Trichome Independent Resistance against Western Flower Thrips in Tomato

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Western flower thrips (WFT) are a major pest on many crops, including tomato. Thrips cause yield losses, not only through feeding damage, but also by the transmission of viruses of which the Tomato Spotted Wilt Virus is the most important one. In cultivated tomato, genetic diversity is extremely low, and all commercial lines are susceptible to WFT. Several wild relatives are WFT resistant and these resistances are based on glandular trichome-derived traits. Introgression of these traits in cultivated lines did not lead to WFT resistant commercial varieties so far. In this study, we investigated WFT resistance in cultivated tomato using a F2 population derived from a cross between a WFT susceptible and a WFT resistant cultivated tomato line. We discovered that this WFT resistance is independent of glandular trichome density or trichome-derived volatile profiles and is associated with three QTLs on chromosomes 4, 5 and 10. Foliar metabolic profiles of F3 families with low and high WFT feeding damage were clearly different. We identified α-tomatine and a phenolic compound as potential defensive compounds. Their causality and interaction need further investigation. Because this study is based on cultivated tomato lines, our findings can directly be used in nowadays breeding programs.

Keywords: Insect resistance • Metabolite profiles • QTL mapping • Tomato • Trichome-derived volatiles • α-Tomatine.

Abbreviations: QTL, quantitative trait loci; WFT, western flower thrips.

Introduction

In cultivated tomato (Solanum lycopersicum), genetic diversity is extremely low due to a genetic bottleneck during domestication (Sim et al. 2011, Menda et al. 2014). Less than 5% of the genetic diversity present in the closest wild relatives of tomato is left in domesticated cultivars (Tanksley and McCouch 1997). This has large consequences for breeding, since variation in insect resistance is almost completely absent within the cultivated tomato gene pool. In the 90s, people started to search for insect resistance in wild relatives of tomato (Krishna Kumar et al. 1995, Maliepaard et al. 1995, Tanksley and McCouch 1997). For many pests, resistant lines of wild relatives were found and breeding efforts were made to introgress these traits into commercial varieties. In most cases this was not successful, because agronomic traits were negatively affected (Hartman and St. Clair 1998). Intensive backcrossing did not always improve results due to linkage drag as a consequence of genomic rearrangements (Mutschler et al. 2005).

Consequently, no cultivar resistant against western flower thrips (WFT, Frankliniella occidentalis) is commercially available nowadays. WFT form a feared pest in tomato cultivation, not only because of yield loss due to feeding damage, but more importantly because WFT can transmit viruses like the tomato spotted wilt virus (Allen and Broadbent 1986). Several wild relatives of the cultivated tomato convey resistance to WFT (Krishna Kumar et al. 1995, Mirnezhad et al. 2010, Voisman et al. 2018) by the presence of type IV glandular trichomes containing acylsugars which after release form a sticky net that may trap insects (Nihoul 1994, Mirnezhad et al. 2010, Lucini et al. 2015, Leckie et al. 2016). Type IV trichome-mediated insect resistance is in practice not preferred due to its negative impact on natural enemies of pest insects (Simmons and Gurr 2005, Riddick and Simmons 2014). In addition, the stickiness of the acylsugars makes fruit picking unpleasant. WFT resistance can also be conveyed by type VI trichomes and is then due to entrapment of insects by polymerization of the phenolic compound rutin (Duffey and Isman 1981, Romero-González et al. 2011) and the production of trichome-derived volatiles that repel WFT (Lin et al. 1987, Koschier et al. 2002, Bleeker et al. 2009, Escobar-Bravo et al. 2017). In cultivated tomato lines, WFT feeding damage can be reduced by activating jasmonic acid-associated defenses that lead to increase of type VI trichome densities (Boughton et al. 2005, Escobar-Bravo et al. 2017). Unfortunately, type VI trichome-mediated WFT resistance is, as far as we know, not strong enough to protect for virus transmission.

Several efforts were made to understand the genetic regulation of trichome-mediated insect resistance (Maliepaard et al. 1995, Mutschler et al. 1996, Blauth et al. 1998, Alba et al. 2009, Mirnezhad et al. 2011) and to introduce this type of resistance in cultivated tomato, either through introgression (Lawson et al. 1997, Mutschler et al. 2005, Smeda et al. 2016) or by
transient expression of the genes involved (Bleeker et al. 2012, Glas et al. 2012, Kortbeek et al. 2016). The success of these efforts was sometimes promising, but still today a WFT resistant tomato cultivar is not commercially available.

In the last decades, WFT resistance research in tomato has focused on trichome-mediated resistance (Glas et al. 2012, Kang et al. 2016, Chen et al. 2018a, and reference herein). Hardly any efforts were made to study WFT resistance based on trichome independent traits (Mirnezhad et al. 2010, Mirnezhad et al. 2011, Chen et al. 2018b, Escobar-Bravo et al. 2019). In this article, we present a cultivated tomato line (TR), which WFT resistance is independent from glandular trichome presence or trichome-derived volatile profiles. Instead, our results suggest that foliar metabolite profiles are involved in WFT resistance. Our findings open the way for marker-assisted breeding of tomato cultivars with trichome independent WFT resistance.

