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ORIGINAL ARTICLE

Role of trichomes in defense against herbivores: comparison of herbivore response to *woolly* and *hairless* trichome mutants in tomato (*Solanum lycopersicum*)

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Abstract Trichomes contribute to plant resistance against herbivory by physical and chemical deterrents. To better understand their role in plant defense, we systemically studied trichome morphology, chemical composition and the response of the insect herbivores *Helicoverpa zea* and *Leptinotarsa decemlineata* (Colorado potato beetle = CPB) on the tomato *hairless* (*hl*), hairy (*woolly*) mutants and wild-type Rutgers (RU) and Alisa Craig (AC) plants. *Hairless* mutants showed reduced number of twisted glandular trichomes (types I, IV, VI and VII) on leaf and stem compared to wild-type Rutgers (RU), while *woolly* mutants showed high density of non-glandular trichomes (types II, III and V) but only on the leaf. In both mutants, trichome numbers were increased by methyl jasmonate (MeJA), but the types of trichomes present were not affected by MeJA treatment. Glandular trichomes contained high levels of monoterpenes and sesquiterpenes. A similar pattern of transcript accumulation was observed for monoterpene *MTS1* (=*TPS5*) and sesquiterpene synthase *SST1* (=*TPS9*) genes in trichomes. While high density of non-glandular trichome on leaves negatively influenced CPB feeding behavior and growth, it stimulated *H. zea* growth. High glandular trichome density impaired *H. zea* growth, but had no effect on CPB. Quantitative real-time polymerase chain reaction (qRT-PCR) showed that glandular trichomes highly express protein inhibitors (*PIN2*), polyphenol oxidase (*PPOF*) and hydroperoxide lyase (*HPL*) when compared to non-glandular trichomes. The SlCycB2 gene, which participates in woolly trichome formation, was highly expressed in the *woolly* mutant trichomes. *PIN2* in trichomes was highly induced by insect feeding in both mutant and wild-type plants. Thus, both the densities of trichomes and the chemical defenses residing in the trichomes are inducible.

Keywords Jasmonate · Plant defenses · Induced defenses · Herbivores · *Leptinotarsa decemlineata* · *Helicoverpa zea*

Abbreviations

woolly	Hairy mutant
hl	Hairless mutant
RU	Rutgers wild-type plants of woolly mutant
AC	Alisa Craig, wild-type plants of hairless mutant
MeJA	Methyl jasmonate
PIN2	Protease inhibitor 2
HPL	Hydroperoxide lyase
SlCycB2	B-type cyclin
PPOF	Polyphenol oxidase
CPB	Colorado potato beetle

Introduction

Trichomes are hair-like protuberances that develop from the aerial epidermis on leaves, stems and other organs on many plant species (Creelman and Mullet 1995; Duffey 1986; Kang et al. 2010a, b; Reeves 1977; Steffens and Walters 1991; Wagner 1991). Trichomes are normally classified as either glandular or non-glandular and may vary in size, shape, number of cells, morphology, and

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chemical composition (Goffreda et al. 1989; Kang et al. 2010a, b; Schilmiller et al. 2010; Nonamua et al. 2008). Numerous studies have shown that trichomes serve multiple functions including defense against herbivores by interfering with their movement and/or by direct toxicity through chemicals they produce and/or release (Arimura et al. 2005; Dimock et al. 1982; Kang et al. 2010a, b; Kennedy 2003; Peiffer et al. 2009; Wagner 1991). Trichome-based plant resistance holds potential to improve sustainability of insect pest management by reducing pesticide use and decreasing the likelihood that pesticide resistance will develop (Dalin and Björkman 2003; Holeski 2007; Simmons and Gurr 2004).

High densities of foliar trichomes may prevent feeding damage by herbivores (Handley et al. 2005; Horgan et al. 2009; Kessler and Baldwin 2002). Several studies have shown that herbivore feeding or mechanical damage to leaves leads to newly formed leaves with higher densities of trichomes (Agrawal et al. 2002; Ågren and Schemske 1994; Valkama et al. 2005; Holeski 2007; Traw and Dawson 2002). In some instances, jasmonic acid regulates trichome production and plant defense (van Schie et al. 2007; Li et al. 2004; Traw and Bergelson 2003). In tomato, application of methyl jasmonate (MeJA) increases densities of glandular trichomes on new leaves (Boughton et al. 2005). The inducibility of trichome density is ecologically significant because trichome density can negatively influence herbivore populations (Agrawal 1999; Horgan et al. 2009; Kessler and Baldwin 2002). While herbivory or wounding is known to affect the quantitative variation in trichome density, it is unknown if herbivory may also influence qualitative variation by altering the expression of defensive chemistry within glandular trichomes.

Because tomato (*Solanum lycopersicum*) is an economically important crop, it serves as a model for studying plant defenses against herbivores and diseases (Green and Ryan 1972; Howe and Jander 2008; Li et al. 1999; Mirnezhad et al. 2010; O'Donnell et al. 1996). Decades of research on tomato has shown that jasmonate signaling (JA) plays an important role in plant defense signaling against insect herbivory (Bostock 2005; Felton et al. 1994; Howe and Jander 2008; Thaler et al. 2001). Trichomes in cultivated tomato and related wild species have been subjects of intense study for many decades (Kennedy 2003; Peiffer et al. 2009; Steffens and Walters 1991). Unlike Arabidopsis, which only has non-glandular trichomes, trichomes in *Solanum* are highly diverse in morphology and chemistry (Kang et al. 2010a, b; Schilmiller et al. 2010). Tomato foliar trichomes are categorized as types I–VII, with types I, IV, VI and VII being glandular and types II, III and V being non-glandular (Kang et al. 2010b; Luckwill 1943). The major distinction between non-glandular and glandular is that glandular trichomes have heads containing various sticky and/or toxic chemicals that may poison or repel herbivores (Schilmiller et al. 2010). Non-glandular trichomes do not have heads and are thought to mainly function in defense by physically hindering insect feeding behavior and movements (Baur et al. 1991).

Insect behavior can be dramatically influenced when trichomes obstructs movement across the plant surface (Simmons and Gurr 2005). Moreover, glandular trichomes may have profound effects on herbivore performance (i.e., growth, survival and fecundity) and host-plant-selection behavior (Duffey 1986; Baur et al. 1991; Kennedy 2003; Amin et al. 2011; Pelletier and Dutheil 2006). The most remarkable feature of tomato glandular trichomes is their capacity to produce and secrete a wide variety of plant secondary compounds including terpenoids (Kang et al. 2010b; Karban and Baldwin 1997; Van Schie et al. 2007; Schilmiller et al. 2010), phenolics (Gang et al. 2001), sucrose esters, methyl ketones (Fridman et al. 2005) and organic acids (Gutiérrez-Alcalá et al. 2000). By studying isolated glands, many of genes involved in the biosynthesis of the chemicals have been characterized, including the roles of monoterpene *MTS1* (=*TPS5*) and sesquiterpene *SST1* (=*TPS9*) synthesis genes in regulating terpene biosynthesis (Besser et al. 2009).

Several methods have been used to study trichome functions in defense including manipulative and genetic methods. For instance, the experimental removal of tomato glandular trichome heads and exudates significantly decreased the mortality of aphids, the tomato fruitworm *Helicoverpa zea*, and the Colorado potato beetle *Leptinotarsa decemlineata* (Dimock et al. 1982; Kennedy 2003; Simmons and Gurr 2004). However, such mechanical treatments of the leaf are likely to induce JA-regulated defenses (Peiffer et al. 2009) and can confound interpretation of results by altering the leaf surface and chemistry in ways unrelated to natural variation in trichome density. The use of mutants is a more rigorous and less ambiguous experimental approach for deciphering the roles of different trichome types in resistance against herbivores (Kang et al. 2010a, b).

In tomato, the effect of trichome-based resistance on insect herbivores has mostly focused on glandular trichomes and there has been considerably less study on the role of non-glandular trichomes against herbivores (Simmons and Gurr 2004; Kang et al. 2010a, b). Because most cultivars possess both glandular and non-glandular trichome, it is difficult to separate trichome function(s) in these commercial varieties. In this current study, we use two mutants with differing trichome types to examine the role of glandular and non-glandular trichomes in resistance to the solanaceous specialist *L. decemlineata* and the generalist *H. zea*. In addition to characterizing the morphologies and densities of the trichomes in these mutants,

we also report their terpene composition. We hypothesize that glandular and non-glandular trichome phenotypes contribute to defense against both herbivorous insect species.

Materials and methods

Plants and insects

Tomato (*S. lycopersicum*) hairless mutant (*hl*, LA3556) and wild-type Alisa Craig (AC, accession number LA2838), hairy mutant (*woolly*, LA0258) and wild-type Rutgers (RU, accession number LA1090) were used in all experiments. Seeds were originally obtained from Tomato Genetics Resource Center (University of California, Davis, CA, USA). Seedlings were grown as described previously (Peiffer and Felton 2005) in Metromix 400 potting mix (Griffin Greenhouse and Nursery Supplies Tewksbury, MA) in greenhouse at Penn State University, University Park, PA, USA). The greenhouse was maintained on a 16-h photoperiod.

