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Quantitative resistance against *Bemisia tabaci* in *Solanum pennellii*: Genetics and metabolomics

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Abstract The whitefly *Bemisia tabaci* is a serious threat in tomato cultivation worldwide as all varieties grown today are highly susceptible to this devastating herbivorous insect. Many accessions of the tomato wild relative *Solanum pennellii* show a high resistance towards *B. tabaci*. A mapping approach was used to elucidate the genetic background of whitefly-resistance related traits and associated biochemical traits in this species. Minor quantitative trait loci (QTLs) for whitefly adult survival (AS) and oviposition rate (OR) were identified and some were confirmed in an F_2BC_1 population, where they showed increased percentages of explained variance (more than 30%). Bulked segregant analyses on pools of whitefly-resistant and -susceptible F_2 plants enabled the identification of metabolites that correlate either with resistance or susceptibility. Genetic mapping of these metabolites showed that a large number of them co-localize with whitefly-resistance QTLs. Some of these whitefly-resistance QTLs are hotspots for metabolite QTLs. Although a large number of metabolite QTLs correlated to whitefly resistance or susceptibility, most of them are yet unknown compounds and further studies are needed to identify the metabolic pathways and genes involved. The results indicate a direct genetic correlation between biochemical-based resistance characteristics and reduced whitefly incidence in *S. pennellii*.

Keywords: Genetic linkage map; life-history; metabolic fingerprinting; parameters; tomato; whitefly
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INTRODUCTION

Bemisia tabaci is a virus-transmitting hemipteran herbivore with a wide host range (Brown et al. 1995). It is among the world's most invasive species (www.issg.org/database) and has devastating effects on many crop and ornamental plant species (Williams et al. 1996; Vázquez et al. 1997). This insect not only inflicts direct damage to plants through phloem consumption, honeydew secretion, and triggering uneven ripening of fruits (Matsui 1992; Schuster 2001), but also causes indirect damage by vectoring more than 100 different viruses and by promoting the growth of saprophytic fungi on the leaves (Oliveira et al. 2001; Valverde et al. 2004).

All publicly available tomato cultivars (*Solanum lycopersicum*) are susceptible to *B. tabaci*, although there is variation in susceptibility level (Heinz and Zalom 1995). Several methods are used to control *B. tabaci*, but these methods are either unsustainable or less effective in the open field. In open field production, the control of *B. tabaci* is predominantly based on the application of insecticides, but the effectiveness of these chemical pest control agents is declining. *Bemisia tabaci* has developed resistance against the most commonly applied insecticides and resistant strains have become more and more abundant (Fernandez et al. 2009; Roditakis et al. 2009; Campuzano-Martinez et al. 2010; Crowder et al. 2010; Feng et al. 2010). In addition, chemical control has negative effects on non-target organisms and ecosystems as a whole (Nash et al. 2010; Cloyd and Bethke 2011; He et al. 2011). Currently, the deployment of biocontrol methods is a successful alternative in protected (greenhouse) tomato production (Van Lenteren and Woets 1988; Van Lenteren et al. 1992; Van Lenteren et al. 1996; Vidal et al. 1998; Van Lenteren 2000; Cuthbertson and Walters 2005; Cuthbertson et al. 2007; Lykouressis et al. 2009; Calvo et al. 2009). However, these methods are difficult to adopt in the open field and semi-field environments. It also does not prevent virus transmission by the whiteflies (Smyrnioudis et al. 2001; Belliure et al. 2011).

A promising alternative to control *B. tabaci* is breeding for durable host-plant resistance (Bruce 2010; Broekgaarden et al. 2011). A number of wild relatives of the cultivated tomato are resistant to whiteflies (Liedl et al. 1995; Nombela et al. 2000; Muigai et al. 2002; Muigai et al. 2003; Baldin et al. 2005; Sanchez-Pena et al. 2006; Firdaus et al. 2012; Firdaus et al. 2013; Lucatti et al. 2013) and can serve as resistance donor in breeding programs. The resistance mechanisms identified so far in the wild relatives of cultivated tomato are based on

chemical compounds produced in the glandular trichomes, including, for example, acyl sugars, methyl ketones, and sesquiterpenes, which affect the host selection behavior (antixenosis) and/or the fitness (antibiosis) of the whiteflies (Liedl et al. 1995; Nombela et al. 2000; Freitas et al. 2002; Antonious and Kochhar 2003; Muigai et al. 2003; Antonious et al. 2005; Resende et al. 2009; Bleeker et al. 2009; Bleeker et al. 2011; Firdaus et al. 2013; Lucatti et al. 2013).

Interspecific crosses between *B. tabaci*-resistant tomato wild relatives and *B. tabaci*-susceptible *S. lycopersicum* enable the development of mapping populations, which can be used for the detection of QTLs for whitefly resistance. Analyzing F_2 populations derived from different tomato wild relative donor plants has resulted in the identification of QTLs related to whitefly resistance (Maliepaard et al. 1995; Momotaz et al. 2010; Firdaus et al. 2013). Metabolite mapping studies performed in F_2 populations with *S. pennellii* LA716 as the donor parent has resulted in the identification of loci related to the biosynthesis of acyl sugars and fatty acids (Mutschler et al. 1996; Blauth et al. 1998; Blauth et al. 1999; Leckie et al. 2013). Although QTLs for these traits could be identified, these studies did not provide a direct link between metabolite and whitefly-resistance QTLs.

The objective of our work was to study the relation between QTLs in an F_2 population derived from an interspecific cross between *S. pennellii* accession LA3791 and *S. lycopersicum*. Two F_2BC_1 populations were used to validate the whitefly-resistance QTLs identified in the F_2 population. We report QTLs for *B. tabaci* life-history parameters in *S. pennellii* and their correlation with metabolite QTLs. We analyzed the metabolic composition of leaf extracts by gas chromatography-mass spectrometry (GC-MS). The untargeted metabolomics approach allowed us to study the relevance of a large number of individual metabolites in whitefly resistance/susceptibility.

RESULTS

Whitefly resistance increases with plant age

An F_2 population ($n = 131$) derived from a cross between an *S. lycopersicum* elite cultivar (*Ec*) and *S. pennellii* LA3791 (*Sp*) was screened for susceptibility/resistance to *B. tabaci* in a no-choice experiment in which AS and OR were monitored. The results are shown in Figure 1. For AS, the percentage of plants on which no *B. tabaci* adults survived (AS = 0) increased from 15% when the plants were 6 weeks old to 64% when the plants were 20 weeks old. The percentage of plants on which no eggs were deposited (OR = 0) increased from 27% on 6-week-old to 51% on 20-week-old plants. Partial resistance to full susceptibility in terms of AS and OR was observed for the remaining genotypes.

