Resistance Mechanisms against *Bemisia tabaci* in Wild Relatives of Tomato

Floor van den Elsen

Thesis committee

Promotor
Prof. Dr M. Dicke
Professor of Entomology
Wageningen University

Co-Promotors
Dr B.J. Vosman
Senior Scientist, Wageningen UR Plant Breeding
Wageningen University and Research Centre

Dr A.W. van Heusden
Senior Scientist, Wageningen UR Plant Breeding
Wageningen University and Research Centre

Other members
Prof. Dr B.J. Zwaan, Wageningen University
Prof. Dr N.M. van Dam, Radboud University, Nijmegen, The Netherlands
Dr W.J. de Kogel, Plant Research International, Wageningen
Prof. Dr L.F.M. Marcelis, Plant Research International, Wageningen and Wageningen University

This research was conducted under the auspices of the Graduate School of Experimental Plant Sciences.

Resistance Mechanisms against *Bemisia tabaci* in Wild Relatives of Tomato

Floor van den Elsen

Thesis

Submitted in fulfilment for the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr M.J. Kropff
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Tuesday 22 October 2013
at 4 p.m. in the Aula.

Floor van den Elsen

Resistance Mechanisms against *Bemisia tabaci* in Wild Relatives of Tomato,
180 pages

PhD thesis, Wageningen University, Wageningen, NL (2013)
With references, with summaries in English and Dutch

ISBN: 978-94-6173-729-8

TABLE OF CONTENTS

Chapter 1: General introduction and thesis outline **7**

Chapter 2: Integrating phenomic and metabolomic data to characterize *S. pennellii* resistance mechanisms against the whitefly *Bemisia tabaci* **25**

Chapter 3: Genetic study of quantitative resistance traits against *Bemisia tabaci* in tomato **51**

Chapter 4: Characterization and genetic analyses of *S. pennellii* trichomes and Acyl sugars associated with whitefly resistance **81**

Chapter 5: Phenotypic screening for *B. tabaci* resistance in a *S. habrochaites* introgression line population **103**

Chapter 6: General discussion **123**

References **133**
Summary **149**
Samenvatting **153**
Dankwoord **158**
Curriculum vitae **161**
Education Statement of the Graduate School **163**
Supplementary Tables **167**

CHAPTER 1

General introduction and thesis outline

Floor van den Elsen[1,2,3]

[1]Wageningen UR Plant Breeding, Wageningen University and Research Centre, P.O. Box 386, 6700AJ, Wageningen, the Netherlands.
[2]Laboratory of Entomology, Wageningen University and Research Centre, P.O. Box 8031, 6700EH, Wageningen, the Netherlands.
[3]Graduate School Experimental Plant Sciences

Tomato systematics in relation to whitefly resistance breeding

Tomato (*Solanum lycopersicum* Solanaceae; *Solanum* sect. *lycopersicon*) is the third most important vegetable crop after potato and onion, with a worldwide gross production value of roughly fifty-five billion US dollars in 2009 (FAOSTAT, 11 Oct 2011). Tomato originates from the Andean region (Nakazato and Housworth 2011) and consists of thirteen closely related species (Peralta et al. 2008). Divergence from the common ancestor species took place approximately six million years ago, which makes it a relatively recent event (Wang et al. 2008; Rodriguez et al. 2009). Nevertheless, there is evidence for niche differentiation (Nakazato et al. 2010). Nakazato and Housworth (2011) suggested that abiotic conditions contributed to a great extent to the formation of the divergent tomato phenotypes. Most likely, also biotic factors elicited selection pressure that resulted in specific phenotypes. Because of their sessile nature plants require rapid adaptation of defense mechanisms towards attackers. Research on phylogenetic relationships between these tomato species revealed that *S. pennellii* and *S. habrochaites* are closely related to each other (Fig 1), but are within the tomato wild relatives the most distant from cultivated tomato. *Solanum pimpinellifolium* is the closest relative of the cultivated tomato based on sequencing data of several genes (Marshall et al. 2001; Peralta et al. 2008; Rodriguez et al. 2009). Screening for insect resistance within the tomato wild relatives showed a large phenotypic variation between different species and accessions within a species (Muigai et al. 2002; Muigai et al. 2003; Firdaus et al. 2013a). With regard to resistance against the whitefly *Bemisia tabaci*, a number of tomato wild relatives possess a resistant phenotype, namely accessions within the species *S. pennellii*, *S. habrochaites* f. typicum, *S. habrochaites* f. glabratum, *S. galapagense*, *S. chilense*, *S. peruvianum*, and *S. pimpinellifolium* (Nombela et al. 2000; Resende et al. 2009; Sánchez-Peña et al. 2006; Heinz and Zalom 1995; Liedl et al. 1995; Firdaus et al. 2012). Since the domesticated tomato *S. lycopersicum* shows high susceptibility against *B. tabaci*, wild relatives of tomato are looked at as important genetic resources for plant breeding programs for improvement of whitefly resistance.

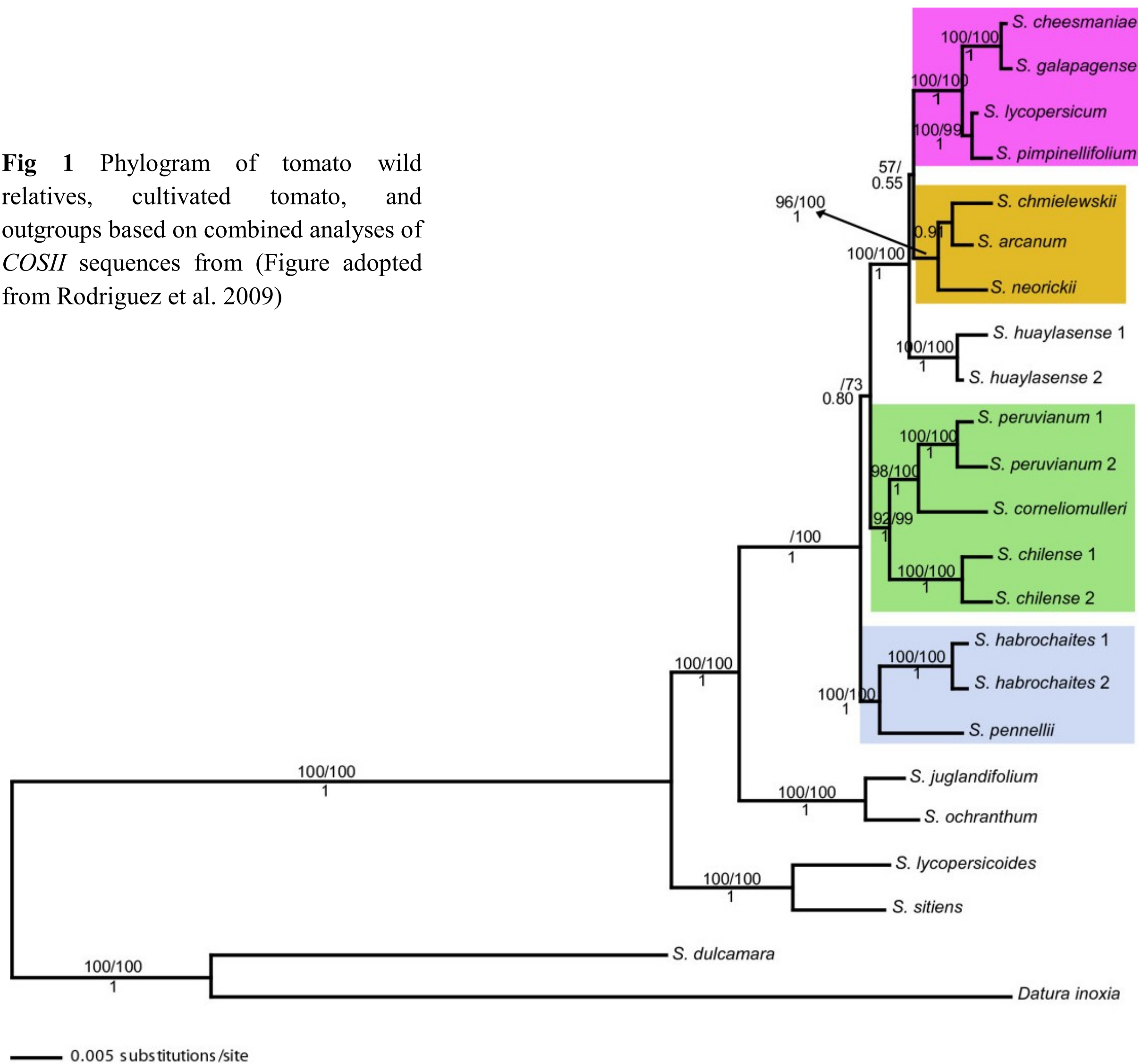


Fig 1 Phylogram of tomato wild relatives, cultivated tomato, and outgroups based on combined analyses of *COSII* sequences from (Figure adopted from Rodriguez et al. 2009)

Bemisia tabaci biology and systematics

Bemisia tabaci (Gennadius)(Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) is the scientific name for a number of herbivorous phloem-feeding whitefly species that pose a serious threat to agriculture as they feed on many plant species and are capable of transmitting diseases among these plants. Until recently, *B. tabaci* was regarded a complex species, but new insights revealed that it is a cryptic species complex consisting of eleven well-defined high-level groups containing at least twenty-four morphologically indistinguishable and reproductively isolated species (Dinsdale et al. 2010; De Barro et al. 2011), which were previously at least partly referred to as biotypes (Frohlich et al. 1999; Boykin et al. 2007). Species identification occurred through sequencing part of the COI gene and the use of a divergence threshold of 3.5% to identify whitefly species (Dinsdale et al. 2010; De Barro

2012; De Barro et al. 2011; Liu et al. 2012a; Liu et al. 2012b; Tay et al. 2012). Since the Dinsdale et al. (2010) publication four new groups have been identified in China (Hu et al. 2011), one (New World 2) in Argentina (Alemandri et al. 2012) and seven others after analyzing all data present in the database by the end of 2010, bringing the total number of groups with a more than 3.5% divergence to 36 (Firdaus et al. 2013a). The new nomenclature proposed by De Barro (2012) links to the geographical region from which the species originates, whereby the most invasive and globally distributed *Bemisia* species, commonly known as *B. tabaci* B biotype, has been renamed Middle East–Asia Minor 1. Both the Middle East-Asia Minor 1 species and the Mediterranean species, formally known as *B. tabaci* biotype Q, are considered invasive species in the Netherlands as well (Fig 2).

Despite their morphological resemblance, there is ample evidence that *B. tabaci* species differ to a great extent in characteristics like efficiency and capability of virus transmission, induction of phytotoxic symptoms, biological control efficacy and feeding behavior (Bedford et al. 1994; Gottlieb et al. 2010; Wintermantel et al. 2008; Wintermantel and Wisler 2006; Jiang et al. 1999; Pan et al. 2012; De Barro et al. 2011). A recent paper by Pan et al. (2012) clearly exemplified the difference in biology between the *B. tabaci* species. In this study the infection frequency of the tomato yellow leaf curl virus (TYLCV) was assessed at fifty-five field sites in China to compare acquisition and transmission capability of TYLCV between Middle East-Asia Minor 1 and the Mediterranean species and it was revealed that both the Middle East-Asia Minor 1 and the Mediterranean species can acquire and transmit the virus, but the Mediterranean species performed significantly better for both traits. Also it was observed that the Mediterranean species was more dominantly present at these sites as forty-three Mediterranean over twelve Middle East–Asia Minor 1 biotypes were identified across eighteen provinces in China (Pan et al. 2012).

Nowadays, molecular markers and gene amplification methods are widely employed to screen *B. tabaci* genotypes for species identification, which is essential when performing biological screening assays to ensure the allocation of biological results to the correct species (Shatters et al. 2009; Bel-Kadhi et al. 2008; Gupta et al. 2010; Teng et al. 2010; Guo et al. 2013; Dinsdale et al. 2010; De Barro et al. 2011).

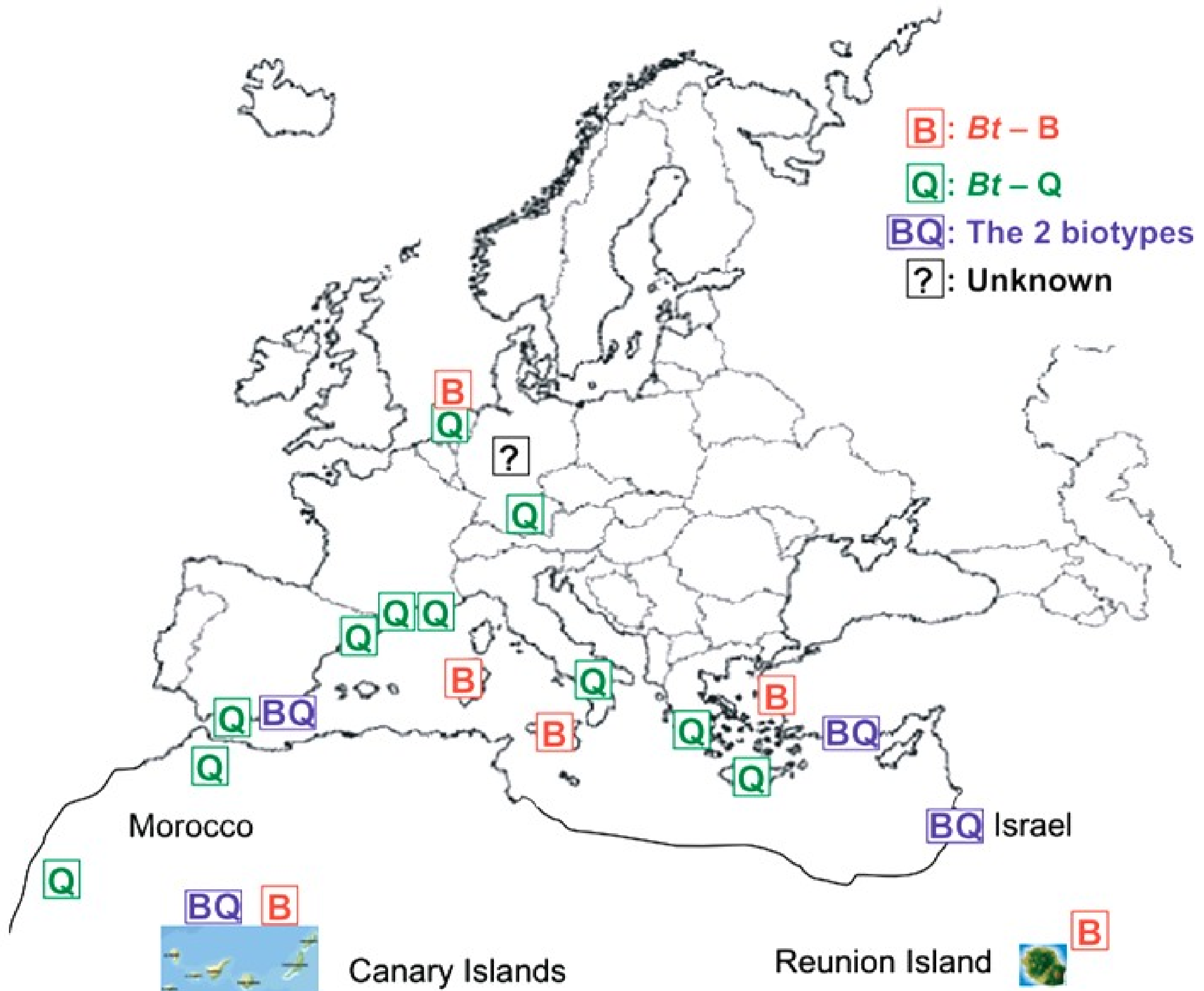


Fig 2 Distribution pattern of the Mediterranean (Q) and the Middle East-Asia Minor I (B) *B. tabaci* species in European, Middle-Eastern, and North African countries. Picture from endure database (Figure adopted from www.endure-network.eu; Arnó et al. 2009).

The lifecycle of *B. tabaci* comprises seven developmental stages (Fig 3). The eggs are deposited on the abaxial leaf side, often in a semi-circular pattern during feeding (McAuslane 2000), but solitary eggs are also found. After hatching, the mobile first-instar nymph will search for a suitable feeding site, where it will ingest phloem sap. Three moulting events take place, in which the immobile nymphs increase in size. After moulting, the fourth-instar nymph will turn red-eyed.

Fig 3 Lifecycle of *Bemisia tabaci.* The eggs (**1**) are oval-shaped and attached to the leaf with a stalk-like structure functioning as a channel for fluid uptake; source: www.bio-bee.com. The 1st nymphal instar (**2**), named crawler, is mobile and seeks a suitable feeding site nearby the eclosion site; source: Charles Olsen, USDA APHIS PPQ, Bugwood.org. The 2nd, 3rd, and 4th nymphal instars (**3**) are sessile. These stages are similar in morphology, but differ in size. The 4th nymphal instars (**4**) become red-eyed (**5**) without occurrence of molting and this stage is commonly referred to as pharate adult stage or pupal stage; sources: www.csiro.au, www.sciencephoto.com. However, the latter term is incorrect because whiteflies are hemimetabolous and hence have incomplete metamorphosis. Adult whiteflies (**6**) emerge from the red-eyed nymph. The remaining shells are transparent; source: Scott Bauer, USDA Agricultural Research Service, Bugwood.org.

The red-eyed stage is often erroneously called the pupal stage, but as *B. tabaci* is a hemimetabolous species, complete metamorphosis does not take place (Gelman et al. 2002; McAuslane 2000). When the final nymphal stage is completed, a mature whitefly will emerge leaving behind an empty transparent shell, which is once again often erroneously named an empty pupa in whitefly resistance screenings.

During *B. tabaci* development, the younger leaves are preferred as oviposition sites. The various nymphal developmental stages take mainly place on the middle aged and older leaves (Cardoza et al. 2000; Chu et al. 2000; Schuster et al. 1998). Females can oviposit as many as 300 eggs during their lifespan when environmental conditions are optimal (Byrne et al. 1990).

Bemisia tabaci has many opportunities to find suitable host plants in the field because of their highly polyphagous nature, feeding on over five-hundred plant species in seventy-four families (McAuslane 2000), in addition, the whitefly is easily spread in the field because of their relatively small size (http://www.issg.org/database).

Bemisia tabaci is an arrhenotokous parthenogenetic species, which can reproduce both sexually and asexually. Reproduction by unfertilized females results in haploid progeny, while eggs of fertilized females can be haploid, resulting in male offspring or diploid, resulting in female offspring (Byrne and Bellows 1991; Blackman and Cahill 1998).

The complete lifecycle of *B. tabaci* takes on average fourteen to twenty-eight days, strongly depending on environmental factors and host plant (sub)species (Fekrat and Shishehbor 2007; Salas and Mendoza 1995). Plants that are partially resistant or plants that have a low nutritional value can negatively affect the duration of the different developmental stages of *B. tabaci*, thereby prolonging the lifecycle of the whitefly (Jindal and Dhaliwal 2009; Muigai et al. 2003). For this reason, monitoring of *B. tabaci* life-history parameters provides whitefly resistance breeding programs with good quality indicators for germplasm selection. Principal life history traits are size at birth, growth pattern, development rate, age at maturity, size at maturity, number, size and sex-ratio of offspring, age- and size specific reproduction, age- and size specific mortality, longevity (Charleston and Dicke 2008). Population growth can be determined by various ways, but literature often refers to the intrinsic rate of increase (r_m) as a measure for population growth (Charleston and Dicke 2008). Examples of commonly used biological indicator parameters for determining *B. tabaci* resistance between different plant genotypes are pre-adult survival, adult survival, oviposition, pre-adult and adult development time (Mann et al. 2008; Tsai and Wang 1996; Carabali et al. 2010; Mansaray and Sundufu 2009; Zhao et al. 2009).

Damage caused by *Bemisia tabaci*

Bemisia tabaci is amongst the world's most invasive agricultural pest species (http://www.issg.org/database). Plant damage caused by this whitefly leads to global losses in vegetable, fibre, and ornamental crop production in field and greenhouse environments (Oliveira et al. 2001; Morgan and MacLeod 1996). The economic impact is invigorated by the fact that whitefly populations have an explosive growth through their short developmental cycle and rapid reproductive potential. Plant damage by the whitefly is caused in a direct and indirect way. Direct damage by whitefly colonization results from the uptake of nutrients

from the phloem, which can cause alterations in plant physiology, resulting in severe phytotoxicity. Consequentially, plants suffer from morphological deformities, like squash silverleaf in *Curcubita* spp., uneven ripening of tomato and white stem disorder in *Brassica* spp. (Lima et al. 2000; Oliveira et al. 2001; Schmalstig and McAuslane 2001; Jimenez et al. 1995; Costa and Brown 1991).

An unfavorable side effect of whitefly infestation is the production of carbohydrate-rich honeydew excretions, which makes the leaves sticky and supports the growth of sooty mold fungi on the plant leaf and fruit surface (http://www.issg.org/database).

Albeit that the direct damage elicited by *B. tabaci* has a vast impact on plant fitness and consequently yield, the indirect damage caused by this whitefly is even more destructive for agriculture. The whitefly can vector at least one-hundred-and-eleven pathogenic viruses that can seriously harm the fitness of the host plant (Jones 2003). Studies on the infection incidence of *B. tabaci*-vectored viruses showed that viral outbreaks occur that infect all field- or greenhouse-grown plants of a crop, resulting in high percentages of yield loss (Alegbejo 2000; Moriones and Navas-Castillo 2000; Papayiannis et al. 2008). Whitefly-vectored viruses are, therefore, of major concern for growers and for that reason research on whitefly-plant interactions is often focused on virus control. Most prevailing whitefly-vectored viruses belong to the Begomovirus genus (ninety percent), Crinivirus (six percent), and the remaining four percent are in the Closterovirus, Ipomovirus, or Carlavirus genera (Jones 2003). The main virus species transmitted by *B. tabaci* are Tomato yellow leaf curl virus (TYLCV; Begomovirus), Tomato yellow leaf curl Sardinia virus (TYLCSV; Begomovirus), Cucurbit yellow stunting disorder virus (CYSDV; Crinivirus), Tomato chlorosis virus (ToCV; Crinivirus), and Cucumber vein yellowing virus (CVYV; Ipomovirus)(ENDURE database; www.endure-network.eu).

Bemisia tabaci: current control methods and host plant resistance

Control methods, like the use of pesticides and cultural measures, do not effectively reduce *B. tabaci* numbers and the frequent application of chemicals increases the development of pesticide resistance (Roditakis et al. 2006; Roditakis et al. 2009; Ahmad et al. 2002; Horowitz et al. 2005; Horowitz et al. 2004; Wilson et al. 2007; Prabhaker et al. 2005; Cahill et al. 1996). *Bemisia tabaci* resistance is confirmed for pesticides of several classes of formally effective compounds, including organophosphates, carbamates, pyrethroids, insect growth regulators, and chlorinated hydrocarbons (Elbert and Nauen 2000). The toxicity of pesticides

to non-target beneficial insects and the environment, the costliness of newly produced pesticides, and the ineffectiveness of sprays to control abaxial whitefly infestations are other disadvantages of pesticide application. An alternative for the use of pesticides is the implementation of natural enemies in pest control programs (de Barro and Cooms 2009; Ellers-Kirk et al. 2000; Moreno-Ripoll et al. 2012). Mainly the parasitoids belonging to the genera *Eretmocerus* and *Encarsia* and predators belonging to the families Coccinellidae (beetles), Miridae (true bugs), Anthocoridae (true bugs), Chrysopidae (lacewings), Coniopterygidae (lacewings), Phytoseiidae (mites), and Araneae (spiders) have been studied and applied for control of *B. tabaci* in greenhouses (De Barro et al. 2007; De Barro et al. 2000; Gerling et al. 2001; Gerling and Kravchenko 1996; Gerling 1986; Goolsby et al. 1996; Qiu et al. 2007). Problems that arise in the application of natural enemies are for example the presence of co-existing whitefly species that are non-hosts (Arnó and Gabarra 1994) that affect the establishment of natural enemies, varying climate conditions that require the use of a variety of natural enemy species that perform under different environmental circumstances, plant morphological barriers, and aversive effects of pesticides on the natural enemies (Naranjo 2001; Gerling et al. 2001). Despite the disadvantages, biological control by using natural enemies has great value for crop protection as it is an effective and environmental friendly system that can be combined with other pest control methods in plant protection programs (Van Lenteren 2000; Van Lenteren and Noldus 1990; Broekgaarden et al. 2011).

Another alternative solution to keep whitefly numbers below economic injury threshold levels can be found in breeding for host plant resistance (HPR)(Broekgaarden et al. 2011). Host plant resistance is observed in wild relatives of many crop species and the level of resistance can be complete or partial when compared to susceptible varieties. Both tomato wild relative species *S. pennellii* and *S. habrochaites* display resistance against whitefly *B. tabaci* in plant preference and toxicity screenings (Baldin et al. 2005; Liedl et al. 1995; Muigai et al. 2002; Muigai et al. 2003; Sanchez-Pena et al. 2006). However, these tomato wild relatives have poor agronomic characteristics and are not suitable for consumption purposes. Insect resistance, as observed in tomato wild relatives, is usually based on heritable traits, which makes it possible to study resistance-related traits of interest in a qualitative and/or quantitative manner in progeny populations (Panda and Khush 1995). As interspecific breeding is possible in tomato, the wild accessions provide as donor material in breeding programs for the development of breeding populations to study whitefly resistance characteristics.

Qualitative insect resistance traits in tomato

A broad-spectrum R-gene has been implicated in insect resistance. The *Mi-1.2* gene, which is located on chromosome VI, encodes a protein with a nucleotide binding site (NBS) and leucine rich repeat (LLR) motif and is involved in whitefly resistance. The *Mi-1.2* gene was originally found to render high levels of resistance against root-knot nematodes (Goggin et al. 2001; Roberts et al. 1986; Medina-Filho and Tanksley 1983) and the potato aphid *Macrosiphum euphorbiae* (Rossi et al. 1998). A transgenic tomato line having the *Mi-1.2* gene in homozygous state showed enhanced resistance against the *B. tabaci* Middle East–Asia Minor 1 and the Mediterranean species in free- and no-choice experiments (Nombela et al. 2003; Nombela et al. 2000). The broad-spectrum resistance perceived for this R-gene in tomato was also found in potato in a later study that demonstrated that the *Mi-1* gene is a homolog of the *Rpi-blb2* gene from *Solanum bulbocastanum* conferring resistance against late blight (Van Der Vossen et al. 2005), showing again the potential of resistance genes against attackers of extremely divergent genetic backgrounds. The background of the resistance mechanism for *Mi-1.2* against *B. tabaci* still remains unclear, but electrical penetration graph (EPG) experiments showed that resistance components are active during early leaf penetration of epidermis and/or mesophyll tissues (Jiang et al. 2001).

A disadvantage with regard to *Mi-1.2* is that resistance is only a partial resistance, which is not very effective in the field (Nombela et al. 2003; Nombela et al. 2001; Nombela et al. 2000) and in general single gene resistance is more prone to breakdown compared to the more complex polygenic resistance traits.

Quantitative whitefly resistance traits in tomato

Many studies on wild tomato accessions have been performed where targeted secondary metabolite compounds were assessed for their effect on whitefly fitness/preference and a few literature reports describe quantitative trait loci (QTL) that are associated with these metabolite compounds or *B. tabaci* fitness parameters. Extensively described categories of resistance of plants against insect attackers are antixenosis and antibiosis.

Both antixenotic and antibiotic mechanisms might involve biophysical and biochemical plant defenses. Antixenosis is defined as a mechanism that is employed by the plant to deter or reduce colonization by insects (Panda and Khush 1995). The antibiotic mechanism is used by the plant after the colonization of insects and the mechanism adversely affects life-history

parameters, like reduced survival, oviposition, and development. These adverse effects of antibiosis on the insect make the insect more prone to natural enemies as there is an increase in exposure time of the insect to its natural enemy (Panda and Khush 1995).

Solanum pennellii secondary metabolites

Liedl et al. (1995) applied purified Acyl sugars from *S. pennellii* accession LA716 on susceptible tomato leaves and found a negative correlation between the presence of Acyl sugars and the settling and oviposition rate of *B. tabaci* adults. The threshold concentration of Acyl sugars required for deterring settling and oviposition were below the amount of Acyl sugars that were reported for control of other insects, including aphid and leafminer species (Liedl et al. 1995). Quantitative trait loci (QTL) analysis identified five genomic regions, two on chromosome II and one each on chromosomes III, IV and XI, which were associated with Acyl sugar production (Mutschler et al. 1996) in an interspecific cross between *S. pennellii* LA716 and *S. lycopersicum*. Backcross populations were developed that contained subsets of the five QTL regions, but this did not result in lines that showed accumulated Acyl sugar levels. Crosses between lines with complementary QTL subsets, resulted in a population of which a small percentage of lines (0.3%) accumulated low levels of Acyl sugars (Lawson et al. 1997). An intraspecific cross between *S. pennellii* LA716 (high Acyl sugar level) and *S. pennellii* LA1912 (low Acyl sugar level) was made for QTL identification of Acyl sugar pathway components (Blauth et al. 1999). Six QTLs were identified on chromosomes II, V, VI, VII, VIII, and XII that correlated with fatty-acid constituents, which are esterified to sucrose or glucose molecules to form Acyl sugars (Blauth et al. 1999), but so far no study has reported about the introgression of these QTLs in tomato cultivars and the correlation with *B. tabaci* resistance. The most recent literature report comes from Leckie et al. (2012) who used an F_1BC_1 population of a cross between *S. pennellii* cultivar LA716 and a breeding line with five *S. pennellii* introgressions on chromosomes II, III, VII, and X that produced moderate levels of Acyl sugars. By using this population additional QTLs for further improvement of Acyl sugar production were identified and their results showed reduced fitness of *B. tabaci* on a number of BC_1F_1 lines possessing additional minor effect QTLs at chromosomes VI and X.

Solanum habrochaites secondary metabolites

In *S. habrochaites* f. glabratum, *B. tabaci* resistance was associated with the presence of methylketones, like 2-undecanone and 2-tridecanone in no-choice toxicity assays (Antonious et al. 2005; Muigai et al. 2002; Fridman et al. 2005; Yu et al. 2010; Ben-Israel et al. 2009). These methylketones were found to be the major constituents in *S. habrochaites* f. glabratum, but these components were also recorded in *S. lycopersicum* at low levels (Antonious 2001).

In a separate study, QTLs were detected on chromosomes I and XII that affected *B. tabaci* oviposition rates and on chromosomes V and IX for trichome type IV segregation in an F_2 population with donor parent *S. habrochaites* f. glabratum (Maliepaard et al. 1995).
The sesquiterpenes zingiberene and curcumene were associated with reduced *B. tabaci* preference in *S. habrochaites* (Bleeker et al. 2009). In a mapping study by Momotaz et al. (2010), in which an F_2 population of donor parent *S. habrochaites* accession LA1777 was phenotyped for *B. tabaci* resistance using no-choice assays QTLs were identified for life-history parameters female survival and oviposition on four different loci. One QTL was detected on chromosome IX, one on chromosome X, and two on chromosome XI (Momotaz et al. 2010). None of these QTLs correspond to the QTLs found for Acyl sugar production (Mutschler et al. 1996) or QTLs found in *S. habrochaites* for *B. tabaci* life-history parameters and type IV glandular trichome production (Maliepaard et al. 1995). So far the few QTL studies on compounds involved in whitefly resistance in tomato have not demonstrated the genetic relationships between resistance traits and metabolites, nor is there direct evidence for the quantitative relationship between individual biochemical compounds and tomato genotypes. Joining data generated from different approaches like biochemical whitefly resistance traits, phenotype traits, and population genetics will complement knowledge on resistance mechanisms and corresponding genetic loci.

The role of glandular trichomes of tomato in insect resistance

Methylketones, mono- and sesquiterpenes as well as Acyl sugars are produced in specific types of secreting structures, the glandular trichomes (Wagner 1991; Schilmiller et al. 2008; Slocombe et al. 2008; Tissier 2012). It is generally accepted that glandular trichomes are essential for tomato resistance against whiteflies (Simmons and Gurr 2005). The role of trichomes as a quantitative trait in *B. tabaci* resistance, often with regard to glandular trichome type and density has been studied in tomato wild relatives (Oriani and Vendramim 2010; Muigai et al. 2003; Heinz and Zalom 1995) and in breeding populations (Maliepaard et al. 1995; Momotaz et al. 2005; Freitas 2002).
Mechanical trichome removal experiments have been carried out in *S. pennellii* and *S. habrochaites* f. typicum and f. glabratum accessions to study the effect on several insect pest species belonging to different orders. The mortality rate of the green peach aphid *Myzus persicae* was reduced on three different *S. pennellii* accessions when the trichomes were removed (Simmons et al. 2003). The number of leaf punctures and mines by the leafminer

Liriomyza trifolii increased on *S. pennellii* LA716 after removal of trichomes (Hawthorne et al. 1992). In trichome removal experiments on *S. habrochaites* f. glabratum, an increased survival of the potato moth *Phthorimaea operculella* was observed (Gurr and McGrath 2002) after removal of the trichomes. Entrapment and mortality of the cotton bollworm *Helicoverpa armigera* was significantly reduced when trichome exudates were removed from accessions of *S.habrochaites* f. typicum and *S. pennellii* (Simmons et al. 2004). Although the evidence for the involvement of glandular trichomes in insect resistance is abundant, the precise contribution of these surface structures to the resistance trait is yet unclear and thus far no literature is available that provides in-depth information on the relation between glandular trichomes and whitefly resistance. It is of interest to study the correlation between trichome type and metabolite content in relation to *B. tabaci* antibiosis traits in breeding populations to obtain detailed information on the effect of biochemical compositions in the leaves, morphological structures on the plant surface and the consequences for *B. tabaci* life-history.

Molecular markers

Molecular markers enable the detection of genetic variation at a specific genome position. The markers used in this thesis are Amplified Fragment Length Polymorphism (AFLP) markers described by Vos et al. (Vos et al. 1995) and Single Nucleotide Polymorphism (SNP) markers. The implementation of SNPs and AFLPs have been described in numerous linkage mapping studies that assessed genetic variation in tomato for various purposes (Shirasawa et al. 2010; Jimenez-Gomez and Maloof 2009; Spooner et al. 2005; Zuriaga et al. 2009) and will not be reviewed in detail here. Statistical software packages like JoinMap 4.1 (Van Ooijen 2006) have been designed to calculate genetic distances between markers within a linkage group and permit the construction of genetic linkage maps. Statistical software like MapQTL® 6 (Van Ooijen 2004) was developed to enable the detection of QTLs in a wide number of population types including first generation backcrosses, F_2 populations, recombinant inbred line families, families of F_1-derived doubled haploids, families of F_2-derived doubled haploids, advanced backcross inbred line families, advanced intermated inbred line families, and outbreeder full-sib families of diploid species.

Combining a genetic marker map with phenomics data from greenhouse whitefly screenings of segregating populations provides the possibility to localize QTLs for resistance parameters and associated traits, like specific trichome types and metabolic constituents. Recently, the tomato genome (*S. lycopersicum* cv. Heinz 1706) was sequenced and made publicly available

(Tomato Genome Consortium 2012), which can serve as a reference to establish the physical positions of genetic markers. Upon the localization of genetic loci that show significant associations with single or multiple resistance traits, further ~omics tools like transcriptome sequencing can be administered to study the region of interest and can be used as a large scale quantification method to compare gene expression levels between genotypes in a study population or subsets thereof.

Introgression Lines

As mentioned before, wild tomato relatives have been used as donor material to develop breeding populations to study phenotypic whitefly resistance traits. An F_2 population is often used to screen for QTLs as the breeding process for obtained F_2 plants is simple and short and the molecular tools are available to perform QTL mapping on this population structure (www.kyazma.nl). This population structure is also the most commonly used population type to study whitefly resistance traits in tomato. However, the genetic contribution of wild donor material is large in F_2 populations and therefore studying an F_2 population has a few drawbacks. A large percentage of wild genetic material in F_2 genotypes is disadvantageous when searching for candidate loci and succeeding populations are imperative to narrow down the genetic factors involved in whitefly resistance often resulting in loss of resistance as epistatic interactions are lost in subsequent populations. Other disadvantages are the wide variation in morphological and physiological traits and the fact that F_2 plants are unique genotypes and repetitive screenings can only be performed on cuttings (Finkers et al. 2007). Introgression lines (ILs), which in theory contain a single DNA fragment of the donor parent, have been developed to overcome these disadvantages. Finkers et al. (2007) developed such an IL population with donor *S. habrochaites* accession LYC4 and recurrent parent *S. lycopersicum* cv. Moneymaker to study *Botrytis cinerea* resistance in tomato (Finkers et al. 2007). Each IL possessed a single, defined chromosome segment of approximately five percent from the donor parent in a uniform genetic background of the recurrent parent and the total population covered ninety-five percent of the genome of the donor parent. Finkers et al. (2007) detected QTLs for *B. cinerea* in this population showing the potential for breeding of *B. cinerea* resistant cultivars. This population structure provides good opportunities for the identification of *B. tabaci* resistance in tomato. However, populations like this have the disadvantage that epistatic interaction may be more difficult to study.

An ~omics approach for whitefly resistance research in tomato

High throughput data generation and the development of statistical tools to process and analyse large datasets provide new opportunities for exploring whitefly resistance traits in tomato. The starting point for finding whitefly resistance alleles in breeding populations is, besides good source material, the deployment of procedures to assess and quantify phenotypes. The experimental design should incorporate the biological characteristics of the pest insect and the resistance mechanism. In whitefly resistance breeding, the emphasis lies on constitutive or rapidly induced resistance rather than gradually induced resistance and on toxic rather than preferential traits as field- and greenhouse-grown crops are often monocultures and under constant pressure of pest invaders. However, accumulation of resistance by employing combined antixenosis and antibiosis traits, thereby exposing the whitefly to a wide range of plant defenses, is likely to provide more durable protection against insects (Zangerl and Rutledge 1996; Anderson et al. 2011; Broekgaarden et al. 2011; Panda and Khush 1995).

Once the plant phenotypic characteristics with regard to *B. tabaci* resistance are established in a breeding population, it is essential to identify the factors underlying this resistance by studying for instance the plant morphological, physiological or metabolic profile, to detect the background of the plant defense mechanism(s). As insect resistance is heritable (Panda and Khush 1995), integrating genotype and phenotype data will result in the localization of the genetic factors contributing to the resistance trait and corresponding mechanism.

Thesis outline

The overall goal of the work described in this thesis is to identify the genetic background of phenomic and metabolomic traits correlating with *B. tabaci* resistance in tomato, which is investigated by using an ~omics approach and to reveal the main mechanisms behind *B. tabaci* resistance in *S. pennellii*.
In chapter 2, quantitative data of life-history parameters of *B. tabaci* on young and old plants of a segregating F_2 population from a *S. pennellii* accession LA3791 x *S. lycopersicum* elite cultivar cross were examined under greenhouse conditions. In addition, the effect of glandular trichomes on *B. tabaci* performance was investigated in the wild *S. pennellii* parent. To explain the resistance mechanism of *S. pennellii*, metabolic fingerprints were made of extreme phenotype bulks and correlations between semi-volatile and volatile (GC-MS) and non-volatile (LC-TOF-MS) components and *B. tabaci* resistance and susceptibility were identified.

In chapter 3, I constructed a genetic linkage map from AFLP and SNP marker data generated from our F_2 population to identify the genetic background of *B. tabaci* survival and oviposition in a six- and 20-week-old population. In addition, metabolic volatile profiles were determined by GC-MS for the whole F_2 population in order to identify QTLs of the metabolic compounds identified through GC-MS that were found to relate to a resistant or susceptible *B. tabaci* phenotype on *S. pennellii* in chapter 2. Furthermore, two F_2 genotypes were selected based on two criteria: 1) resistant phenotype, 2) heterozygosity for phenotypic and metabolite QTLs and backcrossed with their recurrent parent, resulting in F_2BC_1 populations, which were screened under greenhouse conditions in order to confirm the phQTLs from the F_2 population in a next generation.

Chapter 4: Metabolic non-volatile profiles were determined by LC-TOF-MS for the whole F_2BC_1 population that showed strongest divergence for whitefly life-history parameters between genotypes (chapter 3) to identify genetic loci of these compounds and reduce the size of the QTLs that were identified in our F_2 population. A characterization of glandular trichome composition in genotypes of the F_2BC_1 population was performed to identify intra-population correlations between segregation patterns of trichome types in relation to whitefly life-history parameters.

Chapter 5: In this chapter, an Introgression Line (IL) population from *S. habrochaites*, that was developed and screened for *Botrytis cinerea* disease QTLs by Finkers et al. (2007), was evaluated for *B. tabaci* life-history parameters under greenhouse and field conditions. A subset of the population was chemoprofiled using GC-MS for untargeted analyses of known and unknown resistance components.

Chapter 6: In the final chapter, the general discussion, the results from all the chapters will be integrated and general conclusions drawn with respect to resistance mechanism and prospects for breeding. Furthermore, the current status of research of plant breeding for insect resistance will be discussed.

CHAPTER 2

Integrating phenomic and metabolomic data to characterize *S. pennellii* resistance mechanisms against the whitefly *Bemisia tabaci*

Floor van den Elsen[1,4,5], Adriaan W. van Heusden[1], Colette Broekgaarden[1], Roland Mumm[2,3], Ric C.H. de Vos[2,3,6], Marcel Dicke[4], Ben Vosman[1]

[1]Wageningen UR Plant Breeding, Wageningen University and Research Centre, P.O. Box 386, 6700AJ, Wageningen, the Netherlands.
[2]Plant Research International, Business Unit Bioscience, Wageningen University and Research Centre, P.O. Box 619, 6700AP Wageningen, the Netherlands.
[3]Centre for Biosystems Genomics, P.O. Box 98, 6700AB, Wageningen, the Netherlands.
[4]Laboratory of Entomology, Wageningen University and Research Centre, P.O. Box 8031, 6700EH, Wageningen, the Netherlands.
[5]Graduate School Experimental Plant Sciences
[6]Netherlands Metabolomics Centre, Einsteinweg 55, 2333 CC, Leiden, The Netherlands

Abstract

The whitefly *Bemisia tabaci* is a pest insect capable of causing major damage to tomato and consequently reduces yield. Some tomato wild relatives are resistant against this pest and their genetic traits are desired for resistance breeding. An F_2 population was phenotyped for *B. tabaci* life-history traits and extreme phenotypes were selected to study various genetically determined mechanisms of *B. tabaci* resistance from wild tomato relative *Solanum pennellii*. To get more insight into the role of mechanical barriers and resistance- and susceptibility-related metabolites interfering with whitefly life-history, we employed an untargeted metabolomics approach, using complementary platforms, and identified constituents that either negatively or positively correlate with *B. tabaci* fitness. We therefore analyzed the most extreme resistant and susceptible F_2 phenotypes using complementary LC-TOF-MS and GC-MS platforms, enabling the evaluation of plants for their relative abundance of in total 443 different metabolites, and used multivariate analysis techniques to discriminate the resistant and susceptible phenotypes on the basis of their biochemical fingerprints. Moreover, we demonstrated that removal of glandular trichomes nullified the resistance effect of the wild relative *S. pennellii* with regard to oviposition and strongly reduced the resistance effect of the wild parent on whitefly survival. The data may be used to develop novel durable strategies to control this pest.

Keywords*:* *Bemisia tabaci*, *Solanum pennellii*, phenotyping, life-history parameters, glandular trichomes, GC-MS, LC-TOF-MS.

Introduction

One of the most damaging and invasive pest insects is the silverleaf whitefly *B. tabaci* Gennadius (Hemiptera: Aleyrodidae), although recent studies suggest that it in fact is a species complex, consisting of at least 36 cryptic species (Firdaus et al. 2013a). *Bemisia tabaci* is an herbivorous phloem-feeding generalist that poses a serious threat to agriculture as it feeds on over 500 plant species from 74 families (McAuslane 2000). In cultivated tomato (*Solanum lycopersicum*), *B. tabaci* nymphs and adults cause direct damage by the uptake of phloem sap. Indirect damage is caused by this whitefly as it vectors at least 111 pathogenic viruses that can seriously affect the fitness of the host plant (Bedford et al. 1994; Mayer et al. 2002; Jones 2003). Other unfavorable side effects caused by *B. tabaci* are the production of carbohydrate-rich honeydew excretions, which support the growth of sooty mold fungi on the plant leaf surface (Henneberry et al. 1996; Byrne and Bellows 1991), and irregular ripening of the fruits (McKenzie and Albano 2009; Costa and Brown 1991).

Plants possess biochemical and physical traits that protect them against insect herbivory. Accessions of several wild relatives of cultivated tomato have been found to harbor partial or full resistance against *B. tabaci* (Muigai et al. 2003; Muigai et al. 2002; De Ponti et al. 1975; Oriani et al. 2011; Oriani and Vendramim 2010; Baldin et al. 2005; Firdaus et al. 2012). There is ample evidence that one of the modes of action of plant defence against *B. tabaci* is through the synthesis of antibiotic as well as antixenotic compounds. High levels of the methylketones 2-undecanone and 2-tridecanone were detected in different accessions of *Solanum habrochaites* and they have deterrent effects on *B. tabaci* (Yu et al. 2010; Antonious et al. 2005; Antonious 2001; Williams et al. 1980). Other *S. habrochaites* accessions produce the sesquiterpene hydrocarbons zingiberene and curcumene that showed to be repellent as well as toxic for *B. tabaci* (Freitas et al. 2002; Antonious and Kochhar 2003; Bleeker et al. 2012; Bleeker et al. 2009).

The major whitefly-resistance-related constituents in *S. pennellii* accession LA716 are Acyl sugars (Slocombe et al. 2008; Nombela et al. 2000; Blauth et al. 1999; Blauth et al. 1998; Lawson et al. 1997; Mutschler et al. 1996; Liedl et al. 1995). Acyl sugars are composed of 2,3,4-tri-O-Acylglucoses that contain predominantly the branched-chain fatty acids (BCFAs) 2-methylpropanoic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, and 8-methylnonanoic acid (Burke et al. 1987; Shapiro et al. 1994; Li et al. 1999). To a minor extent the glandular trichomes produce straight-chain fatty acids (SCFAs) of short to medium length (C4 to C12)(Ghangas and Steffens 1993; Burke et al. 1987). In *S. pennellii* LA716,

Acyl sugars constitute approximately 90% of the exudate of type IV glandular trichomes (Mutschler et al. 1996) and have, besides an important defensive role against *B. tabaci,* also a toxic effect on a wide range of insects with different feeding strategies, which classifies these Acyl sugars as broad-spectrum insect resistance compounds (Goffreda et al. 1990; Goffreda and Mutschler 1989; Goffreda et al. 1989; Goffreda et al. 1988). Shapiro et al. (1994) studied Acyl sugar compositions in fifteen accessions of *S. pennellii* and found substantial variation among accessions for the level of Acyl sugars produced, the type of sugar (glucose or sucrose), and the incorporated fatty acids. Liedl et al. (1995) showed a direct relation between the presence of Acyl sugars and reduced settling and oviposition of *B. tabaci* in *S. pennellii* LA716. The effect of individual Acyl sugars or other metabolites on resistance against *B. tabaci* has not been studied. Moreover, there is no study documenting the effect of *S. pennellii* metabolites on the susceptibility to *B. tabaci*.

Within *Solanum* section *Lycopersicon*, *S. pennellii* is the most distant relative of the cultivated tomato (Anderson et al. 2010), yet it is possible to obtain fertile interspecific hybrids (Lippman et al. 2007). Segregating populations have proven to be useful for studying quantitative resistance traits against *B. tabaci* in different crops (Jindal and Dhaliwal 2009; Momotaz et al. 2010). In this work, F_2 progeny of a cross between *S. pennellii* LA3791 and an *S. lycopersicum* elite cultivar were used to explore non-, semi-, and volatile compounds correlating with *B. tabaci* resistance and susceptibility. No-choice experiments on young and old F_2 plants were carried out to quantify the levels of toxicity/deterrence against *B. tabaci*. The life-history parameters adult survival and oviposition of *B. tabaci* were scored as these are key parameters to determine *B. tabaci* resistance and susceptibility (Nombela et al. 2000, Mayer et al. 2002; Wilson et al. 2007; Mansaray and Sundufu 2009, Fekrat and Shishehbor 2007). Two groups of F_2 genotypes, a *B. tabaci* susceptible and resistant group, were selected based on the quantitative phenotypic assessments and employed for comparative metabolic studies using Gas Chromatography combined with Mass Spectrometry (GC-MS) and Liquid Chromatography combined with accurate Time-of- Flight-Mass Spectrometry (LC-TOF-MS). This integrative study of combining phenotype and metabolomics data was employed to reveal the main mechanisms behind *B. tabaci* resistance in *S. pennellii* accession LA3791. Complementary, the effect of leaf glandularity on *B. tabaci* resistance was examined by quantifying whitefly performance in *S. pennellii* accession LA3791 with intact trichomes and with glandular trichomes from which the heads were removed.

Materials and Methods

Plant material

An interspecific cross was made between *S. pennellii* accession LA3791 and *S. lycopersicum* elite cultivar To6W_LI0620 (hereafter referred to as *EC*), which was made available by Nunhems NL, Nunhem, The Netherlands. One F_1 plant was selfed to produce an F_2 population, which was sown in potting trays. Hundred and thirty one of 170 F_2 seeds germinated and were grown for phenotyping and chemoprofiling experiments.

One-week-old seedlings were transplanted in pots (Ø 20cm) on soil substrate. Plants were grown under controlled glasshouse conditions (22 ±2°C, L16:D8 photoperiod, RH about 50%), watered daily, and supplemented with nutrients once a week. No chemical pathogen- or pest control was practiced.

For chemoprofiling, six cuttings per individual F_2 genotype were made from ten-week-old unchallenged plants and grown in trays on soil substrate. Subsequently, two cuttings per F_2 genotype were selected, transferred to soil in pots (Ø 20cm), and grown in an insect and pathogen free environment (22 ±2°C, L16:D8 photoperiod, RH about 50%).

Whiteflies

Bemisia tabaci Middle East–Asia Minor 1 was reared on *S. lycopersicum* cv. Moneymaker in a glasshouse under controlled conditions (26 ±2°C, L16:D8 photoperiod, RH 60±10) at the Laboratory of Entomology, Wageningen University. The colony commenced from a single parthenogenetic female. An allelic discrimination real-time PCR assay was performed on randomly sampled individuals to affirm the Middle East–Asia Minor 1 genotype (according to Jones et al. 2008). Detached cv. Moneymaker leaves with synchronized 4th instar nymphs were placed in a gauze insect cage containing three-week-old cv. Moneymaker plants to provide newly emerged adults with young leaves. One-to-three-day-old adults were collected from the insect cage and anaesthetized with a gas mixture (N_2:H_2:CO_2 [80:10:10]; Linde Gas Benelux) to enable selection and transfer of whiteflies to the test plants.

Phenotyping

Environmental parameters were controlled for *B. tabaci* rearing (26 ±2°C, L16:D8 photoperiod, RH 60±10%) one week prior to the beginning of phenotyping experiments. A total of 131 F_2 genotypes were tested for adult survival and oviposition rate in a no-choice experimental design when plants were six- or 20-weeks old. The resistant parent *S. pennellii*

LA3791, *S. pennellii* LA716, *S. habrochaites* LA1777 and cv. Moneymaker were included as reference material. Three plants per reference were screened and these replicates were randomly positioned throughout the greenhouse.

Adult survival Unsexed 1-3 days old adults were selected under a stereomicroscope (Zeiss) and transferred to the abaxial side of a third internode leaf in a fine-meshed clip-on cage (Ø 25mm) with rubber membranes at the leaf interface to prevent mechanical leaf damage. The third internode leaf was chosen as younger leaves are preferred over older leaves by the whitefly for nutrient uptake and oviposition (Liu and Stansly 1995). Each individual F_2 genotype (n=1) and each reference plant (n=3) was challenged with two clip-on cages containing 20 adults each. Adult survival was counted under a stereomicroscope five days post infestation. Adult survival rate was calculated per clip-on cage according to Van Giessen et al. (1995) and Bas et al. (1992) by the following equation:

$$\text{Adult survival rate} = \left(\frac{m}{n}\right)^{1/d} \text{/day}$$

where *d* is the number of days (five days), *n* the total number of females per clip-on cage, *m* the number of whiteflies alive after *d* days.

Oviposition rates. Six-to-eight-day-old females were selected under a stereomicroscope and transferred to the abaxial side of the 3rd-internode leaf. Each individual F_2 genotype (n=1) and each reference plant (n=3) were challenged with two clip-one cages containing five female *B. tabaci* each. Leaves were cut off after five days of infestation and the total number of females, the number of living females, and the number of eggs were counted under a stereomicroscope. Oviposition rates were calculated per clip-on cage according to Van Giessen et al. (1995) and Bas et al. (1992) by the following equation:

$$\text{Oviposition rate} = \frac{2e}{d(m+n)} \text{ eggs/female/day}$$

where *e* is the number of eggs, *d* the number of days (five days), *n* the total number of females per clip-on cage, *m* the number females alive after *d* days.

Life-history parameters on plants with and without glandular trichomes

A no-choice experiment was carried out on donor parent *S. pennellii* LA3791 with and without glandular trichomes. To obtain leaves without glandular cells, a third internode leaf was dipped in 96% EtOH for ten seconds, glandular cells were removed from the abaxial leaf side with a soft brush, and the leaf was rinsed three times for ten seconds in dH_2O. For the control a third internode leaf was rinsed three times for ten seconds in dH_2O. One control and one test leaf were infested per individual plant and six plants of both *S. pennellii* and cv. Moneymaker were used. Once the leaves were dry, ten one-to-three-day-old unsexed adults were anaesthetized and transferred into a transparent clip-on cage on the abaxial side of a third internode leaf with removed or intact glandular trichomes. The number of dead and alive *B. tabaci* was scored by eye every day for four subsequent days. Adult survival was calculated by dividing the number of living adults by the total number of adults.

To determine the reproduction rate, ten six-to-eight-day-old *B. tabaci* females were anaesthetized and transferred in a clip-on cage to the abaxial side of a third internode leaf with removed or intact glandular trichomes. Leaves were cut off after five days of infestation and the total number of females, the number of living females, and the number of eggs were counted under a stereomicroscope. Oviposition rates were calculated by the abovementioned equation of Van Giessen et al. (1995).

For statistical analyses of life-history parameters of *B. tabaci* on *S. pennellii* and cv. Moneymaker, the means and standard error of the mean (SEM) were calculated by one-way ANOVA (SPSS 12.0.1 for Windows). A Bonferroni test using a confidence interval of 95% was performed to compare differences between treatments and between genotypes.