Tomato fruitworm (*Helicoverpa zea*) and Colorado potato beetle (=CPB) (*Leptinotarsa decemlineata*) were reared in the Entomology Department, Penn State University (University Park PA, USA). *Helicoverpa zea* eggs were purchased from Bioserv (Frenchtown, NJ) and reared on a wheat germ and casein-based artificial diet (Chippendale 1970) with ingredients from Bioserv. Colorado potato beetle was collected from tomato plants in Centre County PA and reared on tomato plants as described previously (Chung and Felton 2011).

Morphology and density of different type trichome on mutant

At four-leaf stage, ten plants of each cultivar were sampled to compare trichome morphology and density of the trichome on leaf. Two leaf discs from the youngest fully expanded leaf were cut from each side of the mid-vein. Trichome numbers were counted under a light microscope on each leaf disc. The density of trichomes was calculated as the number per disc/cm^2.

Scanning electron microscopy (SEM) was performed to compare the morphology of trichomes on different mutants and followed the protocol described in Kang et al. (2010b). Briefly, tissues were fixed overnight in a solution of 2.5 % paraformaldehyde, 2.5 % glutaraldehyde buffered with 0.1 M sodium cacodylate, pH 7.0. Samples were dehydrated in a graduated ethanol series, critical point dried, then mounted, and sputter coated. Samples were then examined with a 20 kV accelerating voltage with a JEOL JSM5400 microscope (Tokyo, Japan).

Trichome purification and real-time PCR

Trichomes were purified following well-established methods with slight modifications (Peiffer et al. 2009). To isolate trichomes, leaflets/stems were removed, placed in a 50-ml conical tube containing 1 g glass beads (diameter, 4 mm) (Kimble chase, Vineland, NJ, USA) and liquid N_2, and the tube was shaken vigorously to shear trichomes off the leaf/stem. We then poured the slurry through a 1-mm strainer for non-glandular trichomes and rinsed with liquid N_2 while glandular trichomes need to go through a 100-μm strainer again followed by rinsing with liquid N_2. The purified trichome preparation was used for chemical analyses and quantification of trichome-specific gene expression.

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to compare gene expression. One hundred mg of purified trichomes were homogenized in liquid nitrogen and total RNA was purified with an RNeasy Plus Mini-kit which can remove genomic DNA (Qiagen, Valencia, CA, USA). A High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA) was used for cDNA synthesis. All qRT-PCR reactions used Power SYBR Green PCR Master Mix and ran on 7500 Fast Real-Time PCR system (Applied Biosystems) following standard protocols (10 min at 95 °C, with 40 cycles of 15 s at 95 °C and 60 s at 60 °C). Relative quantifications were calculated with wild-type RU leaf RNA as the reference group. The ubiquitin gene was used as the housekeeping gene to normalize $2^{-\Delta\Delta C(T)}$ (Rotenberg et al. 2006). To validate this analysis method, primer efficiency was analyzed by comparing the normalized $C(T)$ values of five serial dilutions of cDNA. Melting curve analysis was conducted to confirm the specificity of the primers. Three replicates for each varieties. The relative gene expression value was analyzed by one-way ANOVA followed by Fisher-LSD to do the multi-comparisons. The primers used are shown in Table 1.

Terpene analyses

Because terpenes have been reported to be important phytochemical components of tomato trichome-based resistance (Kang et al. 2010a, b; Wagner 1991), we explored terpene content using two methods. In the first method, we dissolved 100 mg of purified leaf or stem trichomes directly in 1 ml methyl *tertiary*-butyl ether (MTBE) buffer. For the second method, we dipped leaves into MTBE to remove surface terpenes. After weighing two leaves sampled from youngest fully expanded, they were incubated at room temperature in 1.0 ml of (MTBE) for 3 min with gentle shaking. For both assays, we collected three replicate samples per treatment.

Table 1 Primers used for real-time PCR assays of relative gene expression

Primer	Gene name/accession number	Primer sequence (5′–3′)
PIN2	Wound-inducible proteinase inhibitor 2 K03271	F: GGA TTT AGC GGA CTT CCT TCT G
		R: ATG CCA AGG CTT GTA CTA GAG AAT
MTS1 (=*TPS5*)	Monoterpene synthase TC191446	F: GTA ACA TAG GGA TGA TGG TGG TCA CCTT
		R: CTG AAC GCC TTG TGG TGG AAAT
SST1 (=*TPS9*)	Sesquiterpene synthase TC197727	F: AGC AAA CCT TAG AAC AAA CAA GCAA
		R: CCA AAC AGT TGG GTG AAA ATT AGC
PPO	Polyphenol oxidase Z12838	F: ATG TGG ACA GGA TGT GGA ACG AGT
		R:ACT TTC ACG CGG TAA GGG TTA CGA
SlCycB2	B-type cyclin	F: TGA GCA GGA GAA ATG GAA
		R: CAT TGT CAG CGA CCT TGT
HPL	Hydroperoxide lyase AY028373	F:CCT CGG CGC TGA TCC ATC TGT CTCC
		R:CTT GCA TAT CCG GGT TTT TCT CAT CTC
Ubi	Ubiquitin X58253	F: GCC AAG ATC CAG GAC AAG GA
		R: GCT GCT TTC CGG CGA AA

For all trichome extractions, we added to each sample 200 ng *n*-octane and 400 ng nonyl acetate as external standards. Trichome extracts were injected in 1-μl aliquots into an Agilent model 7890 gas chromatograph (GC) fitted with a flame ionization detector, using a splitless injector held at 250 °C. The column (HP-5; 30 m long × 320 μm interior diameter × 0.25 μm film thickness; J&W Scientific, Folsom, CA) was maintained at 35 °C for 1 min, then ramped at 10 °C per min to 250 °C. Quantifications of compounds were made relative to the nonyl acetate standard using ChemStation software (Agilent Technologies, Wilmington, DE). Identifications of trichome components were made with GC–MS in electron ionization mode comparing retention times and spectra with that of commercial standards.

Trichome induction by MeJA and bioassay

In order to compare different trichome responses to MeJA treatment, we treated plants with 3.3 mM MeJA (Sigma, St. Louis, MO, USA) in 0.8 % ethanol at fourth leaf stage. Two weeks after the spray treatment, we counted trichomes on the youngest fully expanded leaf as previously described. From the same cohort of plants, the two youngest leaves were collected for bioassays with *H. zea* and CPB. Thirty insects per treatment were used for the bioassay.

Bioassays using the mutants were conducted to compare how different types of trichomes affect insect growth. Detached leaves were used for the bioassay. Newly hatched *H. zea* larvae were reared on artificial diet for 2 days before transfer to tomato leaf for bioassay. Newly hatched CPB larvae were put on tomato leaf directly in a standard 1 oz. cup (BioServ, Frenchtown, NJ) with 2 % agar on the bottom to maintain leaf moisture. Insects were kept at 27 °C, with a 16:8 L:D photoperiod in an incubator. Thirty insects were used for each bioassay. After 5 days, larval weights were recorded using a precision balance and were statistically analyzed by one-way ANOVA followed by Fisher-LSD for multiple comparisons.

In order to compare how different trichome type affects insect feeding behavior, we used third instar *H. zea* or second instar CPB larvae on a detached tomato leaf. We recorded in a 10-min interval the time spent feeding during our observation under microscope. Fifteen larvae were observed for each species and genotype.

We also tested whether the mutants would impact the oviposition of CPB or *H. zea* moths. Ten mated female *H. zea* moths or ten randomly selected CPB adults were released in cages containing one plant from each of the four genotypes. After 24 h, we examined the plants thoroughly and counted the number of eggs on each plant. The experiment was replicated in eight cages. The average number of eggs on plants was compared on different mutant and wild-type plants.

Plant response to insect feeding

At the four-leaf stage, fifth instar *H. zea* were individually caged on the fourth leaf for 6 h, control plants had empty cages. Experiment was replicated nine times. Larvae were removed and after 24 h, the caged leaf was sampled for RNA extraction and qRT-PCR as previous described to compare *PIN2* relative expression with control leaf. Ubiquitin gene was used as internal control to normalize data.

Statistical analyses

Data were analyzed using general linear models for analysis of variance (ANOVA) and where appropriate with post

hoc comparison of means using the Fisher-LSD means separation test using Minitab (University Park, PA).

Results

Morphology and density of trichome on mutants and induction by MeJA

As observed with light microscopy and SEM, the morphology and trichome densities of the mutants and their respective wild types were highly variable (Figs. 1, 2). The *woolly* mutant had abundant trichomes on leaves (Fig. 1a), but primarily non-glandular types II, III and V. Trichome types II and III were similar in length, but differed in their base, which was multi-cellular under type II trichomes, but unicellular base under type III (Fig. 2a). Type V trichomes were shorter and had unicellular bases (Fig. 2a). While trichomes on the *woolly* mutant stem possessed both glandular and non-glandular trichomes, but were dominated by non-glandular trichomes on leaf (Fig. 1a, c).

The *hl* mutant, which has been described in Kang et al. (2010a), had fewer foliar trichomes than wild-type leaves. These mutant leaves had normal type I trichomes but most others were highly twisted and swollen (Fig. 1g). Type VI glandular trichomes have short neck cells that connect to the four-celled gland, and they showed irregular patterns and low density on the leaf surface (Figs. 1g, 2b). The trichomes on stems of the *hl* mutant showed similar twisted, swollen morphology and lower density (Fig. 1g, i). Both wild-type AC and RU leaves and stems possessed all types of trichomes including glandular and non-glandular, but type VI glandular trichomes were the most abundant on leaves (Figs. 1d, f, j, i; 2c, d).

