Host plant preferences of *Hyalesthes obsoletus*, the vector of the grapevine yellows disease 'bois noir', in Switzerland

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Abstract

Bois noir is an important grapevine yellows disease in Europe that can cause serious economic losses in grapevine production. It is caused by stolbur phytoplasma strains of the taxonomic group 16Sr-XII-A. *Hyalesthes obsoletus* Signoret (Hemiptera: Cixiidae) is the most important vector of bois noir in Europe. This polyphagous planthopper is assumed to mainly use stinging nettle [*Urtica dioica* L. (Urticaceae)] and field bindweed [*Convolvulus arvensis* L. (Convolvulaceae)] as its host plants. For a better understanding of the epidemiology of bois noir in Switzerland, host plant preferences of *H. obsoletus* were studied in the field and in the laboratory. In vineyards of Western Switzerland, adults of *H. obsoletus* were primarily captured on *U. dioica*, but a few specimens were also caught on *C. arvensis*, hedge bindweed [*Calystegia sepium* (L.) R. Brown (Convolvulaceae)], and five other dicotyledons [i.e., *Clematis vitalba* L. (Ranunculaceae), *Lepidium draba* L. (Brassicaceae), *Plantago lanceolata* L. (Plantaginaceae), *Polygonum aviculare* L. (Polygonaceae), and *Taraxacum officinale* Weber (Asteraceae)]. The preference of the vector for *U. dioica* compared to *C. arvensis* was confirmed by a second, more targeted field study and by the positioning of emergence traps above the two plant species. Two-choice experiments in the laboratory showed that *H. obsoletus* adults originating from *U. dioica* preferred to feed and to oviposit on *U. dioica* compared to *C. arvensis*. However, *H. obsoletus* nymphs showed no host plant preference, even though they developed much better on *U. dioica* than on *C. arvensis*. Similarly, adults survived significantly longer on *U. dioica* than on *C. arvensis* or any other plant species tested [i.e., *L. draba* and *Lavandula angustifolia* Mill. (Lamiaceae)]. In conclusion, although nymphs of *H. obsoletus* had no inherent host plant preference, adults tested preferred to feed and oviposit on *U. dioica*, which is in agreement with the observed superior performance of both nymphal and adult stages on this plant species. *Urtica dioica* appears to be the principal host plant of *H. obsoletus* in Switzerland and plays therefore an important role in the epidemiology of the bois noir disease in Swiss vineyards.

Introduction

Bois noir is an important grapevine yellows disease in Europe that can cause serious economic losses in the production of grapevine, *Vitis vinifera* L. (Vitaceae) (Caudwell,

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1961). Disease symptoms include downward-rolled margins, interveinal reddening or yellowing of the leaves, deficient shoot lignification, dried inflorescences or, in later infections, shrivelled bunches (Schaerer et al., 2007). Bois noir is provoked by a phytoplasma. Phytoplasmas are phloem-restricted plant pathogenic bacteria lacking a cell wall, usually transmitted by phloem-sucking hemipterous insects such as leafhoppers, planthoppers, and psyllids (Weintraub & Beanland, 2006). The epidemiology of such

phytoplasma-caused diseases is therefore largely determined by the number of insect vectors and their host plant ranges (Lee et al., 1998). Bois noir belongs to phytoplasmas of the stolbur 16Sr-XII-A group (Lee et al., 1998). Besides grapevine, the stolbur 16Sr-XII-A group infects a wide range of other cultivated and wild plant species, such as potato, tomato, eggplant, pepper, tobacco, celery, carrot, strawberry, beet, maize, common lavender [*Lavandula angustifolia* Mill. (Lamiaceae)], stinging nettle [*Urtica dioica* L. (Urticaceae)], field bindweed [*Convolvulus arvensis* L. (Convolvulaceae)], hedge bindweed [*Calystegia sepium* (L.) R. Brown (Convolvulaceae)], and hoary cress [*Lepidium draba* L. (Brassicaceae)] (Valenta et al., 1961; Fos et al., 1992; Marzachi et al., 1999; Del Serrone et al., 2001; Gatineau et al., 2001; Langer & Maixner, 2004; Terlizzi et al., 2006; Jovic et al., 2007; Carraro et al., 2008; Duduk et al., 2008). Overall, stolbur phytoplasmas infect over 77 plant species belonging to at least 14 families (Sforza, 1998).