Selection of resistant and susceptible genotypes from the F_2 population

Phenotypic traits of the F_2 population were ranked to select the ten most resistant and the ten most susceptible genotypes for metabolite analyses. The group of resistant F_2 plants consisted of genotypes that possessed full resistance against *B. tabaci*, i.e. no adult survival and no oviposition on both six- and 20-week-old plants. The group of susceptible F_2 genotypes was selected based on average highest rank for egg deposition on six-week-old plants, the average highest rank for egg deposition on 20-week-old plants, the average highest rank for adult survival on six-week-old plants, and the average highest rank for adult survival on 20-week-old plants, respectively.

Leaf sample preparation for metabolomics

Two cuttings per F_2 genotype plus *S. pennellii* and *S. lycopersicum* cv. Moneymaker were placed in a randomized block design. The environmental parameters were adjusted one week prior to the collection of leaf material for chemoprofiling (26±2 °C, L16:D8 photoperiod, RH 60±10%), to equal the settings used during phenotyping experiments. Third internode leaves of six-week-old uninfested plants were cut off, packed in aluminum foil, thereby minimalizing damage to leaf tissue, and instantly transferred to LN_2. Leaf samples were stored at -80°C until use in Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Time-of-Flight-Mass Spectrometry (LC-TOF-MS) measurements. Samples were prepared according to Maharijaya et al. (2012).

GC-MS metabolic profiling

The GC-MS analysis was performed on the ten most susceptible and resistant individuals plus reference material to identify apolar metabolites that may contribute to *B. tabaci* resistance. The dichloromethane (DCM) extracts were analyzed using an Agilent 7890A GC-MS machine (Agilent Technologies, Amstelveen, The Netherlands) equipped with a 30-m Zebron ZB-5 ms column with 5 m retention gap (0.25 mm i.d., 0.25-lm film thickness; Phenomenex, Torrance, CA, USA) and an Agilent 5975C quadrupole mass analyzer (Agilent Technologies). The GC was programmed from 45 °C for 1 min, raised to 300 °C at 10 °C per min, and held at 300 °C for 5 min. One microliter of sample was injected in splitless mode. The injection port and interface temperatures were 250 and 280 °C, respectively, and the helium inlet pressure was controlled electronically to achieve a constant column flow of 1.0 ml min^{-1}. The column effluent was ionized using electron impact at 70 eV, and scanning was performed from 45 to 400 atomic mass units.

An untargeted data processing approach was applied to process the raw GC-MS data (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 3 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). Reconstructed metabolites were putatively identified by matching the mass spectra to authentic reference standards, and to commercial spectral libraries NIST08 (www.nist.gov), Wiley (www.wiley. com), and to custom made spectral libraries (Wageningen Natural compounds spectral library), and by comparison with

retention indices of the literature calculated using a series of alkanes and fitted using a third-order polynomial function (Strehmel et al. 2008).

Duplicates of each genotype (with the exception of genotype numbers 54, 86, and 101, where only one replicate was available) were inserted in the GC-MS machine in reverse sequence. Controls DCM, *S. pennellii* LA3791, and cv. Moneymaker were included daily in the course of the measurements.

Data analyses were done with MS Excel (2010) software. The data were $\log_{10}$ transformed and a Student's t-test was performed per metabolite between genotype groups and subsequently *p*-values were ranked. A false discovery rate (FDR) control was applied to correct for multiple comparisons. The corresponding *q*-values were calculated according to Benjamini and Hochberg (1995):

$$q\text{-value} = \left(\frac{m}{i}\right) * P_i$$

where q is the FDR-corrected *p*-value for a single metabolite, m the number of variables (metabolites), i the rank of the *p*-value of the variable, P_i the *p*-value.

The metabolites with $q<0.05$ were used for peak annotation.

LC-TOF-MS metabolic profiling

The leaf samples collected for GC-MS analyses were also used to determine the variation in non-volatile metabolites between bulks of F_2 genotypes with extreme phenotypic values for *B. tabaci* resistance. Two biological replicates per F_2 genotype were used, with the exception of genotype numbers 54, 86, and 101, where only one leaf sample was available. Extraction and analysis by accurate mass Liquid Chromatography-Quadrupole Time of Flight-Mass Spectrometry (LC-QTOF-MS, in short LC-TOF-MS) was performed as described previously (De Vos et al. 2007). In short, 250±10mg (FW) of ground leaf powder was weighed in 10ml glass tubes. Sample extraction was done by thoroughly mixing with 750 µl methanol containing 0.125% formic acid (FA) followed by sonication in a water bath (15min). After centrifugation (5min 3000*g*) and filtering (Captiva 0.2 µM PTFE filter plate,Agilent), 5 µl per sample was injected in the LC-TOF-MS system (Waters QTOF Ultima) and separated on a Phenomenex Luna C18 (2) column (2.0 × 150 mm, 3 mm particle size) using a 5–95% ACN gradient in H_2O with 0.1% FA for acidification. Mass signals of *m/z* 80–1,500 were detected

with negative electrospray ionization. Leucine encephalin was used as lock mass for local accurate mass corrections (Moco et al. 2006).

Metalign software (Lommen 2009) was used to automatically extract and align all relevant LC-TOF-MS signals (signal to local noise ratio >3) from the raw data files. Accurate masses of signals were automatically calculated by Metalign by taking into account only those scans with a signal intensity corresponding to the local lock mass intensity plus or minus 50% (Moco et al. 2006). The total of 10449 signals was filtered for signals present in at least four samples and having an amplitude of at least six times the noise value in at least one of the samples. Then, all signals eluting within 3 min of retention time (i.e. the injection peak, mostly consisting of signals from non-retained highly polar compounds) were removed from the dataset. MSClust was used to group mass signals originating from the same molecule, including the molecular ion, natural isotopes and in-source fragments and adducts, into reconstructed metabolites (Tikunov et al. 2012).

A total of 297 LC-TOF-MS and 146 GC-MS metabolites, each defined by mass and scan number, from the selected genotypes were included in the subsequent data analysis. Data was exported into GeneMaths XT (Applied Maths, Sint-Martens-Latem, Belgium) for constructing a heatmap to visualize differences in peak intensity between genotypes and for visualization of correlations between genotypes. Subsequently, GeneMaths XT was employed to perform hierarchical cluster analyses of both the F_2 genotypes and the GC-MS and LC-TOF-MS metabolites by calculating Pearson’s correlation followed by Unweighted Pair Group Method with Arithmetic mean (UPGMA).

Identification of Acyl sugars

Monoisotopic exact masses of negatively charged ions were calculated for a series of possible Acyl chain-sugar combinations, from 7 up to 30 carbons Acylated to either glucose (G) or sucrose (S) as the sugar backbone, i.e. starting from m/z 333.0827 for G4:7 up to m/z 803.5162 for S3:50), as well as their formic acid adducts (additional mass of 46.0055 for CH_2O_2). Under the LC-TOF-MS conditions applied, the Acyl sugars were mainly detectable as their formic acid adducts. Metalign-extracted LC-TOF-MS signals corresponding to the major Acyl sugars were annotated based on their unique monoisotopic accurate mass, using a threshold of 5 ppm deviation of detected masses from calculated masses.

Results

Phenotyping the F_2 population

An F_2 population (n=131) derived from a cross between a *S. lycopersicum* elite cultivar and *S. pennellii* LA3791 was screened for susceptibility/resistance to *B. tabaci* in a no-choice experiment. Adult survival and oviposition rate were measured on six-week-old plants using a clip cage. The results are shown in Figure 1. Fifteen percent of the genotypes was completely resistant with regard to adult survival (Fig 1a) and 27 percent of genotypes scored zero with regard to oviposition during the five day period (Fig 1c). Partial resistance to full susceptibility was observed for the remaining genotypes.

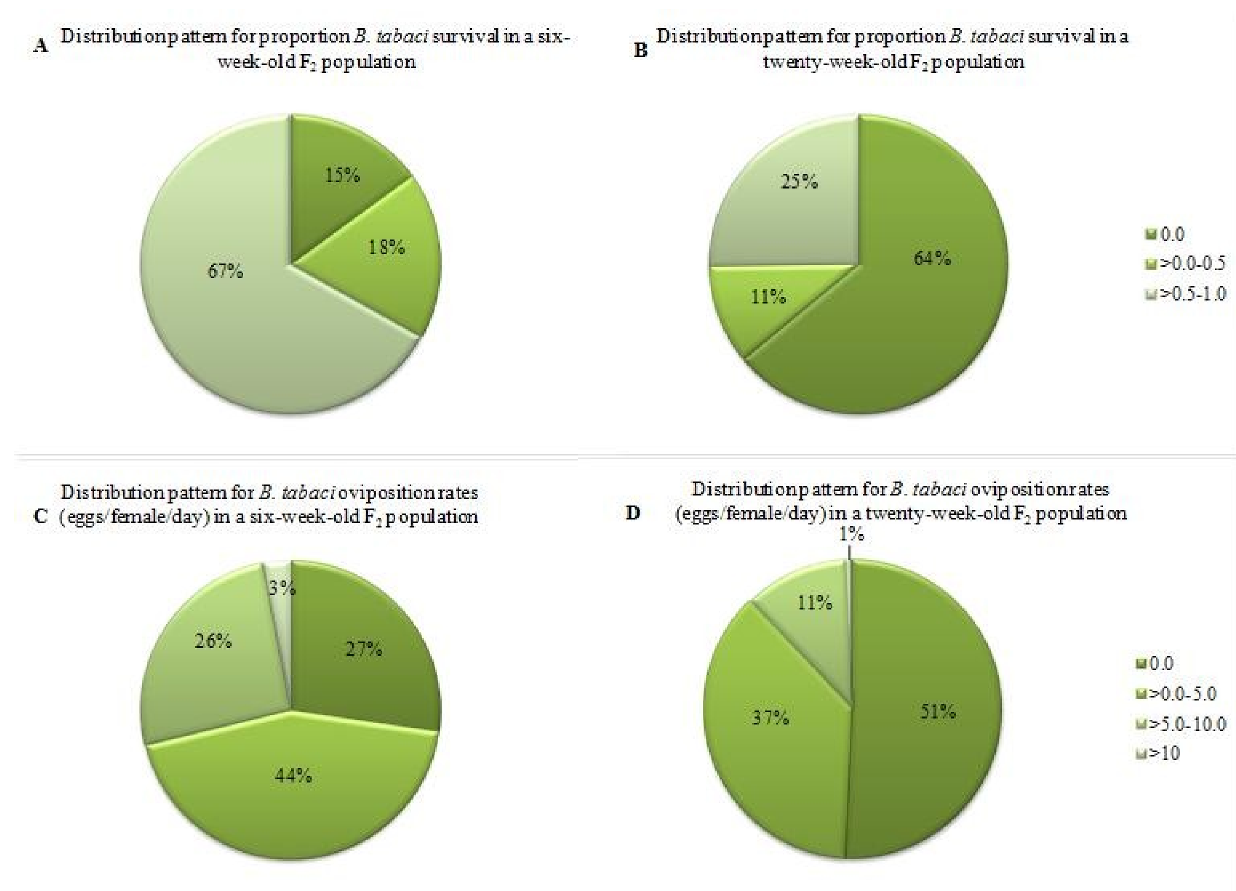


Fig 1 Adult survival and oviposition rate on young (A) and old (B) plants of an F_2 population. The population consisted of 131 plants derived from a cross between *Solanum pennellii* LA3791 and an elite cultivar. Different colors represent different phenotype classes. Phenotype classes are shown in the legend and represent adult survival rate and oviposition rate. This figure shows the percentage of F_2 plants that belong to a specific phenotype class. Phenotype classes are shown in the legend. **A** and **B** show classes for adult survival on younger (six-week-old) and older (20-week-old) plants, respectively. **C** and **D** show classes for oviposition rates of *B. tabaci* on younger and older plants, respectively.

Bemisia tabaci adult survival and oviposition depended on plant age (Fig 1). A more than four-fold increase was observed for the proportion of genotypes with no adult survival on 20-week-old plants compared to six-week-old plants. (Fig 1). There were also many more genotypes in 20-week-old plants on which no eggs were deposited five days after the start of the infestation. The number of 20-week-old genotypes on which no survival was observed was higher than the number of 20-week-old genotypes on which no egg deposition was observed (scoring 69 to 84 out of 131, respectively), meaning that there are plants on which *B. tabaci* was capable of depositing eggs, but resistance factors caused mortality of adults within five days. Parental accession *S. pennellii* LA3791 and reference accessions *S. habrochaites* LA1777 and *S. pennellii* LA716 showed no survival and no egg deposition on both six-and 20-week-old plants (data not shown).

Selection of most resistant and susceptible F_2 phenotypes

Genotypes were ranked according to the phenotyping data on both the six-week-old and 20-week-old plants (see Materials and Methods).This phenotype-based selection resulted in two groups of each ten genotypes that differed in *B. tabaci* oviposition and adult survival rates on both six- and 20-week-old plants (Fig 2). The susceptibility level in the susceptible F_2 genotype group is lower than the susceptibility level of reference cv. Moneymaker (data not shown) indicating that these genotypes still possess some resistance. However, a clear difference in *B. tabaci* survival and oviposition was present between phenotype bulks, enabling the study of important biochemical susceptibility and resistance related factors among these two groups.

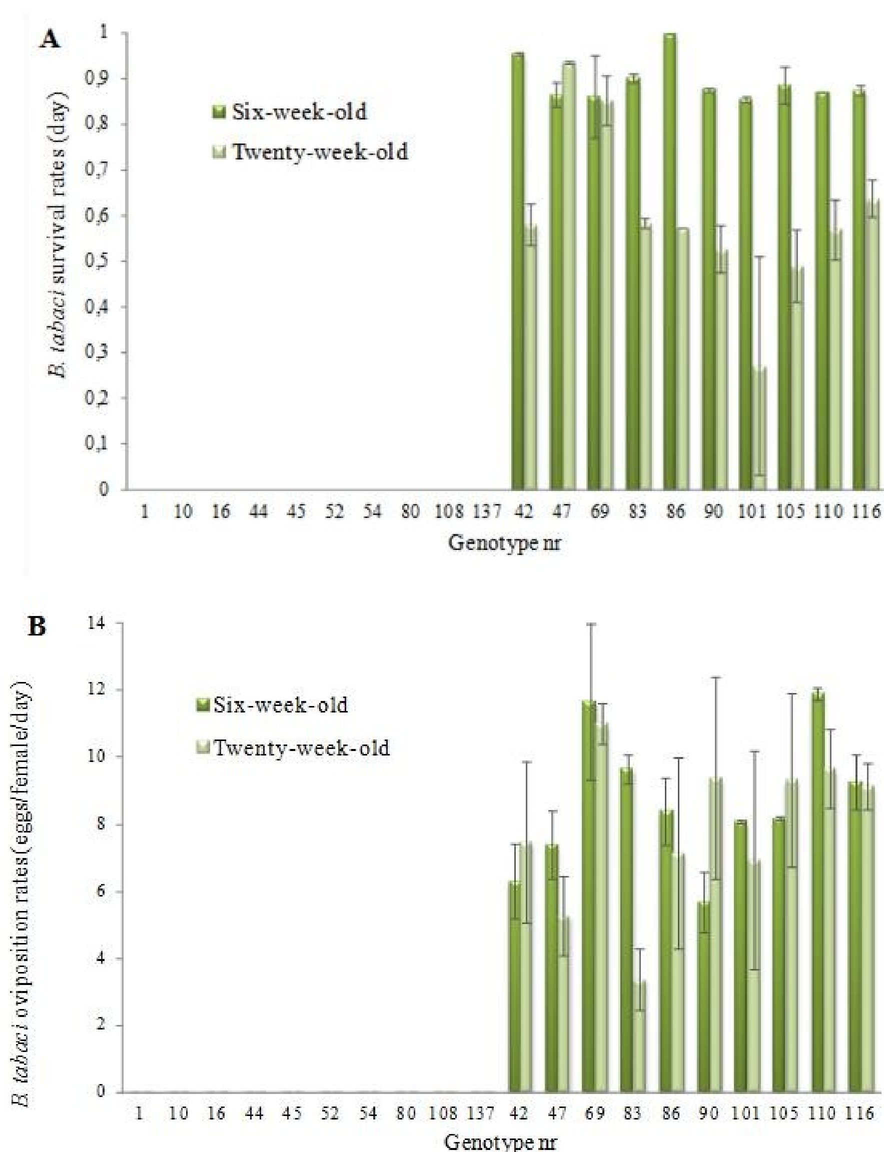


Fig 2 Survival (A) and oviposition (B) rates of adult *Bemisia tabaci* on young and old plants of the selected F_2 genotypes derived from a cross between *Solanum pennellii* LA3791 and an elite cultivar. Genotype numbers 1, 10, 16, 44, 45, 52, 54, 80, 108, and 137 were classified resistant and genotype numbers 42, 47, 69, 83, 86, 90, 101, 105, 110, and 116 were classified susceptible.

Hierarchical clustering analysis based on the metabolomic profiles of resistant and susceptible F_2 genotypes

Bemisia tabaci resistant and susceptible genotypes were subjected to volatile, semi-volatile and non-volatile metabolite analyses. Aqueous methanol extracts were measured using an LC-TOF-MS platform (n=297 compounds), while organic solvent extracts were analyzed on GC-MS platform (n=146 compounds). Datasets were merged to study correlations between chemical components and *B. tabaci* resistance/susceptibility and to identify correlations between compounds measured by the different platforms. A heatmap combined with hierarchical clustering of both F_2 genotypes and chemical compounds from LC-TOF-MS and GC-MS is shown in Figure 3.

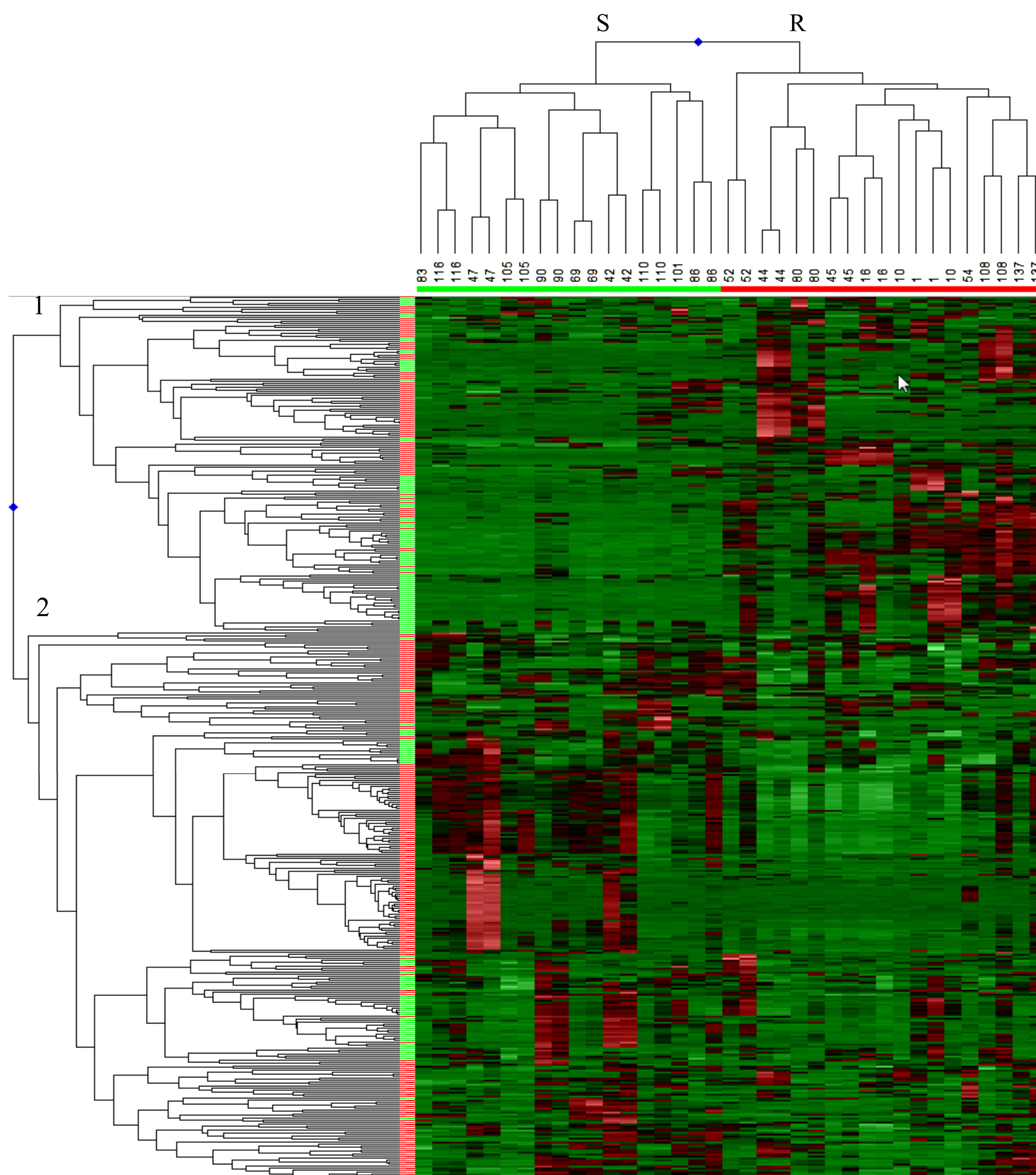


Fig 3 Hierarchical cluster analysis of *B. tabaci* resistant and susceptible F_2 genotypes based on GC-MS and LC-TOF-MS analyses. A heatmap was constructed of concatenated GC-MS and LC-TOF-MS data. Each row of the heatmap represents a single metabolite and each column represents an F_2 genotype. The peak intensity of a biochemical compound is represented as a relative concentration in red (high) and green (low). F_2 plants of the *B. tabaci* susceptible (S-cluster) and resistant phenotypes (R-cluster) are labeled by their genotype numbers on top of the heatmap; equal numbers represent biological duplicates (with exception of genotype numbers 54, 83, and 101). Two independent biological replicates per genotype were employed. Hierarchical cluster analysis was carried out by calculating the Pearson's correlation coefficient followed by UPGMA clustering. The horizontal dendrogram shows the distances between the selected F_2 plants based on their combined untargeted GC-MS and LC-TOF-MS profiles. The vertical dendrogram shows the correlation between individual biochemical compounds from the different platforms LC-TOF-MS (light red boxes in first column) and GC-MS (light green boxes in first column). Numbers one and two of the vertical dendrogram indicate the main branches.

Hierarchical clustering shows discrimination between resistant and susceptible F_2 genotypes based on their total metabolite profile. Nevertheless, besides intergroup differentiation (between the *B. tabaci* resistant and susceptible group), also intragroup differentiation (within a group) was observed. Differences in the relative abundance of metabolites were observed within both F_2 genotype bulks. Not all resistant and susceptible genotypes had the same pattern of highly abundant metabolites (Fig 3). This indicates that most likely not only a few metabolites are responsible for the resistance or/and that more than one resistance mechanism is involved. Metabolic compounds clustered in two main branches and a large number of sub-branches (Fig 3). Metabolites originating from the different analytical platforms, i.e. LC-TOF-MS and GC-MS, were not grouped in separate clusters and are combined positioned in both of the main clusters and in several of the sub-clusters and closely related compounds, meaning that there was no effect of method and that there are metabolic relationships between peaks.

Selection of metabolites involved in *B. tabaci* resistance and susceptibility

Statistical analyses were performed to select metabolites that significantly correlated with either a susceptible or a resistant phenotype. To illustrate the main compounds involved in *B. tabaci* resistance, the results of the ten most significant resistance-related or susceptibility-related metabolic compounds from FDR analyses are shown in tables 1 and 2, respectively. From the GC-MS data 74 out of the 146 compounds showed a significant correlation ($q \leq 0.05$) with resistance (n=62) or susceptibility (n=12) and from the LC-TOF-MS data 123 out of the 297 compounds showed a significant correlation with resistance (n=39) or susceptibility (n=84)(Supplementary Tables 1 and 2, respectively). These data demonstrate that a large part of the biochemical profile of the plant can contribute to the *B. tabaci* resistant/susceptible phenotype. These numbers are probably an underestimation, as intragroup differences in biochemical profiles may lead to extra variance of compounds that are exclusively expressed in a single F_2 genotype or a small part of the F_2 genotypes and thus might not appear as significant in the statistical analysis. An overestimation of correlated resistance/susceptibility compounds is also possible due to co-correlations. The majority of GC-MS compounds in Table 1 could not yet be annotated, due to either the absence of literature references for comparisons of retention times and/or insufficient similarities with confirmed compounds from the NIST Mass Spectral library. The compound that was most significantly related to *B. tabaci* resistance was identified as 2-ethyl-2-methyl butanoic acid. Furthermore, a dehydrated

sugar and dodecanoic acid were among the ten most resistant-related compounds. Susceptibility-related GC-MS compounds were tetramethyl-2-hexadecene, which was most significantly correlated with *B. tabaci* susceptibility, α-humulene, and 3,7,7-trimethyl-1,3,5-cycloheptatriene (Table 1). Table 2 includes five LC-TOF-MS metabolites identified as Acyl sugars and five yet unidentified compounds that were most significantly correlated to resistance. There were no Acyl sugars within the ten compounds most significantly correlated with susceptibility.

Table 1 GC-MS compounds most significantly associated with *Bemisia tabaci* resistance and susceptibility in tomato Comparison of means of metabolic compounds analyzed by GC-MS on two groups with either resistant or susceptible F_2 genotypes originating from a cross between *Solanum pennellii* accession LA3791 and an elite cultivar. The ten metabolic compounds that are most significantly correlated with *Bemisia tabaci* resistance (**A**) and susceptibility (**B**) are presented in order of significance. Mean and SD are given in relative peak area units.

A Top ten GC-MS compounds that significantly correlate with *B. tabaci* resistance

Retention index	CG[a]	Annotation	p-value*	q-value**	Average ± SD R-group	Average ± SD S-group	R=R>S; S=S>R
1005	1	Butanoic acid, 2-ethyl-2-methyl-	<0.0001	<0.0001	5893 ± 1177	1566 ± 151	R
1111	1	Levoglucosenone	<0.0001	<0.0001	1503 ± 379	505 ± 36	R
1536	1	Unknown	<0.0001	<0.0001	1975 ± 476	654 ± 57	R
1555	1	Dodecanoic acid	<0.0001	<0.0001	11704 ± 2899	2263 ± 1141	R
1565	1	Unknown	<0.0001	<0.0001	1206 ± 255	446 ± 26	R
1733	1	Unknown	<0.0001	<0.0001	16646 ± 4039	5657 ± 355	R
1673	1	Unknown	<0.0001	<0.0001	1205 ± 280	383 ± 32	R
1541	1	Unknown	<0.0001	<0.0001	9482 ± 2985	2917.92 ± 96	R
1745	1	Unknown	<0.0001	<0.0001	3199 ± 783	1183 ± 67	R
1070	1	Unknown	<0.0001	<0.0001	1154 ± 338	380 ± 28	R

B Top ten GC-MS compounds that significantly correlate with *B. tabaci* susceptibility

Retention index	CG[a]	Annotation	p-value*	q-value**	Average ± SD R-group	Average ± SD S-group	R=R>S; S=S>R
1839	2	Tetramethyl-2-hexadecene	<0.0001	0.0006	52130 ± 6217	72283 ± 7950	S
2316	2	Unknown	0.0013	0.0055	384 ± 32	690 ± 223	S
2290	2	Unknown	0.0023	0.0083	530 ± 54	856 ± 265	S
2290	2	Unknown	0.0050	0.0157	1178 ± 138	1796 ± 564	S
2106	2	Unknown	0.0060	0.0179	738 ± 91	1108 ± 315	S
1466	2	α-humulene	0.0079	0.0226	1934 ± 458	4816 ± 2440	S
1857	2	Unknown	0.0094	0.0258	207700 ± 17772	239407 ± 18266	S
1856	2	Unknown	0.0097	0.0261	74614 ± 6381	86655 ± 7258	S
973	2	3,7,7-trimethyl-1,3,5-cycloheptatriene	0.0117	0.0309	1184 ± 159	2519 ± 1165	S
1353	2	Unknown	0.0124	0.0322	11319± 1477	14035 ± 1766	S

[a] CG: abbreviation for compound group; numbers indicate different metabolic groups from hierarchical clustering

*p-values were calculated with a Student's t-test (MsExcel v.2010) on a Log10 transformed dataset

**p-values were corrected for multiple testing by Benjamini and Hochberg False Discovery Rate (Benjamini and Hochberg 1995); calculated q-values had a cut-off of 0.05

Table 2 LC-TOF-MS compounds most significantly associated with *Bemisia tabaci* resistance and susceptibility in tomato Comparison of means of metabolic compounds analyzed by LC-TOF-MS on two groups with either resistant or susceptible F_2 genotypes originating from a cross between *Solanum pennellii* accession LA3791 and an elite cultivar. The ten metabolites that are most significantly correlated with *Bemisia tabaci* resistance (**A**) and susceptibility (**B**) are presented in order of significance. Not annotated peaks are compounds different from Acyl glucoses and sucroses.

A Top ten LC-TOF-MS compounds that significantly correlate with *B. tabaci* resistance

Ret(min)	Mass(D)	CG[a]	Annotation	p-value*	q-value**	Average ±SD R-group	Average ±SD S-group	R=R>S; S=S>R
29.93	653	1	S3:16 II	<0.0001	<0.0001	1025± 683	56 ± 20	R
43.30	693	1	Not annotated	<0.0001	<0.0001	1303 ± 355	388 ± 64	R
39.10	132	1	S3:20	<0.0001	<0.0001	4857 ± 1322	1226 ± 457	R
41.86	594	1	Not annotated	<0.0001	<0.0001	232 ± 108	51 ± 3	R
28.12	491	1	S3:15 II	<0.0001	<0.0001	2844 ± 1688	364 ± 41	R
43.20	207	1	S3:22 IV	<0.0001	<0.0001	2381 ± 829	576 ± 93	R
41.74	723	1	S3:21 IV	<0.0001	<0.0001	52852 ± 21475	13226 ± 1563	R
38.89	579	1	Not annotated	<0.0001	<0.0001	4813 ± 1953	1060 ± 140	R
45.06	524	1	Not annotated	<0.0001	<0.0001	409 ± 138	146 ± 4	R
44.77	768	1	Not annotated	<0.0001	<0.0001	356 ± 131	124 ± 11	R

B Top ten LC-TOF-MS compounds that significantly correlate with *B. tabaci* susceptibility

Ret(min)	Mass(D)	CG[a]	Annotation	p-value*	q-value**	Average ±SD R-group	Average ±SD S-group	R=R>S; S=S>R
42.17	771	2	Not annotated	<0.0001	<0.0001	174 ± 34	334 ± 54	S
43.49	777	2	Not annotated	<0.0001	<0.0001	551 ± 103	1060 ± 203	S
39.16	723	2	Not annotated	<0.0001	<0.0001	3488 ± 1182	7392 ± 1313	S
37.44	733	2	Not annotated	<0.0001	0.0001	136 ± 15	218 ± 36	S
49.91	976	2	Not annotated	<0.0001	0.0001	438 ± 82	868 ± 224	S
46.97	759	2	Not annotated	<0.0001	0.0002	2138 ± 275	3945 ± 858	S
49.05	789	2	Not annotated	<0.0001	0.0002	3030 ± 762	6315 ± 1657	S
49.54	761	2	Not annotated	<0.0001	0.0003	485± 131	1056 ± 279	S
45.02	720	2	Not annotated	<0.0001	0.0003	323 ± 73	673 ± 160	S
47.37	946	2	Not annotated	<0.0001	0.0004	208 ± 30	404 ± 118	S

[a] CG: abbreviation for compound group; numbers indicate different metabolic groups from hierarchical clustering

*p-values were calculated with a Student's t-test (MsExcel v.2010) on a Log10 transformed dataset

**p-values were corrected for multiple testing by Benjamini and Hochberg False Discovery Rate (Benjamini and Hochberg 1995); calculated q-values had a cut-off of 0.05

Correlation of Acyl sugars with resistance

Acyl sugars were identified in the LC-TOF-MS chromatograms on the basis of their exact molecular mass (within 5 ppm mass deviation), resulting in a total of 43 different Acyl sugars, including up to five isomeric forms (same exact mass but different retention time) of e.g. S3:21 and S3:22 (data not shown). Only the sucrose type of Acyl sugars was detected in both the F_2 genotypes and parental lines. Table 3 shows the Acyl sugars that were positively correlated with resistance or susceptibility of *B. tabaci* in the selected F_2 genotypes. Sixteen Acyl sugars were more abundant in the resistant bulk, while two were more abundant in the susceptible bulk. The Acyl sugars all belonged to the metabolite cluster number 1 in Figure 3.

Table 3 Acyl sugars associated with *Bemisia tabaci* resistance and susceptibility in tomato Comparison of means of Acyl sugars analyzed by LC-TOF-MS on two groups with either resistant or susceptible F_2 genotypes originating from a cross between *Solanum pennellii* accession LA3791 and an elite cultivar. The Acyl sugars that are significantly correlated with *Bemisia tabaci* resistance (**R**) and susceptibility (**S**) are presented in order of significance.

Acyl sugar	Ret(min)	Mass(D)	p-value*	q-value**	Average ±SD R-group	Average ±SD S-group	R=R>S; S=S>R
S3:16 II	29.93	653	<0.0001	<0.0001	1025 ± 683	56 ± 20	R
S3:20	39.10	1327	<0.0001	<0.0001	4857 ± 1322	1226 ± 457	R
S3:15 II	28.12	491	<0.0001	<0.0001	2844 ± 1688	364 ± 41	R
S3:22 IV	43.20	207	<0.0001	<0.0001	2381 ± 892	576 ± 93	R
S3:21 IV	41.73	723	<0.0001	<0.0001	52852 ± 21475	13226 ± 1563	R
S3:16 I	29.93	653	<0.0001	<0.0001	3376 ± 2060	579 ± 25	R
S3:22 V	43.51	1383	<0.0001	0.0003	10673 ± 4542	2571 ± 671	R
S3:15 I	27.66	630	0.0002	0.0011	2083 ± 1063	568 ± 146	R
S3:22 I	41.63	721	0.0006	0.0028	17260 ± 5216	27961 ± 4562	S
S3:18 IV	34.28	681	0.0010	0.0044	123 ± 121	32 ± 0	R
S3:21 II	40.62	129	0.0014	0.0056	1131 ± 389	455 ± 121	R
S4:22 I	40.67	855	0.0018	0.0068	137 ± 11	202 ± 50	S
S4:24 I	45.04	780	0.0039	0.0129	307 ± 288	58 ± 1	R
S3:22 II	42.04	691	0.0043	0.0139	1075 ± 506	455 ± 93	R
S3:14 I	25.78	495	0.0121	0.0316	979 ± 565	410 ± 73	R
S3:18 II	35.35	682	0.0126	0.0324	94 ± 54	45 ± 1	R
S3:14 II	26.18	626	0.0153	0.03808	569 ± 525	124 ± 35	R
S3:14 III	26.45	579	0.0152	0.0381	2814 ± 2211	571 ± 64	R

*p-values were calculated with a Student's t-test (MsExcel v.2010) on a Log10 transformed dataset
**p-values were corrected for multiple testing by Benjamini and Hochberg False Discovery Rate (Benjamini and Hochberg 1995); calculated q-values had a cut-off of 0.05

Bemisia tabaci life-history parameters for leaves with and without intact glandular trichomes

The role of glandular trichomes in *B. tabaci* resistance was analyzed by studying the effect of trichomes on two different *B. tabaci* life-history parameters. An adult survival curve was made to study the difference between *B. tabaci* adult survival on *S. pennellii* accession LA3791 with and without intact glandular trichomes and to compare these results with the susceptible cv. Moneymaker. Furthermore, egg deposition rates were measured on *S. pennellii* and cv. Moneymaker with and without intact glandular trichomes. Removal of glandular trichome exudates made *S. pennellii* more susceptible to *B. tabaci* and adult survival rates were significantly higher on EtOH-treated *S. pennellii* leaflets on all four scoring days (Fig 4a). The EtOH-treatment did not affect whitefly survival on reference cv. Moneymaker when compared to H_2O-treated plants. A difference in adult survival was observed between EtOH- and H_2O-treated cv. Moneymaker and EtOH-treated *S. pennellii* for every single examination day. Female whiteflies deposited significantly more eggs on *S. pennellii* leaves without trichomes compared to control *S. pennellii* leaves but no differences were observed in oviposition rates on control and trichomeless cv. Moneymaker leaves (Fig 4b).

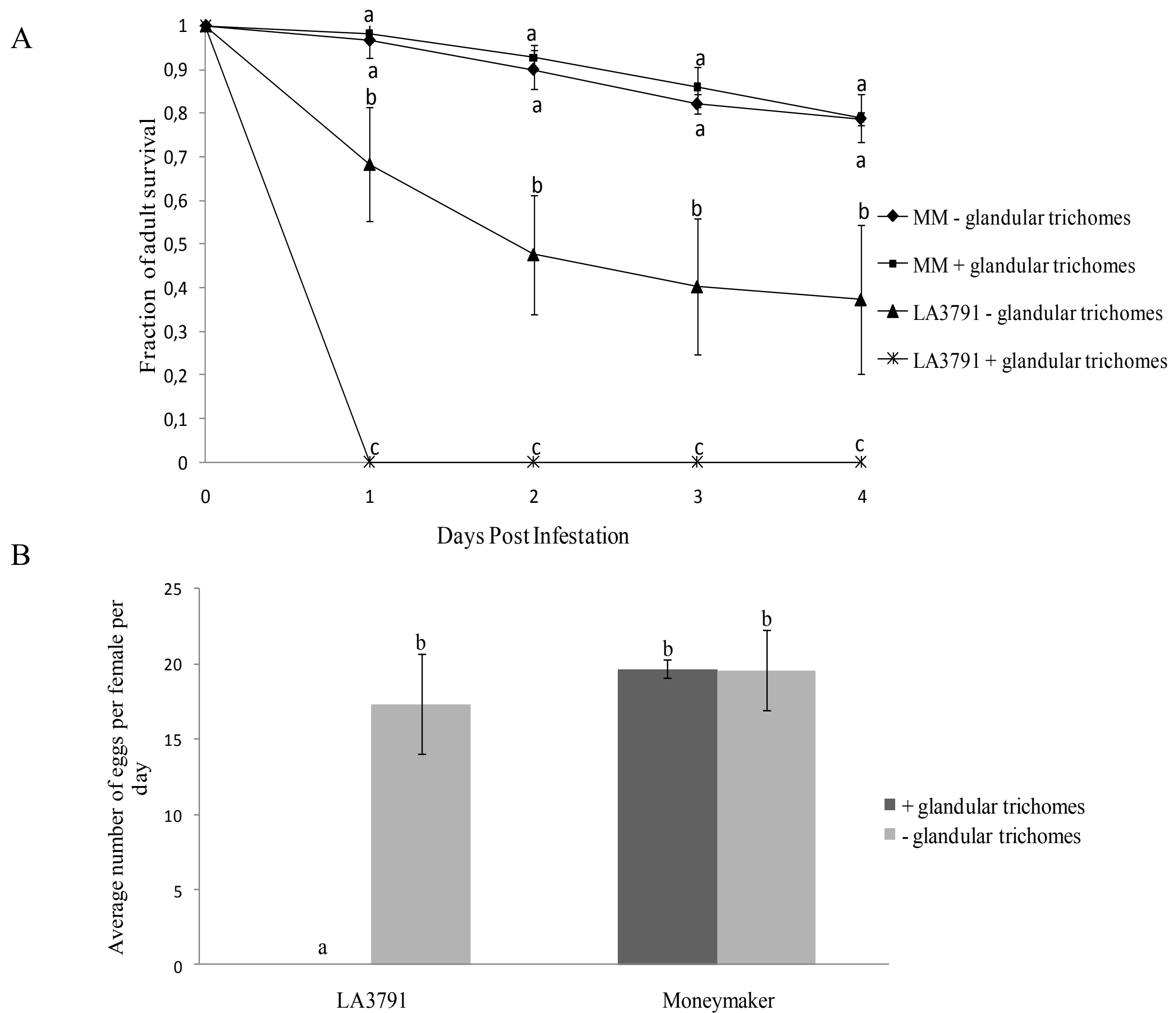


Fig 4a and b. Fig 4a Adult survival curves of *B. tabaci* on *S. pennellii* LA3791 and *Solanum lycopersicum* cv. Moneymaker with and without intact trichomes. Adult survival was monitored during a time frame of four subsequent days. Trichome removal was done by 96% EtOH treatment; controls were rinsed in dH_2O. Values are means ± SEM of the fraction of living adult whiteflies. Different letters indicate significant differences between the numbers of living adults per day. **Fig 4b Effect of trichome removal from *S. pennellii* LA3791 and *S. lycopersicum* cv. Moneymaker on *B. tabaci* oviposition.** Oviposition on cv. Moneymaker and *S. pennellii* with and without intact trichomes after five days of infestation. Values are means ± SD of the number of eggs produced by one female in a period of five days. Different letters indicate significant differences between treatments and species.

Discussion

Antibiosis explains *B. tabaci* resistance in *S. pennellii* cv LA3791

We used an F_2 interspecific cross of *S. pennellii* x *S. lycopersicum* to analyze *B. tabaci* survival and oviposition rates. Quantitative differences were observed in the F_2 population for both parameters (Fig 1). Distribution patterns suggest that the *B. tabaci* resistance of *S. pennellii* LA3791 is under the control of small number of genetic loci, because a large number of F_2 genotypes showed to be partially or completely resistant judging from adult survival and reproduction rates. This assumption is accurate, provided that the alleles segregated according to the Hardy-Weinberg equilibrium (Stern 1943). Distortion of segregation resulting in an overrepresentation of *S. pennellii* genes might result in biased distribution patterns, a phenomenon that often occurs in interspecific crosses (Foolad 1996; Shirasawa et al. 2010). An alternative explanation for the high number of resistant and partially resistant F_2 genotypes can be that several resistance mechanisms are present in *S. pennellii* that act independently and also segregate independently. The latter hypothesis is supported by our GC-MS and LC-TOF-MS analyses that showed differences in metabolic fingerprints among F_2 genotypes within the *B. tabaci*-resistant group and among F_2 genotypes within the *B. tabaci*-susceptible group (Fig 3).
At this stage it cannot be concluded that either a single resistance mechanism or multiple resistance mechanisms underlie the *B. tabaci* resistant phenotype. Partial resistance might indicate that the accumulation of a single toxic or deterrent compound is lower in these genotypes, causing them to be less resistant to *B. tabaci.* Still, it is clear that antibiosis is the main factor explaining the resistance in our population as mortality and reduced oviposition within a short time span indicate the presence of toxic factors and metabolic fingerprinting showed high correlations between resistance and a range of metabolic compounds (Fig 3, Tables 1-3).

The F_2 distribution patterns provided information about the resistance mechanism. *Bemisia tabaci* adult survival was scored after five days of infestation and, therefore, it is not clear whether the resistance mechanism is induced or constitutive. From the survival data it was impossible to conclude upon underlying resistance mechanisms with regard to induced or constitutive resistance. However, when considering the oviposition data, it was observed that there was a substantial number of genotypes on which zero oviposition was scored after five

days of female infestation. *Bemisia tabaci* females do not always reproduce immediately after they emerge from the pupae (McAuslane 2000), but since six- to eight-day-old females were selected for this experiment, this effect does not play a role. Therefore, the results demonstrate that the observed resistance in part of the F_2 genotypes is either constitutive or induced very rapidly because females residing on these genotypes were not able to reproduce from the very beginning.

***Bemisia tabaci* resistance in *S. pennellii* LA3791 F_2 progeny depends on host plant age**

Overall, host-plant resistance strongly differed between young (six weeks) and old (20 weeks) plants of the F_2 population (Fig 1). Our results showed a more than four-fold increase in genotypes with a fully resistant phenotype for *B. tabaci* survival in older plants and an almost two-fold increase in the total number of genotypes where no oviposition was observed. Plant age was the most coherent variable that explained the differentiation in *B. tabaci* life-history parameters as all other factors, including leaf-stage and environmental conditions were standardized. Our observations are in line with results from other studies on the effect of host plant age on resistance and metabolic composition. Leite et al. (2001) studied the effect of plant age on the resistance of *S. habrochaites* to the leafminer *Tuta absoluta* and found that mortality of larvae and length of the larval period were higher on older plants of *S. habrochaites*, which was correlated with an increase in the levels of 2-tridecanone. Slocombe et al. (2008) observed an increase in Acyl sugar accumulation from young to old leaves in *N. benthamiana* and found an increase in Acyl sugar-associated fatty acid accumulation in *S. pennellii* intermediate leaves when compared to the youngest leaves. Broekgaarden et al. (2012) showed that antibiosis against the cabbage whitefly (*Aleyrodes proletella*) was stronger on 12-week-old plants of *Brassica oleracea* cv Rivera compared to six-week-old plants. The source of resistance was assessed by monitoring the feeding behavior of the whitefly with an electrical penetration graph method and it was found that phloem-specific factors, possibly chemically-based, hampered the whiteflies' feeding (Broekgaarden et al. 2012). In our population, it was observed that not all F_2 genotypes showed the age-dependent effect, which might indicate that these plants lack specific genes to elicit the age-dependent response.

From a theoretical point of view, it would be most efficient when plants allocate defense-associated metabolites to valuable plant parts during development to optimally protect themselves and enhance their fitness (López-Gresa et al. 2012; Kaur et al. 2010). It could be

that this also explains the differences in resistance against *B. tabaci* between young and old plants in a substantial number of genotypes in the population that was studied here. Our findings are in line with a study by López-Gresa et al. (2012) who performed metabolic fingerprinting of tomato plants infected with Tomato Mosaic Virus (ToMV) and identified metabolites involved in the plant defence response and metabolites whose accumulation was dependent on the plant's developmental stage. It would be interesting to investigate if *B. tabaci*-transmitted tomato viruses also trigger such defence responses in tomato and how the differences in metabolic composition during different developmental stages relate to the differences observed for *B. tabaci* resistance in *S. pennellii*.

Trichome content of *S. pennellii* LA3791 determines *B. tabaci* survival and oviposition.

We demonstrated that the content of glandular trichomes from *S. pennellii* accession LA3791 highly correlates with *B. tabaci* resistance (Fig 4a and b). Adult mortality was 100% on wild-type plants with intact trichomes. *Bemisia tabaci* survival was much higher on *S. pennellii* with removed glandular trichomes when compared to intact *S. pennellii* plants for every single test day. *Solanum pennellii* LA716 leaflets treated with ethyl- and methyl alcohol are capable of regenerating exudate droplets 48h after treatment (Goffreda et al. 1989; Goffreda et al. 1988), which might explain the lower survival of *B. tabaci* adults on *S. pennellii* LA3791 with removed glandular trichomes after two days of infestation. However, a decline in adult survival was also observed on day one and two after infestation, which might imply the presence of residual amounts of trichome content on the leaves of tomato. Trichomes of *S. pennellii* contain Acyl sugars, which are sticky substances (Fobes et al. 1985), and these compounds may not have been washed away completely due to incomplete solvability. However, it also cannot be completely excluded that additional resistance factors in other tissues besides the glandular trichomes are present in the leaves. Oviposition by *B. tabaci* was zero on water-treated *S. pennellii* LA3791, but the number of eggs deposited per female whitefly per day on ethanol-treated *S. pennellii* was not different from the ethanol- and water-treated reference plants, which indicates that glandular trichome exudates are the sole cause for impaired *B. tabaci* oviposition on *S. pennellii* LA3791. Similar trichome removal experiments have been done on *S. pennellii* for several insect pest species belonging to different insect orders. Upon trichome removal, Potato Aphid *Macrosiphum euphorbiae* on *S. pennellii* LA716 had a reduced settling and a modified feeding behaviour (Goffreda et al. 1988), the mortality rates of Green Peach Aphid *Myzus persicae* were reduced on three

different *S. pennellii* accessions (Simmons et al. 2003), the mortality and entrapment of Cotton Bollworm *Helicoverpa armigera* was lower on different *S. pennellii* accessions (Simmons et al. 2004), and the number of leaf punctures and mines by leafminer *Liriomyza trifolii* were increased on *S. pennellii* LA716 compared to the control plants (Hawthorne et al. 1992).

A large number of metabolites is associated with *B. tabaci* resistance and susceptibility in tomato

Previous work on *S. pennellii* accession LA716 or genotypes derived from this accession targeted solely whole Acyl sugar or fatty acid composition of the plant with respect to whitefly resistance (Resende et al. 2009; Resende et al. 2002; Mutschler et al. 1996; Liedl et al. 1995; Leckie et al. 2012; McDowell et al. 2011). In our study, a clear differentiation between *B. tabaci* resistant and susceptible genotype bulks based on their untargeted metabolic profiles was found (Fig 3). Because all biochemical compounds had an equal weight in the cluster analyses, it can be hypothesized that a substantial part of the studied biochemical components can affect *B. tabaci* life-history parameters, which was evidenced by the large number of metabolic components that contributed to a whitefly resistant or susceptible phenotype (Supplementary Tables 1 and 2) amongst which a number of Acyl sugars (Table 3). Many resistance traits are prone to environmental influences which can cause variation amongst biological replicates. With the exception of genotype numbers one and ten, all biological replicates were within close distance of one another, demonstrating the biochemical resemblance between F_2 material derived from cuttings that were positioned at random locations in the greenhouse, indicating that the genotype effect surpassed the environmental effect for the overall studied metabolic traits.

Hierarchical clustering shows structuring of metabolic groups

Two metabolic groups were formed (Fig 3) by hierarchical clustering of pooled compounds resulting from GC-MS and LC-TOF-MS analysis. Acyl sugars and Acyl sugar precursors were among the metabolites in the upper cluster (group 1), while tetramethyl-2-hexadecene, monocyclic sesquiterpene α-humulene, and 3,7,7-trimethyl-1,3,5-cycloheptatriene were amongst the metabolites in the lower cluster (group 2). Although identification could not be ascertained for the larger part of the metabolites it was clear that the ten most resistance-

related compounds from both platforms were within the upper cluster, while the ten most susceptibility-related compounds were grouped within the lower cluster (Tables 1 and 2).
Acyl sugars were among the ten most resistance-related compounds recorded by LC-TOF-MS. This is in line with previous work that showed a relation between Acyl sugars in relation to *B. tabaci* resistance (Liedl et al. 1995; Leckie et al. 2012). However, previous work addressed the total Acyl sugar content in relation to resistance (Mutschler et al. 1996; Blauth et al. 1998). Here, we show the relation of the individual Acyl sugars from the whole Acyl sugar spectrum in our population and identified 16 Acyl sugars with sucrose groups that were present in higher amounts in the resistant genotype group and two that were present in higher amounts in the susceptible genotype group (Table 3). In contrast to the Acyl glucoses identified by Liedl et al. (1995) we only identify Acyl sucroses in our genotypes. Many other compounds were detected that differ among the resistant and susceptible group. However, many of them cannot be annotated yet; they may be part of unknown novel resistance mechanisms.

Different biochemical profiles can lead to full resistance and susceptibility against *B. tabaci*

Intragroup (within the resistant group and within the susceptible group) differences in metabolic profiles were observed in our dataset (Fig 3). These results give strong indications that resistance/susceptibility mechanisms differ among genotypes. Different combinations of metabolites can result in full resistance. Our data show that not all susceptibility- or resistance-related constituents are essential for the desired phenotype. Unraveling of the resistance mechanisms into its components and limiting the complexity of the trait will facilitate resistance breeding. However, the number of different metabolites associated with resistance is large and we still need to structure these into different pathways to make good choices for breeding targets. This may open new options for breeding of tomato for resistance to *B. tabaci.*

Acknowledgements

This project was financially supported by the Technical Top Institute of Green Genetics (TTI-GG; Resistance mechanisms against whitefly in tomato project: 1C005RP), Monsanto Vegetable Seeds (Bergschenhoek, The Netherlands), Nunhems NL (Nunhem, the Netherlands),

and Wageningen University and Research Centre. The contribution of Dr. Ric de Vos was partially funded by the Netherlands Metabolomics Centre and the Centre of Biosystems Genomics, which are both part of the Netherlands Genomics Initiative / Netherlands Organization for Scientific Research. We also are grateful to Betty Henken for technical assistance in metabolomic and greenhouse work.

CHAPTER 3

Genetic study of quantitative resistance traits against *Bemisia tabaci* in tomato

Floor van den Elsen[1,4,5], Adriaan W. van Heusden[1], Colette Broekgaarden[1], Roland Mumm[2,3], Ric C.H. de Vos[2,3,6], Marcel Dicke[4], Ben Vosman[1]

[1]Wageningen UR Plant Breeding, Wageningen University and Research Centre, P.O. Box 386, 6700AJ, Wageningen, The Netherlands.
[2]Plant Research International, Business Unit Bioscience, Wageningen University and Research Centre, P.O. Box 619, 6700AP Wageningen, The Netherlands.
[3]Centre for Biosystems Genomics, P.O. Box 98, 6700AB, Wageningen, The Netherlands.
[4]Laboratory of Entomology, Wageningen University and Research Centre, P.O. Box 8031, 6700EH, Wageningen, The Netherlands.
[5]Graduate School Experimental Plant Sciences, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.
[6]Netherlands Metabolomics Centre, Einsteinweg 55, 2333 CC, Leiden, The Netherlands

Abstract

Solanum pennellii shows resistance towards the whitefly *Bemisia tabaci*. A mapping approach was employed to elucidate the genetic background of whitefly resistance traits and associated biochemical traits. This was done by phenotyping and metabolic fingerprinting of an F_2 population originating from a cross between a susceptible tomato cultivar and a completely resistant *S. pennellii* accession. Minor quantitative trait loci (QTLs) for adult survival and oviposition were identified on chromosomes IV, VI, X, and XI, which almost all co-localized with resistance-related biochemical traits. The exception was the phenotype QTL on chromosome VI. Some of the QTLs were confirmed in an F_2BC_1 population and showed strongly increased percentages of explained variances. The results demonstrate the direct genetic correlations between biochemical-based resistance characteristics and reduced whitefly incidence in *S. pennellii*.

Keywords: *Bemisia tabaci*, *Solanum pennellii*, metabolic fingerprinting, genetic linkage map, life-history parameters, AFLP and SNP markers.

