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Antagonism between herbivore-induced plant volatiles and trichomes affects tritrophic interactions

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ABSTRACT

We used tomato genotypes deficient in the jasmonic acid (JA) pathway to study the interaction between the production of herbivore-induced plant volatiles (HIPVs) that serve as information cues for herbivores as well as natural enemies of herbivores, and the production of foliar trichomes as defence barriers. We found that *jasmonic acid-insensitive1* (*jai1*) mutant plants with both reduced HIPVs and trichome production received higher oviposition of adult leafminers, which were more likely to be parasitized by the leafminer parasitoids than JA biosynthesis *spr2* mutant plants deficient in HIPVs but not trichomes. We also showed that the preference and acceptance of leafminers and parasitoids to trichome-removed plants from either *spr2* or wild-type (WT) genotypes over trichome-intact genotypes can be ascribed to the reduced trichomes on treated plants, but not to altered direct and indirect defence traits such as JA, proteinase inhibitor (PI)-II and HIPVs levels. Although the HIPVs of WT plants were more attractive to adult insects, the insects preferred trichome-free *jai1* plants for oviposition and also had greater reproductive success on these plants. Our results provide strong evidence that antagonism between HIPV emission and trichome production affects tritrophic interactions. The interactions among defence traits are discussed.

Key-words: agromyzid leafminers; interaction between defence traits; *jai1* mutant; jasmonic acid-deficient mutant plants; leafminer parasitoid; *Liriomyza huidobrensis*; *Opius dissitus*; plant defences; *spr2* mutant; tomato plant.

INTRODUCTION

To optimize resistance against attacks, plants have evolved a variety of defence traits, including chemical defences such as non-volatile metabolites (e.g. toxins, digestibility reducers and deterrents) or herbivore-induced plant volatiles (HIPVs), and physical defence traits, such as trichomes, thorns or waxes (Kessler & Baldwin 2002; Runyon *et al.* 2010). These chemical and physical properties have intricate interactions from genetic to ecological levels (Dicke, van Loon & Soler 2009), and multiple levels of interaction may cause confusion in determining the relative role of each type of defence trait in plants (Rasmann & Agrawal 2009). Tritrophic interactions among plants, herbivores, and natural enemies represent a simplified and suitable model system to unravel the complexity of plant–insect interactions in communities, wherein plant chemistry has been proposed as the most important mediator in the defence systems of below- and above-ground tissues of a plant (Hiltpold *et al.* 2011). Toxic plant secondary metabolites exert not only direct negative effects on the growth and development of herbivores, but also indirect ones on their natural enemies (Kessler & Baldwin 2002; Kessler, Halitschke & Baldwin 2004; Wei *et al.* 2011). In particular, HIPVs are classic examples of plant odours playing a vital role in both attracting herbivores and their natural enemies (Dicke *et al.* 1990; Turlings *et al.* 1995; Kessler *et al.* 2004; Wei *et al.* 2007), as well as mediating plant–plant communication (Engelberth *et al.* 2004; Wei & Kang 2011). Plant volatiles can be released constitutively or after herbivore damage (Pare & Tumlinson 1997); they can vary with plant species and genotype, and with abiotic conditions such as nutrients or light (Gouinguene & Turlings 2002; Hoballah, Turlings & Tamo 2002). Previous studies have shown a reduction in herbivore performance in the presence of parasitoids, along with increased parasitoid attraction towards HIPVs, which act as indirect defences (Dicke & Baldwin 2010). However, the effect of direct plant defence traits on the efficacy of indirect defences and the interaction of both defence types with each other remain largely unexplored (Rasmann & Agrawal 2009; Rasmann *et al.* 2011).

As one of the first structures contacted by approaching arthropods, plant trichomes are crucial for host discrimination and acceptance by arthropods (Muller & Riederer 2005). The tomato plant *Solanum lycopersicum* is an economically important crop worldwide and a model system for studying trichome-based plant defences because of its diversified trichome types and functions (Lin, Trumble & Kumamoto 1987; Kennedy 2003). Polyphagous *Liriomyza* species (agromyzid leafminer), such as *Liriomyza huidobrensis*, *Liriomyza sativa* and *Liriomyza trifolii*, are becoming increasingly serious insect pests of agricultural crops,

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including cultivated tomato plants, worldwide (Kang *et al*. 2009; Liu *et al*. 2009). Leafminer parasitoids can play an important role in the biological control of *Liriomyza* in field crops (Liu *et al*. 2009). In a tritrophic system consisting of tomato plants, the pea leafminer *L. huidobrensis*, and the leafminer parasitoid *Opius dissitus*, HIPVs can attract leafminers and parasitoids (Wei *et al*. 2011). On the other hand, trichomes on the leaf surface can reduce the host-seeking success of these insects. Therefore, in the present study, we postulated an antagonistic interaction between HIPV emission and trichome production in directly and indirectly mediating resistance against herbivores. The tomato jasmonic acid (JA) signalling *jasmonic acid-insensitive1* (*jai1*) mutation results in a loss of function in the *coronatine-insensitive1* (COI1) gene, resulting in abnormal trichome formation (Li *et al*. 2004). The *jai1* and JA biosynthesis *spr2* (the suppressor of *prosystemin-mediated responses2* mutation) mutant plants are also defective in volatile emissions (Li *et al*. 2003, 2004; Sanchez-Hernandez, Lopez & Delano-Frier 2006; Wei *et al*. 2011). Therefore, these mutants provide a useful system for comparing host selection by herbivores and parasitoids among wild-type (WT) plants and their isogenic lines, as well as for examining the interactions between HIPVs and trichomes in a tritrophic system.

Our goals were to understand the interaction between HIPVs and trichomes, as well as to test the hypothesis that plant trichomes play a dominant role in the host acceptance behaviour of a herbivore and its parasitoid. We also tested the hypothesis that there is functional antagonism between HIPVs and trichomes. We used tomato genotypes of WT and JA biosynthesis *spr2* mutant plants, as well as JA signalling *jai1* plants in conjunction with the manual removal of trichomes in *spr2* and WT plants to study the behavioural preference and reproduction of a herbivorous leafminer and its parasitoid.

MATERIALS AND METHODS

Plant materials and insects

Tomato (*S. lycopersicum*) line cv Castlemart was used as the WT parents for all experiments. The seeds of tomato mutant lines *jai1* (Li *et al*. 2004) and *spr2* (Li *et al*. 2003) were derived from cv Castlemart, and the seeds were collected and stored in the same conditions as the WT seed. Given the sterility of *jai1* mutant, we used the screening procedure described by Li *et al*. (2004) to select *jai1* homozygotes. Tomato seedlings were grown in 500 mL pots containing a mixture of peat and vermiculite (4:1) in growth chambers under 16 h of illumination at 28 °C and 8 h of darkness at 18 °C, with 150 μE m^{-2} s^{-1} irradiation during photophase and 60% relative humidity. Plants with four to six fully expanded leaves were used in the experiments. The age and leaf area of each genotype were normalized by sowing in parallel under the same culture conditions.

Colonies of the pea leafminer, *L. huidobrensis*, and the parasitoid, *O. dissitus*, were maintained in environmental chambers as described previously (Wei *et al*. 2007). Two-week-old kidney bean plants (*Phaseolus vulgaris* L. cv Naibai) with two fully developed true leaves were used to culture leafminers. *O. dissitus* females emerging from pupae were mated within 24 h. All *O. dissitus* used in the behavioural assays and parasitism experiments were 2- to 4-day-old females without previous exposure to their host, *L. huidobrensis*, or host plants. Each female was used only once in the experiments.