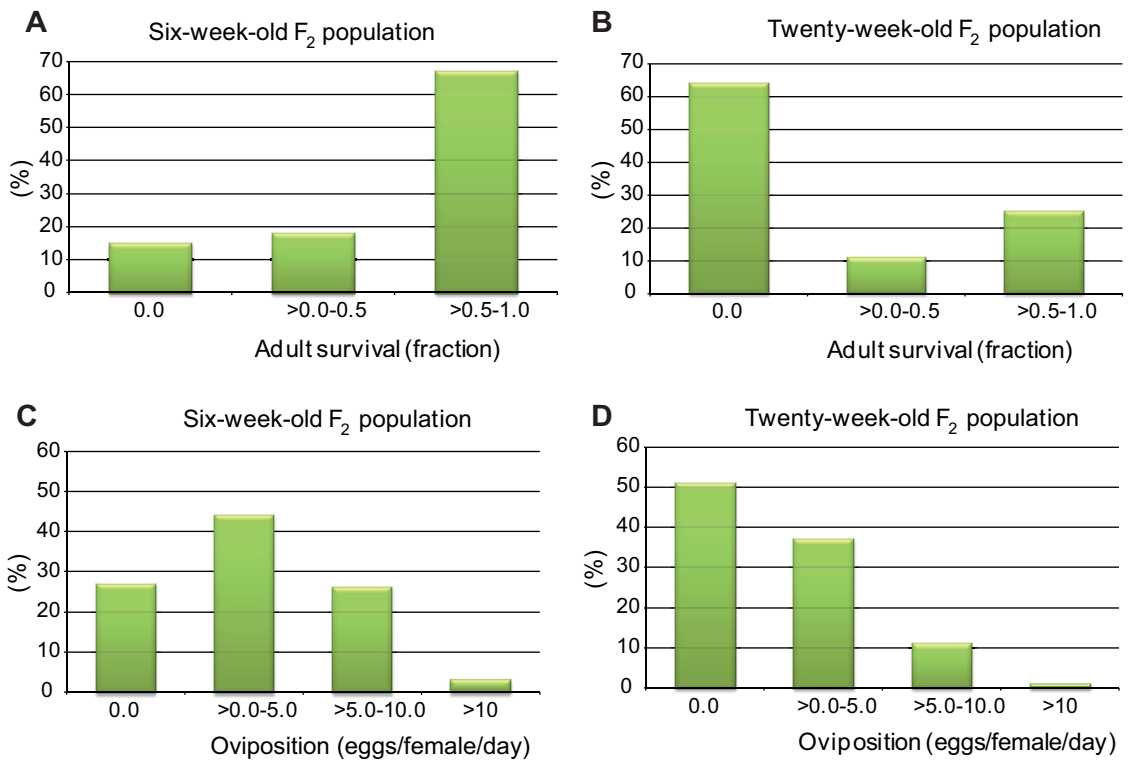

Figure 1. Adult survival and oviposition rate on young and old plants of an F_2 population
The population consisted of 131 plants derived from a cross between *Solanum pennellii* LA3791 and an elite cultivar. Phenotype classes are shown on the x-axis, and the y-axis represents the percentage of F_2 plants in each of the classes. Figure 1**A, B** show the percentage of F_2 plants belonging to each of the classes for AS on younger (6-week-old) and older (20-week-old) plants, respectively. Figure 1**C, D** show the percentages belonging to each of the classes for OR of *Bemisia tabaci* on younger and older plants, respectively.

QTLs for resistance to *B. tabaci* on young and old F_2 tomato plants

For construction of the linkage map 208 markers were used, which enabled the identification of chromosomal regions associated with the whitefly-resistance traits. Quantitative trait segregation for *B. tabaci* AS on 6-week-old plants showed QTLs on Chromosomes IV, VI, X, and XI (Figure 2; Table 1). On 20-week-old plants we identified QTLs at the same locus on Chromosome XI and one just below threshold level (LOD = 3) on Chromosome VI, but the QTLs on Chromosome IV and X were not confirmed (Figure 2). The explained variances for the individual QTLs for AS ranged between 9.6% and 16.4% (Table 1).

Quantitative trait segregation for OR on 6-week-old plants showed QTLs on Chromosome IV, VI, and X (Figure 2; Table 1). On 20-week-old plants we found only the QTL on Chromosome IV back and in addition identified one QTL at Chromosome XI (Figure 2). The QTL on Chromosome XI was visible in the 6-week-old plants, but with a LOD value just below the threshold (LOD = 3). The explained variances of the individual QTLs for OR ranged from 10.0% to 13.9% (Table 1).

The QTLs for OR in 6-week-old plants co-localized with the QTLs for AS on all loci with the exception of the QTL on Chromosome XI where the LOD score for OR was 2.6, which is just below the threshold. The QTLs on Chromosome VI for OR on 6-week-old plants and AS on 20-week-old plants co-localize within the 2-LOD interval, but not within the 1-LOD interval.

QTLs for metabolites associated to whitefly resistance/susceptibility

Chemical profiles of all individuals from the F_2 population were obtained by measuring volatile and semi-volatile compounds in total leaf extracts from 6-week-old plants. A total of 146 metabolites were recorded through GC-MS by an untargeted approach. Quantitative differences in relative abundance between the genotypes were observed. To identify metabolites that were associated with resistance, we compared the relative amount of each metabolite in the ten most resistant and susceptible plants. The abundance of a large number of metabolites was significantly different between pools of resistant and susceptible plants (Table 2) and the majority (>80%) could be mapped (Figure 2; Table 3). Chromosomes IV, X, and XI showed hotspot areas for *B. tabaci* resistance-related compounds with 28, 16, and 25 metabolite QTLs, respectively. Other *B. tabaci* resistance-related metabolite QTLs were detected on almost all chromosomes, except on Chromosomes IX and XII. There were no hotspot areas for *B. tabaci* susceptibility-correlated compounds. The explained variances for the metabolite QTLs varied between 6.8% and 28.1 % (Table 3).

All QTLs positively contributing to whitefly resistance and higher metabolite concentrations had the at least one *Sp* allele.

Evaluation of F_2BC_1 populations

Backcrosses of two resistant plants (numbers 12 and 44) with *Ec* were made to confirm the whitefly-resistance QTLs that were detected in the F_2 population. These F_2 plants showed no AS and (almost) no OR on 6- and 20-week-old plants. The genetic makeup of the plants in the major QTL regions is shown in Figure 3. Combined these two plants have three out of four phenotypic QTLs that were identified in the F_2 population in a heterozygous state, the only exception is on Chromosome VI that was either homozygous for the *S. pennellii* locus in plant 44 or homozygous for the *S. lycopersicum* locus in plant 12.

The size of the F_2BC_1 backcross populations obtained were 154 plants for the population derived from plant 12 ($F_2BC_1(12)$), and 115 plants for the population from plant 44 ($F_2BC_1(44)$). The populations $F_2BC_1(12)$ and $F_2BC_1(44)$ both showed quantitative differences with respect to the *B. tabaci* life-history parameters AS and OR (Figure 4). Parent *S. pennellii* had an AS of zero. None of the plants in population $F_2BC_1(12)$ showed such a high level of whitefly mortality (Figure 4A). However, a clear continuous gradient was observed for OR (Figure 4B). In population $F_2BC_1(44)$, a clear quantitative gradient was observed for AS with nine plants showing an AS of zero (Figure 4C). In this population, 16 plants had an OR = 0. On eight out of the nine plants with no AS there was also no OR (Figure 4D).

Whitefly-resistance QTLs in the F_2BC_1 populations

Single nucleotide polymorphism (SNP) markers were used to construct genetic maps for both F_2BC_1 populations. Based on the physical positions of the SNPs (custom made and SolCap array), it was possible to compare the F_2 and F_2BC_1 maps (Figure 2). A QTL was identified for AS in population $F_2BC_1(12)$ and $F_2BC_1(44)$ on Chromosome I (Figure 2; Table 4). The QTLs for *B. tabaci* AS and OR co-localized in population $F_2BC_1(44)$ on Chromosomes III and IV. In addition, a QTL for OR in population $F_2BC_1(44)$ was mapped on Chromosome VI. Table 4 lists the resistance traits measured, an overview of the QTLs identified per trait, and the percentage of explained variances.

DISCUSSION

Minor effect QTLs determine *B. tabaci* resistance in *S. pennellii* LA3791

Several QTLs that contribute to a reduced AS and OR of *B. tabaci* were identified in an F_2 population of a cross between *S. pennellii* LA3791 and an elite tomato cultivar. These QTLs were mapped to Chromosomes IV, VI, X, and XI.

