



Article

Jasmonic Acid and Salicylic Acid Levels in Defense Response of Azalea (*Rhododendron simsii* Hybrid) to Broad Mite (*Polyphagotarsonemus latus*)

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Abstract: The broad mite (*Polyphagotarsonemus latus* (Banks)) is an important pest in many crops, including azalea (*Rhododendron simsii* Planch. hybrid). Broad mites cause the malformation of shoot tips, leaves and flowers in azalea. It is known that the jasmonic acid (JA)- and salicylic acid (SA)-dependent signaling pathways are related to the presence of herbivorous mites. Here, we describe the levels of the two main plant defense-related hormones, SA and JA, in mite-infested plants. The plant hormones were analyzed using liquid chromatography combined with tandem mass spectrometry (LC-MS/MS). We studied both short-term hormonal responses under controlled conditions with artificial inoculation, and long-term responses under culture conditions with natural infestation. The long-term development of broad mite populations and hormone response were studied during two subsequent growing seasons on 3 and 18 different cultivars, respectively. During the experiments on 18 azalea cultivars under natural infestation, the presence of different species of tarsonemid mites was also examined. JA concentrations only showed variation in the early phase of infestation. Subsequently, the SA levels increased significantly for all the cultivars where broad mites were detected. Based on the observed timing of the defense responses, we suggest that the interaction of the JA and SA pathways as a defense response for pot azalea against *P. latus* involves a primary plant response through the JA pathway. In the presence of the mites, the production of SA increased in the plants in a later phase as the *P. latus* population grew. Our results also show that the hormone response depends on type of mite. Changes in hormone levels were found upon infestation with *P. latus*, but not in the presence of another frequently occurring tarsonemid mite, *Tarsonemus confusus* Ewing.

Keywords: defense suppression; LC-MS/MS; mite defense; mite resistance; plant defense; *Tarsonemus confusus*



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1. Introduction

Pot azalea, commercially sold as a flowering indoor pot plant, is the main ornamental plant produced in Belgium. In Europe, the main producers of azalea and *Rhododendron* are Belgium and Germany, with export values of EUR 36 and 7 million, respectively. The modern pot azalea traces its roots to at least four species, the most important being *Rhododendron simsii* Planch. The introduction of *R. simsii* in Belgium dates back to 1818 [1]. Other parental species are *Rhododendron indicum* (L.) Sweet, *R. mucronatum* G. Don and *R. scabrum* G. Don [2,3]. The production area in Belgium is around 230 ha, and almost all companies are located around Ghent [4,5]. Pot azalea is propagated by cuttings and its

production cycle takes almost two years, combining greenhouse cultivation with outdoor-container field cultivation during summer.

The broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae), is an important pest on many commercial crops and this mite has been reported in most parts of the world. The first record in Europe dates back to 1961 when the broad mite was found in Italy [6]. Since then, broad mites have been found in other European countries, and in Belgium, it was reported on pot azalea in the mid-1980s [7]. Restrictions on the use of broad-spectrum pesticides are the main reason for the significant increase in the pest status of broad mites in pot azalea in recent years. The broad mite causes esthetic damage; on azalea, it is responsible for the bronzing, browning and curling of apical leaves, and can cause leaves to become brittle, rigid and stunted with the edges curled downwards. Flowers also become malformed and discolored. Broad mites are very small (<200 µm) and can only be detected using a microscope. A small number of 20 mites or less can cause serious economic damage in crops such as pepper [8,9], cucumber [10], lime [9] and chili [9,11]. Also in pot azalea, it has been shown that low mite numbers can lead to high damage rates [12]. Even after treatment, symptoms may continue to develop [13] and damage initiated in a plant progresses even when mites are no longer present. In his review on broad mites, Gerson [13] suggested that the symptoms are caused by toxins. This conclusion was based on the persistence of symptoms for some weeks post-treatment. These putative toxins would explain the appearance of injuries very shortly after the initiation of a mite attack, and the later occurrence of symptoms at a distance from the site of mite feeding.

Differences in susceptibility to broad mites have been observed in pot azalea, along with the damage rate, and in relation to the population sizes of *P. latus* on the tested azalea cultivars [12]. Trichomes (plant hairs) are potentially involved in broad mite resistance in pot azalea [14]. Additionally, in *Capsicum* species, trichomes are known to act as a limiting factor for the development of broad mites [15]. In plants, trichomes can act as a physical barrier and chemical repellent to insect or mite attacks [16]. In pot azalea, four types of trichome are described. All pot azaleas have long non-glandular trichomes; in addition, there are three types of short trichomes. They are thorn-like trichomes, short non-sticky trichomes and short trichomes with glandular ends (plants with these trichomes also have sticky leaves). There are indications that the latter are related to broad mite resistance. However, this is not the only discriminative trait, as in certain susceptible cultivars, trichomes with glandular ends were observed [17]. Recently it was shown that wounding or exogenous jasmonic acid (JA) treatments are able to promote trichome development and enhance insect resistance [16].

For decades, researchers have already known that plants have a defense network dependent on phytohormones, which translates stresses of biotic or abiotic origin into plant defense responses. Jasmonic acid (JA) and salicylic acid (SA) are known to serve as the primary signals of the plant's immune response. SA and JA work antagonistically and use other hormones to fine-tune immune responses built on SA and JA [18].

SA is most recognized as primarily being induced in the mediation of attacks by biotrophs [19], whereas JA is involved in defense against necrotrophic pathogens [20] and herbivores [21]. As reviewed by Pieterse et al. [18], well-known trade-offs exist between SA-dependent resistance and JA-dependent defense in disease and pest resistance. However, neutral and synergistic interactions have also been described [18]. Most studies focus on plant pathogens, but in recent years, a large number of studies have also examined plant defense responses for herbivores. For example, in a study on tomato and the two-spotted spider mite, different types of interaction have been described. These different interaction types, including direct and indirect defenses, were reviewed by Blaazer et al. [22]. Their review shows that both JA and SA are related to the presence of herbivorous mites. In brief, (1) the accumulation of SA and JA can be triggered in tomato as a plant response at the start of mite infestation; (2) the levels of both JA and SA can decrease as a result of defense suppression by the mites; and (3) intraspecific variation in natural mite populations,

especially in polyphagous mites, can have an effect on the plant–mite interaction. Not only is the herbivore–plant relationship involved, but so is the attraction of natural enemies by the emission of volatile compounds. Specifically, for *P. latus* in cucumber leaves, both the JA- and SA-dependent pathways are activated, and possibly an oxidative stress response [23]. To examine the possible involvement of the JA signaling pathway in the defense response against *P. latus*, [23] evaluated feeding on wild-type (WT) tomato ‘Castlemart’ and on *def-1*, a mutant impaired in JA biosynthesis. In the WT plants, broad mite feeding did not affect growth or leaf development, but in the *def-1* plants, 50–60% inhibition was observed. Mite populations developed only on *def-1* plants, as was shown by progeny counts. Furthermore, *P. latus* prefers JA-defective mutants, and therefore, is able to actively discriminate between resistant and susceptible plants [23,24]. Therefore, it was concluded by [23] that the JA pathway is likely to play an important role in the resistance response against *P. latus* [23].

Different JA and SA biosynthesis marker genes in azalea have been studied [25]. To obtain insight into the molecular mechanisms behind the interaction between *P. latus* and the *R. simsii* hybrid, the expression levels of genes involved in the infestation of leaves with broad mites were analyzed. The focus was on JA and it was shown that transcript levels of marker genes for the JA biosynthesis pathway were significantly induced upon *P. latus* infestation in the *R. simsii* hybrid.

In this research, we aimed to study the relationship between the presence of broad mites and plant defense hormone levels in pot azalea (*R. simsii* hybrid). The hormonal responses were evaluated shortly after infestation, but the long-term responses were also analyzed. Another aim was to evaluate the variation in susceptibility to broad mites in different pot azalea cultivars. Additionally, we also wanted to observe other mites, such as *Tarsonemus confusus*, on pot azalea, for which it is uncertain if they should be considered harmful.

2. Materials and Methods

2.1. Plant Material and Sampling

2.1.1. Experiment 1—Defense Hormone Analysis after Broad Mite Infestation under Controlled Conditions in Two Cultivars of Pot Azalea Shortly after Infestation (Up to 11 Days)

In a controlled experiment and immediately after infestation with broad mites, the JA and SA levels in azalea plants were analyzed. Two cultivars with well-known resistance—‘Nordlicht’ (susceptible cultivar) and ‘Elien’ (resistant cultivar)—were used [12]. The levels of SA and JA were compared between infested and uninfested plants.