The polyphagous and univoltine planthopper *H. obsoletus* Signoret (Hemiptera: Cixiidae) is assumed to be the most important vector of bois noir (Maixner, 1994; Sforza et al., 1998). Phytoplasma cells are usually acquired by *H. obsoletus* nymphs while feeding and developing on the roots of their host plant in the soil (Kaul et al., 2009). Only the adult stage lives above ground, and adults transmit the acquired phytoplasmas when sucking from the phloem of other plants. In Central Europe, adults commonly emerge in June and fly until the end of August (Kehrli et al., 2010). Over the summer, mated females lay their eggs in the soil at the base of their host plants (Bressan et al., 2007). *Convolvulus arvensis* and *U. dioica* are generally considered to be *H. obsoletus*' primary host plants (Sforza et al., 1999; Bressan et al., 2007; Lessio et al., 2007), and grapevine is only an erroneous food source, on which nymphal development does not take place (Sforza, 1998; Johannesen et al., 2008). Nonetheless, *H. obsoletus* nymphs can develop on various other herbaceous plant species. In France, insects prefer to develop on *C. sepium*, *C. arvensis*, *L. angustifolia*, *L. draba*, and *U. dioica*, but adults were also captured on *Plantago cynops* L. (Plantaginaceae), *Linaria striata* L. (Scrophulariaceae), *Galium verum* L. (Rubiaceae), and *Satureia montana* L. (Lamiaceae) (Sforza et al., 1999). In Germany, *C. arvensis* and *U. dioica* are the most important host plants of *H. obsoletus*, but nymphs can sometimes also develop on *C. sepium* and *Ranunculus bulbosus* L. (Ranunculaceae) (Weber & Maixner, 1998; Langer et al., 2003). In Italy, adults have been observed on *Ambrosia artemisiifolia* L., *Artemisia vulgaris* L., *Tanacetum vulgare* L. (all Asteraceae), *C. sepium*, *C. arvensis*, *Lamium orvala* L. (Lamiaceae), and *U. dioica*, but nymphs develop only on the last four plant species (Alma et al., 2002; Lessio et al., 2007; Picciau et al., 2008;

Forte et al., 2010). In Israel, *Vitex agnus-castus* L. (Lamiaceae) was identified as the favourite host plant of *H. obsoletus*, but adults were also caught on *Amaranthus retroflexus* L. (Amaranthaceae), *C. arvensis*, *Myrtus communis* L. (Myrtaceae), *Olea europaea* L. (Oleaceae), and *V. vinifera* (Sharon et al., 2005). In summary, *C. arvensis* was the favourite host plant of *H. obsoletus* in Central Europe until the hot and dry summer of 2003 (Boudon-Padieu & Maixner, 2007; Maixner et al., 2007; Kast & Stark-Urnau, 2009). Since then, rising populations of *H. obsoletus* have been found on *U. dioica*, previously a neglected host plant species.

Even though bois noir has been present in Switzerland for more than 20 years (Schmid & Emery, 2001), the interaction between phytoplasma, host plants, insect vector, and grapevine is poorly understood. To gain more insight into the epidemiology of the disease, we investigated the abundance of *H. obsoletus* in Swiss vineyards on various potential host plant species, using yellow sticky traps, emergency traps, and a vacuum insect collector. In a first step, we sampled a range of plant species in five conventional, bois noir-infected vineyards, hypothesising that *U. dioica* and *C. arvensis* are favourite host plants of *H. obsoletus*. After establishing that these two plant species are also important host plants for the vector in Swiss vineyards, we thereupon examined the abundance of *H. obsoletus* on *U. dioica* and *C. arvensis* in two more targeted field studies to identify the vector's primary host plant. To better understand the biology of *H. obsoletus*, we complemented our field observations with two laboratory experiments under standardised conditions. First, host plant preferences of *H. obsoletus* adults and nymphs were determined in two-choice trials. Second, adult survival and nymphal development were assessed on potted plant species in a climatic chamber.

