

**Resistance to the two-spotted spider mite (*Tetranychus urticae*) by acylsucroses of wild  
tomato *Solanum pimpinellifolium*. trichomes studied in a recombinant inbred line  
population**

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## Abstract

Trichome-based host plant resistance is a complex mechanism that could be used in tomato breeding to control arthropod pests. The aims of this work were to evaluate the plant traits (density of trichomes and acylsucrose production) and the functional relationships of these traits with mortality, repellence, and oviposition of *Tetranychus urticae* Koch (Acarina: Tetranychidae). We used a population of recombinant inbred lines (RILs) derived from the cross between the wild tomato *Solanum pimpinellifolium* L. ‘TO-937’ and the cultivated tomato *S. lycopersicum* L. Multiple regression analyses showed that high acylsucrose content and high type IV trichome density increased mortality and repellence, and reduced oviposition of *T. urticae*. Single regression analyses showed that logistic model best explained the relationship between mortality or repellence and acylsucrose content, whereas negative-exponential model best described the relationship between oviposition and acylsucrose content. Linear models were the best-fits for the three resistance variables with trichome IV density. Probit analysis was used to estimate acylsucrose effective doses, and revealed that 31 % and 10 % of the RILs produced acylsucrose above the effective doses for 90% mortality or repellence, respectively. Altogether indicates that *S. pimpinellifolium* may be a suitable genetic source of resistance to spider mites to be used in cultivated tomato.

**Keywords:** Acari, Tetranychidae, glandular trichomes, pest resistance, acylsucrose.

## 1   **Introduction**

2   The two-spotted spider mite (*Tetranychus urticae* Koch) (Acarina: Tetranychidae) is a  
3   polyphagous species that causes important economic losses in many crops. In tomato  
4   (*Solanum lycopersicum* L.) this pest causes severe damage on leaves and fruits that result in  
5   yield and quality losses (Berlinger 1986). In contrast to the environmentally friendly practices  
6   (i.e., biological control) that are commonly used to fight this pest in other horticultural crops,  
7   control of *T. urticae* in tomato is generally achieved by application of synthetic pesticides.  
8   This is mainly because tomato plants have foliar and stem pubescence that severely hamper  
9   foraging of commercial natural enemies of spider mites, such as *Phytoseiulus persimilis*  
10   Athias-Henriot (Van Haren et al. 1987; Nihoul 1993; 1994). Yet, trichomes of tomato plants  
11   are also known to confer resistance to arthropod pests.

12         Breeding for resistant cultivars is considered an attractive alternative strategy for crop  
13   protection against pests and diseases (Johnson 1992), provided that durable genetic resistance  
14   is available within the crop. Natural pest resistance based on trichomes has been extensively  
15   studied in tomato (see below). Trichomes of tomato species (genus *Solanum* section  
16   *Lycopersicon*) are classified into eight types according to their morphology (Luckwill 1943;  
17   reviewed by Channarayappa et al. 1992). *Lycopersicon* species have nonglandular (types II,  
18   III, V, and VIII) and glandular trichomes (types I, IV, VI, and VII). Glandular trichomes have  
19   a uni- or multi-cellular glandular head that produces sticky and/or toxic substances for  
20   arthropods. In two green-fruited wild tomato species [*S. habrochaites* S Knapp and DM  
21   Spooner (syn. *Lycopersicon hirsutum* Dunal) and *S. pennellii* Correll], allelochemicals  
22   produced by glandular trichomes are known to severely hamper feeding and reproduction of  
23   many arthropod pests. Type IV and VI glandular trichomes of some accessions of  
24   *S. habrochaites* produce the sesquiterpene zingiberene that confers resistance to the tobacco  
25   spider mite (*Tetranychus evansi* Baker and Pritchard) (Maluf 2001), the two-spotted spider

mite (Guo et al. 1993), the cotton whitefly [*Bemisia tabaci* (Gennadius)] (Freitas et al. 2002), and the South American tomato pinworm [*Tuta absoluta* (Meyrick)] (Azevedo et al. 2003). Type VI trichomes of *S. habrochaites* f. *glabratum* C.H. Müll produce methyl ketones 2-undecanone and 2-tridecanone (Williams et al. 1980), which have antixenotic and antibiotic effects on *T. urticae* (Carter and Snyder 1981; Carter and Snyder 1985; Guo et al. 1993; Chatzivasileiadis and Sabelis 1997), the whiteflies *B. tabaci* (Channarayappa et al. 1992; Snyder et al. 1998) and *Trialeurodes vaporariorum* Westwood (Maliepaard et al. 1995), and *T. absoluta* (Maluf et al. 1997). Type IV trichomes of *S. pennellii* produce acylsugars (Burke et al. 1987) that have deterrence effects on the leafminer *Liriomyza trifolii* (Burgess) (Hawthorne et al. 1992), the green peach aphid [*Myzus persicae* (Sulzer)] (Goffreda and Mutschler 1987), the tomato fruitworm [*Helicoverpa zea* (Boddie)], the beet armyworm [*Spodoptera exigua* (Hubner)] (Juvik et al. 1994), *B. tabaci* (Liedl et al. 1995), and *T. urticae* (Saeidi et al. 2007). Recently, Resende et al. (2008) correlated acylsugars accumulation with *T. evansi* entrapment in glandular trichomes.

