Host plant resistance of tomato plants to western flower thrips

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Chapter 3

Variation in performance and genetics of Dutch western flower thrips populations

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Abstract

Invasion of pests may lead to rapid local adaptation and the development of biotypes specialized to different hosts. In this study we investigated western flower thrips (WFT) *Frankliniella occidentalis*, an invasive pest in Europe. Variation in performance and genetics of thrips from different commercially grown glass house crops within the Dutch Westland and a lab culture kept on chrysanthemum were compared. Genetic barcoding was applied for identification of potential WFT cryptic species in the Netherlands and revealed that all WFT populations belonged to the "glasshouse" strain reported from California. Our study did not indicate the existence of different WFT cryptic species in the Netherlands. Feeding and reproduction parameters in leaf disc and whole plant bioassays were scored. We detected significant differences in thrips feeding and reproduction among host-plants and thrips populations. Host plants differed in average thrips feeding while different thrips populations had similar feeding patterns across host plants. In contrast, the pattern of reproductive success across plant species depended strongly on the origin of the thrips population. The thrips lab culture on chrysanthemum obtained the highest levels of reproduction on the host on which this lab culture was maintained. Differences among the other thrips populations were relatively small. AFLPs were used to study genetic differences between WFT populations. AFLP analyses confirmed that the lab population was also genetically the most different from the other populations. Together the AFLP analyses and the interaction between host-plant and thrips origin in thrips reproduction demonstrate the evolution of a lab biotype specialized to a particular host. This finding has potential relevance for future crop control and breeding programs.

Introduction

Over the last few decades, polyphagous insects have spread worldwide due to a massive increase in the international movement of plant material. This has led to the development of key insect pests in agricultural and horticultural crops. Such an invasion has negative economic and ecological impacts (Sakai et al., 2001). In addition it can exert novel selective pressure on the introduced pest species providing favorable conditions for rapid evolution of the invader possibly leading to local adaptation and formation of biotypes (Suarez & Tsutsui, 2008). Biotypes are defined as insect populations which are morphologically similar, but with different heritable characteristics from other members of the species (Diehl & Bush, 1984). Biotypes have at length been described in whiteflies and aphids (Delatte et al., 2009). One of the characters related to biotypic variation is host plant use (Dolatti et al., 2005; Hebert et al., 2006). Genetic variation for host plant use is the basis for the evolution of better adapted, more aggressive, biotypes, which may rise above the defensive properties of previously resistant plants (Menken & Raijmann, 1996).

Western flower thrips (WFT), *Frankliniella occidentalis*, is one of the most serious agricultural pests worldwide (Jensen, 2000). It originates from the western United States from where it has spread all over the world through international trade in vegetables, fruits and ornamentals (Kirk & Terry, 2003). WFT has first been reported in the Westland of The Netherlands in 1983 (Tommasini & Maini, 1995). Thrips have many traits that predispose them to be successful invaders: polyphagy, small size, affinity for enclosed spaces, short generation time, and a combination of asexual and sexual reproduction leading to a high reproductive potential (Morse & Hoddle, 2006). Thrips have piercing-sucking mouthparts (Hunter & Ullman, 1989) and feeding on actively growing tissue leads to distortion, reduction in plant growth and eventually yield loss. Feeding on expanded tissue results in the characteristic silver leaf scars, which affect product appearance and reduce market quality (de Jager et al., 1995a). In addition, *F. occidentalis* is the primary vector of tospoviruses of which the tomato spotted wilt virus is the economically most important one (Maris et al., 2003).

Control of WFT mainly depends on the use of pesticides. Excessive use of pesticides has lead to problems with pesticide resistance, human health risk, toxicity to beneficial organisms and environmental contamination. Therefore, multiple tactics in the framework of an integrated pest management (IPM) programmer are necessary. One important strategy of IPM is the use of

host plant resistance. However, newly formed biotypes may break through the resistance genes in insect-resistant cultivars (Smith, 2005). For this reason the study of WFT biotypes should be included in breeding programs for thrips resistance.

Host plant resistance to WFT varies within and among different host species such as pepper (Maris et al., 2003), tomato (Kumar et al., 1995; Mirnezhad et al., 2010), chrysanthemum (de Jager et al., 1995a; Leiss et al., 2009a), rose (Gaum et al., 1994) and a hybrid of the wild plant *Jacobaea* (Leiss et al., 2009b). Biotypic variation of reproductive performance of WFT populations, collected worldwide from different hosts and grown on susceptible and resistant cucumber cultivars has been reported by de Kogel, et al. (1997). However, the thrips population which was collected on cucumbers, used as hosts for this experiment, did not perform better on this host in comparison to the other thrips populations.