Results

Trichome density and WFT feeding damage phenotypes

WFT resistance and its relation to type VI trichomes was studied in an F2 population (364 individuals) and in 92 F3 families being progeny of 92 F2 individuals representing the diversity in these two phenotypes. In the F2 and F3 experiment, the resistant (TR) and susceptible (MM) parental lines were grown alongside respectively the F2 population and the F3 families. We observed that the growth and development of both parents was very similar. At the end of the experiment (day 42), the number of leaves did not significantly differ between the two parents (t-test, F2: n = 18, P = 0.20, F3: n = 10, P = 0.85). We observed that the WFT feeding damage scores for the resistant parent TR were lower than for the susceptible parent MM in both the F2 and the F3 experiment (Fig. 1A and Supplementary Fig. S1A). In both experiments, type VI trichome density was for MM (F2: 2.0 mm\(^2\)/C\(^2\), F3: 0.9 mm\(^2\)/C\(^2\)) lower than for TR (F2: 7.0 mm\(^2\)/C\(^2\), F3: 3.3 mm\(^2\)/C\(^2\)) (Fig. 1B, Supplementary Fig. S1B). When comparing the F2 and F3 experiments, we observed that WFT feeding damage was, on average, higher and trichome density was lower for the F3 experiment. These effects are possibly due to differences in the environmental conditions, since our plants were grown in a regular greenhouse in which irradiation, temperature and humidity are influenced by outside weather conditions. Transgressive segregation in the F2 was evident for both WFT feeding damage and type VI trichome density (Fig. 1). Because the resistant and susceptible parent had opposite trichome phenotypes, a coupling was expected between this trait and WFT feeding damage. However, we observed, both in F2 and F3, several lines with low type VI trichome density that were highly WFT resistant, and also lines with high trichome density that were WFT susceptible. In the F2 population, no significant correlation was observed between WFT feeding damage and type VI trichome density (Table 1, Supplementary Fig. S2A). This suggests that WFT resistance was independent from trichome density. Interestingly, a negative correlation between type VI trichome density and WFT feeding damage was observed in the F3 selection when analyzing the family means that were used for QTL mapping (Table 1, Supplementary Fig. S2B). This correlation was not significant when calculations were based on data of individual F3 plants (Supplementary Fig. S2C). The 92 F2 individuals that were chosen to produce F3 progeny were selected based on phenotype. This selection may have unintentionally led to favoring of certain marker combinations, which probably can explain the negatively correlation between the F3 family means. (For details see Supplementary Data S1).

Trichome-derived volatile profiles

Volatile profiles of the trichomes of the 92 F3 families were determined using a leaf-dip in pentane and subsequent gas
Table 1: Spearman’s rank correlation coefficients of all traits measured in F2 and/or F3 plants of the mapping population [F2: 364 lines, F3: 92 F3 families (mean of 5 plants per F3 family)]

| Trait          | F2       | F3       |
|---------------|----------|----------|
| Trichome density |          |          |
| Volatiles     |          |          |
| a-Pinene      |          |          |
| b-Cymene      |          |          |
| b-Pinene      |          |          |
| b-Carene      |          |          |
| a-Phellandrene|          |          |
| Trans-ocimene |          |          |
| WFT density   |          |          |
| Feeding damage|          |          |
| g*(g FW)      |          |          |

Data on trichome-derived volatiles are not corrected for type VI trichome density. Significant positive correlations are marked in blue, significant negative correlations are marked in red (light color: \( p < 0.05 \), dark color: \( p < 0.0005 \) (Bonferroni corrected threshold).

Using \(^1\)H-Nuclear Magnetic Resonance (NMR) we obtained foliar metabolite profiles from 12 of the 92 F3 families. These 12 families represented resistant and susceptible lines with low and high type VI trichome density in equal numbers (Supplementary Table S1). Data reduction by principal component analysis (PCA) and subsequent partial least squares-discriminant analysis (PLS-DA) using WFT feeding damage as grouping factor showed clear differences between metabolic profiles of F3 families with low and high WFT feeding damage (Supplementary Fig. S3), whereas both analyses (PCA and PLS-DA using trichome density as grouping factor) did not lead to separation of F3 families with low and high type VI trichome density. Analysis of the Variable Importance in Projection Score (VIPS) resulted in the identification of two regions of interest in the NMR spectra that were associated with metabolites responsible for the group separation in PLS-DA. Peaks in the first region (0.84 to 1.68 ppm) corresponded with the glycoalkaloid, \( \alpha \)-tomatine. \( \alpha \)-tomatine concentrations varied between 40 and 50 mg/g DW in the F3 families (Fig. 3A). This compound was more abundant in leaves of F3 families with low WFT feeding damage than in F3 families with high WFT feeding damage (Fig. 3A). Peaks in the second region (6.60–6.67 ppm) could not be coupled to any spectra in our libraries, but the chemical shifts and the coupling patterns indicate that this compound is a phenolic compound. This compound was more abundant in F3 families with low WFT feeding damage compared to the F3 families with high WFT feeding damage (Fig. 3B). All earlier lines tested in our laboratory were susceptible to WFT or gained resistance through presence of type IV glandular trichomes, and all these lines did not express this compound at detectable levels (Mirnezhad et al. 2010, Mirnezhad et al.
Levels of α-tomatine and the phenolic compound(s) were positively correlated (Supplementary Table S3). Furthermore, both were strongly negatively correlated with WFT feeding damage while trichome density was not significantly correlated with WFT feeding damage.

**WFT bioassay with α-tomatine**

Thrips larvae were fed with an artificial diet supplemented with α-tomatine at a range of concentrations that were lower (0, 0.02, 0.05%), in the same range (0.2 and 0.5%), and higher (2%) as the fresh weight (FW) concentrations found in leaves (Supplementary Data S2). WFT mortality increased with increasing α-tomatine concentrations (Pearson $r = 0.91$, $P = 0.015$), a mortality of 33% was observed at the highest concentration. The correlation was log-linear for all α-tomatine concentrations except 0.5% (Fig. 4).

**Detection of (interacting) QTLs**

QTL mapping was performed for all traits measured in F2 and F3, except for the foliar metabolites since these were measured
in only 12 F3 families (Supplementary Data S3). For WFT feeding damage, three QTLs were identified: WFT-chr4, WFT-chr5, WFT-chr10 (Table 2, Fig. 5). The QTL on chromosome 4 was only detected for F3, the others for both F2 and F3. WFT-chr10 had the largest effect size (F2: LOD = logarithm (base 10) of odds (LOD) = 11.7, additive effect = 232 mm²/plant). The 1LOD-confidence interval of WFT-chr10 was 0.8 Mb and in this region 101 genes were located. For WFT-chr4 and WFT-chr5 the 1LOD-confidence intervals covered large parts of the chromosome due to low recombination frequencies in the pericentromeric regions. The 1LOD-interval of WFT-chr4 was predicted to contain 998 genes and of WFT-chr5 1,505 genes (Table 2, ITAG3.0). WFT-chr5 and WFT-chr10 were significantly interacting in F3 (Fig. 6A). We observed that if at WFT-chr10 two alleles of the susceptible parent MM were present, WFT feeding damage was high, independent from the alleles at WFT-chr5. In contrast, when one or two TR-alleles were present at WFT-chr10, the presence of an TR-allele at WFT-chr5 was lowering the WFT feeding damage. Note that this interaction was not significant in the F2 population. WFT-chr10 is coinciding with the nine markers on chromosome 10 that showed segregation distortion in our selection for analyses in the F3 families (Supplementary Data S1), revealing that our selection using phenotypes unintentionally favored the presence of the TR-allele at WFT-chr10.

