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Relationships among *Hyalesthes obsoletus*, its herbaceous host plants and "bois noir" phytoplasma strains in vineyard ecosystems in the Marche region (central-eastern Italy)

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Abstract

The relationships between the planthopper *Hyalesthes obsoletus*, its herbaceous host plants (nettle and bindweed), and "bois noir" phytoplasma strains (tuf-type) were studied in five different vineyard areas of the Marche region (Italy). Stolbur phytoplasma was detected by molecular analysis of planthoppers and of their host plants. These planthoppers were caught from bindweed and nettle and separated according to sex. Their mean rates of infection varied from 22.5% to 44.2% in samples from bindweed, and from 7.7% to 14.3% from nettle. All the *H. obsoletus* from bindweed harboured the tuf-type II, with tuf-type I predominant in planthoppers on nettle.

Key words: *Hyalesthes obsoletus*, stolbur phytoplasma type, *Urtica dioica*, *Convolvulus arvensis*, vineyard ecosystems.

Introduction

"Bois noir" (BN) is the most widespread grapevine yellow disease and it is an important limiting factor in grapevine (*Vitis vinifera* L.) growing in Europe and in the Mediterranean basin. It is included in the stolbur (STOL) or 16SrXII group, subgroup A. This phytoplasma is present in a wide range of wild and cultivated herbaceous plant hosts, besides grapevines.

BN phytoplasma is occasionally transmitted to grapevines by the planthopper *Hyalesthes obsoletus* Signoret (Fulgoromorpha, Cixiidae) (Maixner, 2006). Most of the life cycle of *H. obsoletus* is underground, with the eggs and larvae found on plant roots. The aerial stage is limited to winged adults that migrate during the summer (Alma *et al*., 1987).

H. obsoletus is a polyphagous species that ensures the complex transmission cycle of phytoplasma, which have various hosts and a few preferential host plants that act as phytoplasma reservoirs (Maixner, 2006).

In Germany, the host plants reported for *H. obsoletus* are mainly bindweed (*Convolvulus arvensis* L.), hedge bindweed (*Calystegia sepium* L.) and nettle (*Urtica dioica* L.), and the populations of *H. obsoletus* from bindweed and nettle are associated with molecularly distinguishable BN phytoplasma strains (Langer and Maixner, 2004).

In Marche, as in other Italian regions, nettle is the only known host plant of *H. obsoletus*, and this planthopper can also be found in abundance in some vineyards where they feed and mate on bindweed (Alma *et al*., 1987; Riolo *et al*., 2007).

The aim of the present study was to investigate the genetic variability in the BN phytoplasma in *H. obsoletus* and in their herbaceous host plants (nettle and bindweed), to provide a better understanding of both the *H. obsoletus*–herbaceous host plant–"bois noir" relationships and the epidemiological cycle of BN in central-eastern Italy.

Materials and methods

The present study was carried out in July 2006 in the Marche region of Italy. Adult planthoppers and host plants were collected from vineyard ecosystems in five different districts in the Ancona (Castelferretti, Morro D'Alba) and Macerata (Montefano, Morrovalle, Serrapetrona) provinces, where *H. obsoletus* and BN phytoplasma are known to occur.

These adult *H. obsoletus* were collected using a sweep net, separately from bindweed and nettle. They were then identified and separated according to sex.

The number of samples collected were representative of the presence of both *H. obsoletus* and plant (symptomatic and asymptomatic) individuals in the different vineyard ecosystems investigated.

Total DNA from each whole planthopper was obtained using modified CTAB methods (Angelini *et al*., 2001).

Leaf tissue (10 g) from plants was also ground in liquid nitrogen for each plant sample and the DNA was extracted from 2 g of this fine powder, according to methods proposed by Langer and Maixner (2004).

The detection of the stolbur phytoplasma from both kinds of samples was performed by PCR analysis of a non-ribosomal sequence related to elongation factor Tu (the *tuf* gene). The PCR fragments obtained using specific fTufAy/rTufAy primers (Schneider *et al*., 1997) were analysed by PCR-RFLP, using the enzyme HpaII (Langer and Maixner, 2004).

Results

A total of 339 *H. obsoletus* planthoppers, 76 *C. arvensis* and 9 *U. dioica* plants were sampled in the vineyard ecosystems investigated. In some locations there was no nettle or bindweed found in or around the vineyards.

H. obsoletus females were more numerous than males in the samples collected from bindweed, a situation not

Table 1. Collected data from *H. obsoletus* and host-plants analyzed: a) *H. obsoletus* females (f) and males (m) collected from *C. arvensis* and *U. dioica*; b) mean infected *H. obsoletus* collected from *C. arvensis* and *U. dioica* (*mean infected females and males); c) infection according to host plant.

	Hyalesthes obsoletus				*Host plant*
Plant species	Planthopper samples collected, according to sex (% tested) (a)		Infected planthopper samples collected (% positive) (tuf-type) (b)		Infected host-plant samples collected (% positive) (tuf-type) (c)
Convolvulus arvensis	70.5 f	29.5 m	36.6 (tuf-type II) 39.0 f*	27.6 m*	23.7 (tuf-type II)
Urtica dioica	51.9 f	48.1 m	12.3 (65.0 tuf-type I, 35.0 tuf-type II) 9.5 f* (50.0 tuf-type I, 50.0 tuf-type II)	15.4 m* (80.0 tuf-type I, 20.0 tuf-type II)	11.1 (tuf-type I)

seen in the samples collected from nettle (table 1).

Molecular analysis revealed the presence of BN, both in planthopper specimens and in its herbaceous host plants.

The mean levels of infected *H. obsoletus* according to vineyard varied from 22.5% (Montefano) to 44.5% (Castelferretti) in samples collected from bindweed, and 7.7% (Morro D'Alba) to 14.3% (Serrapetrona) in those collected from nettle. The overall mean level of infected *H. obsoletus* collected from bindweed was 36.6% (39.0% infected females and 27.6% infected males), while from nettle it was 12.3% (9.5% infected females and 15.4% infected males) (table 1).

The *H. obsoletus* and their host-plant samples showed the presence of two BN phytoplasma strains: tuf-type I and tuf-type II. The former was detected in nettle and tuf-type II in bindweed; whereby all *H. obsoletus* sampled from bindweed harboured only tuf-type II, with tuf-type I predominating in the *H. obsoletus* sampled on nettle, according to Langer and Maixner (2004) (table 1).

Discussion

BN showed an infection incidence of 22.5% to 44.5% and 7.7% to 14.3% in the *H. obsoletus* populations sampled from bindweed and nettle, respectively. All of the *H. obsoletus* specimens collected from bindweed in the vineyards harboured tuf-type II, while tuf-type I was predominant from *H. obsoletus* sampled on nettle.

The major presence of females caught on bindweed, together with the BN phytoplasma strains detected in planthoppers on nettle, would suggest a different patterns of movement between the host plants by the different sexes.

Recently, studies have shown the predominant presence of tuf-type I in grapevines of the Marche region (Quaglino *et al*., 2007). This situation would suggest that further, different, vectors could be involved in the transmission of stolbur phytoplasma to the grapevines.

The information obtained from these preliminary studies will help in our understanding of the *H. obsoletus*–nettle/bindweed–"bois noir" relationships and the epidemiological cycle of BN disease in central-eastern Italy.

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