Variation in host plant use between populations collected from different host species within a geographical area can reveal patterns of local adaptation (Via, 1989). We, therefore, collected thrips from different hosts within the Dutch Westland, the center of the Dutch vegetable and ornamental production, to study their variation in silver damage and reproduction to reveal adaptation in host plant use. Further evidence for the evolution of biotypes can come from molecular analysis. Brunner et al. (2004) developed six polymorphic microsatellite markers for molecular identification of thrips species including WFT. Using these markers Brunner & Frey (2010) revealed the existence of two WFT habitat specific phylogenetic lines. Also Fang et al. (2005) demonstrated genetic variation between and within populations of WFT using amplified fragment length polymorphism (AFLP). Therefore, besides comparing thrips performance we also compared the genetic variation of the populations collected to indicate possible formation of biotypes using AFLP. Recently, Rugman-Jones et al. (2010) reported the existence of two cryptic species of WFT in California. We performed mitochondrial COI barcoding on the thrips populations collected to investigate the possible presence of cryptic species of WFT in The Netherlands.

We specifically asked the following questions:

- Do cryptic species of WFT occur in the Netherlands?
- Do WFT populations in the Netherlands genetically differ from each other?
- Do WFT populations collected from different hosts differ in their feeding behavior and reproduction?
- If so, do WFT populations prefer hosts where they were collected on?
- Do WFT populations reproduce better on hosts where they were collected on?

Materials and methods

Insect collections

WFT were collected from 3 crops in 2008. These included the commercially grown glasshouse crops lettuce, *Lactuca sativa* (var. Fatima), and chrysanthemum, *Dendranthema grandiflora* (var. unknown) the Dutch Westland and the field crop leek, *Allium porrum* (var. unknown) in Wageningen. WFT collected from our continuous mass-rearing on flowering chrysanthemum, *D. grandiflora* (var. Euro Sunny), deriving from Naaldwijk, was used as a lab culture population. WFT had regenerated about 100 times during five years of cultivation on this plant. The different thrips populations were reared in 0.5 liter glass jars on bean pods, *Phaseolus vulgaris* (var. Prelude) in a climate chamber (L16: D8, 20 °C, 70% RH & 11.36 µmol m^{-2} s^{-1} light intensity), as described by Outchkourov et al. (2004), to diminish possible maternal effects for about 5 generations.

Genotyping

AFLP. To study genetic differences between WFT populations AFLP was used. DNA was isolated using the Dneasy blood & tissue kit (Qiagen) following the manufacturer's protocol with some minor adjustments. Each sample contained 20 randomly collected adult females per population. Bulk extraction was chosen to obtain sufficient genomic DNA for AFLP fingerprinting (Vos et al., 1995). The DNA isolation was modified as follows: Thrips were grinded with a plastic mortar in 35µl ATL, to which 145 µl ATL was added after grinding. The solution was incubated at 56°C overnight. Elution took place in 2 x 60 µl AE Buffer. The extracted DNA was quantified using a nanodrop ND-1000 spectrophotometer (Thermo Scientific).

The level of polymorphism between populations was analyzed with AFLP to see if a unique pattern could be detected for each population. Two bulk DNA extractions were performed for each thrips population. Thus per population 40 adult female thrips were included in the AFLP analysis. The protocol applied was based on the AFLP plant mapping protocol (Applied Biosystems) but adjusted and optimized for thrips. Per sample 500 ng genomic DNA was used for AFLP analysis. The genomic DNA was double digested with *EcoR* I and *Mse* I at 37°C for 16 hours. The digestion mixture contained 500 ng genomic DNA, 5U EcoRI, 5U *Mse* I, 0,01 mg BSA, and 4 µl 10X restriction buffer (New England Biolabs react 4) in a final volume of 40 µl adjusted with distilled H_20. After the digestion step 10 μl ligation mix was added to each sample. The ligation mix contained 5 pmol *EcoR* I adaptor, 45 pmol *Mse* I adaptor, 2,5 pmol ATP, 2 U T4 DNA

