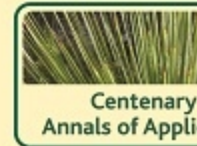


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Vitex agnus-castus cannot be used as trap plant for the vector *Hyalesthes obsoletus* to prevent infections by 'Candidatus *Phytoplasma solani*' in northern Italian vineyards: experimental evidence

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Key Words:	insect vectors, Bois noir, trap plant, transmission trials, stamp gene

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The authors thank the Editor and the anonymous Reviewers for their valuable comments and their time. Following each comment, you'll find authors response (**Answer**).

Comments of Editor:

Title. I agree with Reviewer 1 in that the title could be substantially improved and altered from a question to a definitive statement.

Answer. We modified the title in accordance with the suggestion by the reviewer (see lines 1-3 of the new version of the manuscript).

Approach. Please carefully consider the comments of Reviewer 2 which has suggested that further information on the phytosanitary status of the plants used in the experiments be included to determine whether these may have affected the volatiles. Additional information is requested on the instrumentation used in the experiment, and total numbers of insects analyzed in the field experiments.

Answer. We inserted the requested details (see lines 142 and 153-155 of the new version of the manuscript), we improved the olfactometer description and the phytosanitary status of the plants (see lines 169-171 of the new version of the manuscript)

Methods. Please pay particular attention to include additional information necessary in the methods to outline the robustness of the approach. These include: the method for collection of *H. obsoletus* adults, how many were in each cage, etc.

Answer. We are grateful to the editor and reviewers for these comments allowing the improvement of the manuscript We improved the requested data through the all manuscript

Manuscript Preparation. Please check the entire manuscript for typographical errors noted by both reviewers.

Answer. Done. See the improvement through the manuscript in the "with_truck_change" file

Comments of Reviewers:

Reviewer 1:

This is a very interesting study on the possible role of *Vitex agnus-castus* in BN epidemiology in North Italy, either as attractant plant for vector control strategy or as a common host plant of vector and the pathogen with its own role in the epidemiology. It is a carefully written and well designed study. It gives important new information on the host plant association of *H. obsoletus* and its specialization towards specific natural host plants. Very important are details on the ability of vector originating from one natural host plant to transmit the pathogen onto other host plant. This study opens a new area of study on the role of *V. agnus-castus* as constituent in the epidemiological cycle of BN in the coastal zone of Italy.

Manuscript is well written, results are clearly presented and findings are mostly well discussed. I have only minor suggestions for improvement of the clarity of experimental design and suggestions regarding some aspects of discussion.

Title:

Please consider modifying the title in context that it gives an answer to raised question. This is just a suggestion. It could be something like: "*Vitex agnus-castus* cannot be used as trap plant for the vector *Hyalesthes obsoletus* to prevent infections by '*Candidatus Phytoplasma solani*' in Northern Italy vineyards: experimental evidence"

Answer. We modified the title in accordance with the suggestion by the reviewer (see lines 1-3 of the new version of the manuscript).

Abstract:

- Lines 32 and 35: Please either change "Israel", or change "Eastern Europe". Since you are comparing two geographic regions where *Vitex agnus-castus* is a host plant of *H. obsoletus*, please use comparable geographic qualifier. Meaning, use both "Israel" and "Montenegro", or use "east Mediterranean coast of Israel" and "east Adriatic coast of Montenegro". East Europe is too wide geographical term, while it is important to point out that this plant is only growing in the coastal area.

Answer. We modified the wording in accordance with the suggestion by the reviewer. (see lines 32 and 36 of the new version of the manuscript).

- Lines 43-45: I think you should be careful about statement under point (iv) because of the characterization of the transmitted genotype. It is true that *H. obsoletus* originating from nettle transmitted '*Ca. P. solani*' to chaste tree, but it remains questionable did this genotype originated from nettle. I give more details about these findings in the discussion section, but I would suggest to changing this sentence into: "(iv) *H. obsoletus* originating from nettle is able to transmit '*Ca. P. solani*' to chaste tree (two plants out of 16 were found infected by the BN phytoplasma strain St5 identified in *H. obsoletus* specimens)"

Answer: We modified the sentence at point (iv) in accordance with the reviewer (see lines 44-45 of the new version of the manuscript), and we improved the discussion based on the indication given here below

Introduction:

- Line 91: Replace "recent" with "most recent"

Answer: Done (see lines 97 of the new version of the manuscript).

