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ORIGINAL PAPER

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GENETIC VARIATION OF ONION THRIPS (*Thrips tabaci* Lindeman, Thysanoptera Thripidae) POPULATIONS IN LITHUANIA AND THEIR LINK WITH HOST PLANTS

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ABSTRACT

Thrips tabaci Lindeman is one of the most important polyphagous horticulture crop pests in Lithuania. The aim of this work was to investigate new approach with the geographic distributions and host plant associations between different mtDNA COI haplotypes of *T. tabaci*. Specimens of *T. tabaci* were collected from different horticultural crops in the main horticulture regions across Lithuania, where sustainable plant protection practice is used. Four different haplotypes of *T. tabaci* were detected during investigation, and the greatest haplotype diversity was registered in the northern districts of Lithuania. The mean genetic distance between different haplotypes was 1.6%, and diversity was up to 2.9% comparing with sequences from other European countries (the Netherlands, UK and France) deposited in GenBank. Sequences obtained during this investigation differed by ≥10% comparing with sequences from other countries. *Thrips tabaci* COI gene differences partly reflect the geographic distribution, but results did not reveal the relationship between COI gene polymorphism of *T. tabaci* and different host plants.

Key words: COI diversity, haplotypes, horticultural crops

INTRODUCTION

Onion thrips (*Thrips tabaci* Lindeman) is a polyphagous and an economically harmful insect pest, which has spread to all continents [Liu and Sparks 2003]. The species is very prolific, the generations overlap, oviposition is within plant tissue, natural enemies are lacking and alternate hosts are numerous [Alimousavil et al. 2007]. *Thrips tabaci* are regarded as a pest of onions, leeks, and cabbages in Europe, and in other continents [Theunissen and Schelling 1998, Trdan et al. 2008, Diaz-Montano et al. 2011, Mautino et al. 2012, Pandey et al. 2012, Gombač and Trdan 2014] as well as of garlic in some countries too [Karuppaiah et al. 2018]. In Poland, *T. tabaci* is a major foliage pest in field cultures of onion [Nawrocka 2003], leek [Legutowska and Theunissen 2003] and cabbage [Pobożniak and Wiech 2005]. *Thrips tabaci* was a major pest of leeks [Duchovskienė 2006] and in current period become a harmful pest of onions, garlic and cabbages in Lithuania also. In ahead of recent years, the increase damage caused by *T. tabaci* on cabbage has been more or less attributed to the use of more productive cabbage hybrids, which often are more sensitive

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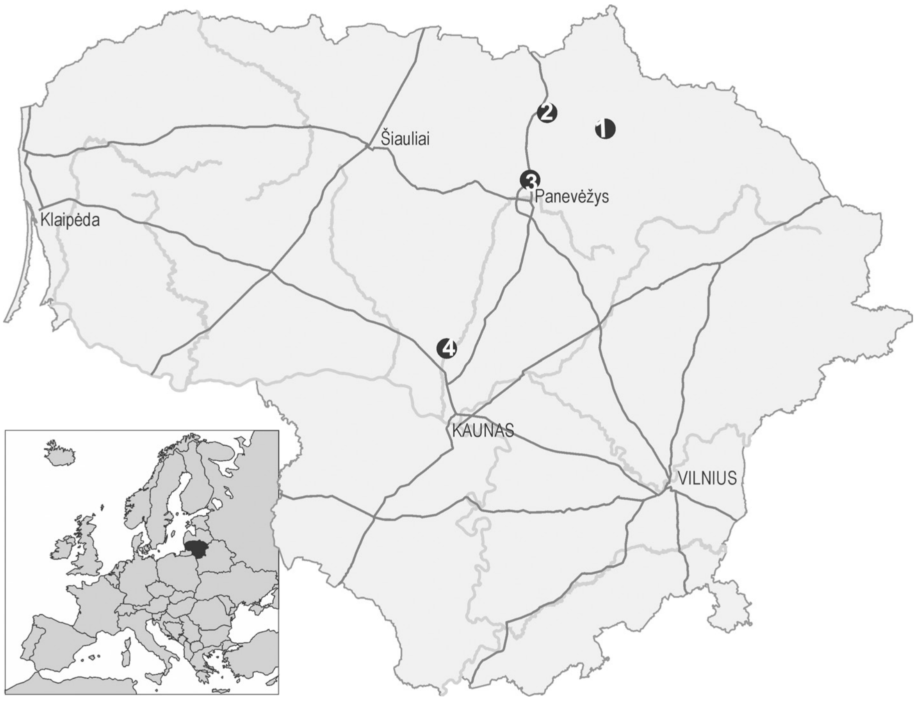