ligase (New England Biolabs) and 1 µl 10x restriction buffer (New England Biolabs react 4) in a final volume of 10 µl adjusted with distilled H_2O. The total end volume per sample, after adding the ligation mixture to the restricted samples, was 50 µl. Ligation/digestion took place at 37°C for 16 hours. The restriction/ligation mixture was diluted (1:2.5) and pre-amplified with AFLP pre-selective primers with one selective nucleotide at the 3' end. Primers used for pre-amplification were M+C (5'-GATGAGTCCTGAGTAAC-3') and E+A (5'-GACTGCGTACCAATTCA-3'). The AFLP core mix of Applied Biosystems was used for amplification and the PCR mix was prepared according to the manufactures protocol. The PCR conditions used for pre-selective amplification were based on a touchdown program including one step at 72°C for 2 minutes, followed by 12 cycli of 30 seconds at 94°C, 30 seconds of annealing, starting at 65°C and an extension step for one minute at 72°C. The annealing temperature was subsequently reduced by 0.7°C for the next 12 cycles and was continued at 56°C for 22 cycles, followed by a final step at 60°C for 30 minutes. The pre-amplification product was diluted 20 times and then used as a template for the selective amplification. The PCR conditions for selective amplification were the same as for the pre-selective PCR except that the first step at 72°C for 2 minutes was replaced by a step at 94°C for 1 minute. Three primer combinations were used for selective amplification as follows:

E-ACA (5'-GACTGCGTACCAATTCACA-3') and M-CTA (5'GATGAGTCCTGAGTAACTA-3'), E-AAG (5'-GACTGCGTACCAATTCAAG-3') and M-CTA, E-AAG and M-CAG (5'-GATGAGTCCTGAGTAACAG-3'). The selective Eco RI side primers were labeled with a fluorescent FAM label at the 5' end of the primer. All reactions were performed in a T-gradient PCR machine (Biometra). Repeatability was checked by repeating the complete AFLP protocol for one sample per population for each primer combination. Samples were then run on a MegaBace 1000 capillary sequencer (Amersham Biosciences). All AFLP bands were scored with Fragment Profiler version 1.2 (Amersham Biosciences) for presence (1) or absence (0). Only clear and unambiguous bands were scored. The AFLP data matrix was analysed with principal coordinates analysis (PCoA) and used to calculate genetic distances applying GenAlex 6 (Peakall & Smouse, 2006).

Mitochondrial COI barcoding. To identify potential WFT cryptic species in the Netherlands mitochondrial COI barcoding was used. For each thrips population, five females were used for individual DNA isolation totalling 20 DNA extractions. DNA extraction followed the method described for AFLP. PCR was used to amplify 571 bp of the mitochondrial gene (mtDNA) cytochrome oxidase c subunit one (COI) with the following primer pair C1-J-1751 (5'-

GGATCACCTGATATAGCATTCCC-3') with C1-N-2329 (5'- ACTGTAAATATATGATGAGCTCAA-3') (Simon et al., 1994). All PCR's were performed in 25 µl reactions in a T-gradient PCR machine (Biometra). The PCR for C1-J-1751 with CI-N-2329 contained 10 ng DNA template, 1x PCR Buffer (Qiagen), 5mM bovine serum albumin (BSA), 5mM of each dNTP, and 1.25U Taq DNA polymerase (Qiagen). Thermocycling comprised 5 minutes at 95°C, then 36 cycles of 30 seconds at 95°C, 30 seconds at 47°C and 1 minute at 72°C, with a final extension of 5 minutes at 72°C. The amplified DNA was cleaned using the Wizard PCR preps DNA purification system (Promega) and sequenced in both directions at Macrogen, Korea. Sequences were analyzed in Sequencer 4.10.1. The consensus sequences of the forward and reverse sequence were further analyzed and aligned in Mega 4.1, and deposited in Genbank (GenBank accessions HQ697596-HQ697598). Existing *F. occidentalis* CO sequences were imported from GenBank and used to determine the mtDNA haplotype (Rugman-Jones et al., 2010).

Plant rearing

In addition to the plant species from which thrips were collected two tomato species were used to test the WFT populations**:** the wild tomato *Solanum pennellii,* and the cultivated tomato, *S. lycopersicum* (var. Moneymaker). In contrast to the cultivated tomato, *S. pennellii* has been shown to be resistant to the WFT lab culture population (Mirnezhad et al., 2010). For all plant species, except chrysanthemum, seeds were directly sown into the soil and seedlings were grown for 5 weeks in a climate chamber (L16: D8, 25 °C, 70% RH & 11.36 µmol m^{-2} s^{-1} light intensity). The chrysanthemum *Dendranthema grandiflora* (var. Polar) has been shown to be partially resistant to the WFT lab culture population (Leiss et al., 2009a). Commercially produced cuttings of this cultivar were derived from the Dutch chrysanthemum breeder Deliflor. Chrysanthemums were kept in a climate chamber as described above, for two weeks, before beginning the experiment, to rule out possible pesticide effects.

