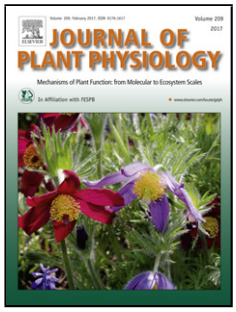

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Local and systemic hormonal responses in pepper (*Capsicum annuum* L.) leaves under green peach aphid (*Myzus persicae* Sulzer) infestation

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Abstract

This study examined the temporal changes in the leaf content of defence-involved phytohormones in pepper (*Capsicum annuum* L.) plants responding to the green peach aphid (*Myzus persicae* Sulzer) infestation, at both local and systemic level. Aphid infestation did not alter the content of cis-12-oxo-phytodienoic acid, the jasmonic acid (JA) precursor, even though endogenous levels of JA and its bioactive isoleucine-conjugated form (JA-Ile) significantly increased from 8 to 96 hours in local infested leaves. Systemic effects in jasmonates were only showed at 48 hours for JA, and 8 and 48 hours in the case of JA-Ile. SA accumulated only in local infested leaves after 96 hours of infestation, when the level of JA-Ile decreased in these leaves. This suggests a possible antagonistic interaction between JA and SA pathways, although other pathways may be also involved. Endogenous level of indole-3-acetic acid was higher in systemic relative to local infested leaves at 3 and 24 hours, although no significant changes in its content were found compared to control leaves. Abscisic acid content was lower in local infested relative to control leaves at 24 hours, but was higher at 48 hours when it also increased systemically. The possible roles of the studied phytohormones in plant defence responses against aphids are discussed.

Abbreviations

ABA, abscisic acid; OPDA, *cis*-12-oxo-phytodienoic acid; hpi, hours post-infestation; IAA, indole-3-acetic acid; JA, jasmonic acid; JA-Ile, jasmonoyl-L-isoleucine; MeJA, methyl jasmonate; MeSA, methyl salicylate; SA, salicylic acid

Keywords
Aphid-plant interaction; UHPLC-MS/MS; plant defence; biotic stress, jasmonates, salicylate, indol-3-acetic acid, abscisic acid

1. Introduction

Although some of the interactions between plants and insects (e. g. pollination) existing in nature are mutually beneficial, a great majority of them involve predation of plant parts and the subsequent reaction of plants to defend themselves (Gatehouse, 2002). Plants have accordingly evolved multiple and highly sophisticated defence systems to cope with herbivore challenges. They can dramatically reshape their transcriptomes, proteomes, and metabolomes in response to herbivory to produce toxins and defensive proteins, which directly target physiological processes in the insect, and also emit volatiles that attract herbivore natural enemies and bolster resistance to future threat (Howe and Jander, 2008; Wu and Baldwin, 2010). These herbivory-induced changes are initiated by the recognition of elicitors contained in insect oral secretions and by signals released from injured plant cells (Bonaventure, 2012; Hogenhout and Bos, 2011) and later mediated by elaborated signalling networks, including calcium influxes, kinase cascades, reactive oxygen species, and phytohormone signalling pathways. Moreover, the defence reaction is activated not only in the wounded region but also in undamaged regions in the attacked leaves and in distal intact (systemic) leaves (Howe and Jander, 2008; Morkunas et al. 2011; Pieterse et al., 2012; Wu and Baldwin, 2010).

The signalling molecules salicylic acid (SA) and jasmonic acid (JA) are known to play, besides ethylene, major roles in regulating plant defence responses against various insects, pathogens and abiotic stresses. It is generally assumed that SA signalling is primarily induced by and involved in defence against biotrophic and hemi-biotrophic pathogens, whereas JA signalling primarily participates in defence against mechanical wounding and insect herbivores and, in conjunction with ethylene, against necrotrophic pathogens (Howe and Jander, 2008; Morkunas et al., 2011; Robert-Seilaniantz et al., 2011; Wu and Baldwin, 2010). However, plant responses to insect herbivores are complex and strongly correlate with their feeding mode and the degree of damage caused at the feeding site (Walling, 2000). Regarding the feeding mode, aphids -the

largest group of phloem-feeding insects- are probably one of the most challenging herbivores. They are the most prevalent vectors of plant viruses and also damage crops by depleting photoassimilates and manipulating growth and nutrient partitioning (Thomson and Goggin, 2006), thus becoming one of the main actual threads to agricultural crops.