For type VI trichome density, two QTLs were detected on chromosome 10: TD-chr10a and TD-chr10b (Table 2, Fig. 5). TD-chr10a was detected for both F2 and F3, TD-chr10b for F3 only. Both QTLs had a small confidence interval (TD-chr10a = 2 Mb, TD-chr10b = 0.9 Mb) in which 191 and 156 genes were located, respectively. For the F3, but not for the F2, one coinciding QTL was observed for trichome density and WFT feeding damage (confidence intervals of TD-chr10b and WFT-chr10 are

![Image](https://via.placeholder.com/150)

**Fig. 4** Log-linear correlation between WFT mortality and α-tomatine concentration. Mortality of WFT larvae when fed with an artificial diet supplemented with α-tomatine in five different concentrations (0, 0.02, 0.05, 0.2, 0.5 and 2%). To visualize the log-linear correlation, ln of α-tomatine concentration was plotted. Least squares regression line

**Table 2:** Details of the QTLs identified for WFT feeding damage, trichome density and trichome volatiles in F2 and/or F3 plants of the mapping population [F2: 364 lines, F3: 92 F3 families (mean of 5 plants per F3 family)].

| Trait                | QTL      | LOD-score Position (cM) | Closest marker Position (Mb) | Additive effect | Dominance effect | 1LOD-interval (cM) | 1LOD-interval (Mb) | No. of genes |
|----------------------|----------|-------------------------|------------------------------|-----------------|-----------------|-------------------|-------------------|--------------|
| WFT feeding damage   | WFT-chr4 | F3 4.2                  | 42.5                         | 52.3            | 23.1            | ~129.4            | 6.5–55            | 998          |
|                      | WFT-chr5 | F2 3.8                  | 40.9                         | 8.6             | 36.9            | 5.6               | 40–60             | 1505         |
|                      |          | F3 3.9                  | 47.0                         | 8.6             | 25.3            | 44.9              | 35–60             | 1505         |
|                      | WFT-chr10| F2 11.7                 | 51.0                         | 61.1            | 54.0            | ~7.9              | 48–54             | 101          |
|                      |          | F3 8.1                  | 53.0                         | 61.1            | 303.3           | 86.1              | 49–61             | 101          |
| Trichome density     | TD-chr10a| F3 6.8                  | 19.0                         | 3.0             | ~11.1           | ~3.8              | 13–31             | 1368         |
|                      |          | TD-chr10b               | 13.3                         | 3.0             | ~14.4           | ~3.4              | 18–25             | 191          |
|                      |          | TD-chr10b               | 8.6                          | 45.9            | ~12.2           | ~4.2              | 42–50             | 156          |
|                      |          |                        |                              |                 |                 |                   |                   |              |
| Volatiles            |          |                        |                              |                 |                 |                   |                   |              |
| α-Pinene             | Vol-chr10a| F3 8.9                 | 19.8                         | 3.0             | ~0.09           | ~0.003            | 16–25             | 91          |
|                      | Vol-chr10b| F3 8.8                 | 49.0                         | 61.0            | ~0.09           | ~0.002            | 39–51             | 188         |
|                      |          | Δ-carene               | Vol-chr10a                   | 9.2             | 21.0            | ~0.088            | 17–33             | 1368        |
|                      |          | Δ-carene               | Vol-chr10b                   | 8.0             | 49.0            | ~0.017            | 42–51             | 116         |
|                      |          | Phellandrene           | Vol-chr10a                   | 9.9             | 21.0            | ~0.011            | 17–28             | 649         |
|                      |          | Phellandrene           | Vol-chr10b                   | 10.2            | 49.0            | ~0.016            | 39–51             | 188         |
|                      |          | Terpinene              | Vol-chr10a                   | 7.7             | 19.8            | ~0.034            | 16–24             | 191         |
|                      |          | Terpinene              | Vol-chr10b                   | 8.0             | 49.0            | ~0.004            | 39–52             | 188         |
|                      |          | β-Phellandrene         | Vol-chr10a                   | 8.9             | 20.0            | ~0.294            | 16–25             | 191         |
|                      |          | β-Phellandrene         | Vol-chr10b                   | 8.5             | 49.0            | ~0.285            | 39–52             | 188         |
|                      |          | Total volatiles        | Vol-chr10a                   | 9.2             | 20.0            | ~0.433            | 16–25             | 91          |
|                      |          | Total volatiles        | Vol-chr10b                   | 8.3             | 49.0            | ~0.404            | 36–51             | 188         |
|                      |          | β-Caryophyllene        | Vol-chr11                    | 5.2             | 64.0            | ~0.017            | 56–end            | 1440        |
overlapping). This is in agreement with the correlations observed between WFT feeding damage and trichome density in the F3, but not in the F2. In addition, TD-chr10a and TD-chr10b were significantly interacting in the F3, but not in the F2 (Fig. 6B). The presence of two alleles of the susceptible parent MM at TD-chr10a results in low type VI trichome density of around 1.5 mm\(^{-1}\) independent from the alleles present at TD-chr10b. If one or two TR-alleles are present at TD-chr10a, the genotype at TD-chr10b does influence the trichome phenotype. This interaction was not reflected in the segregation of WFT feeding damage.

**Fig. 5** QTLs detected for WFT feeding damage, type VI trichome density and trichome-derived volatiles. For each chromosome on which a significant QTL is detected, we represent from left to right: genetic map, 1- and 2-LOD confidence intervals of detected QTLs, LOD-profiles of traits that are significantly associated.