Fig. 1. *Thrips tabaci* collected from various districts in Lithuania: 1 – Biržai, 2 – Pasvalys, 3 – Panevėžys, 4 – Kaunas

to the attacks of pests and diseases [Trdan et al. 2008, Shelton et al. 2008, Gombač and Trdan 2014, Blatt et al. 2015].

Thrips tabaci can cause onion yield loss up to 50% besides direct damage but can be even more problematic when it transmits virus [Diaz-Montano et al. 2011]: *T. tabaci* can transmit *tomato spotted wilt virus* (TSWV) [Zawirska 1976, Riley et al. 2011] and was recently recognized as a transmitter of a new tospovirus, *Iris yellow spot virus* (IYSV) [Tomassoli et al. 2009, Diaz-Montano et al. 2011, Riley et al. 2011].

It is known that climate changes, especially temperature rising, influence the increasing of generations of thrips, spreading of pest and their adaptation to new environmental conditions, which are not favourable for growth of their host plants [Bergant et al. 2005]. It has been noted that there are several thrips strains with different host preferences [Zawirska 1976]. Brunner et al. [2004] investigated cytochrome oxidase c subunit I (COI) gene and suggested that *T. tabaci* is a complex of species in association with host preference. Zawirska [1976] suggested that *T. tabaci* consists of two biotypes, which were associated with different host preferences and their ability to transmit pathogens. The mentioned studies revealed the importance of genetic research of *T. tabaci*.

The aim of this work was to study if the genetic structure (COI sequences) of *T. tabaci* can be related to their horticulture host plants. In this study, we compared and analysed the nucleotide sequences of the COI gene fragment from *T. tabaci* collected from onions *(Allium cepa)*, garlic (*Allium sativum*) and cabbages (*Brassicae oleracea*) in different horticultural crops growing regions of Lithuania.

MATERIALS AND METHODS

Thrips tabaci were collected from four horticultural regions across Lithuania: in Biržai and Pasvalys (northern part of country), Panevėžys and Kaunas (central part of country) districts (Fig. 1).

Thrips tabaci were collected from cabbages (*Brassicae oleracea*), onions (*Allium cepa*) and garlic (*Allium sativum*) grown under sustainable plant pro-

Table 1. Circumstances at sampling time

Sampling location	Crop, variety or hybrid	Date of sampling	Growth stage (BBCH)	Temperature, relative air humidity
Panevėžys district 55°47'22" N 24°21'18" E	garlic ‘Žiemiai’ (local variety)	7th of July	45–47	18°C 81%
Biržai district 55°59'04" N 24°44'26" E	cabbage ‘Lennox’ F1	12th of July	40–41	20°C 78%
Kaunas district 55°07'17" N 23°48'50" E	cabbage ‘Discover ’ F1	15th of July	41–42	24°C 92%
Pasvalys district 56°02'23" N 24°30'33" E	onion ‘Stuttgarter Riesen’	21st of July	41–43	22°C 85%

Table 2. Number of various *Thrips tabaci* haplotypes identified from field samples

Locality	Host plant	*Thrips tabaci* (number of specimens) haplotypes			
		Lt1	Lt2	Lt3	Lt4
Biržai district	*Brassicae oleracea*	5	4		
Kaunas district	*Brassicae oleracea*		3		5
Panevežys district	*Allium sativum*		2		6
Pasvalys district	*Allium cepa*	3		4	

tection system. Plants grown using sustainable plant protection practices must be separated from intensive grown plants in Lithuania. Pesticide use in sustainable plant protection system is restricted: the same active ingredients of plant protection products can’t be used more than two times per season and harvest intervals have to be 1.5 times longer than indicated on the label. Plant protection products labelled as “very toxic” and “toxic” can’t be used. The sampling conditions are described in the table (Tab. 1).