Aphids penetrate plant tissue with their stylet primarily via the apoplastic route to establish feeding sites in the phloem sieve elements (Morkunas et al., 2011; Smith and Boyko, 2007). Given the limited tissue damage and the prolonged stylet interactions with plant cells, plant responses to phloem feeders are distinct from that of chewing insects, eliciting responses in a more similar way to pathogen-elicited defences. In fact, early studies suggested that aphids mainly induced the SA signalling pathway, which largely controls plant defences against pathogens (Fidantsef et al., 1999; Moran and Thompson, 2001; Walling, 2000). More recent studies, however, have shown the involvement of both SA and JA/ethylene signalling pathways in response to aphids, suggesting that the role of SA and JA pathways may vary according to specific aphid-plant interactions (Coppola et al., 2013; Gao et al., 2008; Kuśnierczyk et al., 2008; Martinez de Ilarduya, 2003; Smith et al., 2010; Studham and Macintosh, 2013; Zhu-Salzman et al., 2004). Although transcriptomic studies provide useful information about signalling networks, the identification and quantification of metabolites or end products of metabolic pathways undoubtedly give a clearer understanding of their role in defence responses (Fernie and Stitt, 2012). However, comprehensive analyses of the temporal dynamics of both phytohormones in response to aphid infestation are still scarce (Mai et al., 2014; Stewart et al., 2016).

Some studies have targeted the role of JA and SA signalling as effective defences against aphids by carrying out fitness experiments. Activation of JA signalling correlated with enhanced resistance to aphids (Ellis et al., 2002; Gao et al., 2007; Kuśnierczyk et al., 2011; Mewis et al., 2005) and exogenous application of methyl JA (MeJA) significantly reduced aphid infestation (Ellis et al., 2002; Gao et al., 2007; Zhu-Salzman et al., 2004). On the other hand, the role of SA in plant resistance to aphids is not so straightforward. Whereas the induction of SA-dependent responses did not contribute to resistance in *Arabidopsis* and wheat (Moran and Thompson 2001; Mewis et al., 2005; Smith et al., 2010) in tomato the SA signalling pathway played a role in resistance to aphids (Li et al., 2006; Thaler et al., 2010). Finally, SA treatment had significant increase in aphid resistance in resistant but not in susceptible soybean plants (Studham and Macintosh, 2013) whereas the exogenous application of the SA analogue benzothiadiazole led to reduction in aphid population in wild-type *Arabidopsis* plants as well as in mutants deficient in responsiveness to SA (Moran and Thompson, 2001).

Other studies have shown the implication of different phytohormones in the responses to herbivores modulating the SA-JA backbone of the plant immune signalling network. These hormones include ethylene (Lu et al., 2014; Mantelin et al., 2009; Paudel and Bede, 2015), abscisic acid (ABA; Hillwig et al., 2016; Schaeffer et al., 2018; Studham and Macintosh, 2013), brassinosteroids (Coppola et al., 2013; Campos et al., 2009), gibberellins (Machado et al., 2017; Park et al., 2006), auxins (Machado et al., 2013; 2016; Park et al., 2006), cytokinins (Gilardoni et al., 2010; Hui et al., 2003), peptide hormones (Ren and Lu, 2006) and reactive oxygen species (mainly hydrogen peroxide and nitric oxide; Kuśnierczyk et al., 2008; Mai et al., 2014; Smith et al., 2010). These molecules can act separately or together with antagonistic or synergistic interactions, and the crosstalk between their corresponding signalling pathways may allow plants to choose an optimum defence strategy depending on the type of herbivore (Morkunas et al., 2011; Pieterse et al., 2012, Robert-Seilaniantz et al., 2011).