Without exception, all identified whitefly-resistance QTLs were minor effect QTLs with low explained variances (Table 1). Other QTL studies concerning tomato-whitefly resistance traits on *S. habrochaites* also exclusively showed minor effect QTLs (Maliepaard et al. 1995; Momotaz et al. 2010). Leckie et al. (2012) showed that previously identified QTLs affecting acyl sugar concentration on Chromosomes IV and X also affected whitefly performance. The QTLs that we found on these chromosomes are at similar, if not identical positions, suggesting that they might be the same as the ones identified by Leckie et al. (2012). As these QTLs were found in two studies, using populations based on different parental accessions, it indicates that the QTLs are robust and possibly conserved within *S. pennellii*. The fact that only minor effect QTLs were observed could point to a polygenic inheritance of the resistance, for example, the presence of multiple mechanisms affecting whitefly resistance that individually only have small effects. A bottleneck in high-throughput phenotyping of insect life-history parameters is the difficulty to obtain accurate data

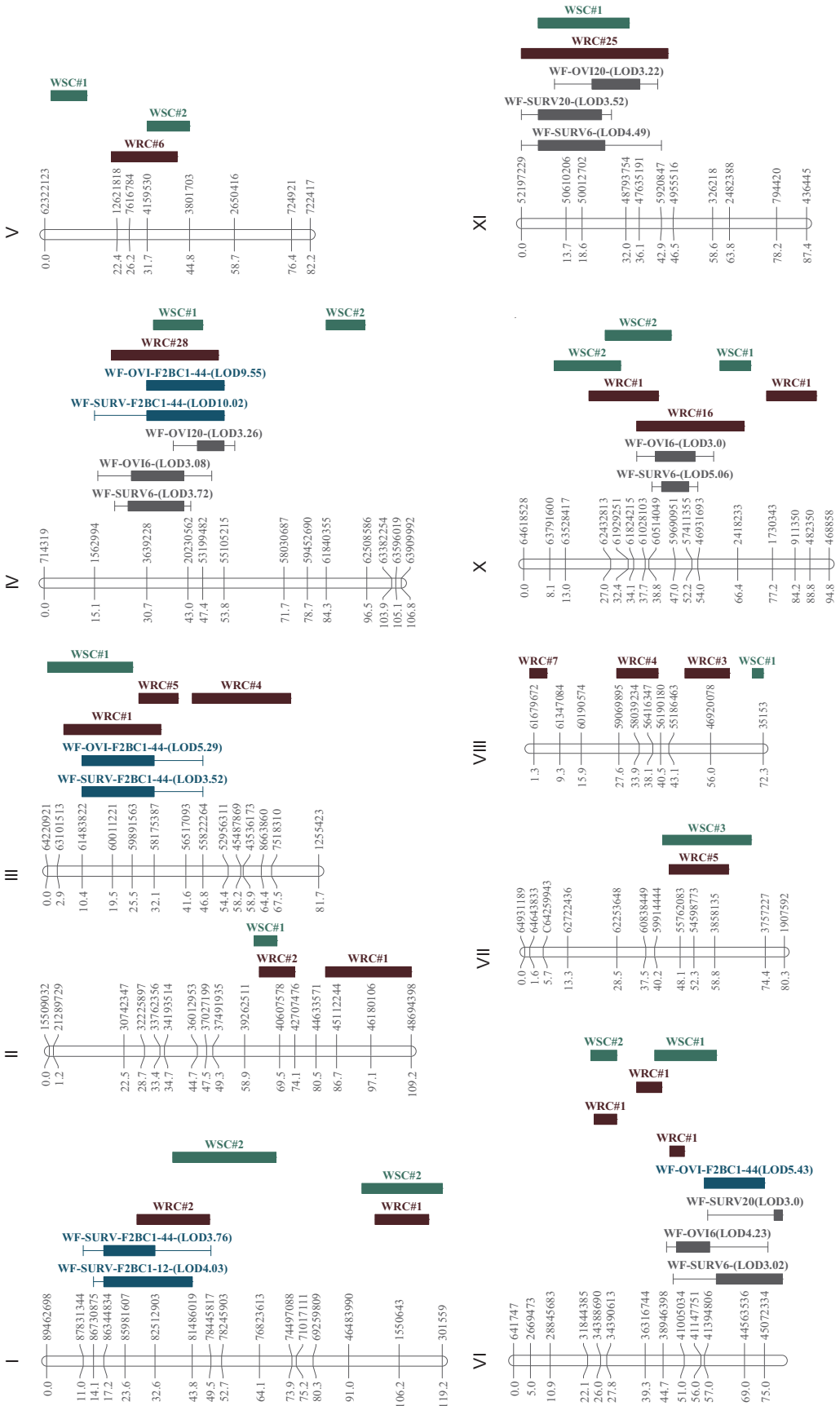

Figure 2. Continued.

Table 1. Quantitative trait loci (QTLs) for *Bemisia tabaci* resistance parameters in 6- and 20-week-old plants

Trait	Trait description	Chromosome	Explained variance (%)
QTL AS_6	AS on 6-week-old plants	IV, VI, X, and XI	12.3, 10.1, 16.4, and 14.7
QTL OR_6	OR on 6-week-old plants	IV, VI, and X	10.3, 13.9, and 10.0
QTL AS_20	AS on 20-week-old plants	VI[a] and XI	9.6 and 12.4
QTL OR_20	OR on 20-week-old plants	IV and XI	10.4 and 10.3

QTLs related to *B. tabaci* AS and OR were identified in an F_2 population of a cross between *Solanum lycopersicum* x *S. pennellii* LA3791 when the plants were 6- or 20 weeks old. Chromosome numbers (column 3) and corresponding percentages of explained variances (column 4) are given in consecutive order. Explained variances show the variance explained by the QTL for the indicated trait. [a] Putative QTL just below threshold level (LOD 2.9).

Table 2. Overview of number of metabolites detected in the gas chromatography-mass spectrometry (GC-MS) analysis and selected by two statistical methods: Orthogonal Partial Least Square-Discriminant Analysis and Student's *t*-test + False Discovery Rate Analyses

Trait	Statistical method	No. components
Number of resistance QTL-related components	OPLS-DA	24
Number of resistance QTL-related components	Student's *t*-Test +FDR	56
Number of susceptibility QTL-related components	OPLS-DA	14
Number of susceptibility QTL-related components	Student's *t*-Test + FDR	13
Resistance QTL-related components in common	OPLS-DA+ Student's *t*-test + FDR	22
Susceptibility QTL-related components in common	OPLS-DA+ Student's *t*-test + FDR	9

Metabolites were profiled in 6-week-old F_2 plant of a cross between *Solanum lycopersicum* x *S. pennellii* LA3791. Bulked Segregant Analyses and multivariate statistical analyses were performed to select metabolites that were discriminatory for resistance or susceptibility against whitefly *Bemisia tabaci*.

from a single plant. This drawback in phenotyping may influence the identification of QTLs and would explain why not 100% variance of the traits was covered.

We observed that some of the QTLs for AS and OR co-localized. This could be due to the same mechanism(s) conferring resistance to both whitefly performance and reproduction. Alternatively, it may be the result of interdependence between survival and oviposition. Strong correlations between AS and OR were observed in other studies as well using other sources of resistance (Firdaus et al. 2012; Lucatti et al. 2013).

QTLs for *B. tabaci* life-history parameters in young and old plants

Overall, 20-week-old plants were more resistant to *B. tabaci* than 6-week-old plants. This increase in resistance was independent of the leaf evaluated as we evaluated the third internode leaf at both plant ages. Similar plant age-dependent increase of resistance to whiteflies was found in other host plants, such as *S. habrochaites* (Bas et al. 1992), *S. lycopersicum* carrying the *Mi*-1.2 gene (Nombela et al. 2003), lettuce and cotton (Byrne and Draeger 1989), and *Brassica oleracea* (Broekgaarden et al. 2012).

Interestingly, the resistance QTLs were not the same in plants of different ages. Some of the QTLs detected in 6-week-old plants could not be detected in 20-week-old plants, which suggests that developmental changes play a role in the expression of the resistance and that different mechanisms may be active at different times. On the other hand, some QTLs were detected at both plant ages, suggesting that the resistance in young and old plants is at least partly based on the same mechanism(s). Interestingly, the number of QTLs detected in the old plants was lower than in the young plants even though old plants were more resistant to whiteflies than young plants and these QTLs had similar explained variances.

QTLs for *B. tabaci* resistance co-localize with resistance-related metabolite QTLs

Metabolic fingerprinting by GC-MS performed on the entire F_2 population revealed a large number of metabolites that

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Figure 2. Quantitative trait loci (QTL) analysis of whitefly resistance and metabolites in *Solanum pennellii*

Whitefly resistance QTLs (dark grey bars) for *Bemisia tabaci* AS and OR on 6- and 20-week-old plants and metabolite QTLs that are associated with resistance (red) or susceptibility (green) in the F_2 population. Whitefly resistance QTLs identified in the backcross populations are shown in blue. All QTLs are shown with 1- and 2-LOD intervals (solid bar resp. line) and are positioned at the right side of the corresponding chromosome. Metabolite QTL coding starts with either WRC (Whitefly Resistance Component) or WSC (Whitefly Susceptibility Component), numbers (# + n) indicate the total number of m-QTLs found. Whitefly resistance QTL coding consists of WF (whitefly), SURV (survival), OVI (oviposition), 6 (6-week-old plants), and 20 (20-week-old plants). The backcross populations are coded $F_2BC_1(12)$ or $F_2BC_1(44)$. Chromosomes IX and XII are not included because no QTLs associated with resistance were identified on these chromosomes.

