

Enhancing Plant Resistance at the Seed Stage: Low Concentrations of Methyl Jasmonate Reduce the Performance of the Leaf Miner *Tuta absoluta* but do not Alter the Behavior of its Predator *Chrysoperla externa*

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Abstract Plants express inducible direct and indirect defenses in response to herbivory. The plant hormone jasmonic acid (JA) and related signaling compounds referred to as jasmonates play a central role in regulating defense responses to a wide range of herbivores. We assessed whether treating tomato seeds with 0.8 mM of methyl jasmonate (MeJA) affected the performance of the leaf miner *Tuta absoluta*, and whether possible changes in volatile profiles altered the behavior of its predator *Chrysoperla externa*. MeJA-treatment significantly lengthened larval development and decreased the pupal weight of *T. absoluta*. Herbivory alone increased the emissions of α -pinene, 6-methyl 5-hepten-2-one, β -myrcene, (*E*)- β -ocimene, isoterpinolene, TMTT, (*Z*)-3-hexenyl butyrate, and hexyl salicylate. MeJA seed treatment significantly decreased the emissions of α -cubebene from undamaged and herbivore-infested plants. In addition, the emissions of several compounds were lower in the absence of herbivory. *Chrysoperla externa* preferred odors from herbivore-infested plants over those from control plants, regardless of the MeJA-treatment, and they did not show any preference for herbivore-infested plants for any of the MeJA-treatments. Our results show preliminary evidence that the treatment of tomato seeds with MeJA can reduce the performance of *Tuta absoluta*, and that the chemical differences observed in plant VOC profiles do not alter the behavior of the model predator.

Keywords Methyl jasmonate · Pest resistance · Volatile organic compounds · Plant-insect interactions · Pest management · Lepidoptera · Gelechiidae · Neuroptera · Chrysopidae

Introduction

Plants have evolved an array of mechanisms to defend themselves against the hostile biotic environment. In addition to constitutive defenses, they can express inducible defenses that are activated in response to adverse conditions such as herbivory (Karban 2011; Karban and Baldwin 1997) that include the increase of secondary metabolites (Mithöfer and Boland 2012) and the induction of structures such as trichomes (Traw and Dawson 2002), which will directly impact the attacking herbivore. Furthermore, inducible defenses include qualitative and/or quantitative changes in the constitutive emissions of volatile organic compounds (VOCs), which are known to attract natural enemies (predators and parasitoids) that provide biological control of herbivores (Pinto-Zevallos et al. 2013; Turlings and Wäckers 2004). The expression of herbivore-induced defenses is mediated primarily by the phytohormones jasmonic acid (JA), ethylene, and salicylic acid (SA) (Smith et al. 2009; Wu and Baldwin 2009). The role of JA has been demonstrated in the induction of several defense traits including secondary metabolites (Van Dam et al. 2004), defensive proteins such as polyphenol oxidase and proteinase inhibitors (Farmer and Ryan 1992; Felton et al. 1989), trichomes (Boughton et al. 2005), and plant VOCs (Ament et al. 2004; van Poecke and Dicke 2002). JA-mediated defenses are particularly activated in response to chewing insects such as lepidopteran caterpillars (Kessler and Baldwin 2002).

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The potential for enhancing plant resistance by using selected chemical molecules (plant elicitors) has been known for several years (Conrath et al. 2006) and may be a good strategy to be incorporated in pest management programs (Pinto-Zevallos and Zarbin 2013; Stout et al. 2002). Induction of resistance at the seed stage by using chemical elicitors, including phytohormones and their derivatives, improves the resistance of the plants to adverse conditions of temperature (Farooq et al. 2008), salinity (Shakirova et al. 2003), water stress (Li et al. 1998), heavy metals (Krantev et al. 2008), and to biotic stresses, particularly microorganisms (Buzi et al. 2004; Pankaj et al. 2013). However, evidence of the potential of seed treatments for improving resistance to arthropod pests is scarce. It recently has been found that treating tomato seeds with JA not only has detrimental effects on herbivores of multiple arthropod taxa, but is also beneficial to the plant since the leaf area grazed by caterpillars was reduced (Worrall et al. 2012).