Given the complexity of plants responses to aphids, it has been pointed out that some of the differences found between plant-aphid systems may be attributed to a variety of experimental factors, with emphasis on aphid density, duration of challenge and collection of locally infested or systemic tissue. It reinforces the need for consideration of these experimental factors when trying to formulate general patterns in plant–aphid interactions (Erb et al., 2012; Stewart et al., 2016; Thompson and Goggin, 2006).

In this context, the present study was aimed to determine the content of defence-involved phytohormones in leaves of pepper (*Capsicum annuum* L.) plants in response to green peach aphid (*Myzus persicae* Sulzer) infestation. We included a detailed time course experiment and the phytohormone analysis was evaluated at both local and systemic level. Our goal therefore was to gain an integrated view on the spatial and temporal dynamics of phytohormone responses in plant-aphid interactions in a non-model plant species, thus extending the current understanding of the complex hormone crosstalk underlying.

2. Materials and methods

2.1. Plant material and aphid culture

Capsicum annuum var. California Wonder seeds (Ramiro Arnedo S.A, Murcia, Spain) were germinated in plastic pots with a 1:1 mixture of peat (Prohumin potting soil, Projar S.A., Valencia, Spain) and vermiculite. Plants were watered three times a week and maintained in a growth chamber under a 16:8 h photoperiod (day/night), 24°C, and 70% relative humidity. Plants were grown for five weeks and used in the experiments before flowering. The green peach aphid *Myzus persicae* Sulzer was derived from a population on greenhouse-grown sweet pepper plants close to Pilar de la Horadada (Alicante), Spain.

Once in the laboratory, aphids were raised on pepper plants under the conditions mentioned above to obtain the aphid stock culture.

2.2. Local and systemic response of plants to aphid infestation

Aphid infestation was made by placing 20 wingless adult aphids on the abaxial surface of a single leaf at the second true leaf pair of leaves. In order to distinguish among local and systemic responses, the aphids were confined into two clip cages (BioQuip Products, Inc. USA) attached to opposite sides of the leaf and clipped together with an angle-shaped staple (Figure 1). These leaves were analysed for local response while the opposite leaf at the same pair received an empty clip cage and were used to evaluate the systemic response. Phytohormone content was evaluated at 3, 8, 24, 48 and 96 h post-infestation (hpi). Uninfested plants also receiving empty clip cages during the same time as aphid-infested plants were used as controls. Photoperiod, temperature and relative humidity in the growth chamber throughout the experiment were the same as indicated above.

Because plants were infested sequentially (the longer times of infestation first), all plant tissue for phytohormone analysis was harvested at the same time (96 h after the start of the experiment, coinciding with the half of the light phase of photoperiod). Aphids were brushed off from local infested leaves and the leaf discs under the cages cut for all treatments (local, systemic and control leaves). Leaf discs were flash frozen in liquid nitrogen and stored at −80ºC prior to freeze-drying. Lyophilized tissue was ground and stored at 4ºC into airtight vials until extraction. Five biological replicates, each consisting in two leaf discs from two plants pooled together, were carried out for each time point and treatment.

2.3. Phytohormone extraction and UHPLC-MS/MD analysis

Sample preparation and analysis was done according to Floková et al. (2014). For quantification, 3 mg (dry weight, DW) were used for each sample. Each biological replicate was extracted and analysed twice, giving two technical replicates, and the results of both were averaged. Six phytohormones were analyzed, including IAA, ABA, SA, and jasmonates (OPDA, JA and JA-Ile). Briefly, the phytohormones were extracted using an aqueous solution of methanol (10% MeOH/H_2O, v/v). A cocktail of stable isotope-labelled standards consisting of: 5 pmol of [$^{13}C_6$]IAA, 10 pmol of [$^2H_6$]JA, [$^2H_2$]JA-Ile, and [$^2H_6$]ABA, 20 pmol of [$^2H_4$]SA and [$^2H_5$]OPDA (all from Olchemim Ltd, Czech Republic) was added to each sample. The extracts were purified using Oasis® HLB columns (30 mg/1 mL, Waters) and the analytes eluted using 80% MeOH. After gently evaporation of MeOH under nitrogen stream, the level of defence-involved phytohormones were determined by ultra-high performance liquid chromatography-electrospray tandem mass spectrometry (UHPLC-MS/MS) using stable isotope-labelled internal standards as a reference. Separation was performed on an Acquity UPLC® System (Waters, Milford, MA, USA) equipped