The plants were grown on ebb and flow tables. The plants were infested artificially by surrounding them with *Hedera helix* L. infested with broad mites, as described in Luybaert et al. [25]. The samples for LC-MS/MS analysis and for the quantification of the number of mites were taken as shoot tips at 4, 6, 8 and 11 days post-inoculation (dpi). Five independent biological replicates were taken at each time point ($n = 5$), with one shoot tip per replicate for LC-MS/MS analysis and another for mite counts. The samples for LC-MS/MS analysis were flash-frozen in liquid nitrogen and stored until analysis at -80°C . The broad mites were extracted from the sampled shoots following the three-step detection method including (1) sampling of shoot tips, (2) isolation of the mites in 70% ethanol and (3) sieving and vacuum filtration to facilitate counting [26]. Subsequently, the mites were counted using a binocular microscope (Olympus SZX16, Antwerp, Belgium; max 115 $\times$).

2.1.2. Experiment 2—Defense Hormone Analysis under Natural Infestation in Three Cultivars of Pot Azalea during the Growing Season (Up to 32 Weeks)

In experiment 2, the natural occurrence of broad mites was monitored and correlated to the hormone levels. Three azalea cultivars were studied during a growing season of 32 weeks. The three cultivars used—‘Nordlicht’ (susceptible cultivar), ‘Mevrouw Gerard Kint’ (intermediate response) and ‘Elien’ (resistant cultivar)—differed in their susceptibility to *P. latus*: [12].

Each cultivar had one replicate with 100 plants and was placed on a separate greenhouse bed comparable to the setup in commercial cultivation. Luypaert et al. [12] tested a randomized cultivar setup and a setup grouped per cultivar. They concluded that randomization leads to a bias caused by the attractiveness of different azalea cultivars to broad mite; thus, in the present experiment, the plants were grouped according to cultivar. In experiment 2, no tarsonemid mites were artificially introduced into the crop. Instead, the natural distribution of *P. latus* was monitored closely.

The plants were sampled between 19 January (W3) and 13 September (W37). Every two weeks, samples were taken for the estimation of mite numbers. The dates and week (W) numbers of sampling were: 19 January (W3), 1 February (W5), 15 February (W7), 3 March (W9), 16 March (W11), 30 March (W13), 13 April (W15), 27 April (W17), 11 May (W19), 8 June (W23), 21 June (W25), 6 July (W27), 19 July (W29), 2 August (W31) and 31 August (W35) (there was no sampling for mite counts in W21 and W33). For the counting of mite numbers at every time point, three biological replicates were taken ($n = 3$) with three replicate shoot tips per replicate.

The shoot tips were sampled for stress hormone analysis every month and, once the presence of broad mites was observed, every two weeks. The dates and week numbers of sampling were: 15 February (W7), 16 March (W11), 13 April (W15), 11 May (W19), 9 June (W23), 22 June (W25), 5 July (W27), 19 July (W29), 2 August (W31), 16 August (W33), 30 August (W35) and 13 September (W37). For ‘Mevrouw Gerard Kint’, samples were taken separately on plants showing symptoms and plants without symptoms in W33 and W35, and for ‘Nordlicht’, separate samples were taken in W31, W33 and W35. Three independent biological replicates were taken per cultivar and per sampling date ($n = 3$) with three to five shoot tips per sample of the different plants. The samples were flash-frozen in liquid nitrogen and stored until analysis at -80°C .

According to standard cultivation procedures, the plants were cut back in W5 (after sampling), W20 and W35 (after sampling). Sampling for mite counts stopped with the last cut in W35. One extra sample for defense hormone analysis was taken from the fresh shoots in W37.

Broad mites were extracted and counted as described in experiment 1. The morphological identification of mites was conducted using a light microscope (Leica DM2000, Diegem, Belgium; max $400\times$).

2.1.3. Experiment 3—Defense Hormone Analysis under Natural Infestation in 18 Cultivars of Pot Azalea and Occurrence of Mite Species during the Growing Season (30 Weeks)

In total, 18 commercial cultivars of pot azalea were used in the experiment. Eight of these cultivars were previously tested for susceptibility [12] (Table 1). For each replicate, 24 plants of one cultivar were placed in a greenhouse. To avoid dispersion of the mites between cultivars, sticky plates (Biobest Bug Scan IVOG system—yellow) were installed between the blocks of plants. No tarsonemid mites were introduced in the crop, but the natural distribution of the different species of tarsonemid was monitored closely.

To estimate mite numbers, the plants were sampled every two weeks between 20 October (W42) and 9 May (W19). On four dates—1 February (W5), 6 March (W10), 28 March (W13) and 27 April (W17)—shoot tips were sampled for JA and SA analysis. Sampling for defense hormones was conducted according to experiment 2: three independent biological samples were taken per cultivar and per sampling date with 3 to 5 shoot tips per sample of the different plants ($n = 3$). Tarsonemid mites were sampled (3 shoot tips per cultivar) and counted using a binocular and identified morphologically using a light microscope, as described in experiment 2. Damage was calculated per cultivar by counting the number of plants with damage.

In this experiment, we looked in more detail at the different mite species known for their occurrence on pot azalea. Besides *Polyphagotarsonemus latus*, identification keys were used for four *Tarsonemus* species: *Tarsonemus confusus*, *T. bilobatus* Suski, *T. floricolus* Canestrini & Fanzago, *T. lacustris* Schaarschmidt, species of the genus *Xenotarsonemus* Beer,

and the species *Phytonemus pallidus* (Banks). For the identification of these mite species, all the collected specimens were mounted on slides for microscopic observation [27–29].

Table 1. *Rhododendron simsii* hybrids used in the different experiments; cultivars marked with * are also used in Luypaert et al. [12].

Cultivar Name	Parentage / Background	* Used in Luypaert et al. [12]
'Amélie'	sport from 'Thesla' ^y	
'Elie'	sport from 'Mistral' ^x	*
'Fluostern'	sport from 'Sachsenstern' ^y	
'Franziska R.'	sport from 'Michelle Marie' ^x	
'Huelsten'	sport from 'Helmuth Vogel' ^x	
'Inka'	sport from a 'Helmuth Vogel' sport ^x	
'Leonardo'	seedling with unknown parents ^y	
'Luntera'	sport from a 'Helmuth Vogel' sport ^x	
'Mevrouw Edmond Troch'	sport from a 'Helmuth Vogel' sport ^x	
'Mevrouw Gerard Kint' (picotee)	sport from 'Glaser Nummer 10' ^x	*
'Mevrouw Gerard Kint' (red)	sport from 'Mevrouw Gerard Kint' ^y	
'Michelle Marie'	seedling x 'Rosali' ^x	*
'Nordlicht'	sport from 'Helmuth Vogel' ^x	*
'Otto'	'Friedhelm Scherrer' x seedling ^x	*
'Renato'	derived from 'Glaser Nummer 10' ^x	
'Sachsenstern'	Sport from unknown seedling ^x	*
'Tamira'	Seedling with unknown parents ^x	*
'Thesla'	Seedling 'Sankt Valentin' x 'Mevrouw Gerard Kint' ^x	*

^x Parentage and background information based on Heursel et al. [2], ^y Parentage and background information obtained from growers.

2.2. JA and SA Analysis

In experiment 1, the extraction and analysis of phytohormones SA and JA were conducted according to Bosco et al. [30]. In brief, at -80°C , frozen leaf samples were kept in liquid nitrogen and were ground using a mortar and pestle before 20 mg of homogenized sample material was extracted with 2 mL solvent mixture methanol:water:acetic acid (10:89:1) and spiked with $2\text{H}^4\text{-SA}$ (Olchemim, Olomouc, Czech Republic) and $2\text{H}^6\text{-JA}$ internal standards (Olchemim, Olomouc, Czech Republic). The samples were extracted at 4°C on a shaker (210 rpm) for 16 h. Subsequently, the samples were vortexed and filtered using a $0.22\ \mu\text{m}$ Millex-GV filter (Millipore, Billerica, MA, USA), and the extract was collected in glass tubes. Then, 1 mL of the extract was loaded onto Oasis MCX $1\ \text{cm}^3$ cartridges (Waters, Milford, MA, USA) (preconditioned with 1 mL pure methanol and 1 mL milliQ water). After loading the samples, 0.5 mL milliQ water was used to wash the cartridges. The flow-through was discarded and the columns were eluted under vacuum with 1 mL methanol. In a warm water bath (35°C , nitrogen atmosphere), the eluates were evaporated to dryness. For resuspension of the eluates, 0.5 mL 75% methanol was added. The extract was again filtered through a $0.22\ \mu\text{m}$ Millex-GV filter and transferred to a micro-vial prior to the injection of $10\ \mu\text{L}$ into the LC-MS/MS. External calibration curves were used to calculate the final concentrations of the samples for JA (Olchemim, Czech Republic) and SA (Sigma-Aldrich, Bornem, Belgium).