Materials and methods

Survey of potential host plants

Plant preferences of *H. obsoletus* adults were studied using yellow sticky traps and a sucking sampler in five vineyards of Western Switzerland, from the end of May until mid-September 2008. Three of the five vineyards were located in Flanthey (46°15.95′N, 7°27.40′E), Noës (46°16.61′N, 7°29.91′E), and Salgesch (46°18.83′N, 7°34.60′E), in the Valais. The other two vineyards were situated in Vallamand (46°55.35′N, 7°1.74′E) and Le Landeron (47°3.57′N, 7°3.32′E), in the Three Lake area. In the three vineyards in the Valais, 2–6% of all grapevine plants were infected with bois noir in autumn 2007, and in Vallamand and Le Landeron, the infection rate was around 15 and 30%, respectively.

In the Three Lake area, yellow sticky traps (10 × 25 cm; Aeroxon Insect Control, Weiblingen, Germany) were placed above *C. sepium*, *C. arvensis*, and *U. dioica*. In Valais, sticky traps were set up above *Clematis vitalba* L. (Ranunculaceae), *C. arvensis,* and *L. draba*. Traps were tacked to a wooden stick 10 cm above the vegetation. Yellow sticky traps were exchanged weekly from 22 May to 25 September 2008, and the number of *H. obsoletus* captured per trap was determined.

Sucking samples were taken with a D-vac vacuum insect collector (Stihl® SH 85C; Andreas Stihl, Weiblingen, Germany) on *A. retroflexus*, *C. sepium*, *C. vitalba*, *C. arvensis*, *Ononis pusilla* L. (Fabaceae), *Plantago lanceolata* L. (Plantaginaceae), *Polygonum aviculare* L. (Polygonaceae), *Solanum nigrum* L. (Solanaceae), *Taraxacum officinale* Weber (Asteraceae), *U. dioica*, and *V. vinifera*. However, none of these plant species sampled, except for grapevine, grew in all five vineyards. For example, *U. dioica* was only present in the two vineyards of the Three Lake area and *C. sepium* was only found at Vallamand. At all five locations, a plant species was sampled for a period of altogether 5 min. Over this period, numerous plants of a single species were sampled across the whole vineyard and each individual was vacuumed for about 5 s. Sucking samples were taken fortnightly from 25 June to 19 September 2008. Samples were frozen in a conventional freezer to kill the collected fauna and to determine the number of *H. obsoletus* captured.

Adult vector populations on *Urtica dioica* and *Convolvulus arvensis*

To specifically study the abundance of *H. obsoletus* adults on *U. dioica* and *C. arvensis*, a survey was carried out in eight bois noir-diseased vineyards of Western Switzerland and the Ticino. Both plant species were present in the vineyards sampled. The eight vineyards were visited once between 26 June and 8 July 2009, and numerous *U. dioica* and *C. arvensis* plants were sampled within each vineyard for a period of altogether 5 min using a D-vac vacuum insect collector. Once again, plants were sampled across the whole vineyard and an individual was vacuumed for 5 s. Samples were frozen, and the number of *H. obsoletus* adults captured was recorded.

Origin of emergence

To study the importance of *U. dioica* and *C. arvensis* for the development of *H. obsoletus* nymphs, emergence traps (photo-eclectors) with a surface area of 0.28 m^2 were placed directly above patches of the two potential host plants of at least 1 m^2. Three photo-eclectors per plant species were set up in the beginning of June 2009 in a commercial vineyard in Russin (46°11.01′N, 6°0.75′E), about 10 km west of Geneva (Switzerland). Photo-eclectors were checked weekly from 16 June to 10 August 2009, the observed period of the emergence of nymphs.