Quantification of trichomes

Type VI trichomes are the most significant anti-insect trait, and exert repellent and toxic effects on a wide range of insects in tomato plants (Lin *et al*. 1987; Kennedy 2003; Li *et al*. 2004). Hence, we measured and compared this type of trichome among three tomato genotypes. All measurements were performed on WT and mutant plants grown parallel under the growth conditions previously described. Leaves were classified into three groups according to their positions (ages) on the stem. Lower leaves referred to the first and second true leaves from the bottom, and they were the oldest leaves on a plant. Similarly, we defined the third and fourth leaves as middle (younger) leaves, and fifth and above ones as upper (youngest) leaves. To measure trichomes, we randomly took two 6-week-old tomato plants of each genotype from a planting area consisting of approximately six plants. One distal and one lateral leaflet were removed from each leaf group of a plant for measurement. One leaf disk (9 mm in diameter) was taken from a leaflet by punching midway between the leaf tip and leaf base (Supporting Information Fig. S1). The most distinct tomato glandular trichomes, type VI (short), were identified as described by Luckwill (1943) (Supporting Information Fig. S1). The morphology and densities of type VI trichomes on the upper surface of each leaf disk were determined using a stereomicroscope (Leica 250C) equipped with a SmartTouch controller, a Leica DFC 290 camera and a LAS Application Suite assembling Interactive Measurement Module (Leica, Wetzlar, Germany). The density of type VI trichomes was measured on a 3 mm × 3 mm area at each disk, which was magnified and captured by a camera. The trichome densities were represented as the number of trichomes per square centimeter (cm^2). The measurements for each genotype were repeated five times. Thus, a total of 60 leaf disks from 10 plants were measured in each genotype.

Plant volatile analysis

Intact and leafminer-damaged tomato genotypes for volatile collection were prepared as described in the *Plant materials and insects* and *Larval performance on tomato plants* sections in this paper, respectively. The headspace volatile collection system was designed as described by Wei *et al*. (2011). Air with emitted plant volatiles was withdrawn from a plastic oven bag that enclosed one or two tomato plants through a glass collector containing 100 mg of Porapak Q (80–100 mesh size, Supelco, Bellefonte, PA, USA) by a

membrane pump at a rate of 400 mL min^{-1} for 5 h. The absorbed volatiles from Porapak Q collectors were then extracted with 700 μL of HPLC-grade dichloromethane (Tedia Company, Fairfield, OH, USA).

The chemical structures of collected volatile compounds were identified as described previously (Wei *et al*. 2011). Briefly, an Agilent gas chromatography (GC) system (6890N) coupled with a mass spectrometry (MS) system (5973 MSD, Agilent Technologies, Inc., Palo Alto, CA, USA) was equipped with a DB-WAX column (60 m × 0.25 mm ID, 0.15 μm film thickness) for chemical identification. The GC-MS electron impact source was operated in the scan mode with the MS source temperature at 230 °C and MS Quad at 150 °C. Volatile compounds were identified by comparing their retention time and spectra with synthetic standards (Wei *et al*. 2011). Different concentrations of external standards were run under the same GC conditions to develop standard curves to quantify volatiles [2-carene for monoterpenes, β-caryophyllene for sesquiterpenes, (*Z*)-3-hexenyl acetate for green leaf volatiles and (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) for TMTT] in headspace samples.

Extraction and quantification of endogenous JA

The extraction and quantification of endogenous JA in each tomato genotype and treatment were according to the protocol described by Ren *et al*. (2010). Briefly, plant tissue (0.5 g) was ground to a fine powder in liquid nitrogen and transferred to a 10 mL screw-top Supelco vial containing 4 mL of 80% methanol (HPLC grade; Xilong Company, Beijing, China). After 12 h of freezing at –20 °C, 300 ng of [9, 10]-dihydro-JA (99% purity; Sigma-Aldrich, St Louis, MO, USA) was added as an internal standard. JA and the internal standard were partitioned to an aqueous phase by centrifugation and vaporization. After three rounds of freezing and thawing, the aqueous phase was centrifuged and the pH of the supernatant was adjusted to 2.5–3.0 using 2 M HCl. Jasmonates and internal standards were extracted from the supernatant with an equal volume of ethyl acetate and then dried. The dried extract was resuspended in 0.1 M acetic acid and loaded onto a C_{18} column (Waters Company, Milford, MA, USA). The C_{18} column was sequentially eluted with a series of solvent mixtures [acetic acid/methanol (v/v) at 83/17, 60/40 and 40/60]. The last 4 mL that was eluted in 40% methanol and the first 3 mL that was eluted in 60% methanol were collected. After evaporation of the solvent and esterification of the residue using excess ethereal diazomethane, the elution sample volume was adjusted to 50 μL with ethyl acetate and analysed using the same GC-MS apparatus described in the *Plant volatile analysis* section. Exactly 1 μL of each sample was injected in the splitless mode. The GC-MS was equipped with an HP-5-MS column (30 m × 0.25 mm × 0.25 μm; 19091S-433, J&W Scientific, Agilent Technologies). Helium was used as the carrier gas. The oven temperature was initially kept at 50 °C and increased at a rate of 20 °C min^{-1} to 180 °C, held for 5 min, and then increased again at a rate of 40 °C min^{-1} to 290 °C held for 5 min. The injector temperature was maintained at 250 °C with a constant flow rate of 0.8 mL min^{-1}. The total ion count or selected ion count of the samples and standard compounds were measured as follows: 8–10.5 min, M^+ *m/z* ions 151 and 224 for methyl jasmonate (MeJA; 95% purity; Sigma-Aldrich), and ions 153 and 226 for methyl dihydro-jasmonate (dh-MeJA). The retention times were 9.91 min for MeJA and 9.97 min for dh-MeJA. JA was quantified by correlating the peak area (extracted ion 224) of the compound with that of the respective internal standard (extracted ion 226) (Ren *et al*. 2010). The plants used for endogenous JA measurements were prepared under the same procedures and conditions described in the *Trichome removal from leaflet of tomato plants*, *Preference assays with leafminers and parasitoids*, and *Larval performance on tomato plants* sections. There were five replications per genotype and treatment.

Trichome removal from leaflet of tomato plants

During trichome removal, the exudates of type VI trichomes on the adaxial surface were gently washed off using a brush pen dipped in distilled water, and then dried with tissue paper. Type I trichomes on the adaxial surface were carefully shaven using a sharp razor blade (Gillette, Boston, MA, USA) to avoid damaging on the other tissues (Supporting Information Figs S1 & S2). Plants sprayed with distilled water served as controls. To reduce the effect of the possible defence response in treated plants, the treated and control plants were held for 24 h under the growth conditions previously described. They were then subjected to experiments on adult leafminer preference, larval performance, parasitoid parasitism, JA and proteinase inhibitor (PI)-II protein level quantifications, and volatile collection under the same procedures and conditions described in the *Larval performance on tomato plants* section.

Preference assays with leafminers and parasitoids

The feeding and oviposition preference of adult leafminers for tomato genotypes or treatments were monitored in a 40 cm × 40 cm × 40 cm cage. The experiments were designed as dual-choice tests. Two plants of each genotype or treatment were paired in the cage. The plants were exposed to 150–200 adult flies (male : female ≈ 1:1) for 5 h under the same conditions as those used for rearing leafminers. After 24 h, the feeding and oviposition punctures on each leaflet of a plant were counted under a stereo microscope (Wild, Heerbrugg, Switzerland). Each assay was repeated at least six times with newly prepared plants.

Parasitism experiments were performed to investigate the incidence of parasitism of leafminer larvae by parasitoids on tomato plants. Two plants of each genotype or treatment were paired in 40 cm × 40 cm × 40 cm cages, each infested with similar numbers and sizes of leafminer larvae

(Fig. 2 legend). The larvae on the WT plants were 12 h older than those on the *jai1* plants, and thus had the same sizes but different ages. The female parasitoids (approximately 10 females to 100 larvae) were released into the cage to parasitize for 24 h. Parasitism was determined by dissecting leafminer larvae 3 d after exposure to adult parasitoids when larval parasitoids had developed into the first-instar larval stage.

Y-tube experiments were performed to investigate the preference of female *O. dissitus* for different odour sources as previously described (Wei *et al*. 2011). Briefly, in Y-tube tests, each odour source was placed inside one of the two arms of a Y-tube olfactometer. A naïve individual female parasitoid was placed in the olfactometer for 5 min. A 'first choice' was recorded when the parasitoid moved more than 5 cm onto either arm. Each experiment was replicated with at least 30 females. We observed the preference of parasitoids for paired intact or leafminer-damaged WT and *jai1* plants, as well as for paired leafminer-damaged trichome-intact (T) and trichome-removed (TR) *spr2* or WT plants.