In experiments 2 and 3, extraction was based on Sanchez-Bel et al. [31] using diethyl ether as solvent. Before analysis, the frozen samples were ground and lyophilized. All the steps were performed on ice. Per sample, 20 mg of lyophilized plant material was placed in a glass tube and spiked with internal standard $2\text{H}^4\text{-SA}$ (Olchemim, Olomouc, Czech Republic) and $2\text{H}^6\text{-JA}$ internal standard (Olchemim, Czech Republic) prepared in MeOH/water (75/25). Subsequently, 2.5 mL ultrapure water was added. The samples were shaken on ice for 30 min (150 rpm) and centrifuged (cooled; $5000\times g$) for 40 min. The supernatant was adjusted to pH 2.8 with 6% acetic acid. The samples were partitioned twice against diethyl ether. Subsequently, the total eluate was evaporated using nitrogen gas at 35°C , and the residues were dissolved in 1 mL water/methanol 25:75 and put on

a 0.22 μm PVDF filter. The filtrate was put in a micro-vial and stored at 4 °C prior to injection (10 μL) into the UHPLC–MS/MS system. In experiments 2 and 3, the level of JA present was calculated using a calibration curve (relative), but without the use of an internal standard.

The advantage of the protocol based on Sanchez-Bel et al. [31] in experiments 2 and 3, compared to Bosco et al. [30] used in experiment 1, is that the extraction is conducted on dry plant material. This results in lower variation in the measured values. Moreover, the protocol using dry plant material is more simple and makes no use of expensive solid-phase extraction columns that can also compromise the recovery of some compounds. Therefore, in experiment 1, the hormone levels are expressed as fresh weight (fw), and in experiments 2 and 3, the hormone levels are expressed as dry weight (dw).

For all the experiments, the analysis was run on an Acquity UHPLC system coupled with a Xevo TQ-S triple quadrupole MS detector (Waters, Milford, MA, USA), as described in Bosco et al. [30].

2.3. Statistical Data Analysis

The LC-MS/MS data were acquired using MassLynx, version 4.1 (Waters, Milford, MA, USA), and data processing of the acquired LC-MS/MS data was performed using Targetlynx (Waters, Milford, MA, USA). The types of statistical test used are mentioned with the different experiments in the Section 3 and in tables and graphs.

3. Results

3.1. Experiment 1—Defense Hormone Analysis after Broad Mite Infestation under Controlled Conditions in Two Cultivars of Pot Azalea Shortly after Infestation (Up to 11 Days)

In experiment 1, the phytohormone level was determined in *R. simsii* ‘Elien’ and ‘Nordlicht’ at four time points upon artificial infestation. On ‘Nordlicht’, the first mites were observed at 6 dpi, while on ‘Elien’, the first observation was at 8 dpi. At 11 dpi, the final day of observations, the number of mites on ‘Nordlicht’ increased to 12.4 ± 4.4 , while on ‘Elien’ at the same time point, 1.4 ± 1.2 mites were counted. Figure 1 shows the levels of JA over the course of 11 days. The infestation of *R. simsii* ‘Nordlicht’ had no effect on the JA level (Figure 1). In contrast, ‘Elien’ had a significantly higher level of JA when comparing the *P. latus*-infested plants to the control plants (mock) at 6 dpi.

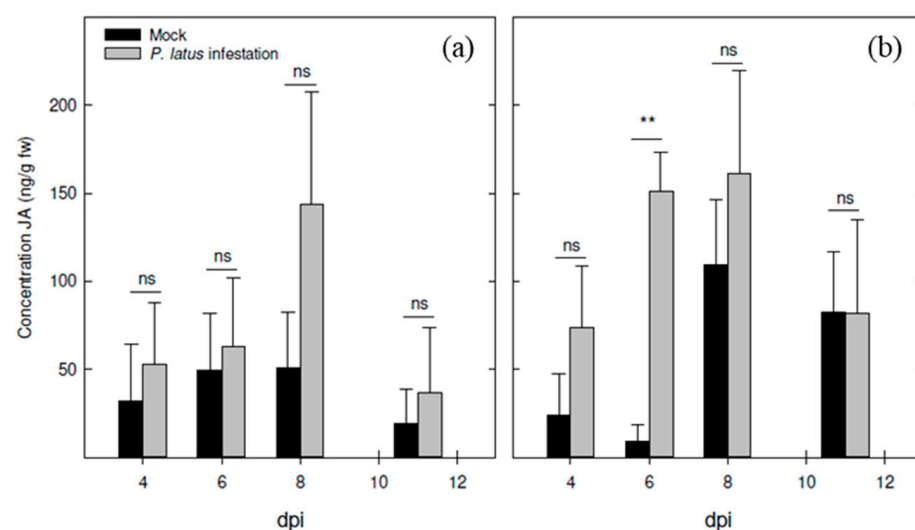


Figure 1. Levels of jasmonic acid (JA) in experiment 1 analyzed 4, 6, 8 and 11 days post-infestation (dpi) in the pot azalea cultivars ‘Nordlicht’ (a) and ‘Elien’ (b). Treatments are mock or *Polyphagotarsonemus latus*-infested leaflets and presented as the average (+SE) ($n = 5$). Significant differences compared to the respective mock are marked: ns = not significant; ** $p < 0.01$ (Mann–Whitney U).

The SA levels are expressed as the relative response: area analyte/area internal standard. As sample preparation required ground fresh leaves, the hormone levels are expressed as fresh weight. The levels of free SA in ‘Nordlicht’ were increased by 1.6, 10.4, 6.3 and 18.7 at 4, 6, 8 and 11 dpi, respectively (Table 2). Statistical differences were only observed at 6 and 11 dpi (Mann–Whitney U, $p < 0.05$). In contrast, in ‘Elie’ the level of free SA remained relatively stable. Across all the time points after *P. latus* infestation, the increase in the level of free SA was 1 to 1.6 times baseline; these differences were not statistically significant (Mann–Whitney U, $p < 0.05$).

Table 2. The relative difference in salicylic acid content in the pot azaleas ‘Nordlicht’ and ‘Elie’. Leaflets are analyzed 4, 6, 8 and 11 days post-infestation (dpi) with *Polyphagotarsonemus latus* or mock treatment (experiment 1). Relative differences are calculated as the ratio of area analyte/internal standard of infested leaves and mock treatment ($n = 5$) (Mann–Whitney U, p -values are given).

Genotype	Time Point (dpi)	Treatment	Area Analyte/Area Internal Standard	Relative Difference of SA Content	Level of Significance (p -Value)
‘Nordlicht’	4	Mock	0.11	1.6	0.09
		<i>P. latus</i> infestation	0.16		
	6	Mock	0.08	10.4	0.01
		<i>P. latus</i> infestation	0.83		
	8	Mock	0.10	6.3	0.09
		<i>P. latus</i> infestation	0.60		
‘Elie’	11	Mock	0.12	18.7	0.01
		<i>P. latus</i> infestation	2.17		
	4	Mock	0.10	1.48	0.19
		<i>P. latus</i> infestation	0.15		
	6	Mock	0.16	1.21	0.69
		<i>P. latus</i> infestation	0.19		
	8	Mock	0.15	0.99	1.00
		<i>P. latus</i> infestation	0.14		
	11	Mock	0.13	1.62	0.31
		<i>P. latus</i> infestation	0.22		

3.2. Experiment 2—Defense Hormone Analysis under Natural Infestation in Three Cultivars of Pot Azalea during the Growing Season (Up to 32 Weeks)

The plants were examined for the presence of mites from January onward, but it took until W19 for the first *P. latus* mite to be observed on ‘Mevrouw Gerard Kint’. A large number of 28 mites was found on ‘Nordlicht’ in W25 (Figure 2). In W29, large numbers of mites, 36.3 (SD $\pm$ 48.5), were found on ‘Mevrouw Gerard Kint’. On ‘Elie’, during the whole experiment, only a limited number of two mites was counted (W29). No mites were extracted on the other dates. When symptoms of mite infection were observed in a cultivar, this was always locally at first, and subsequently spread to a larger area.

A significant increase in SA was found in ‘Mevrouw Gerard Kint’ and ‘Nordlicht’ after observations of mite infestations. In ‘Elie’, the levels of SA were very low during the whole growing period (Figure 2). The differences between the plants of ‘Nordlicht’ with and without visual symptoms were not significant in W31. In W33 and W35, the differences between symptomatic and asymptomatic plants were significant for both ‘Mevrouw Gerard Kint’ and ‘Nordlicht’ (t -test; $p < 0.05$). The levels of JA were low in April but increased in all three cultivars from W19 onward. From W35, a decrease in JA levels was observed.