In previous studies, Fernández-Muñoz et al. (2000; 2003) described another gene source of resistance to *T. urticae*, i.e. the accession ‘TO-937’ from the currant tomato, *S. pimpinellifolium* L. In these studies, the authors examined co-segregation of resistance and trichome density in both the F<sub>2</sub> and backcross populations of a cross of ‘TO-937’ with a susceptible tomato cultivar, and found that resistance to *T. urticae* was related to the density of type IV trichomes. The advantages of accession ‘TO-937’ as source of genetic resistance, compared to those described in *S. habrochaites*, *S. habrochaites* f. *glabratum*, and *S. pennellii*, are (a) that *S. pimpinellifolium* is a red-fruited, self-compatible wild species and very closely related to the cultivated tomato (Rick 1979), therefore having significant potential for commercial breeding; and (b) that in ‘TO-937’ the resistance to *T. urticae* is governed by a major dominant gene (Fernández-Muñoz et al. 2003), therefore minimizing the

1 risk of linkage drag of undesirable traits when attempting the transference of resistance to the  
2 cultivated tomato. In the other three taxa resistance has been repeatedly reported as polygenic.  
3 Recently, we have determined that significant amounts of epicuticular acylsucroses are  
4 produced (presumably by glandular trichomes) on leaves of ‘TO-937’ (unpublished data).

5       The purpose of the research presented here was to assess the role of some traits  
6 present in TO-937 (i.e. acylsucrose accumulation and type IV glandular trichome density) in  
7 the resistance to the two-spotted spider mite. We used a recombinant inbred line (RIL)  
8 population obtained from crossing the susceptible *S. lycopersicum* cv. Moneymaker and the  
9 resistant *S. pimpinellifolium* acc. ‘TO-937’. A RIL population is a collection of homozygous  
10 lines in which traits from both parents are recombined. RILs have some advantages relative to  
11 other segregant populations. This is because a high number of recombination events occur  
12 during the construction of RILs making it easier to determine if two correlated traits are  
13 governed by the same locus or by different, but linked, loci.

14       Laboratory bioassays of mortality, repellence, and oviposition were done on each of  
15 the RILs in order to determine the functional relationship between both the densities of main  
16 types of foliar trichomes and acylsucrose production with resistance to *T. urticae*.

17       In order to emphasize the potential of *S. pimpinellifolium* as gene source for *T. urticae*  
18 resistance to the cultivated tomato, we obtained acylsucrose effective doses on spider mite  
19 mortality and repellence, so that they can be used as selection thresholds in future breeding  
20 programs.

## 21 22 **Materials and methods**

### 23 **Mites**

24 Adult females of *T. urticae* were obtained from colonies reared on French bean plants (cv. ‘F-  
25 15’, Semillas Fitó, Barcelona, Spain) inside a growth chamber at 25 - 18°C, 50% RH., and

L16:D8. The colony originated in 1999 from mites collected on *Ricinus communis* L., *Convolvulus arvensis* L., and *Lavatera* sp. plants, located in the Exp. Sta. La Mayora - CSIC, Málaga, Spain (36°45'N, 4°2'W, 40 m a.s.l.). This population has repeatedly shown to be able to infest and grow on tomato plants.

## **Host plants**

Recombinant inbred lines (RILs) were developed by repetitively selfing the progeny of individual members of an F<sub>2</sub> population obtained from crossing *S. lycopersicum* cv. 'Moneymaker' x *S. pimpinellifolium* acc. 'TO-937' until the F<sub>8</sub> generation. Seeds of the two parental genotypes, the hybrid F<sub>1</sub>, and of 160 RILs were individually sown in pots (60 ml) containing 15% plant-nutrient loaded zeolite and 85% coconut fiber substrate. Plantlets were grown in a glasshouse until they reached the 3-5 leaf stage. Eighteen plants of 'Moneymaker', 'TO-937' and the F<sub>1</sub>, and six plants of each RIL were transplanted to soil in a plastic-house. The transplants were placed 0.4 m apart in a row. Distance between rows was 1 m. Plants were watered at 3-4 day intervals and fed with soluble fertilizer once a week.