A cage experiment was also used to observe the landing preference of parasitoids for paired WT and *jai1* plants. Two WT plants and two *jai1* mutant plants containing second-instar larvae were paired in the cage (the mean numbers of larvae on each genotype are shown in the legend of Fig. 2). A naïve female parasitoid was released at the midpoint between two groups of tomato plants, and its behaviour was monitored for 10 min. At least 30 female wasps were tested in a cage with the same set of plants. The assay was then repeated at least six times with freshly prepared plants.

Larval performance on tomato plants

A no-choice experiment was performed to examine larval performance on WT and *jai1* genotypes. Plant preparation and treatments were as for the *Preference assays with leafminers and parasitoids* section. Plants were exposed to leafminer adults for oviposition and then moved into growth chambers for developmental studies. The larval survival, development and size were determined as described previously (Wei *et al*. 2011). Briefly, larval survivorship (%) was calculated as the ratio of total pupa number to viable eggs. The developmental time, defined as the required time for 50% of the population to reach a certain developmental stage, was recorded for each larval stage and tomato genotype. The larval area (length × width) was used to represent the host size, which was measured with a microscope (Leica M250C). The images were processed using the LAS Interactive Measurement Module (Leica). More than 20 larvae were measured for each genotype and developmental stage.

The proteinase inhibitor (PI)-II protein levels in the leaflets of tomato genotypes or treatments were quantified by a radial immunodiffusion assay (Wei *et al*. 2011). For each leafminer-infested WT or *jai1* plant, the PI-II level was measured on days 4, 5 and 7 after oviposition by adult flies. There were at least five replications per genotype and developmental stage. For each treatment in the trichome removal experiments, the PI-II level was measured in trichome-intact and TR tomato plants at two time points after treatments, namely, 24 h after trichome removal and at the second-instar larval stage from ovipoistion by adult leafminers.

Pupal weight gain was used as an indicator of leafminer performance on trichome-intact and TR tomato plants. Puparia were collected from each plant and weighed immediately after collection using an electronic balance (Mettler AE 240, Shanghai, China). There were at least five replications per treatment and 10 pupae were weighted in each replication.

Data analysis

Data were analysed using the SPSS software package (version 15.0; SPSS Inc., Chicago, IL, USA). The densities of trichomes, amounts of volatile compounds, and JA levels of the different tomato genotypes or treatments were compared by analysis of variance (ANOVA) and Tukey's honestly significant difference test. The levels of volatiles released from each genotype and treatment were normalized to nanogram per hour per 10 g of plant fresh weight. For dual-choice tests, the oviposition preference of adult leafminer as well as the parasitism and landing rates of parasitic wasps were compared by paired *t*-test. Larval survivorship (%), body size, JA and PI-II productions were compared between the two genotypes or treatments using *t*-tests. For parametric analyses, the percentage data (survivorship, parasitism and landing rates) were arcsin ($x^{1/2}$) transformed, whereas the absolute quantity data were log (x + 1) transformed to correct heterogeneity of variances. Chi-square tests were performed to determine the significance of differences in the numbers of parasitoids choosing each olfactometer arm. Parasitoids that did not make a choice were excluded from the statistical analysis. The developmental time of *L. huidobrensis* was compared using the Mann–Whitney *U*-test.

RESULTS

Characterization of trichomes and odour traits in WT and JA-deficient mutant tomato plants

To determine the trichome densities in JA signalling *jai1* mutant, JA biosynthesis *spr2* mutant and WT plants, we examined the density of type VI trichomes in these plants (Supporting Information Fig. S1). Although we found the same number of trichomes between WT and *spr2*, the average density of type VI trichomes on *jai1* plant was 73% less than that in WT plants (Table 1). Therefore, the *jai1* mutant can be considered as a trichome-deficient tomato plant, whereas *spr2* and WT plants were normal in trichome production.

We next characterized the volatile profiles of three tomato genotypes before and after leafminer infestation. Undamaged tomato plants constitutively released five monoterpenes (MTs) and one aromatic compound (AR; *p*-cymene) at different concentrations, whereas WT plant

Table 1. Mean density (no. per cm^2 ± SE) of type VI glandular trichomes on the leaflets of tomato genotypes

Positions[a]	Tomato genotypes and mean trichomes density[b] (no. per cm^2 ± SE)			
	Wild type	*spr2*	*jai1*	*P* value
Average	867.2 ± 44.4a	887.6 ± 61.5a	230.8 ± 14.3b	<0.0001
Upper leaves	1062.5 ± 80.7b	1463.3 ± 72.9a	288.3 ± 16.7c	<0.0001
Middle leaves	1003.9 ± 50.4a	752.2 ± 24.9b	266.5 ± 23.7c	<0.0001
Lower leaves	535.3 ± 22.4a	447.2 ± 25.3b	139.8 ± 17.3c	<0.0001

[a]Type VI trichomes were determined using the morphological descriptions by Luckwill (1943). Leaves were classified into three groups according to their positions (ages) on the stem. Lower (old) leaves referred to the first and second true leaves from the bottom. Middle (younger) leaves were the third and fourth leaves from the base of the plant, and the upper (youngest) were the fifth or higher leaves.
[b]Mean density of type VI glandular trichomes on the upper surface of three genotypes. Densities calculated from counts of trichomes on two leaf disks per plant per position. Ten plants per genotype were examined ($n = 10$). Means within the same row followed by different letters are significantly different by ANOVA and Tukey's honestly significant difference test.

had an additional sesquiterpene (*β*-caryophyllene) in the headspace (Fig. 1). Undamaged JA-deficient mutants emitted significantly lower amounts of MT and AR volatiles than WT plants (ANOVA, $F_{2,14} = 4.76$, $P = 0.032$), whereas there was no difference between the two mutant lines (Fig. 1a). When plants were infested by leafminers, the two mutants released significantly lower amounts of constitutive compounds and inducible volatiles (GLVs and TMTT) compared with WT (amounts of all volatiles among genotypes: ANOVA, $F_{2,14} = 13.72$, $P = 0.0011$), whereas similar amounts of all compounds were released by the mutants (*jai1* versus *spr2*: two tailed *t*-test, $t = 0.608$, d.f. = 8, $P = 0.56$; Fig. 1b). Therefore, *jai1* and *spr2* mutants were characterized as odour-deficient tomato plants.