Densities of trichomes on the mutants were significantly different than their respective wild-type genotypes. The mutant *hl* displayed twisted and low density of glandular trichomes on leaf surface; the glandular trichome density was reduced by 57 % compared to wild AC plants and no non-glandular trichome on the leaf surface. The density of the twisted glandular trichomes on *hl* was induced by MeJA treatment; twisted glandular trichomes on newly

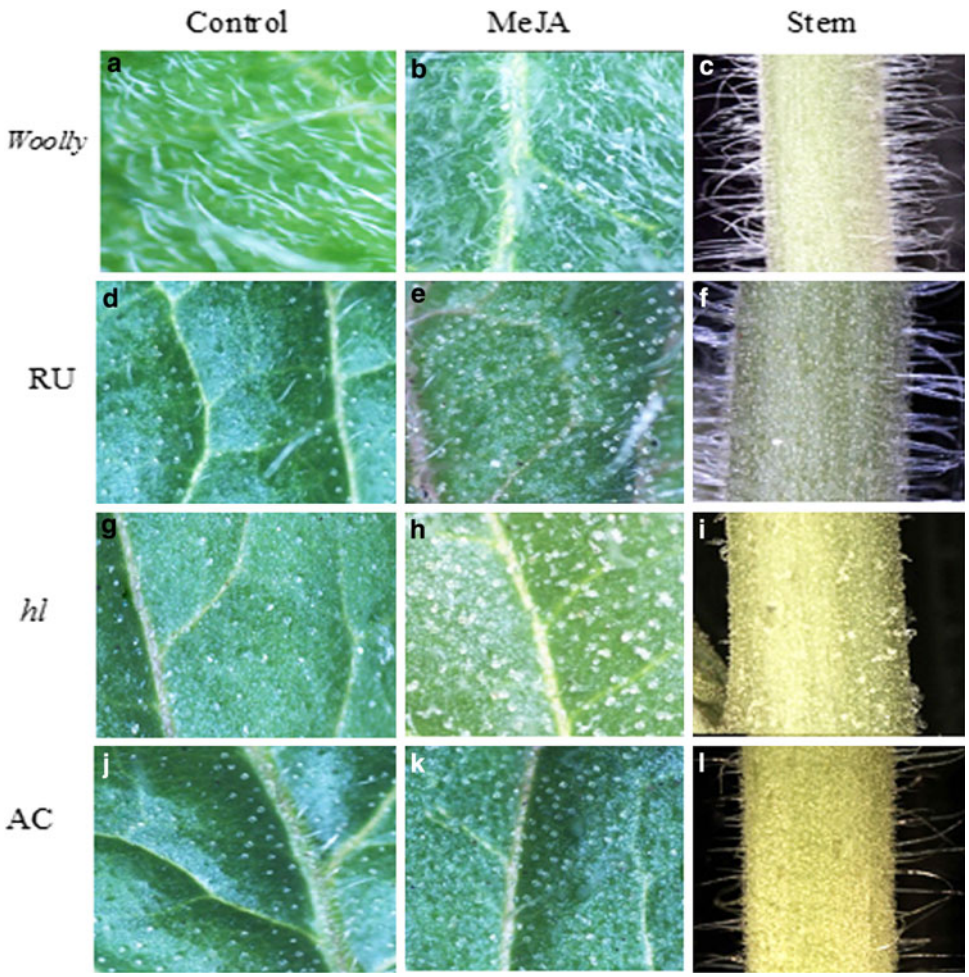

Fig. 1 Morphology of trichomes on mutant and wild-type leaf and stem, and induction by methyl jasmonate (MeJA): **a–c** *woolly* mutant trichome on leaf, stem and MeJA induction **d–f** wild-type Rutgers (RU) trichomes on leaves and stems, **g–i** hairless (*hl*) mutant trichome on leaves and stems and induction by MeJA, **j–l** wild-type Alisa Craig (AC) trichomes

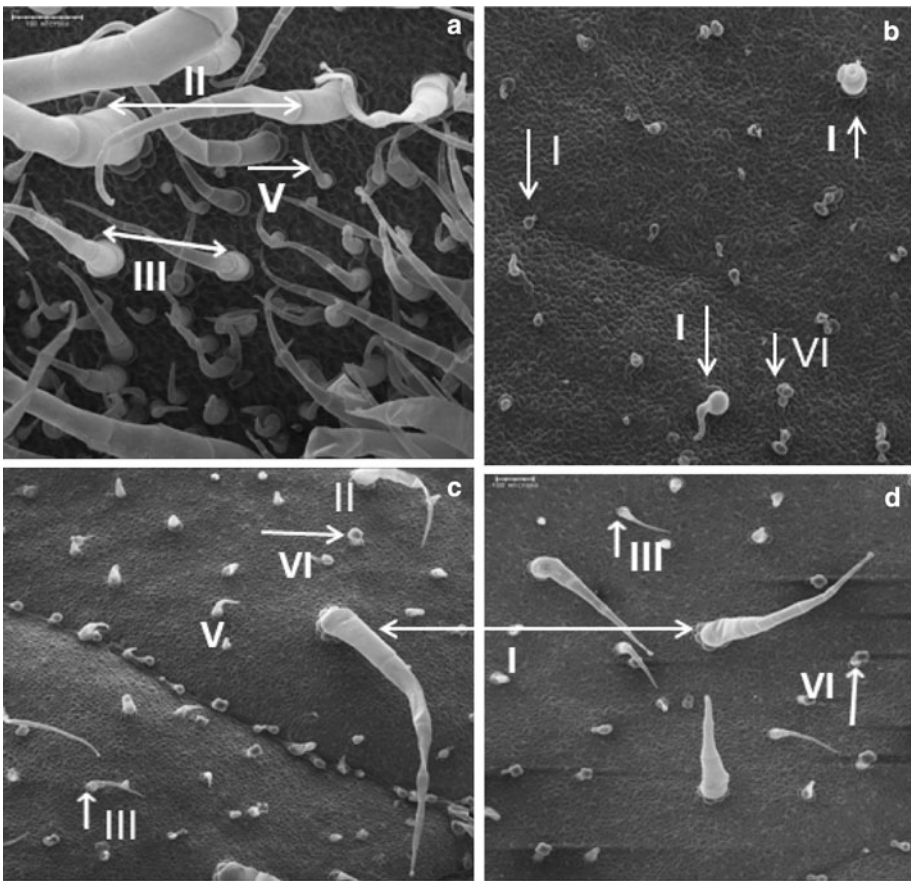

Fig. 2 Trichome morphology on leaves in wild-type and *hl*, *woolly* mutant plants under scanning electron microscope: **a** *woolly* mutant with type I and type III trichomes. **b** *hl* mutant showing fewer trichomes, mostly type VI trichomes, and some distorted type I trichomes. **c** Wild-type RU leaf with type I, II, V and VI trichomes. **d** Wild-type AC leaf with type III, III, and VI trichomes

growth leaf were increased 85.8 % after MeJA treatment. While the *woolly* mutant showed high density of non-glandular trichomes with very few glandular trichome on leaf surface compared to wild-type RU plants (Fig. 3a, b). The non-glandular trichomes on the *woolly* mutant also were induced by MeJA; non-glandular trichome numbers increased 37.8 % following treatment. Also the non-glandular trichome length was increased 71.4 % by MeJA compared to untreated plants (Fig. 3c). The glandular trichome densities on the RU and AC leaf surface were

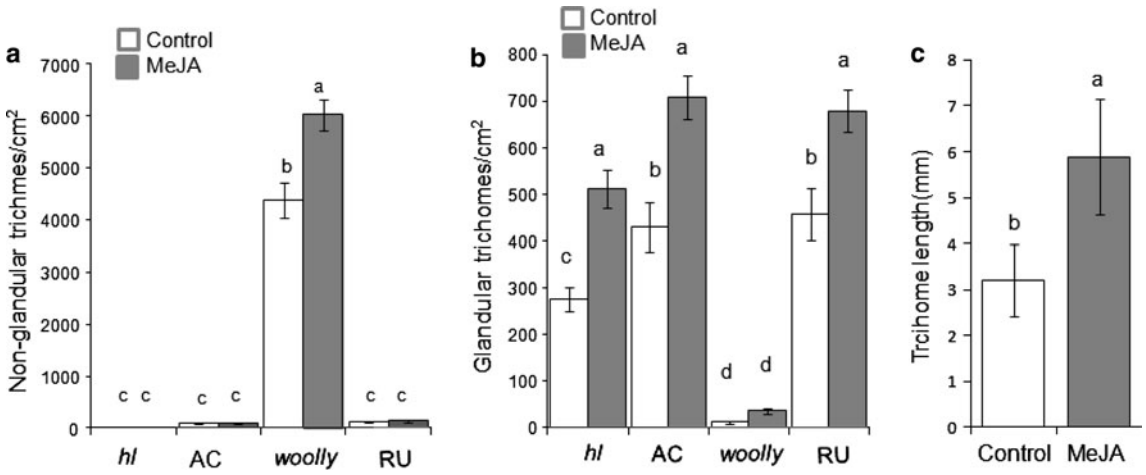

Fig. 3 Trichome density on *hl, woolly* mutant and wild-type plants and induction by MeJA. **a** Density of non-glandular trichome on *hl, woolly* mutant and wild-type leaves, induction by MeJA. **b** Density of glandular trichome on *hl, woolly* mutant and wild-type leaves and induction by MeJA. **c** Trichome length induced by MeJA. Data show trichome density as the mean (±SE) trichome number of 20 replicate leaves of fourth leaf, trichome length is the mean (±SE) mm of 15 non-glandular trichomes on different leaves. Means followed by different letters are significantly different at $P = 0.05$

increased 48.1 and 64.8 % by MeJA (Fig. 3b). Both type trichome densities on the leaf are induced by MeJA treatment.

