Genetic variability in Thrips tabaci (Insecta: Thysanoptera) living on vegetables in Serbia

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The onion thrips (Thrips tabaci Lindeman) belongs to the family Thripidae (Thysanoptera)

- Widely distributed throughout the world

- Highly polyphagous - causes damages on tobacco, alliaceous crops, cabbage, and ornamental plants

- The damage - caused by feeding or by transmitting Tomato spotted wilt virus (TSWV) and Iris yellow spot virus (IYSV)
TSWV and IYSV - tospoviruses with a wide host range, capable of causing serious epidemics and crop losses

The effectiveness of *T. tabaci* as a vector and host-plant preference can vary dramatically among populations

*T. tabaci* consists of two biotypes (Zawirska, 1976)

‘Tabaci type’ - associated with tobacco plants; vector of TSWV

‘Communis type’ - different host plants (not tobacco); couldn’t transmit TSWV; transmitted efficiently IYSV on onion (Chatzivassiliou et al., 2002)
✓ *T. tabaci* is a very small insect that shows a high degree of similarity, particularly in preadult stages

✓ Difficult to identify at the species level

✓ Molecular identification is not hampered by the above factors and can easily be followed

✓ Genetic markers, like mtDNA - a valuable addition or alternative to classical methods of species identification

✓ This study investigates the genetic variability of *T. tabaci* populations on different vegetable crops in Serbia
MATERIALS AND METHODS
Insect collection

Vegetables from 4 sites in Serbia:
a) open field and greenhouse production area around village Donji Vrtogoš (near town Vranje, South Serbia)
b) open field and greenhouse production area located around village Slanci (about 10 km east from Belgrade)
c) main greenhouse and open field production area located near town Ub (Central Serbia)
d) open field bean production area in Zemun

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Locality</th>
<th>Lat/Long</th>
<th>Host plant</th>
<th>Collection date</th>
<th>Accession No.</th>
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<tbody>
<tr>
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<td>Vranje</td>
<td>N42 29.192 E21 49.190</td>
<td>cucumber</td>
<td>01-Sept-2010</td>
<td>JX275861</td>
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<td>Zemun</td>
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<td>S29</td>
<td>Slanci</td>
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<tr>
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<td>Ub</td>
<td>N 44 48 298 E 20 34 228</td>
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<td>S60</td>
<td>Ub</td>
<td>N 44 28 322 E 20 01 278</td>
<td>onion</td>
<td>27 -July-2011</td>
<td>JX275865</td>
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</table>
DNA extraction, PCR amplification and sequencing

✓ DNA was extracted from a single specimen using the QIAGEN DNeasy extraction Kit

✓ The mitochondrial cytochrome oxidase subunit I gene (COI) was chosen as the appropriate gene with good genetic resolution for differentiation at species level

✓ Amplification for the barcode region of the COI gene was performed using LCO1490 and HCO2198 primers (658bp)
Nine COI sequence data on *T. tabaci* submitted by Brunner et al. (2004) were obtained from the GenBank and used for phylogenetic analysis.

The complete mitochondrion sequence data of *T. palmi* (AF378690) and *T. angusticeps* (AF378679) (Brunner et al, 2002) provided from GenBank served as an outgroups.

Phylogenetic analysis - maximum-likelihood (ML) and neighbor-joining method.

500 bootstrap replicates were performed to assess branch support in the resulting tree topology.
Phylogenetic analysis

✓ COI gene of *T. tabaci* individuals collected from five host plants were successfully amplified and sequenced (acc. num. JX275861 to JX275865)

✓ COI sequencing yielded a 617 bp long fragment for four specimens and 571bp long fragment for one individual of *T. tabaci*

✓ Selected sequences were trimmed to 471 bp, the length of the shortest fragment which corresponds to all sequences of *T. tabaci, T. palmi* and *T. angusticeps*
Phylogenetic analysis

✓ The COI sequences showed polymorphism at 18 nucleotide positions (0.4%) among the five Serbian samples. All variations were in the form of silent, single base-pair substitutions, resulting in no amino-acid replacement.

<table>
<thead>
<tr>
<th>Sequence code</th>
<th>Nucleotide position</th>
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<tr>
<td></td>
<td>78</td>
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<tr>
<td>S8</td>
<td>A</td>
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<td>S58</td>
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Phylogenetic analysis

Phylogenetic trees were estimated using the neighbor-joining and maximum likelihood method.

For both analyses, trees showed the same pattern of phylogenetic clustering.
Three different haplotypes were identified among COI sequences from Serbia.

All samples belong to the leek-associated lineage (L1 and L2), proposed by Brunner et al. (2004).

None of them belongs to tobacco-associated lineage (T).
Two haplotypes belong to L2 group, while the third haplotype belongs to L1 group.
Two samples S8 and S58, collected on cucumber and pepper, showed 100% similarity between each other and with H1 of L2 group.

Specimen S13 collected on bean indicate 100% similarity with H5 of L2 group.

Samples S29 and S60, collected on leek and onion, showed 100% homology between each other and with H10 of L1 group.
DISCUSSION AND CONCLUSIONS
Brunner et al. (2004), proposed three distinct major lineages (T, L1 and L2) in *T. tabaci*.

They suggested that T is the tobacco-associated lineage, while L1 and L2 are the leek-associated lineages.

We found three mitochondrial COI haplotypes inside the leek-associated lineage, while none of the analyzed samples belonged to the tobacco-associated lineage (no sample was collected from host plants of the tobacco type).

Our analyses clearly indicate that genetic differentiation is not correlated with host plant preference inside leek-associated group.
The biological relationship of *T. tabaci* with TSWV and IYSV seems to be very complex.

The worldwide distribution of the onion thrips in an extensive range of hosts may have generated specific associations with the tospoviruses that had an impact in their transmission.

Both tospoviruses (IYSW and TSWV) are widely distributed in different host plants in Serbia (Bulajić et al, 2008, Bulajić et al, 2009, Stanković et al, 2012).

The complex relationship between tospoviruses and *T. tabaci* as well as the infection mechanisms involved in this pathosystem remain to be elucidated.

Further testing is required to correlate virus transmission with the presence of different populations in *T. tabaci*. 
THANK YOU FOR ATTENTION