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Authors: Groux R, Hilfiker O, Gouhier-Darimont C, Peñaflor MF, Erb M, Reymond P

Journal: Journal of chemical ecology

Year: 2014 Jul

Volume: 40

Issue: 7

Pages: 754-9

DOI: 10.1007/s10886-014-0470-9

ROLE OF METHYL SALICYLATE ON OVIPOSITION DETERRENCE IN *Arabidopsis thaliana*

RAPHAËL GROUX, OLIVIER HILFIKER, CAROLINE GOUHIER-DARIMONT, MARIA FERNANDA GOMES VILLALBA PEÑAFLOR, MATTHIAS ERB, PHILIPPE REYMOND*

Department of Plant Molecular Biology, University of Lausanne, CH-1015 Lausanne, Switzerland

Raphaël Groux, Olivier Hilfiker, Caroline Gouhier-Darimont, Philippe Reymond
Department of Plant Molecular Biology, Unversity of Lausanne, Biophore Building, CH-1015 Lausanne, Switzerland.

Tel: +41 21 692 42 29
Fax: +41 21 692 41 95
Email: philippe.reymond@unil.ch

Matthias Erb
Laboratory of Fundamental and Applied Research in Chemical Ecology (FARCE), University of Neuchâtel, Institute of Biology, Rue Emile-Argand, 11, CP 158,
CH-2009 Neuchâtel, Switzerland
Present address: University of Bern, Institute of Plant Sciences, Section Biotic Interactions, Altenbergrain 21, CH-3013 Bern, Switzerland

Maria Fernanda Gomes Villalba Peñaflor
Department of Entomology and Acarology, Laboratory of Chemical Ecology and Insect Behavior, University of São Paulo, Escola Superior de Agricultura "Luiz de Queiroz",
Av. Pádua Dias, 11, CP 09, 13418-900 Piracicaba, SP, Brazil

Abstract - Plants attacked by herbivores employ different strategies to fend off their enemies. Insect eggs deposited on leaves have been shown to inhibit further oviposition through visual or chemical cues. In some plant species the volatile methyl salicylate (MeSA) was shown to repel gravid insects but whether it plays the same role in the model species *Arabidopsis thaliana* is currently unknown. Here we showed that *Pieris brassicae* butterflies laid fewer eggs on *Arabidopsis* plants that were next to a MeSA dispenser or on plants with constitutively high MeSA emission than on control plants. Surprisingly, the MeSA biosynthesis mutant *bsmt1-1* treated with egg extract was still repellent to butterflies when compared to untreated *bsmt1-1*. Moreover, the expression of BSMT1 was not enhanced by egg extract treatment but was induced by herbivory. Altogether, these results provide evidence that the deterring activity of eggs on gravid butterflies is independent of MeSA emission in *Arabidopsis* and that MeSA might rather serve as a deterrent in plants challenged by feeding larvae.

Key Words - Oviposition, *Pieris brassicae*, Methyl Salicylate, *Arabidopsis thaliana*.

INTRODUCTION

Plant volatiles play a preponderant role in plant ecology where they serve, among other roles, to inform the surrounding organisms of the plant's physiological status. In response to herbivory, plants trigger complex direct and indirect defenses to ward off their enemies (Howe and Jander 2008; Wu and Baldwin 2010; Mithöfer and Boland 2012). As indirect defenses, attacked plants emit a blend of volatiles that attract parasitoid wasps and insect predators (Dicke and Baldwin 2010). Oviposition by phytophagous insects is known to be tightly dependent on the chemistry of the host plant (Renwick and Chew 1994; Hilker and Meiners 2011). For instance, plant volatiles are used by gravid insects to detect suitable substrate for oviposition (Rothschild and Schoonhoven 1977). On the opposite, the presence of eggs deters butterflies from further oviposition. This behavior is linked to visual and chemical cues from either eggs or plants (Rothschild and Schoonhoven 1977; Schoonhoven et al. 1981; Bergström et al. 1994; Renwick and Chew 1994; Blaakmeer et al. 1994a; de Vos et al. 2008).

We recently discovered that *Arabidopsis thaliana* reacts to *Pieris brassicae* oviposition by accumulating salicylic acid (SA), a signal molecule that is essential for defense against fungal and bacterial pathogens (Bruessow et al. 2010). Early detection of egg-associated elicitors triggers a response similar to basal innate immunity, with the production of reactive oxygen species, callose deposition, local cell death, and activation of the SA pathway, leading to the expression of defense genes (Little et al. 2007; Gouhier-Darimont et al. 2013; Reymond 2013). This finding was unexpected since feeding larvae are known to activate the jasmonic acid (JA) pathway, which is essential for an efficient defense against herbivory (Reymond et al. 2004; Howe and Jander 2008). Accordingly, the transcriptome of oviposited *Arabidopsis* plants was strikingly different from plants challenged with feeding larvae (Little et al. 2007).

Interestingly, MeSA is a common volatile derived from SA through methylation by the enzyme BSMT1 and was shown to repel aphids, moths and thrips in soybean, *Brassica napus*, and cucumber, respectively (Koschier et al. 2007; Ulland et al. 2008; Mallinger et al. 2011). This volatile is released after herbivory in *Arabidopsis* and tomato (Van Poecke et al. 2001; Chen et al. 2003; Ament et al. 2005; Snoeren et al. 2010a) but its role in response to oviposition has never been assessed. In addition, BSMT1 was shown to metabolize other substrates, including benzoic acid, *m*-hydroxybenzoic acid, and anthranilic acid (Chen et al. 2003).

Because *P. brassicae* oviposition on *Arabidopsis* induces the accumulation of SA, we reasoned that MeSA could be produced and deter future oviposition by butterflies. As eggs are inert and represent a non-immediate threat to the plant, this could represent an early response to avoid further increase in egg load.

METHODS AND MATERIALS

Plant and Insects Growth Conditions. All experiments were carried out in *Arabidopsis thaliana* Columbia ecotype (Col-0) background. Plants were grown in soil in growth chambers in short day conditions (8 h light, 20° C, 65 % relative humidity, 100 μmol m^{-2} s^{-1}). The soil consisted of 65% humus, 10% sand, 15% perlite and 10% silt and was not complemented with fertilizer. The *bsmt1-1* mutant (SALK_140496) was kindly provided by Jürgen Zeier (University of Duesseldorf), and OsS6 was obtained from Yang Do Choi (Seoul National University). Individuals of *Pieris brassicae* were reared on *Brassica oleracea* var *gemmifera* in a greenhouse (Reymond et al. 2000).