The tomato leafminer or pinworm *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is an oligophagous herbivore that feeds on Solanaceae species. In its native range of South America, it is considered the major pest of tomato (Villas Bôas et al. 2009). Currently, it is threatening tomato production in Europe where it was introduced a few years ago, and is rapidly spreading towards the Middle East, and North and West Africa (*Tuta absoluta* Information Network 2014). In the absence of effective alternative strategies, multiple applications of chemical pesticides (10–30 application/crop cycle) are used to manage *T. absoluta* (Siqueira et al. 2000). Continuous overuse of pesticides can result in environmental contamination, adverse effects on non-target organisms (El-Wakeil et al. 2013), and rapid development of resistance (Lietti et al. 2005; Silva et al. 2011; Siqueira et al. 2000). Thus, the use of the plants' own defense mechanisms to induce resistance against insect pests may play a role, as a component of an integrated pest management (IPM) approach, in reducing pest damage while also minimizing the adverse effects of pesticide use.

The aim of this study was to assess whether treating the tomato seeds with methyl jasmonate (MeJA) can confer long lasting resistance of tomato plants against *T. absoluta*. Additionally, we assessed whether the MeJA treatment can alter the induction of volatile organic compounds upon herbivory, and whether the foraging behavior of a natural enemy of *T. absoluta* is affected. We chose *Chrysoperla externa* (Neuroptera: Chrysopidae) as the model predator. Immature stages of this species are known as voracious consumers of various herbivores including eggs and young larvae of *T. absoluta* (Ghoneim 2014). *Chrysoperla externa* has been regarded as a good candidate to be incorporated in biological control programs. Mass rearing of this species is easy, and it has already proven its potential as a biological control agent of *T. absoluta* (Embrapa 2014; Ghoneim 2014).

Methods and Materials

Living Material Larvae and pupae of *Tuta absoluta* were collected from a commercial organic tomato cultivar Cereja (cherry tomato) farm in Curitiba (Paraná, South Brazil; (25° 17' 31" S, 49° 13' 26" W) and were further maintained on tomato cultivar Santa Clara in controlled conditions (20±1 °C; 70±10 % RH; L:D 12:12 h) at the Department of Biological Sciences from the Federal University of Paraná. The rearing of *Chrysoperla externa* was started from eggs of a colony established at the Federal University of Lavras (Lavras, Minas Gerais, Brazil), and maintained at 25 °C and a photoperiod of 14:10 h (L:D). Larvae were fed on frozen eggs of *Anagasta kuehniella* until pupation. Adults were kept in a PVC cage with the interior wall lined with paper to support oviposition. Adults were provided with an aqueous solution of honey and yeast. After seed treatment, commercial tomato seeds cv. Santa Clara were sown in pots filled with a commercial substrate (Tropstrato HA, Vida Verde, São Paulo, Brazil). Two to three weeks after plant emergence, they were transferred to individual pots (volume ca. 150 ml). Seedlings were kept at room temperature with artificial lights (L:D cycle 14:10 h). They were watered every 3–4 days and fertilized once a week.

Seed Treatment Before sowing, seeds were soaked in a methyl jasmonate (MeJA) solution of 0.8 mM for 24 h. For this, MeJA (Sigma Aldrich, São Paulo, Brazil) was dissolved in a small amount of ethanol (0.25 % in the final solution) and brought to the desired concentration with distilled water. As a control, another set of seeds was soaked in distilled water with the same amount of ethanol for 24 h. During the treatment, seeds were kept in the dark at room temperature.

Feeding Test 1st instar *T. absoluta* larvae that had emerged within the previous 24 h were transferred in groups of two to plastic cups with a treated or untreated tomato leaflet. The leaflets used were all the 3rd leaves from 4–5-week-old plants. The petioles were wrapped in cotton cloth to maintain turgidity. Every 3 days, leaflets were replaced to ensure fresh food for the larvae. Larvae were observed daily until they pupated. The duration of the larval period (in days) and the pupal weight (in mg) was recorded. The experiment was replicated 100 times ($N=100$) per treatment, all performed at once.