- Lines 86-94: I understand the storyline, which is gradually leading to *Vitex*, but tuf-b BN epidemiological cycle sourced by *Vitex* is evidenced in the east Mediterranean coast of Montenegro (Kosovac et al., 2016). It seems to me that this should be presented alongside with other proven epidemiological cycles of BN.

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3 **Answer:** We modified the introduction in accordance with the reviewer (see lines 99-100 of the
4 new version of the manuscript).

5
6 - Line 121: Please replace "Eastern Europe" with more precise geographic qualifier, such as "east
7 Adriatic coast of Montenegro".

8 **Answer:** We modified the wording in accordance with the reviewer throughout the manuscript (see
9 new version of the manuscript).

10
11
12 Material and Methods:

13 - Lines 138-144: Please describe the method used for collection of *H. obsoletus* adults. Please give
14 details on the number of insects confined on each plant, source of each plant (grown from seeds, or
15 tissue culture, or taken from field as small plant and then potted) and size of each plant species.

16 **Answer:** We inserted the requested details (see lines 142 and 153-155 of the new version of the
17 manuscript).

18
19
20 - Line 156: There is a typo, please replace "stinging nettle vs chaste tree" with "stinging nettle vs
21 grapevine".

22 **Answer:** Done (see lines 170 of the new version of the manuscript).

23
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25 - Lines 163-165: Was 20 *H. obsoletus* adults used per each cage? Please explain.

26 - Line 166: Again typo, please replace "or nettle and chaste tree" with "or nettle and grapevine"

27 - Line 167: Please give details on the size of the plants and source of the plants (from seeds, from
28 nature...)

29 **Answer:** We are grateful to the reviewer for these comments allowing the improvement of the
30 manuscript. According to the questions from line 163-165, 166 and 167, we changed the manuscript
31 (see lines 146-151, 172 and 185-189 of the new version of the manuscript).

32
33
34 - Lines: 179-181: Please give more details on this experiment. If I understood correctly, the nettle
35 was removed from the ditch at the time of the adult flight period; this needs to be better explained.
36 In addition please give details on the size of the plants, distance between the plants within each
37 group and size of the sticky traps used for the monitoring of the *H. obsoletus* adults. It seems that
38 plants must have been fully grown to have enough canopies for sticky traps to be placed within.
39 Please explain the source of plants and condition under which they were grown.

40 **Answer:** We inserted the details requested by the reviewer (see lines 194-197 and 201-205 of the
41 new version of the manuscript).

42
43
44 - Lines 208-209: Please give details on the source of chest tree plants. Were they grown from seeds,
45 or taken from nature and then potted. What was the size of the plants?

46 **Answer:** We inserted the details requested by the reviewer (see lines 214-215 and 218-219 of the
47 new version of the manuscript).

48
49
50 - Lines 216-217: Please explain why limited number of insects was collected from experimental
51 plants.

52 **Answer:** In the case of insects collected from the plant TBS5 we made a typing errors: collected
53 insects were 10 and not 1. The limited number of insects collected after the transmission period was
54 due to the fact that the body of some insects was strongly deteriorated and not suitable for the
55 following molecular analysis (see lines 240 and Table 3 of the new version of the manuscript).

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58 Discussion:

59 - Line 326: Typo, please replace "Serbia" with "Montenegro".

60 **Answer:** Done (see lines 350 of the new version of the manuscript).

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5 - Please give and discuss precise context of the finding that St5 genotype is the one successfully
6 transmitted to *Vitex*. St1, St2 and St30 (previously found associated with *Vitex* or transmitted by
7 *Vitex* associated *H. obsoletus*; Kosovac et al., 2016) and St5 are of the same stamp Cluster b-II,
8 with St5 differing only in 4nt from St2 which is transmitted to grapevine by naturally infected *H.*
9 *obsoletus* originating from *Vitex agnus-castus* from Montenegro. This is especially indicative
10 because St2 is possible natural genotype associated with *Vitex*, because Ho used in this
11 transmission were collected on *Vitex* from natural habitat, hence not associated with
12 agroecosystem.