Table 3. List of the metabolic quantitative trait loci (QTLs) associated with resistance/susceptibility

Chromosome	Metabolite (ID)	Name	Phenotype	Highest-LOD marker	LOD value	Explained variance (%)
	1225	Methyl salicylate	S	P11M54_M413.9	5.60	18.1
	2705	Unknown	R	P11M54_M273.7	3.21	9.8
	3395	Unknown	R	P14M49_M298.9	6.42	14.0
I	3606	Dodecanoic acid	R	P14M50_M237.2	3.61	12.1
	5433	Tetramethyl-2-hexadecene	S	Solcap_snp_sl_15058	4.55	15.0
	7963	Unknown	S	P14M50_M298.8	4.56	15.0
	8626	hydrocarbon	S	Solcap_snp_sl_2234	3.85	11.6
	259	3,7,7-trimethylcyclohepta-1,3,5-triene	S	P14M60_M85.8	3.05	8.4
II	2393	Undecanoic acid	R	Solcap_snp_sl_29891	7.50	23.5
	4486	Unknown	R	CL016576-0377	3.04	9.9
	8563	Unknown	R	Solcap_snp_sl_29891	4.42	13.2
	109	Hexanoic acid	R	P14M49_M177.1	4.71	15.5
	1973	Unknown	R	P14M49_M177.1	3.02	10.2
	3266	Unknown	S	Solcap_snp_sl_36544	3.00	10.2
	3483	Unknown	R	Solcap_snp_sl_62270	3.16	9.7
	3516	Unknown	R	Solcap_snp_sl_62270	3.00	9.2
III	3595	Unknown	R	Solcap_snp_sl_62270	3.26	11.0
	3664	Unknown	R	P14M49_M177.1	4.17	12.5
	3719	Unknown	R	P14M50_M265.5	4.60	15.1
	3767	Unknown	R	P14M50_M265.5	4.78	15.7
	4391	Unknown	R	P14M49_M177.1	3.56	11.9
	4421	Unknown	R	P14M50_M265.5	3.00	6.8
	109	Hexanoic acid	R	Solcap_snp_sl_53136	3.43	11.5
	498	Butanoic acid	R	P14M60_M380.4	3.61	12.1
	947	Unknown	R	P11M50_M118.5	3.41	11.5
	1102	Levoglucosone	R	Solcap_snp_sl_51411	5.72	12.4
	1549	Unknown	R	P14M60_M533.2	3.87	12.9
	1576	Unknown	R	P14M60_M533.2	3.78	12.6
	1973	Unknown	R	Solcap_snp_sl_53136	3.20	10.8
	3114	Unknown	R	Solcap_snp_sl_53136	4.08	13.6
	3449	Unknown	R	P14M49_M189.3	3.70	12.4
	3483	Unknown	R	P14M49_M189.3	3.22	9.9
	3516	Unknown	R	P14M49_M51.5	3.62	12.1
	3595	Unknown	R	P14M49_M51.5	3.26	11.0
	3719	Unknown	R	P14M60_M380.4	4.51	14.9
	3767	Unknown	R	P14M60_M380.4	4.96	16.2
	3878	Unknown	R	P14M60_M380.4	4.65	14.8
IV	3989	Unknown	R	Solcap_snp_sl_53136	3.47	11.6
	4070	Unknown	R	Solcap_snp_sl_53136	3.86	12.9
	4160	Unknown	R	P14M49_M51.5	3.46	11.6
	4391	Unknown	R	P11M50_M118.5	4.52	14.9
	4421	Unknown	R	P14M49_M189.3	3.45	11.6
	4458	Unknown	R	P14M49_M51.5	3.26	11.0
	4531	Unknown	R	P14M60_M380.4	4.15	13.8
	4588	Unknown	R	Solcap_snp_sl_51411	3.38	8.7
	4605	Unknown	R	P14M60_M380.4	3.58	12.0
	4661	Unknown	R	Solcap_snp_sl_51411	3.62	10.2
	4707	Unknown	R	P14M60_M380.4	3.33	11.2
	5223	Unknown	R	P14M60_M380.4	5.03	16.4
	7704	Unknown	R	P14M49_M189.3	3.57	12.0
	7963	Unknown	S	P14M60_M380.4	3.28	11.0
	9234	hydrocarbon	S	P14M50_M195.7	3.11	10.5
	10389	Unknown	S	P14M50_M195.7	3.10	10.5

(Continued)

Table 3. (Continued)

Chromosome	Metabolite (ID)	Name	Phenotype	Highest-LOD marker	LOD value	Explained variance (%)
	3989	Unknown	R	P11M54_M721.1	4.02	13.4
	4531	Unknown	R	P11M54_M721.1	3.83	12.8
	4588	Unknown	R	P11M54_M721.1	3.10	7.4
	4605	Unknown	R	P11M54_M721.1	3.29	11.1
V	5003	Unknown	R	P11M54_M721.1	3.06	10.3
	5223	Unknown	R	P11M50_M169.3	3.16	10.7
	5433	Tetramethyl-2-hexadecene	S	Solcap_snp_sl_23970	5.26	17.1
	5711	Neophytadiene isomer III	S	Solcap_snp_sl_23970	6.44	18.6
	5711	Neophytadiene isomer III	S	P11M54_M127.5	3.73	10.3
	1102	Levoglucosone	R	Solcap_snp_sl_19915	3.86	8.1
	1576	Unknown	R	P11M54_M277.6	3.08	10.4
VI	2552	β-Caryophyllene	S	Solcap_snp_sl_55902	6.45	20.6
	2552	β-Caryophyllene	S	P14M50_M481.8	3.89	13.0
	2807	Guaia-6,9-diene	R	Solcap_snp_sl_55902	4.49	14.8
	2987	α-Humulene	S	Solcap_snp_sl_55902	8.43	20.9
	1102	Levoglucosone	R	Solcap_snp_sl_26437	3.31	6.9
	1283	Unknown	R	Solcap_snp_sl_26437	6.82	17.7
	1920	Decanoic acid	R	P14M49_M159.7	4.88	16.0
VII	3266	Bicyclogermacrene	S	P11M54_M244.9	5.50	17.8
	4270	Tridecanoic acid	R	Solcap_snp_sl_26437	4.50	14.8
	4317	Unknown	R	Solcap_snp_sl_52568	3.39	11.4
	5338	Neophytadiene isomer I	S	P14M49_M159.7	3.73	11.5
	5711	Neophytadiene isomer III	S	P11M54_M244.9	3.10	8.4
	1549	Unknown	R	P11M54_M437.8	8.92	27.3
	1549	Unknown	R	P14M49_M170.6	5.35	17.4
	1576	Unknown	R	P11M54_M437.8	8.86	27.1
	1576	Unknown	R	P14M49_M170.6	5.41	17.6
	1840	Unknown	R	P11M50_M222.4	3.82	12.8
	2705	Unknown	R	P14M60_M442.3	3.36	10.2
	3416	Unknown	R	P14M49_M170.6	3.84	12.8
	3516	Unknown	R	Solcap_snp_sl_10247	3.03	9.9
VIII	4107	Unknown	R	Solcap_snp_sl_10247	4.15	12.6
	4160	Unknown	R	Solcap_snp_sl_10247	3.61	12.1
	4249	Unknown	R	Solcap_snp_sl_10247	3.91	13.0
	4391	Unknown	R	P14M49_M170.6	3.56	11.9
	4531	Unknown	R	Solcap_snp_sl_10247	3.36	11.3
	5003	Unknown	R	Solcap_snp_sl_10247	3.81	12.7
	5047	Unknown	R	Solcap_snp_sl_10247	3.06	9.9
	259	3,7,7-trimethylcyclohepta-1,3,5-triene	S	P11M54_M221.8	5.12	14.9
	1549	Unknown	R	Solcap_snp_sl_3294	3.39	11.4
	1576	Unknown	R	Solcap_snp_sl_3294	3.85	12.8
	2552	β-Caryophyllene	S	P11M54_M684.9	4.21	14.0
	2807	Guaia-6,9-diene	R	P11M54_M684.9	4.33	14.3
	2849	(E)-β-Farnesene	R	Solcap_snp_sl_61131	3.19	10.1
	2987	α-Humulene	S	Solcap_snp_sl_33166	8.22	20.3
	3449	Unknown	R	P11M54_M199.0	3.13	10.6
	3483	Unknown	R	P11M54_M199.0	2.73	9.3
	3516	Unknown	R	P14M49_M166.2	3.04	10.1
	3595	Unknown	R	Solcap_snp_sl_16511	3.06	8.8
X	4160	Unknown	R	P11M54_M199.0	3.65	12.2
	4421	Unknown	R	P11M54_M199.0	3.02	7.0
	4531	Unknown	R	Solcap_snp_sl_16511	3.42	11.5
	4588	Unknown	R	Solcap_snp_sl_16511	3.33	9.6
	4605	Unknown	R	Solcap_snp_sl_16511	3.03	10.2