Accumulation of terpenes in different trichomes

Previous reports indicate that monoterpenes and sesquiterpenes are produced in leaf trichomes (Fridman et al. 2005). Because there is significant variability in the morphology and density of trichomes on the leaf and stem of mutants, we compared the terpenoid composition of trichomes in these mutant lines to their wild types. Trichomes produced a mixture of several monoterpenes with *β*-phellandrene, (*Z*)-3-hexenyl acetate and *α*/*β*-pinene being the main constituents (Fig. 4). Monoterpenes in leaf trichomes were significantly more abundant than in stem trichomes (Fig. 5a). In the *hl* mutant, all the monoterpenes in leaf trichomes were significantly higher than in wild-type trichomes. Compared to the *hl* mutant and wild type, *woolly* mutants showed similar monoterpene profiles, with *β*-phellandrene and (*Z*)-3-hexenyl acetate as the main constituents, whereas *β*-pinene was not detected in the *woolly* mutant leaf trichomes (Table S1). The total monoterpenes in the *woolly* leaf mutant were significantly less than in the wild type (Fig. 5a; Table S1). These results indicate that foliar trichome monoterpene levels were significantly higher than in stems, and also monoterpenes are mainly produced in glandular trichomes.

The profile of sesquiterpenes in trichomes showed the main components are *e*-2-hexenylbutyrate, *β*-caryophyllene and *z*-jasmone (Table S2). Compared to monoterpenes, the quantity of sesquiterpenes was significantly less in the both leaf and stem trichomes (Fig. 5a, b). Most sesquiterpene levels in stem trichomes were less than foliar

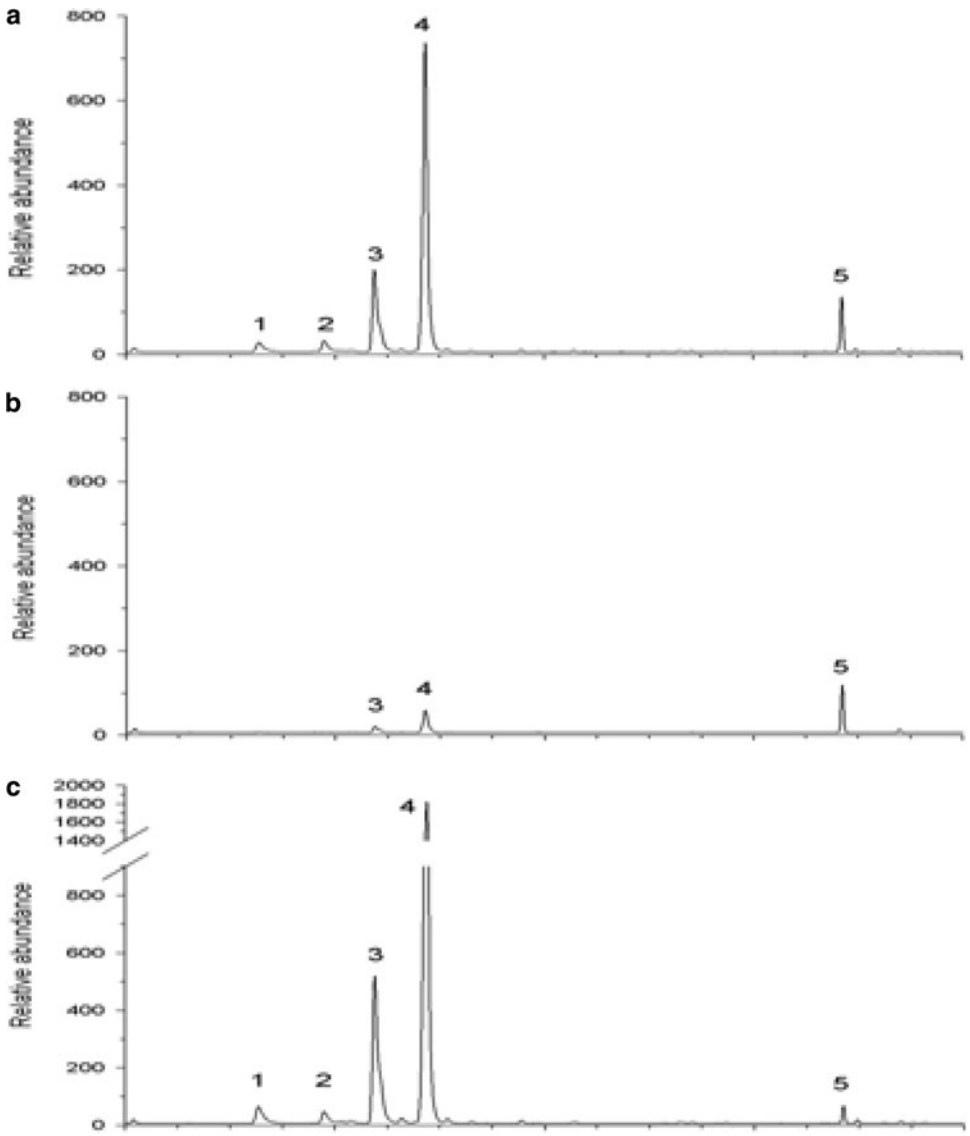

Fig. 4 Representative total ion chromatograms showing MTBE extractions of leaf trichomes from **a** Rutgers (RU; wild type for *woolly*), **b** *woolly* (non-glandular trichomes), **c** *hl* (glandular trichomes). Compounds are: (*1*) *α*-pinene, (*2*) unknown monoterpene, (*3*) *cis*-3-hexenyl acetate, (*4*) *β*-phellandrene, (*5*) nonyl acetate (external standard)

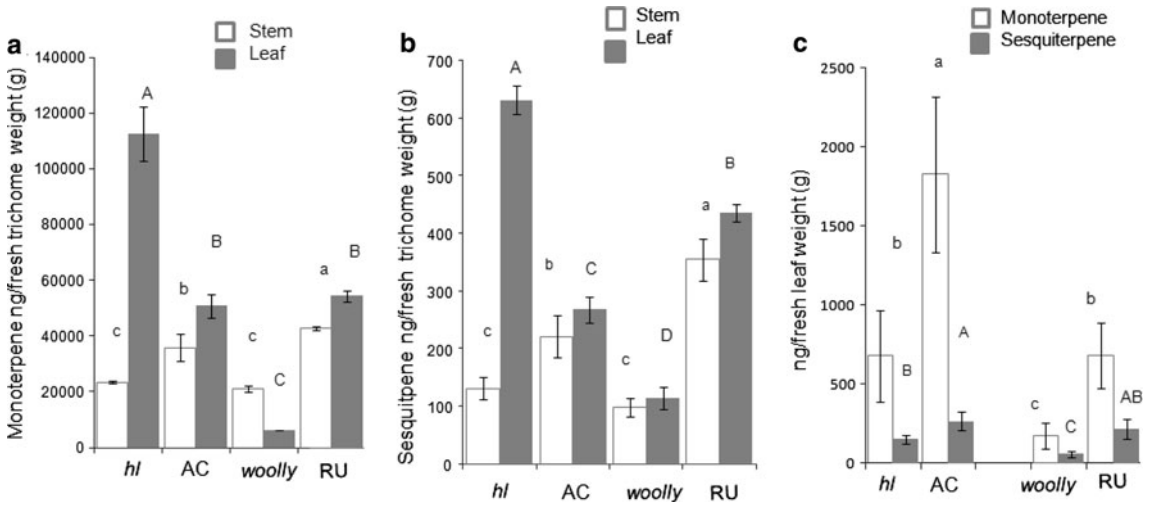

Fig. 5 Comparison of monoterpenes and sesquiterpenes levels in mutant and wild-type leaves and stems. **a** Monoterpenes in stem and leaf. **b** Sesquiterpenes in stem and leaf. **c** Leaf dip method for comparison monoterpenes and sesquiterpenes. Each data point represents the mean ± SE of three replicates. Means followed by different letters are significantly different at $P = 0.05$

trichomes. Also the *z*-3-hexenyl butyrate, α-humulene and β-farnesene were not detected in both *hl* and *woolly* stem trichomes (Table S2). The *hl* glandular trichome sesquiterpene level showed higher quantity than wild-type plants, while *woolly* mutant non-glandular trichome showed lower quantity than wild-type plants.