Cultivation of host plants and laboratory rearing of *Hyalesthes obsoletus*

Convolvulus arvensis, *L. angustifolia*, *L. draba*, and *U. dioica* plants were grown from seeds in pots (12 cm diameter) filled with a peat-based standard substrate containing a slow-release fertilizer in a heated greenhouse. *Hyalesthes obsoletus* adults were captured on a natural *U. dioica* stand in Russin. This founder colony, as well as the following laboratory-born generations, was all reared on potted *U. dioica* in a growth chamber at 25 ± 1 °C, 70 ± 10% r.h., L18:D6 photocycle, and daylight-equivalent light spectra. Potted *U. dioica* plants were exposed to adults of *H. obsoletus* within transparent plastic cages (33 × 44 × 61 cm), and females deposited their eggs in the substrate. After circa 1 month, nymphs hatched and developed above ground at the base of *U. dioica*. The first adults emerged about 2 months later, and they were transferred to a new cage, in which they founded the next generation. However, it should be noted that nymphal mortality was high and that only a small part of the deposited eggs developed into adults.

Host plant preferences in the laboratory

To complement our field observations, host plant preferences of *H. obsoletus* adults and nymphs were studied in two-choice experiments in the laboratory. In the first two-choice experiment, all five nymphal stages of *H. obsoletus* were exposed individually to similar-sized single roots of *C. arvensis* and *U. dioica* of around 6 cm length. Roots were placed at opposite sides in cylindrical Petri dishes (10 cm diameter, 5.5 cm high), which were filled with a 2-cm layer of plaster of Paris. Each nymph was released in the centre of the Petri dish. A wet cotton plug and a covered hole in the dish's lid provided adequate humidification and ventilation. Dishes were stored under controlled conditions in a second growth chamber, at 23 ± 1 °C, 70 ± 10% r.h., and 24-h darkness. The location of live nymphs in the dishes was recorded 1 day after their initial exposure. Eighteen to 32 individuals were tested individually per nymphal stage, and the trials took place between March and May 2009.

In the second two-choice experiment, *H. obsoletus* adults were exposed to entire *C. arvensis* and *U. dioica* plants. Fifteen couples of *H. obsoletus* were kept in the same cages, the same growth chamber, and under the same conditions as previously described for the laboratory rearing of *H. obsoletus*. Each cage contained one potted *C. arvensis* plant and one potted *U. dioica* plant, both of similar size. The location of live insects was recorded once,

2–3 days after the initial exposure. About 3 weeks later, the eggs and nymphs per test plant were counted. The experiment was repeated six times between July 2008 and February 2009, four replicates were conducted each with 15 males and 15 females captured in the field, and the other two each with 15 couples originating from the laboratory rearing.

Development and survival on host plants in the laboratory

In addition to the vector's host plant preference, the survival and development of *H. obsoletus* were studied on a few potential host plant species. Insects and plants were grown in the laboratory as described earlier. In the first experiment, the development of *H. obsoletus* nymphs was tested on *C. arvensis*, *L. angustifolia*, and *U. dioica*. Insects were placed on a potted plant of one of the three species. Thereafter, a plastic cylinder (11 cm diameter, 27 cm tall), sealed with a gauze mesh at the top, was placed over the plant. Plants were incubated in a growth chamber at 23 ± 1 °C, 70 ± 10% r.h., L18:D6 photocycle, and daylight-equivalent light spectra. The emergence of adults was checked weekly. Overall, the experiment was repeated 10 times, 3× with a mixture of altogether 50 eggs and first instars each, 4× with a mixture of altogether 50 second and third instars each, and 3× with a mixture of altogether five-fourth and fifth instars each. The experiment took place between August 2008 and May 2009.

