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Preliminary results on population dynamics and host plants of *Hyalesthes obsoletus* in North-Eastern Italy

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Summary

This work dealt with *Hyalesthes obsoletus*, which is the only known vector of Bois noir disease in grapevine. Investigation centered on its flight activity and occurrence on bindweed and stinging nettle and, to a lesser extent, hedge bindweed and dead-nettle in North-Eastern Italy. The survey underlined the importance of bindweed in the insect lifecycle in Northern Italy, where only data from stinging nettle had previously been recorded. *H. obsoletus* nymphs were found for the first time on the roots of dead-nettle, which proved to be a new host plant. The first observation in Italy of nymphs on hedge bindweed roots was also recorded.

K e y w o r d s : bindweed, *Bois noir*, *Convolvulus arvensis, Calystegia sepium*, flight activity, *Lamium orvala*, nettle, *Urtica dioica*.

Introduction

The planthopper *Hyalesthes obsoletus* Signoret (Homoptera: Cixiidae) is a Palaearctic species, widespread in Central and Southern Europe, in the Near East and in the Mediterranean area (Hoch and Remane 1985). It is a polyphagous and univoltine insect that feeds on a variety of herbaceous plants. However, it showed regional differences in its affiliation to particular host plants and it selects preferred host plant species for development and reproduction. In Germany and France the planthopper seemed to prefer bindweed (*Convolvulus arvensis* L.) to reproduce (Maixner 1994, Sforza *et al.* 1998, Langer *et al.* 2003), though recently stinging nettle (*Urtica dioica* L.) has started to become the predominant host plant species (Maixner *et al.* 2006, Bressan *et al.* 2007). In Italy, however, the whole lifecycle was observed mainly on *U. dioica* (Alma *et al.* 2002, Lessio *et al.* 2007), though adults were found on many plants, including bindweed (Credi *et al.* 2006).

H. obsoletus is the only known vector of *Bois noir* (BN), an endemic grapevine yellow disease that occurs throughout Europe and in the Mediterranean basin. BN is caused by phytoplasmas from the stolbur group (16SrXII-A), which has a broad host range. Three different BN isolates were found which are distinguishable by molecular traits as well as by biological characteristics, such as the association with different natural host plants: the VKI isolate is associated with stinging nettle, the VKII with bindweed, while the VKIII was found in hedge bindweed (*Calystegia sepium* L.) (Langer and Maixner 2004). *H. obsoletus* nymphs can acquire the stolbur phytoplasma by sucking on the roots of infected host plants, enabling the adults to transmit it subsequently to grapevine (Maixner 1994, Sforza *et al.* 1998, Alma *et al.* 2002, Langer *et al.* 2003). These plants often occur in vineyards and at their borders and act as dangerous reservoirs for the disease (Sforza *et al.* 1998, Langer and Maixner 2004, Mori *et al.* 2008).

The vineyard control strategies currently used are ineffective in vineyards, as the insect feeds occasionally on grapevine. Thus BN threatens both commercial vineyards and grapevine nurseries, especially in countries like Italy and Germany, where the occurrence of BN has increased over the last few years (Maixner *et al.* 2006, Lessio *et al.* 2007).

This work focused on the presence of *H. obsoletus* on weeds, in particular bindweed and stinging nettle, growing in some vineyards in the province of Treviso (North-Eastern Italy), and took into account the flight activity of adults and the presence of nymphs on the roots.

Material and Methods

The survey was carried out over three years, 2006, 2007 and 2008, in five selected vineyards with BN infected grapevines and in one nursery in the province of Treviso, in North-Eastern Italy (45° 40′ N, 12° 15′ E). The vineyards were treated at least one time per year - in mid-July - using insecticides for the control of leafhoppers, most of them containing phosphoric esters. The insecticides were applied on grapevine leaves and trunk, but not on the weeds. All vineyards had natural green cover; only grass and weeds in the rows were regularly removed by herbicide or mechanical control. Regular mowing of the grass and weeds was performed inside and outside the vineyards.

The flight activity of *H. obsoletus* adults on weeds was monitored using sticky traps (20 cm X 24.5 cm, glue on both sides, Serbios s.r.l.). The traps were placed at 20 cm above the ground and replaced every 15 days, from July to October in 2006, from April to October in 2007 and from May to September in 2008. The traps were distributed according to the abundance of selected wild plants and were

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placed in the middle of the host plants, inside the vineyards and outside at their borders. The wild plants were selected according to their importance in BN transmission (bindweed, stinging nettle and hedge bindweed) and to previous personal observations (*Lamium orvala* L.). In 2006, 23 traps were set: 20 on bindweed (19 in vineyards and one outside), two on stinging nettle (one in the vineyard and one outside) and one on dead-nettle (*L. orvala*) outside the nursery. In 2007, 29 traps were placed: 17 on bindweed (ten in vineyards and seven outside), ten on stinging nettle (three in vineyards and seven outside) and two on dead-nettle outside the nursery. In 2008, 48 traps were set: 29 on bindweed (26 in vineyards and three outside the nursery), 15 on stinging nettle (six in vineyards and nine outside) and four on hedge bindweed in vineyards. In total, 703 traps were analyzed over the three years: 478 spread within the vineyards and 225 outside.