**Fig. 6** Interactions between QTLs. Effect plots of interacting loci detected in F3 for (A) WFT feeding damage and (B) type VI trichome density (mean ± SE). Note that one of the nine possible allele combinations (TR/TR at TD-chr10a and MM/MM at TD-chr10b) was lacking in the selection for F3 phenotyping but was present in the F2 population at low frequency (0.8%). These interactions were not observed in F2.
For five of the eight monoterpenes (α-pinene, δ-carene, α- and β-phellandrene, and α-terpinene), two QTLs were identified on chromosome 10, Vol-chr10a and Vol-chr10b (Table 2, Fig. 5). The same QTLs were identified for total volatile content. Vol-chr10a coincides with TD-chr10a (Fig. 5). Because higher trichome density results in higher volatile content (these traits are strongly correlated), it is expected that the same gene is causal for both Vol-chr10a and TD-chr10a and that this gene is involved in the regulation of type VI trichome density. Vol-chr10b coincides with TD-chr10b and WFT-chr10 (Fig. 5). The interaction between TD-chr10a and TD-chr10b was also reflected in the segregating patterns of all volatiles correlated with type VI trichome density, but interactions were not significant at $\alpha = 0.05$.

On chromosome 11, a QTL was identified for β-caryophyllene, the only sesquiterpene identified in our experiments (Table 2, Fig. 5). This QTL, Vol-chr11, did not coincide with any of the other QTLs. Probably the causal gene is involved in the regulation of sesquiterpene production, but its function is neither related to trichome density nor to WFT resistance. This QTL is located close to the centromeric region and the 1LOD-confidence interval is therefore large, containing 1,440 genes.

No QTL was detected for the volatiles p-cymene, β-myrcene and trans-ocimene (Table 2), probably due their low heritabilities (Table 3).

Because foliar metabolites were only measured in 12 F3 families, we were not able to perform QTL mapping for these traits. Still, we detected a distinct relationship between the genotype at the WFT-QTLs and the concentration of α-tomatine and the unknown phenolic compound(s) (Fig. 3, Supplementary Table S1 and S2). Concentrations were high when two TR-alleles were present at WFT-chr10, with one exception (F3 family with number 5). But for this F3 family, two MM-alleles were present at WFT-chr5, and one MM-allele at WFT-chr4.

### Heritability and explained variances

Broad-sense heritability was estimated for each trait and medium to low values were obtained for most traits, suggesting that both environmental conditions and genetic variation determine the final phenotype. Although the heritability estimated for trichome density in the F2 was very high, only 8% of the variance in the F2 could be explained by TD-chr10a, the only QTL detected for this trait (Table 3). This suggests the presence of several small QTLs that go undetected. The explained variance for WFT feeding damage in the F2 was not high (18% on basis of WFT-chr5 and WFT-chr10b). In F3 more (interacting) QTLs were detected for trichome density and WFT feeding damage, which explained over 60% of the total variance for both traits. Heritability of most volatiles was estimated to be low to medium (H2: 0.08–0.30). Negative heritabilities indicating no influence of genotype were observed for β-myrcene (H2 = −0.06) and trans-ocimene (H2 = −0.12). The QTLs detected for the volatiles in the F3 explained 42–52% of the variance for each compound (Table 3) with β-caryophyllene as the exception, explaining 21% of the total variance.

### Discussion

**Discovery of foliar WFT resistance, independent from trichome presence**

In both F2 and F3, we discovered lines in which WFT resistance was as strong as in the parent TR, but with much lower type VI trichome density. This indicated that at least large part of the WFT resistance conveyed by the TR line is independent from type VI trichome presence. This finding was confirmed by the lack of a significant correlation between WFT feeding damage and type VI trichome density in F2 (Table 1) and the absence of overlapping QTLs detected for the two traits in this population (Table 2, Fig. 6). Also or other two other species of the genera Solanaceae, potato (Solanum tuberosum × Solanum berthaultii) (Galvez et al. 2005) and bell pepper (Capsicum annuum) (Maharjaya et al. 2015), no correlation was found between trichome density and WFT resistance in segregating populations resulting in the lack of coinciding QTLs. We also detected that trichome-derived volatiles did not play a role in WFT

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**Table 3**: Heritability for each of the measured traits and explained variances for each of the corresponding QTLs and their interactions

| Trait | $H^2$ | Model | Explained variance | Full | 1st | 2nd | 3rd | 4th |
|-------|-------|-------|-------------------|------|-----|-----|-----|-----|
| F2    |       |       |                   |      |     |     |     |     |
| WFT feeding damage | 0.65  | y ~ 5-49 + 10-51 | 18.20 | 4.51 | 13.63 |
| Trichome density | 0.93  | y ~ 10-19   | 8.10  | 8.10 |     |
| F3    |       |       |                   |      |     |     |     |     |
| WFT feeding damage | 0.26  | y ~ 4-42.5 + 5-47 + 10-53 + 5-47: 10-53 | 61.28 | 16.54 | 16.68 | 19.79 | 4.22 |
| Trichome density | 0.21  | y ~ 4-39.2 + 10-21 + 10-45.9 + 10-21: 10-45.9 | 67.27 | 7.78 | 29.62 | 11.07 | 5.19 |
| β-caryophyllene | 0.20  | y ~ 10-19.8 + 10-49 | 47.09 | 12.39 | 10.65 |
| p-cymene | 0.40  | No QTL | 46.31 | 14.27 | 9.22 |
| β-myrcene | −0.06 | No QTL | 51.73 | 12.88 | 12.40 |
| Δα-terpinene | 0.30  | y ~ 10-21 + 10-49 | 42.40 | 10.98 | 10.18 |
| β-phellandrene | 0.29  | y ~ 10-21 + 10-49 | 46.08 | 12.90 | 9.93 |
| Trans-caryophyllene | −0.12 | No QTL | 20.66 | 20.66 |     |
| Total volatiles | 0.28  | y ~ 10-20 + 10-49 | 46.78 | 13.90 | 9.38 |

Each QTL is represented by the closest marker (chromosome-position (cM)). Components of model are given as 1st, 2nd etc.
resistance, as their blend and concentration per trichome did not significantly differ between the parental lines (Fig. 2). Furthermore, in F3 the abundance of most volatiles was highly correlated with trichome number (Table 1). Our observations indicate the existence of a WFT resistance factor that is independent of trichome density and trichome-derived compounds, such as acylsugars and terpenes. The possibility of trichome independent resistance against herbivorous arthropods in tomato was already suggested by Rasmy (1985). He observed that after removal of trichomes in two wild tomato relatives part of the resistance to two-spotted spider mite (Tetranychus urticae) persisted. Recently, reduction of WFT feeding damage was reported in tomato upon infection by Pseudomonas (Chen et al. 2018b) and UV-B (Escobar-Bravo et al. 2019). In both cases, no increase in trichome density or change of volatile blend was observed.