with an Acquity UPLC BEH C18 column (100 x 2.1 mm, 1.7 µm; Waters), and the effluent was introduced into the electrospray ion source of a triple quadrupole mass spectrometer Xevo™ TQ-S MS (Waters, Milford, MA, USA).

2.4. Statistical analysis

The statistical analysis to reveal significant differences was based on Student's T-test, with each time point being analyzed separately. The significance between control and local leaves and between control and systemic leaves was determined by an unpaired T-test, whereas between local and systemic leaves was determined by a paired T-test. In all cases, differences were considered to be significant at $P < 0.05$. SPSS software version 15.0 was used for these analyses.

3. Results

3.1. Effect of aphid infestation on jasmonates (OPDA, JA and JA-Ile) content

The presence of aphids did not alter OPDA content, the JA precursor, even though JA and JA-Ile contents significantly increased in aphid-infested plants (Figure 2). JA and JA-Ile accumulation was mainly restricted to local leaves. JA concentration increased from 8 hpi onwards 2.6 to 3.6-fold in local leaves compared to control leaves but systemic effects were only shown at 48 hpi. Endogenous levels of JA-Ile, the active form of JA, presented a pattern similar to JA, being significantly higher in local leaves compared to control leaves, from 8 hpi onwards. JA-Ile concentration peaked in local leaves at 8 hpi, being increased 6.3-fold compared to control leaves. Systemic effects were shown at 8 and 48 hpi, although endogenous content in systemic leaves was significantly lower than in local leaves.

3.2. Effect of aphid infestation on SA content

SA levels of pepper leaves (Figure 3) remained unaltered as a consequence of aphid infestation until 48 hpi when, although levels in infested plants were similar to control plants, local leaves presented a slightly higher (1.2-fold) SA content than systemic leaves. At 96 hpi, SA content increased 1.6-fold in local leaves compared to control leaves, whereas the content in systemic leaves remained unaltered.

3.3. Effect of aphid infestation on IAA content

Endogenous levels of IAA (Figure 4) in local leaves did not change significantly in response to aphid feeding relative to control leaves. However, systemic leaves presented a higher (1.3-fold) IAA content than local leaves at 3 hpi and 24 hpi.

3.4. Effect of aphid infestation on ABA content

ABA content (Figure 4) was lower (0.8-fold) in local compared to control leaves at 24 hpi, but was higher at 48 hpi (1.5-fold) when it also increased in systemic leaves (1.6-fold). Non-significant differences were found in ABA content between systemic and local leaves throughout the complete period under study.

4. Discussion

Although aphids try to minimize tissue damage during feeding moving their stylets between the cells, probing often results in the disruption of cell wall and membrane integrity, either by the salivary enzymes (e. g. pectinases, pectinmethylesterases, polygalacturonases and cellulases) introduced through the penetrating stylet or simply by mechanical damage following the incursion (Giordanengo et al., 2010; Morkunas et al., 2011; Thompson and Goggin, 2006). This damage is likely to be the first factor that triggers the plant response (Morkunas et al., 2011; Figure 5).

In the present study the “wound hormone” JA, which largely controls plant defences against chewing herbivores (Howe and Jander, 2008; Lortzing and Steppuhn, 2016), accumulated relatively fast (between 3 and 8 hpi) and locally in pepper leaves as a consequence of aphid infestation. This accumulation was moderate (from 2.6 to 3.6-fold in local-infested leaves) and considerably lower to the JA burst commonly induced by chewing insects (Diezel et al., 2009; Stork et al., 2009; Tscharntke et al., 2001; von Dahl and Baldwin, 2004). However, the JA accumulation obtained in this study is similar to the values reported so far in other aphid-plant systems: *M. persicae* in *Solanum tuberosum* (Gosset et al., 2009), and *Acyrthosiphon pisum* in *Medicago truncatula* (Stewart et al., 2016) or *Pisum sativum* (Mai et al., 2014). Interestingly, a positive correlation between the JA content and the production of direct (Baldwin et al., 1997) and indirect (Schmelz et al., 2003) defences against chewing herbivores has been reported. The prevention of the JA burst may partially explain why aphids are so successful colonising and establishing long feeding periods on plants.