Oviposition Dual Choice Assays with P. brassicae Butterflies. Four- to five-week-old plants were used for choice assays. All experiments were performed in a greenhouse under constant

light. Three females and two males butterflies were placed in insect tents (60x60x60 cm, Bugdorm, Taiwan) with four plants of each treatment. During the experiment, butterflies were allowed to mate and drink sugary water. The number of eggs laid was assessed after 12 h. Methyl salicylate (Sigma-Aldrich, purity >99 %) was diluted in hexane to a final concentration of 0.15 mg/µl, and 5 µl was applied to a volatile dispenser consisting of half cotton swabs disposed at the center of the pot. Hexane was applied to the cotton swabs of control plants. The solvent was allowed to evaporate before beginning of the experiment. The amount of MeSA used in the experiment was shown to repel *Mamestra brassicae* moths and corresponds to a release rate of 50-100 ng/h (Ulland et al. 2008).

P. brassicae eggs laid on cabbage leaves were crushed with a pestle in Eppendorf tubes. After centrifugation (15'000 g, 3 min), the supernatant ("egg extract") was stored at -20°C. For egg-extract treatment, 2 x 2 µl of egg extract were applied to the abaxial surface of two leaves per plant for three days.

Each comparison was performed several times in parallel and replicated on different days. Data were analyzed comparing the number of eggs laid on each genotype/treatment using a *Generalized Linear Model* (*GLM*) controlling for tent and temporal effects.

Quantitative Real-Time PCR (QPCR). Egg-extract treatment was performed by applying 2 x 2 µl of *P. brassicae* egg extract on two leaves per plant for five days. For herbivory treatment, two *P. brassicae* neonates were placed on each of four *Arabidopsis* plants and allowed to feed for two days. Tissue samples from local leaves treated with egg-extract were ground in liquid nitrogen. Total RNA was extracted using RNeasy Plant Mini kit and treated with DNaseI according to the manufacturer's instructions (Qiagen). cDNA was synthesized from 500 ng of total RNA using M-MLV reverse transcriptase (Invitrogen) and diluted eightfold with water. Quantitative real-time PCR reactions were performed using Brilliant III Fast SYBR-Green

QPCR Master Mix on a Mx3000P real-time PCR instrument (Agilent) with the following program: 95 °C for 3 min, then 40 cycles of 10 s at 95 °C, 20 s at 60 °C. Values were normalized to the housekeeping gene *EIF4A1* (At3g13920). The expression level of a target gene (TG) was normalized to the reference gene (RG) and calculated as normalized relative quantity (NRQ) as follows: $NRQ = E^{CtRG}/E^{CtTG}$. Primer efficiencies (E) were evaluated by five-step dilution regression. For each experiment, three biological replicates were analyzed. Different genes analyzed were amplified using the following primers: *BSMT1* (AT3G11480) fwd 5'-CATTCAACATGCCGTTTTATG–3' and rev 5'-CATTGGTTCACTAACAGCTC-3'; *PR-1* (AT2G14610) fwd 5'-GTGGGTTAGCGAGAAGGCTA-3' and rev 5'-ACTTTGGCACATCCGAGTCT-3'; *EIF4A1* (At3g13920) fwd 5'-CCAGAAGGCACACAGTTTGA-3' and rev 5'-GACTGAGCCTGTTGAATCAC-3'.

Dynamic Headspace Collection and MeSA Analysis. To verify MeSA emission by *Arabidopsis* with and without *P. brassicae* oviposition, headspace volatiles of individual plants enclosed in glass bottles were collected in a volatile collection system (ARS, Gainesville, FL, USA). Airflow was regulated to 1.0 L/min and volatiles were trapped using SuperQ adsorbent polymer (Alltech Associates Inc., Deerfield, IL, USA). Volatiles were collected for eight plants during 24 h from 48 to 72 h after *P. brassicae* oviposition. Twelve plants without oviposition were used as controls. Headspace collection and analysis were done as described previously (Peñaflor et al. 2011), with the following modifications: after injection, the column temperature was maintained at 40 °C for 3 min and then increased to 100 °C at 8 °C/min and subsequently to 220 °C at 5 °C/min followed by a postrun of 3 min at 250 °C. MeSA was identified by comparing its mass spectra and retention time with MeSA pure standard (Sigma-Aldrich, St. Louis, MO, USA) and with that of the NIST05 library.

Statistical Analyses. All statistical analyses were carried out with R software version 3.0.1 (http://www.R-project.org).

RESULTS

Egg Extract Treatment and MeSA Repel P. brassicae Butterflies. The number of eggs laid on plants pretreated with *P. brassicae* egg extract, which mimics natural oviposition (Little et al. 2007), was significantly lower than on intact plants (Fig. 1a). When applied on a volatile dispenser placed next to *Arabidopsis* plants, MeSA decreased the total number of eggs laid compared to dispensers treated with solvent alone (Fig. 1b). Similarly, butterflies were repelled by *Arabidopsis* OsS6 mutant plants overexpressing a *BSMT1* homolog from rice (*Oryza sativa*) (Fig. 1c). OsS6 plants have been shown to emit MeSA constitutively, even in the absence of stimulus (Koo et al. 2007). Given the repelling role of MeSA and previous observations that oviposited plants accumulate SA (Bruessow et al. 2010), which could be transformed to MeSA by BSMT1, we then tested whether MeSA accumulates after oviposition in *Arabidopsis*. However, we could not detect MeSA in plant volatiles collected between 48 and 72 h after oviposition, with a detection limit < 1 ng (Online resource 1).