In the second performance experiment, the survival of *H. obsoletus* adults was tested on *C. arvensis*, *L. angustifolia*, *L. draba*, and *U. dioica* in the chamber for the laboratory rearing of *H. obsoletus*. Using the same plastic cylinders, freshly emerged adults from the laboratory rearing were individually placed on a single plant species. Insects were checked daily, and the survival of each individual was recorded until death. Between February and May 2009, a total of 10 males and 10 females were tested per plant species.

Statistical analysis

No statistical comparisons were made on the field study identifying potential host plants of *H. obsoletus* because none of the plant species sampled, except for grapevine, grew in all five vineyards. Thus, plant species and sampling locations were partially confounded. Data were therefore analysed descriptively. The comparison between the abundance of vectors on *U. dioica* and *C. arvensis* was analysed by a Wilcoxon signed rank test. Because of the limited number of photo-eclectors set-up per plant species, no statistical comparison was made on the study determining the origin of emergence of *H. obsoletus*. Data were analysed only descriptively.

To test for host plant preferences of *H. obsoletus*, both two-choice experiments were analysed with replicated G-tests for goodness-of-fit. For each nymphal stage, the total number of individuals found on the roots of *C. arvensis* was compared to that found on *U. dioica* roots. In the second two-choice experiment, the number of adults and the number of eggs deposited per plant were compared between the two plant species tested.

The development of *H. obsoletus* on host plants was analysed by a generalised linear model with binominal error distribution and a logit link. The number of emerging adults in relation to the number of unemerged nymphs was treated as the dependent variable, whereas plant species and nymphal instar were used as fixed factors. The experiment testing for the survival of adults on different plants was analysed by a 2-way ANOVA, where the longevity of adults was treated as the dependent variable and the sex of planthoppers and plant species served as fixed factors. The ANOVA was of type III, and means of the various plants species sampled were pairwise compared by Bonferroni post-hoc tests.

Results

Survey of potential host plants

Over the complete field season 2008, 132 *H. obsoletus* adults were captured on yellow sticky traps in the two vineyards of the Three Lake area, whereas only 14 specimens were trapped in the Valais. More adults per sample were captured in traps placed above *U. dioica* than in those placed above *C. sepium*, *L. draba*, *C. vitalba*, or *C. arvensis* (Figure 1A).

A total of 424 *H. obsoletus* adults were identified in the sucking samples, 422 of which were captured in the Three Lake area and just two in the Valais, where *U. dioica* was absent. Altogether, 380 *H. obsoletus* adults were captured on *U. dioica*, whereas only 22 individuals were found on *C. arvensis*, 11 on *C. sepium*, five on *P. aviculare*, four on *T. officinale*, and one on *P. lanceolata* and *C. vitalba* (Figure 1B). Not a single individual of *H. obsoletus* was caught on *A. retroflexus*, *O. pusilla S. nigrum*, or *V. vinifera*. Overall, both sampling techniques indicate that *H. obsoletus* adults are more abundant on *U. dioica* than on any other plant species tested.