## **Pubescence assessment**

Abundant, short trichomes [type IV, V, and VI after Luckwill (1943) classification] were considered for quantifications because these trichome types are the only that have previously demonstrated association with pest resistance traits (see review by Simmons and Gurr 2005). Other trichome types were not counted. For instance, long glandular trichomes such as type I are not likely to interfere with spider mites because their very different relative sizes, and very small and sparse glandular trichomes such as type VII are difficult to count and in our observations in both the *S. lycopersicum* and *S. pimpinellifolium* parents type VII trichomes showed very irregular distributions appearing predominantly on veins (or lamina close to

veins) of adaxial leaf surface. Density of trichomes is known to be affected by leaf age (because of leaf expansion) and environmental conditions (Nihoul 1993; Wilkens et al. 1996; Leite et al. 1999; Gurr and McGrath 2001; Leite et al. 2001). To minimize the interference caused by these effects, all trichome counts were made on leaflets of the same position and developmental stage. Following Gurr and McGrath (2002), pubescence of two leaflets from the third youngest leaf of each plant was assessed when plants were 12 weeks old. Because spider mites usually live on the lower tomato leaf surface, and because abaxial type IV trichome density was by far the variable most related to *S. pimpinellifolium* resistance to the mite (Fernández-Muñoz et al. 2003), leaf pubescence was assessed only on the abaxial surface. First, the area of the leaflets was measured with a Delta-T Area Meter MK2 (Cambridge, UK). Next, trichomes were counted on two 1 mm wide leaflet strips that were cut perpendicularly to the midrib (Gurr and McGrath 2001). Strips were positioned one at a time over a calibrated paper on a microscope slide. Trichomes were observed under stereomicroscope at 40x (Nikon SMZ-10). Type IV and V trichomes were counted on three 1 x 1 mm squares per strip. Type VI trichomes, less abundant, were counted on 15-20 x 1 mm strips. The densities of the three trichome types were recorded as numbers per mm<sup>2</sup>.

### **Acylsugar quantification**

Leaf surface acylsucrose was extracted and quantified using the methods described in Goffreda et al. (1990). Epicuticular sugar esters were extracted by placing two leaflets from the third youngest leaf in a polyethylene scintillation vial (20 ml) with 5 ml of dichloromethane at 4°C and gently shaking the vial for 15 sec. The leaflets were removed and the surface area of the leaflets was measured. The extracts were rinsed with 5 ml of distillate water to dissolve and remove polar compounds and dirty. The two phases were separated by decantation, dichloromethane was evaporated and 2 ml of methanol were added to dissolve

the extracted sugar esters. Aliquots of this extract were used to quantify acylucose contents following Goffreda et al. (1990) protocol that in short consisted of hydrolysis in methanol-NaOH and subsequent free sugar quantification by the Somogyi-Nelson reaction (Nelson 1944; Somogyi 1952). Total sugar content was estimated from a linear regression equation done with sucrose plus invertase solutions at known concentrations.

### **Repellence bioassay**

Repellence was estimated on the abaxial leaf surface with a bioassay based on the thumbtack method (Weston and Snyder, 1990). One leaflet from the third apical leaf per each the six plants of the RILs and six plants randomly selected of parents and F<sub>1</sub> was detached and transported to the laboratory. The leaflet was placed upside-down on a Styrofoam board. A thumbtack was then inserted through the lamina avoiding the main vein, to prevent mites from use veins as an alternative way to reach the surface of the leaf. Ten adult females were transferred with a fine camel hair paintbrush to the head of the thumbtack. Thirty minutes later the trails were observed in a 20x stereomicroscope, and the number of females remaining on the thumbtack or trapped by glandular trichomes on the leaf close to the tack was recorded.

### **Oviposition and mortality bioassay**

Three plants of each RIL, the parents and the F<sub>1</sub> were randomly selected to provide leaflets from the third apical leaf for the assays. The three leaflets of each genotype were detached and placed upside-down in a Petri dish lined with moistened filter paper. A piece of cotton moistened with distilled water was placed on the petiolule to avoid desiccation. Leaflets were placed such as there was no contact among them. Ten adult females were placed per leaflet. The dishes were maintained in a rearing chamber at 25 - 18°C, 50% RH . After 48 h the number of dead and alive mites, as well as the number of eggs laid, were recorded.