Analysis of leaf dip extracts also showed that the *hl* and *woolly* mutants had less monoterpenes and sesquiterpenes than their respective wild-type AC and RU. This is likely due to decreased trichome density on *hl* plants relative to the wild types, and the lack of glandular trichomes on woolly mutants (Fig. 5c). Results from both extraction methods indicate that glandular trichomes are the main source of monoterpenes and sesquiterpenes. Kang et al. (2010a) reports on the trichome terpenoids analysis showed less β-phellandrene and other sesquiterpenes in type VI trichomes. These results showed that compared to glandular trichomes, the non-glandular trichomes accumulate less of these chemicals in the trichomes. Although the trichomes on the *hl* mutant showed distorted morphology, it did not significantly affect the chemical composition in the leaf trichomes.

Comparison of gene expression in different tomato trichomes

Trichomes on many solanaceous species are known to express terpene synthases and produce a variety of terpenes (Schilmiller et al. 2010). Previous study showed that trichome-specific transcripts play a role in controlling terpenoid production (Besser et al. 2009). In this study, we examined the expression of two genes encoding terpenoid synthesis: *MTS1* (=*TPS5*) which encodes for monoterpene synthase and *SST1* (=*TPS9*) which encodes for sesquiterpene synthase. Transcript levels measured by qRT-PCR showed that *MTS1* (=*TPS5*) and *SST1* (=*TPS9*) are highly expressed in glandular trichomes (Fig. 6). The relative expression of *MTS1* (=*TPS5*) and *SST1* (=*TPS9*) in the *woolly* mutant is 9.2- and 3.6-fold lower than the wild type, while in the *hl* mutant *MTS1* (=*TPS5*) and *SST1* (=*TPS9*) are more highly expressed than wild type (Fig. 6a, b). These results are consistent with the monoterpene and sesquiterpene levels in the trichomes.

In addition to the defense provided by terpenes, proteinase inhibitors are another class of defensive proteins in tomato and can accumulate in many plant tissues (Kang et al. 2010a, b; Ryan 1990). Our study showed the relative expression of *PIN2* is highly expressed in *hl* trichomes compared to the non-glandular *woolly* trichomes (Fig. 6c). Polyphenol oxidase (*PPO*) is another important defensive protein which is encoded by seven-member gene family and high expressed tomato trichomes (Yang et al. 2011; Thipyapong and Steffens 1997). Our results showed that *PPOF* gene is highly expressed in glandular trichomes, but has very low expression in the *woolly* mutant, which lacks glandular trichomes. Hydroperoxide lyase (*HPL*) cleaves C18-lipid hydroperoxides to form a C6 aldehyde and a 12-carbon oxoacid. HPL activity has been found in a variety of plants and is associated with plant development and defense. In our experiments, compared with wild-type leaf RNA, the *HPL* was highly expressed in glandular trichomes, but less expressed in non-glandular trichomes (Fig. 6e). Recent study showed that the B-type cyclin (SlCycB2) gene participates in trichome formation (Yang et al. 2011), and our study showed that SlCycB2 was highly expressed in *woolly* mutant trichome, but with lower

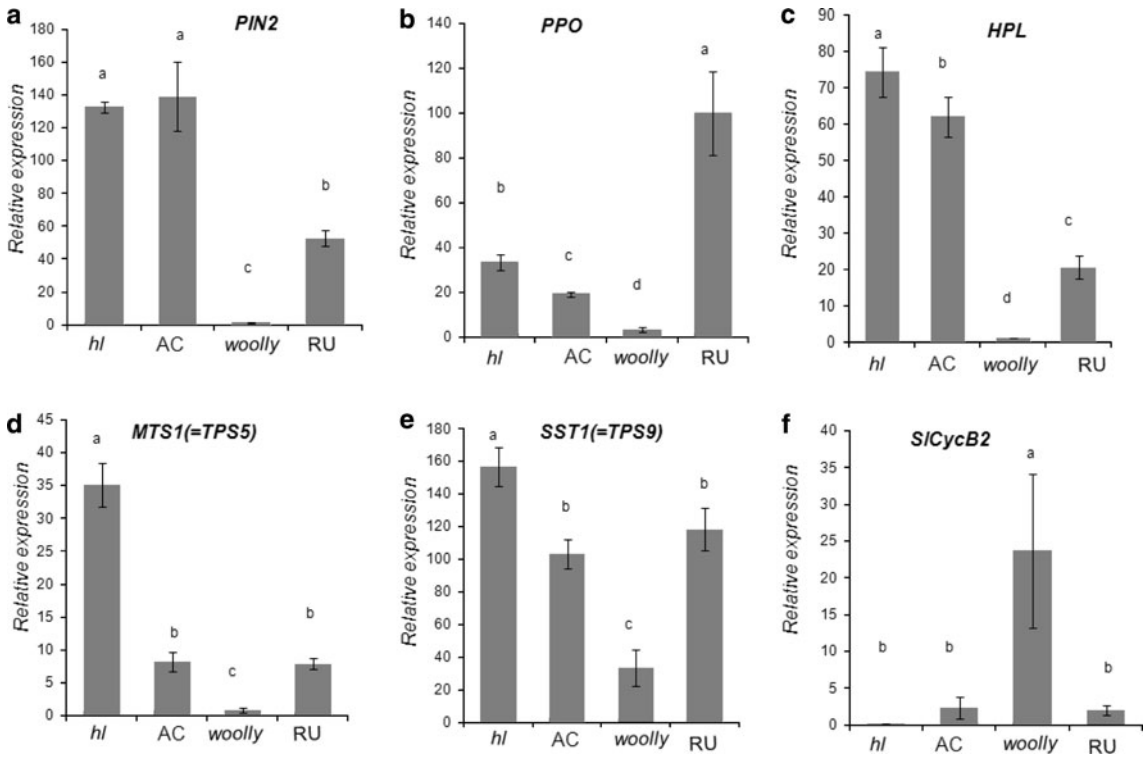

Fig. 6 Relative gene expression in different types of trichomes in *hl, woolly* and wild type. Trichomes were extracted from 30 plant leaves, relative expression is calculated to wild-type RU leaf RNA. *Bars* indicates standard error of three technical replicates. *PIN2* proteinase inhibitor 2 gene, *MTS1* (=*TPS5*) monosesquiterpene synthase 1 gene, *SST1* (=*TPS9*) Sesquiterpene synthase 1, *PPO* polyphenol oxidase gene. *SlCycB2*, B-type cyclin gene. *HPL*, hydroperoxide lyase gene. Means followed by different letters are significantly different at $P = 0.05$

expression in *hl* mutant (Fig. 6d). This result confirmed that SlCycB2 plays an important role in non-glandular trichome formation.

Effects of mutants on insect growth and oviposition

Because the morphology, density and chemical composition of trichomes are different between mutants and their wild types, these varieties may differ in their resistance to insect herbivores. Bioassays showed that *H. zea* larval grew significantly better on *hl* mutants than on wild-type AC plants indicating that glandular trichomes are an important source of resistance to this insect. Both *hl* mutant and wild-type glandular trichomes are highly induced by MeJA treatment and the larval growth on induced leaves was significantly reduced on both *hl* and the AC wild-type plants (Fig. 7a). While *woolly* mutant had high density of non-glandular trichomes, *H. zea* growth was significantly better on this mutant compared to wild-type RU plants. Again, MeJA treatment enhanced resistance to *H. zea* for both woolly mutant and wild-type plants (Fig. 7a). This result confirmed that glandular trichome density is an important factor in resistance to *H. zea*.

In contrast to *H. zea*, Colorado potato beetle (CPB) larvae showed no significant differences in larval growth on *hl* mutants compared to wild type (Fig. 7b). These data indicate that the density of glandular trichomes found on the wild type is not an important mediator of larval growth in CPB. Additionally, MeJA treatment did not affect larval growth on newly formed leaves for the *hl* mutant or its AC wild type. Conversely, the *woolly* mutants significantly reduced CPB growth compared to wild-type plants (Fig. 7b), indicating that the very high densities of non-glandular trichomes on this mutant strongly influenced CPB growth.

To determine if the larval growth data relate to feeding behavior, we recorded the time larval *H. zea* and CPB spent feeding on mutant and wild-type plants. The duration of CPB feeding on the *woolly* mutant was significantly lower than the amount of time spent feeding on wild-type leaves indicating that growth reduction can be, in part, explained by a reduction in feeding due to the high density of non-glandular trichomes. The amount of time *H. zea* spent feeding was not influenced by non-glandular trichomes on *woolly* mutants, but fewer glandular trichomes on woolly leaves appeared to allow *H. zea* to feed longer than on