(Continued)

Table 3. (Continued)

Chromosome	Metabolite (ID)	Name	Phenotype	Highest-LOD marker	LOD value	Explained variance (%)
	4661	Unknown	R	P11M54_M199.0	3.13	8.9
	4707	Unknown	R	P14M49_M166.2	3.11	10.5
	4820	Unknown	R	P11M50_M587.3	3.01	10.2
	5047	Unknown	R	P14M49_M166.2	3.04	9.4
	7963	Unknown	S	Solcap_snp_sl_46475	4.15	13.8
	7963	Unknown	S	P11M54_M221.8	3.28	11.0
	8253	Branched hydrocarbon	R	P11M54_M684.9	3.95	13.2
	498	Butanoic acid	R	P11M54_M90.5	5.96	19.2
	947	Unknown	R	Solcap_snp_sl_5922	4.27	14.1
	1102	Levoglucosone	R	Solcap_snp_sl_5922	6.06	13.2
	1920	Decanoic acid	R	Solcap_snp_sl_5922	6.20	19.9
	2161	Unknown	R	P11M54_M90.5	3.43	10.6
	2393	Undecanoic acid	R	Solcap_snp_sl_56142	4.96	16.2
	3114	Unknown	R	Solcap_snp_sl_5922	4.09	13.6
	3449	Unknown	R	Solcap_snp_sl_5922	4.80	15.7
	3483	Unknown	R	P11M54_M90.5	6.30	18.3
	3516	Unknown	R	Solcap_snp_sl_5922	5.12	16.7
	3595	Unknown	R	Solcap_snp_sl_5922	9.24	28.1
	3664	Unknown	R	P11M54_M160.9	4.05	12.1
XI	3989	Unknown	R	Solcap_snp_sl_5922	3.56	11.9
	4070	Unknown	R	P11M54_M90.5	4.13	13.7
	4421	Unknown	R	P11M54_M90.5	5.02	16.4
	4458	Unknown	R	P11M54_M90.5	4.34	14.3
	4531	Unknown	R	Solcap_snp_sl_56142	5.30	17.2
	4588	Unknown	R	P11M54_M90.5	4.52	13.4
	4605	Unknown	R	Solcap_snp_sl_56142	3.96	13.2
	4661	Unknown	R	P11M54_M90.5	4.86	15.9
	4707	Unknown	R	P11M54_M90.5	4.50	14.8
	4820	Unknown	R	Solcap_snp_sl_5922	3.97	13.2
	5003	Unknown	R	P11M54_M419.7	3.21	10.8
	5433	Tetramethyl-2-hexadecene	S	Solcap_snp_sl_5922	3.47	11.7
	5612	Unknown	R	P11M54_M160.9	4.97	16.2
	7704	Unknown	R	P11M54_M90.5	4.39	14.5
	2416	Unknown	R	n.a.	n.a.	n.a.
	2577	Unknown	R	n.a.	n.a.	n.a.
	2621	Unknown	R	n.a.	n.a.	n.a.
	4195	Unknown	R	n.a.	n.a.	n.a.
	4762	Unknown	R	n.a.	n.a.	n.a.
	5030	Unknown	R	n.a.	n.a.	n.a.
	5517	Neophytadiene isomer II	S	n.a.	n.a.	n.a.
No QTLs	6819	Unknown	S	n.a.	n.a.	n.a.
identified	6819	Unknown	S	n.a.	n.a.	n.a.
	7162	(Z,Z,Z)-9,12,15-Octadecatrienoic acid (Linolenic acid)	S	n.a.	n.a.	n.a.
	7834	Unknown	R	n.a.	n.a.	n.a.
	7844	Unknown	S	n.a.	n.a.	n.a.
	7844	Unknown	S	n.a.	n.a.	n.a.
	7875	Unknown	S	n.a.	n.a.	n.a.
	8588	Unknown	R	n.a.	n.a.	n.a.

Experiments were performed in a 6-week-old F_2 population of *Solanum lycopersicum* x *S. pennellii* LA3791. Student's *t*-test combined with FDR analyses and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) were performed for classification of metabolites as *Bemisia tabaci* resistance QTL components, *B. tabaci* susceptibility QTL components, or components which were not related to *B. tabaci* resistance or susceptibility (not shown). Chromosome number, metabolite, putative identification, resistant/susceptibility-related component, highest corresponding marker, QTL LOD-value, and corresponding percentages of explained variance are given in consecutive order.

Marker	Chromosome nr	Physical map position	SNP genotyping of F_2 nr 12	SNP genotyping of F_2 nr 44
solcap_snp_sl_63976	IV	1,562,994	BB	AB
solcap_snp_sl_21384	IV	2,983,549	BB	AB
solcap_snp_sl_51437	IV	15,097,896	BB	AB
solcap_snp_sl_51334	IV	25,812,609	BB	AB
solcap_snp_sl_51325	IV	29,000,198	BB	AB
solcap_snp_sl_45495	IV	42,190,928	BB	AB
solcap_snp_sl_45378	IV	49,990,085	BB	AB
solcap_snp_sl_53156	IV	53,785,617	AB	AB
solcap_snp_sl_3107	IV	55,105,215	AB	AB
solcap_snp_sl_19915	VI	41,005,034	AA	BB
solcap_snp_sl_57594	VI	41,147,751	AA	BB
solcap_snp_sl_57593	VI	41,147,789	AA	BB
SL10882_924	VI	41,159,856	AA	BB
solcap_snp_sl_24437	VI	41,383,406	AA	BB
solcap_snp_sl_24436	VI	41,394,806	AA	BB
U146140_369c	VI	45,072,334	AA	BB
solcap_snp_sl_8000	X	46,931,693	AB	BB
solcap_snp_sl_5198	X	49,856,593	AB	BB
solcap_snp_sl_18726	X	52,809,001	AB	BB
solcap_snp_sl_16517	X	57,224,189	AB	BB
solcap_snp_sl_24679	X	60,235,795	AB	BB
solcap_snp_sl_59236	X	61,124,385	AB	BB
solcap_snp_sl_24977	XI	6,623,586	BB	BB
solcap_snp_sl_12406	XI	11,933,653	BB	AB
solcap_snp_sl_26262	XI	13,194,095	BB	AB
solcap_snp_sl_59670	XI	19,636,101	BB	AB
solcap_snp_sl_7445	XI	21,374,623	BB	AB
solcap_snp_sl_45043	XI	27,841,963	BB	AB
solcap_snp_sl_45039	XI	30,617,163	BB	AB
solcap_snp_sl_2996	XI	37,689,381	BB	AB
solcap_snp_sl_2989	XI	40,361,385	BB	AB
solcap_snp_sl_6002	XI	49,081,167	BB	AB
solcap_snp_sl_56142	XI	51,359,586	AB	AB

Figure 3. Genotype of F_2 plants numbers 12 and 44 in the quantitative trait loci (QTL) regions for whitefly resistance
Solcap markers, chromosome numbers, physical positions according to the tomato genome sequence (TGC 2012). Heterozygous (AB; green), homozygous *Solanum pennellii* LA3791 (BB; blue), and homozygous *S. lycopersicum* cultivar (AA; yellow).