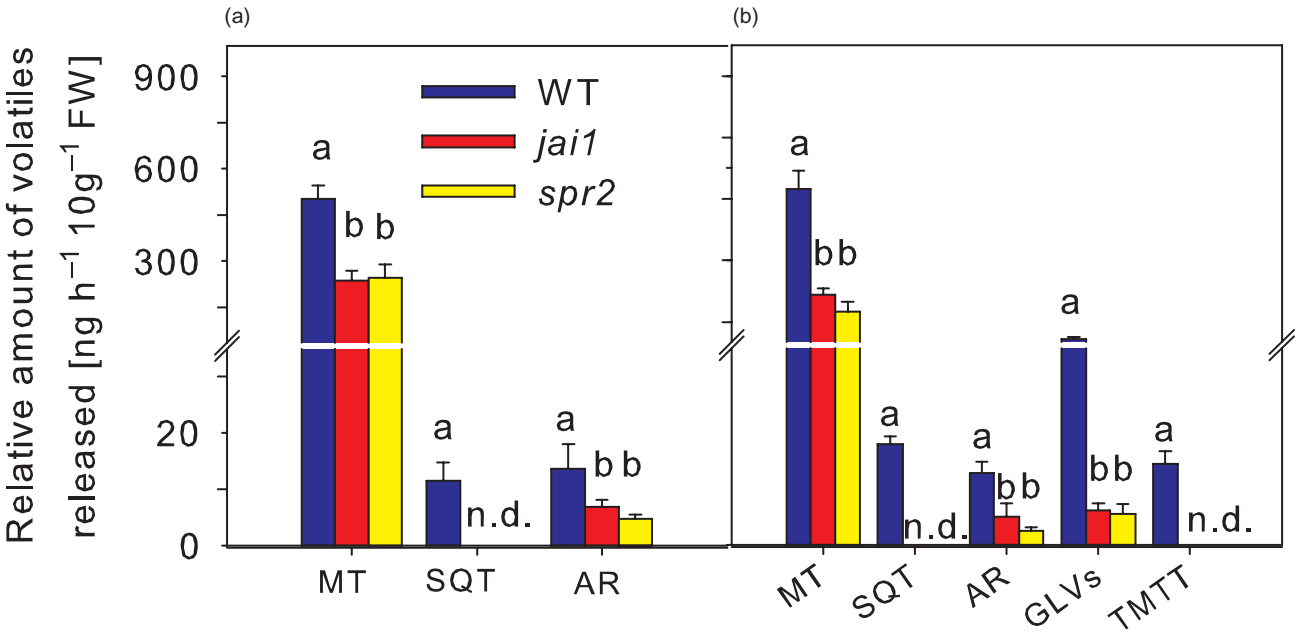

Figure 1. HIPVs are reduced in JA biosynthesis (*spr2*) and perception (*jai1*) mutants. Emission rate of volatiles [ng h^{-1} 10 g^{-1} fresh weight (FW)] from undamaged plants (a) or plants damaged by leafminer larvae (b) among three tomato genotypes. In each panel: MT, monoterpene (*α*-pinene, 2-carene, *α*-phellandrene, limonene and *β*-phellandrene); SQT, sesquiterpene (*β*-caryophyllene); AR, aromatic (*p*-cymene); GLVs, green leaf volatiles [(*Z*)-3-hexenol, (*Z*)-3-hexenyl butyrate, and (*Z*)-3-hexenyl acetate]; and TMTT, (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. There were five replications in each genotype and treatment ($n = 5$); n.d., undetectable. In panel b, the mean numbers of larvae (MNL) in the leaflets of WT, *jai1* and *spr2* plant are 21.3 ± 2.7, 35 ± 4.9 and 35.8 ± 4.7 (mean ± SE; $n = 12$) respectively; *jai1* and *spr2* produce no detectable (*Z*)-3-hexenyl butyrate and (*Z*)-3-hexenyl acetate. Different letters above bars within each compound group indicate significant difference (ANOVA test, $P < 0.05$). HIPV, herbivore-induced plant volatile; JA, jasmonic acid.

JA-mediated direct defence is compromised in JA-deficient mutant tomato plants

In a previous study, we showed that *spr2* mutant plants were compromised in direct defence against leafminer larvae compared with WT plants (Wei *et al*. 2011). Here, we measured the endogenous JA levels of three tomato genotypes before and after leafminer infestation. Undamaged *spr2* plants produced significantly lower amounts of JA than *jai1* and WT plants (ANOVA, $F_{2,14} = 11.51$, $P = 0.0016$), whereas there was no difference between *jai1* and WT plants (Fig. 2a). The JA levels increased approximately onefold to sixfold in second-instar larvae-infested *spr2*, *jai1* and WT plants, respectively. The leafminer-induced JA level in *jai1* plants was comparable to that in WT plants (Fig. 2a), whereas damaged WT plants biosynthesized a higher level of JA than *spr2* plants (ANOVA, $F_{2,14} = 25.43$, $P < 0.0001$).

Leafminer infestation also triggers PI-II production in WT plants, where the PI-II levels accumulated as the insects developed, but such induction was not observed in *jai1* plants at any of the three time periods following oviposition (Fig. 2b). In the no-choice experiments, the performance of larval leafminer on WT and *jai1* plants was significantly different (Fig. 2 c–f). Compared with the *jai1* plants, larvae on the WT plants displayed significantly lower survivorship (%) (two tailed *t*-test: $t = -8.82$, d.f. = 14, $P < 0.001$; Fig. 2d). The developmental time from egg to adult eclosion for larvae on WT plants required significantly longer time than that on *jai1* plants (Mann–Whitney *U* test, $U = 44.5$, $P = 0.0069$; Fig. 2e). Their body size was also nearly 40%

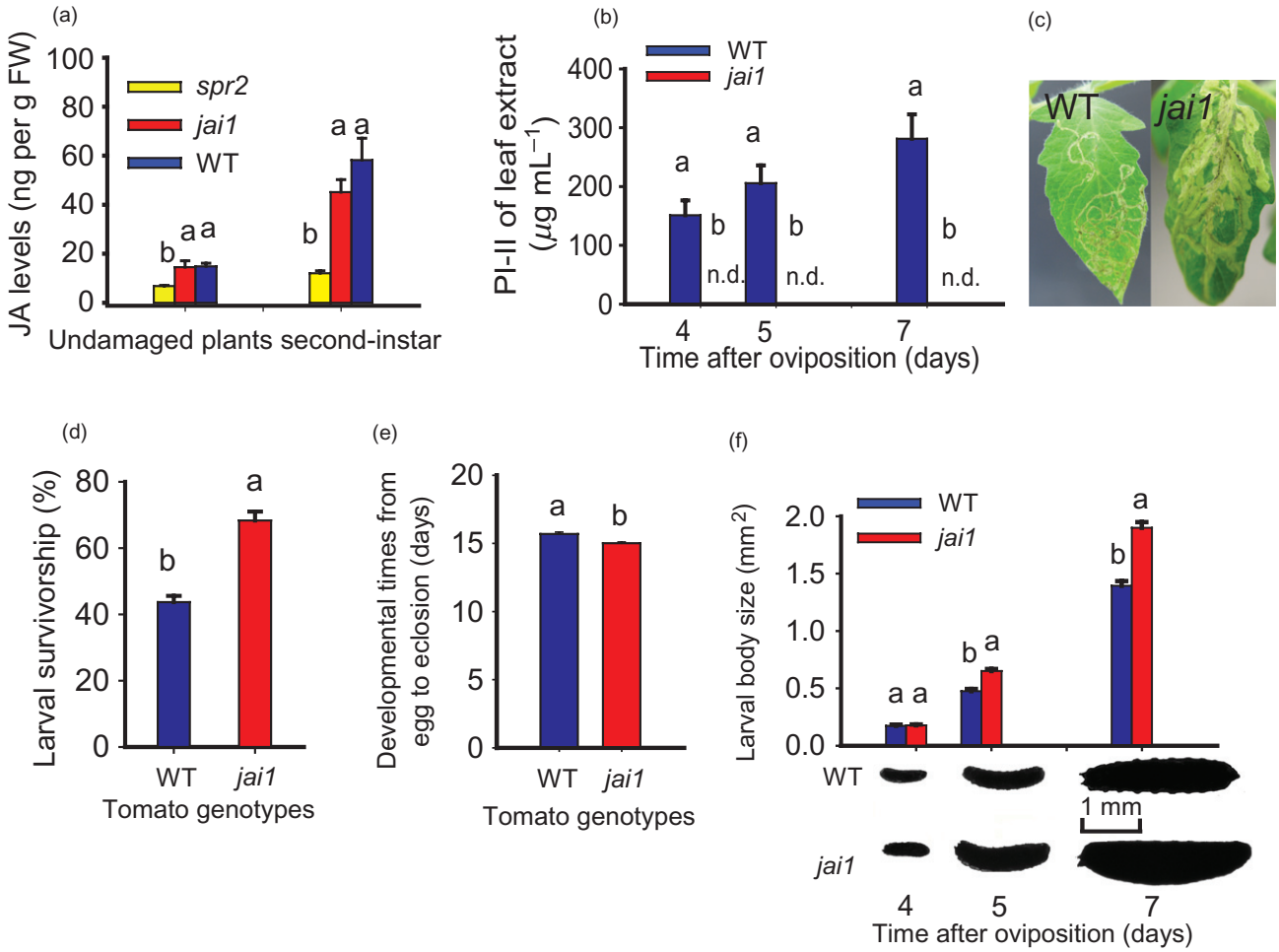

Figure 2. Jasmonate-mediated defence is disrupted in *spr2* and *jai1* mutants. (a) Jasmonic acid (JA) levels in undamaged and second-instar larvae-damaged *spr2*, *jai1* and WT plants (mean ± SE, $n = 5$). The mean numbers of larvae (MNL) in the leaflets of WT, *jai1* and *spr2* plants are 32 ± 3.6, 32 ± 5.3 and 50.8 ± 4.7 (mean ± SE; $n = 10$), respectively. (b) Proteinase inhibitor II (PI-II) accumulates in the leaflets of two genotypes in response to leafminer damages ($n = 5$) at three time points. n.d., undetectable. MNL in the leaflets of WT and *jai1* on day 7: 84.3 ± 7.6 and 91.6 ± 10.3 (mean ± SE; $n = 10$), respectively. (c) Larval mining on the leaflet of two genotypes on day 7 after leafminer oviposition. (d) Larval survival (%) on each genotype. Bars represent the mean values for eight replicates of each genotype ($n = 8$). (e) Development time (days) from egg hatch to pupation on each genotype ($n = 6$). (f) Larval body size (area of length × width, mm ^{2}) on each genotype (above). Bar represents five replicates of each genotype ($n = 5$); images of larval leafminers collected from two genotypes in different developmental stages (down). Scale bar: 1 mm. Significant differences between two genotypes are indicated by letters on each bar (Student's *t*-test, $P < 0.05$). WT, wild-type.

smaller 5 d after oviposition (in the fifth day: *t*-test, $t = -6.69$, d.f. = 77, $P < 0.001$; Fig. 2f). Therefore, our previous study and the present one concurrently show that direct defence is compromised in mutant tomato plants deficient in JA biosynthesis or perception.