The material was collected, by shaking the plants above the white paper in July (2015 – 16) and was stored in 96% ethanol solution. Each sample consisted of 10 specimens adults of thrips, of which *T. tabaci* accounted for from 70 to 90% (Tab. 2). Any plants with symptoms of viruses were not found.

DNA was extracted using the modified method of Robertson and MacLeod [1992]. Briefly: thrips were put in Tris-Borate-EDTA (TBE) buffer for 5 min to wash out the ethanol in which they had been stored. A single specimen was ground between two microscope glass slides, later the homogenate was collected by pipette and incubated at 95°C for 10 min, placed on ice for 3 min and centrifuged at 12 000 rpm for 5 min. The supernatant DNA was relocated to a fresh tube and stored at – 20°C. The mitochondrial COI of *T. tabaci* DNA was amplified with two insect specific primers: C1-N-2191 (5′-CAG GTA AA TTA AAA TAT AAA CTT CTG G-3′) and C1-J-1718 (5′-GGA GGA TTT GGA AAT TGA TTA GT-3′) [Simon et al. 1994]. PCR reactions were performed in a total volume of 50 µl. Each reaction mixture contained 5 µl of 10 × Dream

Taq buffer (Thermo Fisher Scientific, Lithuania), 5 µl of 25 mM MgCl2, 4 µl of 10 mM dNTP's, 4 µl of 10 pmol of each primer, 1 µl of Dream Taq DNA Polymerase (Thermo Fisher Scientific, Lithuania) and 2 µl of DNA genomic template. Amplification was carried out following the thermal cycling parameters: 96°C for 6 min, 36 cycles of 30 s at 94°C, 30 s at 57°C, 90 s at 72°C; 72°C for 10 min.

DNA extraction and PCR reactions were performed in the Nature Research Centre, Vilnius (Lithuania), bidirectional sanger sequencing was performed by Macrogen Inc. (Seoul, South Korea). Editing and analysis of the DNA sequences were performed using *BioEdit* sequence alignment editor. A phylogenetic tree was constructed in Mega 5 using Kimura two-parameter model and neighbour-joining methods. Phylogenetic analyses included *Thrips imaginis* sequence obtained from GenBank as the outgroup.

Pairwise the genetic distances between all sequences were calculated using the Kimura two-parameter model. All unique sequences obtained in this study have been submitted to GenBank (accession numbers KF534480–KF534483 for *T. tabaci*).

RESULTS AND DISCUSION

Investigation of thrips. Of the specimens collected, a fragment of the COI gene was sequenced from 32 individual *T. tabaci*, collected from *Brassicae oleracea* in Kaunas district (n = 8) and Biržai district (n = 9), collected from *Allium* sp. in Panevėžys district (n = 8, collected from *A. sativum*) and Pasvalys district (n = 7, collected from *A. cepa*) (Tab. 2).

Sequences were trimmed to 394 bp, the length of the shortest fragment which corresponds to all sequences of *T. tabaci*. The nucleotide composition averaged over all specimens showed an A-T bias (A = 28.6%, T = 36.9%, C = 18.8%, G = 15.7%). This is typical for the COI sequences of insects.

Four different haplotypes of *T. tabaci* were detected during investigation (Lt1, Lt2, Lt3, Lt4). In total ten parsimony-informative characters were observed. Genetic distance between different haplotypes varied between 1.0% (haplotypes Lt2 and Lt3) and 2.3% (Lt2 and Lt4 as well as Lt3 and Lt4) and was 1.6% in average. Sequences of onion thrips obtained from countries located far away from each other can fall with the same clade not showing the relationship between genetic diversity and geographical distribution (Fig. 2).