Bioassays

Three different bioassays where used to assess the performance of WFT: 1) a whole plant non-choice bioassay, 2) a leaf disc choice bioassay, both to measure feeding damage as well 3) a leaf non- choice bioassay to measure reproduction.

Whole plant non-choice bioassay. Five replicates of each of the five plant species were subjected to each of the four different thrips populations, resulting in a total of 100 plants. The

bioassays were performed as described in Leiss et.al (2009a, b). Plants were placed in individual thrips proof cages consisting of Perspex cylinders (60 cm height, 20 cm diameter) closed on top with nylon gauze of 120 µm mesh size. 20 thrips collected from each WFT population were added to each plant. After a week, Silver damage, expressed as the leaf area damaged per mm^2, was visually scored for each leaf. Since plant size may affect thrips damage, shoot dry mass was recorded. Data were analyzed by an ANOVA using shoot dry mass as a co-variate, followed by a Tukey post-hoc test. Silver damage and shoot dry mass were ln-transformed to obtain normally distributed variables.

Leaf disc choice bioassay. Dual Choice assays as described by Outchkourov et al. (2004) were used to test thrips preference for either of two leaves of different species. Most thrips damage occurs on older leaves (Leiss et al., 2009b; Mirnezhad et al., 2010). Therefore, the leaf discs were taken from older leaves of the host plants. Since host plants differed in their growth habits different leaves were considered as older leaves: a leaflet of the third oldest leaf in tomatoes, the 7^{th} oldest leaf in lettuce, the 5^{th} oldest leaf in chrysanthemum, and one part of the middle of the 4^{th} oldest leaf in leek. The cultivated tomato (var. Moneymaker), which is susceptible to the WFT lab culture population, was used as reference host against which all the other hosts were tested. Each thrips population was tested separately with 5 replicates for each pair of leaf discs, giving a total of 80 tests. For this bioassay, two leaf discs of 19 mm in diameter, punched from the older leaves at the same position, were placed on a thin layer of 1% water agar in a Petri dish. In between the leaf discs a small piece of 10 mm × 10 mm filter paper was placed on which 10 female thrips, that had been starved overnight, were released. The Petri dishes were closed and sealed with parafilm to prevent the thrips from escaping. The dishes were randomly placed in a climate chamber (L16: D8, 20 °C, 70% RH & 11.36 µmol m^{-2} s^{-1} light intensity). The number of thrips on each leaf disc was recorded each hour for the first and the last 5 hours of the experiment respectively. The experiment lasted 25 hours. As the repeated measurements were taken from the same Petri dish, the data were not independent. To circumvent this dependence, data from the multiple time point measurements were averaged into an early period, 0-5 h after the start of the experiment, and a late period, 21 to 25 h after the start of the experiment. Data of the two periods were analyzed with a Wilcoxon signed rank test on the absolute differences of average numbers of thrips on the two leaf discs.

Leaf non-choice bioassay. To measure the reproduction of different WFT populations on different host plants, a leaf bioassay as described by de Kogel et al (1997) was used. Five replicates of each of the five hosts were subjected to each of the four thrips populations amounting to a total of 100 leaf bioassays. The leaves were taken from the same individual plants as used for the choice bioassay. Thus a leaflet of the third oldest leaf of tomatoes, the 6th oldest leaf of lettuce, the 4th oldest leaf of chrysanthemum, and one part of the middle of the third oldest leaf of leek, was placed on a Petri dish (9 cm Ø) filled with 1% water agar for 6 days. A photograph of each leaf was taken and the leaf area measured using ImageJ software (Rasband, 1997-2006). Twenty female thrips were placed in a Petri dish which was closed with a lid containing a hole (Ø 3 cm), covered with fine thrips proof mesh, to prevent water condensation. The dish was sealed with parafilm, to prevent the thrips from escaping and placed randomly in a climate chamber (L16: D8, 20 °C, 70% RH & 11.36 µmol m^{-2} s^{-1} light intensity). The number of larvae per dish was recorded after 6 days. Data were analyzed by an ANOVA using leaf area as co-variate.

Results

Genotyping

AFLP. The three primer combinations resulted in 102 bands of which 30 were polymorphic between the four WFT populations (Table 1). The size of the amplified bands ranged from 65 – 490 bp. The average number of population specific bands was 2.6. Polymorphic band sharing between the populations was 59.4 %. Banding patterns between WFT populations could be distinguished by principal co-ordinate analysis (Fig. 1). The first three axes accounted for 87% of the variance among the thrips populations. The first and second principal component axis as well as the first and third axis separated the lab culture and the chrysanthemum populations from the remaining ones. The number of different polymorphic bands between the WFT populations confirmed genetic differences between the populations (Table 2). The lab culture population showed the largest differences to all other populations, while the leek and lettuce populations were genetically closest.