To further evaluate the involvement of MeSA we used the *Arabidopsis bsmt1-1* mutant that has no detectable MeSA emission (Attaran et al. 2009). Surprisingly, *bsmt1-1* plants treated with egg extract still repelled *P. brassicae* butterflies, as there were significantly more eggs laid on untreated than on treated *bsmt1-1* plants (Fig. 1d). Thus, our results indicate that MeSA emission is able to inhibit oviposition but that this volatile is not responsible for egg extract-induced deterrence of ovipositing butterflies in *Arabidopsis*. However, when butterflies were given the choice between egg extract-treated Col-0 and egg extract-treated *bsmt1-1*, they significantly laid more eggs on Col-0 (Fig. 1e). Finally,

butterflies also preferred non-treated Col-0 over non-treated *bsmt1-1*, suggesting that BSMT1 plays a role in attracting *P. brassicae* (Fig. 1f).

Expression of BSMT1 in Response to Herbivory. To further explore whether MeSA emission was linked to oviposition, we analyzed the expression of *BSMT1* and *PR-1* in plants treated with egg extract or challenged with *Pieris brassicae* larvae. *PR-1* is a marker gene for the SA pathway and is induced by *P. brassicae* egg extract treatment (Little et al. 2007; Gouhier-Darimont et al. 2013). *PR-1* expression increased after egg extract treatment but not after herbivory, which is consistent with previous studies (Reymond et al. 2004; Bruessow et al. 2010) (Fig. 2a). On the contrary, *BSMT1* expression was not induced after five days of egg extract treatment but was strongly induced after herbivory by *P. brassicae* (Fig. 2). In support of this finding, BMST1 was strongly upregulated by *Pieris rapae* feeding in *Arabidopsis* (Snoeren et al. 2010a). Moreover, egg extract treatment did not induce *BSMT1* after 24 h, 48 h, and 72 h (data not shown).

DISCUSSION

P. brassicae oviposition on plants pretreated with egg extract was lower than on untreated plants, confirming earlier observations with other plant species that butterflies can detect oviposited plants and avoid overloading (Rothschild and Schoonhoven 1977; Shapiro 1981; Blaakmeer et al. 1994a). Although egg extract was applied on leaves facing the soil to avoid visual recognition by butterflies, this set-up did not prevent detection of egg-derived cues, suggesting that a chemical response was involved. Interestingly, our results using artificial dispensers and overexpressing lines clearly show that MeSA emission deters oviposition. Previous studies reported a similar effect for the moth *M. brassicae*, the thrips *Frankliniella occidentalis*, and the hemipteran pest *Lygus Hesperus* (Koschier et al. 2007; Ulland et al.

2008; Williams et al. 2010). However, *bsmt1-1* plants lacking MeSA were still able to repel butterflies when treated with egg extract, strongly suggesting that the deterring activity of eggs on gravid butterflies is independent of MeSA emission. In support of this finding, expression of *BSMT1*, which was reported to reflect MeSA emission in *Arabidopsis* (Snoeren et al. 2010a), was not induced by egg extract treatment. Moreover, we could not detect MeSA from the volatile blend of oviposited *Arabidopsis* plants. By comparison, OsS6 plants emit 6 ± 2 ng/g FW/ 24 h (Koo et al. 2007) and *Arabidopsis* infected with *Pseudomonas syringae* pv. *maculicola* emit between 15 to 45 ng/g FW/ h (Attaran et al. 2009), values that are well above the ca. 1 ng detection limit of our instrument. Furthermore, oviposition of *P. brassicae* even reduced MeSA emission in *Brassica oleracea* (Bergström et al. 1994). Finally, it was also reported that plants treated with SA do not release MeSA (Koo et al. 2007). Collectively, these data provide strong evidence that MeSA is not involved in repelling butterflies after oviposition or treatment with egg extract.

Interestingly, we found that *bsmt1-1* plants received fewer eggs than wild-type plants in dual-choice experiments, irrespective of egg extract pre-treatment. This suggests that BSMT1 might have a positive role by producing a compound that attracts female butterflies. BSMT1 belongs to the SABATH family of methyl transferases and *in vitro* analyses have shown that, besides SA, this enzyme catalyzes the methylation of benzoic acid, anthranilic acid, and m-hydrohybenzoic acid, with the highest activity towards benzoic acid (Chen et al. 2003). In addition, we noticed that *bsmt1-1* plants have longer petioles than Col-0 and display leaf epinasty (Online resource 2). Thus, whether any of the methylated metabolites and/or *bsmt1-1* leaf phenotype influence butterflies for their choice of an oviposition site will need further investigations.

Evidence for the absence of a role for MeSA in response to oviposition and the observation that egg extract-treatment repelled butterflies implies that other factor(s) may

render plants less acceptable for females. First, although egg extracts were applied underneath *Arabidopsis* leaves, we cannot formally exclude that visual factors informed butterflies about prior occupancy. Indeed, eggs or egg extract treatment cause chlorosis at the site of deposition in *Arabidopsis* Col-0 (Bruessow et al. 2010; Reymond 2013). An elegant experiment recently demonstrated that *P. rapae* butterflies could discriminate green *Arabidopsis* leaves from variegated green-whitish leaves, obtained after silencing a phytoene desaturase gene (Zheng et al. 2010). Alternatively, infochemicals from either the egg extract or the plant could be detected by gravid females. Avenanthramide alkaloids have been identified in eggs of *P. brassicae* and *P. rapae* and were shown to inhibit oviposition when sprayed on cabbage leaves (Blaakmeer et al. 1994b). However, since cabbage leaves were still repulsive after removal of *P. brassicae* eggs and avenanthramides were no longer detectable, other plant chemicals were postulated to deter oviposition although their nature has not been determined (Blaakmeer et al. 1994a). Glucosinolates (GS) are well-described defense compounds of the Brassicaceae (Halkier and Gershenzon 2006). Many crucifer specialists use GS as signals for oviposition, as larvae are able to detoxify them and thus feed unharmed on the plants (Huang and Renwick 1994; Renwick and Chew 1994; Hopkins et al. 2009). Induction of GS biosynthesis genes and GS accumulation are triggered by herbivory and are regulated by the JA pathway in *Arabidopsis* (Schweizer et al. 2013). Since *P. brassicae* eggs were shown to suppress the expression of JA-dependent defense genes in *Arabidopsis*, including GS-related genes (Bruessow et al. 2010), an intriguing hypothesis is that GS content might be reduced after oviposition and therefore this would lower the attractiveness of *Arabidopsis* plants for further egg laying. In order to test these hypotheses, future studies should aim at measuring leaf chemical changes or emission of volatiles that follow oviposition in *Arabidopsis* and use biosynthesis mutants to identify deterring molecules.