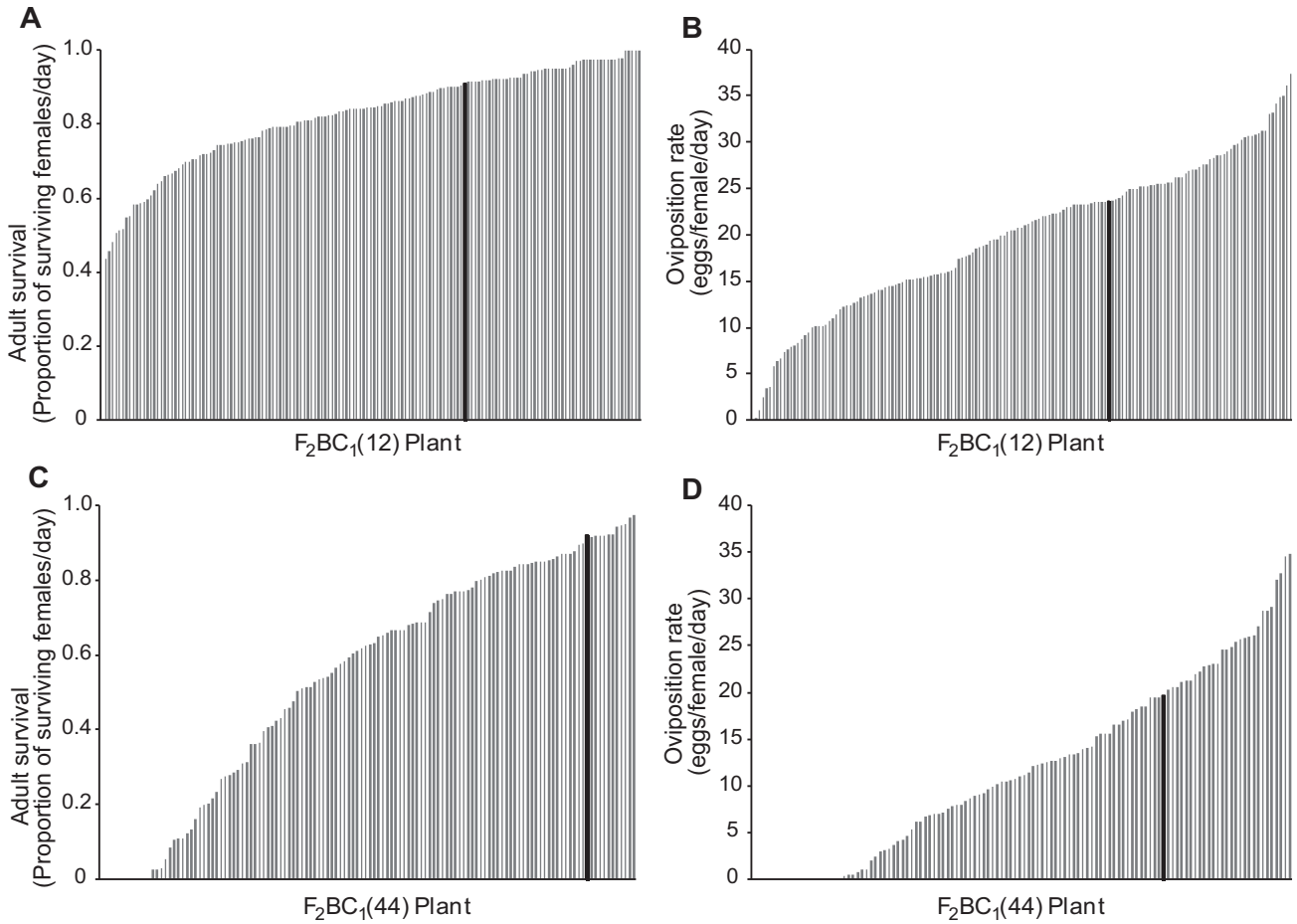

Figure 4. Distribution pattern of adult survival and oviposition rate in the F_2BC populations.
(A) AS and **(B)** OR of *Bemisia tabaci* in population $F_2BC_1(12)$. **(C)** AS and **(D)** OR of *Bemisia tabaci* in population F_2BC_1 (44). The bars represent the average whitefly AS and OR of two replicas ordered from low to high. Black bars are the average of six replicates of the *Solanum lycopersicum* parent. The first bar in each graph represent the average value for *S. pennellii*.

potentially contribute to the resistance/susceptibility of *S. pennellii* to *B. tabaci*. By combining the results of the two statistical methods used, 58 metabolites were associated with the resistant pool and 18 metabolites with the susceptible pool. Most of these metabolites could not be annotated, indicating that a large part of the tomato metabolome involved in resistance towards whitefly is still unknown.

The majority of the metabolites associated to whitefly resistance/susceptibility could be mapped (Figure 2). Hotspots with more than 10 metabolite QTLs associated with resistance were identified on Chromosomes IV, X, and XI. Similar hotspots were found in *Arabidopsis thaliana* (Keurentjes et al. 2006) and *Capsicum sp.* (Wahyuni et al. 2014). Such hotspots may be caused by regulatory genes that control the production of several metabolites or it may be related to the production of glandular trichomes in which the metabolites are synthesized. The positions of these metabolite QTL hotspots were identical to the positions of the identified

Table 4. List of quantitative trait loci (QTLs) related to a *Bemisia tabaci* resistant phenotype. Experiments were performed on F_2BC_1 populations of *Solanum lycopersicum* x *S. pennellii* LA3791 on 6-week-old plants

Trait	Trait description	Chromosome	Explained variance (%)
WFSURV- $F_2BC_1(12)$	QTL for *B. tabaci* survival in population $F_2BC_1(12)$	I	12.0
WFOVI- $F_2BC_1(12)$	QTL for *B. tabaci* oviposition in population $F_2BC_1(12)$	No QTLs identified	n.a.
WFSURV- $F_2BC_1(44)$	QTL for *B. tabaci* survival in population $F_2BC_1(44)$	I, III, and IV	13.7, 12.8, and 32.4
WFOVI- $F_2BC_1(44)$	QTL for *B. tabaci* oviposition in population $F_2BC_1(44)$	III, IV, and VI	12.2, 23.6, and 12.5

Phenotype QTLs were identified in 6-week-old F_2BC_1 populations of a cross between *S. lycopersicum* x *S. pennellii* LA3791. Chromosome numbers (column 3) and corresponding percentages of explained variances (column 4) are given in consecutive order. Explained variances show the variance explained by the QTL for the indicated trait.

whitefly-resistance QTLs on these chromosomes, which suggests that resistance is for the larger part biochemically based, a hypothesis proposed earlier by Liedl et al. (1995).

Multiple resistance-associated metabolite QTLs were identified on Chromosomes I, II, III, V, VI, VII, and VIII, but no co-localization with whitefly-resistance QTLs was found (Figure 2). The explanation for the low explained variances of both whitefly-resistance and metabolite QTLs may in the diversity in biochemical profiles observed among resistant genotypes. This metabolomic diversity may indicate that various, independent resistance mechanisms (metabolites) are present in the resistant genotypes. On Chromosome VI several overlapping resistance QTLs were found but no metabolite QTLs mapped to this region. In *S. pennellii* LA716 this locus was found to be associated with total acyl sugar levels (Leckie et al. 2012). The QTLs related to whitefly resistance identified in our study on Chromosomes IV, X, and XI (Figure 2) co-localized with QTLs found for acyl sugar production and accumulation in *S. pennellii* LA716-derived populations, which may point at causality (Mutschler et al., 1996; Lawson et al. 1997; Blauth et al. 1998; Leckie et al. 2012, 2013). Liedl et al. (1995) tested purified acyl sugars from *S. pennellii* LA716 on susceptible tomato leaves and detected a negative correlation between the presence of acyl sugars and the settling and OR of *B. tabaci* adults. In our study we demonstrate co-localization of whitefly-resistance and metabolite QTLs, among which there are precursors of acyl sugars (Table 3). We also found metabolite QTLs belonging to sesquiterpenes including β-caryophyllene, α-humulene, and bicyclogermacrene which co-localized with whitefly susceptibility on chromosomes VI, VII and X. Interestingly, these compounds are emitted by tomato plants when being damaged, for example, by insects or pathogens (e.g. Bleeker et al. 2009; Farag and Paré 2002; Jansen et al. 2009). Two other sesquiterpenes, (E)-β-farnesene and guaia-6,9-diene, co-localized with resistance against whitefly. Terpenes have been reported to be particularly present in the trichomes of tomato plants (Lange and Turner 2013). Our data indicate that the genome regions associated with the production of at least part of the *B. tabaci* resistance-related metabolites are present in different *S. pennellii* accessions.