Mitochondrial barcoding. A divergence of 2-3% between two sequences in mitochondrial barcoding is seen as an indicative of different insect species (Hebert et al., 2003). The COI sequences of the different thrips populations showed just one variable position at nucleotide 241. The WFT collected from different populations in this study thus belong to one species.

Based on a comparison of the overlap of 571 bp between the COI sequences used for haplotype determination (Rugman-Jones et al., 2010) and the COI sequences in our study the thrips populations collected in The Netherlands all belong to the mtDNA cluster 1 (Rugman-Jones et al., 2010). This mtDNA cluster represents the so-called "glass house strain" or WFTG containing the haplotypes A and B. The thrips population from leek consisted of haplotype A only, while the other three populations consisted of a mixture of 80% haplotype A and 20% haplotype B respectively.

Bioassays

Whole plant non-choice bioassay: this bioassay revealed significant differences in silver damage among host-plants ($F= 53.42$, $df=4$, $P < 0.001$) and origin of thrips populations ($F = 3.39$, $df=3$, $P = 0.024$), (Fig. 2). However, we observed no significant interaction between host-plants and thrips origin ($F= 1.159$, $df=12$, $P = 0.333$). This result suggests a lack of adaptation of thrips to a particular host. The wild tomato *S. pennellii* was highly resistant to WFT showing no damage at all over all WFT populations while the cultivated tomato showed the highest damage. The thrips population from the lab caused the most damage, while the population from lettuce caused the least damage.

Leaf disc choice bioassay. In the choice test each thrips population in the early as well as the late period avoided the wild tomato *S. pennellii* as a host (Fig. 3). We detected no preference for the host plant from which the thrips populations originated. Most thrips, independent from their origin, preferred the cultivated tomato var. Moneymaker as a host.

Leaf non-choice bioassay. The leaf bioassay showed significant differences in thrips reproduction among host plants ($F= 22.7$, $df=4$, $P < 0.001$). No larvae were detected on the wild tomato *S. pennellii* while chrysanthemum sustained the highest number of larvae (Fig. 4). Averaged over all plant species, no differences in number of larvae between the different WFT origins could be detected ($F = 1.432$, $df=3$, $P = 0.241$). Most importantly however, the interaction between host plant and origin of thrips population was highly significant ($F = 3.055$, $df=12$, $P = 0.002$) suggesting an adaptation to host plant use. Although all thrips populations reproduced relatively well on chrysanthemum, the thrips population originating from the lab, which has been maintained on chrysanthemum, produced by far the most larvae. This population also performed far better on chrysanthemum than on the other hosts. In contrast, the remaining thrips populations did not produce more larvae on the host they originated from.

Discussion

Variation in genetics and reproductive parameters of the WFT lab culture indicate the evolution of a lab biotype specialized to a particular host. The lab culture population was genetically the most distant one as shown by the AFLP analysis. At the same time this was the only WFT population with a significantly higher reproductive output on the host plant where it originated from. Such a significant interaction for reproduction between thrips origin and host plant suggests local adaptation to host plant use.

The WFT lab population was genetically the most different from all thrips populations, while the populations from leek and lettuce were closest to each other. Genetic differences between WFT populations based on AFLP have also been reported by Fang et al. (2005). They analysed three WFT populations collected in Israel. It is, however, unknown from what kind of host plants these populations were sampled. Brunner & Frey (2010) using analysis of nucleotide sequence variation and microsatellites, revealed two habitat-specific WFT ecotypes. One is associated with hot and dry climates while the other is restricted to cool and moist climates. Sequence variation of the mitochondrial cytochrome oxidase I gene demonstrated genetic differences in onion thrips (*Thrips tabaci*), another economically important thrips pest, between populations originating from leek and tobacco (Brunner et al., 2004).