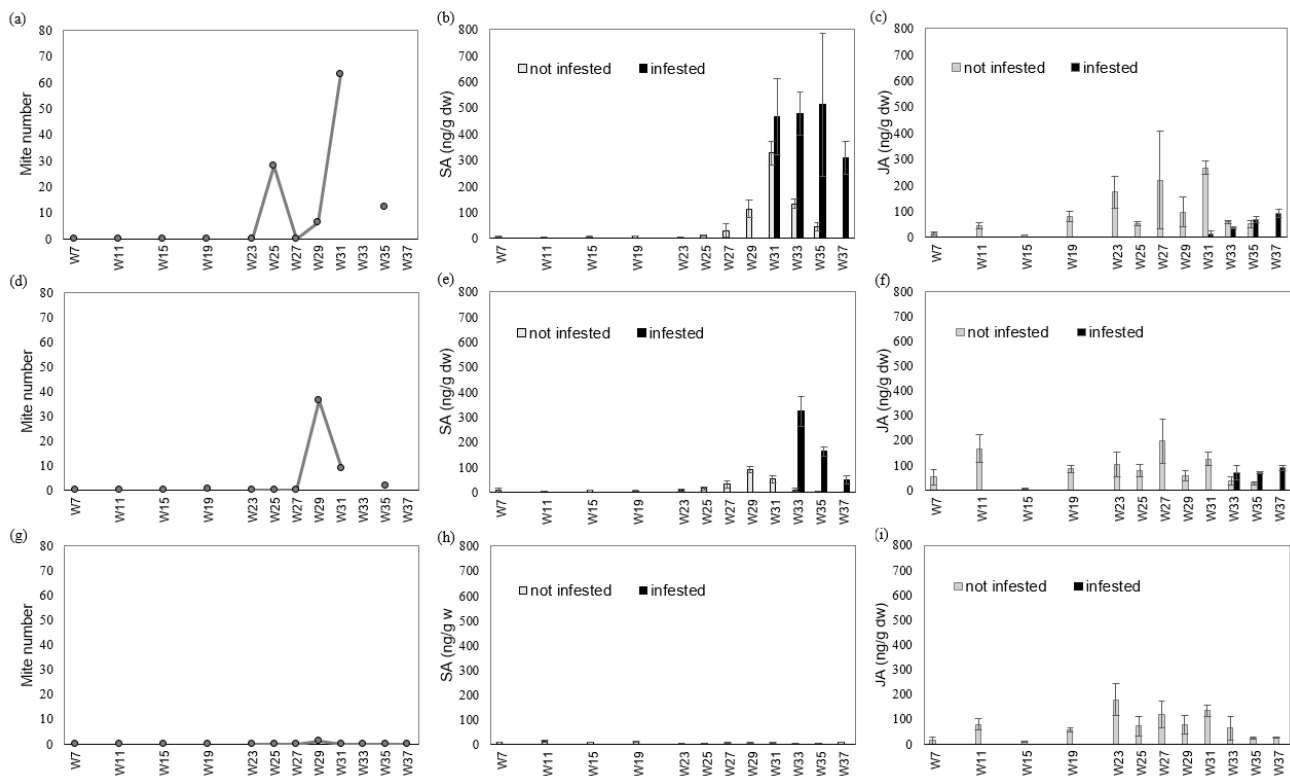


Figure 2. Experiment 2: Mean numbers of the mite *Polyphagotarsonemus latus* ($n = 3$) counted on different sampling dates (12 time points) for ‘Nordlicht’ (a), ‘Mevrouw Gerard Kint’ (d) and ‘Elien’ (g), and respective levels of salicylic acid (SA) (b,e,h) and JA (c,f,i) (data are means $\pm$ SD) ($n = 3$). In ‘Nordlicht’ (weeks 31, 33 and 35) and ‘Mevrouw Gerard Kint’ (weeks 33 and 35) samples were taken separately for plants showing symptoms and plants without symptoms ($n = 3$).

3.3. Experiment 3—Defense Hormone Analysis under Natural Infestation in 18 Cultivars of Pot Azalea and Occurrence of Mite Species during the Growing Season (30 Weeks)

Different mite types were detected during the experiment. Only four mite species occurred; in order of prevalence (total numbers counted during the experiment are given): *P. latus* (counted mites: 3889), *T. confusus* (counted mites: 552), *Xenotarsonemus* spp. (counted mites: 34), *P. pallidus* (counted mites: 10) and *T. floricolus* (counted mites: 4). No specimens were found for *T. bilobatus* or *T. lacustris*.

No correlations (Pearson) were found between the mite numbers of *P. latus* and *T. confusus*. Nevertheless, we observed that the cultivars with no observations of *P. latus* also showed low numbers of *T. confusus*. This results in a moderate Spearman’s rank correlation: $r = 0.55$ ($p < 0.01$). Plant damage was caused by the occurrence of *P. latus*, as shown by a high Pearson correlation between the total number of *P. latus* mites and the number of plants showing damage: $r = 0.89$ ($p < 0.0001$). The same correlation calculated between *T. confusus* and plants showing damage was low and not significant.

The first *P. latus* mites were found at the start of the experiment in W42 on ‘Leonardo’. The first symptoms were only observed in W47 on ‘Franziska R.’. This cultivar showed increasing damage afterwards. Later, symptoms were also seen on the cultivars ‘Michelle Marie’, ‘Fluostern’, ‘Mevrouw Gerard Kint’ (red), ‘Otto’, ‘Leonardo’ and ‘Renato’.

No *P. latus* mites were observed during the experiment on the cultivars ‘Elien’, ‘Inka’, ‘Huelsten’ or ‘Sachsenstern’. On ‘Mevrouw Edmond Troch’, only a single *P. latus* mite was counted on the last sampling date. While *P. latus* was not found on several cultivars, *T. confusus* was found on all the cultivars. *Xenotarsonemus* spp. were present on all the cultivars except ‘Schachsenstern’, ‘Mevrouw Gerard Kint’ (picotée), ‘Luntera’ and ‘Otto’.

The highest total number was counted on ‘Otto’ (counted mites: 741), followed by ‘Leonardo’ (counted mites: 521) and ‘Mevrouw Gerard Kint’ (red) (counted mites: 451). All

these cultivars showed infestation at an early stage of the experiment. On ‘Michelle Marie’, infestation was already present in December (W51), although the mite numbers did not increase considerably compared to the other cultivars. An overview of the occurrence of *P. latus* on the different cultivars is given in Figure 3.