Roles of trichome in host acceptance by leafminer and its parasitoid

To understand the functional interaction between HIPVs and trichomes in WT plants, we observed the behavioural preference and/or reproductive success of adult leafminers and their parasitoids in dual-choice experiments with different combinations of tomato genotypes and treatments. To exclude the influence of plant volatiles on host selection by leafminer and its parasitoid, we compared the oviposition and parasitism in paired *jai1* and *spr2* plants by cage experiments. The *spr2* plants have a higher trichome density than *jai1* plants, but both have similarly reduced volatile emission. The results showed that adult leaminers significantly preferred to feed and oviposit on *jai1* plants over *spr2* plants (paired sample *t*-test, $t = -9.03$, d.f. = 5, $P = 0.00034$; Fig. 3a). Similarly, more leafminer larvae on *jai1* plants were parasitized compared with the larvae on *spr2* plants (*t*-test, $t = -10.53$, d.f. = 5, $P < 0.0001$; Fig. 3b).

To confirm the negative effect of trichomes on the preferences of leafminers and parsitoids, we observed the behavioural choice of both insects to T and TR *spr2* or WT plants. When adult leafminers were given a choice of TR *spr2* versus T *spr2* plants, higher feeding and oviposition frequencies were observed on TR plants (paired *t*-test: $t = 6.96$, d.f. = 5, $P = 0.0008$; Fig. 3c). The parasitism of larvae on TR *spr2* plants was also substantially higher than that on T *spr2* plants (paired *t*-test: $t = 5.90$, d.f. = 5, $P = 0.0011$; Fig. 3d). The oviposition of adult leafminers and parasitism of larval leafminers were significantly higher on TR WT than on T plants (paired *t*-test: oviposition, $t = 4.91$, d.f. = 5, $P = 0.0044$; parasitism, $t = 7.04$, d.f. = 5, $P = 0.00034$; Fig. 3e,f).

Finally, we used paired WT and *jai1* plants to investigate whether HIPVs or trichomes were predominant traits in determining the host preference and acceptance of leafminers and parasitoids. In dual-choice cage experiments, adult leafminers preferred *jail* mutant plants for feeding and oviposition over WT plants (paired *t*-test: $t = 11.10$, d.f. = 5, $P < 0.001$; Fig. 3g) and significantly more larvae were parasitized on *jai1* plants than on WT plants (paired *t*-test, $t = -5.35$, d.f. = 5, $P < 0.01$; Fig. 3h), suggesting a behavioural mechanism by which plant trichomes override the effects of HIPVs in the host acceptance of leafminers and parasitoids. By contrast, Y-tube analyses in which parasitoids were exposed to plant volatiles, but not to the leaf surface, revealed that the female wasps showed no difference in behavioural preference for undamaged WT and *jai1* plants, but the leafminer-damaged WT plants were significantly more attractive to parasitoids than the leafminer-damaged *jai1* mutants ($\chi^2 = 3.903$, $P = 0.048$; Fig. 3i). In a cage experiment, the number of first landings for naïve female parasitoids on WT plants was significantly higher than that on *jai1* mutants (paired sample *t*-test, $t = 9.49$, d.f. = 5, $P < 0.001$; Fig. 3j), supporting the importance of HIPVs in parasitoid attraction. Thus, parasitoids are attracted to HIPVs, but prefer to oviposit in larvae on leaves with low trichome densities.

Direct and indirect defence traits in tomato plants were not altered by trichome removal

To determine whether removing trichomes had a significant impact on direct and indirect plant defence, the endogenous JA production, PI-II accumulation, and volatile emissions of *spr2* and WT plants were measured before and after trichome removal. The behavioural responses of parasitoids to TR and T plants were also observed. For both *spr2* and WT plants, there was no significant difference between the JA levels of TR and T plants at two time points (two tailed *t*-test: 24 h after TR: between *spr2*-T and *spr2*-TR, $t = 0.358$, d.f. = 8, $P = 0.729$; between WT-T and WT-TR, $t = 0.254$, d.f. = 8, $P = 0.805$; at second-instar larval stage: between *spr2*-T and *spr2*-TR, $t = -0.012$, d.f. = 8, $P = 0.99$; between WT-T and WT-TR, $t = 0.132$, d.f. = 8, $P = 0.898$; Fig. 4a,b). By contrast, PI-II production was detectable in TR *spr2* plants compared with T plants (Fig. 4c). However, there was no significant difference between the leafminer-damaged TR and T *spr2* plants (two tailed *t*-test: second-instar larval stage, $t = -0.671$, d.f. = 10, $P = 0.517$; Fig. 4c). The pupal weight gain on TR *spr2* plants did not significantly differ from that on T *spr2* plants ($t = 0.18$, d.f. = 8, $P = 0.85$; Fig. 4e). For WT plants, trichome removal did not significantly affect PI-II production at either of the two time points (two tailed *t*-test: 24 h after TR, $t = -0.395$, d.f. = 10, $P = 0.70$; second-instar larval stage, $t = -0.769$, d.f. = 8, $P = 0.463$; Fig. 4d), and the pupal weight gain on the TR and T plants did not significantly differ ($t = 0.62$, d.f. = 8, $P = 0.55$; Fig. 4f). For both *spr2* and WT plants, there was no significant difference in the total amount of volatile emission between TR and T plants that were either undamaged or leafminer damaged (two tailed *t*-test: between undamaged *spr2*, $t = 1.00$, d.f. = 8, $P = 0.346$; between leafminer-damaged *spr2*, $t = -0.61$, d.f. = 8, $P = 0.56$; Fig. 4g; between undamaged WT, $t = 0.41$, d.f. = 8, $P = 0.78$; between leafminer-damaged WT, $t = -1.52$, d.f. = 8, $P = 0.13$; Fig. 4h). Similarly, in Y-tube assays, female wasps showed no difference in their attraction to HIPVs from leafminer-damaged TR and T plants of either the spr2 or WT genotype (leafminer-damaged *spr2* versus leafminer-damaged *spr2*-TR: $\chi^2 = 0.72$, $P = 0.396$; leafminer-damaged WT versus leafminer-damaged WT-TR: $\chi^2 = 0.107$, $P = 0.68$; Fig. 4i,j). Therefore, the trichome removal treatment did not lead to significant physiological responses in *spr2* or WT tomato plants compared with intact plants in terms of direct defence (endogenous JA production, PI-II protein production and pupal weights) and indirect defence (volatile emissions and parasitoid attractions).