Collection and Analyses of VOCs VOCs from 1) control plants, 2) *T. absoluta*-infested plants, 3) MeJA-treated plants, 4) MeJA-treated+*T. absoluta*-infested plants were collected over a 24 h-period (at 26±1 °C; L:D cycle 12:12 h). Before sampling, plants were removed from the pot, and the substrate was wrapped in aluminum foil to prevent collecting volatiles from the roots or the substrate. To induce volatiles, 15 newly emerged larvae were carefully transferred to the leaves of the

6-7-weeks-old plants and left for 24 h to allow larvae to settle and to enter the leaf parenchyma. On the sampling day (24 to 48 h after infestation), plants were individually placed in 1-L airtight glass chambers. The VOC collection and the analytical procedures were previously described by Martins and Zarbin (2013). Airflow (1 L/min) was introduced into the chamber, and VOCs were pushed and trapped onto 20 mg Hayesep Q 80–100 mesh in a glass tube, and then eluted with 300 μ l of double-distilled HPLC-grade hexane. Ten μ l of tetradecane (50 ppm) were added as an internal standard (IS). Samples were concentrated to 100 μ l by exposing them to a slow flow of synthetic air, and 1 μ l of the extract was injected in splitless mode and analyzed by GC/MS (Shimadzu QP 2010 Plus) with a RTX-5 column (30 m \times 0.25 mm i.d., 0.25 mm film thickness; Restek, Bellefonte, PA, USA). The column oven temperature was held at 40 °C for 1 min, increased to 250 °C at 7 °C min⁻¹, and held for 10 min. Helium was the carrier gas at a column head pressure of 170 kPa. Compounds were quantified based on IS peak area. Identification of individual compounds was achieved by comparing the calculated Kovats Indexes (KI) with the literature. In addition, for 2-hexen-1-ol, α -pinene, β -myrcene, α -phellandrene, α -terpinene, β -phellandrene, nonanal, MeSA, decanal, β -caryophyllene, geranylacetone, and α -humulene, the mass spectra were compared with those from synthetic compounds, and for the others with mass spectra from the literature. A total of 7 replicates ($N=7$) per treatment were analyzed.

Behavioral Tests with *Chrysoperla externa* Dual-choice tests were conducted on a Y-tube olfactometer ($\varnothing=2$ cm; main arm=18 cm; smaller arms=13 cm) with a constant airflow of 0.5 L/min (each arm) previously humidified and passed through an active charcoal filter. Behavioral tests were conducted with the extracts from VOCs collected over 24 h as previously reported by Girling and Hassall (2008). As odor sources, one piece of filter paper (2 \times 2 cm) with 10 μ l of extract (VOC samples concentrated to 100 μ l, as described above), were placed at the end of the smaller arms. To avoid dilution due to evaporation, a new odor source was used for every observation. Third instar larvae of *C. externa* were starved for 24 h and then individually introduced at the distal end of the central Y-tube arm. The response was determined when the insect walked towards one of the odor sources and touched the filtered paper. After every 5 insects, the Y-tube olfactometer was rotated 180°. Larvae were observed for a maximum of 10 min. We chose this time period as a parameter based on preliminary tests. Previous behavioral tests with plant extracts on hexane as odor sources showed that the odor source remains attractive for that period of time (Seenivasagan et al. 2009). Insects that did not respond after this period of time were excluded from the analysis. We compared the response of larvae for the extracts between (i) undamaged

plants vs. *T. absoluta*-infested plants ($N=40$); (ii) undamaged MeJA-treated plants vs. *T. absoluta*-infested-MeJA-treated plants ($N=30$); (iii) *T. absoluta*-infested plants vs. *T. absoluta*-infested MeJA-treated plants ($N=30$). All three comparisons were tested each day in order to avoid results that were the effect of date on behavior.

Statistical Analyses Larval development and pupal weight of *T. absoluta* were analyzed using ANOVA. Data were checked for normality and were analyzed using Minitab 16.0 software (Minitab, State College, PA, USA). Individual VOCs emissions were transformed (Log X+2) and analyzed with a two-way ANOVA that included herbivory, MeJA, and their interaction as main factors. The preference of the predators in the olfactometer was analyzed by applying a two-sided binomial test. The VOC emissions and the preference of *C. externa* were analyzed using SPSS 14.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Feeding Test Duration of the larval stage of *T. absoluta* was significantly longer for larvae fed on tomato plants treated with MeJA at the seed stage in comparison with the control (Fig. 1a) (MeJA: $F=229.61$, $df=1$, $P=0.001$). On average, larvae feeding on leaves of MeJA-treated plants took 1.3 days longer to pupate in comparison with those fed control plants. Similarly, larvae reared on leaves from the treated plants showed lower pupal weights in comparison with those reared on the leaves from untreated plants (Fig. 1b) (MeJA: $F=341$, $df=1$, $P=0.001$).

