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Control of *Hyalesthes obsoletus* nymphs based on chemical weeding and insecticides applied on *Urtica dioica*

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Summary

Bois noir is a grapevine yellows disease associated with *Candidatus* Phytoplasma solani and transmitted to grapevines by means of the planthopper *Hyalesthes obsoletus* Signoret (Homoptera, Cixiidae). The overwintering nymphs of the vector acquire the phytoplasma feeding on roots of herbaceous plants, including *Urtica dioica* L. (stinging nettle). In German and Italian vineyards the possibility to control the *H. obsoletus* nymphs feeding on stinging nettle roots using chemical weeding and insecticides was investigated. In particular, the effect of herbicides, applied in autumn and in different spring timings, and neonicotinoid insecticides on vector adult emergence was evaluated. Trials conducted to control nettle with glyphosate or a mixture of glyphosate+flazasulfuron significantly reduced the density of emerging adult vectors. The efficacy of herbicides was highest when they were applied in autumn or in early spring with the nymphs not older than the fourth instar. Herbicides applied too close to the beginning of the emergence of adults reduced numbers only during the late part of the planthopper flight-period. Although neonicotinoid insecticides applied in early spring gave efficacy comparable to herbicides, their use is not advisable for the negative side effects on non-target arthropods (e.g. honeybees). Overall, the combination of cultural practices and accurately timed applications of selective herbicides might help to refine the current Integrated Pest Management recommendations for controlling nettle, *H. obsoletus* and consequently bois noir.

Key words: Grapevine yellows, Bois noir, planthopper, vector, cultural control, stinging nettle.

Introduction

Bois noir (BN) is a grapevine yellows disease associated with *Candidatus* Phytoplasma solani which causes severe yield losses in European vineyards (Caudwell 1961, Credi 1989, Garau *et al.* 2007, Pavan *et al.* 2012). Two molecularly differentiable strains of BN-phytoplasma, tuf-a and tuf-b, are described as associated with *Urtica dioica* L. (stinging nettle) and *Convolvulus arvensis* L. (bindweed) respectively (Langer and Maixner 2004). BN phytoplasmas were experimentally transmitted to grapevines by means of the planthopper *Hyalesthes obsoletus* Signoret (Homoptera Cixiidae), that was field-collected from bindweed (Maixner 1994, Sforza *et al.* 1998) and stinging nettle (Alma *et al.* 2002, Bressan *et al.* 2007) as the inoculum sources. *H. obsoletus* overwinters as nymphs on the roots of these herbaceous plants and has one generation a year (Brcak 1979, Alma *et al.* 1988). The phenology of the five instar nymphs and adults on bindweed is earlier than on stinging nettle (Maixner 2007, Cargnus *et al.* 2012). The adults, emerging from late spring on, can occasionally invade the vineyards and feed on grapevines.

Spread and disease progress of BN depend on the presence and abundance of natural epidemiological cycles of the associated pathogen *Ca.* Phytoplasma solani that depend on herbaceous plant hosts and the vectoring planthopper *H. obsoletus* (Maixner 2010). In many European grape-growing areas, *U. dioica* is the most important host plant of the phytoplasma associated with BN and its vector *H. obsoletus* (Alma *et al.* 2002, Baric and Dalla Via 2007, Bressan *et al.* 2007, Lessio *et al.* 2007, Maixner 2007, Johannesen *et al.* 2008, Kaul *et al.* 2009, Kessler *et al.* 2011). Stinging nettle is mainly located in vineyard surrounding areas and in particular along hedgerows and ditches, even if nettle bushes can also be scattered inside the vineyards or on the embankments of vineyard terraces. The important role of ditches as sources of infected vectors was shown by a strong edge effect in the spatial distribution of BN infected grapevines and *H. obsoletus* within vineyards (Arzone *et al.* 1993, Cavallini *et al.* 2003, Credi *et al.* 2004, Bressan *et al.* 2007, Mori *et al.* 2008). In indirect confirmation that the main source of infected vectors is the vegetation surrounding the vineyards, insecticides applied to grapevine canopy were shown to be ineffective in controlling the vector and the disease (Pavan 1989, Pavan *et al.* 1989, Sforza and Boudon-Padieu 1998, Weber and Maixner 1998, Maixner 2007, Mori *et al.* 2008). Therefore, BN control attempts need to focus on the immature stages of the insect and on the host plants, which serve as infection

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sources for the root feeding nymphs. For this reason, the management of stinging nettle in the areas surrounding the vineyards is considered crucial (Maixner 2007, 2010). In fact, in northern Italy the reduction of newly symptomatic grapevines in vineyards was proportionate to the reduction of the amount of stinging nettle in areas surrounding the vineyards (Mori *et al.* 2012).