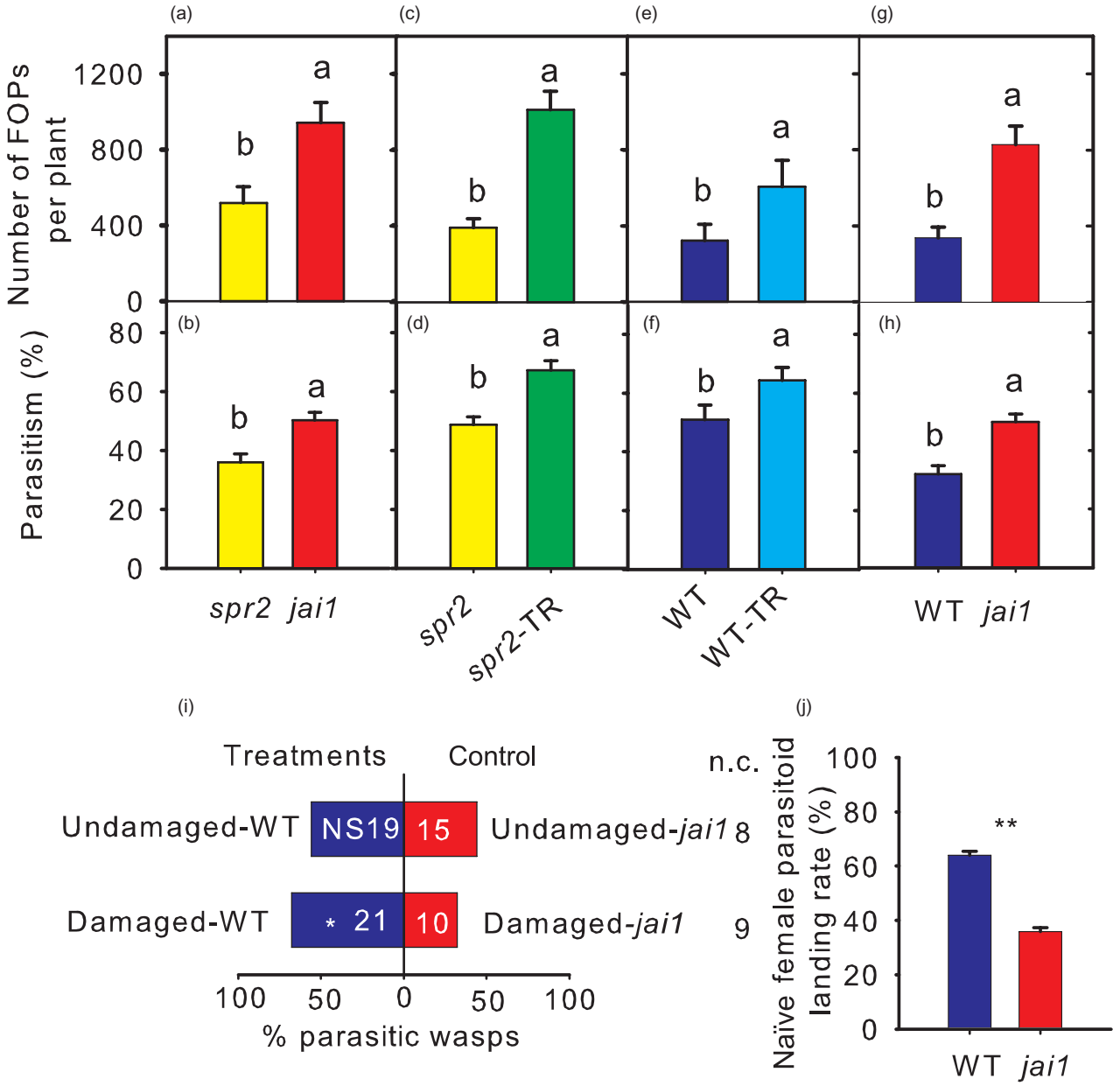

Figure 3. HIPVs attract parasitoids, but trichomes have a strong opposing effect on both parasitoid and herbivore activity. Host selection by adult *Liriomyza huidobrensis* and its parasitoid on tomato plants with genetically modified or artificially modified plant volatiles and/or trichomes in dual-choice experiments. (a) Mean number of feeding and oviposition punctures (FOPs) of adult leafminers on paired *spr2* and *jai1* plants ($n = 6$). (b) Larval parasitism rates (%) on paired *spr2* and *jai1* plants ($n = 6$). The mean numbers of larvae (MNL) in the leaflets of *spr2* and *jai1* are 77.3 ± 5.2 and 85 ± 6.1 (mean ± SE; $n = 12$), respectively. (c) Mean ($n = 6$) number of FOPs on paired *spr2* and trichome-removed *spr2* (*spr2*-TR) plants. (d) Parasitism (%) on *spr2* and trichome-removed *spr2* (*spr2*-TR) ($n = 6$). MNL are 89.8 ± 7.4 ($n = 12$) and 78.3 ± 6.2 ($n = 14$), respectively. (e) Mean ($n = 6$) number of FOPs on paired WT and trichome-removed WT (WT-TR) plants. (f) Parasitism (%) on WT and trichome-removed WT (WT-TR) plants ($n = 6$). MNL are 69.8 ± 5.6 and 64.3 ± 7.7 ($n = 12$), respectively. (g) Mean ($n = 6$) number of FOPs of adult leafminers on paired WT and *jai1* plants. (h) Parasitism (%) on WT versus *jai1* plants ($n = 6$). MNL are 106.5 ± 8.5 and 111.3 ± 6.3, respectively ($n = 12$), and the mean size of larvae (length × width) in the leaflets of WT and *jai1* are 0.904 ± 0.03 ($n = 28$) and 0.847 ± 0.025 ($n = 34$), respectively. (i) Behavioural responses of naïve female wasps to volatile blends emitted by paired undamaged or leafminer-damaged WT and *jai1* plants. Bars represent the percentages of parasitoids choosing either odour source in the Y-tube olfactometer. Numbers in bars are the numbers of parasitoids choosing the indicated odor source; n.c. indicates no choice. (j) Landing preference of *Opius dissitus* naïve females on each of the two tomato genotypes ($n = 6$). MNL in the leaflets of WT and *jai1* are 88.08 ± 11.3 and 97.08 ± 12.3, respectively ($n = 12$). In panels a–h, significant differences between two genotypes or treatments are indicated by different letters above each bar (paired *t*-test, $P < 0.05$). In panels i and j, * $P < 0.05$; ** $P < 0.01$; NS, non-significant. The numbers of parasitoids choosing each olfactometer arm were analysed by a χ^2 test, and landing rates were compared by paired *t*-test. In each panel, WT, wild-type; *jai1*: *jasmonic acid-insensitive1*; *spr2*, *suppressor of prosystemin-mediated responses2*.

DISCUSSION

Impact of trichomes on direct and indirect plant defences

Plant trichomes have long been recognized as physical and chemical defences against herbivore and pathogens. Numerous studies have shown that the density of plant trichomes is negatively correlated with the abundance and effectiveness of natural enemies of herbivores (Luckwill 1943; Kennedy 2003). For instance, type VI glandular trichomes in tomato plants are the most significant anti-insect traits; they exert repellent and toxic effects on a wide range of herbivores and their natural enemies (Lin *et al*. 1987; Farrar & Kennedy 1991; Kennedy 2003; Li *et al*. 2004; Hare 2007; Verheggen *et al*. 2009). The dual feature of trichomes demonstrated in these studies highlights the need to characterize the mechanism of the interactions among trichomes, other direct defence traits and indirect defence traits by synergistic, redundant or antagonistic correlation. Tomato has long been used as a model system to study trichome-based and other plant defences because of its diverse trichome types, trichome-borne chemicals and non-trichome defence chemicals (Kennedy 2003). Terpenes, methyl ketone and acyl sugars are the most abundant secondary metabolites in the tips of type VI glandular trichomes (Muller & Riederer 2005; Kang *et al*. 2010). Therefore, a high density of this trichome type has been associated with high levels of trichome-borne chemicals and resistance against arthropod herbivores. Although constitutive and JA-induced defences other than trichomes have been well studied in *Solanum* species, surprisingly, the interaction between trichome-based and non-trichome plant defences has not been documented so far.

By contrast, there are a few reports in other plant systems. For instance, negative correlations were found between trichomes and gossypol glands in Gossypieae likely reflecting the cost or redundant functions of these constitutive traits, whereas these two direct defence traits were not correlated with extrafloral nectar, an indirect resistance trait, suggesting that these two modes of defences may have evolved independently in this plant family (Rudgers, Strauss & Wendel 2004). In *Arabidopsis lyrata*, a study on the genetic variation and correlation in trichome and glucosinolates (GS) defence traits along with resistance to insect herbivores has demonstrated that the indole GS amount and trichome density act synergistically to increase plant resistance (Clauss *et al*. 2006). An investigation of wild populations of a *Datura* species that differ in trichome production has not found any correlations between HIPV emissions and expressing glandular or non-glandular trichomes among eight lines (Hare 2007). The understanding of the evolution and maintenance of these defence traits in natural conditions is hampered by insufficient laboratory and field observations of behavioural responses of the natural enemies of *Datura wrightii* herbivores to HIPV and trichome traits (Hare 2007). Thus, the evidence provided thus far is not conclusive, with some studies demonstrating negative or positive effects and others finding no effect. These inconsistencies may reflect the complexity of defence traits in a plant or the use of different genotypes with different genetic backgrounds (e.g. cultivars, varieties, and strains) or differences in the mechanism by which defence pathways confer direct and indirect defences in different genotypes and response to herbivores of different feeding guilds (Hoballah *et al*. 2002; Rasmann & Agrawal 2009; Dicke & Baldwin 2010).