Oviposition was referred as the mean number of eggs per female placed at the beginning of the assay.

#### **Data analysis**

Data from acylsucrose content and pubescence were log-transformed [ $\log(x+1)$ ] prior to analysis. Correlations between acylsucrose content and densities of the different trichome types were calculated using the Pearson's  $r$  product-moment coefficient. To determine the effect of density of type IV, V, VI trichomes, and acylsucrose content on repellence and mortality, Generalized Linear Model regression was used assuming a binomial error structure of data and a logit link between the response variables and the linear combination of the explanatory variables. The variables considered were mortality (total number of dead adults) and repellence (total number of adults on or trapped around the thumbtack), in each of the 160 RIL tested. All multiple regressions were performed using stepwise removal of non significant effects. The SAS System v. 8.2 (SAS Institute Inc., Cary, NC, USA) statistical package was used for all the above analyses. Parents and hybrids were excluded from the analysis because we were interested in studying the traits only in the segregating population. To assess the functional relation between either acylsucrose or type IV trichome with mortality, repellence, and oviposition, each pair of variables were plotted, and the function that best explained the variability was chosen. Prior to the analysis, intervals for the corresponding independent variable (type IV trichome density and acylsucrose content) were created to a) remove some variation of the dependent variable, while maintaining the structure of the relationship between the outcome and the independent variable, and b) to allow the regression analysis (Hosmer and Lemeshow 2000). To create the intervals for independent variables, first we pooled all samples corresponding to value 0 for acylsucrose content or type IV trichome density, and then intervals with the same size were created in the log-scale for

each variable. So, independent variable intervals were the same for the analysis of mortality, repellence and oviposition. The mean of all the points of the interval for trichome density and acylsucrose content was used as the value of the independent variable to be used in the regression analysis. The dependent variable was the sum of the total frequency of the events, relative to the number of individuals (total events/total individuals) included in the interval, for repellence and mortality, and the mean of eggs laid, for oviposition. Logistic, linear, and exponential regressions were performed using SPSS v. 14.0 (SPSS Inc., Chicago, IL, USA). Effective doses (ED50, ED90, ED99, and their 95% confidence intervals) of acylsucrose for mortality and repellence were calculated using probit analysis.

## Results

### Pubescence and acylsucrose content

*S. pimpinellifolium* acc. 'TO-937' had high density of type IV trichomes and low density of type VI trichomes (Figure 1, black arrows). The head of Type VI trichomes was four-celled (VIa subtype), as described also in *S. lycopersicum* (Channarayappa et al. 1992). Non-glandular, type V trichomes were absent in 'TO-937', whereas in *S. lycopersicum* cv. 'Moneymaker' they were the most abundant (Figure 1, white arrow). In 'Moneymaker' Type IV glandular trichomes were absent and type VI trichomes were at low density (Figure 1, white arrow). The hybrid F<sub>1</sub> had low densities of both type IV and VI trichomes, and high density of type V trichomes (Figure 1, grey arrows).

The density of trichomes IV and V segregated widely along the RIL population (Figure 1, bars). The range of trichome type IV density was 0 – 129 trichomes/mm<sup>2</sup> and that of type V was 0 – 102 trichomes/mm<sup>2</sup>. Narrower segregation was observed for type VI trichome, with a distribution range of 0.2 – 10 trichomes/mm<sup>2</sup>.

1 In those genotypes where acylsugar production was detected ('TO-937', the F<sub>1</sub>, and  
2 acylsugar-producing RILs), no significant amount of acylglucose was found and only  
3 acylsucrose was detected. Acylsucrose content in the RIL population showed a broad range of  
4 values (0 – 40.3 nmol/cm<sup>2</sup>) (Figure 1, bars).

5 Significant correlations between all leaf traits were found (Table 1). Type IV trichome  
6 density was negatively correlated to type V trichome density, and positively correlated to  
7 acylsucrose content.

### 8 **Mortality, repellence, and oviposition**

10 On 'TO-937' leaves, spider mite mortality was total, repellence almost absolute, and  
11 oviposition negligible (Figure 2, black arrows). In contrast, on 'Moneymaker' leaves spider  
12 mite mortality was very low and repellence null (Figure 2, white arrows). The F<sub>1</sub> hybrid  
13 showed intermediate dominance to 'TO-937' for mortality and repellence, and complete  
14 dominance to 'TO-937' for oviposition (Figure 2, grey arrows). The RIL population  
15 segregated widely for mortality, repellence, and oviposition (Figure 2, bars) and there were  
16 recombinant lines that showed values of these traits similar to those of 'TO-937'.