For the control of *H. obsoletus* on stinging nettle chemical weeding, soil tilling and frequent cuts were proposed (Langer *et al.* 2003, Maixner 2007, Mori *et al.* 2012). The effectiveness of these cultural practices is based on the fact that the death of the nettle host leads to the death of the nymphs that feed on the roots. Selective herbicide applications on stinging nettle during nymphal development showed efficacy in *H. obsoletus* reduction in three cases (Stark-Urnau and Kast 2008, Maixner *et al.* 2010, Mori *et al.* 2012), but not in a trial conducted in Switzerland (Kehrli and Delabays 2011). When stinging nettle control is aimed at BN management, the timing of cultural practices is considered a key aspect. In fact, if the above-ground part of the stinging nettle plants is destroyed during the vector's flight period, the search by adults for alternative hosts could increase the risk of grapevine colonization (Mori *et al.* 2005, 2012, Maixner and Langer 2006, Maixner 2007, 2010, Stark-Urnau and Kast 2008, Kessler *et al.* 2011).

Neonicotinoid insecticides are effective against some planthopper pests (Bae *et al.* 1992, Jian Zhong *et al.* 1996) and their activity against *H. obsoletus* can be supposed. However, while adults can be directly sprayed with insecticides, control of nymphs is more problematic as they feed on the roots in the ground. Foliar application of neonicotinoids on stinging nettle could be interesting in the *H. obsoletus* control because, differently from chemical weeding, it could reduce vector populations without inducing emerged adults to colonize grapevines due to the death of stinging nettle plants. The aims of this study were to: (i) verify the effectiveness of chemical weeding in *H. obsoletus* control in different field conditions and grape-growing areas (ii) compare the efficacy of chemical weeding at different timings in order to establish when the herbicide provides the highest effectiveness, and (iii) compare the effectiveness of chemical weeding with that of foliar application of neonicotinoid insecticides.

Material and Methods

Locality and years. Five trials were conducted during 2006-2011 in Italy and Germany to evaluate the efficacy of chemical weeding and neonicotinoid insecticides to control *H. obsoletus* nymphs feeding on stinging nettle roots (Table). In trial 1 the efficacy of selective herbicide treatment of individuals nettle bushes scattered within a vineyard was tested, whereas in trials 2-5 treatments of continuous nettle stands along ditches or embankments were considered.

Trials 1 and 2 were carried out from April to August 2006 at a viticultural site of the Middle-Rhine region in Germany. In trial 1, 14 nettle bushes in the inter-rows of a vineyard were treated with a mixture of flazasulfuron (Katana® 25 WG, ISK) and glyphosate (Roundup Turbo, Monsanto) at the rate of 200 $g \cdot ha^{-1}$ + 2.52 $L \cdot ha^{-1}$ on April 25, about nine weeks ahead of the emergence of adult *H. obsoletus* with nymphs of third to fourth instar. As a control, 10 more bushes remained untreated. Treatments for trial 2 were carried out on a nettle stand extending between the border of the vineyard and a ditch (approximately 4 × 16 m) at the same time as trial 1. The size of this experimental area allowed the selection of six patches of 2 × 2 m. Two patches each were treated either with the same herbicide mixture as in trial 1, with imidacloprid (Confidor® 70 WDG, Bayer)

Table

Localities, nettle localisation within the vineyard habitat and treatments for the trials conducted in Germany (1-2) and Italy (3-5)

