COST STSM Scientific Final Report – Nicola Mori

COST Action:	FA0807
COST STSM Reference Number:	COST-STSM-ECOST-STMS-FA0807-11024
COST STSM Grant Letter Number:	COST-STSM-ECOST-STMS-FA0807-130712-019494
COST STSM Manager:	Dr. Matthew DISKINSON, e-mail: matthew.dickinson@nottihnham.ac.uk
STSM Title:	Role of Hylesthes obsoletus Signoret in the epidemiology of stolbur phytoplasma subgroup 16SrXII-A) on maize in Serbia and Italy
Applicant:	Mori Nicola Department of Agronomy Food Natural Resources Animals and Environment University of Padova. Viale dell'Università 16 35020 Legnaro (Padova) e-mail: nicola.mori@unipd.it
Host:	Dr. Bojan Duduk; Laboratory of Applied Phytopathology,Institute of Pesticides and Environmental Protection,Banatska 31b, P.O. Box 163, 11080 Belgrade, Serbia e-mail: Bojan.Duduk@pesting.org.rs
Period:	13 - 28 July 2012 (16 days)

Background

Maize redness (MR), was observed for the first time in Europe in the middle south Banat region of Serbia in the late 1950s (Marić *et al.*, 1965). After that, the MR was spreading in other regions of Serbia, in Romania, Bulgaria (Šutić *et al.*, 2002) and recently in Italy (Calari *et al.*, 2010). In 2006, stolbur phytoplasmas (subgroup 16SrXII-A, 'Candidatus phytoplasma solani') was associated with MR disease (Duduk & Bertaccini, 2006). On maize, this pathogen is transmitted by the Cixiids *Hyalesthes obsoletus* Signoret (Aleksić *et al.*, 1967) and *Reptalus panzeri* (Löw, 1883) (Jović *et al.*, 2009).

H. *obsoletus* Signoret plays also an important role as vector of stolbur phytoplasma associated with the grapevine (Maixner, 1994) and tomato (Aleksić *et al.*, 1967) yellow, while R. *panzeri* (Löw), was found positive for stolbur phytoplasma in vineyard infected by the same phytoplasma (Palermo et al., 2004).

The initial aim of this STSM was to study the presence, the ecology and the phenology of *H. obsoletus* where MR is spreading.

In Europe *H. obsoletus* is univoltinous on wild plants, mainly herbaceous. This polyphagous planthopper is assumed to mainly use stinging nettle *Urtica dioica* L. (Alma *et al.*, 2002) and field bindweed *Convolvulus arvensis* L. (Maixner e Reinert, 2000; Langer *et al.,* 2003) as its host plants.

Females laid eggs during the summer in the soil near the roots of host plants on which the nymphs feed and overwinter (Brčak, 1979). Five nymphal instars are reported (Alma *et al*., 1988; Sforza *et al*., 1999). In relation to different areas different overwintering nymphal instars are reported. In north Italy, France and Germany the planthopper overwinters as second-third instar (Alma *et al*., 1988; Sforza *et al*., 1999; Maixner, 2007), but in Bulgaria on *C. arvensis* as fourth instar (Brčak, 1979) and in Turkey as first-second instar (Güclü & Ozbek, 1988). As well as the environment condition, also the host plant species influence the nymphal development time (Maixner, 2007) and consequently the phenology of adult emergence (Pavan et al., 2012). The flight period of *H. obsoletus* adults was earlier on bindweed (from late May to mid-July) than on nettle (from mid-June to early August) (Maixner, 2007, Mori *et al*., 2008) and in general population density on nettle was 10 times higher than on bindweed. (Mori et al., 2008)

Purpose of visit

In some Italian and Serbs districts the MR is spreading but *R. panzeri* was not found. The aim of this STSM was to study the presence, the ecology and the phenology of *H. obsoletus* where the MR is spreading. Moreover the role of the *H. obsoletus* in the MR epidemiology was investigated setting up some specific transmission trials under semi-field condition using wild insect populations and maize seedlings. Molecular biology tests were used to confirm the results of the above experiments.

Description of the work carried out during the visit

<u>Characteristics of the sampled areas</u>

1. Smederevo:

Hilly and low-water region, where grape and wheat are cultivated. In this region there is a high presence into the vineyard and in the surrounding areas of C. *arvensis*. Stolbur phytoplasmas symptomatic grapevines and wild symptomatic *C. arvensis* plants were observed.

2. Udovice:

Hilly region, where the availability of water allows the production of stone and pome fruits. *C. arvensis* and *U. dioca* are very common in this region into the orchards and in the surrounding areas and there is a high variability of wild herbaceous and shrubs plants. Stolbur phytoplasmas symptomatic grapevines and wild symptomatic *C. arvensis* and *U. dioica* plants were observed.

