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Title of STSM:	Identification of potential phytoplasma cixiid vectors in maize and grapevine
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Background

Maize redness (MR) is a severe disease of maize, associated with stolbur phytoplasma (subgroup 16SrXII-A, 'Candidatus phytoplasma solani') (Duduk & Bertaccini, 2006) transmitted by a cixiid planthopper *Reptalus panzeri* (Jović et al., 2007). It was observed for the first time in Serbia in 1957 and has occurred sporadically there ever since, especially in the Banat region. However, in 2002 and 2003 severe outbreaks of the disease took place in late July-early August causing yield losses up to 90% (Bekavac et al., 2007). Concerning geographical distribution of the disease, the presence of stolbur phytoplasma induced symptoms of MR were recently identified in Italy and Hungary (Calari et al., 2010; Acs et al., 2011), whilst the presence of *R. panzeri* was recorded for Serbia, Austria, Bulgaria, Croatia, Hungary, Italy and Romania (EPPO RS, 2012). As maize is an important crop in the EPPO region, since February 2012 the EPPO Panel on Phytosanitary measures have suggested the addition of Maize redness to the Alert List of emerging diseases (EPPO RS, 2012).

Stolbur phytoplasma can be transmitted by the cixiid planthoppers *Hyalesthes obsoletus* Signoret, 1865*, Pentastiridius leporinus* (Linnaeus, 1761) and *Reptalus panzeri* (Löw, 1883) and the leafhoppers *Macrosteles quadripunctulatus* (Kirschbaum, 1868) and *Anaceratagallia ribauti* (Ossiannilsson, 1938). *H. obsoletus* is important for the spread of grapevine yellows 'bois noir' caused by stolbur phytoplasma, while *R. panzeri* is implicated in the spread of MR in maize (Jović *et al*., 2009).

The phytoplasma is transmitted to maize by the *R. panzeri* species and in the years of epiphytotic appearance can cause significant yield losses. The causes midrib, leaf and stalk reddening and abnormal ear development in maize (Jović et al., 2007).

Grapevine yellows (GY) are an increasing problem in several viticultural regions of the world. GY are widespread diseases caused by different types of phytoplasma including 'bois noir' (BN). BN is a GY disease associated with a stolbur phytoplasma which belongs to 16SrXII ribosomal group (Lee *et al*., 1998, cit. Contaldo et al., 2011) and reported in Asia Minor, and in the Mediterranean and European countries (Maixner, 2006, cit. Contaldo et al., 2011). According to Delić et al. (2011) BN phytoplasma is widespread in the main viticulture centers of the Republic of Srpska (Bosnia and Herzegovina (B&H)). *Hyalesthes obsoletus* is considered to be the main vector of BN (16SrXII-A) phytoplasmas, while *Reptalus panzeri* was found to be infected with stolbur phytoplasma in Hungarian vineyards and considered to be a potential vector (Palermo et al., 2004). Recently, another cixiid species *Reptalus quinquecostatus* was found to be infected with the stolbur phytoplasma in vineyards of Tuscany and capable of transmitting this phytoplasma to the artificial feeding medium (Trivellione et al., 2005; Pinzauti et al., 2008).

Among the yellows diseases of grapevine, Flavescence dorée (FD) is the most severe. Its agent belongs to ribosomal subgroups 16SrV-C and 16SrV-D and is transmitted by the leafhopper *Scaphoideus titanus* Ball (Auchenorrhyncha: Cicadellidae). Originally described in France, FD is currently distributed in several European countries (Palermo et al., 2004). FD on grapevine still has not been reported in B&H but it was confirmed to occur in neighboring countries such as Croatia, Serbia and Slovenia (Delić et al., 2011).

Purpose of visit

Identification of phytoplasmas' insect vectors is an important step to understanding the epidemiology of diseases. The identification of the vectors of phytoplasmas is primarily based on morphological characters using keys for the identification. So, the main objective of the action was to perform analyses concerning the taxonomical identification of the vectors of MR and GY phytoplasmas. Proper morphological identification of the planthopper species in question is essential for any epidemiological study concerning transmitted phytoplasma diseases.

Description of the work

The first part of the work was related to the field work during summer 2010 and 2011, when the insects were collected from the vineyards in the area of Popovo Polje, Mostar and Banja Luka (B&H). Also, from potentially MR affected maize fields cixiids were collected in June and July 2012 from three locations in the area of Bijeljina (B&H). Insects were stored in 96% ethanol at +4°C prior to analysis.

Morphological analyses of the individual insects were carried out during the visit from 12th to 23rd November 2012. The first week was dedicated to the training and to the introduction of the laboratory protocols. After this introduction part, in the second week, analyses were performed and results developed.

Insects were identified using cixiidae identification keys (Holzinger et al., 2003, Biedermann & Niedringhaus, 2004). We have gone through several genera of *Cixiinae* subfamily: *Cixius*, *Tachycixius*, *Hyalesthes* and *Reptalus*. On the basis of morphological characteristics some insects were viewed externally, others by dissection. Diagnostic characteristics were different, for example characteristics of the head, pronotum, anntenae, thorax, abdomen, legs, wings; color, size, shape, etc. Some species needed to be separated and macerated so we could examine male genitalia. For this purpose we used KOH (10%), heated them, and then washed in distilled water. After studying them, we put male genitalia in glycerine in micro tubes for storage and later used these as a reference in further identification. Here are results on identified cixiid species collected in maize fields and vineyards in table 1 [1].

Table 1. Cixiid species in maize and grapevine

Location	Date	*R. panzeri*		*R. quinquecostatus*		*R. cuspidatus*	
		Male	Female	Male	Female	Male	Female
BN-GO	June 2012	119	73	17	16	-	-
BN-DV	June 2012	128	57	8	21	-	-
BN-AM	June 2012	156	64	10	3	-	-
	July 2012	101	37	2	8	-	-
PP-RA	June 2011	-	-	27	27	-	-
MO-CV	June 2011	-	-	-	-	-	-
BL-BI	July 2010	-	-	-	-	30	36

[1] besides cixiid species a lot of others Auchenorrhycha species were identified

Conclusion and future collaboration

I learned features needed in morphological identification of insects belonging to the *Cixiinae* subfamily and I was also taught basic techniques of insect collecting, storage and examination. These analyses allowed us to define which cixiid species act as vectors of maize redness and grapevine yellows phytoplasmas in the Republic of Srpska (B&H), but the vector efficiency of cixiidae species and its significance for the spread of phytoplasma diseases also requires further investigation. The results obtained from this scientific mission allowed the start of the epidemiological and vector-role studies of the phytoplasmas infecting maize and grapevine in B&H.

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