Trial	Locality	Nettle localisation	Treatments
1	Bacharach, Middle-Rhine region 50° 04' N, 7° 46' E, 145 m a.s.l.	Within vineyard	Glyphosate+Flazasulfuron April 25th, 2006
2	Bacharach, Middle-Rhine 50° 04' N, 7° 46' E, 145 m a.s.l.	Embankment of vineyard	Glyphosate+Flazasulfuron April 25th, 2006 Imidacloprid April 25th, 2006
3	Castelfranco Emilia, Modena district 44° 66' latitude N, 11° 15' longitude E, 42 m altitude a.s.l.	Along a ditch at vineyard border side	Glyphosate April 21st, 2010 Glyphosate May 21st, 2010 Glyphosate June 18th, 2010 Glyphosate July 22nd, 2010 Control
4	Castelfranco Emilia, Modena district 44° 66' latitude N, 11° 15' longitude E, 42 m altitude a.s.l.	Along a ditch at vineyard border side	Glyphosate October 28th, 2010 Glyphosate April 21st, 2011 Control
5	Ronco all'Adige, Verona district 45° 20' latitude N, 11° 13' longitude E, 35 m altitude a.s.l.	Along a ditch at vineyard border side	Glyphosate April 20th, 2010 Imidacloprid June 8th, 2010 Thiamethoxam June 8th, 2010 Control

at the rate of 160 g·ha^{-1}, or were left untreated as controls. All treatments were conducted with a backpack-sprayer (Solo Port 420) with 4 bar pressure and a nozzle-flow of 1.68 L·min^{-1} with a water amount of 400 L·ha^{-1}.

Trials 3-5 were carried out from April 2010 to August 2011 in three vineyard habitats of Northern Italy with a high amount of nettles and *H. obsoletus* along surrounding ditches. In trial 3, different spring timings of chemical weeding with glyphosate (Roundup Max® 68 WG, Monsanto) were compared (Table). In the trial area the oldest *H. obsoletus* stages were third instar nymphs in April, fourth instar nymphs in May, fifth instar nymphs in June and adults in July (Cargnus *et al.* 2012). In trial 4, the herbicide application timing that showed the highest efficacy in trial 3 was compared with an application in October of the previous year. The herbicide was applied on nettle with a rate of 4 kg·ha^{-1}. In trial 5 the effectiveness of April timing of glyphosate was compared with that of two neonicotinoid insecticides applied before the beginning of *H. obsoletus* flights, thiamethoxam (Actara® 25 WG, Syngenta) and imidacloprid (Confidor® 200 SL, Bayer) applied at a rate of 200 g·ha^{-1} and 0.75 L·ha^{-1}, respectively. All treatments were conducted with a backpack-sprayer (FOX 320) with 6 bar pressure and a nozzle-flow of 1.54 L·min^{-1} with a water amount of 600 L·ha^{-1}. Trials 3-5 were arranged in a randomized complete block design with three replicates. Within each block between three to five plots (2.5 m × 1.5 m) with about the same amount of stinging nettle were selected and each randomly assigned to a treatment.

In both grape-growing regions, neonicotinoid insecticides were applied, although at different dates and with different objectives. In trial 2 in Germany, the insecticide was used as a standard to evaluate the efficiency of chemical weeding against *H. obsoletus*, while trial 5 in Italy focused on the question whether neonicotinoids are suitable to control *H. obsoletus* in late spring, shortly before the start of adult emergence, when herbicide treatments or mowing would be inefficient or even cause an intensified vector migration to grapevine.

Samplings: In trial 1, one yellow sticky trap (13 × 25 cm, cut from commercial 40 × 25 cm traps, Aeroxon, Waiblingen, Germany) was fixed on a woody stake approximately 30 cm above each nettle bush and replaced every week from May 17 to August 8. *H. obsoletus* adults on the traps were counted using a dissection microscope.

Emergence traps were used in trial 2 to collect the adults that emerged from the soil of the treated or control plots. They consisted of a white-cloth tent supported by a metal frame at the top of which a collecting bottle was fixed. The liquid in the collecting bottle was composed of 40 % ethanol, 20 % glycerine and 10 % acetic acid in water. On one replicate of each treatment a smaller trap covering 0.3 m^2 and on the other one a larger trap covering 1.0 m^2 were used. They were positioned in the centre of the patches in June 14 and the collecting liquid was replaced every week until August 8th. *H. obsoletus* adults were counted in the collecting liquid with a dissection microscope and the counts on the different-size traps standardized by calculating the counts per m^2.