### 18 **Relationships of pubescence and acylsucrose with resistance**

19 Variability for mortality, repellence and oviposition, as well as for pubescence density, within  
20 the RIL population, allowed the analysis of the functional relationships among traits by  
21 multiple regression analysis. A first analysis type including all the predictor variables  
22 revealed that acylsucrose content was that best explained variation of mortality, repellence  
23 and oviposition (Table 2) with positive effects on mortality and repellence and negative effect  
24 on oviposition. Among trichome types, type IV ones were those with higher effects. However,  
25 this could be an collinearity artifact because of positive correlation shown by density of this

1 trichome and acylsucrose content. Then, new multiple regression analysis including only  
2 trichome types as predictor variables were performed (Table 2) and these showed that, among  
3 trichomes, type IV was the best predictor of resistance traits. Similarly to acylsucrose, type IV  
4 trichome effects were positive for mortality and repellence and negative for oviposition. Type  
5 V effects were also significant for the three resistance traits and type VI effects were  
6 significant for mortality and repellence and their effects were of opposite sign than those of  
7 type IV.

8 Mortality, repellence, and oviposition were regressed over the two explanative  
9 individual effects, i.e. acylsucrose content and trichome IV density, using different models to  
10 determine the functional relations between traits. Models that best fitted data are shown in  
11 Figure 3. The models that best described the relation between acylsucrose content and spider  
12 mite mortality and repellence were logistic models, and for oviposition was negative-  
13 exponential model. In contrast, type IV trichome density was linearly related to the three  
14 dependent variables and the extent of explained variation for these trichomes was less than for  
15 acylsucrose (Figure 3, see coefficients of determination).

16 The logistic functional relationship found between acylsucrose content and mortality  
17 and repellence made possible the determination of the effective doses of the two traits. Probit  
18 analysis revealed that many RILs had very high levels of resistance. For example, 31% and  
19 10% of RILs had acylsucrose contents above the corresponding ED90 values for mortality  
20 and repellence, respectively (Table 3).

## 22 Discussion

23 Our results indicated that resistance to the two-spotted spider mite in *S. pimpinellifolium* acc.  
24 ‘TO-937’, and derived lines, was due to the acylsucrose trichome IV exudates, which caused  
25 high repellence, high mortality, and reduced oviposition in spider mite females.

1       The RIL population was highly variable for density of type IV and V trichomes,  
2       acylsucrose production, and *T. urticae* resistance. Within the RIL population there were  
3       phenotypes similar to the parents as well as a wide range of intermediate phenotypes. This  
4       variability allowed us to perform the regression analysis among traits presented in this work.

5       Multiple regression revealed that high densities of type IV trichomes and, specially,  
6       high production of acylsucroses were associated with increased mortality and repellence of  
7       adult mites, and reduced oviposition. Simmons et al. (2006) found association between  
8       glandular trichomes of an heterogeneous collection of tomato species and potato moth  
9       resistance. Different *Solanum* section *Lycopersicon* species produce different trichome  
10      exudates compounds. In our study, we observed positive correlation between acylsucrose  
11      content and density of type IV trichomes, suggesting that in ‘TO-937’ and derived lines  
12      acylsucroses are produced only by these trichomes. That acylsucrose production was the most  
13      explicative trait in the complete multiple regression analyses and that type IV was the best  
14      predictor in the analyses including only trichome types indicate that resistance to spider mites  
15      in ‘TO-937’ and derived lines are due to acylsucrose type IV trichome exudates. Furthermore,  
16      single regressions between acylsucrose production and both mortality and repellence  
17      supported this hypothesis. The models that best explained the relationships of both resistance  
18      traits with acylsucrose production were sigmoidal models, and sigmoidal is a common  
19      relation found in dose-response studies.

20      Puterka et al. (2003) found that synthetic sugar esters was functionally related to  
21      mortality of nymphal stages of *T. urticae*. Here, we found similar conclusions for adult mites,  
22      and extended them to repellence and oviposition. In contrast, the relations of resistance traits  
23      with density of type IV trichomes were linear, suggesting correlated more than functional  
24      effects. This is because type IV trichomes are the structures where acylsucrose is produced, and  
25      therefore their density and spatial distribution could contribute to mite mortality due to

1 starvation (because of entrapment). We therefore expect that a high glandular trichome  
2 density line will be more resistant due to higher rates of entrapment.