The WFT population originating from a lab culture kept on chrysanthemum obtained the highest levels of reproduction on this host plant. The short generation time of thrips (Reitz, 2008) and the parthenogenetic reproduction in areas of introduction (Morse & Hoddle, 2006) predispose WFT for rapid evolution under novel selective pressure. In the short time span of approximately 100 generations, in which our thrips culture has been established, the reproductive isolation in a climate chamber has led to genetic differentiation of this thrips population. Our result thus confirms the potential of a rapid biotype formation in WFT. However, whether this will actually occur on commercially grown crops remains the question. Massive WFT outbreaks on ornamentals and vegetables in Dutch glasshouses occur regularly (Vierbergen, 2001). Glasshouse crops such as chrysanthemum are often continuously grown the whole year round forming the basis for reproductive isolation of WFT glasshouse biotypes. However, on this ornamental we could not observe an increased reproduction of the chrysanthemum WFT population. This may suggest migration form thrips deriving from different

host plants into the glasshouses. In contrast, lettuce is a glasshouse crop for a limited period only and leak is a typical field crop in the Netherlands. A limited growing season as well as wind-borne travel of thrips to neighboring fields may hinder the development of host plant adaptation in these crops. In contrast to our findings indications of WFT biotype formations in crops have been reported by de Kogel et al. (1997). They compared reproduction of WFT populations, collected worldwide from different hosts and grown on susceptible and resistant cucumber cultivars. However, the thrips population originating from cucumbers, used as hosts for this experiment, did not show a better reproductive success on this host. Furthermore, it would be of interest whether or not the WFT populations used in this experiment belonged to the same species. The experiment also included a WFT population from New Zealand, which showed significant differences in reproductive output compared to other WFT populations. According to the newest findings of Rugman-Jones et al. (2010) WFT is a complex of two cryptic species, the "lupin strain" from the wilderness of New Zealand and the " glasshouse" strain" which is globally distributed.

Our study showed that the wild tomato *S. pennellii* was the most rejected and most resistant host for feeding and reproduction. In contrast, the cultivated tomato var. Moneymaker was the most preferred and most susceptible host over all feeding bioassays performed. Similarly, Mirnezhad et al. (2010) comparing thrips resistance among wild and cultivated tomatoes reported the least adult feeding damage of WFT on *S. pennellii* and the most on cultivated tomato. Cultivated tomato was the most susceptible host for feeding while chrysanthemum was the preferred host for reproduction, although the var. Polar is partially resistant to WFT feeding. Also Brodbeck et al. (2001) and Reitz (2002) reported that tomato is a less suitable host for WFT reproduction. Thus not all food sources of WFT constitute a suitable environment for breeding. Hosts of relatively low nutrient quality may not be chosen for reproduction but for feeding only. Feeding on these plants may lead to more damage due to the longer food intake needed.

In conclusion: The WFT lab culture population was genetically the most different population. At the same time this populations showed an increase reproductive output on the host plant it originated from. Together, the AFLP analyses and the interaction between host plant and WFT origin in thrips reproduction demonstrate the evolution of a host plant adapted lab biotype. Our results underline the potential of WFT biotype formation and they are thus of relevance for future crop control and breeding programmes.

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Table 1. Analysis of AFLP band patterns between and within populations of WFT, collected from different crops and a lab culture on chrysanthemum.

		Within populations			
	Between populations	Lab culture	Leek	Chrysanthemum	Lettuce
No. Total bands	102	83	88	87	78
No. Polymorphic bands	30	14	18	17	8
% Polymorphic bands total		13.72	17.65	16.67	7.84
% Polymorphic bands per population		16.87	20.45	19.54	10.25
Specific bands in each populations		8	2	3	0

Table 2. Differences in AFLP polymorphic bands between WFT populations collected on different crops and a lab culture on chrysanthemum.

Thrips populations	Lab culture	Leek	Chrysanthemum
Leek	19		
Chrysanthemum	20	15	
Lettuce	23	10	11

Figure. 1. Principal Co-ordinate Analysis (PCoA) separating populations of WFT collected on different crops and a lab culture on chrysanthemum. The first three coordinates explained 86.7 % of the genetic variation in AFLP banding patterns.