Previous microarray experiments on plants oviposited or damaged by herbivory reported that *BSMT1* was induced by *P. rapae* and *S. littoralis* feeding but not by eggs (Reymond et al. 2004; Little et al. 2007). We showed here by QPCR that *BMST1* is indeed not upregulated by egg extract treatment whereas it is strongly induced by herbivory. MeSA emission was reported to occur after herbivory in several plant species including *Arabidopsis*, (Van Poecke et al. 2001; Snoeren et al. 2010a,b), wild tobacco (Kessler and Baldwin 2001), maize (Turlings et al. 1998), rice (Zhao et al. 2010), cotton (Rodriguez-Saona et al. 2001), cucumber (Agrawal et al. 2002), and potato (Bolter et al. 1997). In support of these findings, BSMT1 transcript levels were induced after methyl jasmonate (MeJA) application and herbivory in *Arabidopsis* (Snoeren et al. 2010a; Chen et al. 2003), indicating that MeSA emission is under the control of the JA-pathway.

Interestingly, the production/emission of MeSA as well as the expression of *BSMT1* were also found to be induced after infection with the bacterial pathogen *P. syringae* in *Arabidopsis*. This effect was due to the presence of coronatine (COR), a bacterial effector that mimics JA-Ile, which is the bioactive JA (Attaran et al. 2009; Zheng et al. 2012). It would be interesting to carry out oviposition test with plants inoculated with *P. syringae* to test whether butterflies avoid infected plants to maximize the survival of their progeny. Use of COR^- strains could confirm the role of this effector in oviposition responses.

In conclusion, our results suggest that MeSA emission is not responsible for reduced oviposition by *P. brassicae* on egg-treated *Arabidopsis* plants but that it may rather play a role during larval feeding to block further oviposition. Whether this is a strategy developed by the plant to prevent an excess of attackers or by the insect to control food availability for developing larvae will deserve future studies. A recent meta-analysis offered clear support for the preference-performance hypothesis which states that female insects evolved to oviposit more eggs on plants on which their offspring performs best (Gripenberg et al. 2010). This

indicates that the avoidance of MeSA-emitting plants by females could be linked to a poorer performance on such plants.

REFERENCES

Agrawal AA, Janssen A, Bruin J, Posthumus MA, Sabelis MW (2002) An ecological cost of plant defence:attractiveness of bitter cucumber plants to natural enemies of herbivores. Ecol Lett 5:377–385

Ament K, Kant MR, Sabelis MW, Haring MA, Schuurink RC (2004) Jasmonic acid is a key regulator of spider mite-induced volatile terpenoid and methyl salicylate emission in tomato. Plant Physiol 135:2025–2037

Attaran E, Zeier TE, Griebel T, Zeier J (2009) Methyl salicylate production and jasmonate signaling are not essential for systemic acquired resistance in Arabidopsis. Plant Cell 21:954–971

Bergström G, Rothschild M, Groth I, Crighton C (1994) Oviposition by butterflies on young leaves: Investigation of leaf volatiles. Chemoecology 5-6 147–158

Blaakmeer A, Hagenbeek D, Vanbeek T, DeGroot A, Schoonhoven L, van Loon JJA (1994a) Plant response to eggs vs. host marking pheromone as factors inhibiting oviposition by *Pieris brassicae*. J Chem Ecol 20:1657–1665

Blaakmeer A, Stork A, Vanveldhuizen A, Vanbeek T, DeGroot A, van Loon JJA, Schoonhoven L (1994b) Isolation, identification, and synthesis of miriamides, new hostmarkers from eggs of *Pieris brassicae*. J Nat Prod 57:90–99

Bolter CJ, Dicke M, vanLoon J, Visser JH, Posthumus MA (1997) Attraction of Colorado potato beetle to herbivore-damaged plants during herbivory and after its termination. J Chem Ecol 23:1003–1023

Bruessow F, Gouhier-Darimont C, Buchala A, Metraux J-P, Reymond P (2010) Insect eggs suppress plant defence against chewing herbivores. Plant J 62:876–885

Chen F, D'Auria JC, Tholl D, Ross J, Gershenzon J, Noel J, Pichersky E (2003) An *Arabidopsis thaliana* gene for methylsalicylate biosynthesis, identified by a biochemical genomics approach, has a role in defense. Plant J 36:577–588

de Vos M, Kriksunov KL, Jander G (2008) Indole-3-acetonitrile production from indole glucosinolates deters oviposition by *Pieris rapae*. Plant Physiol 146:916–926

Dicke M, Baldwin IT (2010) The evolutionary context for herbivore-induced plant volatiles: beyond the "cry for help." Trends Plant Sci 15:167–175

Gouhier-Darimont C, Schmiesing A, Bonnet C, Lassueur S, Reymond P (2013) Signalling of *Arabidopsis thaliana* response to *Pieris brassicae* eggs shares similarities with PAMP-

triggered immunity. J Exp Bot 64:665–674

Gripenberg S, Mayhew PJ, Parnell M, Roslin T (2010) A meta-analysis of preference-performance relationships in phytophagous insects. Ecol Lett 13:383–393

Halkier BA, Gershenzon J (2006) Biology and biochemistry of glucosinolates. Annu Rev Plant Biol 57:303–333

Hilker M, Meiners T (2011) Plants and insect eggs: How do they affect each other? Phytochemistry 72:1612–1623

Hopkins RJ, van Dam NM, van Loon JJA (2009) Role of glucosinolates in insect-plant relationships and multitrophic interactions. Annu Rev Entomol 54:57–83

Howe GA, Jander G (2008) Plant immunity to insect herbivores. Annu Rev Plant Biol 59:41–66

Huang X, Renwick JA (1994) Relative activities of glucosinolates as oviposition stimulants for *Pieris rapae* and *P. napi oleracea*. J Chem Ecol 20:1025–1037

Kessler A, Baldwin IT (2001) Defensive function of herbivore-induced plant volatile emissions in nature. Science 291:2141–2144