In trials 3-5 an emergence cage of 1 m^3 (L × l × h, 1 × 1 × 1 m) was located in the middle of each plot. It consisted of a wooden frame supporting a tent of nylon insect-proof net. Inside each cage a yellow sticky trap (SuperColor®, Serbios, 40 × 24.5 cm) was fixed on a woody stake above nettle plants and replaced at intervals varying from one to two weeks (see Figs 3A, 4A, 5A) from mid-late June to late August. *H. obsoletus* adult catches were counted under dissection microscope.

In all trials *H. obsoletus* adults were identified using dichotomous keys (Holzinger *et al.* 2003, Bertin *et al.* 2010).

Data analysis. Count data were analysed statistically after logarithmic transformation. Since at the beginning and the end of the sampling period the catches could be nil or very low, counts of dates with less than one *H. obsoletus* adult per sticky trap or five specimens per emergence trap were combined.

In trial 1 the effects of the treatments were compared with paired t-test with time and treatments as independent factors. In trials 2, 3, 4, 5 the effects of the treatments were compared with Repeated Measures ANOVA and Tukey's test with time and treatment as independent factors.

To know at which sampling dates the treatment differed significantly, the counts of each date in trial 1 were compared by one-sample t-test and in trials 3, 4, 5 by one-way ANOVA and Tukey's test. In trial 2, due to the small number of replications, the weekly data from emergence traps were not analysed statistically.

In trials 1-2, statistical analysis was performed using JMP 9.0.2. In trials 3-5, statistical analysis was performed using GraphPad Instat 3.0 for Macintosh.

Results

Trial 1: The selective treatment of individual stinging-nettle bushes with herbicide in late April had a significant effect on the *H. obsoletus* population inside the vineyard. The cumulative number of catches was significantly reduced (Fig. 1A). With the exception of the beginning and the end of the flight period, the counts on traps exposed on herbicide treated nettle bushes were significantly reduced compared to traps on untreated bushes at each sampling date ($p < 0.049$ to $p < 0.0003$) (Fig. 1B).

Trial 2: Both insecticide and herbicide treatments applied on an extended stinging-nettle stand at the vineyard border significantly reduced the cumulative number of emerging *H. obsoletus* adults (Fig. 2A). The average of 495 vectors emerging per square metre during the capturing period on the control plots was reduced to 17 % and 11 % by the treatment with herbicides and insecticide, respectively. There were no significant differences between the two treatments. The reducing effect appeared similar at the different sampling dates (Fig. 2B).

Trial 3: The treatment of stinging nettle with glyphosate had a significant effect on *H. obsoletus* emergence. The April and May timings significantly reduced the catches, considering both cumulative data (Fig. 3A)