Recent advances in the genetic modification of specific metabolite pathways in WT plants have enabled researchers to disentangle the complexity of defence traits and understand the function of single traits in plant–insect interactions (Kessler *et al*. 2004; Degenhardt *et al*. 2009). Plant trichome formation is directly or indirectly under control of phytohormones, such as JA, brassinosteroids, ethylene, gibberellin and auxin (Li *et al*. 2004; Campos *et al*. 2009). Logically, decreased trichome density in genetically modified plants often correlates with reduced resistance to arthropod herbivores (Fig. 2). A recent study has revealed correlations between trichomes and secondary metabolites along with insect herbivore performance using eight tomato hormonal mutants that have a single genetic background (Campos *et al*. 2009). However, the existence of synergistic, redundant or antagonistic interactions between trichomes, other direct defence traits and indirect defence traits modulating arthropod behaviour in tritrophic context is still unclear. By comparative studies with tomato plants manipulated genetically and artificially in trichome and/or HIPV traits, we extended these findings by showing that although complete volatile blends of WT plants are more attractive to adult insects (Wei *et al*. 2011), higher densities of trichomes have more significant and antagonistic effects, presumably by physically impeding parasitoid movement and restricting further activity. However, the effect of interaction of these traits needs to be studied in agricultural systems with these genetic lines to understand better the significance of this interaction in crop protection.

Plant trichomes determine the host acceptance and reproductive success of herbivore and its parasitoid

We hypothesized that leafminers and their parasitoids are more sensitive to plant trichomes than other insects (Fordyce & Agrawal 2001; Weinhold & Baldwin 2011), because these tiny insects have to circumvent relatively large trichomes to oviposit successfully on leaves or locate the host larvae in leaf tissue (Kang *et al*. 2009). Therefore, we hypothesized that decreased density of trichomes in tomato plant is associated with increased preference and acceptance of the herbivore and of its natural enemy. A dual-choice design with WT plants and otherwise-isogenic lines deficient in JA biosynthesis and perception enabled us to distinguish the effects of plant trichomes and HIPVs in preference and performance assays (WT: trichomes and HIPVs, *spr2*: trichomes and low HIPVs, *jai1*: few trichomes and low HIPVs; Table 1, Figs 1 & 3). Interestingly, adult

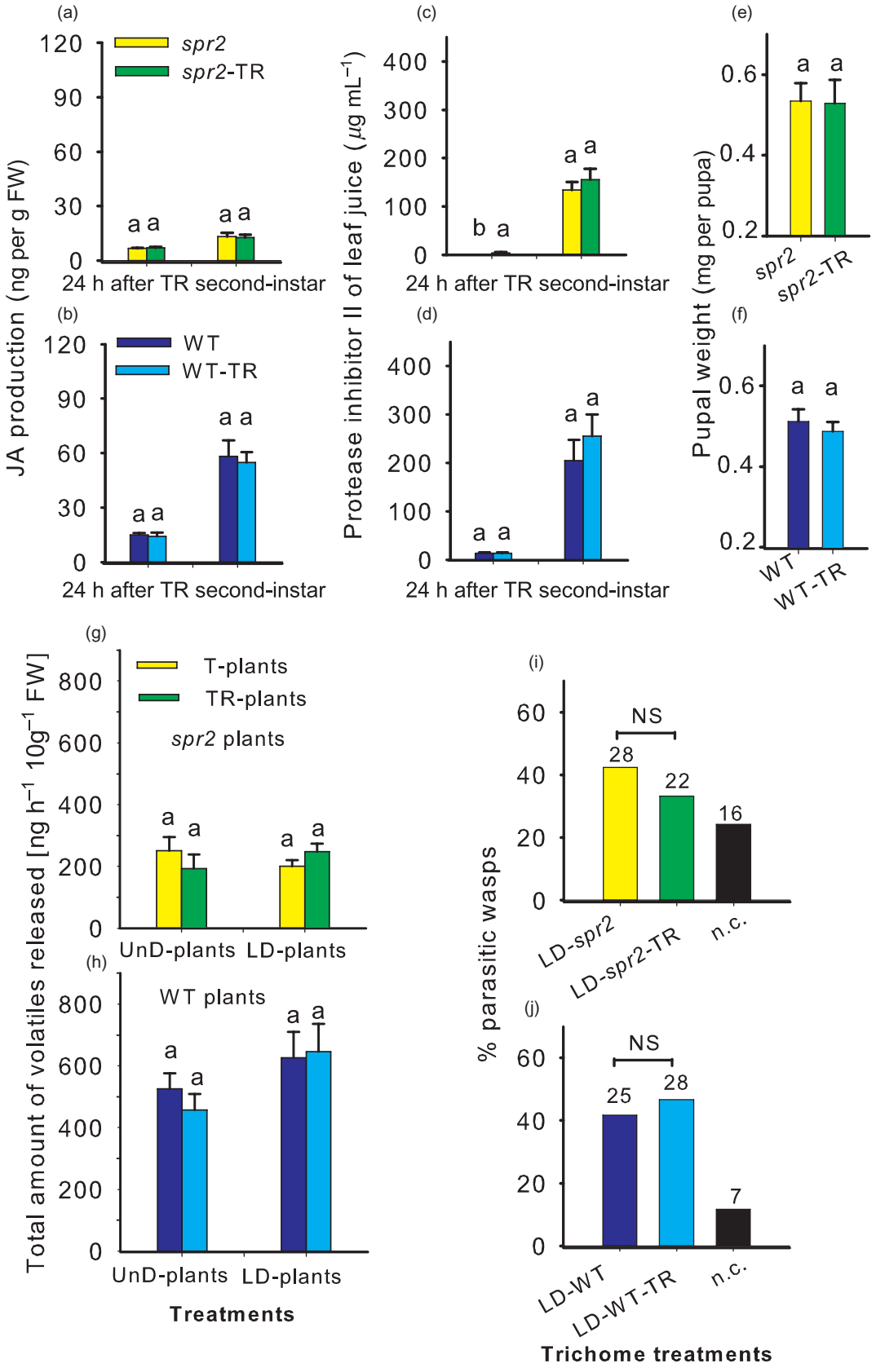
(a)
spr2
spr2-TR
JA production (ng per g FW)
120
90
60
30
0
a a
a a
24 h after TR second-instar
(b)
WT
WT-TR
a a
a a
24 h after TR second-instar
(c)
Protease inhibitor II of leaf juice (μg mL−1)
400
300
200
100
0
b a
a a
24 h after TR second-instar
(d)
a a
a a
24 h after TR second-instar
(e)
Pupal weight (mg per pupa)
0.6
0.4
0.2
a a
spr2
spr2-TR
(f)
a a
WT
WT-TR
(g)
T-plants
TR-plants
spr2 plants
Total amount of volatiles released [ng h−1 10g−1 FW]
800
600
400
200
0
a a
a a
UnD-plants LD-plants
(h)
WT plants
a a
a a
UnD-plants LD-plants
Treatments
(i)
% parasitic wasps
60
40
20
0
NS
28
22
16
LD-spr2
LD-spr2-TR
n.c.
(j)
NS
25
28
7
LD-WT
LD-WT-TR
n.c.
Trichome treatments