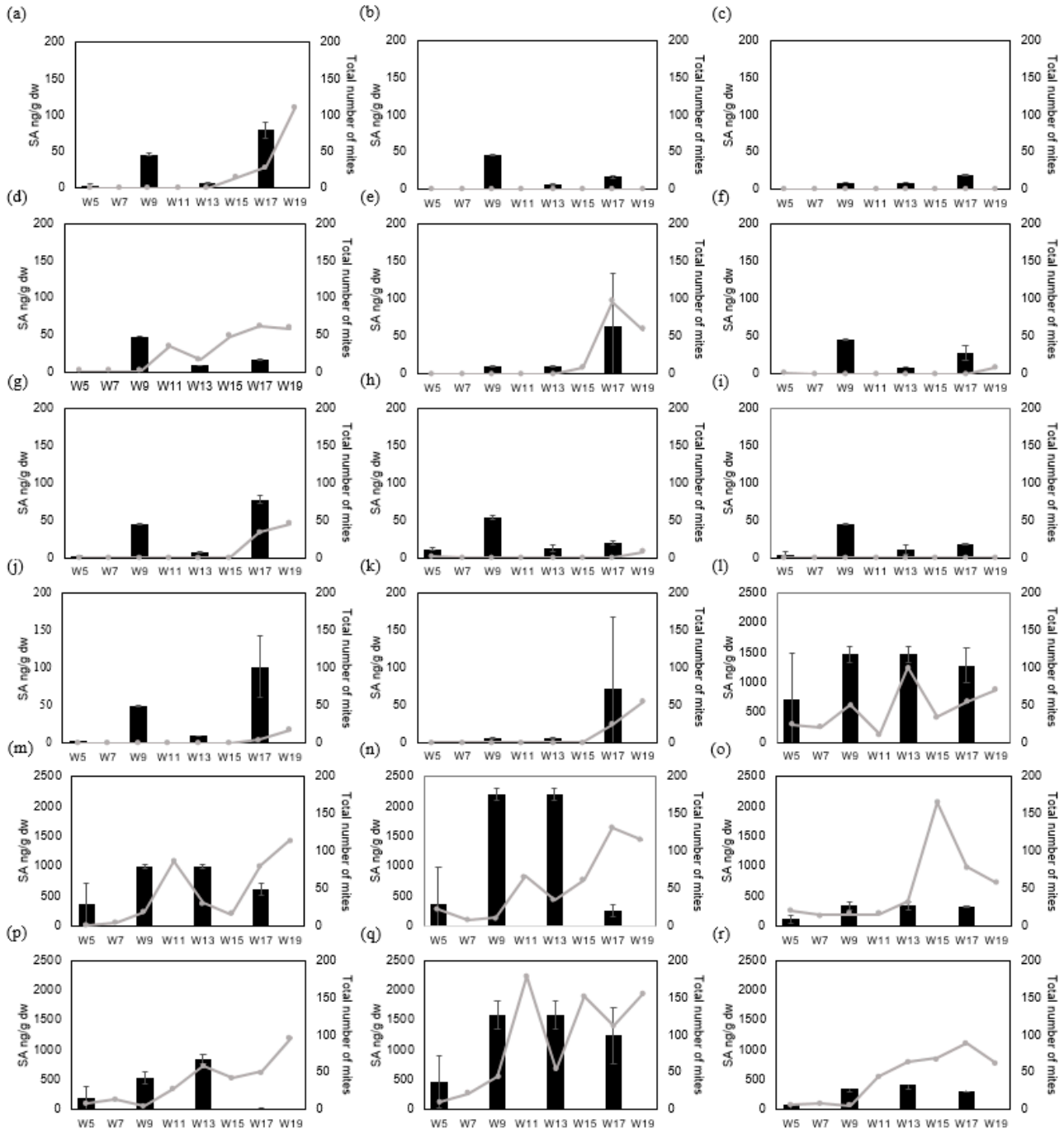


Figure 3. Experiment 3: mite numbers of *Polyphagotarsonemus latus* (total of 3 shoot tips) counted on different sampling dates (every two weeks, 15 time points) and levels of salicylic acid (SA) (+ SD) ($n = 3$) in 18 cultivars of pot azalea. With low SA levels (Y-axis up to 200 ng/g dw): ‘Amélie’ (a), ‘Elien’ (b), ‘Huelsten’ (c), ‘Inka’ (d), ‘Luntera’ (e), ‘Mevrouw Edmond Troch’ (f), ‘Mevrouw Gerard Kint’ (picotee) (g), ‘Nordlicht’ (h), ‘Sachsenstern’ (i), ‘Tamira’ (j) and ‘Thesla’ (k). With high SA levels (Y-axis up to 2500 ng/g dw): ‘Fluostern’ (l), ‘Franziska R.’ (m), ‘Leonardo’ (n), ‘Mevrouw Gerard Kint’ (red) (o), ‘Michelle Marie’ (p), ‘Otto’ (q) and ‘Renato’ (r).

In all the cultivars, the levels of SA increased in March (W9 to W13) compared to W5. A Pearson correlation was calculated between the maximum number of mites and the SA levels in W10. The correlation was high, with, respectively, $r = 0.75$ ($p = 0.0002$) and $r = 0.84$ ($p < 0.0001$) when the mite numbers of W9 and W11 were correlated with the SA levels on W10. Moreover, for all the cultivars, the correlation was very high, with $r = 0.84$ ($p < 0.00001$) when the total mite numbers across the entire experiment were correlated to the SA level found in W10.

For 11 cultivars, no *P. latus* mites were observed before W9. This was the case for: ‘Elien’, ‘Huelsten’, ‘Inka’, ‘Sachsenstern’, ‘Troch’, ‘Nordlicht’, ‘Tamira’, ‘Mevrouw Gerard Kint’ (red), ‘Thesla’, ‘Amélie’, ‘Luntera’ and ‘Mevrouw Gerard Kint’ (picotee). All these cultivars had an SA level between 43.5 and 53.4 ng/g dw in W9. For cultivars with only a few mites, such as ‘Michelle Marie’ (counted mites: 5) and ‘Renato’ (counted mites: 6), in W9, the SA levels rose to 539.9 and 344.8 ng/g dw, respectively. The highest number of mites in W9 was counted on ‘Otto’ (counted mites: 44) and ‘Fluostern’ (counted mites: 50); these cultivars had SA levels of 1498.1 and 1475.1 ng/g dw, respectively (Figure 3).

Although in W13, the non-infected cultivars showed lower levels of SA, the correlation between the SA levels and mite numbers for all cultivars at this time point was still very strong ($r = 0.85$; $p < 0.0001$). Two cultivars showed symptoms only on part of the plant. Therefore, the samples taken from symptomatic vs. asymptomatic plants were analyzed separately. In both cultivars, huge differences in the SA level between symptomatic and asymptomatic plants were observed. For ‘Michelle Marie’, the plants with symptoms had an SA level of 852.0 ng/g dw compared to 15.1 ng/g dw in plants without symptoms. For ‘Leonardo’, symptomatic plants had an SA level of 2204.1 and asymptomatic plants 254.3 ng/g dw. Only these two cultivars, ‘Michelle Marie’ and ‘Leonardo’, had relevant levels of JA when comparing plants with and without symptoms (Figure 4). In both cultivars, the levels of JA in plants showing symptoms were around 10 and 20 times higher in ‘Michelle Marie’ and ‘Leonardo’, respectively, in comparison to plants showing no symptoms.

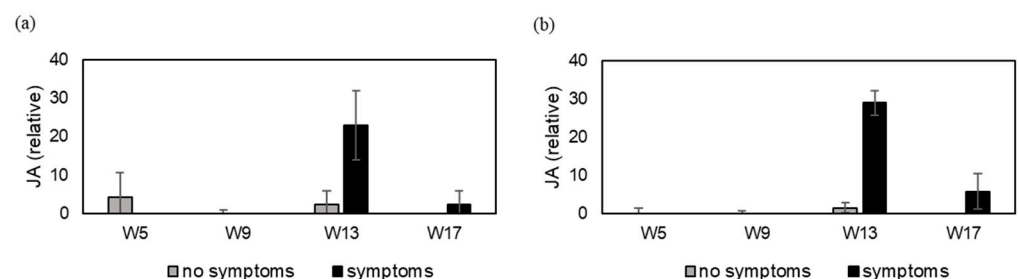


Figure 4. Jasmonic acid (JA) levels on the pot azalea cultivars ‘Michelle Marie’ (a) and ‘Leonardo’ (b). Plants showing symptoms and plants without symptoms of infestation by *Polyphagotarsonemus latus* were sampled separately. Data are means $\pm$ SD ($n = 3$).

4. Discussion

The study of plant–arthropod interactions lags behind the study of plant–pathogen interactions. However, in recent years, plant–herbivore interactions have received increasing attention. For mites, plant reactions to the presence of the two-spotted spider mite (*Tetranychus urticae* Koch) have been studied most, as reviewed by Agut et al. [32] and Blazer et al. [22]. In the present study, we have focused on the relationship between the two main plant defense hormones, SA and JA, in response to broad mite presence in pot azalea. Our results are in line with the general accepted idea that the JA pathway is involved in defense against herbivores [21]. It is known that endogenous bioactive plant defense hormones such as JA are present in low levels in the plant, but accumulate upon herbivore feeding or wounding [33]. The results of experiment 1 under controlled conditions and of experiment 3 under natural infestation during the growing season showed that an increase in JA is only found at specific time points and early after infestation. In experiment 2, no

significant changes in JA levels were observed. As suggested by Luypaert et al. [17], the timing of sampling is essential while measuring hormonal changes. Additionally, infestation pressure at the time of sampling plays a role in the observed hormone levels. The present study confirms the findings of Luypaert et al. [25], that *P. latus* infestation activates JA-dependent pathways most strongly at earlier time points (5–12 dpi) in *R. simsii* hybrids. In preliminary laboratory tests using floating leaf discs, it was shown that after the artificial application of 50 or 100 μ M MeJA, the population growth of *P. latus* on *R. simsii* ‘Nordlicht’ significantly decreased [17]. Luypaert et al. [25] isolated different JA biosynthesis marker genes in azalea to obtain insight into the molecular mechanisms behind the interaction between *P. latus* and the *R. simsii* hybrid. Transcript levels of marker genes for the JA biosynthesis pathway were significantly induced upon *P. latus* infestation in the *R. simsii* hybrid [25]. This strengthens our hypothesis of an early-induced plant defense based on the JA response upon *P. latus* infestation in pot azalea.

The high increase in SA levels upon broad mite infestation is in contrast with the expected plant defense response. The SA pathway is often associated with defense against biotrophic pathogens [19]. Arena et al. [34] found, in *Arabidopsis thaliana* (L.) Heynh., a simultaneous increase in the levels of SA and JA when the plants were infested with false-spider mites of the genus *Brevipalpus* Donnadieu. They also evaluated mite performance on *Arabidopsis* mutants impaired in their SA or JA response and showed a function for the SA-mediated pathway in improved mite reproduction. Therefore, it is suggested that *Brevipalpus* mites manipulate the plant defensive response via the SA-mediated pathway for their own benefit [34].

