

WILD HOST PLANTS OF STOLBUR PHYTOPLASMA AND ITS VECTOR, *HYALESTHES OBSOLETUS*, AT SITES OF GRAPEVINE BOIS NOIR OCCURRENCE IN EMILIA-ROMAGNA, ITALY

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Introduction

Stolbur phytoplasma (16SrXII-A) is a serious pathogen affecting a range of agronomic crops. On grapevine (*Vitis vinifera* L.) it causes bois noir (BN), an important disease which occurs with high incidence levels in most European viticultural regions. *Hylesthes obsoletus* Sign. (Homoptera, Cixiidae) has been identified as the main natural vector of stolbur (Fos *et al.*, 1992); subsequently, the role of this planthopper and some alternate non-crop plant hosts of the phytoplasma in the spread of BN was demonstrated (Maixner *et al.*, 1995; Sforza *et al.*, 1998).

Research into the epidemiology of grapevine BN has recently been started in viticultural areas of Emilia-Romagna (northern Italy). Field surveys demonstrated a wide distribution of *H. obsoletus*; winged adults were present from the end of June to the end of August, planthopper populations peaked around the middle of July, up to 40% of captured adults and immature stages tested positive for stolbur by PCR and some naturally phytoplasma-infected weed species were found in vineyards with the disease (Credi *et al.*, 2002). As in the case of other insect-transmitted plant pathogens, wild plants likely contribute to the spread of stolbur by serving as both a reservoir for phytoplasma infection and as reproductive hosts for the vector. Elimination of these plants may be an important part of an integrated control strategy for BN disease in vineyards. However, these aspects are still not well understood in our ecological conditions. This study was conducted to identify the putative species which could play such a role in grapevine BN epidemiology, through field collections of common weeds and shrubs.

Materials and Methods

Wild plant species, with or without symptoms, were collected randomly in different vineyards located in the Reggio-Emilia, Modena and Ravenna districts of the Emilia-Romagna region. These are important grapevine-growing locations with a known BN history and where high disease incidences have been recorded over the last few years. A systematic botanical inventory was established for all sites; sampling was done from June through September of 2003 and 2004. Total DNA was extracted from leaf veins and petioles. Samples were then screened using the universal primer pair P1/P7 in direct PCR, and the primer pair fStol/rStol to amplify in nested PCR a specific target sequence from the stolbur phytoplasma (Maixner *et al.*, 1995). Plant species were also surveyed from April to May 2005 to obtain information on the biology of *H. obsoletus*. Roots were examined for instar larvae (nymphs) and their presence was considered indicative that the plant would potentially support reproduction of the insect vector.

Results and Discussion

During the surveys, a total of 162 non-crop native plant samples, comprising 30 different species, were collected within and/or in the surroundings of vineyards where a high incidence of BN has been recorded. When PCR tested individually for stolbur, 78 (48.1%) samples of 20 species representing 15 families assayed positive. These included (numbers in parentheses represent number of plants infected/total tested): Amaranthaceae: *Amaranthus retroflexus* L. (3/4), Caryophyllaceae: *Silene alba* (Miller) Krause (2/4), Chenopodiaceae: *Chenopodium album* L. (3/6), Compositae: *Artemisia vulgaris* L. (2/5), *Cirsium arvense* (L.) Scop. (5/7), *Picris echioides* L. (1/4), *Sonchus oleraceus* L. (2/2), *Taraxacum officinale* Wigg. (2/5), Convolvulaceae: *Calystegia sepium* (L.) R. Br. (2/2), *Convolvulus arvensis* L. (11/13), Labiateae: *Mentha arvensis* L. (2/7), Leguminosae: *Medicago sativa* L. (2/6), Malvaceae: *Malva sylvestris* L. (1/2), Plantaginaceae: *Plantago lanceolata* L. (1/6), Poaceae: *Setaria viridis* (L.) Beauv. (2/4), Rosaceae: *Potentilla reptans* L. (3/3), Salicaceae: *Salix alba* L. (3/5), Solanaceae: *Datura stramonium* L. (2/2), Ulmaceae: *Ulmus campestris* L. (5/6), and Urticaceae: *Urtica dioica* L. (24/41). Most of these species appear to be new recordings as hosts for the stolbur phytoplasma. Infected weeds included 5 annual, 1 biennial and 12 perennial species. The phytoplasma was also detected on 2 woody plant species: *S. alba* (white willow) and *U. campestris* (English elm). In general, symptoms on the tested plants consisted of

stunting, resetting, chlorosis, leaf malformation, little leaf, leaf yellowing, reddening and necrosis; some species were however symptomless, including *A. retroflexus* (redroot pigweed), *C. album* (lamb's quarter) and *U. dioica* (stinging nettle). Among the locations sampled, stolbur was commonly found in *C. arvensis* (bindweed) and in *U. dioica*. The two plants are perennial in growth habit and frequently found throughout the survey locations, although their populations vary in abundance spatially. Populations of *C. arvensis* are usually very abundant both in and beside the vineyards; populations of *U. dioica* are instead more prevalent in the field borders, where plants may remain undisturbed for long periods.

Among the stolbur susceptible plant species, when evaluated in the field, only *U. dioica* and *C. arvensis* proved to harbour immature *H. obsoletus*, but were not equal in their ability to support reproducing populations of the insect. The mean proportion of its nymphs on the plant roots varied considerably, indicating a different epidemiological importance of the two weeds. High levels of *H. obsoletus* larval instars were found on *U. dioica*: 16 sites with nymphs over 18 sites surveyed, 80 plants showing nymphs over 103 examined, with a mean of 51 nymphs per plant. On the contrary, *C. arvensis* appeared to support a very low level of the planthopper nymphs: 1 site positive over 14 sites surveyed, only 1 plant with 2 immature insects over 228 observed. At a site in the Reggio-Emilia district, *Humulus lupulus* L. (common hop) was also found to be colonized in the roots by the insect nymphs (7 plants of 8 checked, mean of 4 nymphs per plant), but all the plants tested so far were not positive for stolbur.

The nested-PCR technique was used to identify stolbur reservoir hosts in some major grapevine-growing areas of Emilia-Romagna. Stolbur is a phytoplasma having a wide host range (Garnier 2000; Maixner *et al.*, 1995; Sforza *et al.*, 1998; Credi *et al.*, 2002). Here we report additional wild plant hosts commonly found within and outside vineyards with BN infection. Including these, the phytoplasma now occurs in about 45 species. An understanding of stolbur-vector relationships with specific plant species is important in determining the main reservoir hosts. In the epidemiology of grapevine BN disease, *C. arvensis* has been shown to be a major reservoir for stolbur and *H. obsoletus* in Germany and France (Maixner *et al.*, 1995; Sforza *et al.*, 1998). Although a high percentage of stolbur-infected *C. arvensis* plants were identified in our study, in effect the species was not associated with immature stages of the vector. Of the several plants inspected, only one plant was found harbouring just two nymphs on the roots. On the contrary, *U. dioica* was shown to be the major reservoir for stolbur and the planthopper vector. Hence, plant species that are susceptible to stolbur infection but are poor hosts for reproduction by *H. obsoletus* are less likely to be important epidemiologically than are stolbur-infected plants that support high levels of the planthopper reproduction. In conclusion, results from our surveys demonstrate that only the perennial *U. dioica* is host for both stolbur phytoplasma and its insect vector, confirming the results of Alma *et al.*, (2002) in Piedmont. However, the real impact of this weed and other wild species in BN epidemics needs to be elucidated with further investigations.

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