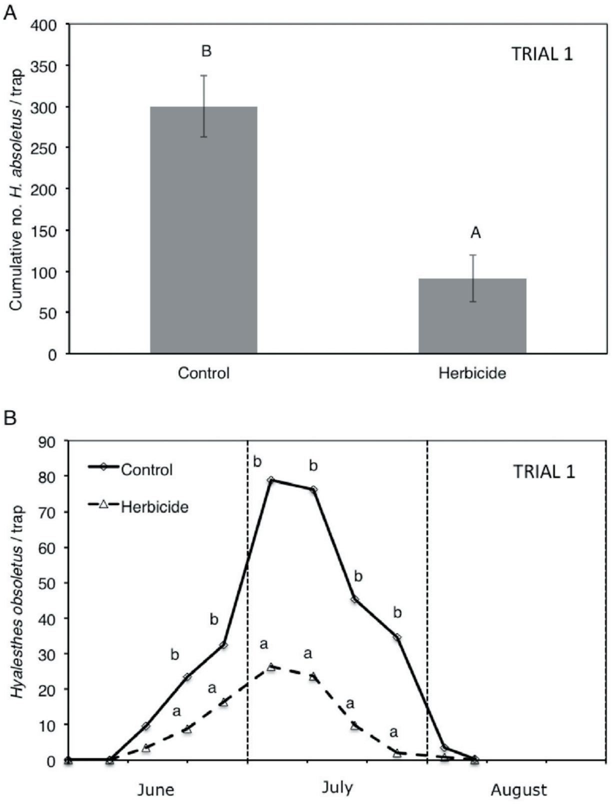

Fig. 1: Catches of *H. obsoletus* on yellow sticky traps positioned above nettle bushes treated with a combination of herbicides (glyphosate and flazasulfuron) or untreated (**A** = cumulative catches over time, **B** = catches over time). In Fig. 1A error bars indicate standard error and different capital letters above columns indicate statistical differences (α = 0.01) (F (1.14) = 4.65; P < 0.05). In Fig. 1B different small letters between control and treatment at the respective sampling date indicate statistical differences (α = 0.05).

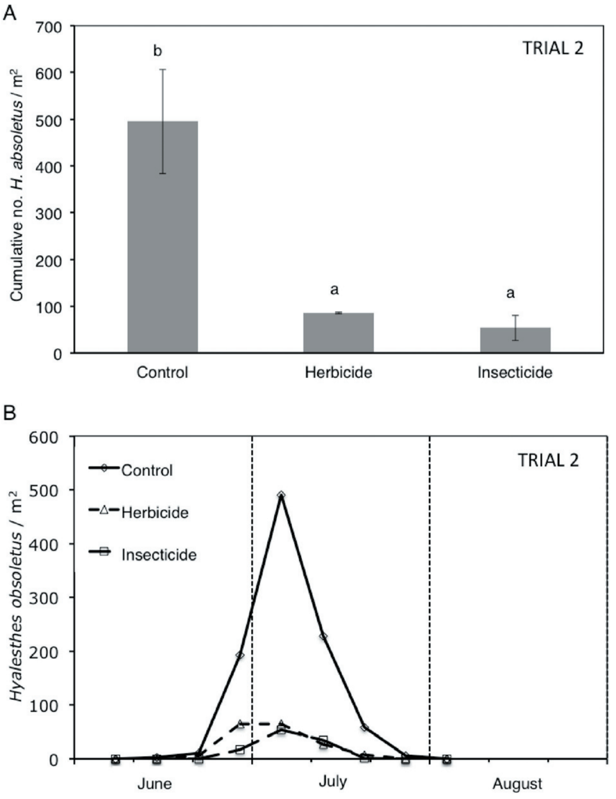

Fig. 2: Catches of *H. obsoletus* in emergence traps in the control and in the treatments characterised by the combined use of glyphosate and flazasulfuron (Herbicide) or imidacloprid (Insecticide) (**A** = cumulative catches over time, **B** = catches over time). In Fig. 2A error bars indicate standard error and different small letters above column indicate statistical differences (α = 0.05) (F (2,9) = 5.47; P < 0.05).

and single sampling dates (Fig. 3B). The April application was significantly preferable to the others with the exception of the May application (Fig. 3B). The July timing did not differ from the control based on cumulative catches (Fig. 3A) or on the single sampling dates (Fig. 3B). The June application did not reduce significantly the cumulative catches in comparison with the control (Fig. 3A), but it showed significantly lower catches than the control a month after treatment, indicating that a reducing effect on the planthopper population occurs during the last part of the adult emergence period (Fig. 3B).

Trial 4: The treatments of stinging nettle with glyphosate had a significant effect on *H. obsoletus* emergence. The autumn timing reduced the catches to the same level as the April timing both considering cumulative data (Fig. 4A) and single sampling dates (Fig. 4B).

Trial 5: The insecticide treatment with neonicotinoids in early June, before the beginning of *H. obsoletus* emergence, significantly reduced the cumulative catches of vector adults in comparison with the control (Fig. 5A). However, the effect was significantly lower than that of glyphosate applied in April considering both cumulative data (Fig. 5A) and single sampling dates (Fig. 5B).

Discussion

Our experiments showed that chemical weeding is effective to reduce *H. obsoletus* populations only when herbicides are applied in autumn or in early spring and systemic insecticides applied on nettle foliage are able to control the vector by killing the root feeding nymphs. The trials conducted both in Italy and Germany showed consistent results.

