting the role of PIs as possible defense against thrips. In our field experiments the soybean cultivar (Williams) with high constitutive and inducible levels of genistin and TPI reduced more thrips survivorship than the less defended cultivar (Charata) (Figs. 1 and 2). Although cv Williams produced flavonol glycosides (QT3 and QD1 + QD2) absent in cv Charata that may have decreased thrips survivorship, our results showed that a 6-day thrips feeding induced the JA-regulated defense TPI and an unknown phenolic compound in soybean (Table 1 and Fig. 2). Furthermore, our field experiments suggest that while JA/JA-Ile plays a key role in regulating soybean defenses against thrips, cis-OPDA accumulated in the attacked tissue. In addition, although ET can be induced by trips damage and act synergistically with JA to induce plant defenses (Abe et al., 2008; Escobar-Bravo et al., 2017), in this study ET showed no response to thrips damage after 24 h of continuous damage (Fig. 3).

Plants grown in open-field conditions benefit from the effects of solar UV-B radiation, by increasing their productivity and resistance against insects, including soybean crops (Zavala et al., 2001; Zavala and Botto, 2002; Zavala et al., 2015; Dillon et al., 2018). Thrips perceived and avoided UV-B radiation, and also preferred to feed on soybean organs not exposed to UV-B (Mazza et al., 1999). However, our field experiments suggest that soybean cultivar traits decreased thrips survivorship rather than defenses induced by solar UV-B radiation (Figs. 1,2, and Table 1). Interestingly, while cell-content feeders, such as thrips induced *cis*-OPDA independent of UV-B environment (Fig. 3), soybean defenses against leaf-chewing caterpillars were regulated by JA/JA-Ile and ET, and modulated by UV-B (Dillon et al., 2018). Since emission of volatiles organic compound are a signal for herbivores and are regulated by jasmonates, there is a possibility that jasmonates induction by solar UV-B radiation produce quantitative and qualitative changes in volatiles emission and affect thrips feeding preference. Although some studies have suggested the role of constitutive defenses against thrips, functional analyses of mechanisms that regulate inducible defensive compounds against thrips damage in field conditions are lacking. In this study we identified TPI and genistin induction after thrips feeding, which can be a potential defense of field-grown soybean against thrips. Recently, a comparative transcriptomic analysis of susceptible and resistance alfalfa genotypes showed that thrips feeding induced gene expression of flavonoid biosynthesis pathway (Tu et al., 2018). Exploiting natural environmental conditions to improve own defense mechanisms of plants against insect herbivores will reduce insecticide application and minimize the effects on non-target organisms.

5. Conclusions

Thrips damage in field-grown soybean strongly induced jasmonates and to a minor extent salicylic acid, together with genistin and TPI activity. This is the first study that profiles all the defensive phytohormones in response to thrips damage associating them with chemical defenses such as TPIs and flavonoids. It also has the added value of being performed in field conditions under contrasting levels of solar UV-B radiation.

Contributions

FMD and JAZ contributed to the conception and design of the study, the analysis and interpretation of data and drafting the manuscript; FMD, HDC, MR and AM contributed to acquisition of data; FMD, HDC, AM, and JAZ contributed in revising the article critically for important intellectual content and for final approval of the version. Jorge Zavala and Francisco Dillon take responsibility for the integrity of the work as a whole, from inception to finished article.

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