We selected 92 F2 plants to produce F3 seeds with the aim to test equal numbers of F3 families with low and high WFT feeding damage and low and high trichome density. It turned out that in this selection some marker combinations were more frequently present than others. We did not intend to increase the amount of segregation distortion, but in hindsight it allowed us to draw interesting additional conclusions. It led to large segregation distortion for markers in the proximity of our strongest QTL for WFT feeding damage (WFT-chr10), and a moderate negative correlation between the F3 family means for WFT feeding damage and type VI trichome density. As a consequence, QTL mapping using F3 families means resulted not only in the detection of two QTLs specific for WFT feeding damage (WFT-chr4 and WFT-chr5), but also to the detection of a third QTL (WFT-chr10) that co-localized with QTLs for trichome density (TD-chr10b) and trichome-derived monoterpenes (Vol-chr10b). This suggests that an increase of trichome density can still add to the resistance phenotype. This is also reflected in the observed interaction between WFT-chr10 and TD-chr10a suggesting that TR-alleles at WFT-chr10 conditionally increase trichome density. However, high type VI trichome density does not always convey WFT resistance as we observed, in both F2 and F3, lines that were equally susceptible to the parent MM, although their type VI trichome densities equaled those of the parent TR. We, therefore, hypothesized that the influence of type VI trichome density on WFT resistance depended on the chemotype of the leaves. These findings question whether activation of jasmonic acid mediated defenses will always lead to more WFT resistance via increase of type VI trichomes (Escobar-Bravo et al. 2017).

**Indication for a role of foliar metabolites in WFT resistance**

Our experimental work indicates a role for foliar metabolites in WFT resistance. A clear difference was observed between the metabolite profiles of F3 families with high and low WFT feeding damage (Supplementary Fig. S3). Two regions of the NMR spectrum were associated with this difference, suggesting x-tomatine and (a) phenolic compound(s) as defensive compounds (Fig. 3).

Only a few other studies compare the metabolite profiles of tomato lines with varying WFT resistance (Mirnezhad et al. 2010, Mirnezhad et al. 2011, Vosman et al. 2018). These studies include in their analyses not only cultivated tomato lines, but also wild relatives. Metabolites mentioned to play a role in WFT resistance were associated with the presence or absence of type IV trichomes. Type IV trichomes contain acylsugars which have a negative impact on WFT performance (Leckie et al. 2016). We did not observe type IV trichomes in any of our F2 lines, F3 lines or our parents. In addition, type IV trichomes are only reported in cultivated tomato in a very early developmental stage (Kim et al. 2012). As far as we know, in tomato only two recent studies report changes in foliar metabolites upon activation of defenses resulting in reduction of WFT feeding damage (Chen et al. 2018a, Xiao et al. 2019). The first study was done with our susceptible parent MM (Chen et al. 2018). As expected the defensive compounds indicated to be important in our study were not associated with reduction of WFT feeding damage in that study. The second study reports a decline in thrips population and an increase of total foliar phenolics in an Japanese cultivar upon the presence of an earthworm (Xiao et al. 2019), which is in line with our finding that (a) phenolic compound(s) as potential defensive compounds.

Besides the phenolic compound(s) also α-tomatine was found as potential defense compounds. Causality of both compounds for WFT resistance is still unclear, and therefore we cannot exclude that additional metabolites play a role in WFT resistance. The negative impact of α-tomatine on WFT was proven in a bioassay (Fig. 4). But this compound cannot solely be responsible for the observed WFT resistance, because the concentration differences in the F3 families are small (ranging from 40 to 50 mg/g DW), and mortality of WFT in the bioassays was max 17% at a concentration comparable to the one found in F3 families with low WFT feeding damage. Also our QTL analyses suggest that more than one metabolite is involved in WFT resistance, as it is unlikely that all three QTLs associated with WFT resistance are involved in the regulation of one defense compound. We, therefore, hypothesize that the identified phenolic compound(s) is (are) responsible for a large part of the observed resistance and that probably the interaction with α-tomatine increases it’s negative impact on WFT. To test this hypothesis the identity of the phenolic compound(s) needs to be revealed.

**QTLs for trichome independent WFT resistance**

In our analyses we detected three QTLs for WFT resistance on chromosome 4, 5 and 10, WFT-chr4, WFT-chr5, WFT-chr10. All other studies reporting QTLs for WFT resistance in tomato are based on populations in which at least one parents is a Solanum pennellii line (Eshed and Zamir 1995, Blauth et al. 1998, Mirnezhad et al. 2011, van den Oever-van den Elsen et al. 2016, Fernandez-Moreno et al. 2017.). Solanum pennellii is a wild tomato with type IV trichomes that are lacking on the plants in our study. Therefore, overlap in genetic regulation of WFT resistance between our results and S. pennellii derived results was not to be expected. No QTLs for WFT resistance...
have been reported in tomato lines for which the WFT resistance is not linked with trichome presence. Lack of literature about the genetic regulation of trichome independent WFT resistance emphasizes that the WFT resistance conveyed by TR is new and based on resistance genes not associated with WFT resistance before.