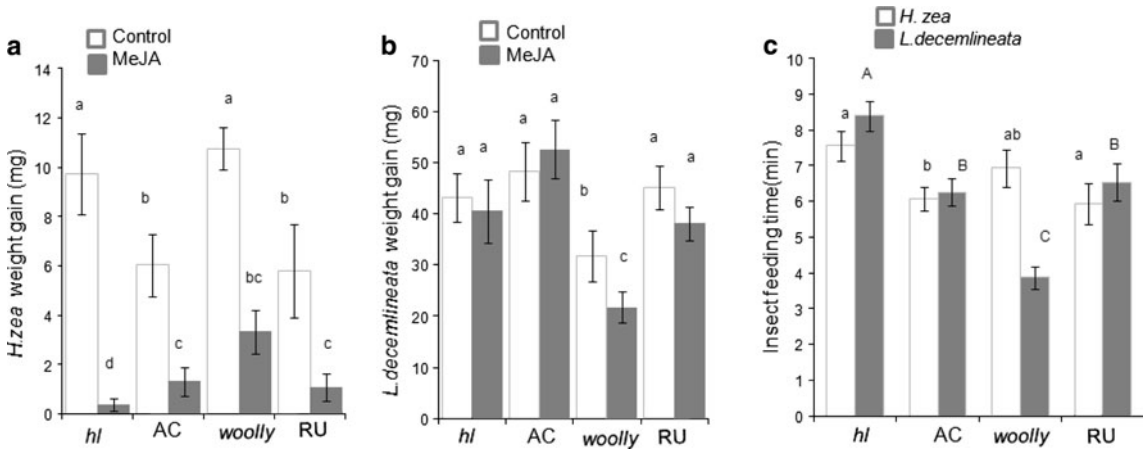

Fig. 7 *hl* and *woolly* mutant affect *Helicoverpa zea* and Colorado potato beetle growth and feeding time. No choice bioassay were performed by placing first instar *H. zea* and CPB for 4 days bioassay. **a** *hl*, *woolly* mutants and wild type influence *H. zea* growth. **b** *hl*, *woolly* mutants and wild type influence CPB growth. **c** Feeding time influenced by different mutants compared to wild-type plants. Data represent mean ± SE of larval weight ($n = 30$); data of feeding time represents mean ± SE of larvae ($n = 15$) feeding on leaves. Means followed by different letters are significantly different at $P = 0.05$

wild-type plants. During our observations of *H. zea* feeding, we found that larvae preferentially ingested non-glandular trichomes prior to feeding on leaf mesophyll. There was no evidence that larvae later regurgitated the non-glandular trichomes. The CPB do not preferentially feed on the trichomes and it appears that the trichomes interfere with their access to the leaf (Fig. 7c).

Because trichomes have been reported to influence insect oviposition (Heinz and Zalom 1995; Horgan et al. 2009; Kessler and Baldwin 2002), we conducted this experiment to determine if tomato trichomes influence ovipositional choice for both insect species. We found no observable differences in ovipositional choice for the different plant varieties (Fig. S1). *H. zea* moths laid eggs randomly on the adaxial leaf surface of all varieties and CPB laid eggs in clumps of 5–20 on the adaxial leaf surface equally across varieties (Fig. S2).

PIN2 induction by insect feeding

Because trichome expression has been shown to be partly dependent upon a functional jasmonate signaling pathway (Peiffer et al. 2009), we tested if induction of the JA-regulated defense gene *PIN2* was influenced by *H. zea* feeding. In all genotypes, the expression of *PIN2* was significantly induced by feeding compared with their unwounded plants. However, no differences in levels of *PIN2* induction were detected among the different mutants and wild-type plants (Fig. 8). These results indicate that JA signaling is activated by insect feeding damage in both mutants and wild-type plants and suggest that differences in insect growth on the mutants were likely not due to significant impairments in jasmonate signaling.

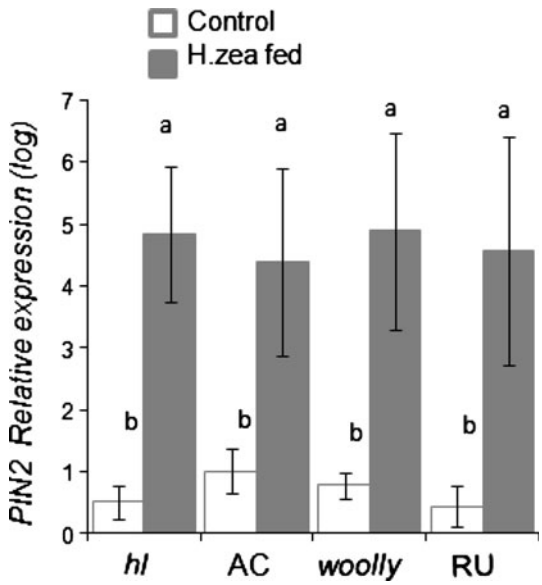

Fig. 8 *H. zea* feeding induced relative expression of proteinase inhibitor 2 (*PIN2*) in mutant and wild-type plants. *woolly* is the woolly (hairy) mutant, *hl* is the hairless mutant, RU and AC are the wild type of *woolly* and *hl* mutant. Relative expression of *PIN2*. Means followed by different letters are significantly different at $P = 0.05$

Discussion

Trichome-based resistance in tomato plants offers a feasible approach to reduce pesticide applications (Simmons and Gurr 2004; Alba et al. 2009; Kang et al. 2010b). Trichome morphology, density and chemical composition are important mechanisms of defense to prevent or decrease herbivore damage. The morphology and density of leaf trichomes vary considerably among plant species and may

also vary among populations and within individual plants (Dalin and Björkman 2003; Kang et al. 2010a; Holeski 2007). However, in plants such as tomato, which possess a variety of trichome types, these specialized cell types can be difficult to isolate separately to study the function of each type of trichome. Thus, the use of mutants provides a good tool to study trichome function. The *hl* mutant was described as hairless, and the phenotypic expression of this mutant at the microscopic level shows a characteristic twisting and shortening of trichomes (Kang et al. 2010b; Reeves 1977). The *woolly* mutant was described as hairy with a high density of type II and III non-glandular trichomes on leaf surface.

Numerous studies have shown that trichomes are capable of synthesizing and either storing or secreting large amounts of specialized metabolites that could influence their interaction with herbivores (Schilmiller et al. 2010). Chemical profiles of type VI trichome in *hl* mutant showed similar profiles of monoterpenes and sesquiterpenes with wild-type plants, but with less β-phellandrene, β-caryophyllene and α-humulene (Kang et al. 2010b). In our experiment, we collected both types of trichome from the *hl* mutant, but the profiles of chemical composition were different from previous results. The β-phellandrene, β-caryophyllene and α-humulene levels were higher in *hl* trichomes than in the AC wild-type trichomes. The leaf dip method showed the same pattern with previous results (Kang et al. 2010a, b). These results indicated that although the trichomes were distorted in the *hl* mutant, the chemical accumulation in the twisted head cells was not affected. Not surprisingly the *woolly* mutant trichomes showed significantly low monoterpenes and sesquiterpenes confirming that that the glandular trichome is the main source of terpenes. This is consistent with the previous characterization of other *woolly* mutants (Yang et al. 2011). Our results also indicated that the foliar trichomes had higher levels of monoterpenes and sesquiterpenes than the stem trichomes.

Our results indicate that both glandular and non-glandular trichomes function in insect defense. The non-choice bioassay showed the growth of *H. zea* larvae was substantially improved by feeding on the *hl* genotype compared to the wild-type parent (cv. Alisa Craig). The bioassay results with the *hl* mutant are consistent with Kang et al. (2010a) who observed similar effects with another caterpillar species, *Manduca sexta*. These results show that caterpillar growth is compromised by the presence of glandular trichomes which contain an arsenal of chemical defenses (e.g., terpenes, polyphenol oxidase, etc.) that may entrap small larvae as they attempt to move on the leaf surface (Simmons and Gurr 2004).

Moreover, the feeding time of *H. zea* was significantly longer on the *hl* genotype compared to the parent wild type (cv. Alisa Craig). Larval *H. zea* spent more time feeding and had enhanced growth on the *woolly* genotype compared to the parent wild type (cv. Rutgers). These results indicate that the absence of glandular trichomes combined with a high density of non-glandular trichomes greatly enhances *H. zea* larval growth. We observed that larvae preferentially ingested the non-glandular trichomes prior to feeding on the remaining leaf tissue. We recognize that other pleiotropic factors (e.g., nutritional differences) could be responsible for the improved growth on this mutant; however, the increased feeding would argue otherwise. By comparison, when *H. zea* feed on leaves with high densities of glandular trichomes, they frequently are observed to remove the trichomes and egest them (personal observations). Our results are quite different from the recent finding in *Nicotiana attenuata* where neonate *M. sexta* preferentially consumes tobacco glandular trichomes as their first meal (Weinhold and Baldwin 2011).

The results with the specialist beetle *L. decemlineata* were markedly different from what we observed with *H. zea*. The *L. decemlineata* growth was not improved when larvae fed on the *hl* genotype compared to the wild-type parent. The *hl* mutant possesses a low density of glandular trichomes and reduced total terpenes in the leaves compared to the wild-type plants. Although beetle larvae were observed to feed for longer bouts on the *hl* mutant leaf compared to wild plants, this did not result in improved growth during the time course of our bioassay. Our bioassay results were different from Kang et al. (2010b). They investigated resistance of the *odorless-2* (*od-2*) mutant and found that larval growth of *L. decemlineata* was significantly increased on *od-2* mutant compared to wild-type plants. Although *od-2* possesses a normal glandular trichome phenotype, the levels of terpenes and flavonoids in the trichomes are compromised. In our study we used *L. decemlineata* which originated from tomato fields and has been reared solely on tomato. Perhaps our colony is better adapted to tomato and is not as sensitive to the level of terpenes and flavonoids in the glandular trichomes. The most surprising results we observed were with the *woolly* genotype which negatively influenced larval beetle weight gain and feeding time. We observed that *L. decemlineata* larvae prefer to feed on leaves with very low density of trichomes. We observed that larval *L. decemlineata* had difficulty finding a suitable feeding site on the *woolly* mutant leaf which contains a very high density of non-glandular trichomes (personal observation). Similar results were found when the larvae of the leaf beetle (*Phratora vulgatissima L.*) fed on willows (*Salix viminalis*), where they appeared to feed at the base of trichomes (Dalin et al. 2004). These results indicate that non-glandular trichomes although harmless or perhaps even beneficial for caterpillar growth, were detrimental to beetle feeding and growth.