Introduction

Bemisia tabaci biotype B, recently taxonomically reclassified as the Middle-East-Asia Minor 1 species (Dinsdale et al. 2010), is a virus-transmitting hemipteran with a wide host range (Brown et al. 1995). It is among the world's one-hundred most invasive species (www.issg.org/database) and has devastating effects on many crop and ornamental plant species (Vazquez et al. 1997; Williams et al. 1996). There is a demand for the development of sustainable control strategies to reduce direct damage of this pest by phloem consumption, honeydew secretion, and uneven ripening of fruits (Matsui 1992; Schuster 2001; Schuster et al. 1995) as well as indirect damage by viral disease transmission and fungal growth on the honeydew (Oliveira et al. 2001). At present, there are several *B. tabaci* control methods, but these are either unsustainable or less effective in the open field. Current control of *B. tabaci* in the field is predominantly based on pesticide application, but the effectiveness of chemical pest control is declining, since *B. tabaci* has become resistant against a broad range of chemical compounds (Crowder et al. 2010; Feng et al. 2010; Fernandez et al. 2009; Roditakis et al. 2009). Also the negative toxic effect of chemicals on beneficial non-target insects, whole ecosystems, and the environment requires the implementation of alternative *B. tabaci* control methods (He et al. 2012; Nash et al. 2010). Currently, deployment of biocontrol agents is a successful alternative strategy in glasshouses to keep the population size at low levels (Lykouressis et al. 2009; Calvo et al. 2009; Cuthbertson et al. 2007; Cuthbertson and Walters 2005; Vidal et al. 1998; Van Lenteren 2000; Roermund and Van Lenteren 1996). However, this method is difficult to adopt in field and semi-field situations and does not prevent viral transmission, although it might lead to reduced disease incidence (Smyrnioudis et al. 2001). Another promising alternative approach for *B. tabaci* control is breeding for durable host-plant resistance (McDonald and Linde 2010). All of the cultivars of tomato (*Solanum lycopersicum*) are susceptible to this pest, although there is variation in susceptibility level (Heinz and Zalom 1995). A number of wild relatives of the cultivated tomato are resistant to whiteflies (Baldin et al. 2005; Liedl et al. 1995; Nombela et al. 2000; Muigai et al. 2003; Muigai et al. 2002; Sanchez-Pena et al. 2006; Firdaus et al. 2012) and can serve as donor material in breeding programs. The resistance mechanisms identified so far are biochemically-based, concerning mostly Acyl sugars, methylketones, and sesquiterpenes with antixenosis (affecting the behavior of an insect) and antibiosis (affecting the fitness of the insect) as modes of action (Antonious and Kochhar, 2003; Bleeker et al. 2009; Bleeker et al. 2011; Freitas et al. 2002; Resende et al. 2009; Liedl et al. 1995; Muigai et al. 2003; Nombela

et al. 2000; Antonious et al. 2005). Since these wild relatives are crossable with tomato it is worth trying to introduce the resistance via introgression breeding. This introgression of quantitative trait loci (QTLs) into elite tomato lines might lead to a sustainable and effective pest control. Effective vector control might also concomitantly result in reduced viral disease incidence (Bellows and Arakawa 1986; Rodriguez-Lopez et al. 2011).

In the case of *Solanum pennellii*, cross-compatibility enables interspecific hybridization with *S. lycopersicum* (Rick 1951; Liedl et al. 1995). Interspecific crosses between *B. tabaci* resistant tomato wild relatives and susceptible cultivars enable the development of mapping populations which can be utilized for the detection of QTLs for whitefly resistance. Analyzing F_2 populations derived from different *S. habrochaites* donor plants has resulted in the identification of QTLs related to whitefly resistance (Momotaz et al. 2010; Maliepaard et al. 1995).

In chapter 2 we demonstrated the importance of secondary metabolites in whitefly resistance and susceptibility in an F_2 population of donor parent *S. pennellii* accession LA3791 crossed with an elite cultivar. The objective of the present study was to explore the genetic background of these traits by using a linkage mapping approach. The above-mentioned F_2 population was employed for metabolite and phenotypic QTL analyses and two F_2BC_1 populations were used to confirm the phenotypic QTLs identified in the F_2 population. This is the first paper that reports on phenotypic QTLs that relate to bionomic traits, like *B. tabaci* life-history parameters, in *S. pennellii* and their association with metabolite QTLs for resistance and susceptibility. The main goals of this study were to identify chromosomal regions associated with resistance/susceptibility to *B. tabaci* and abundance of metabolites as well as to analyze whether there is overlap in metabolic and phenotypic quantitative trait loci. This may suggest a relation between metabolites and resistant/susceptible phenotypes and may provide clues to the underlying mechanism(s) for whitefly resistance. Metabolite mapping studies have been performed earlier in F_2 breeding populations with *S. pennellii* LA716 as the donor parent, resulting in the identification of loci related to the biosynthesis of Acyl sugars and fatty acids (Mutschler et al. 1996; Blauth et al. 1999; Blauth et al. 1998). These studies used targeted approaches, we used an untargeted approach by surveying complete Gas Chromatography-Mass Spectrometry (GC-MS) profiles of tomato genotypes. The untargeted metabolomics approach allowed us to study the biological relevance of a large number of unexplored individual metabolites in whitefly resistance/susceptibility. Also, identifying susceptibility-related loci by mapping of *B. tabaci* susceptibility-related metabolites was another objective of our present study.

Materials and Methods

Plant material

An interspecific cross was made between *S. pennellii* accession LA3791 and an elite tomato (*S. lycopersicum*) cultivar To6W_LI0620 (hereafter referred to as EC), which was made available by Nunhems NL, Nunhem, The Netherlands. One F_1 plant was selfed to produce an F_2 population. Hundred and thirty-one out of 170 F_2 seeds germinated and were grown for phenotyping and chemoprofiling. Two F_2BC_1 populations were produced by backcrossing two fully whitefly resistant F_2 genotypes with EC. One hundred and fifty four plants were grown of one F_2BC_1 population (originating from F_2 genotype 12; hereafter referred to as F_2BC_1(12)) and 115 plants for the other F_2BC_1 population (originating from F_2 genotype 44; hereafter referred to as F_2BC_1(44)). Growing conditions for the F_2 and F_2BC_1 populations were as follows: Seeds were sown in potting trays on soil substrate for flowering plants (Lentse Potgrond®). One-week-old seedlings were transplanted into pots (Ø 20cm) with the same soil substrate. Plants were grown under controlled conditions in a glasshouse (22 ±2°C, L16:D8 photoperiod, RH about 50%) and watered daily. For chemoprofiling, six cuttings per individual F_2 genotype were made from ten-week-old unchallenged plants and grown in trays on soil substrate. Subsequently, two cuttings per F_2 genotype were transferred to soil in pots (Ø 20cm), and grown in an insect- and pathogen-free environment (22 ±2°C, L16:D8 photoperiod, RH about 50%). No chemical pathogen- or pest control was practiced during growing, screening, and sampling of the test plants.

Whiteflies

Bemisia tabaci Middle-East-Asia Minor 1 was reared on *S. lycopersicum* cv. Moneymaker in the glasshouse under controlled conditions (26 ±2°C, L16:D8 photoperiod, RH 60±10) at the Laboratory of Entomology, Wageningen University. The colony commenced from a single parthenogenetic female. An allelic discrimination real-time PCR assay was performed on randomly sampled individuals (according to Jones et al. 2008), which confirmed that the rearing was of the Middle-East-Asia Minor 1species. Detached cv. Moneymaker leaves with 1st to 4th instar nymphs were placed in a gauze insect cage containing three-week-old cv. Moneymaker plants to provide newly emerging adults with young leaves and to facilitate the synchronization of adults for phenotyping experiments. After three days, one-to-three-day-old adults were collected from the insect cage and anaesthetized with a gas mixture (N_2:H_2:CO_2 [80:10:10]; Linde Gas Benelux) to facilitate the selection of either both sexes of adults for

whitefly survival assays or females for whitefly fecundity assays before transfer of whiteflies to the test plants.

Phenotyping

Environmental parameters were optimized for *B. tabaci* (26 ±2°C, L16:D8 photoperiod, RH 60±10) one week prior to the beginning of phenotyping experiments. The F_2 and F_2BC_1 genotypes were tested for *B. tabaci* adult survival and oviposition rates in a no-choice experimental design. Besides the resistant parent *S. pennellii*, also the susceptible tomato cultivar Moneymaker was included as reference material during the F_2 population screening. The F_2BC_1 populations were tested with their recurrent parent EC and *S. pennellii*.
Three plants per reference were screened and these replicas were randomly positioned between the F_2 and F_2BC_1 plants. Survival and oviposition rates of *B. tabaci* were determined on both six- and 20-week-old plants for the F_2 population and six-week-old plants for the F_2BC_1 populations.

Adult survival rate Twenty unsexed one-to-three-days-old *B. tabaci* adults were selected under a stereomicroscope (Zeiss). Selected adults were transferred to the abaxial side of a third internode leaf in a fine-meshed clip-on cage (Ø 25mm) with rubber membranes at the leaf interface to prevent mechanical leaf damage. The third internode leaf was chosen as younger leaves are preferred over older leaves by the whitefly for feeding and oviposition (Liu and Stansly 1995). Each individual F_2 or F_2BC_1 (n=1) genotype and each reference plant (n=3) was challenged with two clip-on cages containing 20 adult *B. tabaci* each. Adult survival was scored under a stereomicroscope five days post infestation. Adult survival was calculated per clip-on cage according to Van Giessen et al. (1995) and Bas et al. (1992) by the following equation:

$$\text{Adult survival} = \left(\frac{m}{n}\right)^{1/d} \text{/day}$$

where *d* is the number of days (five days), *n* the total number of whiteflies per clip-on cage, *m* the number whiteflies alive after *d* days.

Oviposition rate Five six- to eight-day-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 genotype (n=1) and each reference plant (n=3) was challenged with two clip-one cages containing five female *B. tabaci* each. Leaves were cut off after five days of infestation and the total number of females, the number of living females, and the number of eggs were counted under a stereomicroscope. Oviposition rate was calculated per clip-on cage according to Van Giessen et al. (1995) and Bas et al. (1992) by the following equation:

$$\text{Oviposition rate} = \frac{2e}{d(m+n)} \text{ eggs/female/day}$$

where *e* is the number of eggs, *d* the number of days (five days), *n* the total number of females per clip-on cage, *m* the number females alive after *d* days. Averages and standard deviations were calculated for the duplicates per genotype.

Leaf sample preparation for metabolomics

Two cuttings per F_2 genotype plus *S. pennellii* and cv. Moneymaker were distributed over the glasshouse in a Randomized Block design. The environmental parameters were adjusted one week prior to the collection of leaf material for biochemical profiling (26 ±2°C, L16:D8 photoperiod, RH 60±10), to standardize the settings used during phenotyping experiments. The third internode leaves of six-week-old uninfested plants were cut off, packed in aluminum foil thereby preventing damaging the leaf tissue, and instantly transferred to liquid N_2 (-196°C). Leaf samples were stored at -80°C until analysis in GC-MS measurements.

Chemical analysis of leaf material of F_2 population

To identify the variation in volatile and semi-volatile secondary metabolites extracts of leaf material, all individuals of the F_2 population plus references were analyzed by GC-MS. Samples for GC-MS analysis were prepared as follows: frozen leaf material (FDW: 300mg±10mg) 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 (CH_2Cl_2; DCM), including 75 µl (1 mg/ml)/100 ml DCM) heptadecanoic acid methyl ester ($CH_3(CH_2)15COOH$ as internal standard (IS), was added to the frozen leaf powder, vortexed (30s), and centrifuged (10 min. 1500 rpm). The DCM phase was collected into a new 20 ml glass tube. One ml of DCM was added to the residual solid- and water-phase in the initial glass tube, vortexed (30 s), and centrifuged (10 min. 1500 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). Extracts were analyzed by GC-MS (5975C inert Mass. Selective Detector with Triple-Axis Detector and 7890A Gas Chromatograph system, Agilent Technologies, USA) with a splitless program (GS_TERP_10°MIN_SD4,4_5MIN300_splitless). Duplicates of each genotype (with the exception of genotype numbers 54, 86, and 101, for which only one sample was available) were injected in the GC-MS machine in reverse sequence. Controls DCM, DCM plus IS, *S. pennellii*, and cv. Moneymaker were included daily in the course of the measurements.

The GC-MS data were pre-processed, using the software program metAlign (Lommen 2009). This included dataset al.ignment, baseline correction (minimum row value set to 150) and noise elimination (sample above noise set to four). The total ion current was plotted against mass scan number to obtain Total Ion Chromatogram *(*TIC*)* output files, which contained 10910 out of the original 19403 mass peaks. Clustering of mass peaks from the TIC output file into centrotypes (representing putative metabolites) was carried out with MSClust software (Tikunov et al. 2012). Data was pre-processed by filtering the metabolites in the MSClust output file (MsExcel v.2010) for the number of mass peaks per metabolite (≥5) and the centrotype factor (≥0.7), of which the latter indicates the genuineness of a specific centrotype. Centrotypes with accurate masses were extracted from the data after filtering and correcting for the IS by dividing all metabolic peak values per genotype by the IS value measured for that genotype.

Selecting metabolites that play a role in *B. tabaci* resistance and susceptibility

Selection of resistant and susceptible genotypes from the F_2 population Phenotypic data for whitefly performance of the F_2 population was ranked to select the ten most resistant and the ten most susceptible genotypes. The GC-MS profiles of these two groups were subjected to comparative statistical analyses as described in chapter 2.

Metabolites that were significantly different between the two groups were identified. Data analyses were done with Simca P+ version 12.0.1 software for multivariate data analysis (Umetrics, MKS Instruments Inc. Sweden). These analyses enabled a non-targeted selection of metabolites that might be involved in the resistance to *B. tabaci* as quantified in terms of adult survival and reproduction rates. The input data file was $\log_{10}$ transformed and principal component analysis (PCA) was performed to analyze the structure and to detect outliers. An

Orthogonal Partial Least Squares Discriminant Analyses (OPLS-DA) model was used to discriminate between resistant and susceptible genotype classes on the basis of their metabolome spectra. Statistical significance of metabolites belonging to one of the classified groups was determined by calculating their coefficient values. For the identification of individual metabolites that significantly contribute to *B. tabaci* resistance, a Student's t-Test (MsExcel2010) was performed on the metabolites from the pre-processed and log_{10}-transformed MSClust dataset. Metabolites were ranked according to their p-values and q-values were calculated with the Benjamini and Hochberg False Discovery Rate (FDR) multiple comparison procedure to correct for false discoveries (Benjamini and Hochberg 1995; see materials and methods chapter 2 for equation). Metabolites were considered significant when $q \leq 0.05$.

DNA extraction and marker analysis of F_2 backcross populations

The leaves from 131 F_2 seedlings were sampled when plants were in the true-two leaf stage and collected in 96-wells plates. Genomic DNA isolation was performed according to the protocol described by Doyle and Doyle (1990), adjusted for 96-well plates. The Amplified Fragment Length Polymorphism (AFLP) analysis of the 131 F_2 plants and parental lines was performed according to Vos et al. (1995). Single nucleotide polymorphism (SNP) genotyping was done with a custom-made Infinium SNP Array (Illumina Inc., USA). Leaves from 115 F_2BC_1(44) and 154 F_2BC_1(12) plants were sampled when the plants were in the true-two leaf stage and collected on ice in 1.4 ml polypropylene tubes in 96-well format (Micronics) containing two 3 mm stainless steel grinding beads (Retsch GmbH & Co KG). Lysis buffer (300 µl; LGC Genomics, Germany) with 0.5 µl RNase (2 mg/ml) was added per tube and samples were ground with a Retsch mixer mill (1min, 30 rps; MM300 Retsch GmbH & Co KG), centrifuged (1 min 300 rpm), and incubated in a water bath (65°C, 30 min). DNA was extracted with the Kingfisher Flex Magnetic Particle Processor (ThermoScientific). Reagents for the Kingfisher DNA extraction were obtained from LGC Genomics (Germany). The sbeadex® Maxi Plant kit was used according to the protocol of the supplier (LGC Genomics). Two hundred µl of the dissolved plant material was mixed with 520 µl binding buffer and suspended with 60 µl magnetic beads in a 96-Deep Well plate (ThermoScientific). Subsequently, DNA purification was performed with the KingFisher Flex Magnetic Particle Processor (ThermoScientific). Sample concentration and quality was assessed on 1% agarose gel. Samples were normalised to 50 ng/µl by diluting the gDNA concentration in 10 mM Tris/1 mM EDTA pH=8 (TE). Genotyping was carried out by Service XS, Leiden, the

Netherlands with Illumina's Infinium SolCAP Tomato BeadChip (Sim et al. 2012), according to the Illumina Infinium II Protocol (Illumina Inc.).

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. Three out of in total 308 markers were not 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. Distorted markers 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 (Van Ooijen 2004) v.6.0. LOD-score threshold values for phenotypic QTLs and metabolite QTLs were fixed at 3.0. Interval mapping was employed 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 employed for the graphical presentation of linkage maps and QTLs.

Results

QTLs for *B. tabaci* adult survival and oviposition on young and old F_2 tomato plants

Quantitative differences in susceptibility/resistance to *B. tabaci* were observed among 131 F_2 genotypes of a cross between EC and *S. pennellii* with regard to adult survival in a no-choice clip-on cage screening of young (six weeks) and older (20 weeks) plants. Quantitative trait segregation for *B. tabaci* adult survival on six-week-old plants showed QTLs on chromosomes IV, VI, X, and XI (Fig 1, Table 1). On 20-week-old plants we identified QTLs at the same loci on the chromosome XI and one just below threshold level at chromosome VI, but the QTLs on chromosomes IV and X were not found back (Fig 1). The explained variances found for the individual QTLs for adult survival range between 9.6 and 16.4 percent (Table 1).

Quantitative trait segregation for *B. tabaci* oviposition on six-week-old plants showed QTLs on chromosomes IV, VI, and X (Fig 1, 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 (Fig 1), although the latter one was visible in the six-week-old plants, but did not reach the threshold. The explained variances found for the individual QTLs for oviposition range between 10.0 and 13.9 percent (Table 1).

The QTLs for oviposition in six-week-old plants co-localized with QTLs for survival on all loci with the exception of the locus on chromosome XI where the LOD score was only 2.6. The QTLs on chromosome VI for oviposition on six-week-old plants and survival on 20-week-old plants co-localize within the 2-LOD interval, but not within the 1-LOD interval, which may point at different QTLs.

Table 1 QTLs for *B. tabaci* resistance parameters in six- and 20-week-old plants. Phenotypic QTLs were identified in an F_2 population of a cross between *S. lycopersicum* cv Moneymaker 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.

Trait	Trait description	QTL chromosome	Explained variance (%)
Phenotype QTL surv6	survival on 6-wk-old plants	IV, VI, X, and XI	12.3, 10.1, 16.4, and 14.7
Phenotype QTL ovi6	oviposition on 6-wk-old plants	IV, VI, and X	10.3, 13.9, and 10.0
Phenotype QTL surv20	survival on 20-wk-old plants	VI[a] and XI	9.6 and 12.4
Phenotype QTL ovi20	oviposition on 20-wk-old plants	IV and XI	10.4 and 10.3

[a] putative QTL just below threshold level (LOD 2.9).

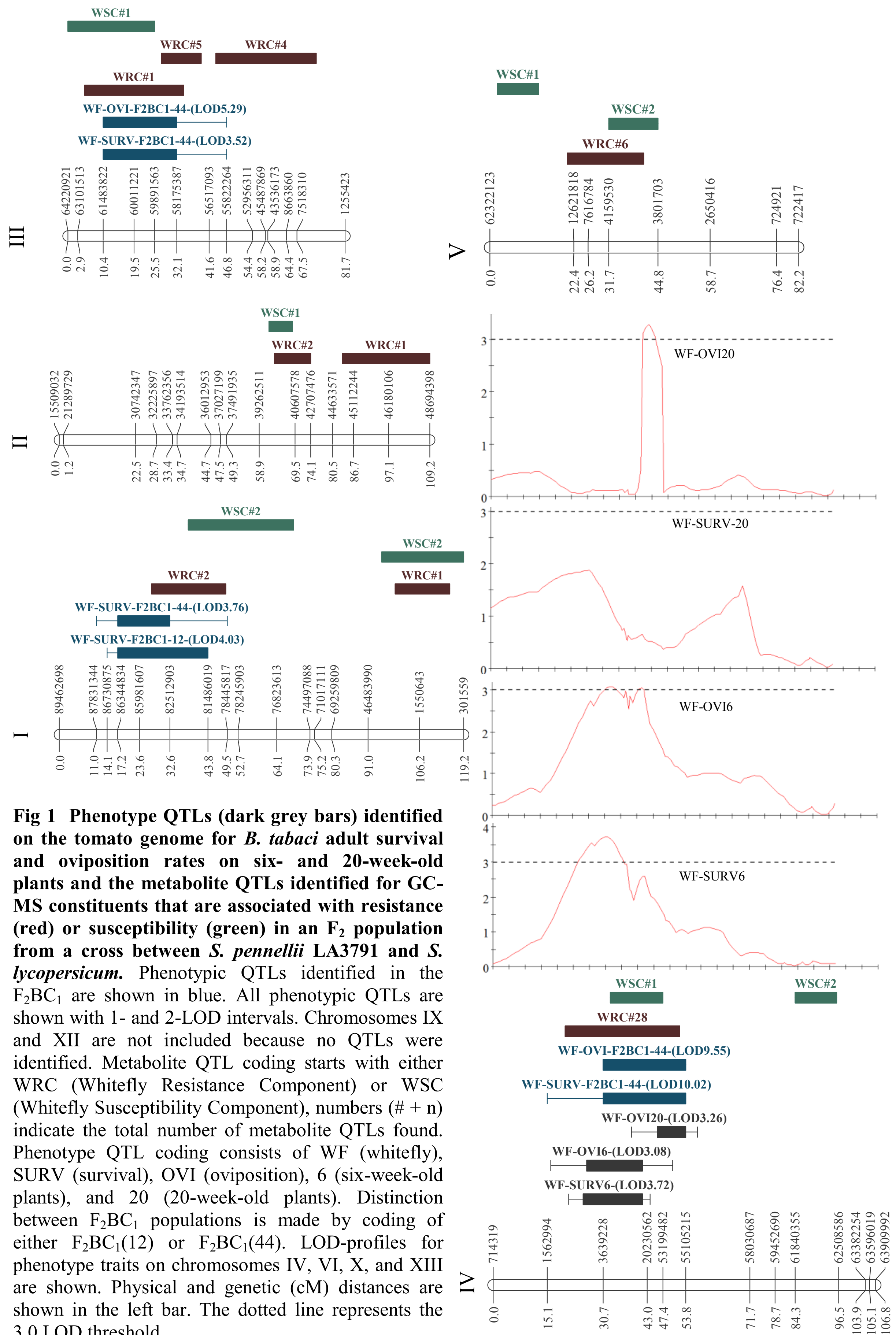


Fig 1 Phenotype QTLs (dark grey bars) identified on the tomato genome for *B. tabaci* adult survival and oviposition rates on six- and 20-week-old plants and the metabolite QTLs identified for GC-MS constituents that are associated with resistance (red) or susceptibility (green) in an F_2 population from a cross between *S. pennellii* LA3791 and *S. lycopersicum*. Phenotypic QTLs identified in the F_2BC_1 are shown in blue. All phenotypic QTLs are shown with 1- and 2-LOD intervals. Chromosomes IX and XII are not included because no QTLs were identified. Metabolite QTL coding starts with either WRC (Whitefly Resistance Component) or WSC (Whitefly Susceptibility Component), numbers (# + n) indicate the total number of metabolite QTLs found. Phenotype QTL coding consists of WF (whitefly), SURV (survival), OVI (oviposition), 6 (six-week-old plants), and 20 (20-week-old plants). Distinction between F_2BC_1 populations is made by coding of either $F_2BC_1(12)$ or $F_2BC_1(44)$. LOD-profiles for phenotype traits on chromosomes IV, VI, X, and XIII are shown. Physical and genetic (cM) distances are shown in the left bar. The dotted line represents the 3.0 LOD threshold.

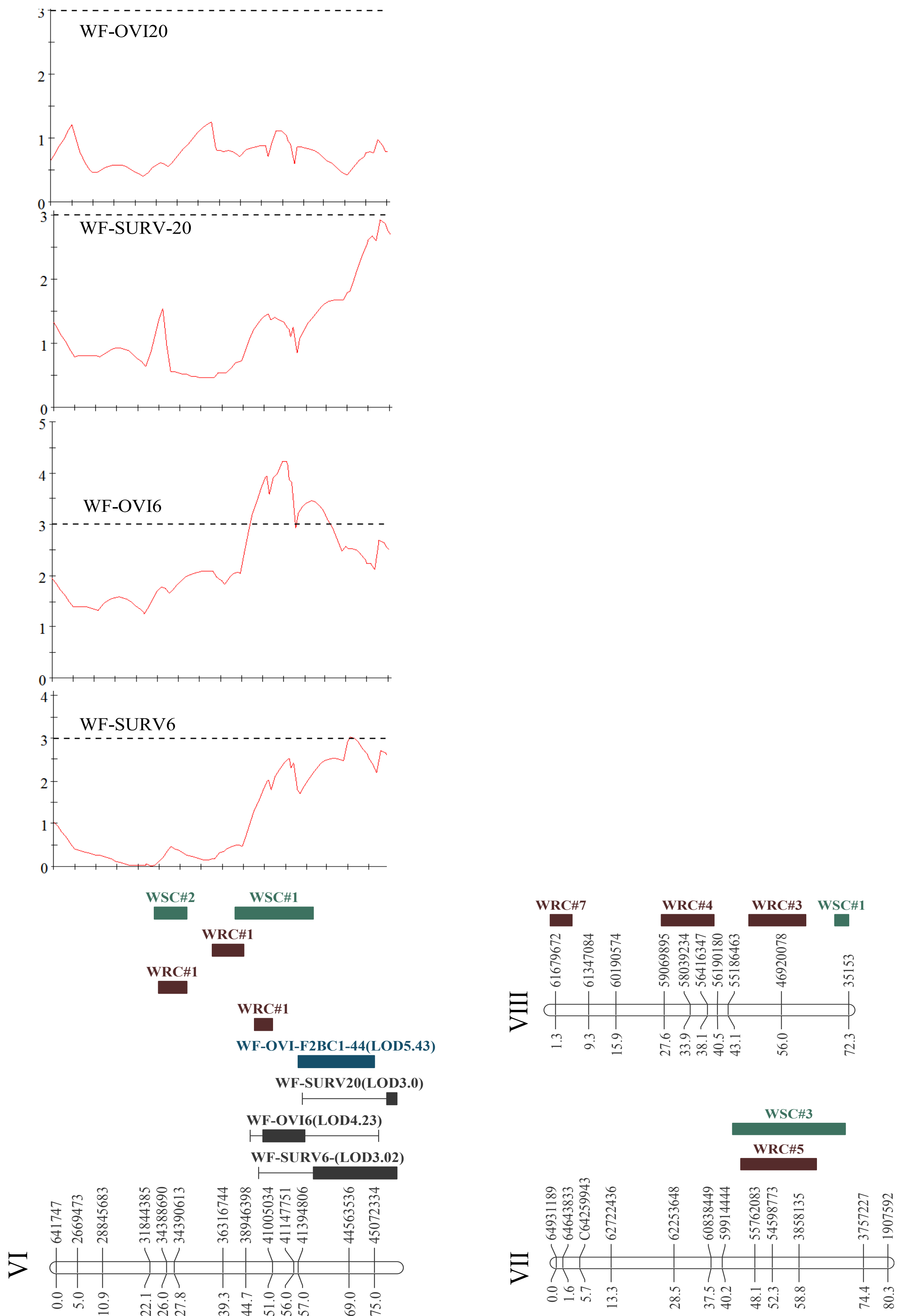


Fig 1 continued Chromosomes VI-VIII

Fig 1 continued Chromosomes X and XI

QTLs for metabolites

Chemical profiles of all individuals from the F_2 population were obtained by measuring total volatile and semi-volatile compounds in leaf extracts from six-week-old plants. Quantitative differences in peak abundance were observed in the GC-MS profiles. To identify centrotypes, putative components, amongst the high number of segregating centrotypes, that are discriminating between *B. tabaci* resistant and susceptible bulks, a statistical approach was taken whereby biochemical profiles of the 10 most resistant and susceptible plants were analyzed by OPLS-DA to determine the total metabolite spectrum explanatory for resistance or susceptibility and by FDR to determine which individual metabolic constituents correlate with *B. tabaci* resistance or susceptibility. A large number of centrotypes were associated with resistance/susceptibility QTLs (Table 2) and the majority (>80%) could be placed on the genetic map (Fig 1). Centrotypes, correlation with *B. tabaci* phenotype (resistance or susceptibility), statistical methodology, highest LOD markers, LOD-values, and percentages of explained variances of the QTLs identified are listed per chromosome in Table 3. Co-localizing metabolite QTLs are presented by a single interval bar in Figure 1 and the number of QTLs at that interval is included in the tag in Fig 1 (WRC#28 stands for 28 metabolite QTLs associated with the resistance QTL at this position). The chromosomes IV, X, and XI show hot-spot areas for *B. tabaci* resistance-related compounds and as many as 28, 16, and 25 metabolite QTLs map to the same region on these chromosomes, respectively. Other *B. tabaci* resistance QTL-related metabolite QTLs were detected on almost all chromosomes, except for the chromosomes IX and XII. Minor hot spots with five or more metabolite QTLs were found on chromosomes III, V, VII, and VIII.

A lower number of centrotypes was identified that were associated with susceptibility in both the FDR (13 susceptibility-related centrotypes) and OPLS-DA (14 susceptibility-related centrotypes) analyses (Table 2) and there were no obvious hot-spot areas. The highest number of susceptibility QTL-related metabolite QTLs that co-localized was three on linkage group VII. The explained variances for the metabolite QTLs ranged between 6.8 and 28.1 percent.

Phenotypic as well as resistance QTL related metabolite QTLs had only the *S. pennellii* allele homozygously present or were heterozygous.

Table 2 Overview of number of metabolic components selected by two statistical methods: Orthogonal Partial Least Square-Discriminant Analyses and Student's t-Test + False Discovery Rate Analyses.

Trait / Statistical methodology	Nr. of 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 + Fl	9

Metabolic components were screened in six-week-old F_2 populations of a cross between *S. lycopersicum* x *S. pennellii* LA3791. Bulked Segregant Analyses and multivariate statistical analyses was performed to select metabolic components that were in composition (OPLS-DA) or individually (Student's t-Test + FDR) explanatory for resistance or susceptibility against whitefly *B. tabaci*.

Evaluation of F_2BC_1 populations

Backcrosses of two resistant plants (numbers 12 and 44) with EC were made to confirm the phenotypic QTLs that were detected in the F_2 population. Life-history traits of these F_2 plants showed zero survival and zero to low oviposition on six- and 20-week-old plants and the genetic makeup of the plants in the major QTL regions is shown in Figure 2. In the combination of these two plants we have the phenotypic QTLs and the majority of hotspot regions that were identified in the F_2 population heterozygously present, the only exception is on chromosome VI that was either homozygous *S. pennellii* (44) or *S. lycopersicum* (12).

The two F_2BC_1 populations were screened for whitefly resistance in a greenhouse phenotyping assay (Fig 3). The populations F_2BC_1(12) and F_2BC_1(44) both showed quantitative differences with respect to the *B. tabaci* life-history parameters adult survival and oviposition rate. Parent *S. pennellii* showed one-hundred percent *B. tabaci* mortality five days after infestation. None of the F_2BC_1(12) genotypes showed such high levels (Fig 3A). A clear quantitative gradient for adult survival was observed for population F_2BC_1(44) and nine of the genotypes had an adult survival score of zero (Fig 3B).

Although there was little variance for adult survival in population F_2BC_1(12), a clear continuous gradient was observed for oviposition (Fig 3C), although none of the F_2BC_1(12) plants showed zero oviposition. In population F_2BC_1(44) sixteen individuals had zero oviposition. Eight out of the nine plants with an adult survival of zero also had zero oviposition (Fig 3D).

Table 3 List of the metabolomic QTLs associated with resistance/susceptibility. Experiments were performed in a six-week-old F_2 population of *S. lycopersicum* x *S. pennellii* LA3791. FDR and OPLS-DA statistical analyses were performed for classification of metabolites as *B. 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, centrotype, putative annotation, resistant/susceptibility-related component, statistical method, highest corresponding marker, QTL LOD-value, and corresponding % of explained variance are given in consecutive order.

Chromosome	Centrotype	Putative ID	S[a] R[b]	Statistics	HLM[c]	LOD	EV[d] (%)
I	1225	Methyl salicylate	S	FDR	P11M54_M413.9	5.60	18.1
I	2705	Unknown	R	OPLS-DA + FDR	P11M54_M273.7	3.21	9.8
I	3395	Unknown	R	FDR	P14M49_M298.9	6.42	14.0
I	3606	Dodecanoic acid	R	OPLS-DA + FDR	P14M50_M237.2	3.61	12.1
I	5433	Unknown	S	OPLS-DA + FDR	Solcap_snp_sl_15058	4.55	15.0
I	7963	Unknown	S	OPLS-DA + FDR	P14M50_M298.8	4.56	15.0
I	8626	Unknown	S	OPLS-DA	Solcap_snp_sl_2234	3.85	11.6
II	259	Unknown	S	FDR	P14M60_M85.8	3.05	8.4
II	2393	Undecanoic acid	R	OPLS-DA + FDR	Solcap_snp_sl_29891	7.50	23.5
II	4486	Unknown	R	FDR	CL016576-0377	3.04	9.9
II	8563	Unknown	R	OPLS-DA	Solcap_snp_sl_29891	4.42	13.2
III	109	Unknown	R	FDR	P14M49_M177.1	4.71	15.5
III	1973	Unknown	R	FDR	P14M49_M177.1	3.02	10.2
III	3266	Unknown	S	OPLS-DA + FDR	Solcap_snp_sl_36544	3.00	10.2
III	3483	Unknown	R	OPLS-DA + FDR	Solcap_snp_sl_62270	3.16	9.7
III	3516	Unknown	R	OPLS-DA + FDR	Solcap_snp_sl_62270	3.00	9.2
III	3595	Unknown	R	OPLS-DA + FDR	Solcap_snp_sl_62270	3.26	11.0
III	3664	1-Dodecyn-4-ol	R	FDR	P14M49_M177.1	4.17	12.5
III	3719	Unknown	R	OPLS-DA + FDR	P14M50_M265.5	4.60	15.1
III	3767	Unknown	R	FDR	P14M50_M265.5	4.78	15.7
III	4391	Unknown	R	FDR	P14M49_M177.1	3.56	11.9
III	4421	Unknown	R	OPLS-DA + FDR	P14M50_M265.5	3.00	6.8
IV	109	Unknown	R	FDR	Solcap_snp_sl_53136	3.43	11.5
IV	498	Butanoic acid	R	OPLS-DA + FDR	P14M60_M380.4	3.61	12.1
IV	947	Unknown	R	OPLS-DA + FDR	P11M50_M118.5	3.41	11.5
IV	1102	Levoglucosone	R	OPLS-DA + FDR	Solcap_snp_sl_51411	5.72	12.4
IV	1549	Unknown	R	FDR	P14M60_M533.2	3.87	12.9
IV	1576	Unknown	R	FDR	P14M60_M533.2	3.78	12.6
IV	1973	Unknown	R	FDR	Solcap_snp_sl_53136	3.20	10.8
IV	3114	Unknown	R	FDR	Solcap_snp_sl_53136	4.08	13.6
IV	3449	Unknown	R	OPLS-DA + FDR	P14M49_M189.3	3.70	12.4
IV	3483	Unknown	R	OPLS-DA + FDR	P14M49_M189.3	3.22	9.9
IV	3516	Unknown	R	OPLS-DA + FDR	P14M49_M51.5	3.62	12.1
IV	3595	Unknown	R	OPLS-DA + FDR	P14M49_M51.5	3.26	11.0
IV	3719	Unknown	R	OPLS-DA + FDR	P14M60_M380.4	4.51	14.9
IV	3767	Unknown	R	FDR	P14M60_M380.4	4.96	16.2
IV	3878	Unknown	R	FDR	P14M60_M380.4	4.65	14.8
IV	3989	Unknown	R	FDR	Solcap_snp_sl_53136	3.47	11.6
IV	4070	Unknown	R	FDR	Solcap_snp_sl_53136	3.86	12.9
IV	4160	Unknown	R	FDR	P14M49_M51.5	3.46	11.6
IV	4391	Unknown	R	FDR	P11M50_M118.5	4.52	14.9
IV	4421	Unknown	R	OPLS-DA + FDR	P14M49_M189.3	3.45	11.6
IV	4458	Unknown	R	OPLS-DA + FDR	P14M49_M51.5	3.26	11.0
IV	4531	Unknown	R	FDR	P14M60_M380.4	4.15	13.8
IV	4588	Unknown	R	FDR	Solcap_snp_sl_51411	3.38	8.7
IV	4605	Unknown	R	FDR	P14M60_M380.4	3.58	12.0
IV	4661	Unknown	R	OPLS-DA + FDR	Solcap_snp_sl_51411	3.62	10.2
IV	4707	Unknown	R	FDR	P14M60_M380.4	3.33	11.2
IV	5223	Unknown	R	FDR	P14M60_M380.4	5.03	16.4
IV	7704	Unknown	R	OPLS-DA + FDR	P14M49_M189.3	3.57	12.0
IV	7963	Unknown	S	OPLS-DA + FDR	P14M60_M380.4	3.28	11.0
IV	9234	Eicosane	S	OPLS-DA	P14M50_M195.7	3.11	10.5
IV	10389	Unknown	S	OPLS-DA	P14M50_M195.7	3.10	10.5
V	3989	Unknown	R	FDR	P11M54_M721.1	4.02	13.4
V	4531	Unknown	R	FDR	P11M54_M721.1	3.83	12.8
V	4588	Unknown	R	FDR	P11M54_M721.1	3.10	7.4
V	4605	Unknown	R	FDR	P11M54_M721.1	3.29	11.1

Table 3 continued

Chromosome	Centrotype	Putative ID	S[a] R[b]	Statistics	HLM[c]	LOD	EV[d] (%)
V	5003	Unknown	R	FDR	P11M54_M721.1	3.06	10.3
V	5223	Unknown	R	FDR	P11M50_M169.3	3.16	10.7
V	5433	Unknown	S	OPLS-DA + FDR	Solcap_snp_sl_23970	5.26	17.1
V	5711	Unknown	S	OPLS-DA + FDR	Solcap_snp_sl_23970	6.44	18.6
V	5711	Unknown	S	OPLS-DA + FDR	P11M54_M127.5	3.73	10.3
VI	1102	Levoglucosone	R	OPLS-DA + FDR	Solcap_snp_sl_19915	3.86	8.1
VI	1576	Unknown	R	FDR	P11M54_M277.6	3.08	10.4
VI	2552	Caryophyllene	S	OPLS-DA	Solcap_snp_sl_55902	6.45	20.6
VI	2552	Caryophyllene	S	OPLS-DA	P14M50_M481.8	3.89	13.0
VI	2807	Unknown	R	FDR	Solcap_snp_sl_55902	4.49	14.8
VI	2987	Unknown	S	FDR	Solcap_snp_sl_55902	8.43	20.9
VII	1102	Levoglucosone	R	OPLS-DA + FDR	Solcap_snp_sl_26437	3.31	6.9
VII	1283	Unknown	R	FDR	Solcap_snp_sl_26437	6.82	17.7
VII	1920	Hexanoic acid	R	OPLS-DA + FDR	P14M49_M159.7	4.88	16.0
VII	3266	Unknown	S	OPLS-DA + FDR	P11M54_M244.9	5.50	17.8
VII	4270	Tridecanoic acid	R	OPLS-DA + FDR	Solcap_snp_sl_26437	4.50	14.8
VII	4317	Unknown	R	OPLS-DA + FDR	Solcap_snp_sl_52568	3.39	11.4
VII	5338	Unknown	S	OPLS-DA + FDR	P14M49_M159.7	3.73	11.5
VII	5711	Unknown	S	OPLS-DA + FDR	P11M54_M244.9	3.10	8.4
VIII	1549	Unknown	R	FDR	P11M54_M437.8	8.92	27.3
VIII	1549	Unknown	R	FDR	P14M49_M170.6	5.35	17.4
VIII	1576	Unknown	R	FDR	P11M54_M437.8	8.86	27.1
VIII	1576	Unknown	R	FDR	P14M49_M170.6	5.41	17.6
VIII	1840	Unknown	R	FDR	P11M50_M222.4	3.82	12.8
VIII	2705	Unknown	R	OPLS-DA + FDR	P14M60_M442.3	3.36	10.2
VIII	3416	Unknown	R	FDR	P14M49_M170.6	3.84	12.8
VIII	3516	Unknown	R	OPLS-DA + FDR	Solcap_snp_sl_10247	3.03	9.9
VIII	4107	Unknown	R	FDR	Solcap_snp_sl_10247	4.15	12.6
VIII	4160	Unknown	R	FDR	Solcap_snp_sl_10247	3.61	12.1
VIII	4249	Unknown	R	FDR	Solcap_snp_sl_10247	3.91	13.0
VIII	4391	Unknown	R	FDR	P14M49_M170.6	3.56	11.9
VIII	4531	Unknown	R	FDR	Solcap_snp_sl_10247	3.36	11.3
VIII	5003	Unknown	R	FDR	Solcap_snp_sl_10247	3.81	12.7
VIII	5047	Unknown	R	OPLS-DA + FDR	Solcap_snp_sl_10247	3.06	9.9
X	259	Unknown	S	FDR	P11M54_M221.8	5.12	14.9
X	1549	Unknown	R	FDR	Solcap_snp_sl_3294	3.39	11.4
X	1576	Unknown	R	FDR	Solcap_snp_sl_3294	3.85	12.8
X	2552	Caryophyllene	S	OPLS-DA	P11M54_M684.9	4.21	14.0
X	2807	Unknown	R	FDR	P11M54_M684.9	4.33	14.3
X	2849	Unknown	R	FDR	Solcap_snp_sl_61131	3.19	10.1
X	2987	Unknown	S	FDR	Solcap_snp_sl_33166	8.22	20.3
X	3449	Unknown	R	OPLS-DA + FDR	P11M54_M199.0	3.13	10.6
X	3483	Unknown	R	OPLS-DA + FDR	P11M54_M199.0	2.73	9.3
X	3516	Unknown	R	OPLS-DA + FDR	P14M49_M166.2	3.04	10.1
X	3595	Unknown	R	OPLS-DA + FDR	Solcap_snp_sl_16511	3.06	8.8
X	4160	Unknown	R	FDR	P11M54_M199.0	3.65	12.2
X	4421	Unknown	R	OPLS-DA + FDR	P11M54_M199.0	3.02	7.0
X	4531	Unknown	R	FDR	Solcap_snp_sl_16511	3.42	11.5
X	4588	Unknown	R	FDR	Solcap_snp_sl_16511	3.33	9.6
X	4605	Unknown	R	FDR	Solcap_snp_sl_16511	3.03	10.2
X	4661	Unknown	R	OPLS-DA + FDR	P11M54_M199.0	3.13	8.9
X	4707	Unknown	R	FDR	P14M49_M166.2	3.11	10.5
X	4820	Unknown	R	OPLS-DA + FDR	P11M50_M587.3	3.01	10.2
X	5047	Unknown	R	OPLS-DA + FDR	P14M49_M166.2	3.04	9.4
X	7963	Unknown	S	OPLS-DA + FDR	Solcap_snp_sl_46475	4.15	13.8
X	7963	Unknown	S	OPLS-DA + FDR	P11M54_M221.8	3.28	11.0
X	8253	Unknown	R	OPLS-DA	P11M54_M684.9	3.95	13.2
XI	498	Butanoic acid	R	OPLS-DA + FDR	P11M54_M90.5	5.96	19.2
XI	947	Unknown	R	OPLS-DA + FDR	Solcap_snp_sl_5922	4.27	14.1
XI	1102	Levoglucosone	R	OPLS-DA + FDR	Solcap_snp_sl_5922	6.06	13.2

Table 3 continued

Chromosome	Centrotype	Putative ID	S[a] R[b]	Statistics	HLM[c]	LOD	EV[d](%)
XI	1920	Hexanoic acid	R	OPLS-DA + FDR	Solcap_snp_sl_5922	6.20	19.9
XI	2161	Decanoic acid	R	FDR	P11M54_M90.5	3.43	10.6
XI	2393	Undecanoic acid	R	OPLS-DA + FDR	Solcap_snp_sl_56142	4.96	16.2
XI	3114	Unknown	R	FDR	Solcap_snp_sl_5922	4.09	13.6
XI	3449	Unknown	R	OPLS-DA + FDR	Solcap_snp_sl_5922	4.80	15.7
XI	3483	Unknown	R	OPLS-DA + FDR	P11M54_M90.5	6.30	18.3
XI	3516	Unknown	R	OPLS-DA + FDR	Solcap_snp_sl_5922	5.12	16.7
XI	3595	Unknown	R	OPLS-DA + FDR	Solcap_snp_sl_5922	9.24	28.1
XI	3664	1-Dodecyn-4-ol	R	FDR	P11M54_M160.9	4.05	12.1
XI	3989	Unknown	R	FDR	Solcap_snp_sl_5922	3.56	11.9
XI	4070	Unknown	R	FDR	P11M54_M90.5	4.13	13.7
XI	4421	Unknown	R	OPLS-DA + FDR	P11M54_M90.5	5.02	16.4
XI	4458	Unknown	R	OPLS-DA + FDR	P11M54_M90.5	4.34	14.3
XI	4531	Unknown	R	FDR	Solcap_snp_sl_56142	5.30	17.2
XI	4588	Unknown	R	FDR	P11M54_M90.5	4.52	13.4
XI	4605	Unknown	R	FDR	Solcap_snp_sl_56142	3.96	13.2
XI	4661	Unknown	R	OPLS-DA + FDR	P11M54_M90.5	4.86	15.9
XI	4707	Unknown	R	FDR	P11M54_M90.5	4.50	14.8
XI	4820	Unknown	R	OPLS-DA + FDR	Solcap_snp_sl_5922	3.97	13.2
XI	5003	Unknown	R	FDR	P11M54_M419.7	3.21	10.8
XI	5433	Unknown	S	OPLS-DA + FDR	Solcap_snp_sl_5922	3.47	11.7
XI	5612	Unknown	R	FDR	P11M54_M160.9	4.97	16.2
XI	7704	Unknown	R	OPLS-DA + FDR	P11M54_M90.5	4.39	14.5
No QTLs identified	2416	Unknown	R	FDR	n.a.	n.a.	n.a.
No QTLs identified	2577	Unknown	R	FDR	n.a.	n.a.	n.a.
No QTLs identified	2621	Unknown	R	FDR	n.a.	n.a.	n.a.
No QTLs identified	4195	Unknown	R	FDR	n.a.	n.a.	n.a.
No QTLs identified	4762	Unknown	R	OPLS-DA + FDR	n.a.	n.a.	n.a.
No QTLs identified	5030	Unknown	R	OPLS-DA + FDR	n.a.	n.a.	n.a.
No QTLs identified	5517	Unknown	S	OPLS-DA + FDR	n.a.	n.a.	n.a.
No QTLs identified	6819	Unknown	S	OPLS-DA + FDR	n.a.	n.a.	n.a.
No QTLs identified	6819	Unknown	S	OPLS-DA + FDR	n.a.	n.a.	n.a.
No QTLs identified	7162	Unknown	S	OPLS-DA	n.a.	n.a.	n.a.
No QTLs identified	7834	Unknown	R	FDR	n.a.	n.a.	n.a.
No QTLs identified	7844	Unknown	S	OPLS-DA + FDR	n.a.	n.a.	n.a.
No QTLs identified	7844	Unknown	S	OPLS-DA + FDR	n.a.	n.a.	n.a.
No QTLs identified	7875	Unknown	S	OPLS-DA + FDR	n.a.	n.a.	n.a.
No QTLs identified	8588	Unknown	R	FDR	n.a.	n.a.	n.a.

[a]S: *Bemisia tabaci* susceptibility component

[b]R: *B. tabaci* resistance component

[c]HLM Highest LOD marker

[d]EV: Explained variance (%)

Chromosome nr	Physical map position	SNP genotyping of F_2 nr 12	SNP genotyping of F2 nr 44
IV	1.562.994	B	H
IV	2.983.549	B	H
IV	15.097.896	B	H
IV	25.812.609	B	H
IV	29.000.198	B	H
IV	42.190.928	B	H
IV	49.990.085	B	H
IV	53.785.617	H	H
IV	55.105.215	H	H
VI	41.005.034	A	B
VI	41.147.751	A	B
VI	41.147.789	A	B
VI	41.159.856	A	B
VI	41.383.406	A	B
VI	41.394.806	A	B
VI	45.072.334	A	B
X	46.931.693	H	B
X	49.856.593	H	B
X	52.809.001	H	B
X	57.224.189	H	B
X	60.235.795	H	B
X	61.124.385	H	B
XI	6.623.586	B	B
XI	11.933.653	B	H
XI	13.194.095	B	H
XI	19.636.101	B	H
XI	21.374.623	B	H
XI	27.841.963	B	H
XI	30.617.163	B	H
XI	37.689.381	B	H
XI	40.361.385	B	H
XI	49.081.167	B	H
XI	51.359.586	H	H

Fig 2 Genotype of F_2 plants nr 12 and 44 in the phenotypic QTL regions. Chromosome numbers, physical positions and genotypes are displayed. Genotypes are colored according to heterozygosity (H; green), homozygosity of *S. pennellii* LA3791 donor parent (B; blue), and homozygosity of *S. lycopersicum* cultivar (A; Orange).

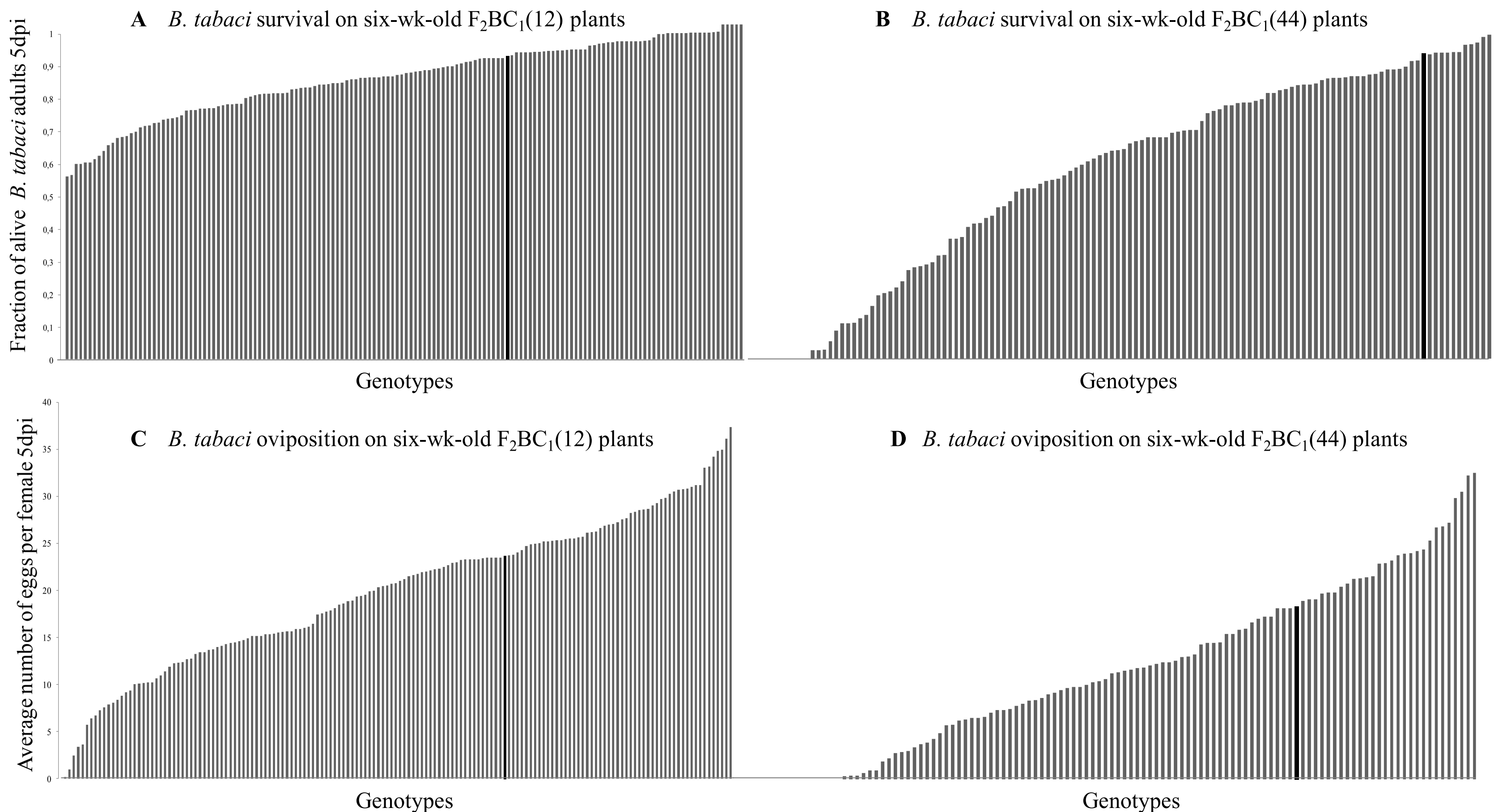


Fig 3A-D Distribution pattern of adult survival of *Bemisia tabaci* in population F_2BC_1(12) (A) and F_2BC_1(44) (B) and oviposition in population F_2BC_1(12)(C) and F_2BC_1(44)(D). Values of graphs **A** and **B** are means of the fraction of living adult whiteflies. Values of graphs **C** and **D** represent the average number of eggs laid per female in five days. The grey bars represent the average whitefly survival and oviposition rates on F_2BC_1 genotypes. Each bar represents two replicas per genotype. The black bars represent the average survival and the average oviposition rates on reference parent *S. lycopersicum* EC, as the average of six replicas. The first data points showing complete absence of adult survival and oviposition represent *Solanum pennellii* (**A-D**) and a number of F_2BC_1 genotypes (**B** and **D**).

Phenotypic QTLs in the F_2BC_1 populations

SNP markers were used to construct genetic maps for both F_2BC_1 populations and the known physical positions of the SNPs on the custom made array and the SolCap array made a comparison possible between the F_2 and F_2BC_1 maps (Fig 1). A QTL was identified for adult survival in population F_2BC_1(12) and F_2BC_1(44) on chromosome I (Fig 1, Table 4). Co-localizing QTLs for *B. tabaci* adult survival and oviposition rate in population F_2BC_1(44) mapped on chromosome III and IV. Finally, a QTL for oviposition in population F_2BC_1(44) mapped on chromosome VI. Table 4 lists the phenotype trait descriptions, an overview of the QTLs identified per trait, and the percentage of explained variances.