The chemical weeding resulted in a significant and, in regard to practical purposes, sufficient reduction of the density of emerging adult vectors. However, reducing the vector's abundance by depriving its nymphs of their food source can only be effective if the immature stages are not able to complete their development to adults. The correct timing of the herbicide application is therefore crucial for the efficiency of the treatment. The efficacy was optimal with early application dates in spring and with the autumn application of the herbicide. At these dates, the *H. obsoletus* populations were in the third and fourth instar of the nymphal development (Kaul *et al.* 2009, Cargnus *et al.* 2012). It can be supposed that application dates close to the time of adult emergence result in a damage of the stinging nettle that is behind the time to prevent the majority of the nymphs from completing their development. In trial

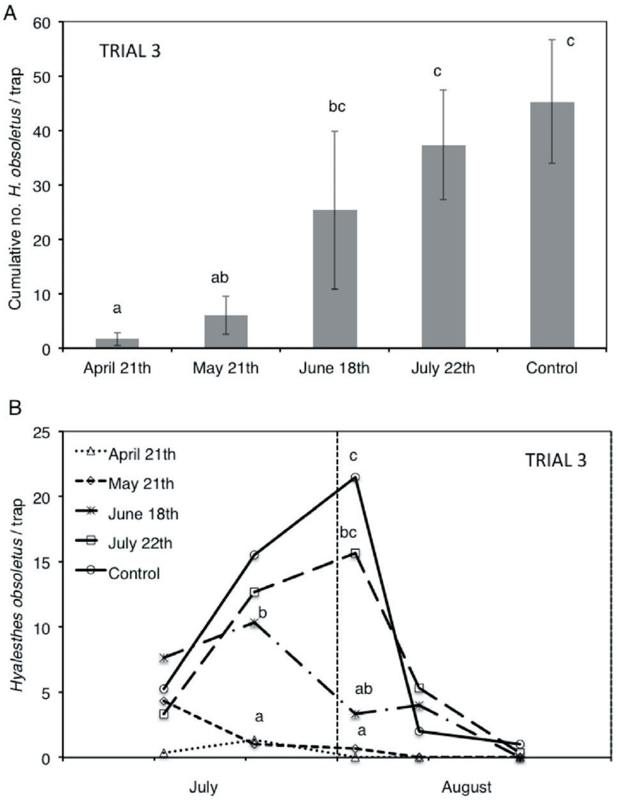

Fig. 3: Catches of *H. obsoletus* on yellow sticky traps in emergence cages and in the treatments characterised by different timings of glyphosate application (**A** = cumulative catches over time, **B** = catches over time). In Fig. 3A error bars indicate standard error and different small letters above column indicate statistical differences ($\alpha = 0.05$) ($F(4,24) = 9.90$; $P < 0.001$). In Fig. 3B different small letters among treatments at the respective sampling date indicate statistical differences ($\alpha = 0.05$).