3 In this study we found that density of glandular type V and VI trichomes showed  
4 negative relationships with *T. urticae* mortality and repellence and that type V showed  
5 positive effect on oviposition. In the case of nonglandular type V, these results can be  
6 explained because of the negative correlations with acylsucrose production and type IV  
7 density. In the case of glandular type VI, the results were surprising because *S. habrochaites*  
8 type VI trichomes have been related with resistance to many herbivore arthropods, and such  
9 resistance has been attributed to trichome secretion of methyl ketones and sesquiterpenes (see  
10 introduction for references). However, in *S. lycopersicum*, type VI trichomes do not secrete  
11 significant quantities of methyl ketones (Williams et al. 1980). Also, the abundance of this  
12 type of trichomes in *S. lycopersicum* and *S. pimpinellifolium* is low. Therefore, the entrapment  
13 of spider mites in this trichome type is probably not common. Hence, the minor although  
14 significant effect of type VI trichome could also be an indirect effect, much probably due to  
15 the negative correlation observed for this trichome type with type IV trichome density and  
16 acylsucrose production as it has also been suggested in other studies (Simmons and Gurr  
17 2005).

18 Probit analysis revealed that low contents of acylsucrose were required to achieve high  
19 values of mortality and repellence. Repellence is an immediate response that can be triggered  
20 by an odor stimulus. Mortality, however, may be caused by entrapment and subsequent  
21 starvation, or by accumulation of toxic compounds (Chatzivasileiadis and Sabelis 1999). Low  
22 acylsucrose levels effect on mortality could be explained by mites being progressively  
23 covered by toxic trichome exudates as they ambulate on the leaf. Indeed, spider mites on  
24 *S. habrochaites* f. *glabratum* and aphids on *Nicotiana* leaves have been reported to be rapidly  
25 coated with trichome exudates (Chatzivasileiadis et al. 1999; Wagner et al. 2004). Therefore,

not only secretion but also density and distribution of the type IV trichome might be important to affect negatively the populations of the spider mite.

In a previous study where parent, F<sub>1</sub>, F<sub>2</sub>, and first backcrosses populations were tested (Fernández-Muñoz et al. 2003), it was shown that resistance to *T. urticae* had a relatively simple genetic control, and that it was most related to abaxial density of type IV glandular trichomes. Here we showed results that are more robust than those obtained previously with earlier segregating populations (e.g., F<sub>2</sub>). This is because in RILs the genetic recombination is higher and, therefore, the effective separation of traits coming from the same parent is easier to achieve. In here, we found that many RILs had levels of acylsucroses above the ED90 value for mortality and repellence. This supports the hypothesis that the trichome-based host plant resistance to *T. urticae* of *S. pimpinellifolium* could be easily transferred to the cultivated tomato. Furthermore, the values we calculated for acylsucrose effective dose could be used as selection thresholds in future tomato breeding programs for resistance against *T. urticae*.

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Table 1. Correlations between log-transformed densities of type IV, V, and VI trichomes and acylsucrose content on leaves of *S. lycopersicum* cv. 'Moneymaker' x *S. pimpinellifolium* acc. 'TO-937' tomato recombinant inbred lines.

	Type IV	Type V	Type VI
Type V	-0.770 (P < 0.0001)		
Type VI	-0.242 (P = 0.0022)	0.267 (P = 0.0007)	
Acylsucrose	0.615 (P < 0.0001)	-0.626 (P < 0.0001)	-0.368 (P < 0.0001)

1 Table 2. Multiple regression parameter estimates of individual effects of acylsucrose content  
2 and density of trichome types on *T. urticae* mortality, repellence, and oviposition on leaves of  
3 *S. lycopersicum* x *S. pimpinellifolium* tomato recombinant inbred lines.

Dependent variable Effect <sup>3</sup>	Complete analysis <sup>1</sup>			Trichome-only analysis <sup>2</sup>		
	Parameter	SE	P	Parameter	SE	P
Mortality <sup>4</sup>						
Intercept	-0.283	0.041	<0.0001	-0.391	0.039	<0.0001
Acylsucrose	1.529	0.062	<0.0001			
Type IV	0.405	0.056	<0.0001	0.842	0.067	<0.0001
Type V				-0.441	0.053	<0.0001
Type VI	-0.346	0.045	<0.0001	-0.588	0.040	<0.0001
Repellence <sup>4</sup>						
Intercept	1.014	0.035	<0.0001	0.669	0.028	<0.0001
Acylsucrose	1.606	0.050	<0.0001			
Type IV	0.882	0.047	<0.0001	1.172	0.044	<0.0001
Type V	0.132	0.049	0.0069	-0.265	0.043	<0.0001
Type VI				-0.275	0.028	<0.0001
Oviposition <sup>5</sup>						
Intercept	0.863	0.050	<0.0001	0.863	0.055	<0.0001
Acylsucrose	-0.419	0.063	<0.0001			
Type IV	-0.209	0.063	0.0011	-0.292	0.086	0.0009
Type V				0.226	0.086	0.0098
Type VI						

<sup>1</sup> All effects were entered in the model and then nonsignificant effects were removed from the model following the stepwise method.