Figure 4. Trichome removal does not affect plant defences. Total jasmonic acid (JA) levels in (a) trichome-intact *spr2* plants [mean numbers of larvae (MNL) = 52.5 ± 4.5] and trichome-removed *spr2* (*spr2*-TR) plants (MNL = 59.5 ± 7.5), and in (b) trichome-intact WT plants (MNL = 32.1 ± 3.6) and trichome-removed WT (WT-TR) plants (MNL = 32.5 ± 4.5) at two time points after treatments (mean ± SE, $n = 5$). Proteinase inhibitor II (PI-II) productions in (c) trichome-intact *spr2* plants and trichome-removed *spr2* (*spr2*-TR) plants, and in (d) trichome-intact WT plants and trichome-removed WT (WT-TR) plants at two time points after treatments (mean ± SE, $n = 6$). At the second-instar larval stage, MNL of *spr2* and *spr2*-TR plants are 71 ± 6.4 and 66.3 ± 6.1 ($n = 12$), respectively; MNL of WT and WT-TR plants are 42.2 ± 2.8 and 50.1 ± 6.4 ($n = 12$), respectively. Pupal weights of leafminers collected from (e) trichome-intact *spr2* plants and trichome-removed *spr2* (*spr2*-TR), and (f) trichome-intact WT plants and trichome-removed WT (WT-TR) (mean ± SE). At least five replications were performed per treatment for pupal weight ($n \geq 5$) and 10 pupae were measured in each replication. Emission rate of volatiles [ng h^{-1} 10 g^{-1} fresh weight (FW)] from undamaged (UnD) and leafminer-damaged (LD) (g) *spr2* or (h) WT plants which had the trichomes removed or were intact (control). For details on the compound list, see Supporting Information Fig. S3. MNL of *spr2* and *spr2*-TR plants are 36 ± 2.4 and 35.8 ± 4.7 ($n = 12$), respectively, whereas those of the WT and WT-TR plants are 25.7 ± 4.4 and 21.3 ± 2.7 ($n = 12$), respectively. (a–h) Different letters above bars within each treatment indicate significant difference (Student's *t*-test, $P < 0.05$). (i, j) Behavioural responses of naïve female wasps to volatile blends emitted by paired LD trichome-intact and trichome-removed (i) *spr2* or (j) WT plants. Bars represent the percentages of parasitoids choosing either odour source in the Y-tube olfactometer. The numbers of parasitoids choosing each olfactometer arm were analysed by a χ^2 test. Numbers in bars are the numbers of parasitoids choosing the indicated odour source; n.c. indicates no choice. NS, non-significant; WT, wild-type.

leafminers showed significant preferences for feeding and oviposition on *jai1* plants over *spr2* plants, and significantly higher numbers of larvae on *jai1* plants were parasitized (Fig. 3). Trichome removal experiments with odour-deficient *spr2* mutants increased feeding and oviposition on TR plants. The parasitism on leafminer-damaged TR *spr2* plants was also substantially higher than that on leafminer-damaged *spr2* plants with intact trichomes. Similar trichome removal experiments using WT plants also showed that decreased density of trichomes in plants was associated with increased preference and acceptance of insects (Fig. 3). Finally, using paired *jail* mutant and WT plants bioassays, we showed the predominant role of plant trichomes in host acceptance and reproductive successes by insects (Fig. 3). Although leafminer-damaged WT plants were significantly more attractive to parasitoids than damaged *jai1* plants in Y-shaped olfactometer and cage experiments (Fig. 3i,j), parasitoids preferred to oviposit in larvae on trichome-deficient *jai1* plants. We have shown in a previous study that adult leafminers utilize HIPVs to locate host plants, and the HIPVs of the WT plant are more attractive to adult leafminers and parasitoids than the HIPVs of JA-deficient *spr2* plants (Kang *et al*. 2009; Wei *et al*. 2011).

Although plant trichomes contribute to constitutive terpene (monoterpenes and sesquiterpenes) biosynthesis (Li *et al*. 2004; Schilmiller *et al*. 2009), previous studies (Sanchez-Hernandez *et al*. 2006; Wei *et al*. 2011) and the present one (Figs 1, 4 & Supporting Information Fig. S3) have demonstrated that herbivore damage, mechanical damage and exogenous JA application trigger some levels of terpene up-regulation, suggesting that terpene productions are directly linked to leaf tissue damage as well as to chemical elicitation such as JA treatment or oral secretion of insects. However, the underlying molecular and chemical mechanisms behind terpene biosynthesis following insect herbivory in plants remain elusive. This study is the first to demonstrate that JA levels, PI-II levels and volatile emissions are not affected by trichome removal in tomato plants, although the removal of trichomes has been proposed by other authors (Fordyce & Agrawal 2001). The variations in the parasitism rates of leafminers on TR and trichome-intact plants are due to the responses of parasitoids to plant trichomes (Fig. 3), not to the variations in attraction to HIPVs or to mortality during the development of the immature stages of the parasitoid (Figs 2 & 3). These strong behavioural responses of insects to trichomes reinforce the suggestion of a physical role of leaf trichomes in the host selection process of small insects.

JA-mediated defences in biosynthesis and signaling mutations

In the JA biosynthesis mutant *spr2*, the reduced JA levels, PI-II levels, and volatile emissions before and after herbivore damage (Fig. 2a) (Wei *et al*. 2011) are due to the loss of function of *ω*-3 chloroplast fatty acid desaturase, which reduces the the lipid-derived fatty acid 18:3 content in leaflets to <10% of WT levels (Li *et al*. 2003). Therefore, *spr2* mutant plants are compromised in direct and indirect defences against larval leafminers compared with WT plants (Wei *et al*. 2011). However, we found that this mutant was less attractive to adult flies compared with WT plants, implying that the reduced attractiveness to foraging herbivores may be due to the reduced HIPV emission (Wei *et al*. 2011). In contrast to the JA biosynthesis mutant *spr2*, the JA signalling *jai1* mutant produces comparable basal and induced levels of JA to that in WT plants (Fig. 2a). Therefore, the reduced PI-II levels, volatile emissions and trichome density as well as the compromise in direct defence against the larval leafminers are not due to JA levels but to JA perception. In *Arabidopsis*, jasmonate ZIM-domain (JAZ) proteins, the substrates of COI1–based SCF^{COI1} complex, negatively regulate trichome formation. The genes that control trichome development belong to a group of well-defined transcription factor complexes, including MYB transcription factor and homeodomain types (Katsir *et al*. 2008; Qi *et al*. 2011). The JA-induced degradation of JAZ proteins disrupts the interaction of JAZ proteins with basic-helix-loop-helix (bHLH) and MYB factors, allowing the transcriptional function of WD-repeat/bHLH/MYB complexes to activate the corresponding downstream signal cascades to modulate trichome initiation (Qi *et al*. 2011).

The overexpression of the MYB transcription factor bHLH factors (GL3 and EGL3) restores trichome initiation in *Arabidopsis thaliana coi1* mutant. The tomato *jai1* mutation is in the tomato homolog of the *Arabidopsis COI1* gene, which is essential for JA-mediated induction of accumulating proteinase inhibitor proteins (Li *et al*. 2004). These results open up the promising perspective of overexpressing *Arabidopsis*-homologous genes that regulate the trichome number or morphology in other plants, such as cultivated tomato. However, in contrast to the relatively simple non-glandular trichome in *Arabidopsis*, much less is known about the underlying molecular mechanism generating the various glandular trichomes of tomato plant (Li *et al*. 2004) and their ecological functions in multitrophic systems or more natural conditions (Kennedy 2003; Kang *et al*. 2010).

CONCLUSIONS

Trichome-based tomato resistance has long been suggested as an environmentally friendly insect-pest management strategy (Lin *et al*. 1987; Kennedy 2003). This study provides strong evidence that antagonism occurs between HIPVs and trichomes in an important crop plant. This finding is essential for the development of trichome-based behaviour-manipulated pest management strategies. The following push and pull strategies are proposed to increase plant herbivore resistance: (1) if repellent rather than toxic functions of trichomes are exerted in genetically modified plants, potential negative impacts on natural enemies would be reduced; and (2) engineered trapping plants with few trichomes can potentially be used to harbour herbivores and their natural enemies to protect crops. The plant fitness effect of interaction of these traits needs to be studied in agricultural systems with various genetic lines to understand better the significance of this interaction for crop protection.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Leaf trichomes on the adaxial surface of WT (a), *spr2* (b) and *jai1* (c) plants.

Figure S2. Removal of trichomes on the adaxial surface of tomato plants.

Figure S3. Volatile profiles of trichome-removed and trichome-intact tomato plants before and after leafminer infestations.