In addition to JA, other relevant components of the JA metabolic pathway including OPDA and JA-Ile, can also act like signalling molecules (Wasternack and Strnad, 2016; Figure 5). We have shown that the endogenous levels of the bioactive JA-Ile, the molecule responsible for the activation of the majority of JA-induced molecular responses (Staswick and Tiryaki, 2004), were also increased in local leaves from 8 hpi onwards and to a greater extent than JA (6.3-fold at 8hpi). Interestingly, the physical interaction of COI1 and JAZ1, which results in the degradation of JAZ proteins and transcription of jasmonate-responsive genes, is stimulated in a dose-dependent manner by JA-Ile (Thines et al., 2007). Conversely, we did not observe significant changes in OPDA content of pepper leaves as a consequence of aphid infestation. OPDA has been shown to increase after aphid attack (Gosset et al., 2009) and is involved in stimulation of plant defence responses to piercing-sucking insects (Guo et al.,

2014). However, a conjugated form of OPDA with isoleucine (OPDA-Ile) has been described (Floková et al., 2016) and data suggest that OPDA specific responses might be mediated upon formation of OPDA-Ile (Arnold et al., 2016), which was not analysed in the present study.

Both, systemic and local effects of aphid feeding on plant chemical induction have been described (reviewed in Moran et al., 2002). Also, prior aphid feeding caused an increase in aphid resistance in a later infestation at systemic level in *Medicago truncatula* (Klingler et al., 2005) but only in local tissue in *Arabidopsis* (De Vos and Jander, 2009) and potato (Dugravot et al., 2007). Although different plant species may have distinct mechanisms to activate systemic responses to insect herbivores, the JA pathway seems to be necessary (reviewed in Wu and Baldwin, 2010). Accordingly, in the present study we observed a transient systemic accumulation of JA and JA-Ile in pepper leaves in response to aphid attack at specific times post-infestation, whereas SA accumulation resulted to be only a local response (Figure 5).

The SA-mediated pathways typically activated in response to pathogens, promote the development of systemic acquired resistance and are crucial for localized plant tissue hypersensitive responses (Morkunas et al., 2011; Smith and Boyko, 2007). Increased SA level has been suggested to be a critical step in the signalling of down-stream defence responses of plants to aphid infestation (Mohase and Van der Westhuizen, 2002). Comparative studies of resistant and susceptible hosts have shown that a faster and stronger induction of SA-responsive genes occurs in resistant cultivars after aphid attack (Gao et al., 2008; Martinez de Ilarduya, 2003; Mohase and Van der Westhuizen, 2002; Studham and Macintosh 2013). Moreover, MeSA has been reported as a strong aphid repellent that may deter aphids from settling on plants with already high aphid densities (Morkunas et al., 2011) and also attracts different aphid predators (Salamanca et al., 2017). It is worth noting that the local aphid-induced SA accumulation described in the present study occurred only late after infestation (at 96 hpi), coinciding with the results found in other plant-aphid interactions (Mai et al., 2014; Mohase and Van der Westhuizen, 2002; Stewart et al., 2016). One possible explanation for this delayed increase in SA can be found in an antagonistic JA-SA crosstalk (Beckers and Spoel, 2006; Thaler et al., 2012), in which the increased levels of endogenous jasmonates suppressed early production of SA. This JA-SA crosstalk has been also suggested to occur in pepper plants in response to pathogen infection (Ueeda et al. 2006) and is commonly reported in plant-insect interactions (Kroes et al., 2015, Schaeffer et al., 2018; Schweiger et al., 2014; Figure 5). However, the JA-SA interaction seems to be dependent upon the concentration of each hormone and the relative timing of induction (Pieterse et al., 2012; Schweiger et al., 2014; Thompson and Goggin, 2006) and also the possibility that other signalling pathways are involved in the observed responses has to be considered. The