Koo YJ, Kim MA, Kim EH, Song JT, Jung C, Moon J-K, Kim J-H, Seo HS, Song SI, Kim J-K, et al (2007) Overexpression of salicylic acid carboxyl methyltransferase reduces salicylic acid-mediated pathogen resistance in *Arabidopsis thaliana*. Plant Mol Biol 64:1–15

Koschier EH, Hoffmann D, Riefler J (2007) Influence of salicylaldehyde and methyl salicylate on post-landing behaviour of *Frankliniella occidentalis* Pergande. J Appl Entomol 131:362–367

Little D, Gouhier-Darimont C, Bruessow F, Reymond P (2007) Oviposition by pierid butterflies triggers defense responses in Arabidopsis. Plant Physiol 143:784–800

Mallinger RE, Hogg DB, Gratton C (2011) Methyl salicylate attracts natural enemies and reduces populations of soybean aphids (Hemiptera: Aphididae) in soybean agroecosystems. J Econ Entomol 104:115–124

Mithöfer A, Boland W (2012) Plant defense against herbivores: chemical aspects. Annu Rev Plant Biol 63:431–450

Peñaflor MFGV, Erb M, Miranda LA, Werneburg AG, Bento JMS (2011) Herbivore-induced plant volatiles can serve as host location cues for a generalist and a specialist egg parasitoid. J Chem Ecol 37:1304-1313

Renwick J, Chew FS (1994) Oviposition behavior in Lepidoptera. Annu Rev Entomol 39:377–400

Reymond P (2013) Perception, signaling and molecular basis of oviposition-mediated plant responses. Planta 238:247–258

Reymond P, Bodenhausen N, Van Poecke RMP, Krishnamurthy V, Dicke M, Farmer EE (2004) A conserved transcript pattern in response to a specialist and a generalist herbivore. Plant Cell 16:3132–3147

Reymond P, Weber H, Damond M, Farmer EE (2000) Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. Plant Cell 12:707–720

Rodriguez-Saona C, Crafts-Brandner SJ, Paré PW, Henneberry TJ (2001) Exogenous methyl jasmonate induces volatile emissions in cotton plants. J Chem Ecol 27:679–695

Rothschild M, Schoonhoven L (1977) Assessment of egg load by *Pieris brassicae* (Lepidoptera: Pieridae). Nature 266:352–355

Schoonhoven LM, Sparnaay T, van Wissen W, Meerman J (1981) Seven-week persistence of an oviposition-deterrent pheromone. J Chem Ecol 7:583–588

Schweizer F, Fernández-Calvo P, Zander M, Diez-Diaz M, Fonseca S, Glauser G, Lewsey MG, Ecker JR, Solano R, Reymond P (2013) Arabidopsis basic helix-loop-helix transcription factors MYC2, MYC3, and MYC4 regulate glucosinolate biosynthesis, insect performance, and feeding behavior. Plant Cell 25:3117–3132

Shapiro AM (1981) Egg-mimics of *Streptanthus* (Cruciferae) deter oviposition by *Pieris sisymbrii* (Lepidoptera: Pieridae). Oecologia 48:142–143

Snoeren TAL, Kappers IF, Broekgaarden C, Mumm R, Dicke M, Bouwmeester HJ (2010a) Natural variation in herbivore-induced volatiles in *Arabidopsis thaliana*. J Exp Bot 61:3041–3056

Snoeren TAL, Mumm R, Poelman EH, Yang Y, Pichersky E, Dicke M (2010b) The herbivore-induced plant volatile methyl salicylate negatively affects attraction of the parasitoid *Diadegma semiclausum*. J Chem Ecol 36:479–489

Turlings T, Bernasconi M, Bertossa R, Bigler F, Caloz G, Dorn S (1998) The induction of volatile emissions in maize by three herbivore species with different feeding habits: Possible consequences for their natural enemies. Biol Control 11:122–129

Ulland S, Ian E, Mozuraitis R, Borg-Karlson A-K, Meadow R, Mustaparta H (2008) Methyl salicylate, identified as primary odorant of a specific receptor neuron type, inhibits oviposition by the moth *Mamestra brassicae* L. (Lepidoptera, Noctuidae). Chem Senses 33:35–46

Van Poecke RMP, Posthumus MA, Dicke M (2001) Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid *Cotesia rubecula*: chemical, behavioral, and gene-expression analysis. J Chem Ecol 27:1911–1928

Williams L, Blackmer JL, Rodriguez-Saona C, Zhu S (2010) Plant volatiles influence electrophysiological and behavioral responses of *Lygus hesperus*. J Chem Ecol 36:467–478

Wu J, Baldwin IT (2010) New insights into plant responses to the attack from insect herbivores. Annu Rev Genet 44:1–24

Zhao N, Guan J, Ferrer J-L, Engle N, Chern M, Ronald P, Tschaplinski TJ, Chen F (2010) Biosynthesis and emission of insect-induced methyl salicylate and methyl benzoate from rice. Plant Physiol Bioch 48:279–287

Zheng S-J, Snoeren TAL, Hogewoning SW, van Loon JJA, Dicke M (2010) Disruption of plant carotenoid biosynthesis through virus-induced gene silencing affects oviposition behaviour of the butterfly *Pieris rapae*. New Phytol 186:733–745

Zheng X-Y, Spivey NW, Zeng W, Liu P-P, Fu ZQ, Klessig DF, He SY, Dong X (2012) Coronatine promotes *Pseudomonas syringae* virulence in plants by activating a signaling cascade that inhibits salicylic acid accumulation. Cell Host Microbe 11:587–596

ACKNOWLEDGEMENTS

We thank Blaise Tissot for taking care of the plants. This work was supported by the Swiss National Science Foundation Grant 31003A_149286 and EUROCORES programme EuroVOL to P. R.