Intra- and interspecies QTLs for *B. tabaci* resistance traits overlap

Solanum habrochaites is the closest relative of *S. pennellii* (Rodriguez et al. 2009) and it is possible that resistance mechanisms between the two species are (partly) conserved. Few QTL studies have been performed on different accessions of *S. habrochaites* in which whitefly resistance was mapped (Maliepaard et al. 1995). In the study by Maliepaard et al. (1995) QTLs for the OR of *Trialeurodes vaporariorum* were identified on Chromosomes I and XII (*Tv-1* and *Tv-2*, respectively). The QTL for OR in *S. habrochaites* on Chromosome I maps at the same position as the QTLs for *B. tabaci* AS in our F_2BC_1(12) and F_2BC_1(44) populations. Two *B. tabaci* resistance-related fatty acid constituents also mapped in this region (Figure 2). Recently, using the same *S. habrochaites* population, a QTL on Chromosome 5 (*OR-5*) was identified that only reduced the OR of *B. tabaci* (Lucatti et al. 2014). That QTL co-localized with a minor hotspot metabolite QTL associated to resistance to *B. tabaci* in our F_2 population.

On *S. habrochaites* LA1777 four QTLs (on Chromosomes IX, X, and two on XI) were identified that were associated with resistance to *B. tabaci* (Momotaz et al. 2010). However, none of these QTLs correspond to the regions in which we found whitefly-resistance QTLs. This may be explained by the difference in resistance mechanism between accessions of *S. habrochaites*. Some accessions (i.e., LA1777, PI-127826) accumulate sesquiterpenes and others accumulate methyl-ketones (i.e., CGN1.1561, PI-134417, PI-134418). On the *S. habrochaites* accessions that accumulate sesquiterpenes, 7-epizingiberene and r-curcumene were associated with resistance to *B. tabaci* (Freitas et al. 2002; Bleeker et al. 2009; Bleeker et al. 2011). We did not detect these compounds in the *S. pennellii* LA3791 F_2 progeny (Table 3).

Enhancement of QTLs for *B. tabaci* AS and OR in F_2BC_1 populations

The population F_2BC_1(44) showed a larger variation for whitefly resistance related traits than the F_2BC_1(12) population, allowing the detection of four QTLs (Chromosomes I, III, IV, and VI) for AS and OR. In this population eight genotypes showed zero AS and OR. Not all resistance QTLs that were mapped in the F_2 population were found back in the backcross populations, which may be attributed to environmental factors. We observed that the explained variances were higher in F_2BC_1(44) than in the F_2 population for the QTLs found on Chromosome IV (Table 4). The increase in explained variance may be due to a reduction in the linkage drag by backcrossing the F_2 plant with the recurrent parent. The population F_2BC_1(12) showed small quantitative differences for both *B. tabaci* life-history parameters (Figure 3A, C) and only a single minor effect QTL was detected for AS. It may be that resistance in this F_2 parent was incorrect determined.

Insect resistance in general, and *B. tabaci* resistance in particular is a complex trait, and it can be hypothesized that many epistatic interactions take place in a resistant plant. The loss of one or a few genetic loci may result in breakdown of resistance in *S. pennellii* crossings (Eshed and Zamir 1995). Therefore, research to better understand the complex mechanisms of insect resistance in wild tomato material will maintain a necessity and all wild genetic resources should be considered as valuable resources for resistance breeding.

MATERIALS AND METHODS

Plant material and growing conditions

An interspecific cross was made between *Solanum pennellii* accession LA3791 (hereafter referred to as *Sp*) and *S. lycopersicum* elite cultivar To6W_LI0620 (hereafter referred to as *Ec*), which was made available by Bayer CropScience Vegetable Seeds, Nunhem, The Netherlands. One F_1 plant was selfed to produce an F_2 population. One hundred and thirty-one F_2 seeds germinated and were grown for phenotyping and chemoprofiling. Two fully whitefly resistant F_2 plants (plant 12 and 44) were backcrossed with *Ec* to produce two F_2BC_1 populations (F_2BC_1(12) and F_2BC_1(44)). One hundred and fifty-four plants were grown of F_2BC_1(12) and 115 plants of F_2BC_1(44).

Seeds were sown in potting trays with soil as substrate (Lentse Potgrond) and transplanted into pots (Ø 20 cm) when the seedling were 1 week old. Plants were grown under controlled conditions in a glasshouse at Wageningen University (22 ± 2 °C, L16:D8 photoperiod, RH about 50%) and watered daily. When the F_2 plants were 10-weeks-old, two cuttings per individual F_2 were made for chemo-profiling. The cuttings were transferred to soil in pots (Ø 20 cm) and grown in an insect- and pathogen-free environment (22 ± 2 °C, L16:D8 photoperiod, RH about 50%) for 6 weeks.

Throughout the experiment (growing, screening, and sampling) no chemical pest or disease control was practiced. One week prior to the beginning of the phenotyping experiments, the greenhouse temperature was optimized for *B. tabaci* (27 ± 2 °C). The temperature was increased gradually over several days to allow plants to acclimatize to the higher temperature.

Insect rearing

A non-viruliferous whitefly rearing (*Bemisia tabaci* Group Mediterranean-Middle East-Asia Minor I) was maintained on the susceptible tomato cultivar Moneymaker (hereafter referred to as cv. MM) at the Laboratory of Entomology, Wageningen UR, The Netherlands. The purity of the colony was regularly checked on a random sample by real-time PCR assay (Jones et al. 2008). For synchronization, cv. Moneymaker leaves with 4th instar nymphs were placed in a gauze insect cage containing a young and clean cv. Moneymaker plant to provide newly emerging adults with fresh leaves for feeding and oviposition.

Whitefly resistance tests

The F_2 and F_2BC_1 plants were tested for *B. tabaci* AS and OR in a no-choice experiment. The F_2BC_1 populations were tested with their recurrent parent *Ec* as reference. Three plants per reference line were used and these plants were randomly positioned between the F_2 and F_2BC_1 plants. For the F_2 population, AS and OR of *B. tabaci* were determined on 6- and 20-week-old plants, whereas for the F_2BC_1 only 6-week-old plants were used.

Adult survival: Twenty unsexed 1–3-d-old *B. tabaci* adults were anaesthetized (N_2:H_2:CO_2 [80:10:10]; Linde Gas Benelux) and put into a fine-meshed clip-on cage (2.5 cm diameter and 1.0 cm high) with a rubber membrane at the leaf interface. The cages were placed on the abaxial side of a third internode leaf. This leaf was used because young leaves are preferred by the whitefly for feeding and oviposition (Liu and Stansly 1995). Each individual F_2 or F_2BC_1 ($n = 1$) plant and each reference plant ($n = 3$) was challenged with two clip-on cages. Five days after inoculation, the number of living and dead whiteflies was recorded. Adult survival was determined according to Bas et al. (1992).

Oviposition rate: Five 6- to 8-d-old *B. tabaci* females were selected under a stereomicroscope and transferred to the abaxial side of the 3th-internode leaf. Each individual F_2 or F_2BC_1 plant ($n = 1$) and each reference plant ($n = 3$) was challenged with two clip-on cages, containing five females each. After 5 days of infestation, the leaves containing the cages were removed and the number of living females and eggs was counted under a stereomicroscope. Oviposition rate was calculated according to Bas et al. (1992).