Adult vector populations on *Urtica dioica* and *Convolvulus arvensis*

Significantly, more adults were aspirated from *U. dioica* than from *C. arvensis* (Z = −2.38, P = 0.017). Overall, 1 203 specimens were captured on *U. dioica* and only 11 on *C. arvensis*. The survey thus confirms that *H. obsoletus* adults are more abundant on *U. dioica* than on *C. arvensis* in Western Switzerland.
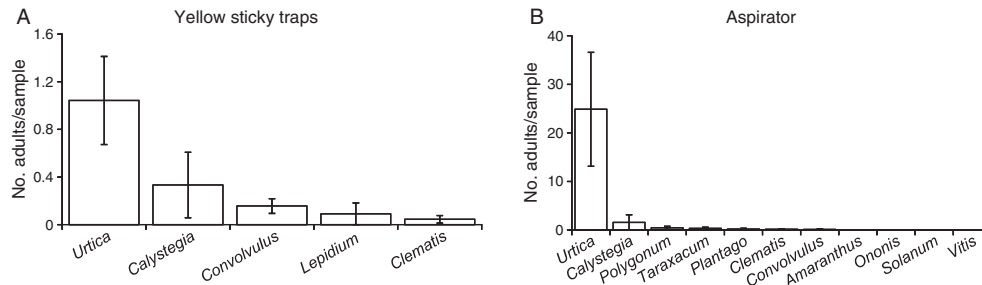
Figure 1 Mean (± SE) number of *Hyalesthes obsoletus* adults captured per sample on the various plant species (genus names refer to plant species tested) with (A) yellow sticky traps, or (B) the vacuum insect collector in five vineyards in Switzerland.
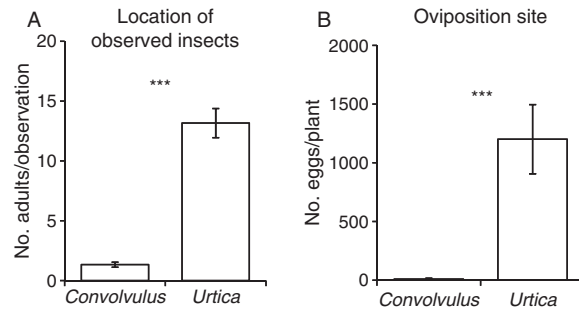
Origin of emergence

Just one *H. obsoletus* adult emerged from the three photo-eclectors positioned above *C. arvensis*, whereas 80, 16, and 0 individuals were trapped by the three photo-eclectors placed above *U. dioica*, indicating an aggregated distribution of nymphs.

Host plant preferences in the laboratory

In the first two-choice experiment, *H. obsoletus* nymphs showed no plant preference (G = 6.76, d.f. = 5, P = 0.24). After 1 day of exposure, about the same proportion of nymphs was found on roots of *U. dioica* and *C. arvensis* (Table 1). This was true for all instars, except for the fifth, which tended to prefer *U. dioica* roots.

In contrast, adults of *H. obsoletus*, which were tested in the second two-choice experiment, were observed significantly more frequently on *U. dioica* than on *C. arvensis* (G = 68.90, d.f. = 6, P<0.001; Figure 2A). Similarly, females deposited significantly more eggs on *U. dioica* (G = 9 538.37, d.f. = 6, P<0.001; Figure 2B). Hence, the

Table 1 Host plant preferences of the five *Hyalesthes obsoletus* instars in the two-choice experiment on *Convolvulus arvensis* and *Urtica dioica*. Until their exposure, all nymphs developed on *U. dioica*. All nymphs were tested individually, and the number of nymphs that showed no preferences (were not on roots) is indicated by the difference between the number of nymphs tested and the sum of nymphs found on the roots of the two plant species. P corresponds to the probability calculated by replicated G-tests for goodness-of-fit

		No. nymphs on		
Instar	No. individually tested nymphs	*C. arvensis* roots	*U. dioica* roots	P
N1	24	8	5	0.40
N2	18	7	7	1.0
N3	31	11	15	0.43
N4	21	13	7	0.18
N5	32	7	16	0.06

Figure 2 (A) Location and (B) oviposition preferences of *Hyalesthes obsoletus* adults in the two-choice experiment on *Convolvulus arvensis* and *Urtica dioica*. Indicated are mean values ± SE (n = 6). Replicated G-tests for goodness-of-fit: ***P<0.001.

two-two-choice experiments reveal that nymphs have no innate host plant preference and that the preference to feed and oviposit on *U. dioica* establishes shortly before or after adult emergence.
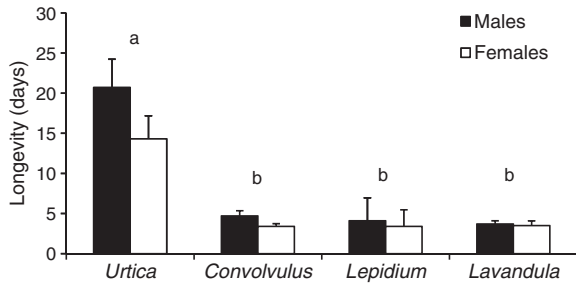
Development and survival on different host plants in the laboratory

A total of 29 *H. obsoletus* adults emerged from the 365 eggs and nymphs placed on *U. dioica*, but only one individual completed its development on *C. arvensis* and none on *L. angustifolia* (Table 2). The difference between the three plant species tested was significant (χ^2 = 64.93, d.f. = 2, P<0.001). In addition, a significant difference was found among the development of the three groups of instars tested (χ^2 = 29.90, d.f. = 2, P<0.001; Table 2), with intermediate instars (i.e., N2 and N3) having a significantly lower proportion of individuals developing into adults.