Emission of Volatile Organic Compounds by Tomato Plants Twenty eight compounds that include aliphatic and aromatic compounds as well as an array of terpenoids were identified in the headspace of tomato plants under the different treatments (Table 1). Herbivory alone significantly increased the emissions of α -pinene ($P=0.016$), 6-methyl 5-hepten-2-one ($P=0.011$), β -myrcene ($P=0.011$), (*E*)- β -ocimene ($P=0.019$), isoterpinolene ($P=0.021$), TMTT ($P=0.017$), (*Z*)-3-hexenyl butyrate ($P<0.001$), decanal ($P=0.032$), and hexyl salicylate ($P=0.009$). The emissions of several compounds from uninfested MeJA-treated plants decreased when compared to uninfested untreated plants. This was not the case for herbivore-infested plants, except for the emission of α -cubebene ($P=0.047$), which was significantly decreased by the MeJA treatment in both uninfested and infested plants. In addition, the MeJA treatment significantly affected α -pinene ($P=0.023$ and) and β -myrcene ($P=0.017$). The results of the interactions suggest that herbivory affected the emissions of α -pinene ($P=0.021$), β -myrcene ($P=0.012$), γ -terpinene ($P=$

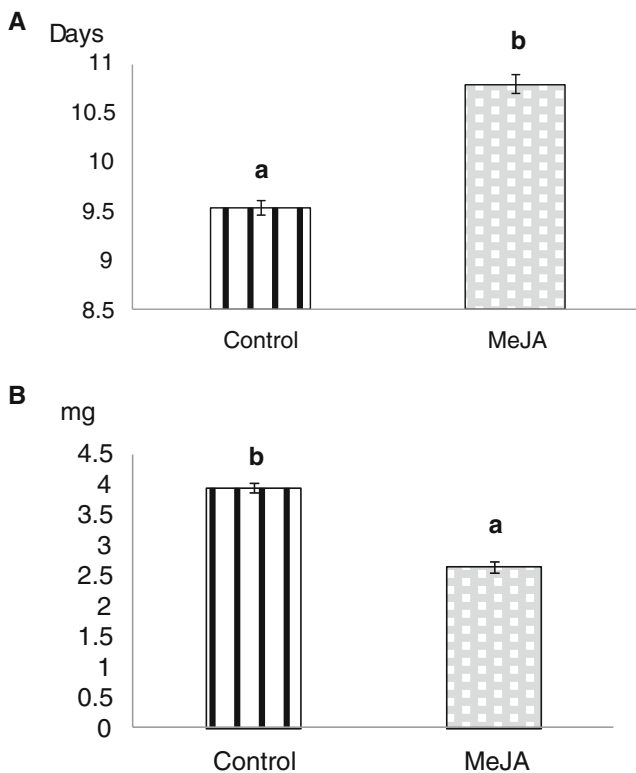


Fig. 1 The effect of MeJA on the performance of *Tuta absoluta*. **a** Length of development to pupation (days) and **b** Pupal weight (mg). Data are shown as means. Error bars represent the standard error of the mean, and the letters indicate statistical different means as determined by Tukey's Test

0.010), and geranyl acetone ($P=0.031$) in the presence of the MeJA treatment (Table 2). While the MeJA treatment tended to reduce the emission from uninfested plants, the treatment tended to increase the emission of these compounds in the presence of herbivory.

Odor Preference of *Chrysoperla externa* Third instar larvae of *C. externa* oriented toward the odor of the extracts from *T. absoluta*-infested tomato plants in both untreated ($P=0.038$) and MeJA-treated ($P=0.001$) plants over their respective controls. There was, however, no difference in the responses of the larvae to infested plant extracts that had been treated with MeJA or not (Fig. 2).

Discussion

The use of biological and chemical elicitors to induce resistance is considered a sound strategy for pest management programs (Stout et al. 2002). A good number of studies have addressed the effects of elicitor treatments at the seed stage to improve pathogen resistance, but only few have explored long lasting induced resistance against herbivores (Worrall et al.