FIGURE LEGENDS

Fig. 1 Dual-choice oviposition tests with *P. brassicae*. Three females and two males *P. brassicae* butterflies were placed in a tent containing two groups of four *Arabidopsis* plants. The number of eggs laid was assessed after 12 h of continuous light. Plants used were wild-type (Col-0) unless otherwise specified. Boxplots represent values from six to twenty-five biological replicates. **a** Number of eggs laid on untreated plants or plants pretreated with *P. brassicae* egg extract (EE) for three days. **b** Number of eggs laid on control plants or plants placed next to a MeSA dispenser. MeSA (0.15 mg/µl in hexane) was applied to a volatile dispenser disposed at the center of the pot. Control plants were placed next to a hexane dispenser. **c** Number of eggs laid on wild-type or *Arabidopsis* OsS6 line that overexpresses the rice BSMT1 gene. **d** Number of eggs laid on untreated or EE-treated *bsmt1-1* plants. **e** Number of eggs laid on EE-treated Col-0 or *bsmt1-1* plants. **f** Number of eggs laid on untreated Col-0 or *bsmt1-1* plants. Oviposition data were analyzed with a *Generalized Linear Model*. Stars indicate a significant difference compared to the control (*** $P < 0.001$).

Fig. 2 Expression of *PR-1* **a** and *BSMT1* **b** after treatment with *P. brassicae* egg extract (EE) for five days or feeding by *P. brassicae* (*P. b.*) for two days. Expression levels were measured by QPCR and are relative to the housekeeping gene *EIF4A1*. Values are mean relative expression ± SE of three technical replicates. Similar results were obtained in two independent experiments. Different letters indicate significant differences at $P < 0.05$ (Tukey's honest significant difference test).

SUPPLEMENTARY MATERIAL

Online resource 1. MeSA analysis of *Arabidopsis* plants with or without *P. brassicae* oviposition.

Online resource 2. Phenotype of Col-0 and *bsmt1-1* plants

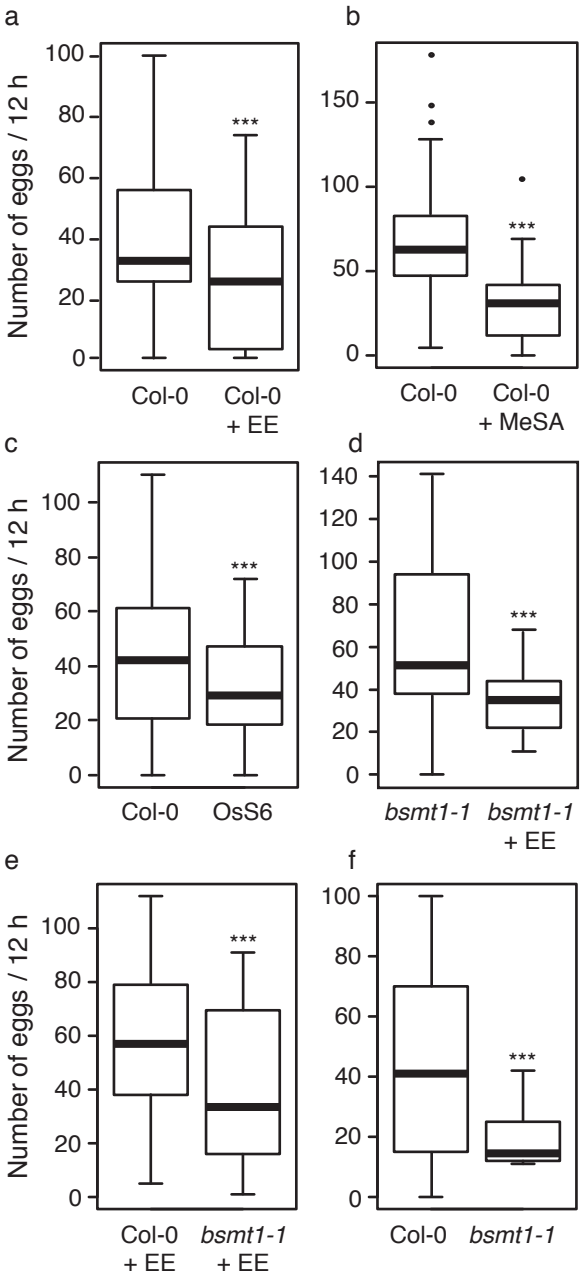

a
b
c
d
e
f
Number of eggs / 12 h
***
Col-0
Col-0
+ EE
Col-0
Col-0
+ MeSA
Col-0
OsS6
bsmt1-1
bsmt1-1
+ EE
Col-0
+ EE
bsmt1-1
+ EE
Col-0
bsmt1-1