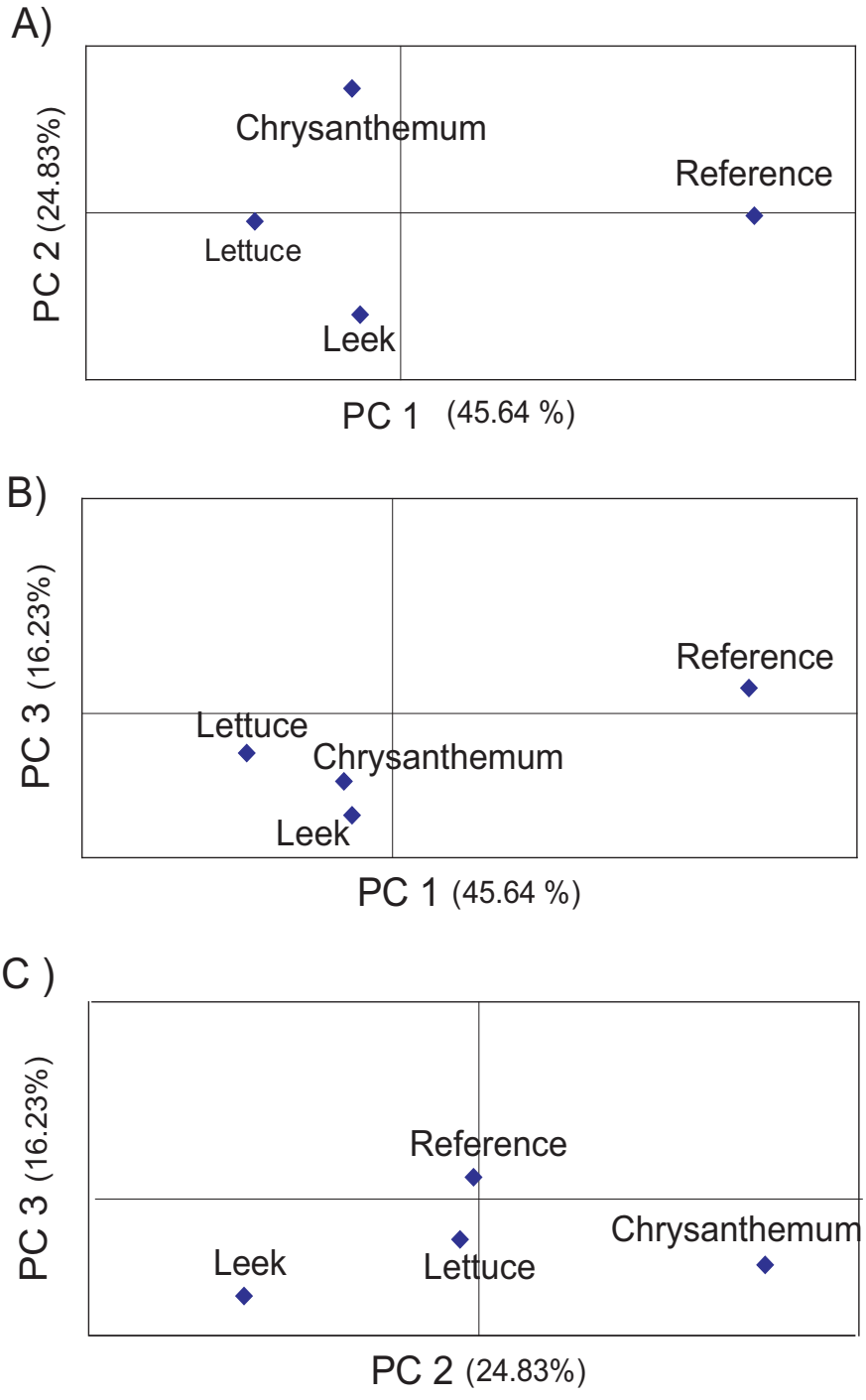

Figure 2. Whole plant non-choice bioassay with different host plants and different populations of WFT collected on different crops and a lab culture on chrysanthemum. Means and SE of silver damage, measured as leaf in mm², are presented. For each thrips population 5 replicates per host plant were used. Asterisks indicate groups that are significantly different from each other *** P≤ 0.001 and * P ≤ 0.05.