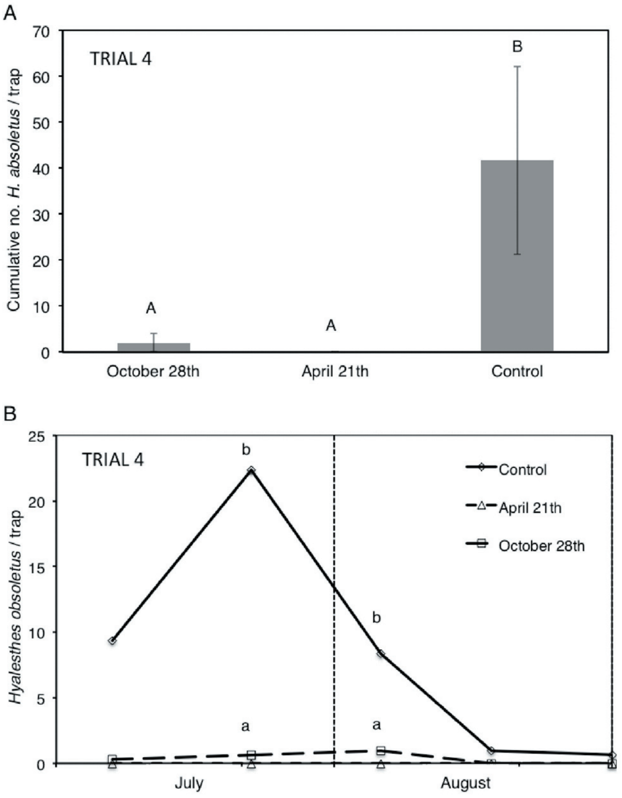

Fig. 4: Catches of *H. obsoletus* on yellow sticky traps in emergence cages in the treatments characterised by two different timings of glyphosate application (**A** = cumulative catches over time, **B** = catches over time). In Fig. 4A error bars indicate standard error and different small letters above column indicate statistical differences ($\alpha = 0.01$) ($F(2,14) = 19.63$; $P < 0.001$). In Fig. 4B different small letters among treatments at the respective sampling date indicate statistical differences ($\alpha = 0.05$).

3 the herbicide treatment in mid of June was carried out when some nymphs were already at the last (fifth) instar. Consequently, there was no effect observed at the beginning of the emergence period, but a significant reduction of the number of late emerging adults. It can be concluded from this observation that herbicide treatments should be applied before the vector's development has proceeded to the fifth instar. In the case of the geographic regions in Italy and Germany where the experiments were carried out, herbicide treatments are therefore advisable before mid May. Autumn and early spring applications led to comparable results. The choice between the two options should be made according to agronomic and environmental considerations.

The corresponding results derived from the experiments in both countries contradict those reported by Kehrli and Delabays (2012) for Switzerland, who suggested that their glyphosate application did not kill nettle roots and therefore did not affect *H. obsoletus* emergence. However, we observed a permanent effect of the herbicide treatments on nettle, since the treated plots remained free from sprouting nettle vegetation. Differences in application rates of the herbicides or the susceptibility of nettle populations might explain the contrasting results in nettle control efficiency. On the other hand, the lack of significant effects on *H. obsoletus* in the Swiss study could also be due to the low overall density of the vector in the experimental plots (Kehrli and Delabays 2012).

Although nettle is growing in high abundance mainly at the vineyard borders, nettle bushes are also scattered inside the vineyards. Selective herbicide treatments of individual nettle bushes were less effective than the treatment of continuous nettle stands. However we achieved a significant reduction of the numbers of *H. obsoletus* adults. Selective point applications are therefore advisable to reduce infection pressure at hot spots inside the vineyards without damaging the additional vineyard vegetation or managed green cover.

Insecticide application resulted in a reduction of emerging adult vectors in both experimental setups. The effect of mid-spring insecticide applications is comparable to herbicide and therefore it is a viable control measure. However, the late application of neonicotenoid insecticides was less effective than mid-spring weeding, but still reduced the number of emerging adults significantly. They might therefore be considered as an "emergency measure".

The efficacy of neonicotinoids against *H. obsoletus* nymphs is likely based on their systemic activity, so it can