Figure 1

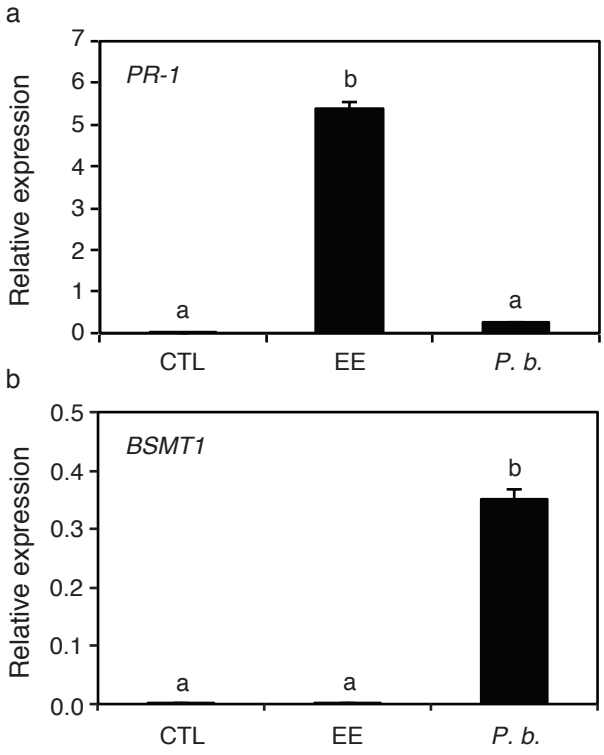
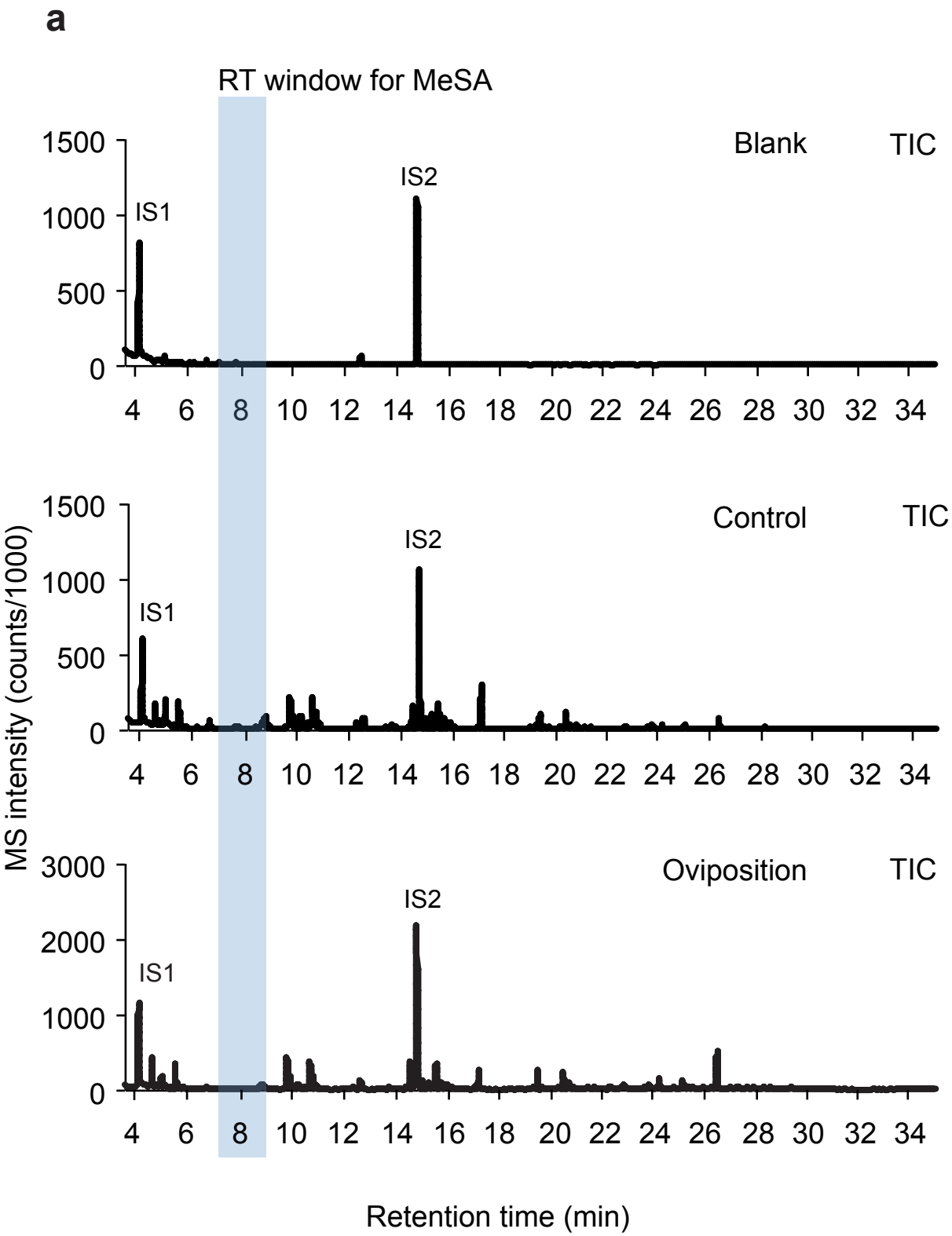
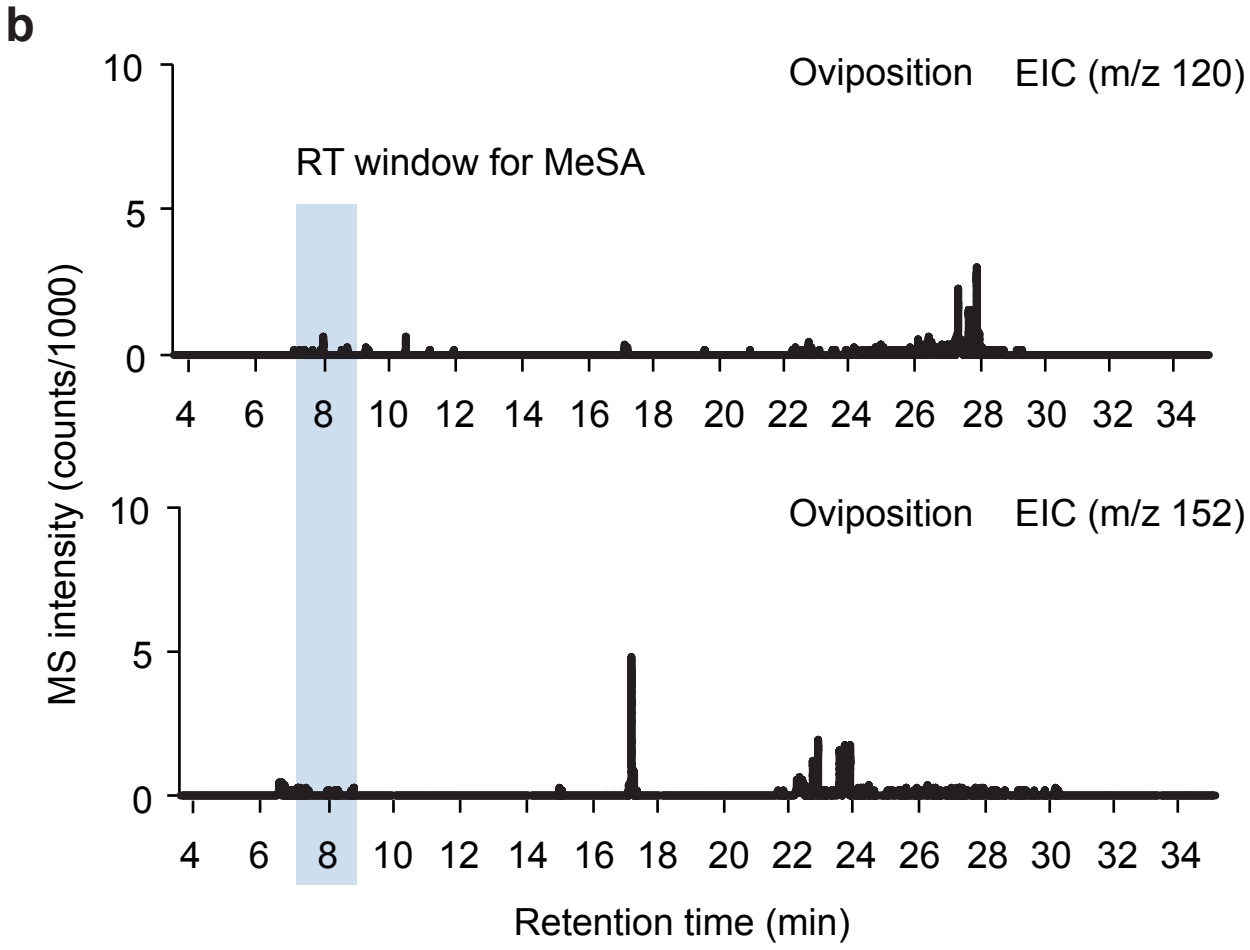