late aphid-induced SA accumulation can be interpreted as well as part of the aphid "decoy" strategy (Thompson and Goggin, 2006) to suppress the more biologically effective JA pathway in the host plant (Kuśnierczyk et al., 2008; Schwartzberg and Tumlinson, 2014; Zhu-Salzman et al., 2004). Increasing evidences accumulated so far suggest that the salivary enzymes that aphids continuously inject during probing and feeding serve mainly to divert or counter responses at the immediate interface between the stylet and plant tissues (Giordanengo et al., 2010; Morkunas et al. 2011). To this respect, a glucose-oxidase has been detected in aphid saliva, which oxidizes D-glucose releasing H_2O_2 and may stimulate SA accumulation (Giordanengo et al., 2010). Also in line with this hypothesis, aphid honeydew has been reported to contain SA and its exogenous application to the leaves induced SA and suppressed JA accumulation (Schwartzberg and Tumlinson, 2014). Moreover, some evidences have highlighted the role of aphid endosymbionts as mediators in the plant-aphid interaction, given that both aphid saliva (Chaudhary et al., 2014) and honeydew (Sabri et al., 2013) contain bacterial proteins. The detection of these bacterial proteins by the plant may trigger the activation of the SA signalling pathway in response to aphid infestation.

Auxin can regulate plant defence responses independently of SA and JA, demonstrating that auxin homeostasis is important in determining plant tolerance against insect herbivory (Erb et al., 2012; Robert-Seilaniantz et al., 2011; Walters, 2015). We did not detect changes in IAA levels as a consequence of aphid infestation compared to control plants throughout the complete experiment. In tobacco plants attacked by *Manduca sexta* IAA strongly accumulated in the first minutes of the interaction preceding the JA burst (Machado et al., 2016). Despite the distinct feeding habits between chewing larvae and the phloem-feeder aphid, which undoubtedly determine the plant responses, we cannot discard the possibility that in our study IAA peaked earlier than 3 hpi. Conversely, the absence of an auxin response in our study could be also part of the plant defence response. Inhibition of auxin signalling is part of the SA-mediated plant defence against biotrophic pathogens (reviewed in Robert-Seilaniantz et al., 2011 and Walters, 2015). Increasing evidences also links an auxin signalling disruption with an enhanced aphid resistance. *Diuraphis noxia* infestation caused an upregulation of genes related to the auxin pathway in susceptible varieties of wheat (Smith et al., 2010) and barley (Marimuthu and Smith, 2010) but not in the corresponding resistant varieties. More recently, miRNA-mediated auxin signalling repression has been reported to occur during Vat-mediated aphid resistance in melon (Sattar et al., 2016). Interestingly, we observed a decrease in IAA content at 3 and 24hpi in local infested leaves compared to systemic infested leaves that may be attributed to IAA transport from local to systemic leaves or to a local suppression of IAA synthesis as a consequence of the higher jasmonates levels in local leaves. In

tobacco leaves, JA negatively regulates wound induced decreases in auxin content (Erb et al., 2012; Figure 5). .