NMR signals associated with α-tomatine and a phenolic compound were detected to be important for profile separation of F3 families with low and high WFT feeding damage (Fig. 3). Although their causal role and their potential interaction still needs further investigation, we reviewed the literature about the regulation and biosynthesis of glycoalkaloids and phenolic compounds in search for candidate causal genes for WFT-chr4, WFT-chr5, WFT-chr10.

α-Tomatine is reported to be present in at least 15 solanum species (Milner et al. 2011) and is toxic to a wide range of organisms (Barbour and Kennedy 1991, Sanford et al. 1996, Weissenberg et al. 1998, Friedman 2002, Chowan´ski et al. 2016), including T. palmi, a relative of WFT (Hirano et al. 1994). In this study we have shown its negative impact on WFT in a bioassay with the pure α-tomatine. α-tomatine is derived from Acetyl-CoA with squalene being one of the first intermediates. Two squalene synthases (SQS) were located within the confidence interval of WFT-chr10 (Soly10g079040 and Soly10g079060). No functional analyses have been performed for those two proteins, but another SQS (Soly10g110290) is known to function in the biosynthesis pathway of α-tomatine (Cárdenas et al. 2015). GAME-genes (GLYCOALKALOID METABOLISM genes) are reported to play an important role in the biosynthesis of α-tomatine in tomato (Alseekh et al. 2015, Cárdenas et al. 2015). None of the GAME-genes were located inside any of our WFT-QTLs, but JRE6 (Soly05g050790) with a sequence highly similar to GAME9 (Thagun et al. 2016) was located inside the confidence window of WFT-chr5. Jasmonate-responsive transcription factors of the ETHYLENE RESPONSE FACTOR (ERF) family (JRE) and the two SQS are, therefore, promising candidates to be tested for their role in α-tomatine accumulation.

Like in our study, phenolic compounds were associated with WFT resistance in bell pepper, chrysanthemum and carrot (Leiss et al. 2009, Maharijaya et al. 2012, Leiss et al. 2013, Maharijaya 2019). Plant phenolics are a very large group of compounds and the biosynthesis pathway can be divided in several branches of which the main players are known (Cheynier et al. 2013). But many enzymes involved in the last steps of the biosynthesis, the steps that mostly determine the bioactivity of the compound, are unknown. Further study on the identification of the phenolic compound may lead us to reveal the genes involved in the accumulation of resistant compounds.

No obvious candidate causal genes in QTLs for trichome density and volatiles

The trichome-derived volatiles detected in our mapping population (Table 1) were also detected in the headspace of several other S. lycopersicum cultivars and S. pennellii accessions (Bleeker et al. 2009). In addition, when type VI trichomes of S. lycopersicum M82 were picked by hand and analyzed for terpene composition, the sesquiterpene β-caryophyllene and seven monoterpenes were detected of which five were also detected by us (A.L. Schilmiller et al. 2010a, A.L. Schilmiller et al. 2010b). These observations suggest that our leaf dipping method resulted in the extraction of trichome-derived volatiles and that contamination was limited. Several of the detected volatiles have been studied earlier for their effect on thrips. Some appeared to be neutral (β-caryophyllene, myrcene and trans-ocimene (Koschier et al. 2000, Tol et al. 2012), whereas for other volatiles a negative impact has been reported (cymene (Janmaat et al. 2002), α-pinene, δ-carene and α-terpinene (Kim et al. 2015)). In our mapping population we detected two QTLs for most monoterpenes, both located on chromosome 10, Vol-chr10a and Vol-chr10b. These two QTLs coincided with the two QTLs detected for type VI trichome density, TD-chr10a and TD-chr10b. Because all volatiles, except for trans-ocimene, were moderately to strongly correlated with type VI trichome density in F3, overlap in QTLs was expected, and the causal gene probably influences trichome abundance, but not trichome-derived volatile profiles. In conclusion, in the present study we observed little evidence for trichome-derived volatiles adding to WFT resistance. Although in tomato many genes involved in the biosynthesis of type VI trichomes and their volatiles are known, the regulatory picture is not complete yet (Li et al. 2004, Kang et al. 2010, Spyropoulou et al. 2014, Stratmann and Bequette 2016). No candidate causal genes could be detected for TD-chr10a (coinciding with Vol-chr10a) and TD-chr10b (coinciding with Vol-chr10b), suggesting that the causal genes are not annotated yet or not present in the Heinz reference genome. Vol-chr11, the third QTL detected for trichome-derived volatiles, was only associated with the sesquiterpene β-caryophyllene. One candidate causal gene was detected in its confidence interval: Soly11g072600. It is an AP2 domain-containing transcription factor possibly involved in terpene biosynthesis. It is unknown whether the function of this gene is specific for sesquiterpenes.

Dependencies between QTLs

The QTLs WFT-chr10 and TD-chr10b coincided. Two interactions were observed for this locus in the F3 generation, but not in the F2 (Fig. 5). For this locus also segregation distortion was observed, suggesting that the observed interactions become detectable in F3 as a combined result of reduction of total variation and skewed selection.

The first interaction we observed was between TD-chr10a and TD-chr10b. This interaction showed that the allele of the susceptible parent MM at TD-chr10a overrules the allele present at TD-chr10b. It is possible that the causal genes of these two QTLs form two sequential steps in a regulatory pathway. If the gene causal for TD-chr10a is the limiting step, due to the presence of the MM-allele, the allele at TD-chr10b has no influence any more on the final phenotype. It is good to note that this interaction merely shows that TD-chr10b, which coincides with WFT-chr10, is involved in the determination of type VI trichome density. It does not show that trichomes play a role in WFT resistance.
In contrast, WFT feeding damage is not determined by TD-chr10a, whatever allele is present at TD-chr10b/WFT-chr10. It seems, therefore, that the causal gene of TD-chr10b/WFT-chr10 has a dual function, it influences both trichome density and another process, probably secondary metabolite accumulation, resulting in WFT resistance. Another explanation for the observed interaction could be that TD-chr10b and WFT-chr10 do not share the same causal gene, but are caused by two genes in close proximity but having different functions.