There was a significant difference between the survival of *H. obsoletus* adults on the four plant species tested ($F_{3,72}$ = 32.53, P<0.001; Figure 3). Adults reared on *U. dioica* survived significantly longer on *U. dioica* than on *C. arvensis*, *L. angustifolia*, or *L. draba*. Average longevity on *U. dioica* was 17.5 days, with some insects living up to 41 days. Males tended to live longer than females

Table 2 Nymphal development of *Hyales-thes obsoletus* on *Convolvulus arvensis*, *Lavandula angustifolia*, and *Urtica dioica*. Before being placed on the experimental plants, all nymphs had developed on *U. dioica*

Developmental instar	No. nymphs exposed per plant species	Total no. adults emerged from		
		C. arvensis	*L. angustifolia*	*U. dioica*
Eggs and N1	3 × 50	0	0	21
N2 and N3	4 × 50	1	0	3
N4 and N5	3 × 5	0	0	5

Figure 3 Mean (+ SE; n = 10) longevity of adult *Hyalesthes obsoletus* on *Convolvulus arvensis*, *Lavandula angustifolia*, *Lepidium draba*, and *Urtica dioica* (genus names refer to plant species tested). Longevity on plant species with different letters was significantly different (2-way ANOVA, followed by Bonferroni post-hoc test: P<0.05).

($F_{1,72}$ = 3.20, P = 0.078), and there was no significant interaction between plant species and sex ($F_{3,72}$ = 1.43, P = 0.24; Figure 3). Thus, our two performance experiments showed that *H. obsoletus* nymphs reared on *U. dioica* developed significantly better on *U. dioica* and that adults survived significantly longer on *U. dioica* than on any other plant species tested.

Discussion

Our studies revealed that *U. dioica* is the most important host plant of *H. obsoletus* in Swiss vineyards. Indeed, we captured significantly more adults on *U. dioica* than on any other plant species examined, and nymphs were found to develop primarily on *U. dioica*. This finding was confirmed by the laboratory experiments. Nymphs developed better on *U. dioica* and adults survived significantly longer on that plant, compared to any other plant species tested. Given the choice, adults also preferred to feed and oviposit on *U. dioica*, whereas nymphs showed no preference.

As in neighbouring countries, *U. dioica* is currently the predominant host plant of *H. obsoletus* in Swiss vineyards (Langer et al., 2003; Bressan et al., 2007; Lessio et al., 2007; Picciau et al., 2008; Kuntzmann et al., 2009). Nonetheless, a few individuals were also found to develop or at least feed on other dicotyledons, in particular on *C. arven-*

sis and *C. sepium*. This was also evident from the fact that molecular analyses showed that *H. obsoletus* adults as well as grapevines do not only harbour the stolbur strain tuf-type-I, which is associated with *U. dioica*, but also the stolbur strain tuf-type-II associated with *C. sepium* and *C. arvensis* (S Schaerer, unpubl. data). Future molecular analyses should help to completely untangle and quantify the importance and contribution of the various plant species for the epidemiology of bois noir in Swiss vineyards. However, at present, we can confidently conclude that *U. dioica* is the preferred host plant of *H. obsoletus* in most parts of Switzerland (Kehrli et al., 2010). Our laboratory experiments on host plant preferences and performance of *H. obsoletus* further substantiate this conclusion. Adults emerging from *U. dioica* clearly preferred *U. dioica* over *C. arvensis*. They not only fed but also deposited their eggs exclusively on this plant species. A similar preference of *H. obsoletus* adults for the plant species on which they developed was also observed by Sharon et al. (2005). Using an olfactometer and electroantennograms, the authors revealed that adults clearly preferred their original host plants *V. agnus-castus* over grapevine and *C. arvensis*.