In our study, significant increases in SA level were strongly related to the presence of broad mites. Levels remained low when no mites were present. In experiment 3 with 18 cultivars tested, an especially strong correlation was found between the rising number of mites in W9 ($r = 0.75$) and W11 ($r = 0.84$) and the SA level in W10. An increase of up to almost 100 times the SA level was measured in the susceptible cultivar ‘Nordlicht’ (experiment 2). These high SA levels were still observed during the following months of infestation. At the same moment, in ‘Mevrouw Gerard Kint’, a cultivar with moderate susceptibility, the increase in SA was lower, while in ‘Elien’, a resistant cultivar, no change in the SA level was observed.

In preliminary tests with a floating disc assay, the application of 50 or 100 μ M SA had no significant effect on the broad mite population size [35]. Other preliminary data suggest that the application of SA can lead to increasing population sizes of broad mites, but this depends on the SA concentration applied. Additionally, the expression of SA marker genes isolated after *P. latus* infestation in the *R. simsii* hybrid was not clear [25]. Our SA analysis, however, could not confirm their conclusion that the SA biosynthesis pathway, led by either RsPAL or RsICS, was suppressed at later time points in response to *P. latus* infestation in pot azalea. If no local increase in transcript levels is observed, a systemic response of signaling molecules in plant defense for stronger and faster defense responses could be a hypothesis [36]. JA, for example, will not only accumulate at the sites of local wounding, but also in undamaged, more distal leaves. This increase can be found within two minutes [33]. For SA, more data are available in relation to pathogen-induced SA, which was also shown to act systemically in the plant and was found in the phloem [37].

In tomato, *P. latus* prefers plants deficient in the JA pathway compared to wild-type plants [38]. Treatment of the JA-deficient plants made them as unattractive as the wild-type plants. The wild-type plants infested by broad mites showed an increase in JA-related transcripts. In a study by Grinberg et al. [9] using Northern blot analysis, it was shown that both the JA and SA pathways are activated in response to broad mites on cucumber. In citrus, *P. latus* infestation activated both the JA- and the SA-dependent pathways in sour orange but no activation was observed in Cleopatra mandarin [39].

From our results, it is probable that in an early phase of infestation, the plant responds by activating the JA-dependent pathway; subsequently, the JA levels drop quickly and a switch is made to the production of SA. Because of the small timeframe, it is possible that

the increase in JA was not observed in some of our experiments under natural infestation, as the exact moment of mite infestation is difficult to determine. In experiment 1, under controlled infestation conditions, it is shown that the JA level can change in a time period of only two days. Under culture conditions, as applied in experiments 2 and 3, not all cultivars and not all plants within one cultivar experience the same infestation pressure. Differences in JA content could be observed when symptomatic and asymptomatic plants were compared at the moment the mite population start to build up, as was the case for ‘Michelle Marie’ and ‘Leonardo’ (experiment 3). Because timing is crucial, further research would be needed to elucidate the switch from the JA- to the SA-dependent pathway.

The key question to be elucidated is that of whether *P. latus* is able to suppress plant defense by modulating the action of SA in response to JA. If so, a plausible answer might be that *P. latus* mites inject effectors, interfering with host immune responses. To date, no data for *P. latus* are available, but this phenomenon is known to occur in phytopathogens [40], aphids [41] and nematodes [42]; additionally, spider mites might secrete effectors via their saliva [43,44]. For example, strains of the spider mites *Tetranychus urticae* and *T. evansi* Baker & Pritchard can suppress the expression of SA and/or JA marker genes [21,44–46]. The genomic data of *P. latus*, in accordance with the whole spider mite genome, would provide evidence if this mite species is also able to encode putative salivary proteins [47]. Another alternative might be that *P. latus* is a vector for phytopathogens that have been implicated in the suppression of plant defenses. The hypothesis of virus transmission by *P. latus* feeding on plants is experimentally rejected [48,49], still leaving the possibility of transmitting bacteria or fungi.

For whitefly, *Bemisia tabaci* (Gennadius),² under laboratory and semi-field conditions, examples are known of JA suppression in correlation with the induction of SA [50–52]. Negative crosstalk between the SA and JA defense pathways is often displayed. For example, the suppression of JA can be an effect of antagonistic crosstalk through strong SA induction [18]. However, suppression can also occur downstream of phytohormone accumulation and independently of JA-SA crosstalk [53].

The conclusion on the role of JA in the defense of pot azalea against broad mite is supported by the findings that the application of MeJA had a negative effect on the fitness of *P. latus* in the pot azalea cultivar ‘Nordlicht’ when compared to a control treatment [35]. This is in accordance with the reports of decreases in spider mite population growth after the application of MeJA [32,54–59]. In pot azalea, the negative effect of MeJA on the population of *P. latus* was related to the concentration applied: for 50 and 100 µM MeJA, the population growth decreased by a factor 3.2 and 2.3, respectively [35]. Choh et al. [60] reported a similar effect as the number of eggs produced by *T. urticae* on lima beans (*Phaseolus lunatus* L.) differs in relation to the JA concentration applied. Additionally, in the *T. urticae*-sensitive Cleopatra mandarin, MeJA treatment significantly reduced the number of eggs produced by the spider mite, whereas salicylic acid had no effect on the oviposition of *T. urticae* [32].

For the eight cultivars used in the study of Luypaert et al. [12] and in experiment 3, correlations between the experiments were found for mite numbers and for damage rates, but the correlations were not significant. Previous studies on pot azalea and broad mite interaction showed that leaf damage does not always correlate well with the number of mites found on the plant [12]. However, our results regarding susceptibility confirmed the study of Luypaert et al. [12], with ‘Elie’ as the most resistant and ‘Otto’ being the most susceptible cultivar. Similar results were found for cultivars with a shared genetic background. ‘Huelsten’, ‘Inka’, ‘Lunterra’, ‘Mevrouw Edmond Troch’ and ‘Nordlicht’ all are sports of ‘Helmuth Vogel’. In these five cultivars, the levels of SA were relatively low upon infestation by broad mites. For ‘Michelle Marie’ and its sport ‘Franziska R.’, both mite numbers and SA levels were high, suggesting that these cultivars are susceptible to broad mites. Similar results are also found for ‘Thesla’ and its sport ‘Amélie’ in mite numbers and SA levels. Both cultivars showed infestation later in the growing season. ‘Mevrouw Gerard Kint’ (red) and (picotee) and ‘Renato’ share a common genetic background and show a relatively low increase in SA levels; nevertheless, higher numbers of mites can

occur. Only on ‘Sachsenstern’ were the level of SA and mite number low, in contrast to the results obtained for its sport ‘Fluostern’. ‘Sachsenstern’ probably avoided infestation. Additionally, ‘Nordlicht’ had lower mite numbers and a lower SA level compared to experiment 2. Additionally, in other crops, a differential response towards broad mite was found, e.g., in citrus, sour orange supported larger densities than Cleopatra mandarin but the levels of injury were similar [39], and in 36 genotypes of common bean (*Phaseolus vulgaris*), differences in *P. latus* infestation were found [61].

The present study showed that not all types of tarsonemid mites cause damage in pot azalea. In culture conditions, the herbivorous and damage-causing mite *P. latus* is most prevalent. Nevertheless, not all pot azalea cultivars are equally attractive to this mite. ‘Elien’, the most resistant cultivar towards *P. latus* mites, possibly exhibits an antixenotic effect based on the presence of glandular trichomes with a sticky end [17]. For the other cultivars, i.e., ‘Huelsten’, ‘Inka’ and ‘Sachsenstern’, the nature of their resistance to *P. latus* is less clear. Another frequently occurring mite, *T. confusus*, was present on all the azalea cultivars without causing any symptoms. Until now, it was not fully understood whether this mite species is herbivorous. This study could only relate the presence of *P. latus* to plant damage. *P. pallidus* could be harmful, but so far, this has not been observed and the number of mites found in our experiments was probably too low.

5. Conclusions

In conclusion, it is clear that the presence of broad mite on pot azalea increases both JA and SA levels. A primary plant response is through the JA pathway, but later, this changes to an increase in the levels of SA. Further efforts, e.g., experiments under controlled conditions, are needed to better understand whether the increase in SA is a result of crosstalk between JA and SA and whether it can help to elucidate the timing and factors influencing the JA and SA responses.

Another conclusion has importance for azalea cultivation. In 18 cultivars, a large variation in susceptibility to broad mite was found, with ‘Otto’ being the most susceptible and ‘Elien’ the most resistant cultivar. This variation in susceptibility to broad mite opens up perspectives for using more resistant genotypes in cultivation and suggests that more genotypes with improved resistance can be developed through breeding.