Table 4 List of QTLs related to a *B. tabaci* resistant phenotype. Experiments were performed on F_2BC_1 populations of *S. lycopersicum* x *S. pennellii* LA3791 on six-week-old-plants.

Trait	Trait description	QTL 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 six-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.

Discussion

QTLs for *B. tabaci* life-history parameters in a *S. lycopersicum* x *S. pennellii* F_2 population

This chapter reports the first QTLs for *B. tabaci* life-history parameters in *S. pennellii*. Several loci that contribute to *B. tabaci* reduced adult survival and oviposition rate were identified in an F_2 population of a cross between *S. pennellii* LA3791 and an elite tomato cultivar. These QTLs mapped to the chromosomes IV, VI, X, and XI (Fig 1). Not all four phenotypic QTLs identified in this study were always present, detection depended on plant age (Table 1). Some of the QTLs found in six-week-old plants could not be detected in 20-week-old plants, which suggests that developmental changes play a role in the expression of resistance to whiteflies.

Finding the same QTLs at different plant ages suggests that resistance is at least partly based on the same mechanism(s). The QTLs identified on chromosomes IV and VI were confirmed in backcross population F_2BC_1(44), which was obtained from a cross between a fully *B. tabaci*-resistant F_2 genotype and EC. Some of the adult survival and oviposition QTLs co-localize, suggesting that also in this case the mechanism(s) that govern these traits are at least partly the same. However, it could also be the result of interdependence between the parameters. Strong correlations between adult survival and oviposition rate have been observed previously (Firdaus et al. 2012). High *B. tabaci* resistance levels were found previously in *S. pennellii* accession LA3791 (chapter 2). No adult survival was observed 24 hours post whitefly infestation and no oviposition took place before they died, which suggests that the mechanism(s) of resistance against *B. tabaci* is either constitutive or rapidly induced. Such complete resistance against *B. tabaci* from the onset of the screening was also found for oviposition on the *S. pennellii* accession LA716 (Heinz et al. 1995; Nombela et al. 2000). The phenotypic QTLs identified in our study on chromosomes IV, X, and XI (Fig 1) co-localized with metabolite QTLs found for Acyl sugar production and accumulation in other studies (Blauth et al. 1998; Mutschler et al. 1996; Lawson et al. 1997) in *S. pennellii* LA716 derived populations. The QTLs for adult survival and oviposition rate on chromosome VI co-localized with previously identified QTLs for total Acyl sugars, an increased density of trichome type IV, and reduced incidence of *B. tabaci* in the field (Leckie et al. 2012).

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

Without exception, all identified phenotypic QTLs in the F_2 population were minor effect QTLs with low explained variances (Table 1), which shows that the resistance is polygenic. Another explanation for the low explained variances of both phenotype and metabolite QTLs can be found in the diversity in biochemical profiles that was observed between *B. tabaci* resistant genotypes (chapter 2), which may indicate that various, independent resistance mechanisms are present in the different resistant genotypes. This also complicates the detection of QTLs. QTL studies in other F_2 populations concerning tomato-whitefly resistance traits also resulted in minor effect QTLs (Momotaz et al. 2010; Maliepaard et al. 1995), but no information is available from other populations that concentrating on a single mechanism leads to higher explained variances. The identification of only part of the *B. tabaci* resistance QTLs is also reflected by the total explained variances of the phenotype QTLs. Together they only explain part of the *B. tabaci* resistance trait with values ranging from 22% to 53.5% for

B. tabaci survival on 20-week-old plants and *B. tabaci* survival on six-week-old plants, respectively (Table 1). The presence of multiple mechanisms with small effects combined with the common incidence of measurement errors might explain that not 100 percent variance of the traits was covered.

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

Biochemical fingerprinting by GC-MS was performed on the entire F_2 population and discriminant analyses revealed that a large number of metabolic constituents potentially contribute to the resistance/susceptibility of *S. pennellii* to *B. tabaci.* The majority of these metabolites could be mapped (Fig 1). Hot-spots with more than ten metabolite QTLs associated with resistance were identified on chromosomes IV, X, and XI. The positions of these metabolite QTL hot-spots were identical to the positions of the identified phenotypic QTLs on these chromosomes, which suggests that resistance is for the larger part biochemically-based, a hypothesis proposed earlier by Liedl et al. (1995).
On chromosome VI multiple overlapping phenotype QTLs were found, which could have a common underlying resistance mechanism, but no metabolite QTLs mapped to this region. This locus was found to be associated with total Acyl sugar levels in a previous *S. pennellii* study (Leckie et al. 2012).
Multiple resistance associated metabolite QTLs were identified on chromosomes I, II, III, V, VI, VII, and VIII, but no phenotypic QTLs mapped to these positions (Fig 1).

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

Some of the phenotype QTLs found in this study localized at the same chromosomal regions as QTLs found for Acyl sugar production in *S. pennellii* LA716 (Blauth et al. 1999; Mutschler et al. 1996). 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 oviposition rate of *B. tabaci* adults. In our study we demonstrate co-localization of phenotype and metabolite QTLs, among which Acyl sugar precursors (Table 3). Our data support the perspective that the genome regions associated with the production of at least part of the *B. tabaci* resistance-related GC-MS constituents are present in different *S. pennellii* accessions.

Solanum habrochaites is the closest relative of *S. pennellii* (Rodriguez et al. 2009) and it is possible that resistance mechanisms are (partly) conserved. Few QTL studies have been performed on different accessions of *S. habrochaites* in which whitefly resistance was mapped. In a study by Maliepaard et al. (1995), QTLs for oviposition rate were identified on chromosomes I and XII in an F_2 population with *S. habrochaites* CGN1.1561. The QTL for oviposition rate in *S. habrochaites* on chromosome I maps at the same position as the QTLs found for *B. tabaci* adult survival in our $F_2BC_1(12)$ and $F_2BC_1(44)$ populations (Fig 1). Two *B. tabaci* resistance-related fatty acid constituents also mapped in this region (Fig 1). Antonious et al. (2005) found that the *B. tabaci* resistance in another *S. habrochaites* accession was associated with the presence of methylketones 2-undecanone and 2-tridecanone in no-choice toxicity assays. It might be that this secondary metabolite-based resistance, underlying the QTL on chromosome I has a similar functionality in the different tomato wild relatives. These two major fatty acid constituents 2-undecanone and 2-tridecanone are present in a number of *S. habrochaites* accessions and are known as precursors that conjugate with sucrose or glucose molecules to form Acyl sugars (Burke et al. 1987; Shapiro et al. 1994; Li et al. 1999). When considering the co-localization of phenotypic QTLs for *B. tabaci* resistance and the proposed underlying resistance mechanism in both *S. habrochaites* and *S. pennellii* LA3791, it is conceivable that in both wild relatives of the cultivated tomato gene homologues are involved in the synthesis of fatty acids, but further studies are needed to verify this hypothesis.

A mapping study by Momotaz et al. (2010), in which an F_2 population of *S. habrochaites* accession LA1777 was phenotyped for *B. tabaci* resistance by means of no-choice assays, identified QTLs for life-history parameters survival rate and oviposition rate, which were mapped at four different loci. One QTL was detected on chromosome IX, one on chromosome X, and two on chromosome XI (Momotaz et al. 2010). None of these QTLs correspond to the regions in which we found phenotypic QTLs. This may be explained by the fact that the resistance mechanism of *S. habrochaites* accession LA1777 has been suggested to be the result of the production of sesquiterpene zingiberene (Freitas et al. 2002). Freitas et al. (2002) selected F_2 genotypes of *S. habrochaites* accession PI-127826 for high zingiberene levels and demonstrated that these genotypes showed similar resistance levels as *S. habrochaites* accession PI-127826 and other whitefly resistant accessions. Zingiberene and its hydrogenation product curcumene were also associated with reduced *B. tabaci* preference in *S. habrochaites* accession PI127826 (Bleeker et al. 2009) showing the potential of biochemical constituents to have different modes of action at different behavioral or fitness

levels of *B. tabaci*. This also demonstrates the need for *B. tabaci* resistance screenings *in planta* to elucidate the full mechanism behind *B. tabaci* resistance and to discover valuable new resistance sources for host plant resistance breeding as was attempted in our study. We did not find sesquiterpenes or zingiberene in the *S. pennellii* LA3791 F_2 progeny (Table 3) that correlated with *B. tabaci* resistance/susceptibility and therefore there is no support for similarities in *B. tabaci* resistance between these sources.

Enhancement of QTLs for *B. tabaci* adult survival and oviposition in F_2BC_1 populations

Backcross population F_2BC_1(12) showed small quantitative differences for both *B. tabaci* life-history parameters (Fig 3A and C). The phenotyping data obtained for population F_2BC_1(12) appeared difficult to use for QTL mapping as no QTLs were detected for *B. tabaci* oviposition rates and only a single minor effect QTL was detected for *B. tabaci* survival rates. It may be that resistance in this F_2 genotype number 12 was incorrectly phenotyped or it could be that the loss of one or more resistance genes caused the abolishment of resistance in this population. As *B. tabaci* resistance is a complex polygenic trait, it can be hypothesized that many epistatic interactions take place and that the loss of one or a few genetic loci results in major breakdown of resistance in *S. pennellii* crossings (Eshed and Zamir 1996). Other literature reports about a comparable screening for Acyl sugar levels in BC_2F_1 and BC_3F_1 selected genotypes that contained subsets of five target QTLs, identified by F_2 screenings, which were associated with Acyl sugar accumulation (Lawson et al. 1997). None of these lines accumulated Acyl sugars and the BC_3F_1 was intermated to obtain homozygotes. From one thousand BC_3F_1-intermated plants, only three plants accumulated Acyl sugars at low levels (Lawson et al. 1997), which shows the complexity of the trait and the necessity of an untargeted metabolomics approach.

Strong segregation was observed in population F_2BC_1(44) for both *B. tabaci* life-history parameters (Fig 3B and D). Eight F_2BC_1(44) genotypes showed zero adult survival and oviposition rate in the no-choice assay, which indicates that these genotypes still possess the genetic profile that fully protects them against *B. tabaci* attack. This resulted in phenotypic QTLs on chromosomes I, III, IV, and VI of which the phenotypic QTLs on chromosome IV showed the highest percentage of explained variances (32.4% and 23.6% for *B. tabaci* adult survival and oviposition, respectively)(Fig 1, Table 4).

Not all phenotypic QTLs that were mapped in the F_2 population were found back in the backcross populations, which may be attributed to environmental factors. When we look at

the data from population F_2BC_1(44), we see that the explained variances are higher in this population for the QTLs found on chromosome number IV (32.4% and 23.6% for adult survival and fecundity, respectively) (Table 4). The increase in explained variances may be due to a combination of resistance mechanisms in the F_2 population that reduced the effect of a specific locus in the QTL mapping. We observed in chapter 2 that the divergence in GC-MS and LC-TOF-MS profiles between resistant F_2 genotypes was high and that genotypes possessed different biochemical fingerprints all resulting in the same complete resistant phenotype. This divergence complicates identification of genetic linkage between traits. Therefore we hypothesize that the complexity of mechanisms was higher in the F_2 population and has been reduced in the backcross lines, resulting in stronger QTLs.

Conclusion

We have identified QTLs for *B. tabaci* resistance in *S. pennellii* and compared these with resistance-related metabolite QTLs and found that the majority of metabolite QTLs are in the same region as the phenotype QTLs and it is therefore likely that these components explain a large part of *B. tabaci* resistance in *S. pennellii*. Our results show that by using F_2 and subsequently F_2BC_1 populations, that were selected on the basis of phenotype and genotype, the complexity of the resistance trait was reduced, thereby reducing the noise/signal ratio and enhancing the QTL power of phenotype traits. The reduction in complexity of the resistance offers potential for future breeding for *B. tabaci* resistance in tomato. Major and minor resistance-based QTLs were identified in our work; however, because minor QTLs with low explained variances offer, from a practical point of view, little perspective for *B. tabaci* resistance breeding and the focus for future resistance breeding should be towards major QTL regions.

By using a non-targeted approach and integrating phenotype, genotype and resistance/susceptibility-related metabolite information we took an important step in elucidating the resistance mechanism(s) behind *B. tabaci* resistance.

Acknowledgements

This project was financially supported by the Technical Top Institute of Green Genetics (TTI-GG; Resistance mechanisms against whitefly in tomato project: 1C005RP), Monsanto Vegetable Seeds (Bergschenhoek, The Netherlands), Nunhems NL (Nunhem, the

Netherlands), and Wageningen University and Research Centre. The contribution of Dr. Ric de Vos was partially funded by the Netherlands Metabolomics Centre and the Centre of Biosystems Genomics, which are both part of the Netherlands Genomics Initiative / Netherlands Organization for Scientific Research. We also are grateful to Betty Henken for technical assistance in metabolomic and greenhouse work.

CHAPTER 4

Characterization and genetic analyses of *S. pennellii* trichomes and Acyl sugars associated with whitefly resistance

Floor van den Elsen[1,4,5], Adriaan W. van Heusden[1], Colette Broekgaarden[1], Roland Mumm[2,3], Ric C.H. de Vos[2,3,6], Marcel Dicke[4], Ben Vosman[1]

[1]Wageningen UR Plant Breeding, Wageningen University and Research Centre, P.O. Box 386, 6700AJ, Wageningen, the Netherlands.
[2]Plant Research International, Business Unit Bioscience, Wageningen University and Research Centre, P.O. Box 619, 6700AP Wageningen, the Netherlands.
[3]Centre for Biosystems Genomics, P.O. Box 98, 6700AB, Wageningen, the Netherlands.
[4]Laboratory of Entomology, Wageningen University and Research Centre, P.O. Box 8031, 6700EH, Wageningen, the Netherlands.
[5]Graduate School Experimental Plant Sciences
[6]Netherlands Metabolomics Centre, Einsteinweg 55, 2333 CC, Leiden, The Netherlands

Abstract

The whitefly *Bemisia tabaci* causes large crop losses in tomato cultivation, which may be prevented by the use of resistant cultivars. The tomato wild relative *Solanum pennellii* LA3791 is completely resistant against this whitefly. Phenotyping of a *S. pennellii* LA3791 derived F_2 and F_2BC_1 mapping populations showed that a substantial part of the genotypes possess the wild relative derived resistant phenotype. Resistance was suggested to be mainly based on the presence of toxic metabolic compounds, predominantly Acyl sugars, and the presence of type I and IV trichomes. Here, we performed a genetic study on whitefly resistance traits and individual Acyl sugars in an F_2BC_1 population and were able to map all *B. tabaci* resistance-related Acyl sugars on chromosomes I, III, IV, and VIII. Exclusively Acyl sucroses were identified in the F_2BC_1 and several of them cosegregated with *B. tabaci* resistance traits on chromosomes I, III, and IV. In addition, correlations between the presence of glandular trichome types I and IV and the whitefly resistance parameters adult survival and oviposition rate were negative and highly significant.

Keywords*:* *Bemisia tabaci*, *Solanum pennellii*, LC-TOF-MS, genetic linkage map, Acyl sucroses, SNP marker, glandular trichomes.

Introduction

The yield of tomato is under constant pressure by biotic stresses, because cultivars are often highly susceptible towards many pests and diseases. Amongst the most harmful pest organisms is the phloem-sucking whitefly *Bemisia tabaci* Middle East-Asia Minor 1 (formally biotype B), which is highly invasive and extremely damaging. It feeds for prolonged periods of time on host photo-assimilates, causes phytotoxic symptoms in tomato fruits, and vectors plant-pathogenic viruses (Brown and Czosnek 2002; Oliveira et al. 2001; Byrne and Bellows 1991). A solution to overcome these problems is breeding for host plant resistance (HPR)(Panda and Khush 1995; Broekgaarden et al. 2011), which involves the transfer of resistance genes from wild relatives into tomato cultivars.

Breeding for HPR against *Bemisia tabaci* in tomato was so far primarily aimed at screening tomato wild relatives for antibiosis and antixenosis (Firdaus et al. 2012; Snyder et al. 1998; Muigai et al. 2003; Muigai et al. 2002), surveying of potentially associated biochemical compounds (Antonious et al. 2005; McKenzie et al. 2004; Liedl et al. 1995), and genetic characterization of such biochemical compounds in mapping populations (Leckie et al. 2012; Mutschler et al. 1996; Blauth et al. 1999; Blauth et al. 1998; Schilmiller et al. 2012; Schilmiller et al. 2010). Only few studies have linked whitefly resistance traits directly with genetics (Freitas et al. 2002; Heinz and Zalom 1995; Momotaz et al. 2010; Leckie et al. 2012; Firdaus et al. 2013b).

Freitas et al. (2002) studied the inheritance of the sesquiterpene zingiberene in a *Solanum lycopersicum* (accession Tom556) x *Solanum habrochaites* (accession PI 127826) F_2 population and found that F_2 plants that produced higher levels of zingiberene were more resistant to the whitefly *B. tabaci*. However, the data was not analyzed by regression mapping and therefore the chromosomal fragment linked to the resistance remained unidentified. Momotaz et al. (2010) performed quantitative trait loci (QTL) analyses on an F_2 population of *Solanum lycopersicum* x *S. habrochaites* accession LA1777 in no-choice assays. They identified four different loci that were associated with resistance. As this study did not include analysis of plant metabolic contents, it cannot be compared directly with the study of Freitas et al. (2002), and other resistance/susceptibility factors may have been involved as different accessions were employed.

Two studies reported on the genetics of *B. tabaci* resistance originating from donor parent *Solanum pennellii* LA716, which produces Acyl glucoses that confer resistance to *B. tabaci* (Liedl et al. 1995; Maluf et al. 2010; Nombela et al. 2000; Muigai et al. 2003). Heinz and

Zalom (1995) used substitution lines of the chromosomes II, III, IV, VI, VIII, and XI and correlations with *B. tabaci* oviposition rates on these lines suggested that the genetic basis is spread across at least five chromosomes (II, III, VI, VIII, and XI). Together, these substitution lines covered almost half of the *S. pennellii* genome and as the role of six chromosomes was not evaluated, there is still limited information on the genetics behind *B. tabaci* resistance. Leckie et al. (2012) used an F_1BC_1 population of a cross between a breeding line and *S. pennellii* LA716. The homozygous breeding line had five known *S. pennellii* introgressions on chromosomes II, III, VII, and X and produced moderate levels of Acyl sugars. They identified additional QTLs for Acyl sugar production and their results showed reduced fitness of *B. tabaci* on a number of BC_1F_1 plants possessing additional minor QTLs on chromosomes VI and X. However, even plants that contained all QTLs did not produce Acyl sugar levels similar to *S. pennellii* LA716, but the increased levels did reduce the incidence of whitefly damage.

Wild and cultivated tomato have morphological structures called trichomes on the epicuticular leaf surface, predominantly at the abaxial leaf side. Different types of trichomes have been described in the literature (Simmons and Gurr 2005; Luckwill 1943). Trichomes can have no, uni- or multicellular heads (Simmons and Gurr 2005) of which the cellular heads are referred to as glandular heads (Luckwill 1943)(Fig 1a and b). Trichomes are known to have a biochemical as well as mechanical mode of defense against herbivorous insects in many plant species (Agrawal and Karban 1999; Antonious et al. 2005; Van Dam and Hare 1998; Steffens and Walters 1991; Simmons et al. 2004). The role of glandular trichomes of tomato and their correlation with *B. tabaci* resistance has been studied extensively in tomato wild relatives with regards to trichome type and density (Snyder et al. 1998; Simmons and Gurr 2005; Sanchez-Pena et al. 2006; Muigai et al. 2003; Muigai et al. 2002; Channarayappa et al. 1992; Antonious et al. 2005; Firdaus et al. 2012). In the case of *S. pennellii*, all trichome types (I, IV, VI, VII; Fig 1b) at the leaf surface area have glandular heads that release sticky/toxic compounds during contact with an insect, that may entrap the insect and/or have toxic effects on the insect (Simmons and Gurr 2005). On most of the *S. lycopersicum* cultivars the trichomes have no heads, with the exception of trichome types I and VI. The non-glandular trichomes have been suggested to act as mechanical barriers against insect pests in tomato (Simmons and Gurr 2005; Muigai et al. 2002) and also in other plant species (Agrawal and Karban 1999; Agrawal 1998; Fordyce and Agrawal 2001; Traw et al. 2003), but at the same time these epidermal leaf structures can provide protection to the pest insect against insect predators/parasitoids and therefore hamper pest control (Dicke 1999; Krips et al. 1999). The

biochemical constituents of trichomes have been studied in tomato mapping populations. In an F_2 population of *S. lycopersicum* x *S. habrochaites* there was segregation for trichome gland shape and synthesis of methylketones in type VI trichomes, which were correlated (Ben-Israel et al. 2009). Type IV trichome density was scored in an intraspecific F_2 population of *S. pennellii* LA716 (high Acyl sugar levels) x *S. pennellii* LA1912 (low Acyl sugar levels) and a significant correlation was observed between type IV trichome density and Acyl sugar concentrations (Blauth et al. 1998). The Acyl sugar concentration mapped to several loci. There are no literature reports so far about the correlation between trichome types and *B. tabaci* resistance directly.

The objectives of this study were to identify the individual Acyl sugars that significantly contributed to a reduced *B. tabaci* survival and oviposition in a BC_1F_2 population derived from a cross between a *S. lycopersicum* elite line and *S. pennellii* LA3791. A correlation study and QTL analyses were performed to demonstrate morphological and genetic associations between *B. tabaci* life-history parameters, the level of Acyl sucrose production and the distribution of glandular and non-glandular trichome types.

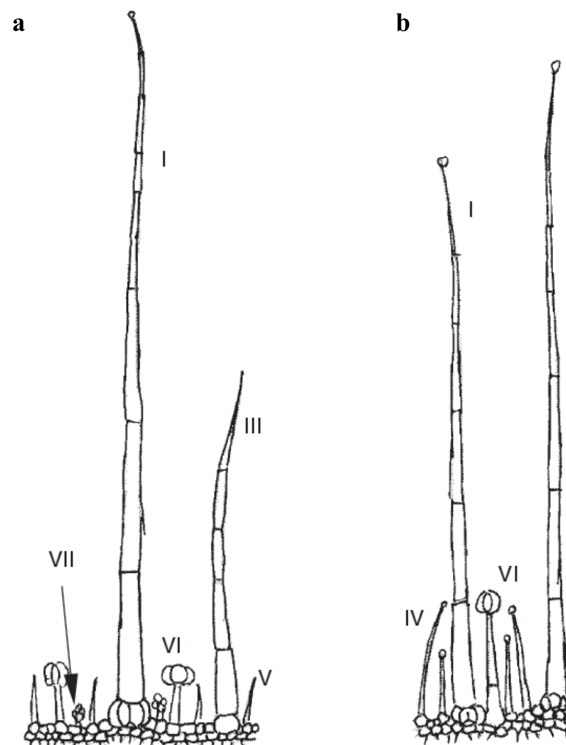


Fig 1a and **b** Trichomes on *S. lycopersicum* (**a**) and *S. pennellii* (**b**) as described by Luckwill (Luckwill 1943). Sources: (Figure adopted from Simmons and Gurr 2005; original figure from Luckwill 1943)

Materials and Methods

Plant material and growing conditions

An interspecific cross was made between *S. pennellii* LA3791 and an elite tomato (*S. lycopersicum*) cultivar To6W_LI0620 (hereafter referred to as EC), which was provided by Nunhems NL, Nunhem, The Netherlands. A single F_1 plant was selfed to produce F_2 seeds. One fully resistant F_2 plant (nr 44; chapter 3) was selected for *B. tabaci* resistance by measuring life-history parameters (chapter 3) and crossed with the EC to produce an F_2BC_1 backcross population. One hundred nineteen F_2BC_1 plants were grown for phenotyping and chemoprofiling experiments. Seeds were sown in potting trays on Lentse Potgrond soil substrate for flowering pot plants (Horticoop, The Netherlands). One-week-old seedlings were transplanted into pots (Ø 20cm) with the same soil substrate. Plants were grown under controlled conditions in a glasshouse (22 ± 2°C, L16:D8 photoperiod, RH about 50%), watered daily, and supplemented with nutrients once a week. For chemoprofiling, one cutting per individual F_2BC_1 genotype was made from six-week-old unchallenged plants and grown in trays on soil substrate. Subsequently, the cuttings were transferred to soil in pots (Ø 20cm), and grown in an insect- and pathogen-free environment (22 ± 2°C, L16:D8 photoperiod, RH about 50%). No chemical control of pathogens or pests was practiced during growing, screening, and sampling of the test plants.

Leaf sample preparation for metabolomics

One cutting per F_2BC_1 genotype plus their *Solanum pennellii* and EC referential genetic sources was grown in a randomized block design. The environmental parameters were adjusted one week prior to the collection of leaf material for chemoprofiling to 26 ± 2°C, L16:D8 photoperiod, RH 60 ± 10%. This was done to use the same environmental conditions as during previously performed *B. tabaci* phenotyping experiments (chapter 2). The third and fourth internode leaves of six-week-old uninfested plants were cut off, petioles were removed, and leaves were pooled, packed in aluminum foil without causing damage to leaf tissue, and instantly frozen in liquid nitrogen (LN_2; -196°C). Leaf samples were stored at -80°C until further analysis.

LC-QTOF-MS metabolic profiling

Extraction and analysis by accurate mass Liquid Chromatography-Quadrupole Time of Flight-Mass Spectrometry (LC-QTOF-MS, in short LC-TOF-MS) was performed as described previously (De Vos et al. 2007). In short, 250 ± 10mg (FW) of ground leaf powder was weighed in 10ml glass tubes. Sample extraction was done by thoroughly mixing with 750 µl methanol containing 0.125% formic acid (FA) followed by sonication in a water bath (15min). After centrifugation (5 min at 3000g) and filtering (Captiva 0.2 µM PTFE filter plate, Agilent), 5 µl per sample was injected in the LC-TOF-MS system (Waters QTOF Ultima) and separated on a Phenomenex Luna C18 (2) column (2.0 × 150 mm, 3 mm particle size) using a 5–95% ACN gradient in H_2O with 0.1% FA for acidification. Mass signals of *m/z* 80–1,500 were detected with negative electrospray ionization. Leucine encephalin was used as lock mass for local accurate mass corrections (Moco et al. 2006).

Metalign software (Lommen 2009) was used to automatically extract and align all relevant LC-TOF-MS signals (signal to local noise ratio >3) from the raw data files. Accurate masses of signals were automatically calculated by Metalign by taking into account only those scans with a signal intensity corresponding to the local lock mass intensity plus or minus 50% (Moco et al. 2006). The total number of signals were filtered for signals present in at least four samples and having an amplitude of at least six times the noise value in at least one of the samples. Then, all signals eluting within 3 min of retention time (i.e. the injection peak, mostly consisting of signals from non-retained highly polar compounds) were removed from the dataset. MSClust was used to group mass signals originating from the same molecule, including the molecular ion, natural isotopes and in-source fragments and adducts, into reconstructed metabolites (Tikunov et al. 2012).

Identification of Acyl sugars

Mono-isotopic exact masses of negatively charged ions were calculated for a series of possible Acyl chain-sugar combinations, from 7 up to 30 carbons Acylated to either glucose (G) or sucrose (S) as the sugar backbone, i.e. starting from m/z 333.0827 for G4:7 up to m/z 803.5162 for S3:50, as well as their formic acid adducts (additional mass of 46.0055 for CH_2O_2). Under the LC-TOF-MS conditions applied, the Acyl sugars were mainly detectable as their formic acid adducts. Metalign software (www.metalign.nl) was used to extract all LC-TOF-MS mass signals and mass peaks corresponding to the major Acyl sugars were subsequently annotated based on their unique mono-isotopic accurate mass, using a threshold of 5 ppm deviation of detected masses from calculated masses.

Data analyses and selection of Acyl sugars

Two genotype bulks, a *B. tabaci* susceptible (n=10) and a resistant (n=10) group, were formed based on adult survival and oviposition life-history data and used for comparative analyses to select for Acyl sugars that significantly differentiated between the two bulks.

Data analyses were done with MS Excel (2010) software. The data were $\log_{10}$ transformed and a Student's t-test was performed per metabolite between genotype groups and subsequently *p*-values were ranked. A false discovery rate (FDR) control was applied to correct for multiple comparisons. The corresponding *q*-values were calculated according to Benjamini and Hochberg (1995):

$$q\text{-value} = \left(\frac{m}{i}\right) * P_i$$

where *q* is the FDR-corrected *p*-value for a single metabolite, *m* the number of variables (metabolites), *i* the rank of the *p*-value of the variable, P_i the *p*-value.

The metabolites with $q<0.05$ were used for peak annotation.

DNA extraction and marker analysis of an F_2BC_1 population

Leaves from 144 F_2BC_1 plants were sampled when the plants were in the true two-leaf stage and collected on ice in 1.4 ml polypropylene tubes in 96-well format (Micronics) containing two 3 mm stainless steel grinding beads (Retsch GmbH & Co KG). Lysis buffer (300 µl; LGC Genomics, Germany) with 0.5 µl RNase (2 mg/ml) was added per tube and samples were ground using the Retsch mixer mill (1 min, 30 rps; MM300 Retsch GmbH & Co KG), centrifuged (1 min 300 rpm), and incubated in a water bath (65°C, 30 min.). DNA was extracted with the Kingfisher Flex Magnetic Particle Processor (ThermoScientific). Reagents for the Kingfisher DNA extraction were obtained from LGC Genomics (Germany). The sbeadex® Maxi Plant kit was used according to the protocol of the supplier (LGC Genomics). Two hundred µl of the dissolved plant material was mixed with 520 µl binding buffer and suspended with 60 µl magnetic beads in a 96-Deep Well plate (ThermoScientific). Sample concentration and quality was assessed on a 1% agarose gel. Samples were normalised to 50 ng/µl by diluting the gDNA concentration in 10 mM Tris/1 mM EDTA pH=8 (TE). Genotyping was carried out by Service XS, Leiden, the Netherlands with Illumina's Infinium SolCAP Tomato BeadChip, according to the Illumina Infinium II Protocol (Sim et al. 2012).

Genetic map construction and QTL mapping

Construction of the genetic map for the F_2BC_1 population was performed with the software package JoinMap v.4.0 (Van Ooijen 2006). JoinMap settings were adjusted for both F_2BC_1 populations to enable the construction of linkage maps with large numbers of SNP markers, as obtained with the SolCap array. Linkage groups were based on recombination frequency with a maximum value of 0.25 and the Haldane mapping function based on the maximum likelihood mapping algorithm. Distorted markers were excluded from the map and markers showing an identical segregation pattern were represented by one marker. Phenotypic and metabolic QTLs in the F_2BC_1 population were calculated using MapQTL (Van Ooijen 2004) v.6.0. The LOD-score threshold values for phenotype QTLs and metabolite QTLs were fixed at 3.0. Interval mapping was employed to determine the interval of the phenotypic QTL using a 1-LOD and 2-LOD drop off interval. The MapChart 2.2 Software (Voorrips 2002) was employed for the graphical presentation of linkage maps and QTLs.

Analyses of trichomes in the F_2BC_1 population

Non-glandular and glandular trichomes on the abaxial side of the leaves of plants from the F_2BC_1 population were classified under a stereomicroscope (Zeiss) according to their morphological characteristics (Simmons and Gurr 2005; Luckwill 1943)(Fig 1). The number of trichomes with and without glandular secretion cells from a particular type was estimated in the whole F_2BC_1 using a quantitative scale (Table 1). The ratios between non-glandular trichome type V and glandular trichome type IV and the ratios between non-glandular trichome type III and glandular trichome type I were determined and divided over seven classes (Table 1). The regression coefficient (R) was calculated by Spearman's rank correlation coefficients in Genstat for Windows (14th edition).

Cryo-Scanning Electron Microscopy imaging

Cryo-Scanning Electron Microscopy was performed to visualize the various trichome types that were studied. The abaxial side of fresh tomato leaves was glued on a brass Leica sample holder by carbon glue (Leit- C, Neubauer Chemicalien, Germany), immediately frozen in liquid nitrogen and simultaneously fitted in the cryo-sample loading system (VCT 100). The Leica sample holder was transferred to a non-dedicated cryo-preparation system (MED 020/VCT 100, Leica, Vienna, Austria) onto a sample stage at -93˚ C. In this cryo-preparation chamber the samples were freeze-dried for 3 min. at -93°C at 1.3 x 10 $^{-6}$ m-Bar to remove water vapour contamination from the surface of the sample. The sample was sputter coated

with a layer of 15 nm Tungsten at the same temperature. The samples were transferred into the field emission scanning microscope (Magellan 400, FEI, Eindhoven, the Netherlands) on the sample stage at -122°C at 4 x 10 $^{-7}$ m-Bar. The analysis was performed with SE at 1 and 2 kV, 13 pA. All images were recorded digitally.

Table 1 Classification scheme for trichome glandularity ratios. Ratios were determined between glandular secreting trichome type I and non-glandular trichome III and between glandular secreting trichome type IV and non-glandular trichome V.

Class	Glandular trichome type I/IV (%)	Non-glandular trichome type III/V (%)
1	0	100
2	1 to 20	80 to 99
3	21 to 40	60 to 79
4	41 to 59	41 to 59
5	60 to 79	21 to 40
6	80 to 99	1 to 20
7	100	0

Results

Relationship between glandular trichome types and resistance against *B. tabaci*

In our F_2 population, variation in the combination and type of leaf trichomes was observed, which was still present in the F_2BC_1 population. Segregation patterns of different trichome types were further analyzed to reveal correlations between whitefly resistance traits and glandularity of trichomes and to reveal correlations between the segregation of specific trichome types. Glandular trichome types I and IV and non-glandular trichome types III and V were identified (Fig 2a-f) and classified in order to detect correlations between *B. tabaci* resistance and trichome composition. While overall trichome density was more or less constant in the F_2BC_1 population (results not shown), segregation in the ratio of trichome type was observed. Correlations were significant ($P<0.001$) for all studied traits although not collinear for all comparisons and strong correlations ($R>0.8$) were absent, indicating that glandularity of trichomes plays an important role, but supplementary factors contribute to *B. tabaci* resistance as well (Table 2).

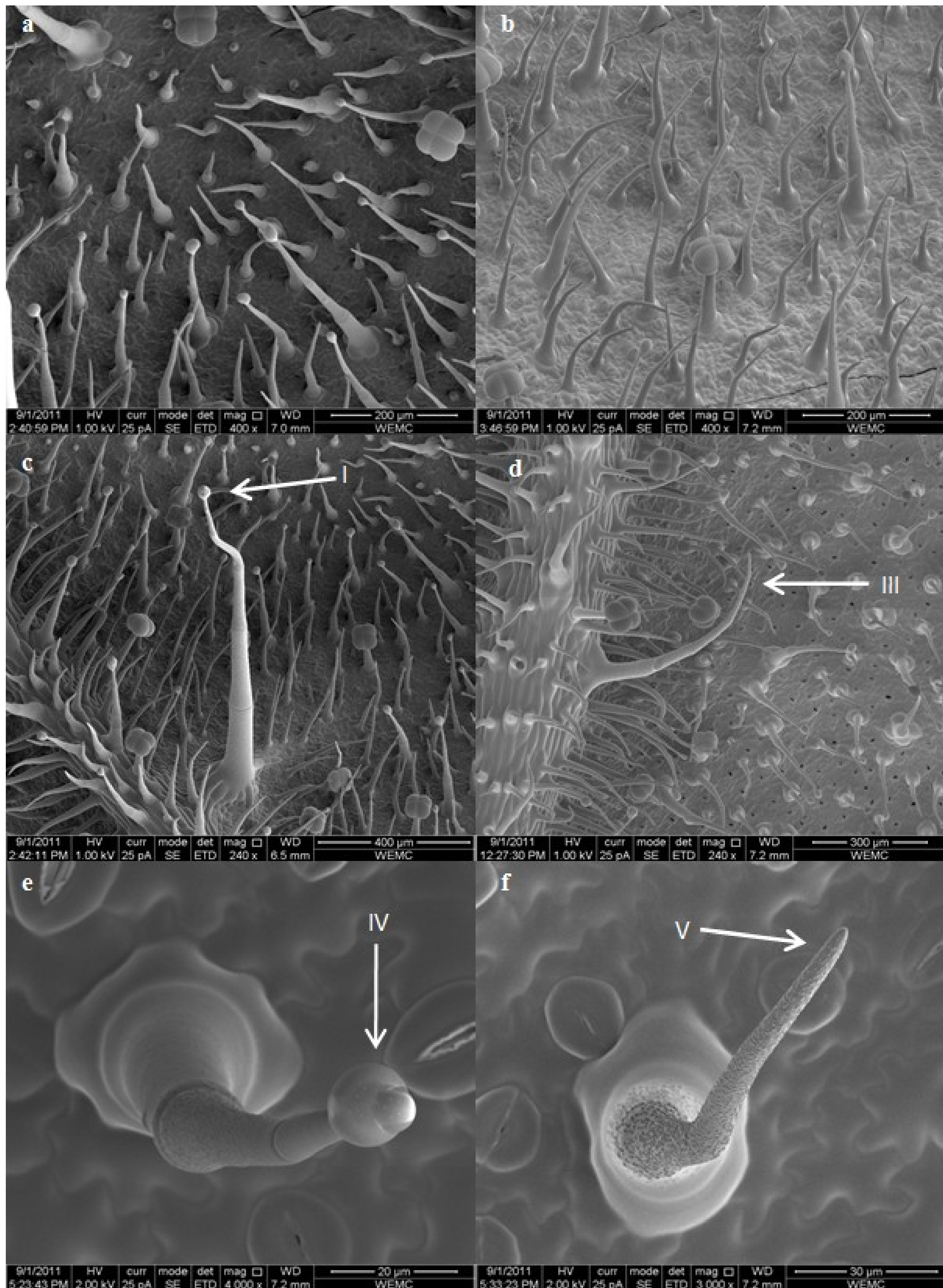


Fig 2a-f Cryo-Scanning Electron Microscopy images of the different glandular and non-glandular trichomes identified in F_2BC_1 plants. Panels a and **b** show the presence of both abaxial epicuticular glandular and non-glandular trichomes. **Panels c-f** show glandular trichome type I, non-glandular trichome type III, glandular trichome type IV, and non-glandular trichome type V, respectively. Images of Cryo-samples were created with a field emission scanning microscope (Magellan 400, FEI, Eindhoven, the Netherlands).

Table 2 Spearman's correlation coefficient matrix showing the R and corresponding P-values of the *B. tabaci* resistance phenotype and plant trichomes in the F_2BC_1 population.

	Bt[a] Survival	Bt Oviposition	Gtt[b] I	Ngtt[c] III	Gtt IV	Ngtt V
Bt Survival	*					
Bt Oviposition	0.62 (>0.001[d])	*				
Gtt I	-0.51 (>0.001)	-0.43 (>0.001)	*			
Ngtt III	0.51 (>0.001)	0.43 (>0.001)	n.a.[e]	*		
Gtt IV	-0.60 (>0.001)	-0.57 (>0.001)	0.70 (>0.001)	-0.70(>0.001)	*	
Ngtt V	0.60 (>0.001)	0.57 (>0.001)	-0.70 (>0.001)	0.70 (>0.001)	n.a.	*

[a] Bt: *Bemisia tabaci* Middle East-Asia Minor 1

[b] Gtt: Glandular trichome type

[c] Ngtt: Non-glandular trichome type

[d] P-values are shown between parentheses

[e] n.a.: not applicable; ratios were determined between these trichome types and cc:1.0.

Correlations between *B. tabaci* resistance and individual Acyl sucroses in an F_2BC_1 population

Intergroup differentiation in the relative abundance of Acyl sugars was studied between the ten most resistant and the ten most susceptible genotypes that are listed in Table 3. The LC-TOF-MS analyses revealed the presence of in total 13 different Acyl sucroses in the F_2BC_1 population (Table 4). Acyl glucoses were not detected, as was expected since Acyl glucoses were absent in our F_2 population (chapter 2). The nomenclature of the different Acyl sucroses is given as e.g. S3-15-I, which describes an Acyl sucrose with three Acyl groups of carbon chain lengths 5, 5, and 5 for a total of 15 carbons (Schilmiller et al. 2012) and the concluding numeral indicates the retention time order in case of isomeric forms.

Nine out of the 13 Acyl sucroses had a significantly higher abundance in our *B. tabaci* resistant group compared to our susceptible group, while none of the Acyl sucroses were associated with susceptibility. This result suggests that these nine Acyl sucroses are directly associated with *B. tabaci* resistance. LC-TOF-MS chromatograms of wild type *S. pennellii* LA3791, F_2 genotype number 44, and an F_2BC_1 genotype (BC of F_2 plant 44) are shown in Fig 3. These three plants were previously identified as fully resistant against *B. tabaci* based on adult survival rate and oviposition rate (chapters 2 and 3). We observed a strong reduction in the complexity of the LC-TOF-MS profile of the resistant F_2BC_1 genotype in comparison to the resistant wild type and F_2 genotype.

Table 3 Trichome classification (class 1 to 7 from Table 1) of the ten most resistant F_2BC_1 genotypes (**R**) and the ten most susceptible F_2BC_1 genotypes (**S**) (ranked according to *B. tabaci* oviposition and survival, respectively) from an F_2 genotype from *S. pennellii* LA3791 x *S. lycopersicum* elite line backcrossed with *S. lycopersicum* elite line.

	Genotype nr	Survival (fraction/5dpi)	Oviposition (eggs/female/5 days)	Gtt[a] I	Gtt IV
R	49	0.00	0.00	6	6
	59	0.00	0.00	6	6
	62	0.00	0.00	6	5
	63	0.00	0.00	3	5
	78	0.00	0.00	6	6
	85	0.00	0.00	6	6
	102	0.00	0.00	3	6
	22	0.03	0.00	4	6
	90	0.03	0.00	4	5
	18	0.05	0.00	4	5
S	29	0.81	25.97	1	1
	1	0.86	26.14	1	1
	23	0.82	27.14	2	2
	42	0.87	28.68	2	2
	92	0.92	28.80	2	2
	48	0.80	29.18	2	2
	26	0.75	32.00	4	4
	7	0.80	32.75	2	2
	71	0.68	34.57	2	2
	51	0.46	34.90	3	3

[a] Gtt: Glandular trichome type

Table 4 Differentiation in individual Acyl sucrose peak intensities between genotype groups associated with *B. tabaci* resistance and susceptibility in an F_2BC_1 population of a cross between an F_2 genotype (*S. pennellii* LA3791 and *S. lycopersicum* elite line) x *S. lycopersicum* elite line.

Acyl sucrose	**P-value**[a]	**Resistant group**[b]	**Susceptible group**[c]	**QTLs**[d]
		Average abundance + SD	Average abundance + SD	
S3-15 I	< 0.001 ***	740.39 ± 269.50	0.18 ± 0.09	Yes
S3-20 II	<0.001 ***	55.6242 ± 22.78	0.65 ± 0.58	Yes
S3-15 II	< 0.01 **	3514.51 ± 1604.48	9.66 ± 4.40	Yes
S3-21 V	< 0.01 **	158.50 ± 75.84	0.12 ± 0.11	Yes
S3-22 III	< 0.01 **	258.82 ± 116.80	0.92 ± 0.94	Yes
S3-20 III	< 0.01 **	323.05 ± 142.06	5.80 ± 4.66	Yes
S3-14 III	<0.05 *	112.79 ± 70.004	0 ± 0	Yes
S3-22 VI	<0.05 *	1079.12 ± 771.47	2.19 ± 2.011	Yes
S3-22 IV	<0.05 *	254.58 ± 120.95	52.90 ± 31.02	Yes
S3-22 V	0.631 n.s.[e]	180.96 ± 187.06	122.21 ± 69.56	No
S3-22 I	0.916 n.s.	0.038 ± 0.06	0.04 ± 0.04	No
S3-20 I	1.000 n.s.	0 ± 0	0 ± 0	No
S3-20 IV	1.000 n.s.	0 ± 0	0 ± 0	No

[a] P-values calculated with Student's t-test; [b] Group of ten genotypes resistant against *Bemisia tabaci* ; [c] Group of ten genotypes susceptible to *B. tabaci* ; [d] Quantitative trait loci identified or not; [e] n.s. not significant

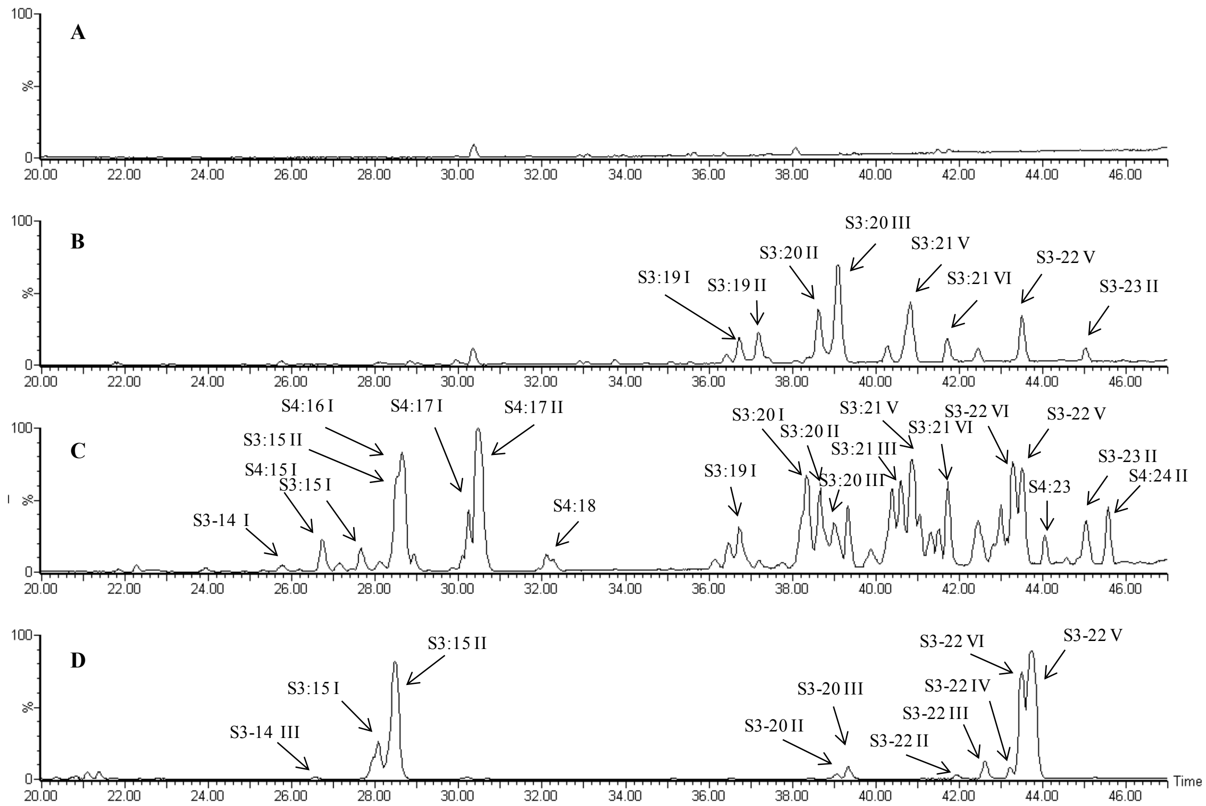


Fig 3 Part of representative LC-QTOF MS chromatograms of crude leaf extracts, showing the presence of Acyl sugars in: A) *S. lycopersicum* Elite Cultivar, B) *S. pennellii* LA3791, C) line $F_2(44)$, and D) line $F_2BC_1(44)$. Y-axes are on the same scale (100% = $3.26x10^4$ ion counts per sec). Acyl sugar peaks are annotated with number of sugars and number of Acyl groups, e.g. S4:15; roman numbers refer to different isomers of the same Acyl sugar.

QTL analyses of Acyl sucroses associated with *B. tabaci* resistance in an F_2BC_1 population

QTLs for Acyl sucroses were found on chromosomes I, III, IV, and VIII and QTLs for *B. tabaci* resistance on chromosomes I, III, and IV (Fig 4). Explained variances, QTLs, and traits are listed in Table 5. Phenotypic and metabolic QTLs co-localize on chromosomes I, III, and IV. The QTL on chromosome III for adult survival co-localized with seven different Acyl sucrose QTLs. There was a minor QTL for *B. tabaci* oviposition on the same position of chromosome III, but it was just below the LOD-threshold value. Two QTLs were identified on chromosome III for Acyl sucrose S3-22-III of which one located within the hotspot area. The highest explained variance on chromosome III was found for Acyl sucrose S3-22-VI, which explained 29% of the trait.

Two closely linked QTLs were found on chromosome IV. At the first QTL seven different Acyl sucrose QTLs co-localized with QTLs for survival and oviposition. At the other QTL,

there were two Acyl sucroses co-localizing (S3-22-III and S3-21-V). A QTL with an explained variance of 43.2% was identified for Acyl sucrose S3-15-I, which is the compound most significantly correlated with *B. tabaci* resistance (Table 4). Other major QTLs were found at this locus for Acyl sucroses S3-22-VI and S3-15-II explaining 40.5 and 49.9 percent of variance, respectively (Table 5). In addition, high explained variances were also identified at this position for adult survival and oviposition with values ranging between 19.8 and 30.7 percent. Three Acyl sucroses were mapped to the same position on chromosome VIII. The LOD-values for phenotype QTLs showed an increase at the same position on this linkage group, but remained below the threshold level.

Table 5 Chromosome numbers, traits, and explained variances in percentages of QTLs. Two QTLs for a single trait on the same chromosome are defined by letters a and b between parentheses.

Chromosome	Trait	Variance explained (%)
I	S3-20-II	10.6
I	*B. tabaci* survival	12.1
III	S3-22-III(a)	25.3
III	S3-22-III(b)	25.2
III	S3-22-IV	26.8
III	S3-20-II	21.6
III	S3-20-III	29.0
III	S3-15-I	16.9
III	S3-21-V	18.9
III	S3-14-III	12.6
III	*B. tabaci* survival	15.6
IV	S3-22-III	12.5
IV	S3-22-IV	17.4
IV	S3-22-VI	40.5
IV	S3-20-II	17.4
IV	S3-15-I	43.2
IV	S3-15-II	49.9
IV	S3-21-V(a)	22.7
IV	S3-21-V(b)	23.3
IV	S3-14-III	16.9
IV	*B. tabaci* survival (a)	30.7
IV	*B. tabaci* survival (b)	29.6
IV	*B. tabaci* oviposition (a)	19.8
IV	*B. tabaci* oviposition (b)	25.9
VIII	S3-22-III	12.8
VIII	S3-22-IV	15.7
VIII	S3-20-II	13.1

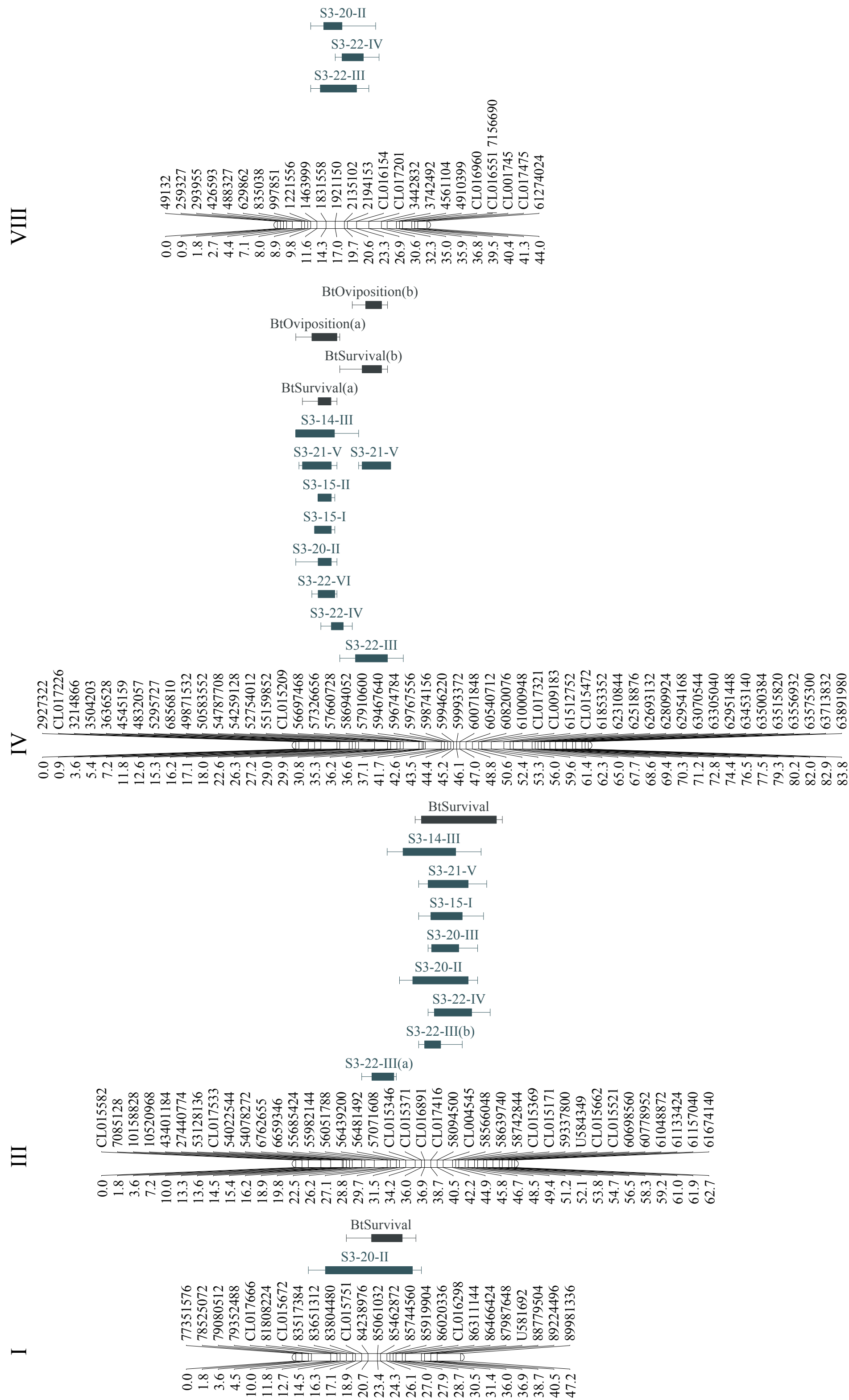


Fig 4 Linkage maps of an F_2BC_1 population of a cross between an F_2 genotype (*S. pennellii* LA3791 and *S. lycopersicum* elite line) x *S. lycopersicum* elite line. Marker names are replaced by their physical position and shown on the right side of the chromosome bar. Genetic positions are shown on the left side of the bar in cM. The QTLs show the localization of QTLs identified for nine different *B. tabaci* resistance-related Acyl sucroses (blue) and of *B. tabaci* survival and oviposition (grey) in 1-LOD and 2-LOD drop off intervals.