<sup>2</sup> Only the trichome type effects were entered in the model and then the stepwise method was followed.

<sup>3</sup> Log-transformed data were standardized to allow direct comparison of the effects.

<sup>4</sup> Generalized linear model (binomial distribution and logit link function).

<sup>5</sup> General linear model.

1 Table 3. Effective doses (ED) and 95% confidence intervals (IC 95%) of acylsucrose content  
 2 on tomato leaflets for mortality and repellence of *T. urticae*, estimated by probit analysis.

3

		Acylsucrose (nmol.cm <sup>-2</sup> ) <sup>1</sup>	
		ED	IC 95%
Mortality			
	ED50	2.03	1.55 – 2.52
	ED90	6.30	4.94 – 9.02
	ED99	13.93	9.72 – 24.94
Repellence			
	ED50	4.14	3.56 – 4.83
	ED90	13.13	10.51 – 17.79
	ED99	31.29	22.39 – 50.05

<sup>1</sup> ED values of acylsucrose content were backtransformed from the  
 obtained from the analyses with log-transformed acylsucrose data

Figure Legends

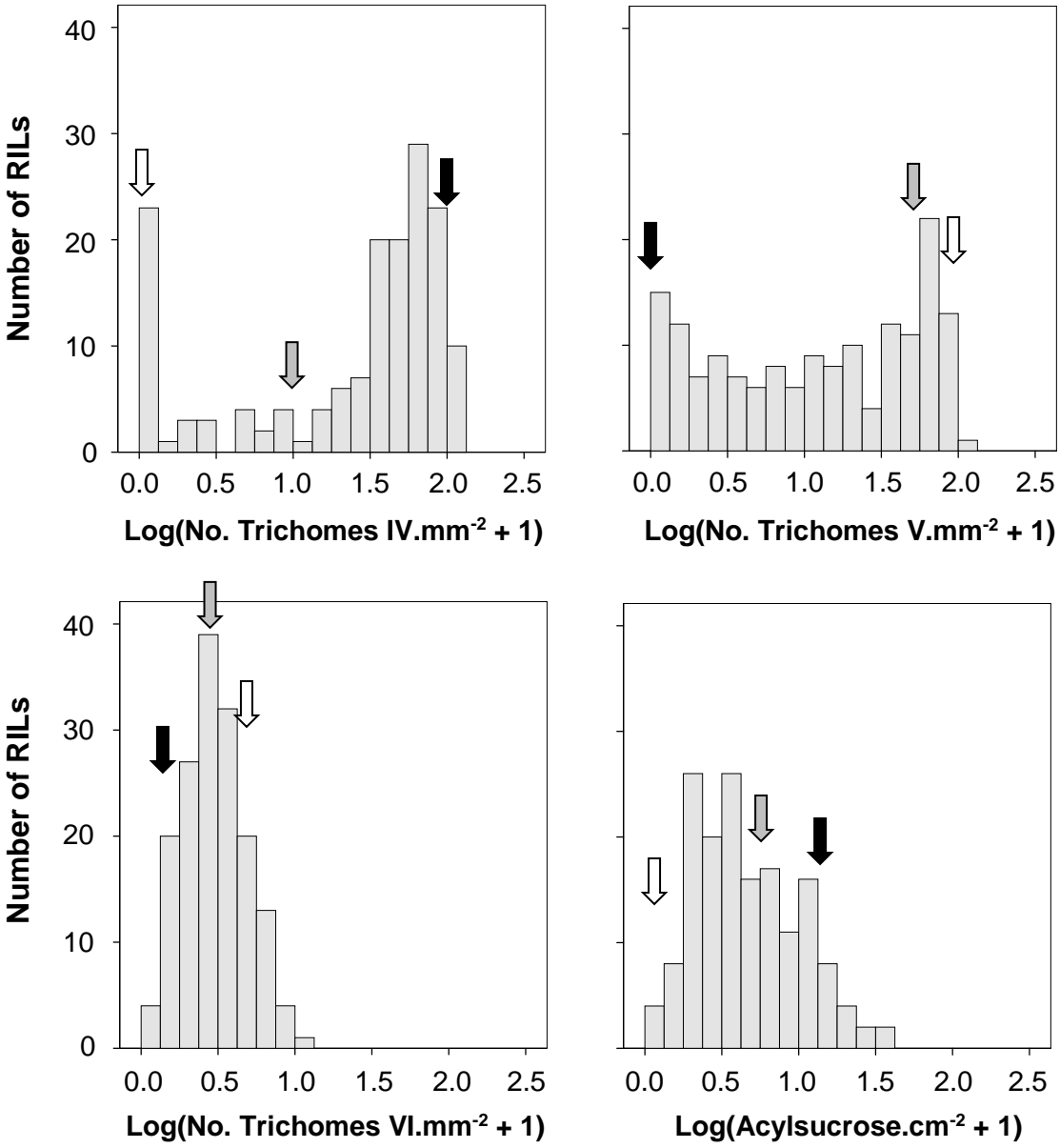
Figure 1. Frequency distributions of log-transformed densities of type IV, V, and VI trichomes and acylsucrose content on leaves of *S. lycopersicum* cv. 'Moneymaker' x *S. pimpinellifolium* acc. 'TO-937' tomato recombinant inbred lines (RILs). Arrows indicate mean values for parents and F<sub>1</sub> (black: 'TO-937'; grey: F<sub>1</sub>; white: 'Moneymaker').