During 2007 and 2008, the sweep net was used every 15 d to monitor *H. obsoletus* on bindweed and stinging nettle in the 5 vineyards. In each vineyard the weeds were swept 50 times and the specimens captured were collected every five sweeps. The *H. obsoletus* specimens collected by traps were counted and divided by sex using a stereomicroscope.

The presence of *H. obsoletus* nymphs was checked in May 2007 and 2008 by means of visual inspection of the roots of 14 stinging nettles, five bindweeds, one hedge bindweed and one dead-nettle. The statistical analyses of the data were carried out using the non-parametric Mann-Whitney-U-test.

Results and Discussion

The vineyards surveyed had previously shown a high occurrence of stolbur phytoplasma in grapevine and in the weeds. In particular, occurrence of BN in the vineyards ranged between 2 and 20 %; bindweed, hedge bindweed and stinging nettle plants infected with stolbur phytoplasmas were found inside and/or at the border of the five vineyards investigated. Moreover, previous analyses on a few *H. obsoletus* specimens collected in three of the five vineyards have shown that some of them were stolbur-infected. Thus, a work focused on the presence of the vector on the weeds started. Bindweed and stinging nettle were investigated because of their key role in the spreading of phytoplasma associated with BN (Sforza *et al.* 1998, Alma *et al.* 2002, Langer and Maixner 2004, Bressan *et al.* 2007, Mori *et al.* 2008). Hedge bindweed and dead-nettle were studied because of their wide occurrence in the monitored areas; hedge bindweed is also known to be infected with the phytoplasma associated with BN (Langer and Maixner 2004).

The sex ratio of *H. obsoletus* on traps recorded in 2007 and 2008 in hedge bindweed was 1:1, while it always had a 3:1 male-bias in bindweed, stinging nettle and dead-nettle, in agreement with previous findings (Weber and Maixner 1998, Lessio *et al.* 2007). This could be explained by the higher flight activity of males, since the sex ratio of *H. obsoletus* collected by sweep-net is usually balanced (Weber and Maixner 1998).

The mean number of *H. obsoletus* specimens captured on stinging nettle was statistically different between traps placed inside (2.82 ± 4.81 sp./trap) and outside (1.02 ± 3.42 sp./trap) the vineyards during the three years ($p < 0.0001$) (Table). Also in the case of bindweed the difference was significant during the three years ($p < 0.0001$), being the mean number of *H. obsoletus* specimens caught inside the vineyards (0.93 ± 2.45 sp./trap) ten times higher than that observed outside (0.09 ± 0.45 sp./trap). This means that insecticide treatments on grapevines - mandatory in these areas for the control of the leafhopper *Scaphoideus titanus* - and regular mowing of the grass were not sufficient to reduce the *H. obsoletus* population inside the vineyards. It is therefore of critical importance to monitor the host plants outside and inside the vineyards, in order to know the spreading of the vector and the disease in the two ecosystems and to apply more adequate control strategies.

The trend of the flights monitored by the traps was similar over the three years (Figure). In the first year, the traps were exposed when the flight had already started. In each year, two different flight peaks were noticed, associated with different host plants: the first was observed on *C. arvensis* and *C. sepium* and the second approximately one month later on *U. dioica* and *L. orvala*. The captures

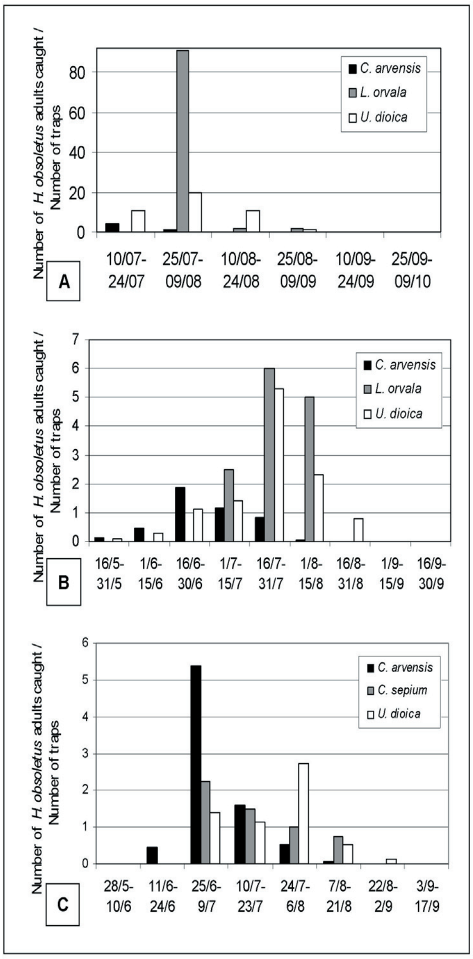

Figure: Number of *H. obsoletus* specimens per trap, caught in 2006 (A), 2007 (B) and 2008 (C) on different host plants.