Discussion

Glandular trichome types I and IV show corresponding segregation and have a role in *B. tabaci* resistance

A linear correlation between the presence of glandular trichome types I and IV was observed in the F_2BC_1 population. These glandular trichome types differ in morphology (Luckwill 1943) as well as in density on the leaf surface of the wild tomato relative *S. pennellii* (Simmons and Gurr 2005; Dowell et al. 2011). In addition, we observed positive correlations between a higher ratio of trichome types I and IV present on the abaxial leaf surface and reduced performance of *B. tabaci*. In a functional genomics comparison of *S. habrochaites* LA1777 trichome types I and IV, McDowell et al. (2011) showed minor differences in transcript abundance and metabolic content between these trichome types, suggesting that they are essentially the same and only differ in stalk length For *S. pennellii* LA3791 this may explain the corresponding segregation between these trichome types and the overall positive correlation with *B. tabaci* resistance. However, since type I is sparsely present on *S. pennellii* the effect of this trichome type on *B. tabaci* resistance might be smaller, which was also apparent from a lower R^2 for adult survival and oviposition and as type I is highly correlated with type IV the R^2 in relation to resistance could be overestimated. Further studies need to be done to confirm if trichome type I synthesizes the same compounds as type IV in *S. pennellii* and what the effect of type I on whitefly resistance is when type IV is absent, which will probably be highly dependent on trichome density and concurrently abundance of toxic metabolites.

Individual Acyl sucroses play a major role in preventing/reducing *B. tabaci* incidence

Previous work focused on the mapping of compounds from untargeted metabolomics profiling (chapter 3) and resulted in the identification of genetic cold- and hotspot QTL areas for these metabolites, of which some co-localize with *B. tabaci* resistance QTLs. The untargeted metabolomics approach applied allowed the detection of new, yet unknown metabolites that correlate with resistance and susceptibility traits. As many of these unknowns (approximately 80%) could be assigned to a specific locus on the tomato map, we were able to study the genetics behind these metabolic traits and were able to assign their functionality by taking an integrative approach. In this approach the metabolite QTLs and the phenotypic

QTLs of whitefly resistance-related parameters were jointly mapped to identify co-localizations between the various traits. Previous studies in *Arabidopsis thaliana* and apple proved that mapping of whole untargeted metabolite profiles on the genetic map can be successfully applied to identify metabolite QTLs (Keurentjes et al. 2006; Khan et al. 2012). With regard to *B. tabaci* resistance in tomato, we found a predominant role for Acyl sucroses and Acyl sucrose derivatives out of several hundreds of metabolites detected by using both LC-TOF-MS and GC-MS-based untargeted profiling platforms (chapter 2).

In the present chapter we studied the role of individual Acyl sugars in tomato on *B. tabaci* resistance and susceptibility and we were able to show their relevance for resistance and the genetic loci involved in their accumulation. Total Acyl sugar content has previously been studied in *S. pennellii* in relation to whitefly resistance (Liedl et al. 1995). However, the correlation of single Acyl sucroses/glucoses with *B. tabaci* resistance has not been studied before. Nine different Acyl sucroses were identified that correlate with *B. tabaci* resistance. Remarkably, we did not detect Acyl glucoses, neither in the wild parental line *S. pennellii* LA3791 nor in the F_2 and F_2BC_1 progeny (chapter 2 and 3). Other studies either confirm or contradict these findings. In a number of studies it was suggested that *S. pennellii* accession LA716 synthesizes a mixture of Acyl glucoses and Acyl sucroses of which the Acyl glucoses were prevailing, amounting up to 85% of the total Acyl sugar content (McDowell et al. 2011; Eggleston et al. 1995). However, in other studies using accurate mass LC-TOF-MS, Acyl sucroses were identified as the most dominant Acyl sugars present in *S. pennellii* LA716 (Schilmiller et al. 2012; Schilmiller et al. 2010).

The total Acyl sugar content of *S. pennellii* LA716 was found to confer resistance against *B. tabaci* (Liedl et al. 1995), but it was not unraveled what the correlation between individual compounds and the resistance was and whether all compounds were required for this resistance. All resistance-related Acyl sucroses that were identified here are composed of three Acyl groups (S3) and have 14 to 22 carbon atoms attached. Our QTL study showed co-localization between nine individual Acyl sucroses and *B. tabaci* resistance factors, indicating a genetic co-correlation between traits, but this can only be confirmed upon the identification of candidate genes.

Reduced complexity of chemoprofiles in F_2BC_1 *B. tabaci* resistant genotypes

Liquid Chromatography-Mass Spectrometry visualized the metabolic fingerprint of F_2 and F_2BC_1 resistant genotypes and it was perceived that the number of metabolites involved in *B.*

tabaci resistance was reduced in the more advanced F_2BC_1 genotypes compared to resistant F_2 genotypes, which was shown as example for genotype 44 in Figure 3. A backcross with the recurrent parent reduced the number of resistance correlated peaks without losing the desired resistant phenotype. The phenomenon of reduced complexity of metabolic profiles resulted in a higher level of resolution of QTLs for the remaining Acyl sugars, which is desired for breeding as major QTLs can be easier adopted in breeding programs and the total number of QTLs should be limited as otherwise breeding becomes too complex and introgression of minor QTLs in commercial tomato might not give the desired level of resistance (Mammadov et al. 2012).

Major QTLs for *B. tabaci* resistance in *S. pennellii* LA3791

Completely whitefly resistant genotypes were identified in a greenhouse trial in our F_2BC_1 population at levels equal to the resistant donor line. A QTL analysis revealed four phenotypic QTLs for adult survival on three different chromosomes. Two minor QTLs were identified for *B. tabaci* survival on chromosome I and III and one major QTL was identified on chromosome IV for adult survival and oviposition rate. On chromosomes I and III no QTLs for oviposition rate were detected, although on chromosome III there may be one that remained undiscovered due to the LOD-score threshold of 3.0. The two QTLs for adult survival on linkage group IV co-localized with the two QTLs for *B. tabaci* oviposition rate. This is in agreement with results in chapter 2 of this thesis in which QTLs were mapped for these resistance parameters at the same location and where we hypothesized that the same biochemical defense mechanism may affect both fitness parameters.
Quantitative trait loci for *B. tabaci* resistance parameters have not been identified in *S. pennellii* accession LA3791. Introgressions of chromosomes II, III, VII, and X of another accession, *S. pennelii* LA716, in a *S. lycopersicum* background showed reduced *B. tabaci* incidence (Leckie et al. 2012). Thus, assuming that no intraspecific chromosomal rearrangements have occurred, only the QTL on linkage group III was found in our study as well as in that of Leckie et al. (2012). Furthermore, quantitative trait loci for oviposition rate of another whitefly species, the greenhouse whitefly *Trialeurodes vaporariorum*, have been mapped on chromosomes I (*tv-1*) and XII (*tv-12*) in *S. habrochaites* for oviposition rate (Maliepaard et al. 1995). We did not detect any QTLs on chromosome XII in our F_2BC_1 population, but the location of the QTL on chromosome I was the same between the two studies. The major known biochemical constituents conferring resistance against *B. tabaci* in

S. habrochaites are the fatty acids 2-undecanone and 2-tridecanone (Antonious et al. 2005). As *S. habrochaites* is the closest relative of *S. pennellii* within the tomato clade (Rodriguez et al. 2009; Marshall et al. 2001; Peralta et al. 2008) and assuming that no major chromosomal rearrangements occurred between these wild tomato relatives (Anderson et al. 2010), it can be hypothesized that identical/comparable resistance mechanism(s) is/are involved and that genes that are part of the same biochemical pathway are located within this QTL region. Acyl sucrose 3S-20-II, which has a highly significant correlation with *B. tabaci* resistance, and a QTL for *B. tabaci* adult survival map at the same position as *tv-1* providing evidence for a biochemically-based resistance gene at chromosome I. In the F_2 population we have previously identified two metabolite QTLs from Gas Chromatography-Mass Spectrometry (GC-MS) analysis, amongst which was the fatty acid dodecanoic acid, located at the same position as *tv-1* (chapter 3). Fatty acids are known to conjugate with sucrose or glucose molecules to form Acyl sugars in *S. pennellii* (Burke et al. 1987; Shapiro et al. 1994). It can be hypothesized that with regard to this biochemical-based resistance, the resistance gene(s) underlying the QTL on chromosome I have a similar functionality in *B. tabaci* resistance. When considering the co-localization of phenotype QTLs for *B. tabaci* resistance and the proposed underlying resistance mechanism in both *S. habrochaites* and *S. pennellii* LA3791, it is conceivable that in both wild relatives of the cultivated tomato, gene homologues are involved in the synthesis of Acyl sugars. The QTL studies confirmed the correlation between nine individual Acyl sucroses and *B. tabaci* resistance factors.

Acknowledgements

This project was financially supported by the Technical Top Institute of Green Genetics (TTI-GG; Resistance mechanisms against whitefly in tomato project: 1C005RP), Monsanto Vegetable Seeds (Bergschenhoek, The Netherlands), Nunhems NL (Nunhem, the Netherlands), and Wageningen University and Research Centre. The contribution of Dr. Ric de Vos was partially funded by the Netherlands Metabolomics Centre and the Centre of Biosystems Genomics, which are both part of the Netherlands Genomics Initiative / Netherlands Organization for Scientific Research. We thank Adriaan van Aelst from Wageningen Electron Microscopy Centre for performing the CRYO-SEM imaging. We also are grateful to Betty Henken for technical assistance in metabolomic and greenhouse work.

CHAPTER 5

Phenotypic screening for *B. tabaci* resistance in a *S. habrochaites* introgression line population

Floor van den Elsen[1,2,3], Adriaan W. van Heusden[1], Colette Broekgaarden[1], Marcel Dicke[2], Roland Mumm[4,5], Ric C.H. de Vos[4,5,6], Ben Vosman[1]

[1]Wageningen UR Plant Breeding, Wageningen University and Research Centre, P.O. Box 386, 6700AJ, Wageningen, the Netherlands.
[2]Laboratory of Entomology, Wageningen University and Research Centre, P.O. Box 8031, 6700EH, Wageningen, the Netherlands.
[3]Graduate School Experimental Plant Sciences
[4]Plant Research International, Business Unit Bioscience, Wageningen University and Research Centre, P.O. Box 619, 6700AP Wageningen, the Netherlands.
[5]Centre for Biosystems Genomics, P.O. Box 98, 6700AB, Wageningen, the Netherlands.
[6]Netherlands Metabolomics Centre, Einsteinweg 55, 2333 CC, Leiden, The Netherlands

Abstract

Tomato (*Solanum lycopersicum*) is susceptible to the whitefly *Bemisia tabaci*. A number of tomato wild relatives are highly resistant against this whitefly and can be used as donor in breeding programs to produce elite tomato lines with the desired *B. tabaci* resistance. Many studies on whitefly resistance in tomato have focussed on resistance, but no resistance has yet been introduced in cultivated tomato. In our study, Introgression Lines (ILs) of *S. habrochaites* LYC4 were screened for *B. tabaci* resistance. Lines possessing some resistance can be utilized as donor in breeding programs. In this work, we performed multiple no-choice resistance screenings on the whole set of ILs in order to identify the variation in resistance between the individual ILs. We identified five ILs that showed a significantly reduced susceptibility towards *B. tabaci*. The introgressions in these lines were on respectively chromosome II (2x), III, V, and IX (LYC2.2, LYC2.3, LYC3.1, LYC5.2, LYC9.1). The ILs LYC2.3 and LYC3.1 expressed respectively, one and three GC-MS peaks (metabolites), at higher levels in the IL compared to recurrent parent cv. Moneymaker and these peaks might be involved in the resistance against *B. tabaci*.
We conclude that breeding for resistance against whiteflies by screening ILs can facilitate the breeding process, but might not deliver the level of resistance required for breeders to implement in their breeding programs and is a less sensitive approach for detecting resistance QTLs than employing an F_2 population.

Keywords*:* *Bemisia tabaci*, *Solanum habrochaites* LYC4, GC-MS, no-choice bio-assays, Introgression Lines (ILs), glandular trichomes.

Introduction

The fundamental principles behind plant resistance against insects have attracted much scientific attention in the last decades as insect feeding on crop plants can be devastating, resulting in substantial yield losses (Haile et al. 1998; Sétamou et al. 2000). Moreover, a large number of insects serve as vector of plant pathogenic viruses, which is the major concern of crop growers (Pan et al. 2012; Hohn 2007; Hogenhout et al. 2008). The virus-transmitting whitefly *Bemisia tabaci* Middle East-Asia Minor 1 is amongst the world's most devastating pest insects (Perring et al. 1993; Palumbo et al. 2001; Oliveira et al. 2001). This whitefly has a broad plant host range, amongst which is tomato (*Solanum lycopersicum* L.)(Cohen and Nitzany 1966; Stansly and Naranjo 2010). In contrast to cultivated tomato, there are a number of related wild tomatoes that show resistance against *B. tabaci.* However, little progress has been made with regard to introducing whitefly resistance into cultivated tomato (Broekgaarden et al. 2011). Some studies suggested that monogenic whitefly resistance can be conferred by the *Mi1.2* gene, but extensive analyses indicated only partial resistance and introduction of the gene has not led to durable whitefly resistant tomato cultivars (Nombela and Muñiz 2010; Nombela et al. 2003; Nombela et al. 2001; Nombela et al. 2000). In addition, polygenic quantitative resistance traits have been assessed in segregating mapping populations by identifying quantitative trait loci (QTLs), but such loci have so far not been introgressed into commercial tomato cultivars and the underlying mechanisms of resistance are still poorly understood, although it is generally accepted that the presence of specific glandular abaxial leaf trichomes and the synthesis of toxic constituents, amongst which Acylsugars, in these glandular structures play a major role in resistance (Rodríguez-López et al. 2012; Liedl et al. 1995; Leckie et al. 2013; Mutschler et al. 1996; Blauth et al. 1999; Blauth et al. 1998; Schilmiller et al. 2012; Schilmiller et al. 2010; Leckie et al. 2012; Firdaus et al.; 2012).

Genetic mapping studies have been performed in F_2 populations derived from interspecific crosses with different *S. habrochaites* accessions to identify the genetic basis of whitefly resistance (Momotaz et al. 2010; Maliepaard et al. 1995). Momotaz et al. (2010) identified QTLs in *S. habrochaites* LA1777 on chromosomes IX, X, and XI based on no-choice bioassays. Maliepaard et al. (1995) phenotyped an interspecific population derived from donor parent *S. habrochaites* CGN1.1561 and recurrent parent *S. lycopersicum* for resistance to *Trialeurodes vaporariorum* (greenhouse whitefly) and found two QTLs affecting oviposition rate that mapped to chromosome I (*Tv*-1) and XII (*Tv*-2). Besides segregating populations, ILs

were developed to characterize and define individual loci of a donor genotype (Lippman and Zamir 2007). Ideally, an IL population exists of multiple lines with each a homozygous single introgression of the donor parent in a recurrent parent background, of which the complete set of lines theoretically represent a hundred percent of the donor parent genome (Eshed and Zamir 1996; Lippman and Zamir 2007), although the practical feasibility of introgressing specific chromosomal regions can be hampered by reduced recombination and/or linkage drag (Finkers et al. 2007).

The advantages of ILs when compared to segregating populations of tomato are manifold as these allow a more reliable phenotyping, promote the identification of QTLs (Eshed and Zamir 1995; Rousseaux et al. 2005), the fine mapping of QTLs (Eshed and Zamir 1996; Monforte et al. 2001; Monforte and Tanksley 2000), and the cloning of QTLs (Frary et al. 2000; Fridman et al. 2000; Liu et al. 2002; Finkers et al. 2007). The disadvantage of such lines is that epistatic interactions are lost (Eshed and Zamir 1996; Tanksley and Nelson 1996).

Introgression line populations have been utilized for identifying QTLs for pathogen resistance (Finkers et al. 2007; Jeuken et al. 2008; Jeuken et al. 2004) and might likewise be useful sources for identification of insect resistance loci. Finkers et al. (2007) developed an IL population from a cross between *S. lycopersicum* cv. Moneymaker × *S. habrochaites* LYC4 (Fig 1), which was employed in this study for *B. tabaci* resistance screenings. The major goal was to identify constitutive durable defense with a toxic mode of action affecting the fitness of the whitefly and hampering the insect development and colonization. This was achieved by performing no-choice bioassays, whereby different life-history parameters of the whitefly were measured. The presence of complete or partial resistance in introgression lines is interesting for breeding purposes as the lines are mainly domesticated tomato and can directly be implemented in breeding programs.

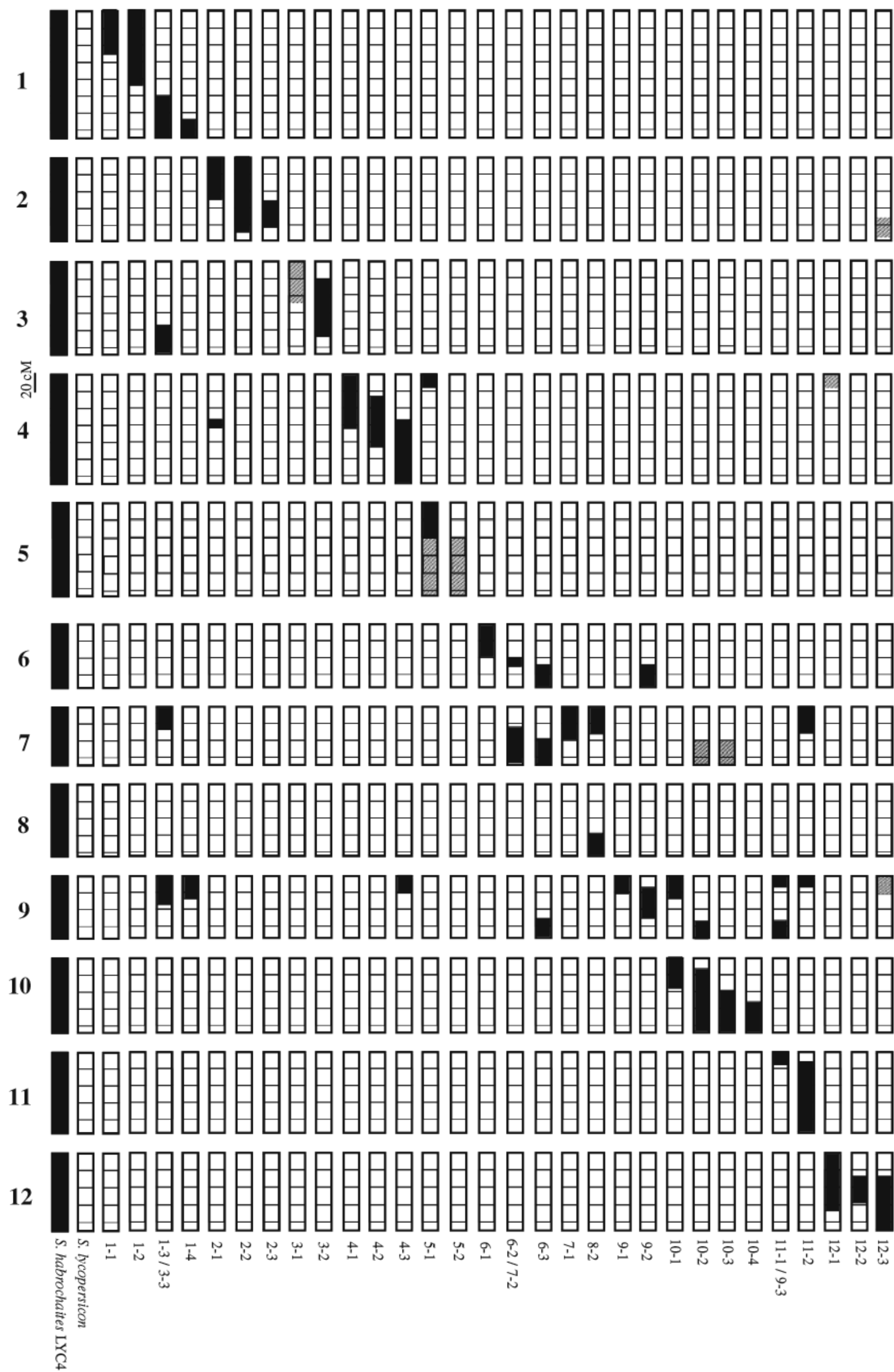


Fig. 1 Graphical representation of the genotypes of the *S. lycopersicum* cv. Moneymaker × *S. habrochaites* LYC4 introgression line population (n=30; Figure adopted from Finkers et al. 2007). All chromosomes are drawn to scale in 20 cM segments or estimated using the *S. lycopersicum* × *S. pennellii* linkage map (Tanksley et al. 1992; http://www.sgn.cornell.edu). Homozygous introgressions from *S. habrochaites* are in black and heterozygous introgressions in gray.

Materials and Methods

Plant material

An IL population was developed by Finkers et al. (2007) between *S. habrochaites* LYC4 and *S. lycopersicum* cv. Moneymaker and was made available by Monsanto Vegetable Seeds, The Netherlands. All ILs and reference genotypes were sown in potting trays. Seeds germinated and were grown in triplicate per genotype for phenotyping and chemoprofiling experiments.
One-week-old seedlings were transplanted in pots (Ø 20cm) on soil substrate. Plants were grown under controlled glasshouse conditions (22 ± 2 °C, L16:D8 photoperiod, RH about 50%), watered daily, and supplemented with nutrients once a week. No chemical pathogen or pest control was practiced.
For chemoprofiling, three biological replicates per individual IL and per reference genotype (*S. habrochaites* LYC4 and *S. lycopersicum* cv. Moneymaker) were made from six-week-old unchallenged plants and grown in trays on soil substrate. Subsequently, the cuttings were transferred to soil in pots (Ø 20cm), and grown in an insect and pathogen free environment (22 ± 2 °C, L16:D8 photoperiod, RH about 50%).

Whiteflies

Bemisia tabaci biotype B was reared on *S. lycopersicum* cv. Moneymaker in a glasshouse under controlled conditions (26 ± 2°C, L16:D8 photoperiod, RH 60 ± 10%) at the Laboratory of Entomology, Wageningen University. The colony commenced from a single parthenogenetic female. An allelic discrimination real-time PCR assay was performed on randomly sampled individuals to affirm biotype B (according to Jones et al. 2008). Detached leaves from cv. Moneymaker plants with synchronized 4th instar nymphs were placed in a gauze insect cage containing three-week-old cv. Moneymaker plants to provide newly emerged adults with young leaves. One-to-three-day-old adults were collected from the insect cage and anaesthetized with N_2:H_2:CO_2 [80:10:10] (Linde Gas Benelux) to enable selection and transfer of whiteflies to the test plants.

Phenotyping

Environmental parameters were optimized for *B. tabaci* rearing (26 ±2°C, L16:D8 photoperiod, RH 60 ±10 %) one week prior to the beginning of phenotyping experiments. The

total IL population and reference genotypes were tested for adult survival and oviposition rate in a no-choice experimental design when plants were six-weeks old. The resistant donor *S. habrochaites* LYC4, *S. pennellii* LA716, *S. habrochaites* LA1777, *S. pimpinellifolium* CGN15528, and recurrent parent *S. lycopersicum* cv. Moneymaker were included as reference material. Three plants per reference were screened and these replicates were randomly positioned throughout the greenhouse.

Adult survival Unsexed one- to three-day-old adults were selected under a stereomicroscope (Zeiss) and transferred to the abaxial side of a third internode leaf in a fine-meshed clip-on cage (Ø 25mm) with rubber membranes at the leaf interface to prevent mechanical leaf damage. The third internode leaf was chosen as younger leaves are preferred over older leaves by the whitefly for feeding and oviposition (Liu and Stansly 1995). Each individual IL line (n=3) and each reference genotype (n=3) was challenged with two clip-on cages containing 20 adults each. Adult survival was counted under a stereomicroscope five days post infestation. Adult survival rate was calculated per clip-on cage according to Van Giessen et al. (1995) and Bas et al. (1992) by the following equation:

$$\text{Adult survival rate} = \left(\frac{m}{n}\right)^{1/d} \text{/day}$$

where d is the number of days (five days), n the total number of females per clip-on cage, m the number of whiteflies alive after d days.

Oviposition rates. Six- to eight-days-old females were selected under a stereomicroscope and transferred to the abaxial side of the 3rd internode leaf. Each individual IL line (n=3) and each reference genotype (n=3) were challenged with two clip-one cages containing five female *B. tabaci* each. Leaves were cut off after five days of infestation and the total number of females, the number of living females, and the number of eggs were counted under a stereomicroscope. Oviposition rates were calculated per clip-on cage according to Van Giessen et al. (1995) and Bas et al. (1992) by the following equation:

$$\text{Oviposition rate} = \frac{2e}{d(m+n)} \text{ eggs/female/day}$$

where *e* is the number of eggs, *d* the number of days (five days), *n* the total number of females per clip-on cage, *m* the number females alive after *d* days.

Semi-field phenotyping trials were performed in Spain and Israel on the IL LYC4 population plus donor *S. habrochaites* LYC4 and recurrent parent cv. Moneymaker as references, using a free-choice bio-assay with natural *B. tabaci* infestation in semi-open polyethylene tunnels. The number of eggs was counted per $3.8 cm^2$ abaxial leaf area on the fifth and seventh leaf internode and two plants per IL were tested and per leaf internode two samples were taken, providing eight replicas in total. The average number of eggs plus larvae (all stages) of the replicas per IL per $3.8 cm^2$ abaxial leaf area was calculated. For statistical analyses, a one-way ANOVA was performed, followed by Bonferroni's post-hoc test ($p<0.05$)(SPSS 12.0.1 for Windows) to compare differences between treatments and between genotypes. In addition, whiteflies were collected from the plants and an allelic discrimination real-time PCR assay was performed on randomly sampled individuals to affirm biotype B (according to Jones et al. 2008).

Life-history parameters on plants with and without glandular trichomes

A no-choice experiment was carried out on a subset of ILs and reference genotypes (*S. habrochaites* LYC4 and *S. lycopersicum* cv. Moneymaker) with and without glandular trichomes. To obtain leaves without glandular cells, a third internode leaf was dipped in 96% EtOH for ten seconds, glandular cells were removed from the abaxial leaf side with a soft brush, and the leaf was rinsed three times for ten seconds in dH_2O. For the control a third internode leaf was rinsed three times for ten seconds in dH_2O. One control and one test leaf were infested per individual plant and six plants of both *S. habrochaites* LYC4 and *S. lycopersicum* cv. Moneymaker were used and seven plants were used per IL. Once the leaves were dry, ten one to three-day-old unsexed adults were anaesthetized and transferred into a transparent clip-on cage on the abaxial side of a third internode leaf with removed or intact glandular trichomes. The number of dead and alive *B. tabaci* was scored by eye every day for four subsequent days. Adult survival was calculated by dividing the number of living adults by the total number of adults.

To determine the reproduction rate, ten six-to-eight-day-old *B. tabaci* females were anaesthetized and transferred in a clip-on cage to the abaxial side of a third internode leaf with removed or intact glandular trichomes. Leaves were cut off after five days of infestation and the total number of females, the number of living females, and the number of eggs were

counted under a stereomicroscope. Oviposition rates were calculated by the abovementioned equation of Van Giessen et al. (1995). For statistical analyses of life-history parameters of *B. tabaci* on *S. pennellii* and cv. Moneymaker, a one-way ANOVA was performed, followed by Bonferroni's post-hoc test ($p<0.05$)(SPSS 12.0.1 for Windows) to compare differences between treatments and between genotypes.

Leaf sample preparation for metabolomics

Three biological replicates per IL plus *S. habrochaites* LYC4 and *S. lycopersicum* cv. Moneymaker were placed in a randomized block design. The environmental parameters were adjusted one week prior to the collection of leaf material for chemoprofiling (26 ±2 °C, L16:D8 photoperiod, RH 60 ±10%), to equal the settings used during phenotyping experiments. Third internode leaves of six-week-old uninfested plants were cut off, packed in aluminum foil, thereby minimizing damage to leaf tissue, and instantly transferred to LN_2. Leaf samples were stored at -80°C until use in Gas Chromatography-Mass Spectrometry (GC-MS). Samples were prepared according to Maharijaya et al. (2012).

GC-MS metabolic profiling

The GC-MS was performed on a subset of ILs plus reference material to identify apolar metabolites that may contribute to *B. tabaci* resistance. The dichloromethane (DCM) extracts were analysed using an Agilent 7890A GC-MS machine (Agilent Technologies, Amstelveen, The Netherlands) equipped with a 30-m Zebron ZB-5 ms column with 5 m retention gap (0.25 mm i.d., 0.25-lm film thickness; Phenomenex, Torrance, CA, USA) and an Agilent 5975C quadrupole mass analyzer (Agilent Technologies). The GC was programmed from 45 °C for 1 min, raised to 300 °C at 10 °C per min, and held at 300 °C for 5 min. One microliter of sample was injected in splitless mode. The injection port and interface temperatures were 250 and 280 °C, respectively, and the helium inlet pressure was controlled electronically to achieve a constant column flow of 1.0 ml min^{-1}. The column effluent was ionized using electron impact at 70 eV, and scanning was performed from 45 to 400 atomic mass units.

An untargeted data processing approach was applied to process the raw GC-MS data (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 3 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). Reconstructed metabolites were putatively identified by matching the mass spectra to authentic reference standards, and to commercial spectral libraries (NIST08 (www.nist.gov)), Wiley (www.wiley.com), and to custom made spectral libraries (Wageningen Natural compounds spectral library), and by comparison with retention indices of the literature calculated using a series of alkanes and fitted using a third-order polynomial function (Strehmel et al. 2008).

Triplicates of each IL were injected into the GC-MS machine in reverse sequence. Controls DCM, *S. habrochaites* LYC4, and *S. lycopersicum* cv. Moneymaker were included daily in the course of the measurements.

Data analyses were done with MS Excel (2010) software. The data were $\log_{10}$ transformed and a Student's t-test was performed per metabolite between genotype groups and subsequently *p*-values were ranked. A false discovery rate (FDR) control was applied to correct for multiple comparisons. The corresponding *q*-values were calculated according to Benjamini and Hochberg (1995):

$$q\text{-value} = \left(\frac{m}{i}\right) * P_i$$

where *q* is the FDR-corrected *p*-value for a single metabolite, *m* the number of variables (metabolites), *i* the rank of the *p*-value of the variable, P_i the *p*-value.

The metabolites with $q<0.05$ were used for peak annotation.

Results

Screening the Introgression Line population of LYC4 for adult survival and oviposition rate

The whole IL population and some reference accessions were screened to determine their resistance levels towards *B. tabaci*. The results are presented in Figure 2. No ILs differed from cv. Moneymaker with regard to whitefly adult survival (Fig 2A), but on three ILs *B. tabaci* showed a lower oviposition, namely LYC2.2, 2.3, and 9.1 (Fig 2B). None of the introgression lines showed high resistance levels comparable to the donor parent *S. habrochaites* LYC4, which had zero adult survival and zero oviposition (Fig 2A-B) or *S. pennellii* LA716 and *S. habrochaites* LA1777 which also showed complete resistance. The susceptible reference *S. pimpinellifolium* CGN15528 was not different from cv. Moneymaker.

Semi-field trials on the whole IL population were performed in Spain and Israel, whereby infestation rates were scored on the abaxial young tomato leaves in a free-choice assay. None of the ILs differed from the recurrent parent cv. Moneymaker with regard to the number of adults, juveniles, or eggs on the leaves with the exception of IL LYC6.3, which had a significantly lower number of eggs and nymphs at the leaf surface in the semi-field trials in Israel (Fig 3).

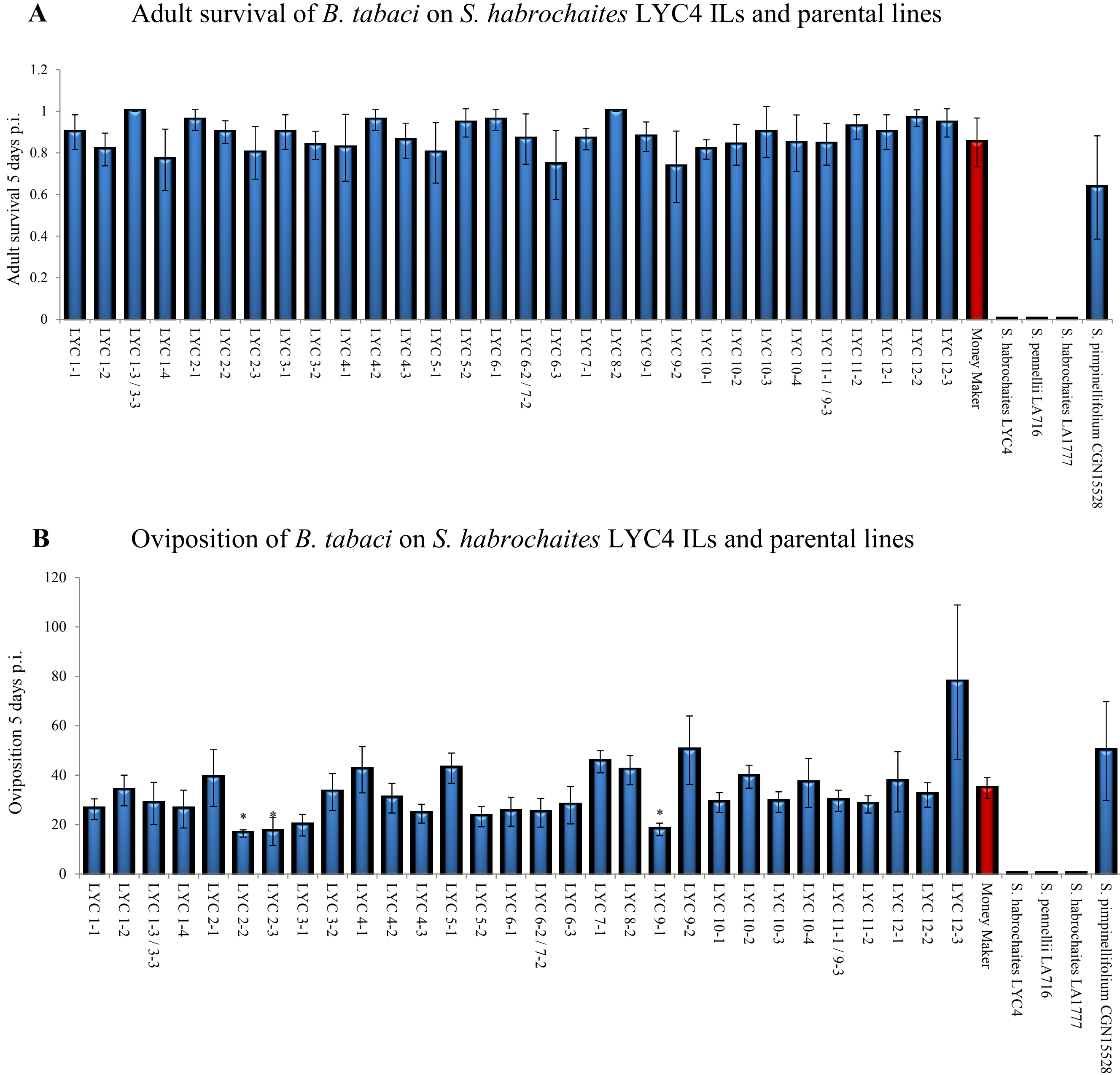


Fig 2A and B *Bemisia tabaci* survival (proportion of females surviving after 5 days of incubation; bars show mean ± SEM)(**A**) and oviposition (number of eggs per whitefly per five days; bars show mean ± SEM)(**B**) data of an introgression line population of *Solanum habrochaites* LYC4 and recurrent parent *S. lycopersicum* cv. Moneymaker (Red bar). Asterisks indicate the ILs that had significantly different values compared to cv. Moneymaker. Additional references of tomato wild relatives are shown at the right side of the figure.

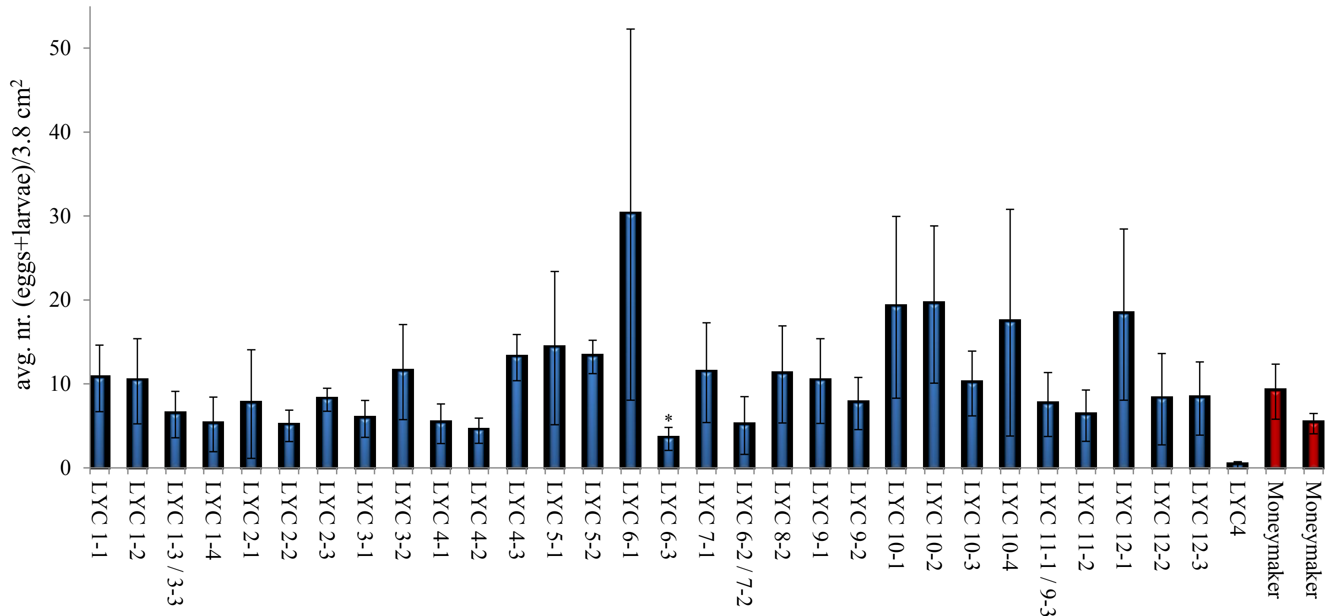


Fig 3 Count of *Bemisia tabaci* eggs and larvae (all stages) on a confined abaxial leaf area of individual lines of an IL population of donor parent *Solanum habrochaites* LYC4 and recurrent parent *S. lycopersicum* cv. Moneymaker (red bar)(bars show mean ± SEM). Data were collected from a semi-field bio-assay in Israel.

Re-evaluation of selected ILs for adult survival and oviposition

The ILs that showed significantly lower oviposition or a trend towards lower oviposition in the greenhouse screening were rescreened and the number of replicates was increased (n=14) (Fig 4A-B). Again, no lines significantly differed from cv. Moneymaker with regard to adult survival (Fig 4A), which confirmed our previous results (Fig 2A). Rescreening of our lines for whitefly oviposition revealed significant differences in five ILs LYC2.2, LYC2.3, LYC3.1, LYC5.2, LYC9.1 (Fig 4B) on chromosomes II (2 ILs), III, V, and IX. The lower oviposition on ILs LYC2.2, LYC2.3, and LYC9.1 were confirmed, LYC3.1 and LYC5.2, with a tendency towards lower oviposition in the previous screening (Fig 2B), became significant, indicating that genes might be present that reduce the fitness of whiteflies but also that variability and the low level of reduced susceptibility make it necessary to include many replications (Fig 4B).

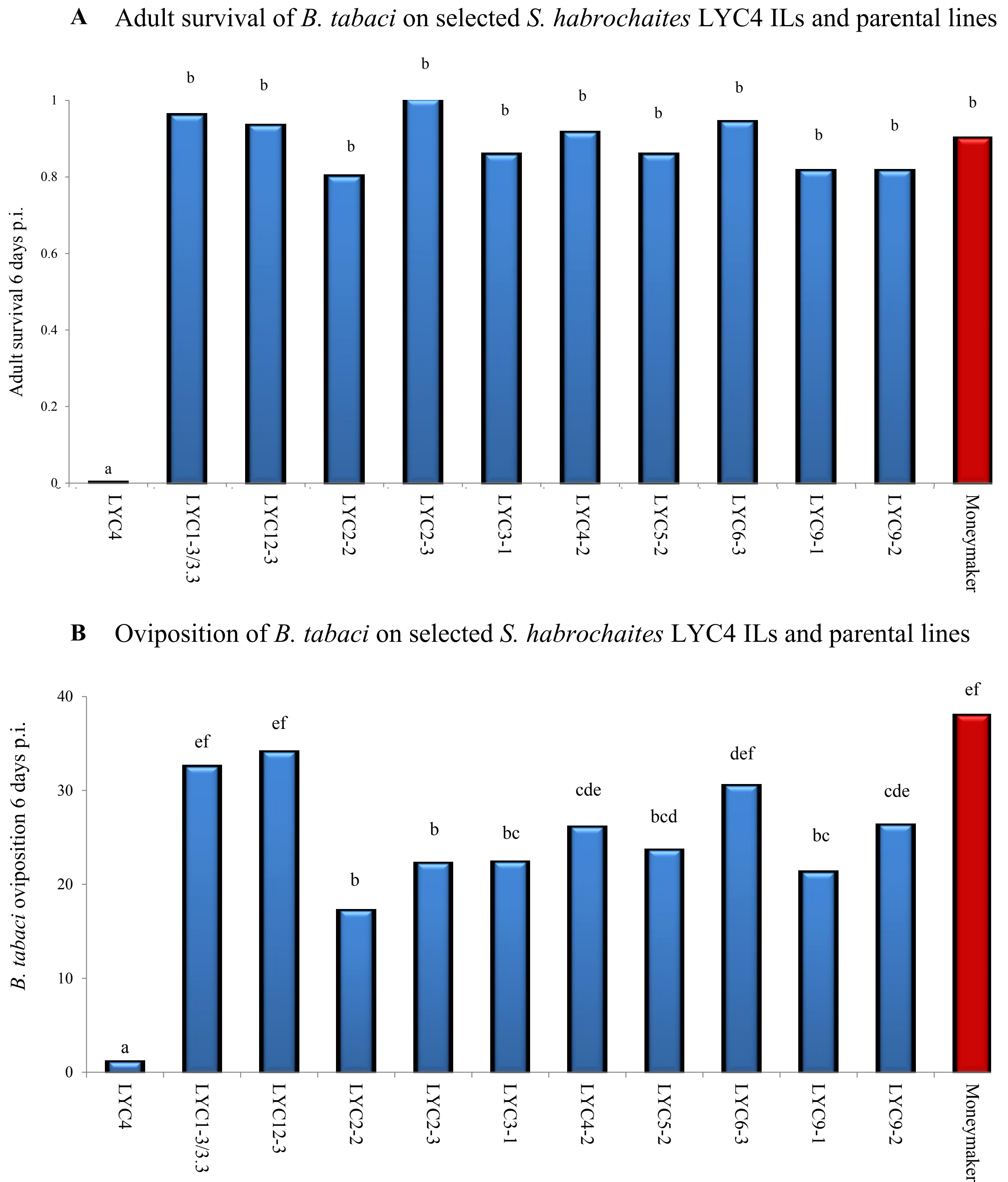


Fig 4A and B *Bemisia tabaci* survival (**A**) and oviposition (**B**) data (n=14) on the subset of ten ILs and donor parent *Solanum habrochaites* LYC4 and recurrent parent *S. lycopersicum* cv. Moneymaker (red bar). Different letters indicate differences in significance p<0.05). The datapoint for *B. tabaci* survival on LYC4 is zero.

The effect of glandular trichome removal on *B. tabaci* survival and oviposition

A trichome removal experiment was performed to evaluate the effect of glandular trichomes in the five ILs with partial resistance against *B. tabaci* and IL LYC6.3 from the free-choice bioassay in the semi-field trial in Israel.

Removal of the glandular trichomes in the selected ILs did not result in significant differences in whitefly adult survival (Fig 5A) and whitefly oviposition (Fig 5B). Furthermore, whitefly oviposition on only two of the selected ILs (LYC2.3 and LYC5.2) was significantly lower than on cv. Moneymaker showing the difficulty to confirm the small reduction in oviposition.

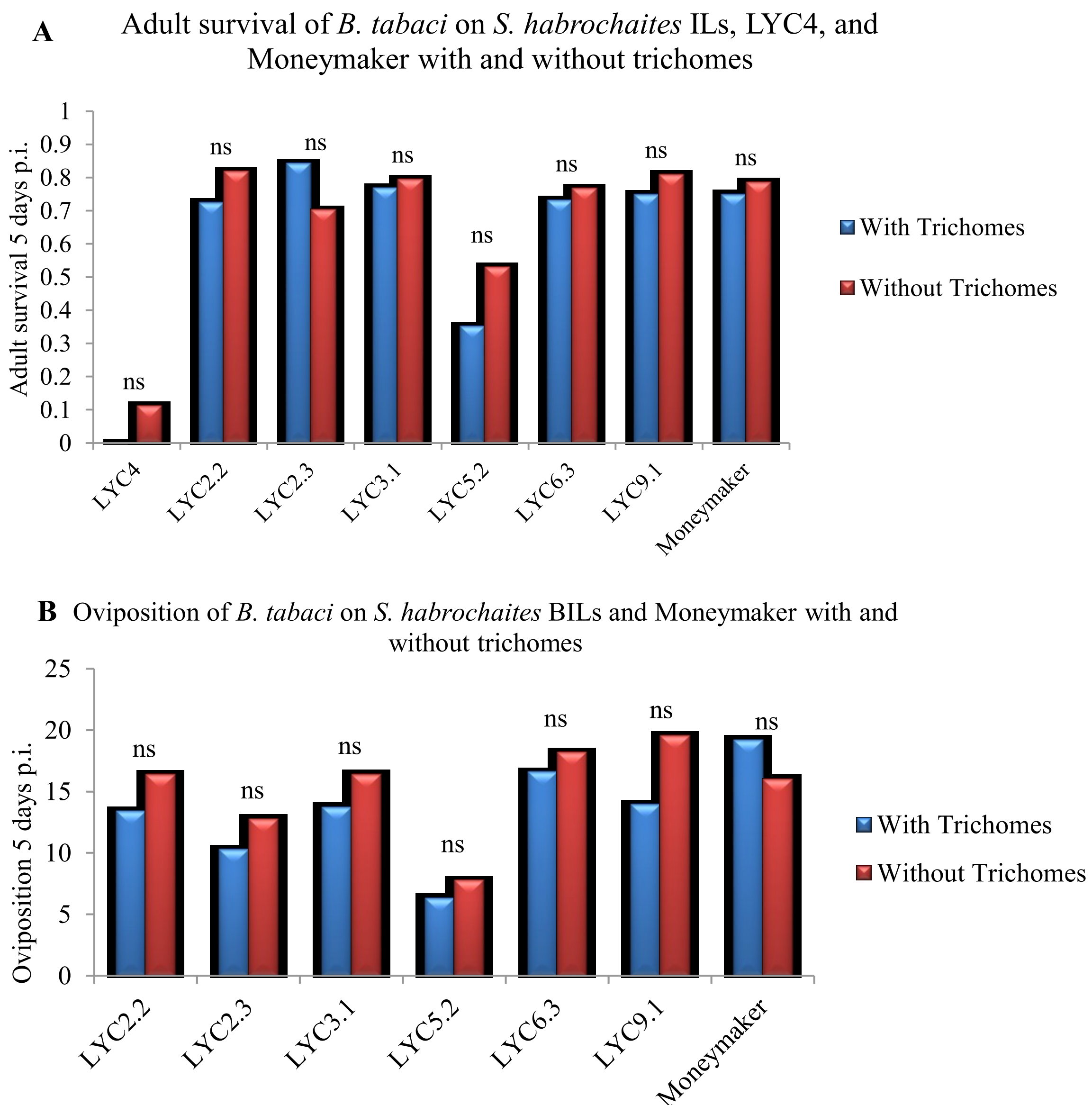


Fig 5A and B *Bemisia tabaci* survival (**A**) and oviposition (**B**) data on an introgression line population of donor parent *Solanum habrochaites* LYC4 and recurrent parent *S. lycopersicum* cv. Moneymaker on plants with intact trichomes (blue bars) and plants with removed trichomes (red bars). Abbreviation ns stands for not significant.

Untargeted GC-MS profiling of LYC4 ILs

An untargeted metabolomics analysis was carried out on all IL lines (data not shown). Table 1 shows the putative metabolites from GC-MS chemoprofiling of ILs LYC2.3 and LYC3.1 that were more abundant in LYC4 and in the IL compared to cv. Moneymaker. No other ILs that were selected based on reduced susceptibility against *B. tabaci* (LYC2.2, LYC5.2, LYC6.3, and LYC9.1) differed from recurrent parent cv. Moneymaker with respect to average abundance of the individual metabolic peaks from GC-MS chemotyping. The IL LYC2.3 possessed one putative metabolite that was higher compared to cv. Moneymaker and was present in LYC4. This peak had a 30-fold higher abundance in LYC4 compared to LYC2.3. The IL LYC3.1 had three peaks with higher abundance compared to cv. Moneymaker of which two peaks were approximately 40- and a 130-fold higher in abundance in LYC4, the other peak was almost a 100-fold higher in LYC3.1 compared to LYC4.

LYC2.3 constituent associated with *Bemisia tabaci* resistance

Id	peak nr	scan	retention	mass	Average abundance cv Moneymaker	Average abundance LYC4	Average abundance LYC2-3	FDR[a] q-value LYC2.3 versus all remaining LYC lines
18	12	2303	14102600	59	7.79 ± 6.74	916.25 ± 516.92	30.48 ± 13.24	0.004

LYC3.1 constituents associated with *Bemisia tabaci* resistance

Id	peak nr	scan	retention	mass	Average abundance cv Moneymaker	Average abundance LYC4	Average abundance LYC3.1	FDR q-value LYC3.1 versus all remaining LYC lines
16	35	2273	13977330	82	0.07 ± 0.06	3260.75 ± 535.73	25.42 ± 0.61	0.033
17	21	2280	14006570	198	5.08 ± 4.40	365.48 ± 151.02	8.68 ± 0.81	0.006
52	12	4640	23860279	243	0 ± 0	9.61 ± 8.32	933.49 ± 784.66	0.015

Table 1 Gas Chromatography-Mass Spectrometry (GC-MS) data of four metabolic peaks that were higher in average abundance in introgression lines (ILs) LYC2.3 and LYC3.1 originating from donor parent *Solanum habrochaites* LYC4 and recurrent parent *S. lycopersicum* cv. Moneymaker. The average peak abundance and standard deviation (SD) is shown for the ILs and parental lines.
[a] False Discovery Rate

Discussion

Some *S. habrochaites* LYC4 ILS show a reduced oviposition of *Bemisia tabaci*

Wild, crossable relatives of tomato are often useful sources of genetic material for breeders. The genetic variation among these wild relatives is high and quantitative resistance against whitefly has been observed for a number of them (Firdaus et al. 2012; Leckie et al. 2012; Bleeker et al. 2009; Momotaz et al. 2010). Introgression of parts of the genome of the related wild species is possible via classical breeding, but barriers like hybrid inviability or sterility can be present in the progeny of the interspecific crosses (Finkers et al. 2007; Rick 1982; Eshed and Zamir 1995). Despite these potential difficulties, the introgression of many new traits has been successful in the past, however so far not for insect resistance (Zamir et al. 1994; Labate and Robertson 2012).

In this study we have used an IL population, based on *S. habrochaites* LYC4, to screen for whitefly resistance. Such an IL population can assist molecular breeders to identify QTLs and relatively rapidly introduce these loci into elite tomato material (Finkers et al. 2007) .

In our work, we identified five ILs with reduced oviposition after several screenings. The introgressions are on four different chromosomes, namely on chromosome II (two ILs), III, V, and IX. The introgression at the bottom of chromosome II in line LYC2.3 completely overlaps with the introgression in line LYC2.2, which indicates that there is probably only one QTL on chromosome II. The IL LYC3.1 has a heterozygous introgression at the top of chromosome III showing that the effect is dominant and some factors seem to prevent that this introgression becomes homozygous for the *S. habrochaites* allele. The IL LYC5.2 has a single heterozygous introgression at the bottom of chromosome V at the same location as the heterozygous introgression in LYC5.1, however, no reduced susceptibility with regard to oviposition was observed in this IL. The difference in phenotype for *B. tabaci* resistance between LYC5.1 and LYC5.2 could have two explanations. First, phenotyping is prone to variability which might have provided an inaccurate oviposition rate for LYC5.1, which was only measured in one test, while LYC5.2 was measured in multiple tests; such variability is often observed in phenotyping assays because environmental factors can interfere with an accurate outcome (Luna and Ton 2012; Rasmann et al. 2012; Gómez-Díaz et al. 2012). Second of all, genome coverage by markers is limited and might not detect all introgressions in a line (Viquez Zamora et al. 2013).

The same explanation holds for the results of line LYC9.1. The introgression of LYC9.1 is located on top of chromosome IX and this region is also present in several other ILs (Fig 1). However, only in line LYC9.1 we did find a significantly reduced whitefly oviposition rate. All other lines show a trend towards lower oviposition rates, but none of these were significantly different from the recurrent parent cv. Moneymaker.
The same IL population was used to screen for *Botrytis cinerea* resistance by measuring disease incidence, lesion size, and lesion growth rate (Finkers et al. 2007). Our QTLs for *B. tabaci* resistance on LYC2.2, LYC3.1, and LYC9.1 co-localize with *B. cinerea* resistance QTLs *Rbcq*2, *Rbcq*3, and *Rbcq*9, respectively. This overlap might be coincidental but may also point at a common genetic factor affecting resistance to both pathogens.