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References

1. Scheerlinck, H.; Baumann, J.; De Somer, F.; Haerens, A.; Van Laere, A. *Tuinbouwencyclopedia I. De Azalea Indica L. De Sikkil*; Wereldbibliotheek: Amsterdam, The Netherlands, 1938; 527p.
2. Heursel, J.; Lootens, P.; Mertens, M.; Saverwijns, A. *Azalea's. Oorsprong, Veredeling en Cultivars*; Lannoo: Tielt, Belgium, 1999; p. 192.
3. Van Trier, H. *Gentse Azalea*; Stichting Kunstboek: Oostkamp, Belgium, 2012; 144p.
4. VLAM 2020. Belgische Export Van Azalea's en Rhododendrons (2011–2020)—Duitsland Sierteelt 2021—Belgische Sierteelt oppervlakte (2011–2020). Available online: https://www.vlaanderen.be/vlam/kennisbank?f%5B0%5D=facet_sector%3A58 (accessed on 25 February 2022).
5. VLAM 2021. Belgische Export Van Azalea's en Rhododendrons (2011–2020)—Duitsland Sierteelt 2021—Belgische Sierteelt oppervlakte (2011–2020). Available online: https://www.vlaanderen.be/vlam/kennisbank?f%5B0%5D=facet_sector%3A58 (accessed on 25 February 2022).
6. Navajas, M.; Migeon, A.; Estrada-Pena, A.; Mallieux, A.C.; Servigne, P.; Petanovic, R. Chapter 7.4: The Acari. Invasive terrestrial invertebrates in Europe. *BioRisk* **2010**, *4*, 149–192. [\[CrossRef\]](#)
7. Heungens, A. Soft-skinned mites in azalea culture and comparable control results on other host plants. *Verb. Voor De Belg. Sierteelt* **1986**, *30*, 257–269.
8. De Coss-Romero, M.; Peña, J.E. Relationship of broad mite (Acari: Tarsonemidae) to host phenology and injury levels in *Capsicum annum*. *Fla. Entomol.* **1998**, *81*, 515–526. [\[CrossRef\]](#)
9. Grinberg, M.; Perl-Treves, R.; Palevsky, E.; Shomer, I.; Soroker, V. Interaction between cucumber plants and the broad mite, *Polyphagotarsonemus latus*: From damage to defense gene expression. *Entomol. Exp. Appl.* **2005**, *115*, 135–144. [\[CrossRef\]](#)
10. Peña, J.E. Relationships of broad mite (Acari: Tarsonemidae) density to lime damage. *J. Econ. Entomol.* **1990**, *83*, 2008–2015. [\[CrossRef\]](#)
11. Dhooria, J.C.; Bindra, O.S. *Polyphagotarsonemus latus* (Banks) a mite pest of chilli and potato in Punjab. *Acarol. Newsl.* **1977**, *4*, 7–9.
12. Luypaert, G.; Van Huylenbroeck, J.; De Riek, J.; De Clercq, P. Screening for broad mite susceptibility in *Rhododendron simsii* hybrids. *J. Plant Dis. Protect.* **2014**, *121*, 260–269. [\[CrossRef\]](#)
13. Gerson, U. Biology and control of the broad mite, *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae). *Exp. Appl. Acarol.* **1992**, *13*, 163–178. [\[CrossRef\]](#)
14. Van Huylenbroeck, J.; Calsyn, E.; De Keyser, E.; Luypaert, G. Breeding for biotic stress resistance in *Rhododendron simsii*. *Acta Hort.* **2015**, *1104*, 375–379. [\[CrossRef\]](#)
15. Nasrin, M.; Amin, M.R.; Miah, M.R.U.; Akanda, A.M.; Miah, M.G.; Kwon, O.; Suh, S.J. Occurrence and severity of mite *Polyphagotarsonemus latus* (Tarsonemidae) on chili plants: Analysis of pest-weather and host plant characteristics. *Entomol. Res.* **2020**, *51*, 273–281. [\[CrossRef\]](#)
16. Wang, T.; Jia, Q.; Wang, W.; Hussain, S.; Ahmed, S.; Adnan; Zhou, D.X.; Ni, Z.; Wang, S. GCN5 modulates trichome initiation in *Arabidopsis* by manipulating histone acetylation of core trichome initiation regulator genes. *Plant Cell Rep.* **2019**, *38*, 755–765. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Luypaert, G.; Van Huylenbroeck, J.; De Riek, J.; Mechant, E.; Pauwels, E.; De Clercq, P. Opportunities to breed for broad mite resistance in *Rhododendron simsii* hybrids. In Proceedings of the XXV International EUCARPIA Symposium Section Ornamentals: Crossing Borders 1087, Melle, Belgium, 28 June 2015; pp. 479–484.
18. Pieterse, C.M.; Van Der Does, D.; Zamioudis, C.; Leon-Reyes, A.; Van Wees, S.C. Hormonal modulation of plant immunity. *Annu. Rev. Cell Dev. Biol.* **2012**, *28*, 489–521. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Vlot, A.C.; D'Maris, A.D.; Klessig, D.F. Salicylic acid, a multifaced hormone to combat disease. *Annu. Rev. Phytopathol.* **2009**, *47*, 177–206. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Glazebrook, J. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* **2005**, *43*, 205–227. [\[CrossRef\]](#)
21. Howe, G.A.; Jander, G. Plant immunity to insect herbivores. *Annu. Rev. Plant Biol.* **2008**, *59*, 41–66. [\[CrossRef\]](#)
22. Blaazer, C.J.H.; Villacis-Perez, E.A.; Chafi, R.; Van Leeuwen, T.; Kant, M.R.; Schimmel, B.C. Why do herbivorous mites suppress plant defenses? *Front. Plant Sci.* **2018**, *9*, 1057. [\[CrossRef\]](#)
23. Grinberg, M.; Alagarmalai, J.; Sharon, L.; Perl-Treves, R.; Soroker, V. The effect of jasmonic acid signalling pathway on tomato defense to broad mite behaviour. *Isr. J. Entomol.* **2007**, *37*, 389–390.
24. Alagarmalai, J.; Grinberg, M.; Reneh, S.; Sharon, L.; Perl-Treves, R.; Soroker, V. Host selection behavior of broad mite, *Polyphagotarsonemus latus* (Acari: Tarsonemidae). *IOBC-WPRS Bull.* **2007**, *30*, 1–7.
25. Luypaert, G.; Witters, J.; Van Huylenbroeck, J.; De Clercq, P.; De Riek, J.; De Keyser, E. Induced expression of selected plant defence related genes in pot azalea, *Rhododendron simsii* hybrid. *Euphytica* **2017**, *231*, 227. [\[CrossRef\]](#)
26. Mechant, E.; Luypaert, G.; Van Delsen, B.; Pauwels, E.; Witters, J.; Van Huylenbroeck, J.; Gobin, B. Development and validation of a three-step detection protocol for broad mites (*Polyphagotarsonemus latus*) in pot azalea (*Rhododendron simsii* hybrids). *Entomol. Exp. Appl.* **2015**, *156*, 99–104. [\[CrossRef\]](#)
27. Vacante, V. *The Handbook of Mites of Economic Plants: Identification, Bio-Ecology and Control*; CABI: Wallingford, UK, 2015.
28. Krantz, G.W.; Walter, D.E. *A Manual of Acarology*; Texas Tech University Press: Lubbock, TX, USA, 2009; 807p.