3. Banat Region

Plain region, plenty of water available for irrigation. where corn, wheat, sunflower, sugar beet and soybean are cultivated. In this region there is a high presence in the headlands and in the drains areas of *U. dioca.* Stolbur phytoplasmas symptomatic maize plants were observed.

H. obsoletus sampling

In each regions the population of *H. obsoletus* nymphal instars were sampled, digging at the roots of host plants. At least ten holes were made.

The population of *H. obsoletus* adults on herbaceous vegetation were monitored using sweep nets. At each sampling the herbaceous vegetation was swept 60 times, collecting the specimens captured in the net every six sweeps. The captured specimens were caged and transported in the transmission field.

Transmission trial

In order to investigate the role of the *H. obsoletus* in the MR epidemiology, a specific transmission trials was setting up using wild insect populations and maize seedlings. In table 1, the transmission scheme was reported:

Acquisition source plant	Insect source	Acquisition period	Latent period	Transmission period
Wild symptomatic *C. arvensis* and *U. dioca* plants	Wild H. obsoletus population	None	None	On maize seedlings until the insect death

Table 1: transmission scheme

At the end of May, 10 asymptomatic maize seedlings were isolated in a cage (1.0m x 1.0m x1.6m). During the STSM period, 50 *H. obsoletus* adults, collected in the three different regions, were put into the cage. 8 cages were made: 2 for each regions and 2 as control without insect.

Molecular biology tests

In the investigated regions symptomatic grapevines, maize (the maize seedlings of the transmission trials), wild plants and *H. obsoletus* adults were collected and tested to verify phytoplasma's presence and identity. Nucleic acid was extracted according to the established procedures for grapevines and herbaceous samples by Prince *et al*. 1993, and for insects by Angelini *et al*. 2001. Nested-PCR followed by restriction fragment length polymorphism (RFLP) analyses on 16S ribosomal gene and on tuf gene for phytoplasma molecular characterization was performed as described by Duduk *et al*. 2004. Informative restriction enzymes employed were *Tru*I and *Taq*I on 16S rDNA gene and *Hpa*II on tuf gene.

Conclusion and future collaboration

Presence, ecology and phenology of *H. obsoletus*

H. obsoletus in Serbia is widespread on the agro-ecosystem and on uncultivated plots. Its ecology is mainly influenced by host plants (Table 2). As reported in north Italy, Germany and Switzerland (Maixner, 2007; Mori *et al*., 2008; Kessler *et al*., 2011) the population density was lower on *C. arvensis* than on *U. dioca* and the flight period of adults was earlier on bindweed (probably end of June - early July in Serbia) than on nettle (mid-July - early August).

Locality	Population density (specimens captured in the net every six sweeps)	Host plant	Catches Male/Female ratio	Presence of nymphal instars	period of the catches in relation of the peak of flight
Smederevo	0-2	*C. arvensis*	0.2-0.4	no	after peak of flight
Udovice	3-5	*C. arvensis* *U. dioca* *A. vulgaris*	1.0-1.1	yes	on peak of flight
Banat	20-30	*U. dioca*	1.0-1.2	yes	on peak of flight

Table 2: characteristics of investigated *H. obsoletus* population

Moreover, nymphs are found on the roots of *Artemisia vulgaris* in Udovice locality; this finding indicates that *H. obsoletus* has been adapted to the Serbian environmental conditions.

Regardless of the host plant species the distribution of *H. obsoletus* is extremely irregular on a small scale. While numerous vectors are found on some plants or plant stands, other plants growing nearby are not colonized at all. The planthopper clearly prefers sparse vegetation, probably because of a better microclimate due to a more intense insolation of the soil (Maixner et al., 2010).

Role of the *H. obsoletus* in the MR epidemiology

The first results of the molecular biology tests showed that some of the captured H. obsoletus were infected. The analysis on maize, maize seedlings wild U. dioca and C. arvensis plants, are in progress.

References

Aleksic Z., Sutic D., Aleksic D., 1967. Transmission intensity of stolbur virus by means of *Hyalesthes obsoletus* Sign. on some host plants. *Zastita bilja* 93-95: 67-73.

Alma, A., Arnò, C., Arzone, A. & Vidano, C. 1988. New biological reports on Auchenorrhyncha in vineyards. pp. 509-516 in *Proeedings of 6th Auchenorrhyncha Meeting*, *Turin, Italy*, 7-11 September 1987, (Ed.) C. Vidano & A. Arzone.

Alma A., G. Soldi,, R. Tedeschi, C. Marzachì, 2002. Ruolo di *Hyalesthes obsoletus* Signoret (Homoptera, Cixiidae) nella trasmissione del Legno nero della vite in Italia. *Petria*, 12 (3), 411-412.