2012). Seed treatments may offer advantages over foliar applications (Thaler 1999) or root drenches (Hodge et al. 2011) as they can be more cost-effective and reduce the negative consequences associated with pesticide use. Here, we addressed whether treating tomato seeds with MeJA affects the performance of *T. absoluta*, one of the major pests of tomato in South America, and whether the treatment can affect the preference of one of its main predators *C. externa* in this region. The results from the feeding test showed preliminary evidence that MeJA increases larval developmental time, which would reduce the number of generations per growing season. Because of the lengthened larval period, the larvae would also be exposed longer to predators and parasitoids, which would reduce pest populations (Price et al. 1980). In addition to increasing larval development time, the MeJA treatment reduced pupal weight. Pupal weight may be directly correlated with adult weight, which in turn is often correlated with fecundity and longevity of adult females as previously reported (Honěk 1993; Leather 1988), although this is not always the case (Fenimore 1977). Further studies are needed to understand how longer development and lower pupal weight caused by MeJA may affect *T. absoluta* population dynamics. Reduced performance of the herbivore may directly affect the performance of natural enemies due to decreased food quality. Treating plants of *Vicia faba* with a soil drench of β -amino butyric acid adversely affected the size of the endoparasitoid *Aphidius ervi* that developed in aphids fed treated plants (Hodge et al. 2011). Nevertheless, Hodge et al. (2011) suggest that predatory arthropods such as chrysopids may not be affected, as they may be able to compensate for lower food quality by increasing food consumption.

Among other factors, herbivory can increase plant volatile emissions (Dudareva et al. 2006). In tomato, significant increases of (*E*)- β -ocimene and TMTT occurred after herbivory by *Tetranychus urticae* (Acari), in addition to increases of linalool, (*E*)-nerolidol, and MeSA (Ament et al. 2004). In our study, the feeding by *T. absoluta* increased the emission of the terpenoids (*E*)- β -ocimene, isoterpinolene and TMTT and resulted in a slight, but not significant, increase of MeSA. We did not detect linalool or (*E*)-nerolidol in the headspace of tomato plants, which probably is due to the genetic characteristics of the studied cultivar. These two compounds have not been reported previously in the headspace volatiles from tomato cultivar Santa Clara (Proffit et al. 2011). Our results, however, contrast with those of Thaler et al. (2002) who reported an increase in several compounds after herbivory by the polyphagous Lepidopteran species *Spodoptera exigua*. In addition to plant genotype and possibly different herbivore-derived elicitors that lead to specific plant responses (De Moraes et al. 1998) that may result from differing feeding habits among herbivore species (Turlings and Wäckers 2004). *Tuta absoluta* is restricted to Solanaceae species and feeds on the parenchyma of the leaves, which results in leaf mines.

Table 1 Emissions of individual volatile organic compounds (Ng G Dw⁻¹ 24 h⁻¹) by undamaged and *Tuta absoluta*-infested plants subjected to a methyl jasmonate treatment at the seed stage (Mean±S.E.M; N=7)