29. Zhang, Z.Q.; Bejakovich, D.; Martin, N.A. *Key to Tarsonemidae of New Zealand*; Final report to MAF Science Policy for Project FMA 102; Ministry of Business, Innovation and Employment: Wellington, New Zealand, 2000; pp. 1–35.
30. Bosco, R.; Daeseleire, E.; Van Pamel, E.; Scariot, V.; Leus, L. Development of an Ultrahigh-Performance Liquid Chromatography–Electrospray Ionization–Tandem Mass Spectrometry Method for the Simultaneous Determination of Salicylic Acid, Jasmonic Acid, and Absciscic Acid in Rose Leaves. *J. Agric. Food Chem.* **2014**, *62*, 6278–6284. [[CrossRef](#)] [[PubMed](#)]
31. Sánchez-Bel, P.; Sanmartín, N.; Pastor, V.; Mateu, D.; Cerezo, M.; Vidal-Albalat, A.; Pastor-Fernández, J.; Pozo, M.J.; Flors, V. Mycorrhizal tomato plants fine tunes the growth-defence balance upon N depleted root environments. *Plant Cell Environ.* **2018**, *41*, 406–420. [[CrossRef](#)] [[PubMed](#)]
32. Agut, B.; Pastor, V.; Jaques, J.A.; Flors, V. Can plant defence mechanisms provide new approaches for the sustainable control of the two-spotted spider mite *Tetranychus urticae*? *Int. J. Mol. Sci.* **2018**, *19*, 614. [[CrossRef](#)] [[PubMed](#)]
33. Wang, J.; Wu, D.; Wang, Y.; Xie, D. Jasmonate action in plant defense against insects. *J. Exp. Bot.* **2019**, *70*, 3391–3400. [[CrossRef](#)]
34. Arena, G.D.; Ramos-González, P.L.; Rogerio, L.A.; Ribeiro-Alves, M.; Casteel, C.L.; Freitas-Astúa, J.; Machado, M.A. Making a Better Home: Modulation of Plant Defensive Response by Brevipalpus Mites. *Front. Plant Sci.* **2018**, *9*, 1147. [[CrossRef](#)]
35. Luypaert, G. The Broad Mite, *Polyphagotarsonemus latus* (Acari: Tarsonemidae), and Its Interactions with Pot Azalea, *Rhododendron simsii* Hybrid. Ph.D. Thesis, Ghent University, Ghent, Belgium, 2015.
36. Heil, M.; Ton, J. Long-distance signalling in plant defence. *Trends Plant Sci.* **2008**, *13*, 264–272. [[CrossRef](#)]
37. Lim, G.-H.; Sine, M.B.; de Lorenzo, L.; Yu, K.; Cui, W.; Navarre, D.; Hunt, A.G.; Lee, J.Y.; Kachroo, A.; Kachroo, P. Plasmodesmata localizing proteins regulate transport and signaling during systematic acquired immunity in plants. *Cell Host Microbe* **2016**, *19*, 541–549. [[CrossRef](#)]
38. Grinberg-Yaari, M.; Alagarmalai, J.; Lewinsohn, E.; Perl-Treves, R.; Soroker, V. Role of jasmonic acid signaling in tomato defense against broad mite, *Polyphagotarsonemus latus* (Acari: Tarsonemidae). *Arthropod-Plant Interact.* **2015**, *9*, 361–372. [[CrossRef](#)]
39. Cabedo-López, M.; Cruz-Miralles, J.; Peris, D.; Ibáñez-Gual, M.V.; Flors, V.; Jaques, J.A. The response of citrus plants to the broad mite *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae). *Agric. For. Entomol.* **2021**, *23*, 411–419. [[CrossRef](#)]
40. De Jonge, R.; Bolton, M.D.; Thomma, B.P. How filamentous pathogens co-opt plants: The ins and outs of fungal effectors. *Curr. Opin. Plant Biol.* **2011**, *4*, 400–406. [[CrossRef](#)]
41. Rodriguez, P.A.; Bos, J.I.B. Toward understanding the role of aphid effectors in plant infestation. *Mol. Plant-Microbe Interact.* **2013**, *26*, 25–30. [[CrossRef](#)] [[PubMed](#)]
42. Haegeman, A.; Mantelin, S.; Jones, J.T.; Gheysen, G. Functional roles of effectors of plant-parasitic nematodes. *Gene* **2012**, *492*, 19–31. [[CrossRef](#)] [[PubMed](#)]
43. Alba, J.M.; Glas, J.J.; Schimmel, B.C.J.; Kant, M.R. Avoidance and suppression of plant defenses by herbivores and pathogens. *J. Plant Interact.* **2011**, *6*, 221–227. [[CrossRef](#)]
44. Alba, J.M.; Schimmel, B.C.; Glas, J.J.; Ataíde, L.M.; Pappas, M.L.; Villarroel, C.A.; Schuurink, R.C.; Sabelis, M.W.; Kant, M.R. Spider mites suppress tomato defenses downstream of jasmonate and salicylate independently of hormonal crosstalk. *New Phytol.* **2015**, *205*, 828–840. [[CrossRef](#)] [[PubMed](#)]
45. Kant, M.R.; Sabelis, M.W.; Haring, M.A.; Schuurink, R.C. Intraspecific variation in a generalist herbivore accounts for differential induction and impact of host plant defences. *P. Roy. Soc. B Biol. Sci.* **2008**, *752*, 443–452. [[CrossRef](#)]
46. Sarmento, R.A.; Lemos, F.; Bleeker, P.M.; Schuurink, R.C.; Pallini, A.; Oliveira, M.G.A.; Lima, E.R.; Kant, M.; Sabelis, M.W.; Janssen, A. A herbivore that manipulates plant defence. *Ecol. Lett.* **2011**, *14*, 229–236. [[CrossRef](#)]
47. Grbić, M.; Van Leeuwen, T.; Clark, R.M.; Rombauts, S.; Rouzé, P.; Grbić, V.; Osborne, E.J.; Dermauw, W.; Thi Ngoc, P.C.; Ortego, F.; et al. The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. *Nature* **2011**, *479*, 487–492. [[CrossRef](#)]
48. Higa, S.Y.; Namba, R. Vectors of the papaya mosaic virus in Hawaii. *Proc. Hawaii. Entomol. Soc.* **1971**, *21*, 93–96.
49. Aubert, B.; Lossois, P.; Marchal, J. Mise en évidence des dégâts causés par *Polyphagotarsonemus latus* (Banks) sur papayers à l'île de la Réunion. *Fruits* **1981**, *36*, 9–24.
50. Zarate, S.I.; Kempema, L.A.; Walling, L.L. Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. *Plant Physiol.* **2007**, *143*, 866–875. [[CrossRef](#)]
51. Zhang, P.J.; Li, W.D.; Huang, F.; Zhang, J.M.; Xu, F.C.; Lu, Y.B. Feeding by whiteflies suppresses downstream jasmonic acid signaling by eliciting salicylic acid signaling. *J. Chem. Ecol.* **2013**, *39*, 612–619. [[CrossRef](#)] [[PubMed](#)]
52. Zhang, P.-J.; He, Y.-C.; Zhao, C.; Ye, Z.-H.; Yu, X.-P. Jasmonic Acid-Dependent Defenses Play a Key Role in Defending Tomato Against *Bemisia tabaci* Nymphs, but Not Adults. *Front. Plant Sci.* **2018**, *20*, 1065. [[CrossRef](#)] [[PubMed](#)]
53. Schimmel, B.C.J.; Alba, J.M.; Wybouw, N.; Glas, J.J.; Meijer, T.T.; Schuurink, R.C.; Kant, M.R. Distinct Signatures of Host Defense Suppression by Plant-Feeding Mites. *Int. J. Mol. Sci.* **2018**, *19*, 3265. [[CrossRef](#)]
54. Omer, A.D.; Granett, J.; Kerban, R.; Villa, E.M. Chemically-induced resistance against multiple pests in cotton. *Int. J. Pest Man.* **2001**, *47*, 49–54. [[CrossRef](#)]
55. Li, C.; Williams, M.M.; Loh, Y.T.; Lee, G.I.; Howe, G.A. Resistance of cultivated tomato to cell content-feeding herbivores is regulated by the octadecanoid-signaling pathway. *Plant Physiol.* **2002**, *130*, 494–503. [[CrossRef](#)]
56. Warabieda, W.; Miszczak, A.; Olszak, R.W. The influence of methyl jasmonate (JA-Me) and β glucosidase on introduction of resistance mechanisms of strawberry against two-spotted spider mite (*Tetranychus urticae* Koch). *Commun. Agric. Appl. Biol. Sci.* **2005**, *70*, 829–836.

-
57. Warabieda, W.; Olszak, R.W. Aspects of the influence of exogenous methyl jasmonate on strawberry plants and on the population size of the two-spotted spider mite (*Tetranychus urticae* Koch). *IOBC-WPRS Bull.* **2012**, *83*, 7–13.
 58. Rohwer, C.L.; Erwin, J.E. Spider mites (*Tetranychus urticae*) perform poorly on and disperse from plants exposed to methyl jasmonate. *Entomol. Exp. Et Appl.* **2010**, *137*, 143–152. [[CrossRef](#)]
 59. Agut, B.; Gamir, J.; Jacas, J.A.; Hurtado, M.; Flors, V. Different metabolic and genetic responses in citrus may explain relative susceptibility to *Tetranychus urticae*. *Pest Manag. Sci.* **2014**, *70*, 1728–1741. [[CrossRef](#)]
 60. Choh, Y.; Ozawa, R.; Takabayashi, J. Effects of exogenous jasmonic acid and benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), a functional analogue of salicylic acid, on the egg production of a herbivorous mite *Tetranychus urticae* (Acari: Tetranychidae). *Appl. Entomol. Zool.* **2004**, *39*, 311–314. [[CrossRef](#)]
 61. Androcioli, H.G.; Hoshino, A.T.; Ventura, M.U.; Hata, F.T.; Brugnerotto, M.d.R.; Constantino, L.V.; Marques, F.d.A. Resistance of Common Bean Genotypes to the Broad Mite, *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae): Offspring Development and Biochemical Basis. *Insects* **2021**, *12*, 910. [[CrossRef](#)] [[PubMed](#)]