We did not find that trichomes impacted oviposition by either insect species in the genotypes we tested. Levin (1973), Dalin et al. (2008) and others have reported that trichomes influence insect oviposition in a wide range of insects and that trichome length on the leaf surface is often negatively correlated with the number of eggs laid. But in our study, using a choice-test, *H. zea* and CPB adults did not exhibit ovipositional preference for any of the genotypes tested. While we cannot conclude that trichomes have no effect on oviposition for these insects, at least for these genotypes there was not sufficient variation in trichome type or density to uncover differences in ovipositional preference.

Trichome density is often inducible by insect herbivory or by plant hormones (Kessler and Baldwin 2002; Traw and Dawson 2002). Trichome density is regulated by jasmonates, brassinosteroids and ethylene in tomato (Boughton et al. 2005; Campos et al. 2009). In fact, induction of glandular trichomes by herbivory, wounding, or MeJA treatment in the parent generation persists in the offspring of the parents (Rasmann et al. 2012). In this study, both glandular and non-glandular types of trichomes were up-regulated by MeJA, although the specific trichome type was not induced by hormone treatment. When plants were treated with MeJA, *H. zea* growth was significantly reduced on treated plants of all genotypes. The growth of *L. decemlineata* was not affected by y MeJA treatment in the *hl*, AC or RU genotypes, except in the case of the *woolly* mutant where the non-glandular trichome length was significantly increased by MeJA treatment. The increase in the length of non-glandular trichomes may further impair larval feeding. These results provide further support for the role of non-glandular trichomes in resistance to *L. decemlineata*.

Trichomes are initiated in the epidermis of developing leaves; previous studies on *Arabidopsis* have revealed several key regulators that participate in trichome formation and induction (Ishida et al. 2008; Yoshida et al. 2009). The mechanisms underlying trichome formation in tomato are less clear. Previous study on *hl* mutant showed that the Hl gene plays an important role in the synthesis of new cell wall material in developing trichomes. Cloning of the gene is needed to understand the precise function of Hl in trichome development (Kang et al. 2010b). The *woolly* (*Wo*) gene is responsible for trichome formation and regulates SlCyB2 gene expression that participates in trichome formation. Suppression of *SlCyB2* using RNAi on the mutant (LA3186) produced low densities of trichomes on the leaf surface (Yang et al. 2011). In our study, we used a different *woolly* mutant (LA0258) from the previous study and our results showed the SlCyB2 gene was also highly expressed in *woolly* trichomes.

While the induction of high densities of trichomes may impact herbivores, the secondary metabolites produced by glandular trichomes may also negatively impact herbivore feeding and growth. Transcriptional co-regulation is an important hallmark of genes involved in secondary metabolite pathway and our results confirmed that there is high degree of association between monoterpene and sesquiterpene content and gene expression [i.e., *MTS1* (=*TPS5*) and *SST1* (=*TPS9*)]. In addition to terpenes, glandular trichomes contained other defenses including anti-nutritive proteins and phenolics, thus it is not surprising that tomato glandular trichomes have been implicated in resistance to a variety of herbivore species including caterpillars and aphids (Larocca et al. 2011; Kang et al. 2010b). For instance, trichomes of many *Solanum* species accumulate significant levels of polyphenol oxidase and play roles in the oxidation of phenolics that may defend against insects and pathogens (Thipyapong et al. 1997). Proteinase inhibitors are another class of defensive proteins in tomato and can accumulate in many plant tissues which affect insect growth (Kang et al. 2010a, b; Ryan 1990). HPL is also associated with plant development and defense. Our results confirmed that *PIN2*, *PPOF* and *HPL* are highly expressed in glandular trichomes. High expression of these proteins in the trichomes may reduce the nutritional quality of dietary protein available to *H. zea*, but not that available to CPB, due to differences in the physical–chemical environment of their digestive systems (Johnson and Felton 1996; Zhu-Salzman et al. 2008). Moreover, the expression of the *PIN2* gene in the trichomes is induced by *H. zea* feeding indicating that herbivory may impact both the density of trichomes as well as their chemical composition.

Trichomes of the cultivated tomato are an important source of constitutive and inducible resistance to insect herbivores and offer potential for exploitation in plant breeding programs. Novel secondary metabolites that are present in the glandular trichomes of wild species of tomato and could be used for sources of host resistance have been shown to produce numerous arthropod defensive compounds (Karban and Baldwin 1997; Li et al. 2004). Our results with *H. zea* provide further substantiation that glandular trichomes play a role in resistance; however, our study provides several important caveats for development of host-plant resistance programs. Although, *H. zea* growth was compromised on the *hl* mutant, the growth of *L. decemlineata* was not. The use of non-glandular trichomes may be useful in resistance programs to *L. decemlineata*, but could inadvertently enhance susceptibility to some insects such as *H. zea*. Thus, our findings underscore the necessity to clarify the roles of specific trichomes to different herbivorous insect species before embarking on a comprehensive plant breeding program.

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References

Agrawal AA (1999) Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. Ecology 80: 1713–1723

Agrawal AA, Conner JK, Johnson MTJ, Wallsgrove R (2002) Ecological genetics of an induced plant defense against herbivores: additive genetic variance and costs of phenotypic plasticity. Evolution 56:2206–2213

Ågren J, Schemske DW (1994) Evolution of trichome number in a naturalized population of *Brassica rapa*. Am Nat 143(1):1–13

Alba JM, Montserrat M, Fernandez-Munoz R (2009) Resistance to the two-spotted spider mite (*Tetranychus urticae*) by acylsucroses of wild tomato (*Solanum pimpinellifolium*) trichomes studied in a recombinant inbred line population. Exp Appl Acarol 47:35–47

Amin MR, Tithi DA, Kwon YJ (2011) Characteristics of three cotton varieties and their impact on feeding and growth of cotton armyworm. Entomol Res 41:151–156

Arimura GI, Kost C, Boland W (2005) Herbivore-induced, indirect plant defences. Biochim Biophys Acta (BBA) Mol Cell Biol Lipids 1734:91–111

Baur R, Binder S, Benz G (1991) Non-glandular leaf trichomes as short-term inducible defense of the grey alder, *Alnus incana* (*L.*), against the chrysomelid beetle, *Agelastica alni* L. Oecologia 87:219–226

Besser K, Harper A, Welsby N, Schauvinhold I, Slocombe S, Li Y, Dixon RA, Broun P (2009) Divergent regulation of terpenoid metabolism in the trichomes of wild and cultivated tomato species. Plant Physiol 149:499–514

Bostock RM (2005) Signal crosstalk and induced resistance: straddling the line between cost and benefit. Annu Rev Phytopathol 43:545–580

Boughton AJ, Hoover K, Felton GW (2005) Methyl jasmonate application induces increased densities of glandular trichomes on tomato, *Lycopersicon esculentum*. J Chem Ecol 31:2211–2216

Campos ML, de Almeida M, Rossi ML, Martinelli AP, Litholdo CG, Figueira A, Rampelotti-Ferreira FT, Vendramim JD, Benedito VA, Peres LEP (2009) Brassinosteroids interact negatively with jasmonates in the formation of anti-herbivory traits in tomato. J Exp Bot 60:4346–4360

Chippendale G (1970) Development of artificial diets for rearing the Angoumois grain moth. J Econ Entomol 63:844–848

Chung SH, Felton GW (2011) Specificity of induced resistance in tomato against specialist Lepidopteran and Coleopteran species. J Chem Ecol 37:378–386

Creelman RA, Mullet JE (1995) Jasmonic acid distribution and action in plants: regulation during development and response to biotic and abiotic stress. Proc Natl Acad Sci USA 92:4114–4119

Dalin P, Björkman C (2003) Adult beetle grazing induces willow trichome defence against subsequent larval feeding. Oecologia 134:112–118

Dalin P, Björkman C, Eklund K (2004) Leaf beetle grazing does not induce willow trichome defence in the coppicing willow *Salix viminalis*. Agric For Entomol 6:105–109

Dalin P, Ågren J, Björkman C, Huttunen P, Kärkkäinen K (2008) Leaf trichome formation and plant resistance to herbivory. In: Schaller A (ed) Induced plant resistance to herbivory, pp 89–105

Dimock MB, Kennedy GG, Williams WG (1982) Toxicity studies of analogs of 2-tridecanone, a naturally-occurring toxicant from a wild tomato. J Chem Ecol 8:837–842

Duffey SS (1986) Plant glandular trichomes: their partial role in defence against insects. In: Juniper BE, Southwood TE (eds) Insects and the plant surface Arnold. England, London, pp 151–172

Felton GW, Workman J, Duffey SS (1992) Avoidance of antinutritive plant defense—role of midgut pH in Colorado potato beetle. J Chem Ecol 18:571–583

Felton GW, Bi JL, Summers CB, Mueller AJ, Duffey SS (1994) Potential role of lipoxygenases in defense against insect herbivory. J Chem Ecol 20:651–666