	K.I.	Control			Herbivory			
		MeJA-untreated	MeJA-treated		MeJA-untreated	MeJA-treated		
(E)-2-hexen-1-ol	n. c.	12.50 ± 7.92	7.18 ^a		5.51 ^a		4.53 ^a	
(Z)-3-hexen-1-ol	n. c.	18.92 ± 12.22	1.79 ^a		32.01 ± 20.72		11.06 ± 9.81	
α-pinene	n. c.	120.02 ± 25.41	25.17 ± 10.51		121.52 ± 27.52		134.32 ± 31.29	
6-methyl-5-hepten-2-one	951	32.02 ± 9.28	9.67 ± 4.35		35.31 ± 5.83		34.18 ± 7.74	
β-myrcene	963	43.90 ± 7.05	14.29 ± 6.96		42.10 ± 5.98		49.65 ± 12.16	
2-carene	1,003	2,290.15 ± 493.48	500.86 ± 98.45		1,979.13 ± 497.13		1,939.53 ± 336.02	
α-phellandrene	1,009	144.82 ± 48.97	32.08 ± 9.41		174.76 ± 56.55		190.45 ± 53.47	
α-terpinene	1,019	83.70 ± 31.98	14.68 ± 6.85		107.16 ± 38.49		101.36 ± 28.99	
Limonene	1,032	768.42 ± 253.39	219.92 ± 52.58		871.43 ± 207.82		894.33 ± 234.26	
β-phellelandrene	1,035	4,202.77 ± 980.61	1,004.36 ± 203.72		3,194.73 ± 921.45		3,758.19 ± 729.59	
(E)-β-ocimene	1,048	9.08 ± 4.49	3.80 ± 2.47		36.12 ± 13.38		25.58 ± 10.56	
γ-terpinene	1,062	13.41 ± 3.75	3.23 ± 1.57		13.57 ± 5.78		19.32 ± 2.69	
Isoterpinolene	1,086	10.84 ± 7.03	1.32 ^a		16.38 ± 6.65		23.79 ± 7.96	
Nonanal	1,107	174.92 ± 61.06	118.28 ± 41.13		193.03 ± 58.85		305.31 ± 72.91	
(Z)-3-hexenyl butyrate	1,184	1.36 ^a	0.52 ^a		38.11 ± 14.50		26.40 ± 11.72	
MeSA	1,197	48.75 ± 21.94	72.24 ± 53.70		93.62 ± 25.07		140.13 ± 52.35	
Decanal	1,206	352.76 ± 78.46	160.38 ± 58.48		570.53 ± 248.23		454.77 ± 127.48	
Δ-elemene	1,344	63.00 ± 25.36	47.44 ± 40.55		82.50 ± 35.02		156.85 ± 82.48	
α-cubebene	1,385	8.12 ± 2.77	0.24 ^a		11.93 ± 5.67		4.79 ± 1.87	
Longifolene	1,397	14.80 ± 6.95	6.87 ± 4.50		12.84 ± 6.66		14.70 ± 5.47	
β-caryophyllene	1,433	445.05 ± 109.91	207.93 ± 144.17		296.80 ± 82.58		542.32 ± 263.86	
γ-elemene	1,438	7.46 ± 4.82	4.41 ± 2.89		6.76 ± 3.32		7.31 ± 3.90	
Geranyl acetone	1,448	34.80 ± 14.33	8.03 ± 3.45		15.57 ± 5.61		31.36 ± 8.60	
α-gurjunene	1,452	14.54 ± 3.94	4.29 ± 2.74		10.20 ± 4.38		6.37 ± 2.46	
α-humulene	1,470	81.83 ± 18.91	60.16 ± 35.22		56.57 ± 20.26		112.40 ± 53.46	
TMTT	1,575	1,102.53 ± 394.14	615.73 ± 323.18		2,277.27 ± 486.01		1,897.53 ± 655.25	
Hexyl salicylate	1,685	17.26 ± 11.17	5.32 ± 3.46		19.15 ± 2.44		15.30 ± 3.07	
(Z)-nerolidol	n. c.	4.83 ^a	6.29 ± 4.33		41.50 ± 35.03		13.90 ± 10.60	

S.E.M. Standard Error of the Mean, K.I. calculated Kovats Index, n.c. not calculated

^a Compound emitted by only one sample. S.E.M. was not calculated

Infestation period duration (Kant et al. 2004) and the low density of the infestation chosen (Girling et al. 2011), may also have affected the emission of induced compounds.

Surprisingly, the MeJA-treatment tended to decrease the emissions by undamaged tomato plants (Table 1). These results contrast with those observed in another study where tomato plants were treated at the seed stage with JA and showed increased emissions of TMTT compared to untreated plants as well as induction of methyl salicylate (Smart et al. 2013). In addition to the genotype, one major difference between the two studies in tomato is the concentration of elicitor used. Whereas we used 0.8 mM MeJA for inducing resistance of the future plants, Smart et al. (2013) used a much higher concentration (3 mM JA) of the elicitor. The MeJA concentration used in our study was selected based on previous studies

showing negative effects on the performance of *H. zea* and an assessment of physiological responses (germination percentage, seedling growth, days to ripeness, and average fruit weight/plant) of tomato cultivar Micro-Tom at Penn State University (Paudel et al. unpublished). Plants may respond differently to various elicitor concentrations. The response of parasitoids to JA-induced Brussels sprout plants, for instance, is dose-dependent (Bruinsma et al. 2009), which suggests that induced VOCs vary depending on the concentration of the elicitor applied. At the lower dose of 0.8 mM, plants may have allocated the exogenous MeJA to direct chemical or mechanical defenses, or to other metabolic processes that resulted in lower volatile emissions in the absence of herbivory.