The diversity of ecosystems, in which pest species are found, raises the question of genetic variation in the use of resources by different populations [Brunner et al. 2004]. The development of molecular genetic techniques has contributed to an understanding of natural genetic diversity [Mehle and Trdan 2012]. Four different haplotypes of *T. tabaci* were detected during this investigation and these haplotypes diverse up to 2.9% compared with other sequences from Europe (the Netherlands, UK and France). Within Lithuania the genetic distance was 1.6%; similarly, the difference between thrips collected in Netherlands from leek was up to 1.8% [Toda and Murai 2007]. There are more than 650 sequences of *T. tabaci* COI gene fragments available in the GenBank. Comparing our data to some of these sequences, very high genetic distances were observed and they reached up to 10%. Some authors pointed out that the differences between *T. tabaci* populations can reach up to 12.9% and genetic differences between species can be 16–27.5% [Brunner et al. 2004].

Differences between *T. tabaci* populations and host-plant preference are of great practical importance because they can be related to the effectiveness of *T. tabaci* as a vector for Tomato spotted wilt virus (TSWV) [Zawirska 1976]. Zawirska [1976] suggested that *T. tabaci* consists of two biotypes: the "tabaci type", which can be found on tobacco plants, and the "communis type", which can be found on a variety of host-plants (but not tobacco). Only the "tabaci type" is known as a vector of TSWV [Zawirska 1976]. Most popular host-plants for the *T. tabaci* genetic diversity investigation were tobacco and onion [Fekrat et al. 2009] or tobacco and leek [Brunner et al. 2004]. Brunner et al. [2004] had conducted molecular phylogenetic analysis accordingly to COI gene sequences, and proposed three distinct major lineages (T, L1 and L2) in *T. tabaci*. They suggested that T is the tobacco-associated lineage (Fig. 2, AY196847-AY196849), and L1 and L2 are the leek-associated lineages (Fig. 2, AY196844). Using more sequences from GenBank, the question of thrips specialization according to host-plant became more complicated. Brunner et al. [2004] have found some adult females that were