Differences in metabolites between introgression lines and *S. lycopersicum* cv. Moneymaker

The GC-MS profile of ILs with a reduced susceptibility for *B. tabaci* was studied and compared to both the recurrent and donor parental line. Several metabolites were absent in both the donor parent *S. habrochaites* LYC4 as well as the recurrent parent cv. Moneymaker and were newly synthesized in the IL lines (data not shown), a phenomenon that occurs more often in progeny lines possible due to recombination resulting in new pathways for synthesis of metabolites (Keurentjes et al. 2006). Three out of the four differentially abundant metabolites showed a significant higher abundance of at least 30-fold in LYC4 compared to the IL, these metabolites might have a role in *B. tabaci* resistance and resistance levels against this pest might become higher upon higher abundance of the metabolite. This study cannot conclude on the role of these metabolites in *B. tabaci* resistance and further studies are needed to identify the role of these metabolites in resistance and if relevant, determine the level of expression required to induce complete resistance. One of the peaks that differed in IL LYC3.1 had an almost 100-fold higher abundance in the IL compared to LYC4. It is unlikely that this metabolite is dominant in whitefly resistance, as a higher level of resistance would have been expected, although the presence of this compound might be required for basic defense.

Conclusion

Data on life-history parameters presented in this chapter showed that an IL population is not suitable for the identification of QTLs involved in a high level of resistance against *B. tabaci*. Most likely, a lack of epistasis hampered the identification of resistance-related loci as specific gene interactions were lost in an introgression line population. However, we identified partial resistance against *B. tabaci* in a number of ILs that can be used for breeding purposes. As the IL lines are mainly domesticated tomato they can be efficiently implemented in commercial tomato breeding programs.

Acknowledgements

This project was financially supported by the Technical Top Institute of Green Genetics (TTI-GG; Resistance mechanisms against whitefly in tomato project: 1C005RP), Monsanto Vegetable Seeds (Bergschenhoek, The Netherlands), Nunhems NL (Nunhem, the Netherlands), and Wageningen University and Research Centre. The contribution of Dr. Ric de Vos was partially funded by the Netherlands Metabolomics Centre and the Centre of Biosystems Genomics, which are both part of the Netherlands Genomics Initiative / Netherlands Organization for Scientific Research. We thank Paul Maris and Sjoerd van den Ent from Monsanto Vegetable Seeds, The Netherlands for providing the semi-field phenotyping data from Israel. We also are grateful to Betty Henken for technical assistance in metabolomic and greenhouse work.

CHAPTER 6

General Discussion

Floor van den Elsen[1,2,3]

[1]Wageningen UR Plant Breeding, Wageningen University and Research Centre, P.O. Box 386, 6700AJ, Wageningen, the Netherlands.
[2]Laboratory of Entomology, Wageningen University and Research Centre, P.O. Box 8031, 6700EH, Wageningen, the Netherlands.
[3]Graduate School Experimental Plant Sciences

Preface

The silverleaf whitefly *Bemisia tabaci* Middle-East minor I is a major problem for tomato growers worldwide. It causes direct damage by uptake of photoassimilates and induction of phytotoxicity (Brown 2007; McCollum et al. 2004) as well as indirect damage by vectoring plant pathogenic viruses (Idris et al. 2001; Navas-Castillo et al. 2011; Belén Picó et al. 1996). However, the main concern for tomato growers is the ability of this whitefly species to transmit viruses to plants, which can reduce crop yield up to a hundred percent (Oliveira et al. 2001; Naranjo et al. 1996; Stansley and Naranjo 2010). There are two approaches to prevent virus infection: breeding for virus resistance or breeding for vector resistance. Breeding for vector resistance has the advantage that it will be effective against a range of different viruses. Virus resistance is often monogenic and successful against a single or few specific viruses only (Stevens et al. 1992; Roselló et al. 1996; López et al. 2011). In practice, both approaches are studied and ideally resistance genes or alleles acting against whiteflies and viruses will be combined to acquire a more durable resistance against a broad range of viruses. Breeding for resistance against both the vector and the virus will also diminish the risk of having mutualistic relationships between vector and virus resulting in increased vector performance/fitness on virus-infected plants; a system evolved to promote the multiplication and spread of viruses (Luan et al. 2013).

In tomato, vector resistance is often polygenic and has proven to be challenging for breeders (Leckie et al. 2012; Mutschler et al. 1996; Maliepaard et al. 1995). To find ways to prevent vectoring of viruses by *B. tabaci*, researchers have studied the life-history parameters and incidence of *B. tabaci* for decades (Costa and Brown 1991; Drost et al. 1998), as well as the effect of viruses on life-history parameters of the vectoring insects by addressing virus-vector relationships (Rubinstein and Czosnek 1997; McKenzie et al. 2002; Czosnek and Ghanim 2011). All of this research is aimed at developing more effective control measures. In this thesis I combine *B. tabaci* life-history assessments with genetic profiling in plant populations obtained from crossings between a tomato cultivar and a whitefly-resistant tomato wild relative. Moreover, large-scale metabolomic data were generated for the different populations and an integrative approach was taken to link chemotyping, phenotyping, and genotyping data in order to comprehend the major mechanisms behind the *B. tabaci* resistance traits in wild tomato relatives *Solanum pennellii* LA3791 and *S. habrochaites* LYC4; this is a systems

biology approach that has proven to be successful in other study systems (Keurentjes 2009; Carreno Quintero et al. 2012; Macel et al. 2010).

Molecular breeding for *B. tabaci* resistance

Nowadays, genetic markers, also referred to as molecular markers, are commonly used in breeding to select indirectly for agronomically interesting traits (Mohan et al. 1997). During the last two decades, research on whitefly resistance in tomato breeding has developed more and more towards the use of segregating populations followed by the breeding of resistant cultivars by taking a marker-assisted approach (Leckie et al. 2012; Schilmiller et al. 2009, Mutschler et al. 1996; Blauth et al. 1999; Blauth et al. 1998; Firdaus et al. 2013). So far, no resistant tomato cultivar has been commercialized, most likely because the complexity of *B. tabaci* resistance is high and the underlying mechanisms of resistance are poorly understood, although the consensus holds that the synthesis of specific Acyl sugars, quantity of total Acyl sugars in trichomes, and the presence of specific glandular abaxial leaf trichomes play a major role in resistance (Rodríguez-López et al. 2012; Firdaus et al. 2012; Mutschler et al. 1996; Liedl et al. 1995; Leckie et al. 2012), and recent research (Firdaus et al. 2013) has shown that in some cases the resistance inherits in a less complex way for *S. galapagense* x *S. lycopersicum* than observed in earlier studies for different tomato wild relatives (Leckie et al. 2012; Nombela and Muñiz 2010; Mutschler et al. 1996; Blauth et al. 1999; Blauth et al. 1998). This opens the door for introgression breeding with as donor *S. galapagense*, a close relative of *S. lycopersicum*.

Mono- versus polygenic resistance mechanisms in tomato

Tomato defense against pathogens and herbivorous insects can be either monogenic (simple qualitative trait), oligogenic (intermediate quantitative trait), or polygenic (complex quantitative trait; several genes contribute to the phenotype)(Agrios 2005). Monogenic traits are preferred by breeders as they can be introgressed into tomato cultivars relatively easily using marker assisted selection, although, single-gene based traits often have a higher chance to be broken by the pathogen or pest, and so do not necessarily provide durable control against plant attackers (Palloix et al. 2009; Quenouille et al. 2013). Monogenic resistance providing complete resistance against *B. tabaci* has not been discovered in tomato wild relative *S. pennellii* or any of the other wild tomato species yet, with the exception of the

resistance recently discovered in *S. galapagense*, which may actually be monogenic as a locus on Chromosome II gives complete resistance (no survival of juvenile and adult whiteflies) against *B. tabaci* when in homozygous state (Firdaus et al. 2013).

A well-known source of whitefly resistance is *S. habrochaites*, which confers a high level of resistance against *B. tabaci* (Firdaus et al. 2012; Heinz and Zalom 1995; Muigai et al. 2002; Berlinger 1986). However, the resistance in *S. habrochaites* LYC4 appears to be under multigenic control and the expression of a gene can be dependent on other genes, a phenomenon called epistasis. Epistatic effects interfere with the process of acquiring a highly or completely resistant phenotype in introgression lines (ILs), which is a frequently perceived outcome in studies on interactions between plants and biotic stress (Finkers et al. 2007; Eshed and Zamir 1996; Lippman and Zamir 2007). The data presented in chapter 5 demonstrate that a single introgression of *S. habrochaites* LYC4, in a *S. lycopersicum* cv. Moneymaker background can give reduced susceptibility. However, no actual resistance against *B. tabaci*, either partial or complete, was observed which may be due to epistasis of genes located on different positions in the genome resulting in the impossibility to get resistance in a single introgression line. This showed that this IL population of *S. habrochaites* was unsuitable for identifying genetic factors underlying *B. tabaci* resistance. Crossings between individual ILs with increased resistance towards *B. tabaci* might improve the resistance level. However, as no background information on the mechanism behind the resistance and interactions between loci is available, the process of intercrossing of ILs would be random and would not guarantee the development of *B. tabaci* resistant lines. This demonstrates the need for a different population type to study *B. tabaci* resistance in *S. habrochaites* LYC4. Combining mapping data from an F_2 population with the presently used IL population may result in the identification of ILs that, once combined, will provide a higher level of resistance against the whitefly. Possible this also counts for resistance against other insect species of different orders and different feeding modes as we did not find evidence for strong chemical-mediated resistance traits in any of the lines when these were characterized by Gas Chromatography-Mass Spectrometry (GC-MS). Pyramiding genes involved in quantitative resistance is complex and requires knowledge about the genetic mechanism(s) underlying the trait, which were investigated in this thesis for *S. pennellii* LA3791 (chapters 2 to 4).

Compounds in glandular trichomes provide the plant with an efficient defense mechanism

To further deepen our knowledge about tomato defense mechanisms, I have zoomed in on the glandular trichomes of the resistant wild relative of tomato, *S. pennellii* LA3791 and studied segregation patterns of glandular and non-glandular trichome types in relation to *B. tabaci* resistance in an F_2 (chapters 2 and 3) and F_2BC_1 population (chapter 4). In both the F_2 and the F_2BC_1 population no resistant individuals were found in the absence of glandular trichomes type I and IV. However, the presence of glandular trichomes I and IV alone, does not result in resistance (chapter 4). This implies that also the composition and quantity of biochemical compounds in the glandular cells are important for resistance. A study with a series of Acyl sugar breeding lines has shown that the presence of modest levels of Acyl sugars resulted in a significantly lower incidence of *B. tabaci* in no-choice field assays (Leckie et al. 2012). Firdaus et al. (2013) demonstrated in an F_2 population resulting from a cross between whitefly resistant tomato wild relative *S. galapagense* and a tomato cultivar that a high level of whitefly resistance was associated with high numbers of glandular trichomes on the leaf surface. I confirmed that plants from which glandular trichome types I and IV had been removed, became susceptible. Experiments with a segregating population demonstrated that glandular trichomes did not act as structural barriers, by interfering with whitefly behavior. The total number of trichomes on the abaxial leaf surface, the area where whiteflies preferably reside, was comparable for all individual genotypes in our populations and the only difference observed was in the ratio of glandular versus non-glandular trichomes (chapter 4). Our work adds to the work by Dimock and Kennedy (1983), Snyder and Carter (1984)(1985), and Channarayappa et al. (1992) who investigated insect behavior in relation to glandular trichomes on wild tomato species and recorded that the presence of glandular trichomes confers resistance. In addition, Firdaus et al. (2012) observed that the number of type I and the number of type IV trichomes were positively correlated, which resembles my findings (chapter 4). The strong correlation between the two trichome types makes it difficult to conclude on the relative contribution of the individual trichome types to resistance, but recent work showed that glandular trichome types I and IV highly resemble one another in terms of metabolic contents and were in fact suggested to be the same (McDowell et al. 2011), but were originally differentiated from one another as the length and morphology of the trichome stalks differ (Luckwill 1943).

Variation in level of whitefly resistance in *S. pennellii* F_2 progeny reveals the involvement of several genes

I identified multigenic resistance in F_2 and F_2BC_1 populations (chapters 3 and 4), of which single QTLs for *B. tabaci* life-history parameters and corresponding resistance-related Acyl sucroses in the F_2BC_1 population had highly explained variances and were considered major QTLs (chapter 4). Such major QTLs are of added value in current breeding programs. The highest explained variance was 49.9% for Acyl sucrose S3-15-II, which co-localized with a *B. tabaci* resistance QTL on chromosome IV that explained 30.7% of the variance. The causal link between these two QTLs can only be confirmed by fine mapping and functional analyses on candidate genes, which was not done in this thesis work. However, there is a strong indication that the genetic background of the mQTLs and phQTLs could be identical because biochemical constituents were identified on the basis of higher abundance in a *B. tabaci*-resistant bulk compared to a susceptible bulk (chapter 4). Although, it cannot be excluded that closely positioned genes with different functionality might have resulted in co-localization of phQTLs and mQTLs without having a common genetic bases (chapter 4). Leckie et al. (2013) also studied backcross lines (F_1BC_1 and F_2BC_1) of breeding line CU071026 x *S. pennellii* LA716 for production and level of Acyl sugars and found QTLs on chromosomes III, IV and XI, which partially differs from the four loci that I identified for Acyl sucrose abundance on linkage groups I, III, IV, and VIII (chapter 4), but since *S. pennellii* accessions have a narrow genetic basis, the loci and corresponding genes identified for whitefly resistance in the different genotypes could possibly have a genetic communality (Spooner et al. 2005). As my study concerns the role of individual biochemical constituents, it is difficult to confirm the congruity in whitefly resistance traits in different accessions as no comparative studies have been published yet. Furthermore, all studies performed so far relate to the role of total Acyl sugar content on *B. tabaci* fitness parameters (Leckie et al. 2012; Liedl et al. 1995). The study of Leckie et al. (2013), who studied QTLs for the level of production of Acyl glucoses in an F_2 population of breeding line CU071026 with *S. pennellii* LA716, differed from my study as in my thesis Acyl sucroses instead of Acyl glucoses were selected for QTL mapping, and moreover, selection of Acyl sucroses was based on *B. tabaci* resistance/susceptibility traits and directly linked with whitefly resistance traits in tomato (chapters 2 and 4).

Considering both *B. tabaci* life-history parameters adult survival and oviposition, it was observed that only a small number of F_2 genotypes were as susceptible as reference cv. Moneymaker (chapter 2); the vast majority of genotypes were fully or partially resistant,

indicating that multiple mechanisms are active against *B. tabaci* and multiple genes could be involved, which was later on confirmed by a genetic mapping study (chapter 3). The majority of whitefly resistance studies found polygenic resistance in different wild species of tomato (Leckie et al. 2012, 2013; Momotaz et al. 2010) and the underlying mechanisms were all based on chemical defense. Congruity between these wild tomato species in whitefly-resistance traits has not been studied yet and might provide interesting information about similarities of resistance traits between species e.g. by studying homologues of candidate genes between species.

In chapter 4, crossing populations were made between cv. Moneymaker and two resistant F_2 individuals originating from the interspecific cross of tomato with *S. pennellii*. One of these populations showed that part of the F_2BC_1 progeny still was completely resistant and part of the QTLs identified in the F_2 population could be retraced and confirmed, while the metabolic and QTL fingerprints corresponding with *B. tabaci* resistance of the F_2 donor parent did not fully resemble other resistant F_2 genotypes. These findings confirmed the hypothesis that multiple resistance mechanisms can lead to the same resistance level and the hypothesis was further strengthened by the fact that percentages of explained variances of the phQTLs had increased considerably (two- to three fold) in the F_2BC_1 population (chapter 4).

Advantages of constitutive resistance

Constitutive defense is efficient as the resistance is always there but it is costly for the plant as the plant continuously has to synthesize defense compounds (Strauss et al. 2002; Wittstock and Gershenzon 2002; Schoonhoven et al. 2005). However, when acting against a broad range of insect species it might be the most desired defense mechanism for the plant to possess. In addition to constitutive defenses, plants have inducible defenses, that can be switched on upon insect attack, to provide the plant with a less costly and more flexible defense mode, which impedes adaptation of attackers (Bennett and Wallsgrove 1994). The risk of breeding for a constitutive defense mechanisms is the chance of resistance breakthrough by specialist or even generalist insect herbivores (Howe and Jander 2008). However, from a breeder's perspective, constitutive defense against viral transmission by *B. tabaci* is preferred over induced defense as the trait is not dependent on specific herbivore or pathogen inducers, or on the environment (Pieterse et al. 2001; Anderson and Agrell 2005; Karban and Baldwin 1997; Agrawal 1998) and resistance breakdown can be intercepted by combining multiple resistance mechanisms/genes e.g. acting against *B. tabaci* as well as the viruses vectored by this pest.

Trichomes, therefore, provide the plant with an intrinsic armor to protect it against whiteflies; however, the stickiness of the Acyl sugars might entail undesired properties for growers in a practical sense during fruit picking (Elle et al. 1999), which should be considered when implementing such a resistance trait in practice.

Our study showed that next to the Acyl sugars a large number of other, yet unidentified metabolites, are negatively affecting *B. tabaci* fitness (chapter 2), which in the future should be further characterized to understand the full mechanism behind the resistance.
With regard to *S. pennellii* it can be assumed that constitutive resistance is not insect species-specific, but acts against a broad range of pests which may compensate for the costs of this defense mode. For *S. pennellii* LA716, the Acyl sugar-related broad-range resistance has been documented for insect species of various orders with divergent feeding strategies. Diverse insect species like the Green Peach Aphid *Myzus persicae* (Hemiptera)(Rodriguez et al. 1993), the Western Flower Thrips *Frankliniella occidentalis* (Thysanoptera)(Mirnezhad et al. 2010), the Serpentine Leafminer *Liriomyza trifolii* (Diptera)(Hawthorne et al. 1992), the Cotton Bollworm *Helicoverpa zea* (Lepidoptera), and the Beet Armyworm *Spodoptera exigua* (Lepidoptera)(Juvik et al. 1994) show reduced fitness when Acyl sugars are present. As the *S. pennellii* accessions LA716 and LA3791 are phylogenetically closely related based on the number of single nucleotide polymorphisms (SNP)(Viquez Zamora et al. submitted BMC Genomics 2013) and *S. pennellii* LA3791 resistance is likely based on the synthesis of specific Acyl sucroses (chapters 2 and 4), it is reasonable to assume that the Acyl sucrose-mediated resistance acts against multiple insect species. To confirm this and to determine which specific species are affected by this mechanism, future studies are required.
Besides constitutive defense, induced defense may also play a role in *B. tabaci* resistance. Puthoff et al. (2010) studied RNA expression levels upon feeding by *B. tabaci* nymphs on tomato and found that expression levels were higher for basic β-1,3-glucanase (*GluB*), basic chitinase (*Chi9*), and pathogenesis-related protein-1 (*PR-1*), a marker for SA-mediated defense. The latter demonstrates the intricacy of *B. tabaci* resistance as in Arabidopsis SA-responsive genes are induced by *B. tabaci* to repress jasmonic acid- (JA) and ethylene-induced defenses to enhance their performance (Zarate et al. 2007). Such SA-JA crosstalk has also been recorded in lima bean plants after *B. tabaci* feeding (Zhang et al. 2009).

Future perspectives

This thesis showed the integrative approach of using phenotypic, metabolomic, and genotypic analyses leads to insights into the complex resistance of tomato against *B tabaci*. The identified QTLs with high explained variances and associated markers on chromosomes I, III, and IV provide breeders with tools to further introduce into their elite material.
Future work should focus on the fine mapping of the major QTLs identified for whitefly resistance as well as for metabolite QTLs via Marker Assisted BackCross Breeding (MABCB). Reducing the size of the introgressions is necessary for introgressing the trait of interest without transferring undesired traits of the donor parent simultaneously. By tracking the whitefly resistant phenotype during MABCB it will be possible to maintain the resistant phenotype throughout the MABCB process.

Furthermore, future studies could aim for losing undesired traits that are directly correlated with the presence of Acyl sugars, like the stickiness. It could well be possible that the toxicity within *S. pennellii* is still present in absence of the sugar groups conjugating to the fatty acid tails, since another studies wild tomato relative, *S. habrochaites*, employs such a system to defend itself against multiple insect attackers (Yu et al. 2010).

The IL population for LYC4 did not possess resistance in the individual ILs; to identify whitefly resistance in the LYC4 parent, it would be advisable to develop a Recombinant Inbred Line (RIL) population, which consists of multiple populations build up from a mosaic of homozygous genomic regions of the two parental lines, which will assist in studying complex whitefly resistance traits without coping with the drawbacks of epistasis (Alonso-Blanco et al. 1981).

Taking the progress of resistance breeding against *B. tabaci* over the last two decades in mind, it is likely that one or multiple commercial lines will be available in the future that harbor a considerable level of resistance against *B. tabaci*.

References

Agrawal AA (1998). Induced responses to herbivory and increased plant performance. Science 279:1201-1202

Agrawal AA, Karban R (1999). Why induced defenses may be favored over constitutive strategies in plants. In R Tollrian, CD Harvell, eds, The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, pp 45-61

Agrios GN (2005). Plant Pathology. 5th ed. Elsevier Academic Press. pp 922

Ahmad M, Arif MI, Ahmad, Denholm I (2002). Cotton whitefly (*Bemisia tabaci*) resistance to organophosphate and pyrethroid insecticides in Pakistan. Pest Management Science 58: 203-208

Alegbejo MD (2000). Whitefly transmitted plant viruses in Nigeria. J. Sustain. Agri. 17(2): 99-109

Alemandri V, De Barro P, Bejerman N, Argüello Caro EB, Dumón AD, et al (2012). Species Within the *Bemisia tabaci* (Hemiptera: Aleyrodidae) complex in soybean and bean crops in Argentina. J Econ Entomol 105: 48–53

Alonso-Blanco C, Peeters AJ, Koornneef M, Lister C, Dean C (1998). Development of an AFLP based linkage map of L*er*, Col and Cvi *Arabidopsis thaliana* ecotypes and construction of a L*er*/Cvi recombinant inbred line population. Plant Journal 14:259-271

Anderson LK, Covey PA, Larsen LR, Bedinger P, Stack SM (2010). Structural differences in chromosomes distinguish species in the tomato clade. Cytogenet. Genome Res. 129:24-34

Anderson P, Agrell J (2005). Within plant variation of induced defence in developing leaves of cotton plants. Oecologia 144: 427-434

Antonious GF (2001). Production and quantification of methyl ketones in wild tomato accessions. J Environ Sci Health B 36: 835–848

Antonious GF, Kochhar TS (2003). Zingiberene and curcumene in wild tomato. J Environ Sci Health B 38: 489–500

Antonious G, Tejinder K, Simmons AM (2005). Natural products: seasonal variation in trichome counts and contents in *lycopersicon hirsutum* f. glabratum. Journal of Environmental Science and Health. 40:619-631

Arnó J, Gabarra R (1994). Whitefly species composition in winter tomato greenhouses. IOBC/wprs Bull. 17:104-109

Arnó J, Gabarra R, Estopà M, Gorman K, Peterschmitt M, Bonato O, Vosman B, Hommes M, Albajes R (2009). Implementation of IPM programs on EUropea, greenhouse tomato production areas: Tools and constraints. Edicions de la Universitat de Lleida

Baldin ELL, Vendramin JD, Lourencao AL (2005). Resistance of tomato genotypes to the whitefly *Bemisia tabaci* (Gennadius) biotype B (Hemiptera: Aleyrodidae). Neotropical Entomology 34:435-441

Bas N, Mollema C, LINDHOUT P (1992). Resistance in *Lycopersicon hirsutum* f. glabratum to the greenhouse whitefly *(Trialeurodes vaporariorum*) increases with plant age. Euphytica 64: 189-195

Bedford ID, Pinner M, Liu S, Markham PG (1994). *Bemisia tabaci* potential infestation, phytotoxicity and virus transmission within European Agriculture. Proceedings of the Brighton Crop Protection Conference: Pests and Diseases 3. The British Crop Protection Council, Farnham, UK., pp. 911–916

Bellows TS, Arakawa K (1986). Modeling the Relationship Between Transient Vector Densities and Plant Disease Incidence with Special Reference to *Bemisia tabaci* (Homoptera: Aleyrodidae) and Lettuce Infectious Virus Yellows. Journal of Economic Entomology 79(5):1235-1239(5)

Bel-Kadhi MS, Onillon JC, Cenis JL (2008). Molecular characterization of *Bemisia tabaci* biotypes in Southern Tunisia. Tunisian Journal of Plant Protection 3(2): 79-86

Benjamini Y, Hochberg Y (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Roy. Statist. Soc. Ser. B 57:289-300

Bennett RN, Wallsgrove RM (1994). Secondary metabolites in plant defence mechanisms, Tansley Review No. 72. New Phytol. 127:617-633

Ben-Israel I, Yu G, Austin MB, Bhuiyan N, Auldridge M, Nguyen T, Schauvinhold I, Noel JP, Pichersky E, Fridman E (2009). Multiple biochemical and morphological factors underlie the production of methylketones in tomato trichomes. Plant Physiol 151: 1952–1964

Berlinger MJ (1986). Host Plant Resistance to *Bemisia tabaci*. Agric.Ecosystems Environ. 17: 69-82

Blackman RL, Cahill M 1998. The karyotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae). Bull. Entomol. Res 88: 213-215

Bleeker PM, Diergaarde PJ, Ament K, Guerra J, Weidner M, Schutz S, Both MT, Haring MA, Schuurink RC (2009). The role of specific tomato volatiles in tomato-whitefly interaction. Plant Physiol. 151(2):925–935

Bleeker PM, Diergaarde PJ, Ament K, Schütz S, Johne B, Dijkink J, Hiemstra H, de Gelder R, de Both MTJ, Sabelis MW, et al Tomato-produced 7-epizingiberene and R-curcumene act as repellents to whiteflies. Phytochemistry 2011 *72*, 68–73

Bleeker PM, Mirabella R, Diergaarde PJ, Vandoorn A, Tissier A, Kant MR, Prins M, de Vos M, Haring MA, Schuurink RC (2012). Improved herbivore resistance in cultivated tomato with the sesquiterpene biosynthetic pathway from a wild relative. Proc. Natl. Acad. Sci. USA 109:20124-20129

Blauth, SL, Steffens JC, Churchill, GA, Mutschler MA (1999). Identification of QTLs controlling acylsugar fatty acid composition in an interspecific population of *Lycopersicon pennellii* (Corr.) D'Arcy. Theor. Appl. Genet. 99:373-381

Blauth SL, Churchill GA, Mutschler MA (1998). Identification of quantitative trait loci associated with acylsugar accumulation using interspecific populations of the wild tomato, *Lycopersicon pennellii*. Theor. Appl. Genet. 96:458-467

Boykin LM, Shatters RG, Rosell RC Jr., McKenzie CL, De Barro P, Frohlich DR (2007). Global relationships of *Bemisia tabaci* (Hemiptera: Aleyrodidae) revealed using Bayesian analysis of mitochondrial COI DNA sequences. Mol. Phylogenet. Evol. 44: 1306-1319

Broekgaarden C, Riviere P, Steenhuis-Broers MM, Cuenca M, Kos M, Vosman B (2012). Phloem-specific resistance in *Brassica oleracea* against the whitefly *Aleyrodes proletella*. Entomologia Experimentalis et Applicata 142(2):153-164

Broekgaarden C, Snoeren, TAL, Dicke M , Vosman, B (2011). Exploiting natural variation to identify insect-resistance genes. Plant Biotechnology Journal 9(8):819 – 825

Brown JK (2007). The *Bemisia tabaci* Complex: Genetic and phenotypic variability drives Begomovirus spread and virus diversification. Online. APS*net* Features. doi: 10.1094/APSnetFeature-2007-0107

Brown JK, Czosnek H (2002). Whitefly Transmitted Viruses. In Advances in Botanical Research, Volume 36: 65-100

Brown JK, Frohlich D, Rosell R (1995). The sweetpotato/silverleaf whiteflies: biotypes of *Bemisia tabaci* (Genn.), or a species complex? Ann. Rev. Entomol. 40:511-534

Burke BA, Goldsby G, Mudd JB (1987). Polar epicuticular lipids of *Lycopersicon pennellii*. Phytochemistry 26: 2567–2571

Byrne DN, Bellows TS Jr (1991). Whitefly biology. Annual Review of Entomology 36: 431-458

Byrne DN, Bellows TS Jr, Parrella MP (1990). Whiteflies in agricultural systems. In Whiteflies: their Bionomics, Pest Status, and Management, ed. D Gerling, pp. 227-261. Andover, Hants, UK: Intercept Ltd

Cahill M, Jarvis W, Gorman K, Denholm I (1996). Resolution of baseline responses and documentation of resistance to buprofezin in *Bemisia tabaci* (Homoptera, Aleyrodidae). Bulletin of Entomological Research 86: 117-122

Calvo J, Bolckmans K, Stansly PA, Urbaneja A (2009). Predation by *Nesidiocoris tenuis* on *Bemisia tabaci* and injury to tomato. BioControl 54: 237-246

Carabalí A, Belloti AC, Lerma JM (2010). Biological parameters of *Bemisia tabaci* (Gennadius) biotype B (Hemiptera:Aleyrodidae) on Jatrophagossypiifolia, commercial (Manihotesculenta) and wild cassava (Manihotflabellifolia and *M. carthaginensis*) (Euphorbiaceae). Neotrop.entomol. 39(4): 562-567

Cardoza YJ, McAuslane HJ, Webb SE (2000). Factors affecting oviposition site selection by *Bemisia argentifolii* in *Cucurbita pepo* L. Environmental Entomology 29(2): 220-225

Carreno-Quintero N, Acharjee A, Maliepaard C, Bachem CWB, Mumm R, Bouwmeester H, Visser RGF, Keurentjes JJB (2012). Untargeted metabolic quantitative trait loci analyses reveal a relationship between primary metabolism and potato tuber quality. Plant Physiol. 158: 1306-1318

Channarayappa SG, Muniyappa V, Frist RH (1992). Resistance of Lycopersicon species to *Bemisia tabaci,* a tomato leaf curl virus vector. Canadian Journal of Botany 70: p.2184-2192

Charleston DS and Dicke M (2008). Designing experimental protocols to investigate the impact of GM crops on non-target arthropods. Commission on Genetic Modification, Bilthoven, The Netherlands

Chu C, Freeman T, Natwick ET, Buckner JS, Nelson DR, Henneberry TJ (2000). *Bemisia argentifolii* adult, nymph and egg densities and egg distribution on selected upland cottons. Journal of Entomological Science 35:39-47

Cohen S, Nitzany FE (1966). Transmission and host range of tomato yellow leaf curl virus. Phytopathology 56: 1127-1131

Costa HS, Brown JK (1991). Variation in biological characteristics and esterase patterns among populations of *Bemisia tabaci*, and the association of one population with silverleaf symptom induction. Entomol. Exp. Appl. 61: 211-219

Crowder DW, Horowitz AR, De Barro PJ, Liu SS, Showalter AM, Kontsedalov S, Khasdan V, Shargal A, Liu J, Carrière Y (2010). Mating behaviour, life history and adaptation to insecticides determine species exclusion between whiteflies. Journal of Animal Ecology 79: 563-570

Cuthbertson AGS, Walters KFA, Northing P, Luo W (2007). Efficacy of the entomopathogenic nematode, *Steinernema feltiae*, against sweetpotato whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) under laboratory and glasshouse conditions. Bulletin of Entomological Research 97: 9-14

Cuthbertson AGS, Walters KFA (2005). Pathogenicity of the entomopathogenic fungus, *Lecanicillium muscarium*, against the sweetpotato whitefly *Bemisia tabaci* under laboratory and glasshouse conditions. Mycopathologia 160: 315-319

Czosnek H, Ghanim M (2011). *Bemisia tabaci* - Tomato yellow leaf curl virus interaction causing worldwide epidemics. In: The whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) interaction with geminivirus-infected host plants - *Bemisia tabaci*, host plants and geminiviruses. Thompson WMO Ed. Springer. pp. 51-67

De Barro PJ (2012). The *Bemisia tabaci* Species Complex: Questions to Guide Future Research. Journal of Integrative Agriculture. 11 (2): 187-196

De Barro PJ, Coombs MT (2009). Post-release evaluation of *Eretmocerus hayati* Zolnerowich and Rose in Australia. Bulletin of Entomological Research 99:193-206

De Barro PJ, Driver F, Naumann ID, Clarke GM, Schmidt S, Curran J (2000). Descriptions of three species of *Eretmocerus* Haldeman (Hymenoptera: Aphelinidae) parasitising *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) in Australia based on morphological and molecular data. Australian Journal of Entomology 39: 259-269

De Barro PJ, Edwards O, Sunnucks P (2007). Applications of Molecular Ecology to IPM – What Impact? In: Jepson P, Kogan M. (eds). Perspectives in ecological theory and integrated pest management. Cambridge University Press. Pp 469-521

De Barro PJ, Liu SS, Boykin LM, Dinsdale AB (2011). *Bemisia tabaci*: a statement of species status. Annu. Rev. Entomol. 56:1-19

De Ponti OMB, PET G, Hogenboom NG (1975). Resistance to the glasshouse silverleaf whitefly (*Trialeurodes vaporariorum* Westw) in tomato *(Lycopersicon esculentum* Mill) and related species. Euphytica 24: 645-649

De Vos RCH, Moco S, Lommen A, Keurentjes JJB, Bino RJ, Hall RD (2007). Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. Nature Protocols 2(4):778–791

Dicke M (1999) Specificity of herbivore-induced plant defences. In: Insect-Plant Interactions and Induced Plant Defence. Novartis Foundation Symposium 223. - Chichester : John Wiley & Sons, 1999 - p. 43-55

Dinsdale, A, Cook L, Riginos C, Buckley YM, De Barro PJ *(*2010). Refined global analysis of *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) *mitochondrial cytochrome oxidase 1* to identify species level genetic boundaries. Ann. Entomol. Soc. Am. 133:196-*208*

Dimock MB, Kennedy GG (1983). The role of glandular trichomes in the resistance of *Lycopersicon hirsutum* f. glabratum to *Heliothis zea.* Entomol. exp. appl. 33:263-268

Doyle JJ and Doyle JL (1990). A rapid total DNA preparation procedure for fresh plant tissue. Focus 12:13-15

Drost YC, van Lenteren JC, Roermund HJW van (1998). Life-history parameters of different biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in relation to temperature and host plant: a selective review. Bull. Entomol. Res. 88: 219-229

Eggleston SL, Lawson DM, Mutschler MA (1995). Genetic Analysis of Acylsugar Production in Intraspecific Populations of the Wild Tomato, *Lycopersicon pennellii.* HortScience 30 (4): 801

Elbert A, Nauen R (2000). Resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides in southern Spain with special reference to neonicotinoids. Pest. Manag. Sci. 56: 60–64

Elle E, Dam NM, Hare JD (1999). Cost of glandular trichomes, a “resistance” character in *Datura wrightii* Regel (Solanaceae). Evolution 53:22-35

Ellers-Kirk CD, Fleischer SJ, Snyder RH, Lynch JP (2000). Potential of entomopathogenic nematodes for biological control of *Acalymma vittatum* (Coleoptera: Chrysomelidae) in cucumbers grown in conventional and organic soil management systems. Journal of Economic Entomology 93: 605–612

Eshed Y, Zamir D (1996). Less than additive epistatic interactions of QTL in tomato. Genetics 143:1807-1817

Eshed Y, Zamir D (1995). An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141: 1147-1162

FAOSTAT. (2011). Statistical database; Online reference. Accessed 31/10/2011

Fekrat L, Shishehbor P (2007). Some Biological Features of Cotton Whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae) on Various Host Plants. Pakistan Journal of Biological Sciences, 10: 3180-3184

Feng Y, Wu Q, Wang S, Chang X, Xie W, Xu B, Zhang Y (2010). Cross resistance study and biochemical mechanisms of thiamethoxam resistance in B-biotype *Bemisia tabaci* (Hemiptera:Aleyrodidae). Pest Management Science 66: 313-318

Fernandez E, Gravalos C, Javier Haro P, Cifuentes D, Bielza P (2009). Insecticide resistance status of *Bemisia tabaci* Q-biotype in south-eastern Spain. Pest Manag Sci 65: 885-891

Finkers R, Heusden AW van, Meijer-Dekens RG, Kan JAL van, Maris PC, Lindhout P(2007). The construction of a *Solanum habrochaites* LYC4 introgression line population and the identification of QTLs for resistance to *Botrytis cinerea*. Theoretical and Applied Genetics 114 (6): 1071- 1080

Finkers R, Bai Y, Berg P van den, Berloo R van, Meijer-Dekens F, Have A ten, Kan JAL van, Lindhout P, Heusden AW van (2008). Quantitative resistance to *Botrytis cinerea* from *Solanum neorickii*. Euphytica 159: 83-92

Firdaus S, Vosman B, Hidayati N, Supena EDJ, Visser RGF, Heusden AW van (2013a). The *Bemisia tabaci* species complex: Additions from different parts of the world. Insect Science DOI: 10.1111/1744-7917.12001

Firdaus S, Heusden AW van, Hidayati N, Supena E, Mumm R, Vos RH de, Visser RGF, Vosman B (2013b). Identification and QTL mapping of whitefly resistance components in *Solanum galapagense*. Theor. Appl. Genet. 126: 1487-1501

Firdaus S, Heusden AW van, Hidayati N, Supena EDJ, Visser RGF, Vosman B (2012). Resistance to *Bemisia tabaci* in tomato wild relatives. Euphytica 187: 31-45

Fobes JF, Mudd JB, Marsden MPF (1985). Epicuticular lipid accumulation on the leaves of *Lycopersicon pennellii* (Corr.) D’Arcy and *Lycopersicon esculentum* Mill. Plant Physiol 77: 567–570

Foolad MR (1996). Unilateral incompatibility as a major cause of skewed segregation in an interspecific cross of tomato. Plant Cell Reports 15: 627-633

Fordyce JA, AA Agrawal (2001). The role of plant trichomes and caterpillar group size on growth and defence of the pipevine swallowtail *Battus philenor*. Journal of Animal Ecology 70: 997-1005

Frary A, Nesbitt TC, Grandillo S, Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert KB, Tanksley SD (2000). *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit size. Science 289: 85-88

Freitas JA, Maluf WR, Graças Cardoso M, Gomes LAA, Bearzotti E (2002). Inheritance of foliar zingiberene contents and their relationship to trichome densities and whitefly resistance in tomatoes. Euphytica 127: 275–287

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

Fridman E, Pleban T, Zamir D (2000). A recombination hotspot delimits a wild-species quantitative trait locus for tomato sugar content to 484 bp within an invertase gene. Proc Natl Acad Sci USA

Frohlich DR, Torres-Jerez I, Bedford D, Markham PG, Brown JK (1999). A phylogeographical analysis of the *Bemisia tabaci* species complex based on mitochondrial DNA markers. Mol Ecol 8: 1683-1691

Gelman DB, Blackburn MB, Hu JS, Gerling D (2002). Timing and regulation of moling/metamorphosis in the whitefly: cues for the development of its parasitoid. *Encarsia formosa* pp. 12-21

Gerling D (1986). Natural enemies of *Bemisia tabaci*, biological characteristics and potential as biological control agents. A review. Agric. Ecosystems and Environ. 17: 99-111

Gerling D, Alomar O, Arno J (2001). Biological Control of *Bemisia tabaci* using predators and parasitoids. Crop Protection. 20:779-799

Gerling D, Kravchenko V (1996). Pest management of *Bemisia* out of doors, In: Gerling, D. and R.T. Mayer.1996.(eds) *Bemisia*: 1995 Taxonomy, Biology, Damage, Control and Management. Intercept, Andover, UK. : 667-681

Ghangas GS, Steffens JC (1993). UDPglucose:fatty acid transglucosylation and transacylation in triacylglucose biosynthesis. Proc Natl Acad Sci USA 90:9911–9915

Goffreda, JC, Mutschler MA (1989). Inheritance of potato aphid resistance in hybrids between *Lycopersicon esculentum* and *L. pennellii.* Theoretical and Applied Genetics 78: 210–216

Goffreda JC, Mutschler MA, Tingey WM (1988). Feeding behaviour of potato aphid affected by glandular trichomes of wild tomato. Entomologia Experimentalis et Applicata 48: 101-107

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

Goffreda JC, Steffens JC, Mutschler MA (1990). Association of epicuticular sugars with aphid resistance in hybrids with wild tomato. Journal of the American Society of Horticultural Science 115: 161–165

Gómez-Díaz E, Jordà M, Peinado MA, Rivero A (2012). Epigenetics of Host–Pathogen Interactions: The Road Ahead and the Road Behind Plos Pathogens, 8 (11): e1003007

Goolsby J, Legaspi JC, Legaspi BC (1996). Quarantine evaluation of exotic parasitoids of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius). Southwestern Entomologist 21: 13-21

Guo Q, Tao Y, Chu D (2013). Characterization and Comparative Profiling of miRNAs in Invasive *Bemisia tabaci* (Gennadius) B and Q. PLoS One. 8(3): e59884. doi:10.1371/journal.pone.0059884_PMCID: PMC3603954

Gupta VK, Sharma R, Jindal V and Dilawari VK (2010). SCAR markers for identification of host plant specificity in whitefly, *Bemisia tabaci* (Genn.) Indian Journal of Biotechnology 9: 360-366

Gurr GM, McGrath D (2002). Foliar pubescence and resistance to potato moth, *Phthorimaea operculella*, in *Lycopersicon hirsutum.* Entomologia Experimentalis et Applicata (2002) 103, 35-41

Haile FJ, Higley LG, Specht JE (1998). Soybean cultivars and insect defoliation: yield loss and economic injury levels. Agron. J. 90: 344–352

Hawthorne DJ, Shapiro JA, Tingey WM, Mutschler MA (1992). Trichome-borne and artificially applied acylsugars of wild tomato deter feeding and oviposition of the leafminer *Liriomyza trifolii*. Entomol Exp Appl. 65:65-73

He Y, Zhao J, Wu D, Wyckhuys KAG, Wu K (2011). Sublethal Effects of Imidacloprid on *Bemisia tabaci* (Hemiptera: Aleyrodidae) Under Laboratory Conditions. Journal of Economic Entomology 104 (3): 833-838

Henneberry TJ, Toscano NC, Faust RM, Coppedge JR (Eds.)(1996). Silverleaf Whitefly (Formerly Sweetpotato Whitefly, Strain B): 1996 Supplement to the 5-year National Research and Action Plan-Fourth Annual Review. US Dept. Agric., Agric. Res. Serv,1996-01, 243pp

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

Hogenhout SA, Ammar E-D, Whitfield AE, Redinbaugh MG (2008). Insect Vector Interactions with Persistently Transmitted Viruses. Annu. Rev. Phytopathol. 46: 327-359

Hohn T, 2007. Plant virus transmission from the insect point of view. Proc. Natl. Acad. Sci., 104: 17905-17906

Horowitz AR, Kontsedalov S, Khasdan V, Ishaaya I (2005). Biotypes B and Q of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. Archives of Insect Biochemistry and Physiology 58: 216-225

Horowitz AR, Kontsedalov S, Ishaaya I (2004). Dynamics of resistance to the neonicotinoids, acetamiprid and thiamethoxam, in *Bemisia tabaci* (Hemiptera: Aleyrodidae). J. Econ. Entomol. 97: 2051-2056

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

Hu J, De Barro P, Zhao H, Wang J, Nardi F, Liu SS (2011). An extensive field survey combined with a phylogenetic analysis reveals rapid and widespread invasion of two alien whiteflies in China. PLoS One 6: 1-9

Idris AM, Smith SE, Brown JK (2001). Ingestion, transmission, and persistence of Chino del tomate virus (CdTV), a New World begomovirus, by Old and New World biotypes of the whitefly vector *Bemisia tabaci.* Annals of Applied Biology (2001) 139: 145-154

Jeuken MJW, Pelgrom K, Stam P, Lindhout P (2008). Efficient QTL detection for nonhost resistance in wild lettuce: backcross inbred lines versus F2 population. Theor Appl Genet 116:845-857

Jeuken MJW, Lindhout P (2004). The development of lettuce backcross inbred lines (BILs) for exploitation of the Lactuca saligna (wild lettuce) germplasm. Theor Appl Genet 109: 394-401

Jiang YX, De Blas C, Barrios L, Fereres F (1999). Correlation between whitefly (Homoptera: Aleyrodidae) feeding behavior and transmission of tomato yellow leaf curl virus. Ann Entomol Soc Am 93, 573-579

Jimenez DR, Yokomi RK, Mayer RT, Shapiro JP (1995). Cytology and physiology of silverleaf whitefly-induced squash silverleaf. Physiol. Mol. Plant Pathol. 46:227–242

Jimenez-Gomez JM, Maloof JM (2009). Sequence diversity in three tomato species: SNPs, markers, and molecular evolution. BMC Plant Biology (9):85

Jindal V, Dhaliwal G (2009). Elucidating resistance in cotton genotypes to whitefly, *Bemisia tabaci*, by population buildup studies. Phytoparasitica 37:137-145

Jones CM, Gorman K, Denholm I, Williamson MS (2008). High-throughput allelic discrimination of B and Q biotypes of the whitefly, *Bemisia tabaci*, using TaqMan allele-selective PCR. Pest Manag. Sci. 64: 12–15

Jones DR (2003). Plant viruses transmitted by whiteflies. European Journal of Plant Pathology 109: 195-219

Juvik JA, Shapiro JA, Young TE, Mutschler MA (1994). Acylglucoses for wild tomatoes alter behavior and reduce growth and survival of *Helicoverpa zea* and *Spodoptera exigua* (Lepidoptera: Noctuidae) J Econ Entomol. 87:482-492

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

Kaur H, Heinzel N, Schoettner M, Baldwin IT, Galis I (2010). R2R3-NaMYB8 regulates the accumulation of phenylpropanoid-polyamine conjugates, which are essential for local and systemic defense against insect herbivores in *Nicotiana attenuata*. Plant Physiol. 152: 1731-1747

Keurentjes JJB (2009). Genetical metabolomics: closing in on phenotypes. Curr. Opin. Plant Biol. 12: 223-230

Keurentjes JJB, Fu J, De Vos RCH, Lommen A, Hall RD, Bino RJ, Plas LHW van der, Jansen RC, Vreugdenhil D, Koornneef M (2006). The genetics of plant metabolism. Nat. Genet. 38: 842–849

Krips OE, Kleijn PW, Willems PEL, Gols GJZ, Dicke M (1999). Leaf hairs influence searching efficiency and predation rate of the predatory mite *Phytoseiulus persimilis* (Acari: Phytoseiidae) Exp. Appl. Acarol. 23:119-131

Labate JA, Robertson LD 2012. Evidence of cryptic introgression in tomato (*Solanum lycopersicum* L.) based on wild tomato species alleles. Biomed Central (BMC) Plant Biology. 12: 133

Lawson DM, Lunde CF, Mutschler MA (1997). Marker-assisted transfer of acylsugar-mediated pest resistance from the wild tomato, *Lycopersicon pennellii*, to the cultivated tomato *Lycopersicon esculentum*. Mol. Breed. 3:307–317

Leckie BM, DeJong DM, Mutschler MA (2013). Quantitative trait loci regulating sugar moiety of acylsugars in tomato. Molecular Breeding 31(4): pp 957-970

Leckie BM, DeJong DM, Mutschler MA (2012). Quantitative trait loci increasing acylsugars in tomato breeding lines and their impacts on silverleaf whiteflies. Molecular Breeding. Advance online publication DOI:10.1007/s11032-012-9746-3

Leite GLD, Picanço M, Guedes RNC, Zanuncio JC (2001). Role of plant age in the resistance of Lycopersicon *hirsutum* f. *glabratum* to the tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae). Scientia Horticulturae Amsterdam 89(2):103-113

Liedl BE, Lawson DM, White KK, Shapiro JA, Cohen DE, Carson WG, Trumble JT, Mutschler MA (1995). Acylsugars of wild tomato *Lycopersicon pennellii* alters settling and reduces oviposition of *Bemisia argentifolii* (Homoptera,Aleyrodidae). J Econ Entomol 88(3): 742-748

Lippman ZB, Semel Y, Zamir D (2007). An integrated view of quantitative trait variation using tomato interspecific introgression lines. Curr Opin Genet Dev 17: 545–552

Liu J, Van Eck J, Cong B, Tanksley SD (2002). A new class of regulatory genes underlying the cause of pear-shaped tomato fruit. Proc Natl Acad Sci USA 99: 13302-13306

Liu SS, Walling LL, Wang XW (2012a). Special issue introduction-The whitefly *Bemisia tabaci* species complex and begomoviruses: research progress and future prospects. Journal of Integrative Agriculture 11: 171-175

Liu SS, Colvin J, De Barro PJ (2012b). Species Concepts as Applied to the Whitefly *Bemisia tabaci* Systematics: How Many Species Are There? Journal of Integrative Agriculture, 11 (2): 176-186

Liu TX, Stansly PA (1995). Toxicity and repellency of biorational insecticides to *Bemisia argentifolii* on tomato plants. Entomol. Exp. Appl. 74: 137-143

López C, Aramburu J, Galipienso L, Soler S, Nuez F, Rubio L (2011). Evolutionary analysis of tomato Sw-5 resistance breaking isolates of Tomato spotted wilt virus. Journal of General Virology 92: 210-215

López-Gresa MP, Lisón P, Kim HK, Choi YH, Verpoorte R, Rodrigo I, Conejero V, Bellés JM (2012). Metabolic fingerprinting of Tomato Mosaic Virus infected *Solanum lycopersicum.* J Plant Physiol 169(16):1586-1596

Luan JB, Yao DM, Zhang T, Walling LL, Yang M, Wang YJ, Liu SS (2013). Suppression of terpenoid synthesis in plants by a virus promotes its mutualism with vectors. Ecol. Lett. 16 (3): 390-398

Luna E and Ton J (2012). The epigenetic machinery controlling transgenerational SAR. Plant Signalling and Behaviour 7(6): 1-4

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

Lima LHC, Návia D, Inglis PW, Oliveira MRV (2000). Survey of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) biotypes in Brazil using RAPD markers. Genet. Mol. Biol. 23:1-5

Lippman, Z B and Zamir D (2007). "Heterosis: revisiting the magic." Trends in Genetics 23(2): 60-66

Lommen A (2009). MetAlign: Interface-driven, versatile metabolomics tool for hyphenated full-scan mass spectrometry data preprocessing. Analytical Chemistry 81:3079-3086

Luckwill LC (1943). The genus *Lycopersicon*: An historical, biological, and taxonomical survey of the wild and cultivated tomatoes. Aberdeen Univ. Stud. 120: 1-44

Lykouressis DP, Perdikis DC, Konstantinou AD (2009). Predation rates of *Macrolophus pygmaeus* (Hemiptera: Miridae) on different densities of eggs and nymphal instars of the greenhouse whitefly *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) Entomol. Gen. 32:105–112

Macel M*,* Van Dam NM, Keurentjes JJB *(*2010*).* Metabolomics: the chemistry between ecology and genetics. Molecular Ecology Resources 10*:* 583-593

Maharijaya A, Vosman B, Verstappen F, Steenhuis-Broers G, Mumm R, Purwito A, Visser RGF Voorrips RE (2012). Resistance factors in pepper inhibit larval development of thrips (*Frankliniella occidentalis*). Entomologia Experimentalis et Applicata 145: 62–71

Maliepaard C, Bas N, Heusden AW van, Kos J, Pet G, Verkerk R, Vrielink R, Zabel P, Lindhout P (1995). Mapping of QTLs for glandular trichome densities and *Trialeurodes vaporariorum* (greenhouse whitefly) resistance in an F_2 from *Lycopersicon esculentum: Lycopersicon hirsutum* f. glabratum. Heredity 75:425-433

Maluf WR, Maciel GM, Gomes LA, Cardoso MG, Gonçalves LD, Silva EC, Knapp M (2010). Broad-spectrum arthropod resistance in hybrids between high- and low-acylsugar tomato lines. Crop Sci 50(2): 439-450

Mammadov J, Aggarwal R, Buyyarapu R, Kumpatla S (2012). “SNP Markers and Their Impact on Plant Breeding,” International Journal of Plant Genomics vol. 2012

Mann RS, Sidhu JS, Butter NS, Sohi AS, Sekhon PS (2008). Performance of *Bemisia tabaci* (Hemiptera: Aleyrodidae) on Healthy and Cotton Leaf Curl Virus Infected Cotton. Florida Entomologist 91 (2)

Mansaray A, Sundufu AJ (2009). Oviposition, development and survivorship of the sweet potato whitefly *Bemisia tabaci* on soybean, Glycine max, and the garden bean, *Phaseolus vulgaris*. Journal of Insect Science 9(1):1-6

Marshall JA, Knapp S, Davey MR, Power JB, Cocking EC, Bennett MD, Cox AV (2001). Molecular systematics of Solanum section Lycopersicum Lycopersicon) using the nuclear ITS rDNA region. Theoretical and Applied Genetics 103: 1216-1222

Matsui M (1992). Control of the sweetpotato whitefly, *Bemisia tabaci* Gennadius, on tomato in small glasshouse by releasing *Encarsia formosa* Gahan. Proceedings of the Kansai Plant Protection Society. 34: 53-54

McAuslane HJ (2000). Sweetpotato whitefly B Biotype of silverleaf whitefly, *Bemisia tabaci* (Gennadius) or *Bemisia argentifolii* Bellows and Perring (Insecta: Homoptera: Aleyrodidae). University of Florida, IFAS Extension EENY129

Mayer RT, Inbar M, McKenzie C, Shatter R, Borowicz, V, Albrecht U, Powell CA, Doostda H (2002). Multitrophic interactions of the silverleaf whitefly, host plants, competing herbivores, and phytopathogens. Arch. Insect Biochem. Physiol. 51: 151-169

McCollum TG, Stoffella PJ, Powell CA, Cantliffe DJ, Hanif-Khan S (2004). Effects of silverleaf whitefly feeding on tomato fruit ripening. Postharvest Biology and Technology 31(2):183–190

McDonald BA, Linde C (2010). Pathogen Population Genetics, Evolutionary Potential, and Durable Resistance. Annual Review of Phytopathology 40: 349-379

McDowell ET, Kapteyn J, Schmidt A, Li C, Kang JH, Descour A, Shi F, Larson M, Schilmiller A, An L, et al (2011). Comparative functional genomic analysis of *Solanum* glandular trichome types. Plant Physiol 155: 524-539

McKenzie CL, Anderson PK, Villarreal N (2004). An extensive survey of *Bemisia tabaci* (Homoptera: Aleyrodidae) in agricultural ecosystems in Florida. Fla Entomol 87: 403–407

McKenzie CL, Shatters RG JR, Doostdar H, Lee SD, Inbar M Mayer RT (2002). Effect of geminivirus infection and *Bemisia* infestation on accumulation of pathogenesis-related proteins in tomato. Arch Insect Biochem Physiol 49: 203-214

McKenzie CL, JP Albano (2009). The effect of time of sweetpotato whitefly infestation on plant nutrition and development of tomato irregular ripening disorder. HortTechnology 19: 353–359