Table

Mean number (± standard deviation) of *H. obsoletus* specimens per trap, captured on bindweed and stinging nettle inside and outside the vineyards in 2006, 2007 and 2008. Results of the statistical test (Mann-Whitney-U-test) are reported. NS: non significant

Year	Bindweed			Stinging nettle		
	Inside vineyards	Outside vineyards	Mann-Whitney-U-test	Inside vineyards	Outside vineyards	Mann-Whitney-U-test
2006	0.89 ± 2.34	0.00	NS	1.00 ± 1.67	4.38 ± 8.87	NS
2007	0.69 ± 1.53	0.14 ± 0.54	p<0.005	1.93 ± 3.34	0.97 ± 2.82	p < 0.005
2008	1.09 ± 2.90	0.00	p<0.01	3.91 ± 5.95	0.49 ± 1.51	p < 0.005

with the sweep net confirmed the same trend (data not shown). Traps are commonly used to study the flight of *H. obsoletus* (Bressan *et al.* 2007); the results of this work showed that they can provide reliable data on the flights of *H. obsoletus* if they are placed in the middle of the host plants. On the whole, the results showed wide occurrence of *H. obsoletus* on bindweed and highlighted the important role of this host plant in Italy, thus confirming recent observation (Riolo *et al.* 2007, Mori *et al.* 2008). The flight activity on stinging nettle was in agreement with that recorded in other regions (Bertaccini *et al.* 2003, Maixner *et al.* 2006, Lessio *et al.* 2007), taking into account the influence of the year and the latitude. The data on the flight activity on dead-nettle and hedge bindweed was very interesting because, to our knowledge, it was the first time they have been recorded.

During 2006, a single trap placed in the middle of *L. orvala* captured more than 90 specimens in the second part of July (Figure, A). As this species had not previously been recorded as a preferred host plant of *H. obsoletus*, the plant was uprooted in the following spring and its roots were inspected, together with the roots from stinging nettle, bindweed and hedge bindweed, collected where *H. obsoletus* had previously been captured. On two stinging nettle plants, three and 22 *H. obsoletus* nymphs were counted respectively, four on one bindweed, nine on one hedge bindweed and 15 on one dead-nettle; the other plants inspected did not host any nymphs. In spring 2008, the roots of ten stinging nettles were observed: on three plants the number of *H. obsoletus* nymphs was 30, three and one, respectively. These inspections revealed a new host plant for *H. obsoletus* nymphs: the dead-nettle, never previously recorded, and the first observation of nymphs on hedge bindweed roots in Italy. The presence of *H. obsoletus* nymphs on the roots of these two species confirmed that they are a host for the vector in the regions investigated and suggested that dead-nettle and hedge bindweed could play an important role in the spread of the insect in the province of Treviso, possibly together with that of the phytoplasma associated with BN given their high occurrence. In particular, studies need to be performed on the presence of the phytoplasma on *L. orvala*, as no data has been recorded up to the present time. Moreover, the finding of nymphs on bindweed roots demonstrated, once again, the importance of this plant species in the ecology of the insect in Italy. The lifecycle of *H. obsoletus* on *C. arvensis* had only previously been demonstrated in Italy under laboratory conditions (Milanesi *et al.* 2005), while in nature only two nymphs had been observed in the roots of one bindweed out of a total collection of 228 (Credi *et al.* 2006).

In conclusion, this work showed that in the investigated regions the number of *H. obsoletus* specimens captured inside the vineyards was significantly higher than that observed outside. The high occurrence of *H. obsoletus* adults and nymphs on bindweed allowed to confirm the whole lifecycle on this host plant in the investigated regions of North-Eastern Italy, differently from North-Western Italy, where the leafhopper was found only on stinging nettle. The discovery of a new plant, *L. orvala*, which can be a host to nymphs and adults, was reported for the first time. The data on flight activity of the insect on stinging nettle and bindweed confirmed the observation carried out in other European regions, while the flight activity on dead-nettle and hedge bindweed was reported for the first time. The observation of nymphs on the roots of stinging nettle and bindweed confirmed the important role of these plants as a host. Moreover, the first observation of nymphs in hedge bindweed roots in Italy was reported. Indeed, all the information gained from this work will be useful in the planning of future control strategies against the spreading of BN and its insect vector in vineyards.

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