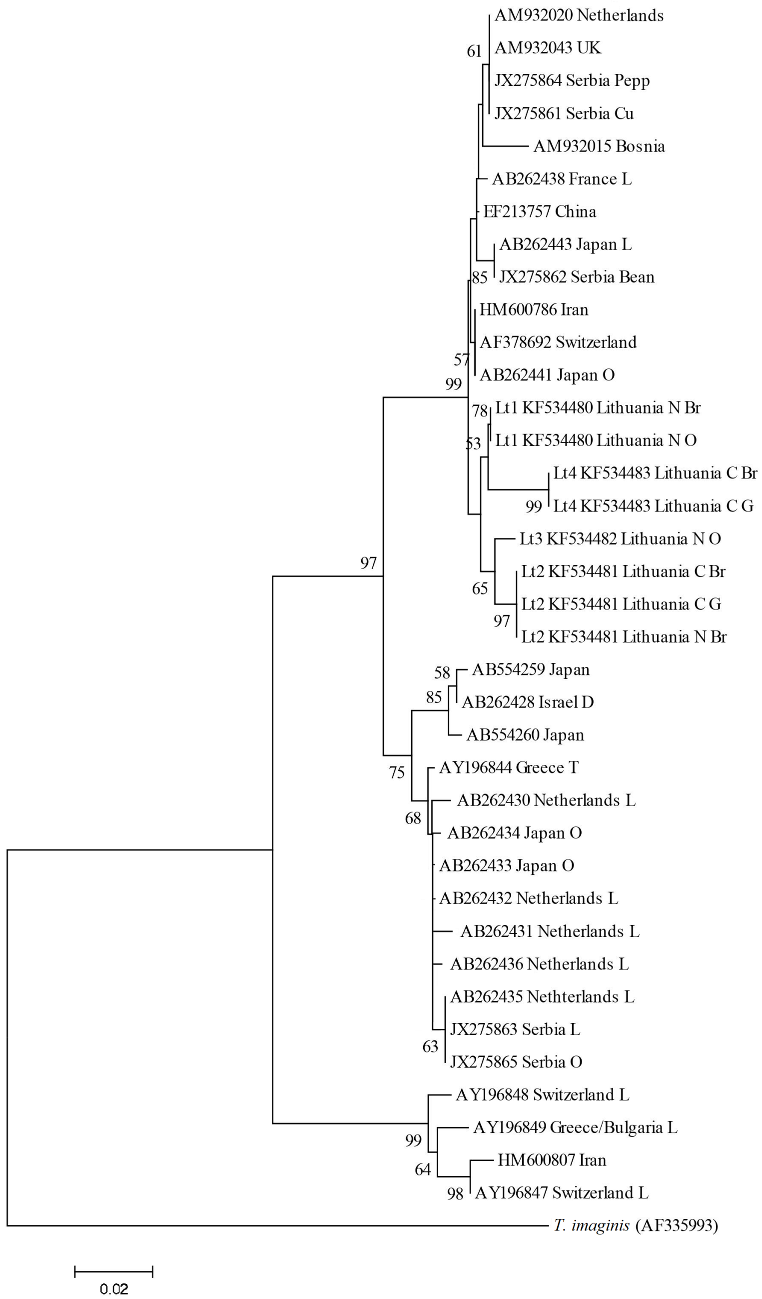

Fig. 2. Phylogenetic relationships of *Thrips tabaci* cytochrome oxidase I (COI) haplotypes. Method – neighbour-joining, model Kimura 2 parameter distances, bootstrap replications 5000, only those exceeding 50% are indicated; *T. imaginis* – the outgroup; sequences from the GenBank are used: accession numbers, the country of origin and the host plants, if they are known, are shown (Br – *Brassicae oleracea*, D – *Daucus* sp., G – *Allium sativum*, L – *Allium porrum*, T – *Nicotiana sylvestris*, O – *Allium cepa*, Bean – Fabaceae, Cu – *Cucumis sativus*, Pepp – *Capsicum* sp.). Lt1–Lt4 – sequences obtained from this study: N – North, C – Central Lithuania

collected on the "wrong" host plant. They explained these host-mismatches as accidental migrants to the reciprocal host. Thrips are weak flyers and can be blown by the wind over far great distances. It was revealed that the ability to transmit TSWV is closely linked to the host preference, and lineage T can transmit TSWV more efficiently comparing with other *T. tabaci* lineages [Brunner et al. 2004, Toda and Muray 2007]. Sequences obtained during this study (Fig. 2) differed from all three types of sequences obtained by Brunner et al. [2004], so we found no samples belonging to lineage T, and that means we could suggest that Lithuanian onions thrips haplotypes may have low possibilities to transmit TSWV (viruses). *Thrips tabaci* COI gene differences did not reflect the difference in host plants even though these differences did not deny it. It is only a limited number of reports that could be used to establish the true host range of the different *T. tabaci* lineages [Fail 2016], sympatric populations of at least two lineages of *T. tabaci* has been also reported [Li et al. 2014]. It is thought that the leek-associated (L1 and L2) lineages are polyphagous and the tobacco-associated (T) lineage has the narrowest host plant range [Fail 2016]. The different lineages of *T. tabaci* differ in their ability to transmit TSWV: the T lineage was reported to be highly effective in transmitting TSWV, whereas the L lineages transmit TSWV inefficiently [Chatzivassiliou et al. 2002]. On the other hand, it has also been reported that the L2 form of *T. tabaci* transmit TSWV with varying efficiency [Fail 2016]. So, the knowledge on the genetic structure of the *T. tabaci* populations should be studied, as it can help to find an efficient control method of *T. tabaci* in different plant hosts-like cabbages, onions and garlic. It is of great importance for growing plants using a sustainable plant protection system, when the usage of pesticides is restricted.

CONCLUSIONS

1. According to sequences of the COI gene four *Thrips tabaci*, haplotypes were determined in Lithuania.
2. The haplotypes of Lithuanian onion thrips may have low possibilities to transmit TSWV.
3. The greatest haplotype diversity was detected in the north of Lithuania.
4. The results did not confirm the relationship between COI gene polymorphism of *T. tabaci* and their preference to different host plants.

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