To determine the effect on trichome density, we made a preliminary counting of the total numbers per mm² in 4 plants

Table 2 *P*-values for main effects of the MeJA-treatment to seeds and herbivory (by *Tuta absoluta*) and their interactions on the emissions of individual compounds

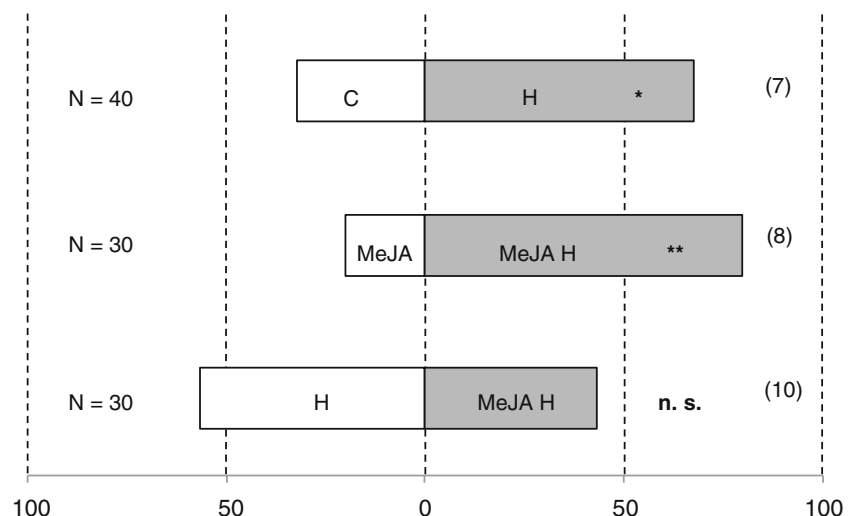
	MeJA	Herbivory	MeJA x Herbivory
(<i>E</i>)-2-hexen-1-ol	0.542	0.493	0.579
(<i>Z</i>)-3-hexen-1-ol	0.333	0.607	0.791
α-pinene	0.023	0.016	0.021
6-methyl-5-hepten-2-one	0.102	0.011	0.124
β-myrcene	0.017	0.011	0.012
2-carene	0.123	0.090	0.082
α-phellandrene	0.441	0.168	0.157
α-terpinene	0.463	0.122	0.203
Limonene	0.742	0.054	0.789
β-phellelandrene	0.171	0.133	0.051
(<i>E</i>)-β-ocimene	0.278	0.019	0.841
γ-terpinene	0.792	0.072	0.010
Isoterpinolene	0.898	0.021	0.276
Nonanal	0.330	0.152	0.597
(<i>Z</i>)-3-hexenyl butyrate	0.636	< 0.001	0.837
MeSA	0.926	0.061	0.710
Decanal	0.123	0.032	0.123
Δ-elemene	0.805	0.204	0.491
α-cubebene	0.047	0.300	0.226
Longifolene	0.708	0.617	0.180
β-caryophyllene	0.054	0.435	0.149
γ-elemene	0.741	0.688	0.613
Geranyl acetone	0.953	0.376	0.031
α-gurjunene	0.064	0.908	0.248
α-humulene	0.190	0.369	0.099
TMTT	0.377	0.017	0.584
Hexyl salicylate	0.279	0.009	0.880
(<i>Z</i>)-nerolidol	0.967	0.242	0.576

from each treatment, but we did not find a clear difference between both MeJA treatments (control upper side 5.2 ± 1.1 trichomes/mm²; lower side 15.6 ± 5.3 trichomes/mm²; MeJA upper side 7.4 ± 2.3 trichomes/mm²; lower side 16.8 ± 5.0 trichomes/mm² by Scanning Electron Microscopy). This suggests that induction of trichomes did not occur in MeJA treated plants. Nevertheless, treating the seeds of tomato cultivar Micro-Tom with MeJA, has resulted in increased levels of polyphenol oxidase in the leaves, with higher levels at higher concentrations (tested up to 1 mM) (Paudel *et al.* unpublished), while low doses of MeJA may have triggered stomatal closure, which may restrict the release of volatile compounds (Akter *et al.* 2013). The fact that some volatile compounds decreased in MeJA-treated plants is interesting because it may result in tomato plants that are less apparent to herbivores that rely on volatile cues for host location (Bleeker *et al.* 2009; Proffit *et al.* 2011). This was not within the scope of our study, but deserves further investigation as it may also be exploited to manipulate herbivore behavior and reduce pest pressure.