Figure 2. Frequency distributions of mortality, repellence, and oviposition on leaves of *S. lycopersicum* cv. 'Moneymaker' x *S. pimpinellifolium* acc. 'TO-937' tomato recombinant inbred lines (RILs). Arrows indicate mean values for parents and F<sub>1</sub> (black: 'TO-937'; grey: F<sub>1</sub>; white: 'Moneymaker').

Figure 3. Functional relationships between acylsucrose content and type IV trichome density, and *T. urticae* repellence, mortality and oviposition on leaves of tomato recombinant inbred lines ( $R^2$ , coefficient of determination; n, number of value pairs). The regression analyses were made using intervals for the independent variables as described in Materials and Methods.



Figure 1



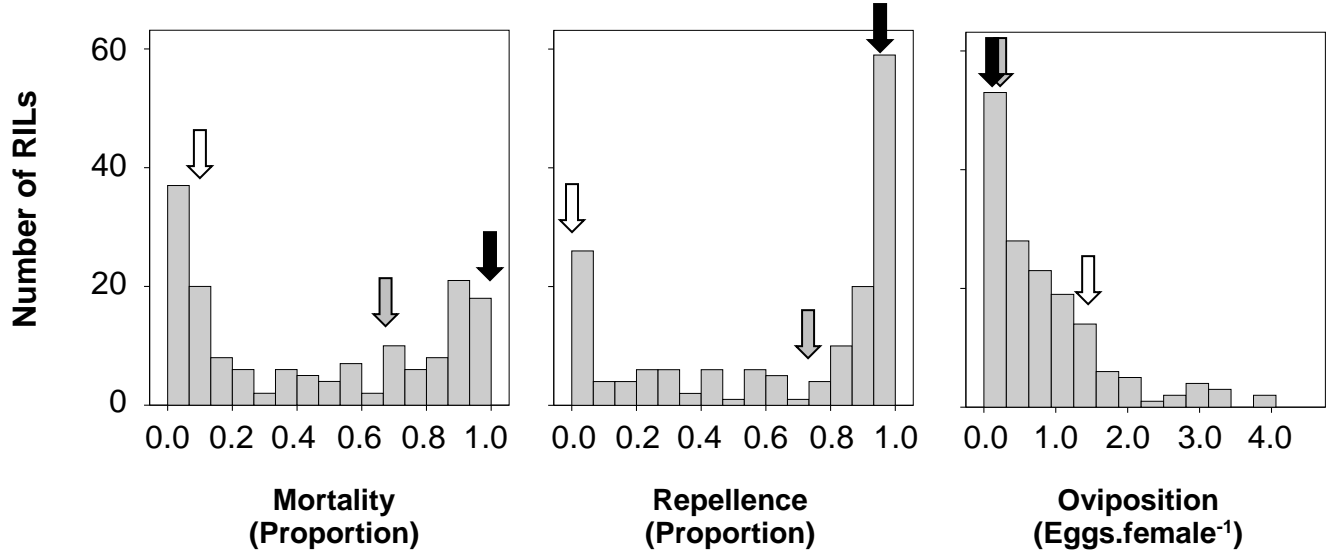
1    Figure 2

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Figure 3