The role of ABA in plant-aphid interactions is still controversial. Some transcriptional studies have shown a strong induction of ABA-related genes (Kerchev et al., 2013; Studham and Macintosh, 2013; Zhu-Salzman et al., 2004), suggesting that ABA is part of a common response to aphid feeding. It was proposed that ABA signalling is induced as a consequence of the water stress associated with aphid infestation (Zhu-Salzman et al., 2004); however, infiltration of aphid saliva by itself induces characteristic expression of ABA-regulated genes (De Vos and Jander, 2009). There is increasing evidence that ABA regulates plant defences responses through effects on callose deposition, production of reactive oxygen species and regulation of defence gene expression (Walters, 2015). Conversely, it has been suggested that the induction of the ABA pathway may be part of the decoy strategy implemented by the aphid to suppress effective salicylic acid- and jasmonate-related defences, exploiting the phytohormone crosstalk (Hillwig et al. 2016; Studham and Macintosh, 2013). Consistent with this hypothesis, in soybean plants an important increase in the expression of ABA biosynthetic and signalling transcripts occurred during the later stages of aphid colonization, coinciding with the suppression of JA responses (Studham and Macintosh, 2013). Moreover, *Arabidopsis* mutants that are defective in ABA signalling are more resistant to aphids (Kerchev et al.,2013; Hillwig et al., 2015) and aphids show a significant preference for wild-type plants compared with the mutant (Hillwig et al. 2015). However, direct ABA quantification has revealed divergent results, with increase (Hillwig et al., 2015), decrease (Stewart et al., 2016) or no effect (Donovan et al., 2013) in ABA content depending on the specific plant-aphid interaction. In our study, we observed a local reduction in ABA levels at 24 hpi, but a local and systemic increase at 48 hpi, which may reflect the dynamic interaction between plant defence responses and aphid counter-defence (Figure 5).

In summary, this is to the best of our knowledge the first attempt to study the temporal and spatial responses of defence and growth-related phytohormones in pepper leaves against an insect herbivore, specifically aphids. Our findings demonstrate that both SA and JA pathways are involved in the molecular responses of *C. annuum* to the green peach aphid *M. persicae*. ABA and IAA also showed spatial-temporal changes which may contribute to the fine-tuning of the plant response. The time lag observed between JAs and SA accumulation in pepper leaves suggest an antagonistic interaction between both signalling pathways, although other pathways may be also implicated. Most of the observed responses were mainly produced at local level, which could be explained accordingly to the stealthy feeding style of aphids that may limit the induction of defence responses to a minimal number of cells.

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Figure captions

Figure 1. Experimental system used for aphid-infested plants (A) Pepper plant with local aphid-infested leaf and the opposite systemic leaf (B) Clip cage consisting in two plastazote foam rings (36.5 mm outside diameter, 25.4 mm inside diameter, and 9.5 mm thickness) covered by a screen and clipped together with an angle-shaped staple (C) Leaf disc collected for phytohormone analysis.

Figure 2. Effect of *Myzus persicae* infestation on local (L) and systemic (S) content of (A) OPDA, (B) JA and (C) JA-Ile in pepper leaves. Means of five biological replicates ± SD are shown. Groups not sharing a letter code were separated using T-test values at $P < 0.05$.

Figure 3. Effect of *Myzus persicae* infestation on local (L) and systemic (S) content of SA in pepper leaves. Means of five biological replicates ± SD are shown. Groups not sharing a letter code were separated using T-test values at $P < 0.05$.

Figure 4. Effect of *Myzus persicae* infestation on local (L) and systemic (S) content of (A) IAA and (B) ABA in pepper leaves. Means of five biological replicates ± SD are shown. Groups not sharing a letter code were separated using T-test values at $P < 0.05$.

Figure 5. A model summarizing the signalling molecules (OPDA, JA-Ile, JA, SA, ABA and IAA) modulated in *Capsicum annuum* leaves in response to *Myzus persicae* infestation, at local and systemic level. Green and red arrows indicate, respectively, the possible positive or negative interactions between the signalling molecules. The numbers in brackets correspond to the following references: [1] Giordanengo et al. (2010); [2] Morkunas et al. (2011); [3] Thompson and Goggin (2006); [4] Howe and Jander (2008); [5] Lortzing and Steppuhn (2016); [6] Schwartzberg and Tumlinson (2014); [7] Thaler et al. (2012); [8] Ueeda et al. (2006); [9] Kroes et al. (2015); [10] Schaeffer et al. (2018); [11] Schweiger et al. (2014); [12] Sattar et al. (2016); [13] Erb et al. (2012); [14] De Vos and Jander (2009); [15] Hillwig et al. (2016); [16] Studham and MacIntosh (2013) and [17] Wu and Baldwin (2010).