a
PR-1
Relative expression
7
6
5
4
3
2
1
0
a
b
a
CTL
EE
P. b.
b
BSMT1
Relative expression
0.5
0.4
0.3
0.2
0.1
0.0
a
a
b
CTL
EE
P. b.

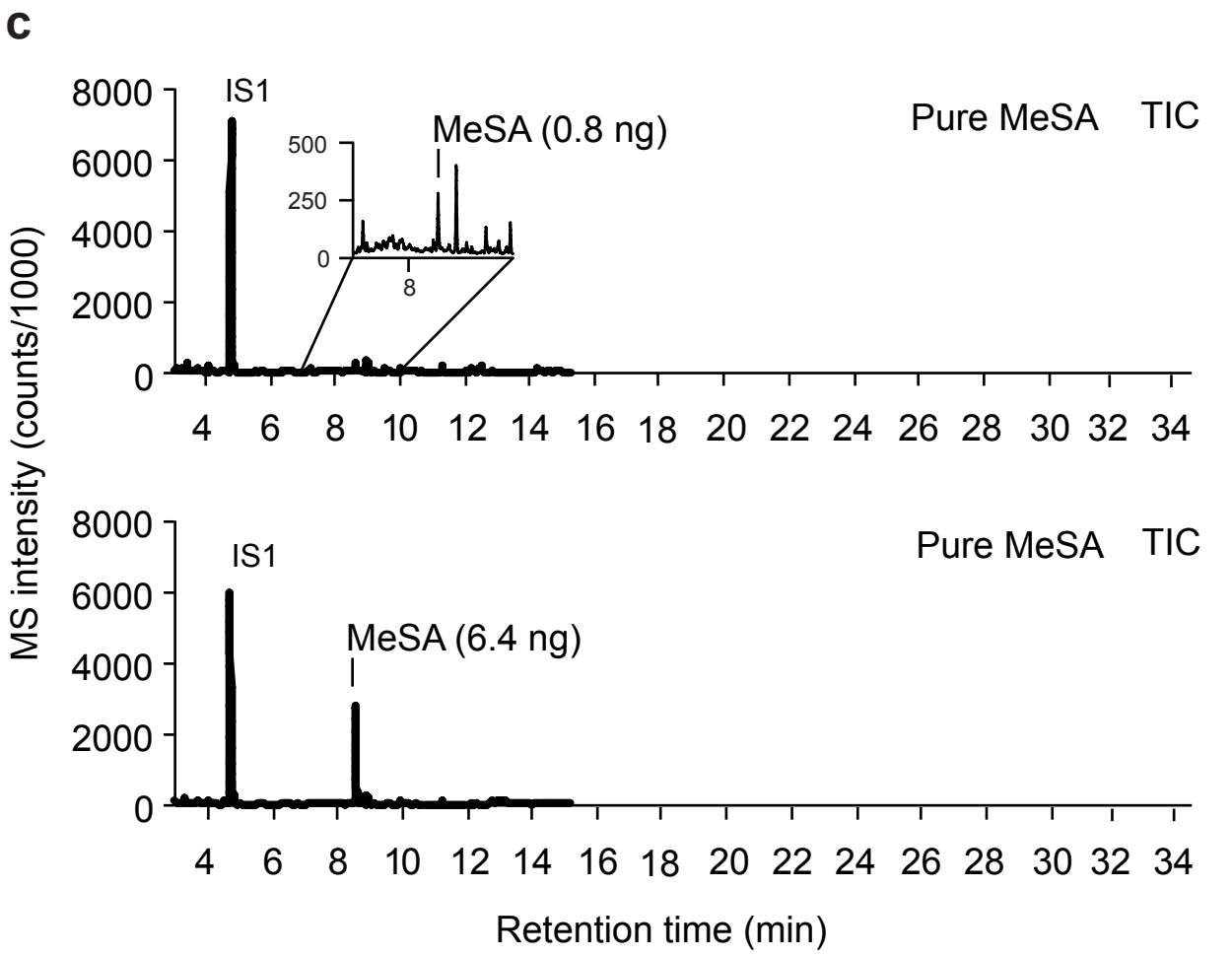
Figure 2

SUPPLEMENTARY MATERIAL

Online resource 1. MeSA analysis of *Arabidopsis* plants with or without *Pieris brassicae* oviposition. Volatiles were collected from 48 h to 72 h after oviposition. **a** Representative total ion chromatograms (TIC) from a blank sample (empty bottle), control plants and oviposited plants. **b** Extracted ion chromatogram (EIC) for ions characteristic of MeSA (mass spectra obtained from NIST05). **c** Total ion chromatogram of pure MeSA (Sigma Aldrich). IS1: internal standard 1 (n-octane); IS2: internal standard 2 (n-nonyl acetate).




b
Oviposition EIC (m/z 120)
RT window for MeSA
Oviposition EIC (m/z 152)
MS intensity (counts/1000)
Retention time (min)


c
IS1
MeSA (0.8 ng)
Pure MeSA TIC
IS1
MeSA (6.4 ng)
Pure MeSA TIC
MS intensity (counts/1000)
Retention time (min)

Online resource 2. Phenotype of 5-week-old Col-0 and *bsmt1-1* plants.

Col-0 *bsmt1-1*