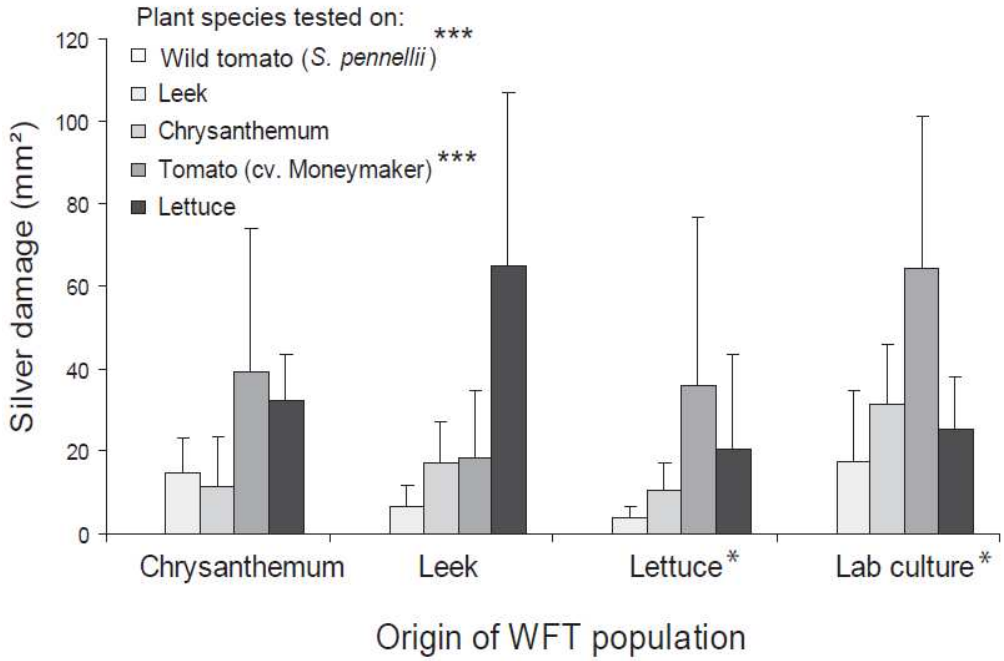

Figure 3. Leaf disc choice bioassay with different populations of WFT collected on different crops and a lab culture on chrysanthemum: A) Lab culture, B) Chrysanthemum, C) Lettuce and D) Leek. On the x-axis, the percentage of thrips choosing the cultivated tomato (var. Moneymaker) (grey) or another host plant (white) is presented for an early 0-5 h and a late 21-25 h period as depicted on the Y- axis. Asterisks indicate significant differences at * $P \leq 0.05$.

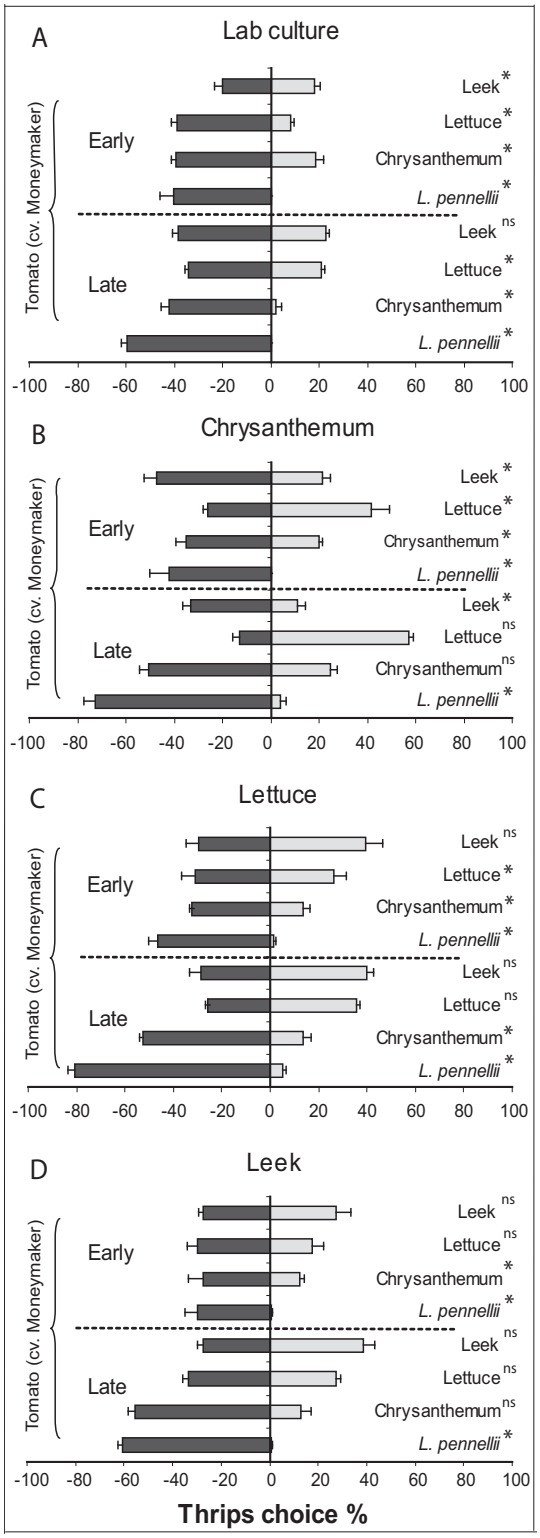

Figure 4. Leaf non-choice bioassay with different host plants and different populations of WFT collected on different crops and a lab culture on chrysanthemum. Means and SE of larval counts are presented. For each thrips population 5 replicates per host plant were used. Asterisks indicate groups that are significantly different from each other at *** P≤ 0.001.