Fridman E, Wang JH, Iijima Y, Froehlich JE, Gang DR, Ohlrogge J, Pichersky E (2005) Metabolic, genomic, and biochemical analyses of glandular trichomes from the wild tomato species *Lycopersicon hirsutum* identify a key enzyme in the biosynthesis of methylketones. Plant Cell 17:1252–1267

Gang DR, Wang J, Dudareva N, Nam KH, Simon JE, Lewinsohn E, Pichersky E (2001) An investigation of the storage and biosynthesis of phenylpropenes in sweet basil. Plant Physiol 125: 539–555

Goffreda JC, Mutschler MA, Ave DA, Tingey WM, Steffens JC (1989) Aphid deterrence by glucose esters in glandular trichome exudate of the wild tomato, *Lycopersicon pennellii*. J Chem Ecol 15:2135–2147

Green TR, Ryan CA (1972) Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. Science 175:776–777

Gutiérrez-Alcalá G, Gotor C, Meyer AJ, Fricker M, Vega JM, Romero LC (2000) Glutathione biosynthesis in Arabidopsis trichome cells. Proc Natl Acad Sci USA 97:11108–11113

Handley R, Ekbom B, Agren J (2005) Variation in trichome density and resistance against a specialist insect herbivore in natural populations of *Arabidopsis thaliana*. Ecol Entomol 30:284–292

Heinz KM, Zalom FG (1995) Variation in trichome-based resistance to bemisia-argentifolii (*Homoptera*, *Aleyrodidae*) oviposition on tomato. J Econ Entomol 88:1494–1502

Holeski LM (2007) Within and between generation phenotypic plasticity in trichome density of *Mimulus guttatus*. J Evol Biol 20:2092–2100

Horgan FG, Quiring DT, Lagnaoui A, Pelletier Y (2009) Effects of altitude of origin on trichome-mediated anti-herbivore resistance in wild andean potatoes. Flora 204:49–62

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

Ishida T, Kurata T, Okada K, Wada T (2008) A genetic regulatory network in the development of trichomes and root hairs. Annu Rev Plant Biol 59:365–386

Johnson KS, Felton GW (1996) Potential influence of midgut pH and redox potential on protein utilization in insect herbivores. Arch Insect Biochem Physiol 32:85–105

Kang JH, Liu G, Shi F, Jones AD, Beaudry RM, Howe GA (2010a) The tomato odorless-2 mutant is defective in trichome-based production of diverse specialized metabolites and broad-spectrum resistance to insect herbivores. Plant Physiol 154:262–272

Kang JH, Shi F, Jones AD, Marks MD, Howe GA (2010b) Distortion of trichome morphology by the hairless mutation of tomato affects leaf surface chemistry. J Exp Bot 61:1053–1064

Karban R, Baldwin IT (1997) Induced responses to herbivory. University of Chicago Press, Chicago

Kennedy GG (2003) Tomato, pests, parasitoids, and predators: tritrophic interactions involving the genus *Lycopersicon*. Annu Rev Entomol 48:51–72

Kessler A, Baldwin IT (2002) Plant responses to insect herbivory: the emerging molecular analysis. Annu Rev Plant Biol 53:299–328

Larocca A, Fanti P, Molinaro A, Mattia MF, Battagli D (2011) Aphid performance on *Vicia faba* and two southern Italy *Phaseolus vulgaris* landraces. Bull Insectol 64:101–106

Levin DA (1973) The role of trichomes in plant defense. Quart Rev Biol 48:3–15

Li AX, Eannetta N, Ghangas GS, Steffens JC (1999) Glucose polyester biosynthesis. Purification and characterization of a glucose acyltransferase. Plant Physiol 121:453–460

Li L, Zhao YF, McCaig BC, Wingerd BA, Wang JH, Whalon ME, Pichersky E, Howe GA (2004) The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. Plant Cell 16:126–143

Luckwill LC (1943) The genus *Lycopersicon*: a historical, biological and taxonomic survey of the wild and cultivated tomato. Aberd Univ Stud 120:1–44

Mirnezhad M, Romero-Gonzalez RR, Leiss KA, Choi YH, Verpoorte R, Klinkhamer PGL (2010) Metabolomic analysis of host plant resistance to thrips in wild and cultivated tomatoes. Phytochem Anal 21:110–117

Nonamua T, Xu L, Wada M, Kawamura S, Miyajima T, Nishitoni A, Kakutan K, Takikawa Y, Matsuda Y, Toyoda H (2008) Trichome exudates of *Lycopersicon pennellii* form a chemical barrier to suppress leaf-surface germination of *Oidium neolycopersici* conidia. Plant Sci 176:31–37

O'Donnell PJ, Calvert C, Atzorn R, Wasternack C, Leyser HMO, Bowles DJ (1996) Ethylene as a signal mediating the wound response of tomato plants. Science 274:1914–1917

Peiffer M, Felton GW (2005) The host plant as a factor in the synthesis and secretion of salivary glucose oxidase in larval *Helicoverpa zea*. Arch Insect Biochem Physiol 58:106–113

Peiffer M, Tooker JF, Luthe DS, Felton GW (2009) Plants on early alert: glandular trichomes as sensors for insect herbivores. New Phytol 184:644–656

Pelletier Y, Dutheil J (2006) Behavioural responses of the Colorado potato beetle to trichomes and leaf surface chemicals of *Solanum tarijense*. Entomol Exper Appl 120:125–130

Rasmann S, De Vos M, Casteel CL, Tian D, Halitschke R, Sun JY, Agrawal AA, Felton GW, Jander G (2012) Herbivory in the previous generation primes plants for enhanced insect resistance. Plant Physiol 158(2):854–863

Reeves AF (1977) Tomato trichomes and mutations affecting their development. Am J Bot 64:186–189

Rotenberg D, Thompson TS, German TL, Willis DK (2006) Methods for effective real-time RT-PCR analysis of virus-induced gene silencing. J Virol Methods 138:49–59

Ryan CA (1990) Proteinase inhibitors in plants: genes for improving defenses against insects and pathogens. Annu Rev Phytopathol 28:425

Schilmiller A, Shi F, Kim J, Charbonneau AL, Holmes D, Jones AD, Last RL (2010) Mass spectrometry screening reveals widespread diversity in trichome specialized metabolites of tomato chromosomal substitution lines. Plant J 62:391–403

Simmons AT, Gurr GM (2004) Trichome-based host plant resistance of *Lycopersicon* species and the biocontrol agent *Mallada signata*: are they compatible? Entom Exper Appl 113:95–101

Simmons AT, Gurr GM (2005) Trichomes of *Lycopersicon* species and their hybrids: effects on pests and natural enemies. Agric Forest Entomol 7:265–276

Steffens JC, Walters DS (1991) Biochemical aspects of glandular trichome-mediated insect resistance in the *Solanaceae*. ACS Symp Ser 449:136–149

Thaler JS, Stout MJ, Karban R, Duffey SS (2001) Jasmonate-mediated induced plant resistance affects a community of herbivores. Ecol Entomol 26:312–324

Thipyapong P, Steffens JC (1997) Tomato polyphenol oxidase-differential response of the polyphenol oxidase F promoter to injuries and wound signals. Plant Physiol 115:409–418

Thipyapong P, Joel DM, Steffens JC (1997) Differential expression and turnover of the tomato polyphenol oxidase gene family during vegetative and reproductive development. Plant Physiol 113:707–718

Traw MB, Bergelson J (2003) Interactive effects of jasmonic acid, salicylic acid, and gibberellin on induction of trichomes in *Arabidopsis*. Plant Physiol 133:1367–1375

Traw MB, Dawson TE (2002) Reduced performance of two specialist herbivores (Lepidoptera: *Pieridae*, Coleoptera: *Chrysomelidae*) on new leaves of damaged black mustard plants. Environ Entomol 31:714–722

Valkama E, Koricheva J, Ossipov V, Ossipova S, Haukioja E, Pihlaja K (2005) Delayed induced responses of birch glandular trichomes and leaf surface lipophilic compounds to mechanical defoliation and simulated winter browsing. Oecologia 146:385–393

Van Schie CCN, Haring MA, Schuurink RC (2007) Tomato linalool synthase is induced in trichomes by jasmonic acid. Plant Molec Biol 64:251–263

Wagner GJ (1991) Secreting glandular trichomes—more than just hairs. Plant Physiol 96:675–679

Weinhold A, Baldwin IT (2011) Trichome-derived O-acyl sugars are a first meal for caterpillars that tags them for predation. Proc Natl Acad Sci USA 108:7855–7859

Yang CX, Li HX, Zhang JH, Luo ZD, Gong PJ, Zhang CJ, Li JH, Wang TT, Zhang YY, Lu YE, Ye ZB (2011) A regulatory gene induces trichome formation and embryo lethality in tomato. Proc Natl Acad Sci USA 108:11836–11841

Yoshida Y, Sano R, Wada T, Takabayashi J, Okada K (2009) Jasmonic acid control of GLABRA3 links inducible defense and trichome patterning in Arabidopsis. Development 136:1039–1048

Zhu-Salzman K, Luthe DS, Felton GW (2008) Arthropod-inducible proteins: broad spectrum defenses against multiple herbivores. Plant Physiol 146:852–858