Chemical profiling of leaf material

Sample preparation

Two cuttings per F_2 genotype plus *Sp* and cv. Moneymaker were distributed over the glasshouse in a randomized block design. The environmental parameters were adjusted to 26 ±2 °C, L16:D8 photoperiod, and RH 60% 1week prior to the collection of leaf material for biochemical profiling. These conditions are similar to the conditions used during the whitefly resistance assay. The third internode leaf of 6-week-old uninfested plants was cut off, carefully packed in aluminum foil, and instantly transferred to liquid nitrogen (−182 °C). Leaf samples were stored at −80 °C until analyses.

GC-MS analysis

To determine the variation in secondary metabolites in the F_2 population, leaf extracts of all individuals plus parental lines were analyzed by gas chromatography-mass spectrometry (GC-MS), essentially as described by Firdaus et al. (2013). Per plant, 300 mg of frozen leaf material was ground in a liquid N_2-cooled basic analytical mill (IKA, Werke Staufen/Germany) and transferred to liquid N_2-cooled 20 mL glass tubes. For component extraction, 2.0 mL of dichloromethane (DCM), including 75 μg/mL heptadecanoic acid methyl ester as internal standard (IS) was added to the frozen leaf powder. The samples were homogenized for 30 s using a vortex and then centrifuged for 10 min at 1,500 rpm. The supernatant was collected into a new 20 mL glass tube. One mL of DCM+IS was added to the residual solid- and water-phase in the initial glass tube, vortexed (30 s), and centrifuged (10 min 1,500 rpm). The DCM-phase was pipetted off and pooled together with the DCM-phase obtained from the first extraction. The pooled DCM-fraction was transferred to a Na_2SO_4-column with glass-wool filter to obtain anhydrous samples. Filtered samples were transferred to 1.5 mL crimp neck insertion vials (Grace Davison Discovery Sciences, USA) and sealed with 11-mm rubber caps (Grace Davison Discovery Sciences, USA). Samples were injected splitless using a 7683 series B injector (Agilent Technologies) into a 7890 A GC (Agilent Technologies) coupled to a 5975 C MSD (Agilent Technologies). Chromatography was performed using a Zb-5MS column (Phenomenex, 30 m, 0.25 mm inner diameter, and 0.25 μm film thickness) with 5 m retention gap. Injection temperature was 250 °C, and column temperature was programmed at 45 °C for 1 min, increased by 10 °C /min to 300 °C, and kept at 300 °C for 7 min. Column flow was set at 1 mL/min, using Helium as carrier gas. The column effluent was ionised by electron impact at 70 eV and mass spectra were obtained from m/z 35–400. Duplicates of each genotype (with the exception of genotype numbers 54 and 86, for which only one sample was available) were injected reverse sequence.

An untargeted metabolomics approach was applied to process the raw GC-MS data as described by Maharijaya et al. (2012). MetAlign software (Lommen 2009) was used to extract and align all mass signals (s/n >3). Absent mass signals were randomized between 0.1 and three times the noise. Mass signals that were present in less than four samples were discarded; signal redundancy per metabolite was removed using clustering and mass spectra were reconstructed using MsClust software (Tikunov et al. 2012). The major ions detected can be found in Table S1.

Reconstructed metabolites were putatively identified by matching the mass spectra to authentic reference standards, or by comparing them to commercial spectral libraries (NIST08 (www.nist.gov), Wiley (www.wiley.com), to custom made spectral libraries (Wageningen Natural compounds spectral library), and by comparison with retention indices calculated using a series of alkanes and fitted using a third-order polynomial function (Strehmel et al. 2008) to those published in the literature.

Metabolites involved in *B. tabaci* resistance and susceptibility

For the selection of the candidate metabolites, that play a role in *B. tabaci* resistance and susceptibility two statistical tests were used; a Student *t*-test between resistant and susceptible bulks followed by FDR analysis, and a multivariate analysis on metabolites between resistant and susceptible bulks. For the Student *t*-test the phenotypic data for whitefly performance of the F_2 population were ranked to select the 10 most resistant and the 10 most susceptible genotypes. The resistant F_2 bulk consisted of 10 plants with zero AS and zero OR on both 6- and 20-week-old plants. The susceptible F_2 bulk consisted of 10 plants with the highest AS and OR on both 6- and 20-week-old plants. Metabolites were considered significantly different between the groups when $q \leq 0.05$. For the multivariate data analysis, the data were $\log_{10}$ transformed and principal component analysis (PCA) was performed to analyze the structure and to detect outliers. Finally, an Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) was used to discriminate between resistant and susceptible genotype classes on the basis of their metabolome spectra. Data analyses were done with Simca P+ version 12.0.1 software for multivariate data analysis (Umetrics, MKS Instruments, Sweden).

Genomic DNA isolation and genotyping

The leaves from 131 F_2, 115 $F_2BC_1(44)$ and 154 $F_2BC_1(12)$ plants were sampled from young leaflets and collected in 1.4 mL polypropylene tubes in 96-well format (Micronics) containing two stainless steel grinding beads (Retsch GmbH & Co KG). Genomic DNA isolation of the F_2 plants was performed according to the protocol described by Doyle and Doyle (1990), adjusted for 96-well plates. Genomic DNA of the $F_2BC_1(44)$ and $F_2BC_1(12)$ plants was extracted with the Kingfisher Flex Magnetic Particle Processor (ThermoScientific) following manufacturer protocol. DNA concentration and quality was assessed on 1% TBA-agarose gel. DNA was adjusted to a final concentration of 50 ng/μL.

The 131 F_2 plants as well as the parental plants were genotyped by 142 Amplified Fragment Length Polymorphism (AFLP) markers (Vos et al.1995) and supplemented with 166 SNP markers. The $F_2BC_1(44)$ and $F_2BC_1(12)$ populations were genotyped using Illumina's Infinium SolCAP Tomato BeadChip (Sim et al. 2012), according to the Illumina Infinium II Protocol (www.illumina.com). Marker analysis was carried out by Service XS Leiden, The Netherlands.

Genetic map construction and QTL mapping

Construction of the genetic map for the F_2 population was performed with the software package JoinMap v.4.0 (Van Ooijen 2006) using the independence LOD score for linkage group formation and the Haldane mapping function based on regression mapping. A calculated SNP map was used as a fixed order backbone and co-dominantly scored AFLP markers were added by regression mapping. In total 305 markers were included in the final genetic map. JoinMap settings were adjusted for both F_2BC_1 populations to enable the construction of linkage maps with high numbers of SNP markers obtained with the SolCap array. Linkage groupings were based on recombination frequency and the Haldane mapping function based on maximum likelihood mapping algorithm. Markers with odd segregation patterns were excluded from the map and markers showing an identical segregation pattern were represented by one marker. Phenotypic QTLs in the F_2 and F_2BC_1 populations and metabolic QTLs in the F_2 population were calculated using MapQTL v.6.0 (Van Ooijen 2004). LOD-score threshold values for phenotype QTLs and m-QTLs were fixed at 3.0. Interval mapping was used to determine the interval of the phenotypic QTL using a 1-LOD and 2-LOD drop off interval. MapChart 2.2 Software (Voorrips 2002) was used for the graphical presentation of linkage maps and QTLs. A region is considered a hotspot when more than 10 metabolites map to the region.

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AUTHOR CONTRIBUTIONS

F.v.d.O. lead scientist of the project, and is responsible for execution of metabolomics work, QTL analyses, phenotyping experiments, data analyses, and writing of the article. A.F.L. is a co-writer. S.v.H. contributed to the data-analyses of mapQTL data and interpretation of data, advised on the employment of mapping populations, contributed to the experimental designs of phenotyping experiments, and revised the article and assisted in writing. C.B. advised on the experimental design of phenotyping experiments, revised the article and assisted in writing. R.M. contributed to the experimental set-up, data-analyses, interpretation of metabolomics data, and revised the article. M.D. contributed to the experimental set-up of the phenotyping and metabolomics work, and revised the article. B.V. contributed to the experimental set-up of the phenotyping and metabolomics work, contributed to data-analyses of mapQTL data and interpretation of data, advised on the employment of mapping populations, and revised the article.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Table S1. List of the most abundant m/z peaks of the metabolites for which a mQTL was detected as shown in Table 3

The 10 most abundant mass peaks and their relative intensity is given.