Jasmonic acid is involved in the activation of both direct and indirect responses of plants to herbivores, in addition to its role in plant physiological functions. For MeJA-treated and untreated plants, similar emissions were observed for most of the compounds after herbivory (except for α-cubebene that decreased significantly in both uninfested and infested plants treated with MeJA). This suggests that MeJA treatment did not affect the storage pools and the induction of VOCs upon herbivory. Hypothetically, plant species whose response is based on quantitative differences between volatile blends emitted from herbivore-damaged and mechanically damaged plants have a high level of direct defenses (Dicke *et al.* 1998), which is indeed the case for tomato (Kennedy 2003).

The development of new techniques such as the induction of resistance should be assessed in a multitrophic context (Stenberg *et al.* 2010). The results from the bioassay suggest

Fig. 2 Preference of *Chrysoperla externa* towards the odors of control (C) vs. herbivore-infested plants (H); MeJA treated plants (MeJA) vs. MeJA-treated herbivore-infested plants (MeJA H) and herbivore-infested vs. MeJA-treated herbivore-infested plants. Asterisks show the level of significance $P < 0.05$ *; $P < 0.01$ **, n.s. means “not significant”. Numbers on the left show the total number of insects tested in each treatment (*N*). The number of non responsive insects is shown on the right side in brackets



that chemical differences recorded in the VOC emissions do not interfere with the foraging behavior of the predator *C. externa*. We did not find any enhanced attraction of the predator towards the extracts of MeJA-treated plants when compared to those from untreated plants after infesting them with *T. absoluta*. It is possible that the compounds that differed between treatments were relevant for the predator. Additionally, no effect on behavior was observed for a parasitoid wasp in olfactometer tests using plants treated (through soil drenches) with β -amino butyric acid (Hodge et al. 2011).

Smart et al. (2013) found that the treatment of tomato seeds with a higher dosage of JA resulted in the emissions of volatiles that attract predatory mites in the absence of herbivory. Based on the volatiles produced in our study (no induced volatile compounds in uninfested plants), we did not assess the response of *C. externa* to the extracts from control and MeJA-treated, uninfested plants. Increasing volatile emissions has been regarded as an approach to boost biological control in crop fields. Nevertheless, the inducibility of both direct and indirect defenses has been favored by evolution as it reduces physiological and ecological costs for the plant (Agrawal and Karban 1999), and therefore the induction of VOCs in the absence of the herbivore should be cautiously assessed.

For behavioral tests, extracts from VOCs collected over 24 h were used, and thus they were a mixture of VOCs collected over the photo- and scotophase. The biosynthesis and emission of several compounds is known to be light-dependent (Kesselmeier and Staudt 1999; Loughrin et al. 1994), and variations of day and night VOC emissions have particular ecological functions (De Moraes et al. 2001). With the exception of TMTT and methyl salicylate that were emitted in a light-dependent fashion, Faraq and Paré (2002) found that undamaged and herbivore-damaged tomato plants emit similar amounts of terpenoids and GLVs during the photo and scotophase. This suggests that release of terpenoids occurs from storage pools (e.g., compounds stored in trichomes). For the aim of our study, we do not consider that having collected VOCs over 24 h affected the results (all samples collected under the same lighting conditions). The fact that the predators were able to discriminate between the extracts of undamaged and damaged plants supports our results.

Our data provide evidence that a seed treatment with a low concentration of MeJA can be used to improve pest resistance, and that the chemical differences in VOCs do not alter the behavior of the predator *C. externa*. However, further studies are needed to understand whether the predator can be indirectly affected (e.g., reduced development or fecundity due to reduced quality of the prey). The array of plant responses to chemical elicitors such as JA depends upon elicitor dosage, how it is applied to the plant, the plant genotype and age, and possibly, the intensity of herbivory (e.g., herbivore density) that challenges the plant. Further research is needed to clarify

these responses in order to successfully integrate elicitor treatments into integrated pest management systems.

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