It is generally accepted that associative learning of chemical and physical cues can influence feeding and oviposition behaviour in adult insects (Papaj & Prokopy, 1989; Barron, 2001). However, insects often establish such plant affiliations shortly before or after adult emergence (Jaenike, 1983; Corbet, 1985; Barron & Corbet, 1999). This is in accordance with our own experiments. Only the last nymphal instar tended to prefer the host plant on which it was reared. The first four instars showed no clear plant preference, even though they developed better on *U. dioica*, the host plant on which they and their parents were reared. *Convolvulus arvensis* and *L. angustifolia*, two important host plants in France (Sforza et al., 1999), were completely unsuitable for nymphal development. Except for one individual, no adult emerged from the more than 700 nymphs placed on these two plant species. Similarly, longevity of *H. obsoletus* adults was almost 4× higher on *U. dioica* than on any other plant species tested. This finding is in accordance with previous studies (Lessio et al., 2007; Mori et al., 2008; Maixner et al., 2009). The two latter studies even tested field-collected adults from

C. arvensis and *U. dioica* and it was shown that both *H. obsoletus* populations lived significantly longer on their original host plant species. Similarly, our attempts to permanently rear *H. obsoletus* collected from *U. dioica* on another host plant failed. Overall, this indicates that *H. obsoletus* is strongly adapted to its original host plant species (Maixner et al., 2009). This implies that *H. obsoletus*' plant affiliation is not only based on associative learning of chemical and/or physical cues, but that it is also determined by genetic factors. Indications of such genetic differentiation among populations of *H. obsoletus* from distinct host plants were found by Johannesen et al. (2008). Hence, even though *H. obsoletus* is reported to be a polyphagous species, it appears that individuals are rather well adapted to a single host plant species. Such differences among individuals in plant species preference as well as in performance on potential host plants may indicate an ongoing host plant specialisation, which may lead in the medium term to a genetic diversification among populations and in the long term to speciation (Powell et al., 2006; Johannesen et al., 2008; Hendry, 2009; Hernandez-Vera et al., 2010; Peccoud & Simon, 2010). Ongoing host plant specialisation in *H. obsoletus* is expected to affect the epidemiological cycles of the two stolbur strains, which in turn might influence the control of bois noir disease.

From the current evidence, it is clear that *H. obsoletus* is the most important insect vector of bois noir in Switzerland, that the insect prefers to develop and to feed on *U. dioica*, and that *U. dioica* plays a major role in the epidemiology of the bois noir disease in Swiss vineyards. First, molecular analyses conducted on more than 30 potential vector taxa revealed that only specimens of *H. obsoletus* carried the stolbur 16Sr-XII-A group (S Kessler, unpubl. data). Moreover, there is a strong positive correlation between the distribution of *H. obsoletus* and that of bois noir disease in Switzerland (Kehrli et al., 2010). Secondly, with the exception of the canton of Valais, the vector is primarily found on *U. dioica* throughout Switzerland. And thirdly, bois noir infected vines are almost exclusively found in vineyards harbouring *U. dioica* (Kehrli et al., 2010). These findings emphasise the important role of *U. dioica* in the phytoplasma–host plant–vector interaction.

Because bois noir disease can hardly be cured and direct control measures against the vector are ineffective, viticultural control practices should target *U. dioica*, the actual reservoir and source of the bois noir phytoplasma and the vector. However, cultural control practices against *U. dioica*, such as weeding and herbicide application, should not be carried out during the flight period of *H. obsoletus* to prevent the vectors' accidental movement towards grapevine. As *U. dioica* is also an important host plant for many beneficial insects, including natural enemies of known pests, such control measures should be implemented cautiously.

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