Medina-Filho HP, Tanksley SD (1983). Breeding for nematode resistance. Pp. 904-923 in D. A. Evans, W. R. Sharp, P. V. Ammirato, and Y. Yamada, eds. Handbook of Plant Cell Culture,Vol. 1. New York: Macmillan

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

Moco S, Bino RJ, Vorst O,Verhoeven HA, de Groot J, van Beek TA, Vervoort J, De Vos RCH (2006). A liquid chromatography-mass spectrometry-based metabolome database for tomato. Plant Physiology 141, 1205–1218

Mohan M, Nair S, Bhagwat A, Krishna TG, Yano M (1997). Genome mapping, molecular markers and marker-assisted selection in crop plants. Molecular Breeding 3:87-103

Momotaz A, Scott JW, Schuster DJ (2005). Searching for silverleaf whitefly and geminivirus resistance genes from *Lycopersicon hirsutum* accession LA1777. Acta Hort. 695:417–422

Momotaz A, Scott JW, Schuster DJ (2010). Identification of Quantitative Trait Loci Conferring Resistance to *Bemisia tabaci* in an F_2 Population of *Solanum lycopersicum* x *Solanum habrochaites* Accession LA1777. J. AMER. SOC. HORT. SCI. 135(2):134-142

Monforte AJ, Tanksley SD (2000). Fine mapping of a quantitative trait locus (QTL) from *Lycopersicon hirsutum* chromosome I affecting fruit characteristics and agronomic traits: breaking linkage among QTLs affecting different traits and dissection of heterosis for yield. Theor Appl Genet 100:471-479

Monforte AJ, Friedman E, Zamir D, Tanksley SD (2001) Comparison of a set of allelic QTL-NILs for chromosome 4 of tomato: Deductions about natural variation and implications for germplasm utilization. Theor Appl Genet 102:572-590

Moreno-Ripoll R, Gabarra R, Symondson WO, King RA, Agustí N (2012).Trophic relationships between predators, whiteflies and their parasitoids in tomato greenhouses: a molecular approach. Bull Entomol Res 7:1-9

Morgan D, MacLeod, A 1996. Assessing the economic threat of *Bemisia tabaci* (Gennadius) and tomato yellow leaf curl virus to the tomato industry in England and Wales. In *Proceedings of the 1996 Brighton Crop Protection Conference - Pests and diseases, II*, pp. 1077-1082. Alton, UK, BCPC Publications. 1242 pp

Moriones E, Navas-Castillo J (2000). Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. Virus Res 71: 123-134

Muigai SG, Bassett MJ, Schuster DJ, Scott JW (2003). Greenhouse and field screening of wild Lycopersicon germplasm for resistance to the whitefly *Bemisia argentifolii.* Phytoparasitica 31:27–38

Muigai, SG, Schuster DJ, Snyder JC, Scott JW, Bassett MJ, McAuslane HJ (2002). Mechanisms of resistance in Lycopersicon germplasm to *Bemisia argentifolii* (Homoptera: Aleyrodidae) Phytoparasitica 30:347–360

Mutschler MA, Doerge RW, Liu SC, Kuai JP, Liedl BE, and Shapiro JA (1996). QTL analysis of pest resistance in the wild tomato *Lycopersicon pennellii*: QTLs controlling acylsugar level and composition. Theor. Appl. Genet. 92:709-718

Nakazato T, Housworth EA (2011). Spatial genetics of wild tomato species reveals roles of the Andean geography on demographic history. Am J Bot. 98(1):88-98

Nakazato T, Warren D, Moyle LC (2010). Ecological and geographic modes of species divergence in wild tomatoes. American Journal of Botany 97 : 680

Naranjo SE (2001). Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. Crop Prot. 20: 835–852

Naranjo SE (1996). Sampling *Bemisia* for research and pest management applications. In: D. Gerling & T. Mayer (eds), *Bemisia* 1995: Taxonomy, Biology, Damage, Control and Management.Intercept, Andover, Hants, UK.pp. 209-224

Nash MA, Hoffmann AA, Thomson LJ (2010). Identifying signature of chemical applications on indigenous and invasive non target arthropod communities in vineyards. Ecological Applications 20:1693-1703

Navas-Castillo J, Fiallo-Olive E, Sanchez-Campos S (2011). Emerging virus diseases transmitted by whiteflies. Annu Rev Phytopathol 49: 219-248

Nombela G, F. Beitia, Muñiz M (2000). Variation in tomato host response to *Bemisia tabaci* (Hemiptera: Aleyrodidae) in relation to acyl sugar content and presence of the nematode and potato aphid resistance gene *Mi*. Bulletin of Entomological Research 90: 161–167

Nombela G, Beita F, Muñiz M (2001). A differential interaction study of *Bemisia tabaci* Q-biotype on commercial tomato varieties bearing or not bearing the *Mi* resistance gene, and comparative host responses with the B-biotype. *Entomol. Exp. Appl.* 98(3): 339-344

Nombela G, Muñiz M (2010). Host Plant Resistance for the Management of *Bemisia tabaci*: A Multi-crop Survey with Emphasis on Tomato. *Bemisia*: *Bionomics and management of a global pest*. Philip A. Stansly & Steven Naranjo (eds.). Springer Science & Business Media (ISBN: 978-90-481-2459-6). Chapter 14: 357-383

Nombela, G, Williamson, VM, Muñiz, M (2003). The root-knot nematode resistance gene *Mi-1.2* of tomato is responsible for resistance against the whitefly *Bemisia tabaci*. Mol. Plant-Microbe Interact.16:645-649

Oliveira MRV, Henneberry TJ, Anderson P (2001). History current status, and collaborative research projects for *Bemisia tabaci*. Crop Protection 20: 709-723

Oriani MAD, Vendramim JD (2010). influence of trichomes on attractiveness and ovipositional preference of *Bemisia tabaci* (Genn.) B biotype (Hemiptera: Aleroydae) on tomato genotypes. Neotrop Entomol 39: 1002–1007

Oriani MAG, Vendramim JD, Vasconcelos CJ (2011). Biology of *Bemisia tabaci* (Genn) B biotype (Hemiptera, Aleyrodidae) on tomato genotypes. Sci. Agric. 68 (1): 37-41

Palloix A, Ayme V, Moury B (2009). Durability of plant major resistance genes to pathogens depends on the genetic background: experimental evidence and consequences for breeding strategies. New Phytol. 183:190-199

Palumbo JC, Horowitz AR, Prabhaker N (2001). Insecticidal control and resistance management for *Bemisia tabaci*. Crop Prot. 20: 739-765

Panda N, Khush GS (1995). Host plant resistance to insects. 448 p. Oxford University Press, Oxford, UK

Pan H, Li X, Ge D, Wang S, Wu Q, et al (2012). Factors Affecting Population Dynamics of Maternally Transmitted Endosymbionts in *Bemisia tabaci*. PLoS ONE 7(2): e30760. doi:10.1371/journal.pone.0030760

Papayiannis LC, Brown JK, Hadjistylli M, Katis NI (2008). *Bemisia tabaci* biotype B associated with tomato yellow leaf curl disease epidemics in Rhodes Island, Greece. Phytoparasitica 36: 20-22

Peralta IE, Spooner DM, Knapp S (2008). Taxonomy of wild tomatoes and their relatives (Solanum sect Lycopersicoides, sect. Juglandifolia, sect. Lycopersicon; Solanaceae) Systematic Botany Monographs. 84:1-186

Perring TM, Cooper AD, Rodriguez RJ, Farrar CA, Bellows TS (1993). Identification of a whitefly species by genomic and behavioral studies. Science 259: 74-77

Picó B, Díez MJ, Nuez F (1996) Viral diseases causing the greatest economic losses to the tomato crop. II. The Tomato yellow leaf curl virus - a review. Scientia Horticulturae 67 (3-4): 151-196

Pieterse CMJ, Ton J, Van Loon LC (2001). Cross-talk between plant defence signalling pathways: boost or burden? AgBiotechNet 3:ABN 068

Prabhaker N, Castle S, Henneberry TJ, Toscano NC (2005). Assessment of cross resistance potential to neonicotinoid insecticides in *Bemisia tabaci* (Hemiptera, Aleyrodidae). Bulletin of Entomological Research 95: 535-543

Puthoff DP, Holzer FM, Perring TM, Walling L (2010). Tomato pathogenesis-related protein genes are expressed in response to *Trialeurodes vaporariorum* and *Bemisia tabaci* biotype B feeding. J. Chem. Ecol. 36: 1271-1285

Quenouille J, Montarry J, Palloix A, Moury B (2013). Farther, slower, stronger: how the plant genetic background protects a major resistance gene from breakdown. Mol. Plant Pathol. 14 (2): 109-18

Qiu BL, De Barro PJ, Ren SX, Xu CX (2007). Effect of temperature on the life history of Eretmocerus sp nr. furuhashii, a parasitoid of *Bemisia tabaci*. Biocontrol 52: 733-746

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

Resende JTV, Maluf WR, Cardoso MG, Gonçalves LD, et al (2009). Resistance of tomato genotypes to the silverleaf whitefly mediated by acylsugars. Hort. Bras. 27: 345-348

Resende JTV, Maluf WR, Cardoso MG, Nelson DL, Faria MV (2002). Inheritance of acylsugar contents in tomatoes derived from an interspecific cross with the wild tomato *Lycopersicon pennellii* and their effect on spider mite repellence. Genetics and Molecular Research 1: 106-116

Rick CM (1951). Hybrids between *Lycopersicon esculentum* Mill. and *Solanum lycopersicoides* Dun. Proc. Natl. Acad. Sci. (USA) 37: 741-744

Roberts PA, May D, Matthews WC(1986). Root-knot nematode resistance in processing tomatoes. California Agriculture 40:24-26

Goggin FL, Williamson VM, Ullman DE (2001). Variability in the response of *Macrosiphum euphorbiae* and *Myzus persicae* (Hemiptera: Aphididae) to the tomato resistance gene *Mi*. Environ. Entomol. 30:101-106

Roditakis E, Grispou M, Morou E, Kristoffersen JB, Roditakis N, Nauen R, Vontas J and Tsagkarakou A (2009). Current status of insecticide resistance in Q biotype *Bemisia tabaci* populations from Crete. Pest Management Science 65: 313-322

Roditakis E, Tsagkarakou A, Vontas J (2006). Identification of mutations in the parasodium channel of *Bemisia tabaci* from Crete, associated with resistance to pyrethroids. Pesticide Biochemistry and Physiology 85: 161-166

Rodríguez-López MJ, Garzo E, Bonani JP, Fernández-Muñoz R, Moriones E, Fereres A (2012). Acylsucrose-Producing Tomato Plants Forces *Bemisia tabaci* to Shift Its Preferred Settling and Feeding Site. PLoS ONE 7(3): e33064. doi:10.1371/journal.pone.0033064

Rodriguez F, Wu F, Ane C, Tanksley S, Spooner DM (2009). Do potatoes and tomatoes have a single evolutionary history, and what proportion of the genome supports this history? BMC Evol Biol.9:191

Rodriguez AE, Tingey WM, Mutschler MA (1993). Acylsugars of *Lycopersicon pennellii* deter settling and feeding of the green peach aphid (Homoptera: Aphididae). Journal of Economic Entomology 86: 34-39

Rodríguez-López MJ, Garzo E, Bonani JP, Fereres A, Fernández-Muñoz R, Moriones E (2011). Whitefly resistance traits derived from the wild tomato *Solanum pimpinellifolium* affect the preference and feeding behavior of *Bemisia tabaci* and reduce the spread of Tomato yellow leaf curl virus. Phytopathology 101(10):1191-201

Roermund HJW van, Van Lenteren, JC (1996). Factors affecting whitefly control by the parasitoid *Encarsia formosa* in tomato. Proc. Exp. & Appl. Entomol. NEV Amsterdam 7:101-108

Rosello S, Diez MJ, Nuez F (1996). Viral diseases causing the greatest economic losses to the tomato crop. I. The tomato spotted wilt virus-a review. Sci. Hortic. 67: 117-150

Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM (1998). The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. Proceedings of the National Academy of Sciences, USA 95: 9750–9754

Rousseaux MC, Jones CM, Adams D, Chetelat R, et al (2005). QTL analysis of fruit antioxidants in tomato using *Lycopersicon pennellii* introgression lines. Theor. Appl. Genet. 111: 1396-1408

Rubinstein G., Czosnek H. (1997). Long-term association of tomato yellow leaf curl virus (TYLCV) with its whitefly vector *Bemisia tabaci:* Effect on the insect transmission capacity, longevity and fecundity. J. Gen. Virol. 78: 2683-2689

Sabaz AK, Chibon PY, De Vos RCH, Schipper BA, Walraven E, Beekwilder J, Dijk T van, Finkers R, Visser RGF, Weg EW van de, Bovy A, Cestaro A, Velasco R, Jacobsen E, Schouten HJ (2012). Genetic analysis of metabolites in apple fruits indicates an mQTL hotspot for phenolic compounds on linkage group 16. J Exp Bot. 63(8): 2895–2908

Sanchez-Pena P, Oyama K, Nunez-Farfan J, Forfoni J, Hernandez-Vertugo S, Marquez-Guzman J, Garzon-Tiznado JA (2006). Sources of resistance to whitefly (Bemisia spp.) in wild populations of *Solanum lycopersicum* var. cerasiforme (Dunal) spooner G.J. Anderson et R.K. Jansen in Nortwestern Mexico. Genetic Resources and Crop Evolution 53: 711-719

Salas J, Mendoza O (1995). Biology of the sweetpotato whitefly (Homoptera:Aleyrodidae) on tomato. Florida Entomologist 78: 154-160

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

Schilmiller AL, Charbonneau AL, Last RL (2012). Identification of a BAHD acetyltransferase that produces protective acyl sugars in tomato trichomes. PNAS 109(40):16377-16382

Schilmiller AL, Last RL, Pichersky E (2008). Harnessing plant trichome biochemistry for the production of useful compounds. Plant Journal 54: 702-711

Schmalstig JG, McAuslane, HJ (2001). Developmental anatomy of zucchini leaves with squash silverleaf disorder caused by the silverleaf whitefly. J. Amer. Soc. Hort. Sci. 126:544–554

Schoonhoven LM, Van Loon JJA, Dicke M (2005). Insect-Plant Biology. (Oxford: Oxford University Press)

Schuster DJ, Evans GA, Bennett FD, Stansly PA, Jansson RK, Leibee GL, Webb SE (1998). A survey of parasitoids of *Bemisia* spp. whiteflies in Florida, the Caribbean and Central and South America. Int. J. Pest Manag. 44(4): 255-260

Schuster DJ (2001). Relationship of silverleaf whitefly density to severity of irregular ripening of tomato. HortScience 36:1089–1091

Schuster DJ, Stansly PA, Polston JE (1995). Expressions of plant damage. D. Gerling R. T. Mayer *Bemisia* taxonomy, biology, damage, control and management 1996. 153-165. Intercept Andover, United Kingdom

Sétamou M, Schulthess F, Poehling H-M, Borgemeister C, 2000. Monitoring and modeling of field infestation and damage by the maize earborer *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) In Benin, West Africa. West Africa. Journal of Economic Entomology 93: 650-657

Shapiro JA, Steffens JC, Mutschler MA (1994). Acyl sugars of the wild tomato *Lycopersicon pennellii* in relation to geographic distribution of the species. Biochem Syst Ecol 22: 545-561

Shatters RG Jr, Powell CA, Boykin LM, Liansheng H, McKenzie CL (2009). Improved DNA barcoding method for *Bemisia tabaci* and related Aleyrodidae: development of universal and *Bemisa tabaci* biotype specific mitochondrial cytochrome oxidase I polymerase chain reaction primers. J. Econ. Entomol. 102: 750-758

Shirasawa K, Isobe S, Hirakawa H, Asamizu E, Fukuoka H, Just D, Rothan C, Sasamoto S, Fujishiro T, Kishida Y, Kohara M, Tsuruoka H, Wada T, Nakamura Y, Sato S, Tabata S (2010). SNP discovery and linkage map construction in cultivated tomato. DNA Research 17: 381-391

Shirasawa K, Koilkonda P, Aoki K, Hirakawa H, Tabata S, Watanabe M, Hasegawa M, Kiyoshima H, Suzuki S, Kuwata C, Naito Y, Kuboyama T, Nakaya A, Sasamoto S, Watanabe A, Kato M, Kawashima K, Kishida Y, Kohara M, Kurabayashi A, Takahashi C, Tsuruoka H, Wada T, Isobe S (2012). *In silico* polymorphism analysis for the development of simple sequence repeat and transposon markers and construction of linkage map in cultivated peanut. BMC Plant Biol 12:80

Sim S-C, Durstewitz G, Plieske J, Wieseke R, Ganal MW, et al (2012). Development of a Large SNP Genotyping Array and Generation of High-Density Genetic Maps in Tomato. PLoS ONE 7(7): e40563

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

Simmons AT, Gurr GM, McGrath D, Nicol HI, Martin PM (2003). Trichomes of Lycopersicon spp. and their effect on *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). Aust J Entomol 42: 373-378

Simmons AT, Gurr GM, McGrath D, Martin PM, Nicol HI (2004). Entrapment of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on glandular trichomes of *Lycopersicon* species. Australian Journal of Entomology 43: 196–200

Slocombe SP, Schauvinhold I, McQuinn RP, Besser K, Welsby NA, Harper A, Aziz N, Li Y, Larson TR, Giovannoni J, et al 2008) Transcriptomic and reverse genetic analyses of branched-chain fatty acid and acyl sugar production in *Solanum pennellii* and *Nicotiana benthamiana*. Plant Physiol 148: 1830–1846

Smyrnioudis IN, Harrington R, Clark SJ, Katis N (2001). The effect of natural enemies on the spread of barley yellow dwarf virus (BYDV) by Rhopalosiphum padi (Hemiptera: Aphididae). Bull. Entomol. Res. 91: 301-306

Snyder JC and Carter CD (1985). Trichomes on leaves of *Lycopersicon hirsutum*, *L. esculentum* and their hybrids. Euphytica 34: 53-64

Snyder JC, Simmons AM, Thacker RR (1998). Attractancy and ovipositional response of adult *Bemisia argentifolii* (Homoptera: Aleyrodidae) to type IV trichomes density on leaves of *Lycopersicon hirsutum* grown in three day-length regimes. J. Entomol. Sci. 33: 270–281

Snyder JC and Carter CD (1984). Leaf trichomes and resistance of *Lycopersicon hirsutum* and *L. esculentum* to spider mites. J. Am. Soc. Hortic. Sci. 109: 837-843

Spooner DM, Peralta IE, Knapp S (2005). Comparison of AFLPs with other markers for phylogenetic inference in wild tomatoes [Solanum L. section Lycopersicon (Mill.) Wettst.]. Taxon 54(1): 43-61

Stansly PA, Naranjo SE [eds.](2010). Bemisia, Bionomics and Management of a Global Pest. Springer, Dordrecht. pp.540

Stern C (1943). The Hardy-Weinberg law. Science 97:137-138

Steffens JC, Walters DS (1991). Biosynthesis of glucose esters by glandular trichomes of Lycopersicon pennellii. In PA Hedin, ed, Naturally Occurring Pest Bioregulators. American Chemical Society, Washington, DC pp 136-149

Stevens MR, Scott SJ, Gergerich RC (1992): Inheritance of a gene for resistance to tomato spotted wilt virus (TSWV) from *Lycopersicon peruvianum* Mill. Euphytica 59: 9-17

Strauss SY, Rudgers JA, Lau JA, Irwin RE (2002). Direct and ecological costs of resistance to herbivory. Trends Ecol. Evol. 17: 278-285

Strehmel N, Hummel J, Erban A, Strassburg K, Kopka J (2008). Retention index thresholds for compound matching in GC-MS metabolite profiling. Journal of Chromatography B Analytical Technology and Biomedical Life Sciences 871:182–190

Tanksley S, Nelson J (1996). Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92: 191-203

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, et al. (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132: 1141–1160

Tay WT, Evans GA, Boykin LM, De Barro PJ (2012). Will the Real *Bemisia tabaci* Please Stand Up? PLoS ONE 7(11): e50550. doi:10.1371/journal.pone.0050550

Teng X, Wan F-H, Chu D (2010). *Bemisia tabaci* biotype Q dominates other biotypes across China. Fla Entomol 93: 363–368

The Tomato Genome Consortium (2012). The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485: 635-641

Tikunov YM, Laptenok S, Hall RD, Bovy A, de Vos RC (2012). MSClust: a tool for unsupervised mass spectra extraction of chromatography-mass spectrometry ion-wise aligned data. Metabolomics 8(4):714-718

Tissier A (2012). Glandular trichomes: What comes after expressed sequence tags? *Plant J.* 70:51–68

Traw MB, Kim J, Enright S, Cipollini DF, Bergelson J (2003). Negative cross-talk between salicylate and jasmonate-mediated pathways in the Wassilewskija ecotype of *Arabidopsis thaliana*. Molecular Ecology 12: 1125–1135

Tsai JH, Wang K (1996). Development and reproduction of *Bemisia argentifolii* (Homoptera: Aleyrodidae) on five host plants. Environ. Entomol. 25(4):810-816

Van Dam NM, Hare JD (1998). Differences in distribution and performance of two sap-sucking herbivores on glandular and non-glandular trichomes in *Datura wrightii*. Ecological Entomology 23: 22-32

Van der Vossen EAG, Gros J, Sikkema A, Muskens M, Wouters D, Wolters P, Pereira A, Allefs S (2005). The *Rpi-blb2* gene from *Solanum bulbocastanum* is an *Mi-1* gene homolog conferring broad-spectrum late blight resistance in potato. Plant. J. 44:208-222

Van Giessen WA, Mollema C, Elsey KD (1995).Design and use of a simulation model to evaluate germplasm for antibiotic resistance to the greenhouse whitefly (*Trialeurodes vaporariorum*) and the sweetpotato whitefly (*Bemisia tabaci*). Entomol. Exp. Appl. 76: 271-286

Van Lenteren JC (2000). A greenhouse without pesticides: fact or fantasy? Crop Protection 19: 375-384

Van Lenteren JC, Noldus LPJJ (1990). Whitefly-plant relationships: behavioural and ecological aspects. Whiteflies: their bionomics, pest status and management. D Gerling, ed. Andover, United Kingdom, Intercept Ltd., 1990, 47-89

Van Lenteren, JC, Szabo P, Huisman PWT (1992). The parasite-host relationship between *Encarsia formosa* Gahan (Hymenoptera, Aphelinidae) and *Trialeurodes vaporariorum* (Westwood) (Homoptera, Aleyrodidae) XXXVII. Adult emergence and initial dispersal pattern of *E. formosa*. *Journal of Applied Entomology 114: 392-399*

Van Ooijen (2004). MapQTL ® 5, Software for the mapping of quantitative trait loci in experimental populations. Kyazma B.V., Wageningen, Netherlands

Van Ooijen (2006). JoinMap ® 4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma B.V., Wageningen, Netherlands

Vázquez LL, Jiménez R, de la Iglesia M, Mateo A, Borges M (1997). Host plants of *Bemisia tabaci* (Homoptera: Aleyrodidae) in Cuba. Rev Biol Trop. 44-45:143-8

Vidal C, Osborne LS, Lacey LA, Fargues J, 1998. Effect of host plant on the potential of *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) for controlling the silverleaf whitefly, *Bemisia argentifolii* (Homoptera: Aleyrodidae) in greenhouses. Biol. Control 12: 191–199

Viquez Zamora et al submitted BMC Genomics 2013

Voorrips RE (2002). MapChart: Software for the graphical presentation of linkage maps and QTLs. The Journal of Heredity 93 (1): 77-78

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 11, 4407–4414

Wagner GJ (1991). Secreting glandular trichomes: more than just hairs. Plant Physiology 96: 675-679

Wang Y, Diehl A, Wu F, Vrebalov J, Giovannoni J, Siepel A, Tanksley SD (2008). Sequencing and comparative analysis of a conserved syntenic segment in the Solanaceae. Genetics 180: 391–408

Williams MC, Bedford ID, Kelly A, Markham PG (1996). *Bemisia tabaci*: Potential infestation and virus transmission within the ornamental plant industry. Brighton Crop Protection Conference: Pests and Diseases 2B:63-68

Williams WG, Kennedy GG, Yamamoo RT, Thacker JD, Bordner J (1980). 2-tridecanone: A naturally occurring insecticide from the wild tomato *Lycopersicon hirsutum* f. glabratum. Science 207: 888-889

Wilson M, Moshitzky P, Laor E, Ghanim M, Horowitz AR, Morin S (2007). Reversal of resistance to pyriproxyfen in the Q biotype of *Bemisia tabaci* (Hemiptera: Aleyrodidae). Pest Manag. Sci. 63: 761-768

Wintermantel WM, Cortez AA, Anchieta AG, Gulati Sakhuja AN, Hladky LL (2008). Co-infection by two criniviruses alters accumulation of each virus in a host-specific manner and influences efficiency of virus transmission. Phytopathology 98:1340–1345

Wintermantel WM, Wisler GC (2006). Vector specificity, host range, and genetic diversity of Tomato chlorosis virus. Plant Dis. 90:814–819

Wittstock U, Gershenzon J (2002). Constitutive plant toxins and their role in plant defense. Curr Opin Plant Biol 5: 300-307

www.endure-network.eu (Publication: DR1.11)

www.issg.org/database

Yu G, Nguyen TT, Guo Y, Schauvinhold I, Auldridge ME, Bhuiyan N, Ben-Israel I, Iijima Y, Fridman E, Noel JP, et al. (2010). Enzymatic functions of wild tomato methylketone synthases 1 and 2. Plant Physiol 154: 67–77

Yu H, Kowalski SP, Steffens JC (1992). Comparison of polyphenol oxidase expression in glandular trichomes of *Solanum* and *Lycopersicon* species. Plant Physiol 100: 1885–1890

Zamir D, Ekstein-Michelson I, Zakay Y, Navot N, Zeidan M, Sarfatti M, Eshed Y, Harel E, Pleben H, Van-Oss H, Kedar N, Rabinowitch D, Czosnek H (1994). Mapping and introgression of a tomato yellow leaf curl virus tolerance gene, *Ty-1*. Theor. Appl. Genet. 88: 141–146

Zangerl AR, Rutledge CE (1996). The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. American Naturalist 147: 599-608

Zarate SI, Kempema LA,Walling LL (2007). Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. Plant Physiol. 143:866–875

Zhang PJ, Zheng SJ, Van Loon JJA, Boland W, David A, Mumm R, Dicke M (2009). Whiteflies interfere with indirect plant defense against spider mites in Lima bean. Proc Natl Acad Sci USA 106: 21202–21207

Zhao JW, He YX, Weng QY, WU DD (2009). Effects of host plants on selection behavior and biological parameters of *Bemisia tabaci* Gennadius biotype B. The Journal of Applied Ecology. 20(9): 2249-2254

Zuriaga E, Blanca J, Nuez F (2009). Classification and phylogenetic relationships in Solanum section *Lycopersicon* based on AFLP and two nuclear gene sequences. Genet Resour Crop Evol 56: 663–678

Summary

The silverleaf whitefly (*Bemisia tabaci* Genn.) poses a serious threat to tomato cultivation. A large part of the damage is done directly through heavy host plant colonization. Colonization has a negative impact on the plant, as the whitefly takes up nutrients from the phloem and induces phytotoxic responses, which result in irregular ripening of the fruits. However, most damage is done indirectly as the silverleaf whitefly vectors a broad range of plant pathogenic viruses.

The silverleaf whitefly can successfully be controlled biologically in greenhouse cultivations, but control of the whitefly in the field is mainly based on the application of pesticides. The use of pesticides can have a negative effect on non-harmful or beneficial organisms in the field. Moreover, the effectiveness of pesticides can decline or even completely disappear through adaptation of the whitefly. An effective alternative for the use of pesticides could be the deployment of resistant cultivars. Nowadays, genetic factors responsible for whitefly resistance can be transferred faster and more efficiently into tomato cultivars through marker-assisted backcross breeding programs. Complete resistance against the whitefly is present in some crossable wild relatives of the cultivated tomato and the literature reports extensively about accessions with a high level of resistance against the whitefly.

In this work, I have studied different populations that were developed by interspecific crosses between cultivated tomato and the tomato wild relatives *S. habrochaites* LYC4 and *S. pennellii* LA3791. By integrating datasets from different research disciplines, I have studied the background of whitefly resistance in these populations. Furthermore, these data were used to identify the chromosomal loci in the wild tomato relatives that harbor genes responsible for the resistance and that can be bred into cultivated tomato.

The mechanisms underlying the resistance in *S. pennellii* LA3791 were studied through phenotypic resistance assays that demonstrated that survival and oviposition of the whitefly were not possible on this wild relative. Through removal of glandular cells, present on the leaf trichomes, the resistance was almost completely lost and only adult survival was still significantly different from the wild type. This result led to the hypothesis that glandular

trichomes play an important role in the resistance. This was confirmed in a segregating population based on a cross between *S. pennellii* LA3791 and a susceptible cultivated tomato. Plants that lacked glandular trichomes type I and IV, had the same resistance level as the susceptible parent. Further analyses of the segregating population showed that the presence of glandular trichomes was not the only factor determining resistance, but that the composition and quantity of the metabolites in the glandular trichomes also played an important role. To gain more knowledge on the role of individual metabolites on whitefly resistance and susceptibility, we analyzed the total metabolite content of extreme phenotypes of the F_2 population. Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography Time-Of-Flight Mass Spectrometry (LC-TOF-MS) were employed for the analyses of the total metabolite content. Analyses revealed that on basis of the total metabolite profiles the extreme phenotypes (susceptible versus resistant for the silverleaf whitefly) could be discriminated into two groups that were correlated with resistance or susceptibility. A number of these metabolites could be annotated, but for the majority of the components this was not possible on the basis of available literature and databases. Subsequently, I have studied the genetic basis of the phenotypic resistance parameters as well as the genetic basis of the metabolites from the GC-MS and LC-TOF-MS analyses. A genetic linkage map of the F_2 mapping population was developed using DNA markers (Amplification Fragment Length Polymorphisms, AFLPs and Single Nucleotide Polymorphisms, SNPs). QTLs (Quantitative Trait Loci) were identified between the majority of the metabolites and the genetic markers (>90%) and also we found genetic linkages between whitefly resistance parameters and markers. The QTLs for metabolites and phenotypic parameters partly co-localized at the same positions on the genetic map. Several metabolite QTLs (mQTLs) co-localized with each other in so-called ‘hotspots’. Remarkably, the results of the individual phenotypic QTLs (phQTLs) for adult survival and oviposition as well as the mQTLs for the individual components did not give high explained variances (<20%), which was supported by an analysis of individual metabolite profiles, that showed a high variation in composition between F_2 genotypes with an identical resistance level.

On the basis of these results I hypothesized that resistance could not be explained by a specific composition of metabolites, but that multiple metabolic profiles can result in the same level of resistance in a plant. To support this hypothesis, a backcross population was developed, an F_2BC_1, by backcrossing a completely resistant F_2 plant with the recurrent parent. The complete F_2BC_1 population was analyzed by LC-TOF-MS to characterize the metabolite content of the progeny lines alongside resistance assays for adult survival and

oviposition on these plants. Again, in this population we identified genotypes that possessed a level of resistance equal to the *S. pennellii* LA3791 donor parent. From the analyses it became clear that the complexity of the chemical profiles was reduced and that only a few components were correlated with whitefly resistance or susceptibility. A genetic linkage map with a large number of SNP markers enabled the identification of new QTLs alongside the QTLs from the previous F_2 mapping that were confirmed in the F_2BC_1 populations. The reduction in complexity of the chemical profile was accompanied by an increase in explained variances of both the phenotypic as well as the metabolite QTLs. The results indicate that performing phenotyping assays by scoring resistance parameters in a population along with analyzing the chemical profiles is required to identify resistance loci, which can subsequently be used in marker-assisted breeding programs.

Finally, I have studied an Introgression Line (IL) population, consisting of 30 lines, which each contained a different introgression of *S. habrochaites* LYC4, a whitefly-resistant wild relative of cultivated tomato. Survival and oviposition assays of the whole population revealed that there were a few lines that showed a slightly reduced susceptibility for the silverleaf whitefly. Completely resistant lines were not identified, which indicates that the resistance in this wild relative is complex and governed by the interaction of several genes at different locations on the tomato genome. Such genetic interactions, also referred to as epistatic interactions, complicate the identification of genes involved in resistance and the underlying resistance mechanisms. Therefore, I concluded that IL populations are not suitable for the elucidation of a complex trait as whitely resistance in tomato.

In conclusion, this thesis demonstrates the most important aspects of susceptibility and resistance against the silverleaf whitefly in a *S. pennellii* accession and provides strong evidence for the underlying resistance mechanisms. Furthermore, we were capable of reducing the complex phenotypic and genotypic variation, which was present in the F_2 population, via a backcross with the recurrent parent. This made it possible to identify three genetic loci in *S. pennellii* that play a role in whitefly resistance. A logical next step of this research would be the fine mapping of these three loci in order to enable the transfer of these loci/genes into cultivated tomato lines. By doing so, an important step towards sustainable control of the silverleaf whitefly in tomato cultivation could be made.

Samenvatting

De tabakswittevlieg (*Bemisia tabaci* Genn.) vormt een ernstige bedreiging voor de teelt van tomaten. Een groot gedeelte van de schade wordt rechtstreeks veroorzaakt door massale plantkolonisatie. Kolonisatie heeft een negatieve impact op de plant, omdat de wittevliegen zich voeden met nutriënten uit floëem- en xyleemsap uit de vaatbundels; daarnaast veroorzaakt de tabakswittevlieg ook fytotoxische reacties in de plant welke resulteren in het onregelmatig rijpen van vruchten. Echter, de meeste schade wordt veroorzaakt doordat tabakswittevliegen als vector fungeren van een breed scala aan plantpathogene virussen.

Tabakswittevlieg kan in kasteelten succesvol biologisch worden bestreden met natuurlijke vijanden, maar in de buitenteelt gebeurt dat hoofdzakelijk met behulp van insecticiden. Het gebruik van insecticiden kan nadelige effecten hebben op niet-schadelijke of nuttige organismen in de omgeving en tevens kan als gevolg van adaptatie door de wittevlieg de effectiviteit van een insecticide afnemen of zelfs geheel verdwijnen. Een goed alternatief voor het gebruik van insecticiden kan het gebruik van resistente tomatenplanten zijn. De erfelijke factoren welke verantwoordelijk zijn voor de resistentie kunnen middels moderne merkergestuurde terugkruisingsprogramma's sneller en efficiënter in tomatenrassen ingekruist worden. Volledige resistentie tegen wittevliegen is aanwezig in enkele wilde verwanten van de cultuurtomaat en in de literatuur is uitgebreid beschreven welke accessies van wilde verwanten een hoge resistentie tegen wittevliegen hebben. Het is mogelijk om gewenste eigenschappen van zo'n verwante wilde soort in te kruisen in de cultuurtomaat. Daarvoor is het echter noodzakelijk dat de wilde soort kruisbaar is met de cultuurtomaat en dat nadelige eigenschappen niet mee ingekruist worden. In de praktijk is het inkruisen van tabakswittevliegresistentie uit wilde verwanten echter problematisch gebleken en tot op heden bestaat er geen tomatenras dat volledig resistent is. Ook partiële resistentie zou al een verbetering opleveren voor de huidige teelt en hoewel er variatie is in de mate van vatbaarheid binnen verschillende cultuurtomaten, zijn de minst vatbare cultivars niet voldoende resistent om interessant te zijn voor telers.

In deze studie heb ik verschillende populaties onderzocht die zijn gemaakt via soortskruisingen tussen de cultuurtomaat en de wilde verwanten, *Solanum habrochaites*

LYC4 en *S. pennellii* LA3791. Door datasets verkregen met verschillende onderzoeksdisciplines te integreren heb ik de achtergrond bestudeerd van wittevliegresistentie. Daarnaast zijn deze gegevens gebruikt om inzicht te krijgen in de chromosoomlocaties in de wilde verwanten van de cultuurtomaat waarop genen aanwezig zijn die verantwoordelijk zijn voor de resistentie en die ingekruist kunnen worden in de cultuurtomaat.

In dit proefschrift werd het onderliggend mechanisme van resistentie in *S. pennellii* LA3791 onderzocht via resistentietoetsen die aantoonden dat overleving van adulten en eileg niet mogelijk was op deze wilde verwant. Door de kliercellen, aanwezig op de bladharen, te verwijderen ging de resistentie grotendeels verloren en was er enkel nog een significant effect waarneembaar voor overleving van volwassen wittevliegen. Dit resultaat leidde tot de hypothese dat klierharen een belangrijke rol spelen in de resistentie. Dit werd bevestigd in een splitsende populatie gebaseerd op een kruising tussen *S. pennellii* LA3791 en een vatbare cultuurtomaat *S. lycopersicum* cv. Moneymaker. Planten waarop bepaalde klierhaartypes (I en IV) afwezig waren, hadden eenzelfde resistentieniveau als de vatbare ouderplant. Waarnemingen aan de splitsende populatie toonden ook aan dat de aanwezigheid van klierharen niet per definitie resistentie veroorzaakt, wat erop duidde dat de structuren op het bladoppervlak niet alleen bepalend waren voor de resistentie, maar dat de samenstelling en kwantiteit van de metabolieten in de klierharen ook een belangrijke rol speelden. Om meer inzicht te krijgen in het belang van de individuele metabolieten voor zowel resistentie als vatbaarheid voor wittevliegen werden extreme fenotypen van de F_2 populatie geanalyseerd op totale metabolietinhoud. Deze waren geselecteerd op basis van hoogste en laagste waarden voor overleving van wittevlieg adulten en mate van eileg. Voor de analyse van de totale metabolietinhoud zijn Gas Chromatography-Mass Spectrometry (GC-MS) en Liquid Chromatography Time-Of-Flight Mass Spectrometry (LC-TOF-MS) gebruikt. Analyses toonden aan dat op basis van complete metabolietprofielen van de extreme fenotypen (resistent versus vatbaar voor tabakswittevlieg) daadwerkelijk onderscheid gemaakt kon worden in twee groepen en dat met statistische methoden chemische componenten geïdentificeerd konden worden die gecorreleerd zijn met resistentie dan wel vatbaarheid. Enkele van de componenten konden benoemd worden, maar voor het overgrote gedeelte was dat niet mogelijk op basis van de beschikbare literatuur. Vervolgens heb ik de genetische basis van zowel de resistentiekenmerken overleving en eileg, alsook de aan resistentie- en vatbaarheid gecorreleerde metabolieten uit de GC-MS en LC-TOF-MS analyses onderzocht. Een genetische koppelingskaart van de F_2 karteringspopulatie werd ontwikkeld met behulp

van DNA merkers (Amplification Fragment Length Polymorphisms, AFLPs en Single Nucleotide Polymorphisms, SNPs). Er werden koppelingen (QTLs) gevonden tussen het merendeel van de metabolieten en de genetische merkers (>90%) en ook werden er correlaties, genaamd QTLs (Quantitative Trait Loci), gevonden voor resistentiekenmerken en merkers. De QTLs voor metabolieten en fenotypische kenmerken lokaliseerden grotendeels op dezelfde posities op de genetische kaart; naast meerdere metaboliet QTLs (mQTLs) welke co-localiseerden en ook wel 'genetische hotspots' genoemd worden. Opvallend aan de resultaten was dat de individuele fenotypische QTLs (fQTLs) voor overleving van adulten en eileg en mQTLs voor de individuele componenten geen hoge verklaarde variantie gaven (<20%), wat ondersteund werd door de analyse van individuele metabolietprofielen, die een grote variatie in samenstelling vertoonden tussen genotypen met eenzelfde resistentieniveau. Op basis van deze resultaten werd de hypothese gesteld dat resistentie niet verklaard wordt door een specifieke samenstelling van metabolieten, maar dat meerdere metabolietprofielen kunnen leiden tot eenzelfde resistentieniveau in de plant. Om deze hypothese te ondersteunen werd een terugkruisingspopulatie ontwikkeld op basis van een volledig resistente F_2 plant, een F_2BC_1, welke door middel van LC-TOF-MS geanalyseerd werd op metabolietinhoud en overleving van wittevlieg adulten en eileg. In deze populatie troffen we wederom genotypen aan die eenzelfde resistentieniveau hadden als de *S. pennellii* LA3791 ouder. Uit de analyse werd duidelijk dat de complexiteit van de chemische profielen gereduceerd was en dat slechts enkele componenten gecorreleerd waren met resistentie dan wel vatbaarheid. Een genetische koppelingskaart met een groot aantal SNP merkers maakte het mogelijk om naast de reeds bekende QTLs ook nieuwe te identificeren. De reductie in complexiteit van de resistentie-eigenschappen werd teruggevonden in een toegenomen verklaarde variantie van zowel fenotypische QTLs als ook van de metaboliet QTLs. Deze resultaten wijzen er op dat het uitvoeren van zowel fenotyperingstoetsen door middel van het meten van resistentieparameters in een populatie als het meten van chemische profielen noodzakelijk is om resistentie-loci te identificeren en vervolgens te gebruiken voor merkergestuurde inkruisingsprogramma's.

Uiteindelijk heb ik een Introgression Line (IL) populatie bestudeerd, die bestaat uit 30 lijnen die ieder een andere introgressie bevatten van *S. habrochaites* LYC4, een wittevliegresistente wilde verwant van de cultuurtomaat. Overlevings- en eilegtoetsen aan de hele populatie laten zien dat er enkele lijnen in de populatie aanwezig zijn die een verminderde vatbaarheid vertonen voor de tabakswittevlieg. Echter, volledig resistente lijnen zijn niet gevonden, wat er op duidt dat de resistentie in deze wilde soort complex is en veroorzaakt wordt door een

interactie tussen verschillende genen op verschillende locaties op het tomatengenoom. Zulke genetische interacties, ook wel epistatische interacties genoemd, compliceren het identificeren van bij resistentie betrokken genen en de achterliggende mechanismen. Daarom concludeerden wij dat IL populaties niet geschikt zijn voor het ophelderen van een complexe eigenschap als wittevliegresistentie in tomaat.

Concluderend, dit proefschrift laat zien dat belangrijke aspecten van vatbaarheid en resistentie tegen de tabakswittevlieg in een *S. pennellii* accessie in kaart zijn gebracht en sterke aanwijzingen voor de onderliggende resistentiemechanismen werden verkregen. Tevens waren wij in staat om via een terugkruising met de cultuurouder de complexe fenotypische en genotypische variatie, welke aanwezig was in de F_2 te reduceren. Dit maakte het mogelijk drie genetische gebieden in *S. pennellii* met een rol in de tabakswittevliegresistentie te identificeren. Een logische vervolgstap op dit onderzoek zou zijn om deze drie loci te fijnkarteren zodat deze loci heel gericht in cultuurtomaat ingekruist kunnen worden. Daarmee zou een belangrijke stap gezet kunnen worden naar het beschermen van tomaat tegen tabakswittevlieg.

Dankwoord

‘Samenwerking overtreft de som der delen’

Dit dankwoord is geen pure wetenschap, maar zonder deze inspirerende en motiverende mensen was dit proefschrift nooit tot stand gekomen.

…en hier ligt dan na veel bloed, zweet en tranen een boek…een echt boek waar doorheen gebladerd, over gediscussieerd en gefilosofeerd kan worden. Nu is het dan eindelijk mijn beurt om middels het dankwoord mijn waardering voor hen uit te spreken, die daar op onmiskenbare wijze aan bijgedragen hebben.

Ik werd gedurende het traject bijgestaan door een gevarieerd kwartet van begeleiders; Marcel, Ben, Sjaak en Colette: ieder van jullie heeft op zijn/haar unieke wijze een kleur aan dit proefschrift gegeven.
Marcel, jij hebt mij lang geleden aangestoken met jouw passie voor insecten en daarmee ook voor een belangrijk deel mijn wetenschappelijke pad beklinkerd. Ik vond het een eer dat jij de begeleider van mijn Ph.D.-project wilde zijn. Ik heb veel geleerd van jouw inhoudelijke inbreng op ecologisch, chemisch en entomologisch gebied, maar heb minstens zoveel waardering voor de wijze raad die jij mij tijdens roerige momenten gedurende het traject gaf. Ben, ons eerste contact was tijdens mijn telefonische sollicitatie vanuit Australië en een dag later was de samenwerking beklonken. Als pas afgestudeerde plantenziektekundige kwam ik in de wereld van de plantenveredeling terecht en heb hier mede dankzij jou veel over geleerd. Tijdens de laatste fase van het project, de beruchte schrijffase, waarbij het einde voor mijn gevoel nooit in zicht kwam, wist jij mij gelukkig altijd te motiveren en mij voor de zoveelste keer te overtuigen dat het écht niet meer zoveel werk was… Sjaak, met de nodige humor voorzag jij mij van kennis op het gebied van moleculaire merkers, genetische kaarten en natuurlijk het meest intrigerende gewas op aarde, de tomaat! Jouw hulp met het leren werken met ‘mapping software’ en jouw inbreng tijdens de revisies van het proefschrift stel ik zeer op prijs. Colette, naast collega’s waren we natuurlijk bovenal voor lange tijd kamergenoten. Jij was destijds net zelf gepromoveerd en was mijn vraagbaak als het ging om praktische Ph.D.-

zaken, maar ook een sterke sparringpartner tijdens inhoudelijke discussies. Ik heb het altijd erg gezellig gevonden om met jou samen te werken met als hoogtepunt ons ‘uitje’ naar het IOBC-congres in Granada. Ik hoop dat we elkaar in de toekomst nog vaker treffen! Marcel, Ben, Sjaak en Colette: dankjewel voor alles!
Ric en Roland, jullie hebben een grote bijdrage geleverd aan dit proefschrift. De dataverwerking van GC-MS en LC-TOF-MS data, identificaties van componenten en wijze adviezen voor het analyseren van resultaten en verbeteren van mijn proefschrift werden door jullie gedaan en gegeven. Jullie hebben mij beetje bij beetje wegwijs gemaakt in de complexe wereld van de chemie en ik waardeer jullie inbreng in dit proefschrift zeer. Renate, Henk, Vincent, Johan, Paul en Sjoerd, vanuit de verdelingsbedrijven zijn jullie allen direct betrokken geweest bij mijn project. Tijdens onze bijeenkomsten leverden jullie een belangrijke bijdrage door jullie kennis en visies met mij te delen. Daarnaast leverden jullie wezenlijke contributies middels merkeranalyses, wereldwijde veldproeven en natuurlijk het doneren van het plantenmateriaal wat de basis vormde voor dit onderzoek. Ik heb met veel plezier met jullie samengewerkt en daarvoor: grote dank et merci beaucoup.

Betty, tijdens mijn promotie ben jij mijn paranimf, mijn ‘rechterhand’, symbolisch voor de enorme hoeveelheid werk die jij voor mij hebt verricht tijdens dit project of dat nu bij snikhete temperaturen in de kas of in de door jou meer geliefde omgeving van het chemische of moleculaire lab was. Bedankt voor al je goede adviezen en harde werk, maar bovenal bedankt voor de enorme gezelligheid en steun! Een vriendschap was al snel ontstaan en ik ben ervan overtuigd dat dit ook in de toekomst zo zal blijven.
Greet, jij hebt mij menig uur geholpen met het monnikenwerk dat fenotyperen heet. Tevens hebben we samen deelgenomen aan InsectenExperience en dat werd een heus succes; bedankt! Also thanks to Koen, Alejandro, Syarifin, and Awang for your help during phenotyping experiments, I wish you all the best with your own scientific careers.
Martijn, mijn dank voor je bijdrage in het werk met uitermate complexe RNAseq data, die, ook al zijn deze niet in het uiteindelijke proefschrift terecht gekomen, wel een waardevolle bijdrage aan het project hebben geleverd.
Part of the most memorable moments during the project were the many conversations, laughs, and cries that were shared in our ‘women-infested’ office: Marleen, Brigitte, Hulya, and again Colette…we never ever had ‘saai’ moments… thanks for the good times and I hope we keep in touch! When Hulya left, a new era arose with the first male moving into the office. Andres, I think you managed very well between the ladies. Thanks for the many humorous moments

en lively conversations. Also thanks to all Ph.D. students, Post-Docs, and staff from Plant Breeding and Entomology for sharing your work, feedback, and interest during the many meetings. Paul, Clemens, René en Niels, het restaurant van de toekomst ligt voor mij in het verleden, maar ik koester goede herinneringen aan onze levendige discussies gedurende de gezellige lunches.
Zonder insecten geen leven op aarde en al helemaal geen Ph.D.-project: Leon en André van de vakgroep Entomologie, bedankt voor het opkweken van de wittevliegen, waar jullie zonder mitsen en maren altijd in slaagden ondanks dat ik vanwege mijn karteringspopulaties gigantische aantallen behoefde; en zonder planten geen wittevliegen: André, Alex en Henk van Unifarm, hartelijk dank dat jullie zulke goede zorg hebben gedragen voor het opkweken en verzorgen van mijn tomatenplanten.

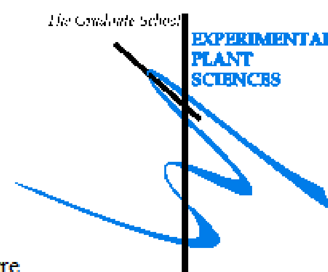
De laatste paragraaf wil ik wijden aan de mensen die mij erg dierbaar zijn en mij op persoonlijk vlak enorm gesteund hebben de afgelopen jaren. Allereerst wil ik mijn ouders bedanken voor de vrijheid die ik van jongs af aan heb gekregen om mijn eigen keuzes te maken en of het nou verstandig of onverstandig was, met vallen en opstaan lieten jullie mij mijn eigen pad bewandelen. Pap en mam, ik ben trots op jullie en prijs mij gelukkig dat jullie mijn ouders zijn! Freek en Nard, mijn 'kleine' broertjes, dit proefschrift is er ook dankzij jullie. Nard, ik wens jou veel succes met het afronden van je studie in Nijmegen en Freek, ik verheug me nu alweer op een bijzonder lekkere kerstmaaltijd! Gelukkig heb ik naast Betty nog een 'rechterhand', Loes, ook jij bent tijdens de promotie met recht mijn paranimf. De afgelopen vijf jaar ben jij ook privé mijn 'rechterhand' geweest en was je altijd bereid mij uit de brand te helpen als dat nodig was. Loes, ik ben erg blij dat jij mij tijdens de promotie op het podium bij wilt staan. Zusje, bedankt!
Ik heb me afgevraagd of ik dit dankwoord met jou zou beginnen, Bram, maar zoals je ziet, krijg jij het laatste woord, wat je vast niet erg vindt…
Zomaar een zin in Brabantse tongval op zaterdagochtend: 'Floortje, nie te veel mauwe en gewoon werken' …typerend voor een scala aan motivatiepogingen die ik het afgelopen jaar op mij afgevuurd kreeg. In de oren van de buitenstaander wellicht wat ongenuanceerd, maar voor mij de perfecte spreekwoordelijke 'schop onder de kont' om weer voor de zoveelste keer een weekend aan het schrijven op te offeren. Lieve Bram, dit proefschrift is er mede door jou; jij betekent de wereld voor mij!

Curriculum vitae

Floor Hubertina Wilhelmina van den Elsen werd geboren op 21 mei 1981 te Venray en groeide op in Meerlo. In 1999 slaagde zij voor het Gymnasium op het Dendron College in Horst. In 2003 begon zij met een Bachelor of Science in de biologie bij Wageningen Universiteit met een specialisatie in de plantenbiologie. Zij specialiseerde zich tijdens haar studie onder andere in entomologie, fytopathologie en virologie. Na haar B.Sc. afgerond te hebben, volgde een Master of Science in de Biologie bij Wageningen Universiteit. Een afstudeervak en stage werden binnen de vakgroep Entomologie voltooid. Tijdens haar eerste afstudeervak werd gekeken naar het effect van 'priming' door *Pseudomonas fluorescens* op directe en indirecte insectenresistentie in kool. Haar stage voltooide ze binnen de fytopathologiegroep van het plantenveredelingsbedrijf Nunhems NL. Een tweede afstudeervak werd volbracht binnen de vakgroep Fytopathologie en uitgevoerd aan La Trobe University, in Bundoora, Australië. Hierbij werd onderzoek gedaan naar een gen van de pathogene schimmel *Sclerotinia sclerotiorum* met een mogelijke rol in necrose-inductie in tabaksplanten. Tussen 2008 en 2013 werkte Floor bij de vakgroepen Plantenveredeling en Entomologie van Wageningen Universiteit aan haar promotieonderzoek, getiteld: 'Resistance Mechanisms against *Bemisia tabaci* in Wild Relatives of Tomato'. Sinds november 2012 is Floor werkzaam als onderzoeker en aspergeveredelaar bij het veredelingsbedrijf Limgroup te Horst.