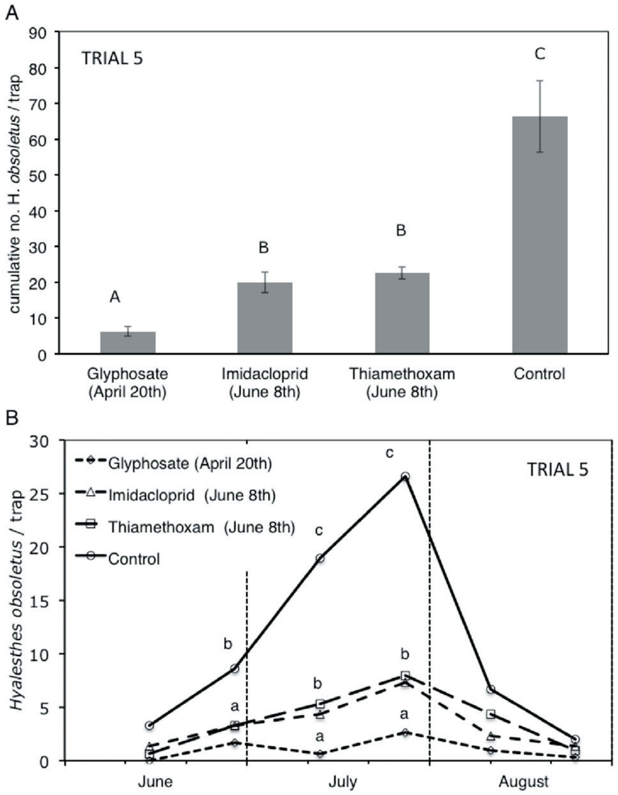

Fig. 5: Catches of *H. obsoletus* on yellow sticky traps in emergence cages in the treatments characterised by the use of glyphosate and two neonicotinoid insecticides (**A** = cumulative catches over time, **B** = catches over time). In Fig. 5A error bars indicate standard error and different capital letters above column indicate statistical differences ($\alpha = 0.01$) (F (3,19) = 42.45; $P < 0.0001$). In Fig. 5B different small letters among treatments at the respective sampling date indicate statistical differences ($\alpha = 0.05$).

be expected that the active ingredient will move from the sprayed aerial parts to the roots of stinging nettle where it is ingested by *H. obsoletus* nymphs that feed on the phloem. This hypothesis contrasts with the poor phloem translocation of neonicotinoids (Maienfisch *et al.* 2001, Sur and Stork 2003). Some authors (Jeschke *et al.* 2011, Van Timmeren *et al.* 2011, Stoner and Eitzer 2012) hypothesized that neonicotinoids deposited at the soil after spraying the leaf surface could be absorbed by the roots. Further studies are necessary to understand which mechanism is involved.

The neonicotinoid strategy implies the risk of negative effects on honeybees because these insecticides are translocated into plant pollen and nectar after being adsorbed by aerial vegetation or roots (Stoner and Eitzer 2012). Therefore, in countries where the use of neonicotinoids could be an option for *H. obsoletus* control, it has to be accompanied by frequent mowing of the flowering vegetation. Regarding the use of neonicotinoids in Italian vineyards to control *Scaphoideus titanus* (Pavan *et al.* 2005), side effects on *H. obsoletus* can be expected. In particular, in young plantings and on vertical training systems with vegetation close to the soil surface, as well as when shoots growing on vertical cordons have to be sprayed, there is the possibility that the insecticide would also be sprayed onto the stinging nettle grown along grapevine rows.

The prevention of grapevine Bois noir infection depends on indirect measures targeting the host plants as reservoirs and the immature vectors as vehicles of the inoculum. The widespread occurrence of the host plant-vector system in the vineyard agroecosystem, although rarely in the vineyards themselves, is a further obstacle to an efficient reduction of infection pressure. Neonicotinoid insecticides proved to be efficient even if applied shortly before the emergence of adult vectors, when the window for chemical weeding is closed. In any case, autumn or spring herbicide applications to control nettle appears the more practical measure to reduce *H. obsoletus* populations, with respect to efficiency as well as probable side effects to the vineyard agroecosystem. However, the use of herbicides for stinging nettle control along ditches and hedgerows is not always feasible because of possible negative effects on contiguous crops and the potential risk of water contamination. Mechanical weeding based on soil tilling is also not applicable on ditches because of soil landslide that reduces their bearing capacity. To avoid undesirable side effects of these cultural practices, frequent cuts of ditch vegetation have been considered as an alternative (Mori *et al.* 2012). Unfortunately, frequent cuts do not result in a rapid and direct control of stinging nettle, because its reduction is a consequence of the scarce capacity to withstand multiple cuts. Thus, a complete control of stinging nettle is expected only over a long time period. Consequently, the nymphs of *H. obsoletus* can continue to feed on roots after cuts and adult emergence is not prevented. In particular, the higher the frequency of cuts, the higher the reduction of the above-ground part of the stinging nettle plants and, as a consequence, the higher propensity of the vectors emerging from roots to colonize the vineyards (Mori *et al.*, submitted). In Integrated Pest Management context, considering the environmental negative effects of herbicides and the slower control of stinging nettle by frequent cuts in comparison with chemical weeding, frequent cuts and occasional selective herbicide applications could be combined to an integrated control strategy of stinging nettle as a host of *H. obsoletus*.

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