The interaction between WFT-chr5 and WFT-chr10 showed that the resistance caused by the TR-allele at WFT-chr5 depends on the presence of a TR-allele at WFT-chr10. Genes causal for WFT-chr5 and WFT-chr10 are thus probably members of the same genetic network.Introgression of WFT-chr5 into another background to enhance WFT resistance should be done carefully, because it might be possible that simultaneous introgression of WFT-chr10 is needed to confer resistance.

**Trichome independent WFT resistance in breeders’ perspective**

WFT are one of the main vectors for the spread of several plant viruses in tomato. WFT acquire and transmit the viruses when they puncture plant tissue and suck the cell content (Rotenberg et al. 2015). To prevent fast build-up of thrips populations within the crop, a cultivar with foliar WFT resistance would be very welcome on the market. Earlier attempts to breed such a cultivar were always based on resistance sources from wild relatives depending on trichome presence. A disadvantage of this approach is that high numbers of trichomes may have negative impact on biological control (Riddick and Simmons 2014) and cause unease during fruit picking. In addition, introgression from wild to cultivated tomato is a long track with a lot of obstacles, among others because of linkage drag. Trichome independent WFT resistance is, therefore, an important step forward. Furthermore, if causality of the phenolic compound and -tomatine for WFT resistance are proven, these compounds may function as biomarkers that might replace laborious infestation experiments with WFT. Only one leaflet of plant material is needed to determine the concentration of the biomarkers, the rest of the plant can be used to determine other traits, such as yield or plant architecture. It also allows to monitor the concentration of defense compounds in the fruit, which is relevant in case these compounds are toxic to human. Another advantage is that the WFT resistance we describe is already located in cultivated tomato. The QTLs we detected can be incorporated right away in marker-assisted breeding programs. They give the opportunity to fully uncouple trichome density and resistance by simultaneous selection in favor of markers associated with resistance and selection against markers associated with trichome density.

**Material and Methods**

**Plant material and insects**

When performing WFT resistance experiments with *S. lycopersicum* cv. Money Maker (MM), we discovered a cultivated tomato line that was highly resistant against WFT (TR). This line is the basis of the present study. Because the origin of TR is unknown, the genetic relationship between the lines MM and TR is unknown. Plant length until first flower emergence, fruit weight and number of seeds per fruit does not differ between the two lines (Supplementary Data S4), but fruit shape differs.

An F2 population (364 individuals) was generated from a cross between MM and TR. From this population a subset of 92 individuals was selected for recovery to produce F3 seed. During this selection process we ensured the presence of resistant and susceptible lines with both low and high type VI trichome densities.

WFT were obtained from a colony reared on cucumber plants grown under standard greenhouse conditions with no additional light and a temperature of around 25°C in the day (minimum set to 20°C) and 19°C in the night (minimum set to 17°C).

**Experimental set-up**

The F2 screen took place February–April 2015 and the F3 screen March–May 2016. Plants were grown at the greenhouse facilities of Rijk Zwaan, The Netherlands B.V., location De Lier. Seeds were sown in a Quick Plug growing system and covered with vermiculite scattered on top to cover the seeds. Seedlings were transplanted in commercial potting soil in 12 cm round plastic pots after 14 d. After 28 d plants were transferred to 30 cm round plastic pots and moved to the greenhouse for experiments. Plants were grown under the same standard greenhouse conditions as the cucumber plants used for WFT rearing.

For the F2 screen: 364 F2 plants and 50 plants of each parent were completely randomized and placed on tables in a greenhouse. Four weeks after germination (day 28) the third fully expanded leaf from the apex was taken from each plant to determine type VI trichome densities. Thereafter, plants were infested with WFT. After 14 d of infestation, WFT feeding damage was determined. WFT feeding damage and type VI trichome density were determined for 18 and 7 plants per parental line, respectively. For the F3 screen, 5 seeds of each of the 92 F3 families were sown, together with 10 seeds of each parent. Plants were placed on tables in a greenhouse in five completely randomized blocks and were treated in the same way as in the F2 screen. Four weeks after germination (day 28) the third fully expanded leaf from the apex was taken from each plant to determine type VI trichome densities and trichome-derived volatile profiles. For 12 F3 families, this leaf was also used for the determination of foliar metabolite profiles. After 14 d of infestation (day 42) WFT feeding damage was quantified.

**Phenotyping**

For each individual leaf the WFT feeding damage (i.e. silver damage) was estimated by eye and expressed in mm². Estimates of each leaf were added to get the total damaged leaf area per plant (Leiss et al. 2009). Type VI trichome density was measured on the adaxial surface of a leaflet. A Zeiss Axio Zoom V16 stereomicroscope equipped with camera was used to take pictures of an area of 12 mm² at both sides of the main vein of the leaflet. Type VI trichomes were counted with ImageJ software (http://imagej.nih.gov/ij/) using the Cell Counter plugin. Data are presented in Supplementary Data S5.

**Volatile profiling of F3 families**

Volatile production by trichomes was analyzed in leaf exudates collected from one leaflet. Before extraction FW of the leaflet (ca. 0.2 g) was measured. Leaf exudates were obtained by dipping the leaflet in 2 ml pentane and incubated for 2 min with gentle shaking before removing the leaf material (Kang et al. 2010, Sallaud et al. 2012). As an internal standard, n-tetradecane (Sigma-Aldrich) was added at a concentration of 1 μg/ml. From the resulting pentane leaf extract 1 μl was injected into an Agilent model 7890 gas chromatograph fitted with a 5975C inert XL MSD Triple Axis Detector using a split ratio of 20:1. Injector temperature was 250°C. The temperature of the initial column (30 m × 0.25 mm, 0.25 μm film thickness, DB-5MS, Agilent Technologies) was held at 40°C for 3 min, then ramped to 150°C at 15°C/min and finally to 220°C at 6°C/min. The helium carrier gas flow was set at 3.0 mL/min. Volatiles were identified...
by comparison with authentic standards when possible or by comparison with retention times and spectral information available in Agilent GC/MSD ChemStation. Compounds were quantified on the basis of the internal standard n-tetradecane. First, calibration curves of known concentrations (five data points in the range of 0.01–15 μg) of synthetic external standards were generated to calculate the internal response factor (IRF). For this, α-pinene and β-caryophyllene (Sigma-Aldrich) were used as external standards to determine the IRF of monoterpenes and sesquiterpenes, respectively. Volatile concentrations were calculated from the formula: Amount of specific compound = (Amount IS × Area SC × IRFSC)/Area IS, where IS corresponds to the internal standard, SC to the specific compound of interest and IRFSC to the IRF. Volatile content was normalized to leaflet weight and expressed as μg per g FW.