Education Statement of the Graduate School

Experimental Plant Sciences



Issued to: Floor van den Elsen
Date: 22 October 2013
Group: Plant Breeding and Entomology, Wageningen University & Research Centre

1) Start-up phase	date
► **First presentation of your project**	
PhD-project presentation at PSG Entomology, WUR	Mar 2009
PhD-project presentation at PSG Plant Breeding, WUR	Mar 2009
► **Writing or rewriting a project proposal**	
Resistance mechanisms of whitefly in tomato	Jan 2009
► **Writing a review or book chapter**	
► **MSc courses**	
► **Laboratory use of isotopes**	
Subtotal Start-up Phase	*7.5 credits**

2) Scientific Exposure	date
► **EPS PhD student days**	
EPS PhD student days, University of Leiden	Feb 26, 2009
EPS PhD student days, University of Utrecht	Jun 01, 2010
► **EPS theme symposia**	
EPS Theme 2 symposium 'Interactions between Plants and Biotic Agents', Utrecht University	Jan 22, 2009
EPS Theme 2 symposium 'Interactions between Plants and Biotic Agents', Utrecht University	Jan 15, 2010
► **NWO Lunteren days and other National Platforms**	
NWO-ALW Meetings 'Experimental Plant Sciences', Lunteren	Apr, 2009-2011
Netherlands Annual Ecology Meetings (NERN), Lunteren	Feb 2009-2012
20th-23th Nederlandse Entomologendagen, Ede	Dec 2008-2012
► **Seminars (series), workshops and symposia**	
Workshop Chemical Plant Insect Interactions, Leiden University	Oct 29, 2008
3th National Ecogenomics Day, NERO, Amsterdam	Apr 21, 2010
5th workshop Plant-Insect Interactions, WUR	Nov 11, 2010
6th workshop Plant-Insect Interactions, WUR	Nov 23, 2011
Bi-weekly seminars 'abiotic and biotic stress', PSG Plant Breeding, WUR	2008-2012
Bi-weekly Plant Breeding seminars, PSG Plant Breeding, WUR	2008-2012
Plant-Insect Interactions seminars, PSG Entomology, WUR	2008-2012
Plant Research Day, PSG Plant Breeding, WUR	2008-2012
Ecogen seminars, Resource Ecology Group, WUR	2010-2012
► **Seminar plus**	
► **International symposia and congresses**	
5th Meeting of the IOBC Working Group, Spain	May 12-16, 2009
10th conference of EFPP "IPM 2.0-Towards future-proof crop protection in Europe, Wageningen	Oct 04, 2012
► **Presentations**	
Poster presentation Netherlands Annual Ecology Meeting, Lunteren	Feb 09, 2010
Oral presentation TTI GG Networking Event, Nieuwegein's Business Center Utrecht	Sep 22, 2010
Poster presentation TTI GG Networking Event, Nieuwegein's Business Center Utrecht	Sep 22, 2010
Poster presentation TTI GG Networking Event, Nieuwegein's Business Center Utrecht	Sep 21, 2011
Poster presentation 6th Workshop Plant-Insect Interactions, University of Amsterdam	Nov 23, 2011
Oral presentation 23th Nederlandse Entomologendagen, Ede	Dec 16, 2011
Oral presentation IPM 2.0 – 'Towards future-proof crop protection in Europe', Wageningen	Oct 04, 2012
► **IAB interview**	Feb 18, 2011
► **Excursions**	
Subtotal Scientific Exposure	*22.7 credits**

3) In-Depth Studies	*date*
► **EPS courses or other PhD courses**	
Bioinformatics - A Users Approach	Mar 15-19, 2010
Kyazma; QTL analysis	Mar 29-31, 2010
Systems Biology: Statistical Analysis of ~Omics Data'	Dec 13-17, 2010
► **Journal club**	
PhD discussion group, PSG Entomology, WUR	2008-2012
Literature Discussion Group, PSG Plant Breeding, WUR	2008-2012
► **Individual research training**	
Subtotal In-Depth Studies	*6.9 credits**

4) Personal development	*date*
► **Skill training courses**	
PhD assessment	Oct 2008
Working with Endnote X2	Dec 09, 2009
Techniques for Writing and Presenting Scientific Papers	Feb 16-19, 2010
► **Organisation of PhD students day, course or conference**	
Organized biweekly PhD colloquia for 'Non Host and Insect Resistance' cluster group	Sep 2009-Oct 2010
Member of organizing committee for 'InsectenExperience'	Jan-May 2011
► **Membership of Board, Committee or PhD council**	
Subtotal Personal Development	*4.8 credits**

TOTAL NUMBER OF CREDIT POINTS*	**41,9**

Herewith the Graduate School declares that the PhD candidate has complied with the educational

** A credit represents a normative study load of 28 hours of study.*

Supplementary Tables

Supplementary Table 1 Total GC-MS data for *B. tabaci* resistant (R) and susceptible (S) groups of F_2 genotypes from a cross between *S. pennellii* LA3791 and an Elite Cultivar (EC) of *S. lycopersicum*.

Ret(umin)	Mass (uD)	Annotation	p-value*	q-value**	Hypothesis	Average R-group	SD R-group	Average S-group	SD S-group	R=R>S; S=S>R
6990000	69	Butanoic acid. 2-ethyl-2-methyl-	0.000	0.000	TRUE	5893.47	1177.66	1566.49	151.40	R
8620000	70	Levoglucosenone	0.000	0.000	TRUE	1503.90	379.22	505.24	36.66	R
14500000	53	Unknown	0.000	0.000	TRUE	1975.38	476.61	654.22	57.82	R
15900000	157	Unknown	0.000	0.000	TRUE	1206.56	255.09	446.06	26.59	R
14700000	183	Dodecanoic acid	0.000	0.000	TRUE	11704.46	2899.75	2263.60	1141.32	R
16700000	57	Unknown	0.000	0.000	TRUE	16646.18	4039.70	5657.54	358.35	R
16100000	122	Unknown	0.000	0.000	TRUE	1205.59	280.80	383.80	32.76	R
14500000	86	Unknown	0.000	0.000	TRUE	9482.41	2985.15	2917.92	96.31	R
16900000	84	Unknown	0.000	0.000	TRUE	3199.15	783.05	1183.35	67.15	R
8010000	54	Unknown	0.000	0.000	TRUE	1154.07	338.45	380.64	28.05	R
16400000	126	Unknown	0.000	0.000	TRUE	2625.63	685.81	1081.73	70.11	R
13300000	57	N.a.	0.000	0.000	TRUE	11437.71	3861.14	3148.36	114.65	R
14900000	157	N.a.	0.000	0.000	TRUE	3197.74	833.14	1178.59	96.31	R
14600000	97	N.a.	0.000	0.000	TRUE	8457.25	2711.19	2954.86	90.06	R
13800000	101	N.a.	0.000	0.000	TRUE	1485.12	413.81	614.93	46.84	R
21200000	69	N.a.	0.000	0.000	TRUE	1487.29	514.38	507.83	25.68	R
14700000	200	N.a.	0.000	0.000	TRUE	52110.16	17031.84	15498.46	10792.29	R
14800000	54	N.a.	0.000	0.000	TRUE	1301.36	450.31	389.82	38.99	R
16300000	53	N.a.	0.000	0.000	TRUE	1817.94	959.32	430.20	12.05	R
12200000	88	N.a.	0.000	0.000	TRUE	4932.44	1721.49	1288.50	668.82	R
12500000	109	N.a.	0.000	0.000	TRUE	713.02	225.49	359.11	13.48	R
16500000	126	N.a.	0.000	0.000	TRUE	1133.14	403.30	442.32	17.83	R
17600000	69	N.a.	0.000	0.000	TRUE	1833.18	587.65	772.91	31.25	R
17300000	98	N.a.	0.000	0.000	TRUE	4644.02	1710.31	1732.92	71.24	R
16300000	52	N.a.	0.000	0.000	TRUE	2855.35	1441.11	800.45	17.02	R
17200000	73	N.a.	0.000	0.000	TRUE	3338.08	1556.88	1276.18	41.22	R
14700000	91	N.a.	0.000	0.000	TRUE	7434.40	3195.43	1812.18	152.04	R
14800000	112	N.a.	0.000	0.000	TRUE	9050.72	4422.88	1482.90	109.31	R
17200000	69	N.a.	0.000	0.000	TRUE	695.36	330.89	261.59	9.52	R
16200000	58	N.a.	0.000	0.000	TRUE	1910.31	880.39	645.89	19.43	R
17900000	85	Tetramethyl-2-hexadecene	0.000	0.001	TRUE	52130.43	6217.53	72283.98	7950.38	S
13000000	100	N.a.	0.000	0.001	TRUE	7453.53	3105.68	1873.45	584.91	R
13400000	56	N.a.	0.000	0.001	TRUE	2319.16	1280.88	931.17	35.79	R
13300000	61	N.a.	0.000	0.001	TRUE	1813.69	947.89	583.76	20.61	R
13100000	70	N.a.	0.000	0.001	TRUE	6532.29	4209.06	1102.67	200.81	R
15400000	126	N.a.	0.000	0.001	TRUE	694.40	344.19	284.37	10.35	R
12200000	82	N.a.	0.000	0.002	TRUE	2063.50	916.92	929.77	607.69	R
15300000	56	N.a.	0.001	0.003	TRUE	1787.10	822.04	852.56	23.22	R
12200000	52	N.a.	0.001	0.003	TRUE	2712.78	981.50	1214.62	214.63	R
16400000	123	N.a.	0.001	0.004	TRUE	1232.97	244.74	907.14	35.78	R
18100000	211	N.a.	0.001	0.004	TRUE	2095.80	890.10	1032.00	31.49	R
13200000	71	N.a.	0.001	0.004	TRUE	3403.55	1527.31	1240.90	330.63	R
15800000	168	N.a.	0.001	0.004	TRUE	814.78	426.98	384.61	41.11	R
15200000	53	N.a.	0.001	0.004	TRUE	1459.79	697.23	659.23	31.73	R
15300000	98	N.a.	0.001	0.004	TRUE	11054.44	5873.45	4740.28	93.52	R
22300000	154	Unknown	0.001	0.005	TRUE	384.15	32.46	690.74	223.37	S
16100000	85	N.a.	0.001	0.006	TRUE	2106.86	771.17	1059.02	57.76	R
13200000	98	N.a.	0.001	0.006	TRUE	3117.45	1477.78	1127.03	207.11	R
15200000	115	N.a.	0.002	0.007	TRUE	3860.97	1997.62	1681.29	72.57	R
21800000	227	N.a.	0.002	0.007	TRUE	498.78	205.41	264.42	18.46	R
15100000	57	N.a.	0.002	0.007	TRUE	6246.31	3141.80	2904.71	424.87	R
22000000	54	Unknown	0.002	0.008	TRUE	530.81	54.72	856.27	265.96	S

Supplementary Table 1 continued

Ret(umin)	Mass (uD)	Annotation	p-value*	q-value**	Hypothesis	Average R-group	SD R-group	Average S-group	SD S-group	R=R>S; S=S>R
10300000	57	N.a.	0.002	0.008	TRUE	9488.44	4436.17	3957.55	319.41	R
23000000	55	N.a.	0.004	0.012	TRUE	557.25	115.74	411.68	18.25	R
14300000	69	N.a.	0.004	0.012	TRUE	1568.87	648.18	879.08	24.76	R
17000000	55	N.a.	0.004	0.014	TRUE	613.03	229.50	396.69	36.59	R
11800000	112	N.a.	0.005	0.015	TRUE	587.28	357.81	196.98	7.79	R
22100000	135	Unknown	0.005	0.016	TRUE	1178.48	138.36	1796.43	564.49	S
20500000	72	Unknown	0.006	0.018	TRUE	738.74	91.15	1108.07	315.25	S
11700000	85	N.a.	0.007	0.022	TRUE	4515.69	2813.87	1485.04	20.69	R
13600000	110	a-humulene	0.008	0.023	TRUE	1934.48	458.75	4816.36	2440.31	S
18100000	56	Unknown	0.009	0.026	TRUE	207700.00	17772.19	239407.17	18266.48	S
18300000	151	Unknown	0.010	0.026	TRUE	74614.40	6381.27	86655.54	7258.84	S
16600000	103	N.a.	0.011	0.028	TRUE	2226.87	704.01	1500.03	256.43	R
6010000	87	N.a.	0.011	0.029	TRUE	4347.75	3416.98	1838.61	57.22	R
6490000	79	3.7.7-trimethyl- 1.3.5-cycloheptatriene	0.012	0.031	TRUE	1184.21	159.73	2519.88	1165.85	S
12100000	97	Unknown	0.012	0.032	TRUE	11319.74	1477.75	14035.66	1766.74	S
15500000	97	N.a.	0.014	0.035	TRUE	1534.00	1037.74	681.50	15.09	R
18800000	57	N.a.	0.016	0.039	TRUE	1868.08	304.89	1485.35	121.37	R
14300000	55	N.a.	0.017	0.041	TRUE	2114.21	1091.95	1193.65	42.60	R
13100000	131	N.a.	0.017	0.042	TRUE	7864.07	3492.39	21557.36	11784.36	S
9900000	64	N.a.	0.019	0.046	TRUE	482.61	120.59	917.19	362.51	S
18800000	93	N.a.	0.020	0.046	TRUE	11801.40	2642.01	8605.17	1275.60	R
25400000	81	N.a.	0.021	0.047	TRUE	470.02	170.57	323.09	12.82	R
17800000	77	N.a.	0.024	0.054	FALSE	579367.00	51106.11	661749.67	56847.58	S
21200000	239	N.a.	0.028	0.060	FALSE	2202.04	367.63	1758.91	244.30	R
27500000	183	N.a.	0.028	0.061	FALSE	6910.58	1173.59	9325.00	1850.69	S
15400000	74	N.a.	0.031	0.066	FALSE	3951.15	2461.16	1992.89	62.91	R
13900000	93	N.a.	0.033	0.068	FALSE	395.90	83.06	649.40	239.87	S
12700000	147	N.a.	0.034	0.070	FALSE	932.39	185.65	1626.70	626.53	S
14200000	56	N.a.	0.035	0.071	FALSE	3106.14	1801.23	1691.72	36.60	R
25500000	113	N.a.	0.041	0.081	FALSE	2587.60	388.46	3043.52	298.73	S
11900000	134	N.a.	0.042	0.082	FALSE	2385.48	850.31	4563.45	1960.67	S
30500000	167	N.a.	0.047	0.091	FALSE	22941.56	4862.99	17403.11	4072.05	R
9830000	137	N.a.	0.071	0.129	FALSE	1191.43	539.90	805.30	45.22	R
12100000	65	N.a.	0.074	0.131	FALSE	1087.00	245.66	1690.33	627.95	S
20400000	112	N.a.	0.080	0.139	FALSE	447.90	209.18	307.25	19.92	R
13000000	97	N.a.	0.085	0.145	FALSE	12976.50	5984.58	4772.62	2850.47	R
19700000	55	N.a.	0.089	0.149	FALSE	164808.68	16263.65	149724.94	5469.22	R
17800000	125	N.a.	0.108	0.175	FALSE	14661.13	1712.21	16538.01	1821.82	S
7830000	67	N.a.	0.109	0.175	FALSE	2579.89	1030.28	4981.91	2766.83	S
8480000	91	N.a.	0.112	0.180	FALSE	220.44	20.13	345.34	146.28	S
21400000	61	N.a.	0.116	0.186	FALSE	758.36	120.78	671.28	22.15	R
21000000	169	N.a.	0.118	0.188	FALSE	3490.61	1728.01	2417.08	197.72	R
24100000	149	N.a.	0.121	0.193	FALSE	14820.03	3211.44	11917.91	2138.76	R
13100000	87	N.a.	0.125	0.196	FALSE	128868.36	108983.35	31685.01	10281.66	R
28100000	56	N.a.	0.128	0.201	FALSE	6574.65	1197.21	7945.55	1534.41	S
25600000	99	N.a.	0.129	0.202	FALSE	387.67	175.85	282.99	14.75	R
11900000	77	N.a.	0.131	0.204	FALSE	476.51	151.20	771.39	326.20	S
11800000	83	N.a.	0.135	0.210	FALSE	1453.25	917.18	817.59	134.01	R
20700000	53	N.a.	0.137	0.211	FALSE	19680.53	4830.80	27682.57	9700.85	S
7320000	65	N.a.	0.140	0.213	FALSE	2322.89	470.11	3787.48	1805.91	S
29400000	197	N.a.	0.143	0.218	FALSE	12547.56	2401.16	15343.00	3117.56	S
14100000	122	N.a.	0.190	0.278	FALSE	2324.65	825.15	1766.95	533.71	R
26200000	124	N.a.	0.191	0.278	FALSE	4537.89	1447.38	3702.25	1919.84	R
14200000	119	N.a.	0.223	0.319	FALSE	241.48	21.36	300.95	93.53	S
26500000	126	N.a.	0.222	0.319	FALSE	4254.86	1494.01	3217.80	865.57	R
23100000	83	N.a.	0.228	0.323	FALSE	334.70	15.88	322.48	13.68	R

Supplementary Table 1 continued

Ret(umin)	Mass (uD)	Annotation	p-value*	q-value**	Hypothesis	Average R-group	SD R-group	Average S-group	SD S-group	R=R>S; S=S>R
5760000	79	N.a.	0.233	0.327	FALSE	1194.88	450.56	1911.72	939.07	S
19300000	91	N.a.	0.250	0.347	FALSE	549.97	297.69	341.03	80.65	R
27300000	197	N.a.	0.257	0.355	FALSE	6296.44	1833.80	5141.33	1492.41	R
25200000	57	N.a.	0.285	0.389	FALSE	5694.41	798.11	6084.30	615.92	S
10300000	91	N.a.	0.291	0.396	FALSE	296.57	54.98	495.94	271.44	S
22900000	340	N.a.	0.340	0.445	FALSE	779.04	101.51	729.06	114.68	R
20600000	95	N.a.	0.352	0.458	FALSE	4362.52	1606.01	3446.02	1256.83	R
19000000	152	N.a.	0.359	0.463	FALSE	16186.75	4816.68	19557.04	6283.39	S
28800000	353	N.a.	0.388	0.496	FALSE	7588.19	2898.57	5778.92	1308.46	R
7020000	92	N.a.	0.395	0.502	FALSE	62700.79	26476.47	88034.26	44052.87	S
30100000	224	N.a.	0.437	0.541	FALSE	7253.35	2741.29	5814.57	1731.55	R
20700000	235	N.a.	0.485	0.588	FALSE	17640.91	6318.81	20080.76	6782.89	S
23800000	71	N.a.	0.525	0.633	FALSE	2515.97	660.11	2245.61	303.01	R
14900000	68	N.a.	0.534	0.642	FALSE	304.17	68.29	451.92	271.94	S
18500000	57	N.a.	0.570	0.672	FALSE	928.98	87.91	895.44	44.50	R
20900000	60	N.a.	0.575	0.674	FALSE	14401.07	3446.67	15867.80	4192.93	S
27200000	155	N.a.	0.614	0.707	FALSE	1670.51	520.21	1622.99	678.73	R
7190000	136	N.a.	0.610	0.707	FALSE	1707.75	747.80	1845.11	736.35	S
26000000	57	N.a.	0.611	0.707	FALSE	61923.35	13129.48	66206.81	13584.92	S
30200000	95	N.a.	0.626	0.715	FALSE	4859.86	1728.75	4241.02	1239.78	R
29000000	71	N.a.	0.652	0.742	FALSE	67869.59	21573.24	62002.38	17308.79	R
8440000	71	N.a.	0.656	0.745	FALSE	701.22	181.08	903.56	409.43	S
7380000	67	N.a.	0.673	0.759	FALSE	32317.26	15783.99	35310.34	14196.41	S
6160000	136	N.a.	0.719	0.789	FALSE	713.84	336.22	761.15	292.70	S
18200000	69	N.a.	0.725	0.791	FALSE	7642.02	1424.67	7127.93	1043.29	R
8260000	121	N.a.	0.770	0.836	FALSE	2076.50	841.78	2016.50	811.95	R
7450000	109	N.a.	0.773	0.837	FALSE	599.61	295.34	624.52	248.71	S
18100000	205	N.a.	0.784	0.843	FALSE	1895.61	188.48	1896.63	89.35	S
6910000	62	N.a.	0.806	0.860	FALSE	4934.64	2496.60	4314.19	1744.47	R
26700000	224	N.a.	0.809	0.861	FALSE	31460.03	8105.40	32525.49	6971.15	S
28000000	323	N.a.	0.818	0.866	FALSE	36981.38	12654.65	33724.14	5226.74	R
7420000	61	N.a.	0.823	0.868	FALSE	4728.98	2644.10	4793.72	2129.04	S
24600000	57	N.a.	0.825	0.868	FALSE	4167.49	1538.50	3790.15	798.74	R
29100000	153	N.a.	0.862	0.900	FALSE	25874.02	5350.62	26302.86	4714.38	S
28400000	223	N.a.	0.899	0.927	FALSE	21938.13	4268.92	22663.47	4126.35	S
28700000	175	N.a.	0.971	0.983	FALSE	50087.87	11058.89	50852.04	12689.86	S
5880000	106	N.a.	0.971	0.984	FALSE	1434.21	696.95	1386.55	539.51	R
25300000	224	N.a.	0.970	0.986	FALSE	10798.10	3643.46	10350.97	2239.69	R

[a]N.a. : Not annotated

*p-values were calculated with a Student's t-test (MsExcel v.2010) on a Log10 transformed dataset

**p-values were corrected for multiple testing by Benjamini and Hochberg False Discovery Rate (Benjamini and Hochberg 1995); calculated q-values had a cut-off of 0.05

Supplementary Table 2 Total LC-TOF-MS data for *B. tabaci* resistant (R) and susceptible (S) groups of F_2 genotypes from a cross between *S. pennellii* LA3791 and an Elite Cultivar (EC) of *S. lycopersicum*.

Ret(umin)	Mass(uD)	Annotation	p-value*	q-value**	Hypothesis	Average R-group	SD R-group	Average S-group	SD S-group	R=R>S; S=S>R
29933399	653304993	S3:16 II	0.000	0.000	TRUE	1025.89	683.55	56.37	20.74	R
43307865	693405945	N.a.[a]	0.000	0.000	TRUE	1303.44	355.24	388.11	64.92	R
39102917	1,328E+09	S3:20	0.000	0.000	TRUE	4857.27	1322.11	1226.44	457.84	R
41863918	594322937	N.a.	0.000	0.000	TRUE	232.84	108.13	51.71	3.66	R
28128450	491215698	S3:15 II	0.000	0.000	TRUE	2844.01	1688.40	364.94	41.30	R
42171318	771459412	N.a.	0.000	0.000	TRUE	174.99	34.32	334.26	54.27	S
43200718	207051697	S3:22 IV	0.000	0.000	TRUE	2381.90	829.92	576.73	93.96	R
41738899	723386414	S3:21 IV	0.000	0.000	TRUE	52852.99	21475.16	13226.86	1563.95	R
38886700	579302307	N.a.	0.000	0.000	TRUE	4813.26	1953.96	1060.00	140.05	R
43488365	777472046	N.a.	0.000	0.000	TRUE	551.90	103.81	1060.70	203.05	S
45059250	524246216	N.a.	0.000	0.000	TRUE	409.67	138.57	146.98	4.04	R
44769684	768429443	N.a.	0.000	0.000	TRUE	356.54	131.17	124.59	11.28	R
29933399	653304993	S3:16 I	0.000	0.000	TRUE	3376.23	2060.91	579.63	25.60	R
34049049	101061615	N.a.	0.000	0.000	TRUE	339.99	104.99	109.09	1.70	R
39156483	723430298	N.a.	0.000	0.000	TRUE	3488.59	1182.45	7392.10	1313.92	S
37442734	733397400	N.a.	0.000	0.000	TRUE	136.32	15.18	218.80	36.37	S
49914749	976599182	N.a.	0.000	0.000	TRUE	438.62	82.02	868.26	224.69	S
46973251	759465942	N.a.	0.000	0.000	TRUE	2138.99	275.02	3945.78	858.39	S
40872150	1,404E+09	N.a.	0.000	0.000	TRUE	3210.55	1290.44	679.30	69.44	R
49047985	789511719	N.a.	0.000	0.000	TRUE	3030.65	762.54	6315.59	1657.98	S
49535900	761453491	N.a.	0.000	0.000	TRUE	485.30	131.73	1056.00	279.00	S
45023518	720466370	N.a.	0.000	0.000	TRUE	323.74	73.53	673.20	160.53	S
43506233	1,384E+09	S3:22 V	0.000	0.000	TRUE	10673.10	4542.94	2571.60	671.89	R
36449066	695360779	N.a.	0.000	0.000	TRUE	11876.17	5456.41	2512.98	357.14	R
47369949	946586853	N.a.	0.000	0.000	TRUE	208.85	30.58	404.39	118.79	S
39644402	693387146	N.a.	0.000	0.001	TRUE	11805.16	3349.26	21157.03	3593.96	S
40798801	963596680	N.a.	0.000	0.001	TRUE	496.47	54.51	894.61	240.24	S
39319134	771444031	N.a.	0.000	0.001	TRUE	588.31	137.27	979.43	149.99	S
37063885	495210022	N.a.	0.000	0.001	TRUE	266.61	114.46	105.43	2.98	R
47532585	759462402	N.a.	0.000	0.001	TRUE	569.64	108.10	1073.47	242.22	S
43994133	702453308	N.a.	0.000	0.001	TRUE	133.84	34.26	248.94	56.93	S
50311451	927571594	N.a.	0.000	0.001	TRUE	306.58	47.04	603.86	180.18	S
47080399	735439270	N.a.	0.000	0.001	TRUE	663.97	142.08	1208.11	247.58	S
35294666	887495483	N.a.	0.000	0.001	TRUE	695.92	202.34	1195.12	190.26	S
37460602	884475952	N.a.	0.000	0.001	TRUE	4540.22	1428.67	8216.16	1460.91	S
41881767	862492981	N.a.	0.000	0.001	TRUE	1593.10	574.19	2940.62	589.22	S
46612251	852521851	N.a.	0.000	0.001	TRUE	283.12	72.60	583.92	171.38	S
27660299	630264709	S3:15 I	0.000	0.001	TRUE	2083.85	1063.38	568.96	146.67	R
2894883	566053101	N.a.	0.000	0.001	TRUE	931.78	111.60	1244.87	122.99	S
38091385	739392944	N.a.	0.000	0.001	TRUE	5060.95	1584.21	8394.85	1135.00	S
31486416	1,032E+09	N.a.	0.000	0.001	TRUE	100.28	18.00	175.91	37.87	S
35510868	885486145	N.a.	0.000	0.001	TRUE	1716.09	723.84	3558.85	891.45	S
40168018	999592224	N.a.	0.000	0.002	TRUE	171.00	32.95	310.20	78.14	S
45654301	952577209	N.a.	0.000	0.002	TRUE	694.66	139.36	1186.51	283.75	S
35078449	885486389	N.a.	0.000	0.002	TRUE	1497.38	449.91	2725.34	610.95	S
43055935	1,378E+09	N.a.	0.000	0.002	TRUE	478.08	239.91	161.15	46.20	R
49752102	928537415	N.a.	0.000	0.002	TRUE	64.23	6.58	133.98	58.87	S
2625100	152994522	N.a.	0.000	0.002	TRUE	1005.74	168.45	1388.30	131.45	S
46376282	185155533	N.a.	0.000	0.003	TRUE	365.23	137.50	133.26	31.17	R

Supplementary Table 2 continued

Ret(umin)	Mass(uD)	Annotation	p-value*	q-value**	Hypothesis	Average R-group	SD R-group	Average S-group	SD S-group	R=R>S; S=S>R
41502918	637334167	N.a.	0.001	0.003	TRUE	351.11	83.84	518.46	68.93	S
41629833	721420044	S3:22 I	0.001	0.003	TRUE	17260.22	5216.87	27961.05	4562.17	S
39463902	837458984	N.a.	0.001	0.003	TRUE	300.92	19.46	390.98	56.24	S
48470783	199171021	N.a.	0.001	0.004	TRUE	605.86	262.73	212.56	72.17	R
42748535	1,015E+09	N.a.	0.001	0.004	TRUE	152.05	16.99	252.68	78.08	S
44751835	822494873	N.a.	0.001	0.004	TRUE	264.65	38.30	407.97	87.72	S
39608685	443193756	N.a.	0.001	0.004	TRUE	257.58	49.66	577.09	249.23	S
34283134	681336914	S3:18 IV	0.001	0.004	TRUE	123.89	121.79	32.76	0.93	R
39481766	770442993	N.a.	0.001	0.004	TRUE	1085.49	354.87	1849.57	392.33	S
38073517	513308960	N.a.	0.001	0.005	TRUE	940.14	167.52	1830.93	686.83	S
48290283	989573730	N.a.	0.001	0.005	TRUE	501.95	77.66	771.64	166.30	S
28308933	263115448	N.a.	0.001	0.006	TRUE	1091.30	679.83	306.41	5.44	R
40618317	128961166	S3:21 II	0.001	0.006	TRUE	1131.30	389.76	455.47	121.45	R
32442467	723383484	N.a.	0.001	0.006	TRUE	300.08	50.29	434.04	64.80	S
35655651	741414490	N.a.	0.001	0.006	TRUE	4436.23	1262.22	6711.61	937.07	S
38976002	864505371	N.a.	0.001	0.006	TRUE	56.96	11.35	82.41	14.31	S
37188900	697389160	N.a.	0.002	0.006	TRUE	1412.14	659.07	529.16	55.45	R
46521069	765427368	N.a.	0.002	0.006	TRUE	647.65	397.56	113.45	34.33	R
40673782	855463928	S4:22 I	0.002	0.007	TRUE	137.02	11.62	202.32	50.52	S
38670483	625310669	N.a.	0.002	0.007	TRUE	785.16	160.16	512.59	121.80	R
39860615	786440430	N.a.	0.002	0.007	TRUE	127.67	40.54	195.76	37.15	S
35691368	884476563	N.a.	0.002	0.008	TRUE	100.82	14.63	402.15	327.29	S
45473816	941519714	N.a.	0.003	0.009	TRUE	536.06	39.75	855.05	276.03	S
13581700	727200745	N.a.	0.003	0.010	TRUE	158.01	19.24	282.31	86.11	S
17822384	1,216E+09	N.a.	0.003	0.011	TRUE	2208.59	563.93	5116.67	1912.97	S
46556782	277217712	N.a.	0.003	0.011	TRUE	76.20	11.72	114.25	30.05	S
31720484	1,05E+09	N.a.	0.003	0.012	TRUE	72.60	19.35	158.88	59.39	S
28832567	298048859	N.a.	0.004	0.012	TRUE	138.78	69.84	51.98	2.31	R
36685032	565288147	N.a.	0.004	0.012	TRUE	392.17	253.70	119.70	1.97	R
41304565	979588196	N.a.	0.004	0.012	TRUE	155.16	16.82	201.86	30.69	S
45041382	780415955	S4:24 I	0.004	0.013	TRUE	307.31	288.41	58.83	1.34	R
33110867	566339172	N.a.	0.004	0.013	TRUE	203.78	28.72	270.98	39.25	S
42044399	691400452	S3:22 II	0.004	0.014	TRUE	1075.74	506.13	455.16	92.58	R
50347168	651379883	N.a.	0.005	0.015	TRUE	327.06	103.95	510.74	102.86	S
47947166	199171356	N.a.	0.005	0.016	TRUE	180.96	76.45	89.89	19.56	R
2299817	439085205	N.a.	0.005	0.017	TRUE	22174.23	3507.95	28233.27	2837.44	S
46792751	806506531	N.a.	0.005	0.017	TRUE	239.54	22.49	388.34	158.10	S
2335550	391090851	N.a.	0.005	0.017	TRUE	1073.97	139.42	1348.40	163.10	S
46124352	824483337	N.a.	0.005	0.017	TRUE	737.88	76.92	1027.25	231.96	S
40023251	879498474	N.a.	0.006	0.017	TRUE	499.60	86.39	1046.38	484.41	S
14771833	595166443	N.a.	0.006	0.018	TRUE	178.93	21.76	324.31	135.92	S
18056450	1,084E+09	N.a.	0.007	0.019	TRUE	178.84	139.91	805.05	608.13	S
15079233	965522339	N.a.	0.007	0.020	TRUE	98.31	19.02	253.91	124.77	S
42964748	590329590	N.a.	0.007	0.022	TRUE	579.75	127.13	414.58	77.45	R
2571517	346057709	N.a.	0.008	0.022	TRUE	302.44	31.49	350.16	23.59	S
23742983	653319031	N.a.	0.008	0.023	TRUE	181.27	82.29	333.45	116.95	S
32912498	477283264	N.a.	0.008	0.023	TRUE	415.11	91.27	595.13	108.80	S
45384518	860514160	N.a.	0.009	0.024	TRUE	121.06	11.76	219.18	108.41	S

Supplementary Table 2 continued

Ret(umin)	Mass(uD)	Annotation	p-value*	q-value**	Hypothesis	Average R-group	SD R-group	Average S-group	SD S-group	R=R>S; S=S>R
30764433	474264679	N.a.	0.009	0.024	TRUE	275.90	46.50	377.68	65.93	S
46322701	485277161	N.a.	0.009	0.024	TRUE	717.56	161.60	1001.68	181.46	S
41520782	819482544	N.a.	0.009	0.025	TRUE	413.78	56.87	508.52	61.58	S
33110867	425169037	N.a.	0.010	0.026	TRUE	131.98	63.81	57.71	5.38	R
39590816	774453613	N.a.	0.010	0.028	TRUE	386.06	16.03	463.53	65.04	S
13671000	610150024	N.a.	0.012	0.031	TRUE	7615.09	2052.64	13490.08	4549.16	S
25782017	495209778	S3:14 I	0.012	0.032	TRUE	979.95	565.66	409.52	72.73	R
48867500	762474060	N.a.	0.012	0.032	TRUE	61.30	3.32	89.58	28.64	S
35348232	682340027	S3:18 II	0.013	0.032	TRUE	94.59	54.04	45.21	1.33	R
29030916	373094849	N.a.	0.014	0.035	TRUE	208.60	83.63	115.14	17.69	R
38706200	1,001E+09	N.a.	0.014	0.035	TRUE	212.66	10.37	247.60	27.42	S
48669132	681410522	N.a.	0.015	0.038	TRUE	364.36	35.21	665.05	326.37	S
26178717	626276123	S3:14 II	0.015	0.038	TRUE	569.77	525.54	123.83	34.78	R
26450417	579267700	S3:14 III	0.015	0.038	TRUE	2814.01	2211.42	571.38	64.19	R
47713085	769508545	N.a.	0.017	0.041	TRUE	2649.74	645.36	3445.79	451.40	S
16431999	888461792	N.a.	0.018	0.042	TRUE	123.26	4.12	133.52	7.94	S
49462551	770499451	N.a.	0.018	0.043	TRUE	134.44	4.75	204.57	75.73	S
33723782	855496521	N.a.	0.018	0.044	TRUE	158.05	4.42	180.47	19.94	S
40511150	889520752	N.a.	0.019	0.044	TRUE	159.74	12.09	197.40	37.70	S
17263033	1,49E+09	N.a.	0.020	0.046	TRUE	83.01	27.72	127.53	33.66	S
19609467	453249542	N.a.	0.020	0.047	TRUE	677.27	237.47	445.35	284.08	R
11631984	1,217E+09	N.a.	0.021	0.047	TRUE	182.46	11.97	352.22	181.69	S
10999300	402151031	N.a.	0.021	0.047	TRUE	113.71	7.38	197.89	89.77	S
49752102	955558716	N.a.	0.021	0.047	TRUE	4580.66	978.59	6202.61	900.84	S
39860615	721432007	N.a.	0.021	0.047	TRUE	1191.27	297.81	2440.39	1261.37	S
18346001	919492737	N.a.	0.021	0.047	TRUE	431.79	209.09	939.78	460.66	S
36088085	857438538	N.a.	0.022	0.050	FALSE	138.40	9.02	231.22	94.13	S
36268566	749372986	N.a.	0.024	0.053	FALSE	350.82	39.21	632.35	281.48	S
44571335	1,392E+09	S3:23 III	0.024	0.053	FALSE	570.93	417.42	159.57	39.03	R
36901249	650350891	S3:19	0.027	0.059	FALSE	554.50	449.78	154.24	18.73	R
17100401	1,349E+09	N.a.	0.028	0.061	FALSE	109.94	15.64	180.60	61.18	S
2317683	612150452	N.a.	0.028	0.061	FALSE	223.15	49.07	334.95	86.34	S
14898750	285041656	N.a.	0.030	0.063	FALSE	46.01	2.46	57.05	11.42	S
35907585	883472046	N.a.	0.030	0.063	FALSE	471.01	159.14	1802.18	1374.95	S
20313583	555231079	N.a.	0.030	0.064	FALSE	331.55	242.40	91.34	2.28	R
15386650	1,067E+09	N.a.	0.030	0.064	FALSE	1051.44	280.27	679.87	302.15	R
21955900	867389709	N.a.	0.033	0.069	FALSE	173.61	19.28	320.20	168.35	S
11342417	388171021	N.a.	0.034	0.070	FALSE	291.57	75.15	430.29	101.65	S
30927067	562317627	N.a.	0.034	0.070	FALSE	542.94	99.69	730.58	148.66	S
9555333	904249268	N.a.	0.035	0.071	FALSE	90.36	9.01	109.55	16.96	S
29590267	899468567	N.a.	0.036	0.072	FALSE	56.39	1.18	83.86	29.76	S
12407534	694356323	N.a.	0.035	0.072	FALSE	941.27	1104.88	254.15	240.21	R
43777916	883542603	N.a.	0.037	0.074	FALSE	324.21	70.35	497.04	166.64	S
45221882	967566467	N.a.	0.040	0.080	FALSE	116.23	29.18	272.70	189.93	S
44047718	977609619	S3:23 II	0.040	0.080	FALSE	255.81	95.11	339.07	96.10	S
21180349	834389893	N.a.	0.040	0.080	FALSE	124.68	17.51	250.01	142.74	S
15241867	1,097E+09	N.a.	0.042	0.083	FALSE	7682.21	2702.37	13344.18	5429.39	S
22281166	1,156E+09	N.a.	0.043	0.083	FALSE	588.65	245.68	306.18	142.39	R
10205883	191056030	N.a.	0.047	0.091	FALSE	14759.68	7945.72	6510.86	1334.35	R
30060316	897454041	N.a.	0.050	0.095	FALSE	108.02	2.17	167.00	68.53	S

Supplementary Table 2 continued

Ret(umin)	Mass(uD)	Annotation	p-value*	q-value**	Hypothesis	Average R-group	SD R-group	Average S-group	SD S-group	R=R>S; S=S>R
31125416	916466370	N.a.	0.052	0.098	FALSE	105.01	2.06	133.22	31.46	S
30024584	929481201	N.a.	0.052	0.099	FALSE	103.96	10.49	227.12	140.33	S
34356468	754411072	N.a.	0.052	0.099	FALSE	137.08	3.32	187.17	57.35	S
41016918	495209564	S4:22 II	0.054	0.102	FALSE	121.48	72.86	58.50	6.89	R
16306984	293088806	N.a.	0.054	0.102	FALSE	96.77	2.24	121.78	33.10	S
23941351	611257141	N.a.	0.055	0.103	FALSE	167.50	77.82	106.92	6.05	R
32406750	737412903	N.a.	0.056	0.105	FALSE	205.70	8.16	327.27	138.86	S
22677883	849378662	N.a.	0.057	0.105	FALSE	140.41	14.08	282.21	174.29	S
22985283	672335571	N.a.	0.059	0.108	FALSE	250.92	40.47	543.51	349.39	S
11487200	191056992	N.a.	0.062	0.113	FALSE	678.68	445.21	262.63	72.08	R
33075150	735398315	N.a.	0.063	0.116	FALSE	187.94	2.73	343.82	191.31	S
23941351	1,196E+09	N.a.	0.067	0.122	FALSE	320.67	212.15	148.39	47.71	R
2353400	592183594	N.a.	0.070	0.128	FALSE	196.97	35.65	230.62	26.63	S
15206150	898483643	N.a.	0.072	0.130	FALSE	1109.97	408.61	783.65	313.29	R
39319134	853448303	N.a.	0.072	0.130	FALSE	91.01	3.75	102.31	12.93	S
19736383	449147278	N.a.	0.074	0.131	FALSE	77.91	3.79	88.84	12.54	S
43073799	968569336	N.a.	0.073	0.131	FALSE	53.95	0.97	76.28	33.14	S
17875950	1,344E+09	N.a.	0.075	0.133	FALSE	58.96	2.46	67.73	10.76	S
22840517	653319641	N.a.	0.077	0.135	FALSE	108.21	34.82	298.20	231.33	S
21124866	435240295	N.a.	0.080	0.138	FALSE	265.99	126.21	369.56	139.14	S
23905634	670318665	N.a.	0.079	0.138	FALSE	213.93	4.25	539.18	412.60	S
15224017	1,154E+09	N.a.	0.079	0.138	FALSE	133.71	19.98	188.24	57.12	S
37153183	751428589	S3:23 I	0.082	0.140	FALSE	32.56	1.14	51.17	22.59	S
39824883	737370789	S4:21 II	0.082	0.141	FALSE	1165.56	1486.80	129.41	107.82	R
28941616	681299927	S4:17 III	0.084	0.143	FALSE	349.90	423.70	45.33	17.89	R
20890800	661309204	N.a.	0.085	0.145	FALSE	55.23	8.30	101.52	57.49	S
21757549	713283691	N.a.	0.086	0.146	FALSE	210.76	15.04	373.19	214.17	S
16469616	1,136E+09	N.a.	0.088	0.148	FALSE	2428.69	506.29	2054.53	867.97	R
41756748	891545105	N.a.	0.091	0.153	FALSE	52.64	1.17	56.84	5.66	S
32226250	487292969	N.a.	0.092	0.154	FALSE	105.25	14.35	94.66	1.35	R
44085335	767400757	S4:23 II	0.094	0.156	FALSE	300.57	228.32	106.54	24.63	R
31341633	726381775	N.a.	0.098	0.162	FALSE	177.56	2.84	217.83	55.44	S
30655367	727393188	N.a.	0.098	0.162	FALSE	86.72	3.58	106.79	25.11	S
34536968	682341980	S3:18 I	0.101	0.166	FALSE	87.06	28.76	66.54	16.39	R
16955633	944488586	N.a.	0.103	0.168	FALSE	87.46	10.69	168.83	109.63	S
24734766	651303467	N.a.	0.104	0.170	FALSE	81.46	18.79	177.74	127.36	S
26793550	653269592	S4:15	0.108	0.175	FALSE	4119.03	2932.53	2105.12	27.53	R
19085850	163077225	N.a.	0.124	0.197	FALSE	188.92	8.77	182.39	2.28	R
17641884	1,21E+09	N.a.	0.128	0.201	FALSE	2076.78	1326.19	3388.57	1810.92	S
43651001	891542175	N.a.	0.136	0.209	FALSE	129.38	28.11	149.07	25.92	S
12570167	338155090	N.a.	0.137	0.210	FALSE	114.74	40.97	87.84	42.78	R
15079233	1,076E+09	N.a.	0.144	0.218	FALSE	2641.11	1238.08	2278.57	1564.64	R
34699600	723366333	N.a.	0.147	0.223	FALSE	637.55	272.84	404.77	64.30	R
22082817	825355469	N.a.	0.151	0.226	FALSE	185.84	2.31	226.11	58.99	S
32279835	695314941	S4:18	0.151	0.227	FALSE	665.25	713.61	138.85	51.67	R
18707001	1,199E+09	N.a.	0.154	0.230	FALSE	1104.35	578.58	593.66	179.97	R
28525150	580270142	N.a.	0.162	0.241	FALSE	138.93	93.04	66.93	15.98	R
44914467	171136581	N.a.	0.164	0.244	FALSE	98.74	40.79	69.68	17.89	R
30240801	682299805	S4:17 I	0.166	0.245	FALSE	2083.70	1208.78	1343.93	63.01	R
42460884	692398010	S3:22 III	0.168	0.248	FALSE	2633.91	730.70	1946.15	435.59	R

Supplementary Table 2 continued

Ret(umin)	Mass(uD)	Annotation	p-value*	q-value**	Hypothesis	Average R-group	SD R-group	Average S-group	SD S-group	R=R>S; S=S>R
33616634	476280121	N.a.	0.169	0.248	FALSE	429.37	126.81	485.98	84.75	S
42694950	425168304	N.a.	0.170	0.249	FALSE	289.48	85.97	241.68	72.69	R
28652067	496212891	S4:16 I	0.174	0.254	FALSE	612.98	436.46	276.49	79.23	R
21828983	328223297	N.a.	0.213	0.307	FALSE	80.23	29.74	88.81	20.66	S
18201233	1,112E+09	N.a.	0.213	0.308	FALSE	4961.60	2896.18	6885.22	2803.62	S
41502918	621314148	S4:22 III	0.224	0.318	FALSE	490.39	287.01	299.90	82.05	R
38254017	999556580	N.a.	0.223	0.319	FALSE	2033.01	132.32	2245.01	303.06	S
2030033	632730591	N.a.	0.226	0.321	FALSE	801.23	121.40	874.50	95.25	S
42026550	293213165	N.a.	0.229	0.323	FALSE	949.58	28.45	1080.87	208.03	S
15404516	1,156E+09	N.a.	0.231	0.325	FALSE	255.76	72.00	353.80	133.37	S
36250717	515323120	S4:20 I	0.245	0.342	FALSE	87.56	13.03	97.72	13.99	S
45600735	780417053	S4:24 II	0.250	0.348	FALSE	25749.39	11797.06	18280.71	2950.27	R
29951250	532286316	N.a.	0.260	0.359	FALSE	1162.17	313.03	935.35	225.89	R
43182850	677378479	N.a.	0.279	0.384	FALSE	310.52	106.65	255.52	90.15	R
14357250	1,049E+09	N.a.	0.281	0.385	FALSE	468.51	148.10	538.71	116.18	S
17985016	1,066E+09	N.a.	0.285	0.389	FALSE	7090.66	4642.15	9836.36	4569.15	S
30637516	977501465	N.a.	0.293	0.396	FALSE	604.59	313.59	878.54	452.56	S
15349033	494103302	N.a.	0.292	0.396	FALSE	278.02	313.91	106.94	69.83	R
15061383	1,096E+09	N.a.	0.295	0.398	FALSE	1768.27	580.19	1482.16	546.12	R
17659750	1,051E+09	N.a.	0.300	0.402	FALSE	554.04	110.49	722.65	226.15	S
18111933	1,11E+09	N.a.	0.300	0.402	FALSE	1044.08	654.87	1465.61	716.87	S
43073799	887539551	N.a.	0.307	0.410	FALSE	1883.49	300.50	1981.44	141.61	S
20908649	1,197E+09	N.a.	0.311	0.414	FALSE	97.40	38.87	75.43	24.54	R
2030033	112984413	N.a.	0.329	0.437	FALSE	370.72	39.60	347.49	41.24	R
42207050	291198364	N.a.	0.335	0.443	FALSE	230.92	28.80	262.18	56.12	S
17153984	1,215E+09	N.a.	0.337	0.444	FALSE	585.59	93.23	681.15	152.19	S
37153183	723346741	S4:20 II	0.339	0.446	FALSE	77.67	49.57	53.23	32.00	R
2480317	275020050	N.a.	0.342	0.447	FALSE	2799.87	720.41	2402.48	600.86	R
30474884	673269165	S4:17 II	0.356	0.463	FALSE	1508.32	1071.30	797.40	429.85	R
31107567	397136780	N.a.	0.358	0.464	FALSE	271.21	78.29	224.02	60.94	R
29536684	1,109E+09	N.a.	0.362	0.466	FALSE	80.17	29.11	110.65	56.50	S
49157051	1,463E+09	N.a.	0.380	0.488	FALSE	352.55	93.82	274.23	53.83	R
40402100	677377441	S3:21 I	0.396	0.501	FALSE	119819.44	92910.50	85374.93	47094.00	R
34445766	513308777	N.a.	0.395	0.503	FALSE	237.58	53.68	260.42	50.59	S
12895433	149046204	N.a.	0.394	0.504	FALSE	86.24	2.11	88.22	4.21	S
37658951	663363281	N.a.	0.408	0.514	FALSE	196.64	16.72	187.94	11.50	R
49210617	928582458	N.a.	0.412	0.518	FALSE	156.63	11.04	161.72	11.23	S
18816050	1,08E+09	N.a.	0.427	0.535	FALSE	271.78	92.43	285.69	64.25	S
16685833	883491577	N.a.	0.430	0.535	FALSE	234.87	184.33	334.39	234.87	S
14880883	897472290	N.a.	0.427	0.536	FALSE	563.47	241.77	675.00	616.60	S
13635283	1,096E+09	N.a.	0.430	0.536	FALSE	155.24	68.00	178.45	148.34	S
16792984	1,342E+09	N.a.	0.434	0.538	FALSE	703.12	258.39	592.89	250.90	R
12498734	625141907	N.a.	0.445	0.549	FALSE	193.49	4.46	190.44	12.65	R
18851767	447224396	N.a.	0.447	0.550	FALSE	7026.86	3028.11	7994.19	2165.41	S
36052368	649344604	N.a.	0.464	0.570	FALSE	891.06	363.02	928.44	628.75	S
2210533	341106995	N.a.	0.471	0.575	FALSE	18574.28	3276.21	20631.39	3976.82	S
14194633	1,113E+09	N.a.	0.470	0.575	FALSE	379.62	143.24	444.13	135.16	S
16955633	1,08E+09	N.a.	0.478	0.582	FALSE	59770.94	12843.82	64481.29	11981.47	S
17497116	1,034E+09	N.a.	0.523	0.633	FALSE	683.09	488.13	999.03	667.54	S
15692166	1,215E+09	N.a.	0.543	0.652	FALSE	207.70	85.64	215.87	66.42	S
35782566	608337708	N.a.	0.550	0.658	FALSE	178.04	84.92	224.04	206.75	S
17804516	1,053E+09	N.a.	0.555	0.663	FALSE	145.99	34.54	171.38	50.11	S

Supplementary Table 2 continued

Ret(umin)	Mass(uD)	Annotation	p-value*	q-value**	Hypothesis	Average R-group	SD R-group	Average S-group	SD S-group	R=R>S; S=S>R
33759499	560317871	N.a.	0.564	0.669	FALSE	2750.78	626.17	2419.83	377.92	R
40745232	723383606	S3:21 V	0.566	0.670	FALSE	10881.37	6898.40	5361.91	2588.19	R
16757267	1,138E+09	N.a.	0.570	0.670	FALSE	10802.71	2782.06	9655.86	2104.35	R
16919901	1,032E+09	N.a.	0.563	0.670	FALSE	609.04	494.42	467.42	434.71	R
20529800	496264526	N.a.	0.570	0.673	FALSE	150.10	61.46	160.28	60.19	S
16576784	1,345E+09	N.a.	0.579	0.677	FALSE	196.55	44.33	214.79	51.43	S
16937767	1,034E+09	N.a.	0.595	0.694	FALSE	656.92	202.69	811.57	466.36	S
30367716	676363708	N.a.	0.604	0.702	FALSE	10437.40	2351.27	9243.79	1844.32	R
13310000	191055618	N.a.	0.617	0.708	FALSE	132.71	40.28	118.03	24.33	R
41340282	411151917	S3:21 III	0.614	0.708	FALSE	415.00	289.17	261.07	33.33	R
12750667	741187805	N.a.	0.623	0.713	FALSE	19235.33	7682.36	19609.18	5198.21	S
22606449	1,067E+09	N.a.	0.659	0.746	FALSE	117.25	64.12	83.81	26.02	R
11883917	431193604	N.a.	0.667	0.754	FALSE	570.16	113.71	532.12	104.02	R
1811917	403919312	N.a.	0.676	0.759	FALSE	490.59	154.92	440.50	106.22	R
7696816	371063110	N.a.	0.676	0.760	FALSE	757.52	433.95	583.70	182.33	R
20115232	765266846	N.a.	0.684	0.765	FALSE	102.14	21.07	95.11	10.15	R
2589383	176935120	N.a.	0.695	0.774	FALSE	1458.54	188.72	1499.19	181.13	S
13851500	1,05E+09	N.a.	0.695	0.776	FALSE	90.95	17.27	113.07	48.96	S
1776200	387940826	N.a.	0.704	0.776	FALSE	4612.28	395.75	4691.96	354.56	S
3202300	111008102	N.a.	0.704	0.778	FALSE	232.59	75.92	211.76	72.29	R
10549000	529157532	N.a.	0.703	0.779	FALSE	947.73	308.66	821.69	166.77	R
17153984	930511047	N.a.	0.702	0.779	FALSE	501.54	120.24	584.20	248.36	S
15061383	736429504	N.a.	0.709	0.779	FALSE	130.56	25.52	138.05	32.60	S
2444600	209028931	N.a.	0.723	0.791	FALSE	3536.19	992.65	3718.02	1019.61	S
46991100	592266296	N.a.	0.762	0.829	FALSE	356.95	103.44	384.15	117.27	S
48091934	822477173	N.a.	0.782	0.843	FALSE	877.70	207.43	908.70	194.97	S
17263033	1,034E+09	N.a.	0.780	0.843	FALSE	12110.01	1875.06	12430.23	1714.54	S
18399584	1,331E+09	N.a.	0.799	0.854	FALSE	296.74	138.36	349.56	167.52	S
16576784	1,032E+09	N.a.	0.798	0.856	FALSE	14900.37	3158.77	16092.83	4095.68	S
18597933	469229797	N.a.	0.809	0.860	FALSE	358.61	111.05	406.37	159.29	S
2137183	145060883	N.a.	0.819	0.866	FALSE	425.81	71.17	451.89	120.58	S
14682533	801450256	N.a.	0.848	0.891	FALSE	255.65	109.70	300.78	164.39	S
30457016	726364258	N.a.	0.857	0.897	FALSE	186.62	73.47	180.19	53.77	R
16650116	1,004E+09	N.a.	0.869	0.906	FALSE	121.78	47.12	151.02	90.88	S
14031983	191024673	N.a.	0.886	0.917	FALSE	97.04	17.42	93.51	10.44	R
9898467	181051102	N.a.	0.886	0.919	FALSE	110.42	40.52	97.90	24.87	R
38904549	739380737	S4:21 I	0.884	0.920	FALSE	135.51	26.55	136.75	23.22	S
44716118	780415894	N.a.	0.897	0.926	FALSE	259.24	99.55	230.11	48.82	R
38434517	510229065	N.a.	0.906	0.931	FALSE	141.20	59.55	141.26	53.04	S
43020218	766395569	S4:23 I	0.919	0.942	FALSE	3647.82	1231.46	4869.70	3508.82	S
19230618	1,051E+09	N.a.	0.928	0.950	FALSE	74.53	31.05	68.06	14.53	R
42946884	888544617	N.a.	0.956	0.975	FALSE	1933.96	399.12	1772.71	123.89	R
30096033	1,064E+09	N.a.	0.980	0.985	FALSE	666.02	496.38	459.09	215.18	R
13653133	592234741	N.a.	0.980	0.986	FALSE	141.53	30.50	152.03	40.15	S
11957250	337093628	N.a.	0.977	0.986	FALSE	126.36	18.06	127.40	20.49	S
19266333	769403076	N.a.	0.970	0.988	FALSE	673.34	526.86	604.59	363.77	R
27045483	647329468	N.a.	0.988	0.990	FALSE	129.00	35.87	130.43	36.90	S
17046816	959509949	N.a.	0.992	0.992	FALSE	77.96	18.78	79.65	22.95	S

[a]N.a. : Not annotated

*p-values were calculated with a Student's t-test (MsExcel v.2010) on a Log10 transformed dataset

**p-values were corrected for multiple testing by Benjamini and Hochberg False Discovery Rate (Benjamini and Hochberg 1995); calculated q-values had a cut-off of 0.05

Colophon

The research of this thesis was funded by:

Technical Top Institute of Green Genetics (TTI-GG; Resistance mechanisms against whitefly in tomato project: 1C005RP)

Monsanto Vegetable Seeds (Bergschenhoek, The Netherlands)

Nunhems NL (Nunhem, the Netherlands)

Wageningen University and Research Centre

Printed by: Ipskamp Drukkers BV Amsterdam, The Netherlands

Layout and Cover design:

Betty Henken (cover pictures) and Floor van den Elsen