Angelini, E.; Clair, D.; Borgo, M.; Bertaccini, A.; Boudon-Padieu, E; 2001: Flavescence dorée in France and Italy - Occurrence of closely related phytoplasma isolates and their near relationships to Palatinate grapevine yellows and an alder yellows phytoplasma. Vitis 40 (2), 79-86.

Brčak, J. (1979) Leafhopper and planthopper vectors of plant disease agents in central and southern Europe. pp. 97-146 in Maramorosch, K. & Harris, K.F. (Ed.) *Leafhopper vectors and plant disease agents*. London, Academic Press.

Calari A. et al. (2010). Phytoplasma detection in corn with reddening in Italy. Atti convegno *"Current status and prospectives of phytoplasma disease research and management"*. February 1st and 2nd 2010 Sitges, Spain, pg 5.

Duduk B., Bertaccini A., 2006.- Corn with symptoms of reddening: new host of stolbur phytoplasma.-
Plant Disease, 90: 1313-1319.

Duduk, B.; Botti, S.; Ivanović, M.; Krstić, B.; Dukić, N.; Bertaccini, A.; 2004: Identification of phytoplasmas associated with grapevine yellows in Serbia. Journal of Phytopathology 152, 575-579

Güclü, S. & Ozbek, H. 1988. Some studies on the biology of *Hyalesthes obsoletus* Signoret (Homoptera: Cixiidae) in the conditions of Erzurum. *Turkiye Entomoloji Dergisi* 12, 103-111 (in Turkish).

Jović J., Cvrković T., Mitrović M., Krnjajić S., Petrović A., Redinbaugh M. G., Pratt R. C., Hogenhout S. A., Toševski I., 2009.- Stolbur phytoplasma transmission to maize by *Reptalus panzeri* and the disease cycle of maize redness in Serbia.- *Phytopathology,* 99: 1053-1061.

Kessler S., Schaerer S., Delabays N., Turlings Ted C.J., Trivellone V., Kehrli P. 2011 Host plant preferences of Hyalesthes obsoletus, the vector of the grapevine yellows disease "bois noir", in Switzerland. Entomologia Experimentalis et Applicata 139:60-67.

Langer M., Darimont H., Maixner M., 2003. Control of phytoplasma vectors in organic viticulture.
IOBC/ WPRS Bulletin 26 (8): 197-203.

Maixner M., 1994. Transmission of German grapevine yellows (Vergilbungskrankheit) by the planthopper *Hyalesthes obsoletus* (Auchenorrhyncha: Cixiidae). *Vitis*, 33 (2), 103-104.

Maixner M., 2007. Biology of *Hyalesthes obsoletus* and approaches to control this soilborne vector of Bois noire disease. *IOBC/WPRS Bulletin*, 30 (7), 3-9.

Maixner M., Reinert W., 2000. Monitoring of planthopper vectors in vineyards: an aid for grapevine yellows management decisions. IOBC/ WPRS Bulletin 23 (4): 123-124.

Maixner M., Weintraub P., Barbara Jarausch B.. 2010 Grapevine Yellows Vector Sampling and Monitoring Training School Bernkastel-Kues, Germany 5th to 9th of July

Marić A.and Savić R.. 1965 – Dosadašnji rezultati istrazivanja crvenile kukuruza. Dokumentacija za tehnologiju i tehniku u poljoprivedi: 8

Mori, N., Pavan, F**.,** Bacchiavini, M., Reggiani, N., Bonomi, F., Bertaccini, A. 2008 Fenologia di *Hyalesthes obsoletus* Signoret su convolvolo e ortica. *Petria* 18, 229-231.

Palermo S., Elekes M., Botti S., Ember I., Alma A., Orosz A., Bertaccini A., Kölber M., 2004.
Presence of Stolbur phytoplasma in Cixiidae in Hungarian vineyards. Vitis 43: 201-203.

Prince, J.P.; Davis, R.E.; Wolf, .K.; Lee, I.-M.; Mogen, B.D.; Dally, E.L.; Bertaccini, A.; Credi, R.; Barba, M.; 1993: Molecular detection of diverse mycoplasmalike organisms (MLOs) associated with grapevine yellows and their classification with aster yellows, X-disease, and elm yellows MLOs. Phytopathology 83, 1130-1137.

Sforza, R., Bourgoin, T., Wilson, S.W. & Boudon-Padieu, E. (1999) Field observation, laboratory rearing and description of immatures of the planthopper *Hyalesthes obsoletus* (Hemiptera: Cixiidae). *European Journal of Entomol*ogy 96, 409-418.

Sutic D., Tosic M., Starovic M., Stankovic R., Tomic T..2002 – Redness of Maize. Plant protection 53: 57-73