**Metabolite profiling of F3 families**

NMR sample preparations, spectra recording (standard ‘H-NMR) and data analysis was done according to Kim et al. (2010). The starting material was 25 mg grinded freeze-dried leaf material from 4 or 5 randomly chosen individuals of 12 F3 families. 3-(Trimethylsilyl)propionic-2,2,3,3-d<sub>4</sub> acid was used as an internal standard. Spectra were integrated to bins of 0.04 ppm using AMIX software and residual solvent signals (water and methanol) were removed. SIMCA-P software (v14.1) was used to perform PCA and PLS-DA analyses (Pareto scaling including a log-transformation when needed). The resulting data are presented in Supplementary Data S5. The PLS-DA model was validated using the permutation test.

**WFT bioassay of pure α-tomatine**

A WFT bioassay was performed as described by Liu et al. (2017). Second instar larvae of the WFT, *F. occidentalis* Perigane (Thripidae), were obtained from a lab culture reared on chrysanthsium flowers in a growth chamber at 23°C, 12 h L/D photoperiod, 60% Relative humidity (RH). WFT bioassays were conducted in adapted 96-well plates filled with 55 μL test solution covered with parafilm. Single second instar larvae of thrips were placed into each lid of an 8 cup flat-cup strip. Each lid was sealed with parafilm and then put on top of the 96 well plates. The 96 wells plates were placed upside down so that thrips were able to feed from the offered test solutions by piercing through the parafilm. The plates were placed into a growth chamber with standard thrips rearing conditions (12h L:D, 23°C). The test solution consisted of 10% fructose and α-tomatine concentration (0.002, 0.05, 0.2, 0.5 and 2%). For these plates, the six rows and an additional row without test solution were covered with flat-cup strips containing larvae. After 5 d, the numbers of surviving larvae were counted with a stereo microscope. Mortality was calculated as one minus corrected survival. Corrected survival is the number of larvae surviving at a certain α-tomatine concentration divided by the number of larvae surviving in the control (test solution without α-tomatine). Resulting data are presented in Supplementary Data S2.

**Genotyping**

RNA was isolated from leaf material of the parental lines with Qiagen RNAeasy kit. RNAseq was performed by BaseClear. Paired-end sequence reads were generated using the Illumina HiSeq500 system. FASTQ sequence files were generated using the Illumina Casava pipeline version 1.8.3. Both genomes were mapped against the reference genome *S. lycopersicum* var. Heinz 1706 (accession assembly GCF_000188115.3, sequence version SL2.50) using programme TopHat 2 for alignment and Picard to mark duplicates. GATK 3.5 was used for variant calling. Informative SNPs equally distributed over the chromosomes with a distance of ±5 Mb, were selected. SNPs were genotyped in all lines of the F2 population using the allele specific KASP method. Based on our results, we increased the coverage on chromosome 10 to 1 marker per ±2.8 Mb.

**Construction genetic map**

The genetic map was constructed using the R package R/qtl (Broman et al. 2003). Recombination frequencies were calculated using R-function ‘estri’. Markers were grouped in 12 linkage groups using the R-function ‘formLinkageGroups’ with maximal recombination frequency = 0.35 and minimal LOD-score = 6. Marker order within the groups was constrained to the order on the physical map of the Heinz genome sequence (Tomato Genome Consortium 2012). Distances in cM between the markers were calculated using the Lande–Green algorithm and the Haldane map function (R-function ‘est.map’). The resulting data are presented in Supplementary Data S1 and Supplementary Figs. S4, S5.

**QTL mapping**

A genome scan with a single QTL model was performed in R (R-function ‘scancor’) for each of the traits individually, using Haley–Knott regression (Haley and Knott 1992). For F3, for each trait, family means were coupled to corresponding F2 genotypes. Results were visualized using MapChart v. 2.30 (Voorrips 2002). These data are given in Supplementary Data S3. One thousand permutations were performed to determine the significance threshold. The effect of each marker on each trait was calculated using the R-function ‘effectscan’. For each trait, a multiple QTL model containing all possible interactions between the detected traits was tested using the R-function ‘fitqtl’. Non-significant terms were dropped from the model. We searched for additional conditional QTLs (R-function ‘addqtl’), but did not detect any. Thereafter, the model with the significant terms was used to calculate the explained variance using the drop-one option of the R-function ‘fitqtl’. Broad-sense heritabilities were estimated using the formula: $H^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2)$, where $\sigma_g^2$ is the between genotype variance component and $\sigma_e^2$ is the within residual (error) variance component. For F2, parental data were used to perform a one-way ANOVA. Total phenotypic variance ($\sigma_{\text{tot}}^2$) was then calculated as the variance between the parental lines, denoted by MSB. Total phenotypic variance ($\sigma_{\text{tot}}^2$) was then taken for each trait as the variance between the F2 lines. $\sigma_g^2$ was calculated as $\sigma_g^2 = \text{var}(F2) − \text{MSB}$.

For F3, phenotypic data were analyzed using a one-way ANOVA. According to the model $\text{ij} = \mu + G_i + e_{ij}$, the total phenotypic variance for each trait was partitioned and the mean sum of squares between F3 families (MSF) and the mean sum of square within F3 families (MSW) were obtained. With these estimates $\sigma_g^2$ was then calculated as $\sigma_g^2 = (\text{MSF} - \text{MSW})/n$, where n is the number of plants within each F3 family. To analyze genes within the confidence intervals of the QTLs the tomato genome and gene annotation of the Sol Genomics Network was used (Tomato Genome version SL3.0 and Annotation ITAG3.10).

**Supplementary Data**

Supplementary data are available at PCP online.

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**Disclosures**

The authors have no conflicts of interest to declare.

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