13 - There is an additional epidemiological importance of the St5 genotype which you transmitted with
14 Ho originating from *Urtica* to *Vitex*. It is relevant that this genotype is so far known to be associated
15 only with *Convolvulus* as source plant, *Hyalesthes obsoletus* from *Convolvulus* as vectors, and
16 from grapevine, in wide geographic area from Germany, Italy, Austria, Slovenia, to Macedonia
17 (Pierro et al., 2018, *Phytopathol*). Also, it is one of the dominant stamp genotypes of the recent
18 epidemics in Tuscany (Pierro et al., 2018, *Ann App Biol*). Hence, this strain was never associated
19 with *Urtica* as host plant.

20
21 Please, give a short discussion on this finding and on the possibility that all other genotypes which
22 are not transmitted to *Vitex* in your study could be (as expected) those that are strictly associated
23 with *Urtica* (stamp a1 and a2 clusters) and that it is probably why they couldn't be transmitted. Of
24 course, this all requires further (future) investigations, but it would be very informative and
25 lucrative to discuss this situation in context of present findings.

26
27 **Answer:** We are grateful to the reviewer for these comments allowing the improvement of the
28 discussion of the obtained results. We modified the discussion inserting new sentences focused on
29 the points raised by the reviewer (see lines 350-357 and 369-371 of the new version of the
30 manuscript).
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Comments of Reviewers:

Reviewer 2:

The aim of this works was to verify if chaste tree is attractive for *Hyalesthes obsoletus*, the vector of *Candidatus phytoplasma solani*. In this work was show that the chaste tree plant can be host to both the insect vector *H. obsoletus* and the *Ca. phytoplasma solani*. However previous works, as indicated by the authors, have shown this although in separate papers. About the experiments to verify the attractiveness of chaste tree and grapevine for *H. obsoletus* from stinging nettle among the experiments conducted, some procedural gaps must be filled. In particular in the laboratory and semi-field experiments no phytosanitary status of the plants was indicated. It is not clear whether these plants have been tested for the presence of pathogens and / or phytoplasmas. This information is important because could be affect the volatiles elements from the plants. While in the transmission test was indicated that the plants, was PCR-negative to '*Ca. P. solani*'. Furthermore, regarding to the test with the olfactometer some information about the instrument must be added. About the semi-field experiment, the study seem based on a low number of insects. Also, in the field experiments it is not indicated how many insects were analyzed in this experiment. However, the news of this work is that *H. obsoletus* was able to transmit from nettle to chaste tree.

See below for detail:

Introduction

Line 86: Add that several other insects are referred as suspected vectors of *Ca. solani* phytoplasma

Answer: We are grateful to the reviewer for these comments allowing the improvement of the introduction. We insert new references (see lines 83-91 of the new version of the manuscript).

Lines 115-116: 'In both olfactometric and field studies chaste tree resulted more attractive than grapevine for *H. obsoletus* adults (Sharon et al., 2005; Zahavi et al., 2007).'

Other more recent tests show a significant attraction of male *H. obsoletus* to chaste tree, and of the females to nettle (Riolo et al., 2012).

Answer: We insert the suggested citation (see lines 123-124 of the new version of the manuscript).

Line 124: 'Considering such contradictory data'

These are not contradictory data, it is better to write that the epidemiological cycle involving both, the plant insect vector and the pathogen has not yet been shown

Answer: Due to the modifications inserted throughout the introduction, we re-phrased the sentence by deleting the words "Considering such contradictory data" (see lines 98-99 of the new version of the manuscript).

Materials and Methods

Line 137: 'Survival of *Hyalesthes obsoletus* from stinging nettle on chaste tree and grapevine.'

Indicate on how many insects were tested in the survival experiment form each plant species

Answer: See answer Reviewer 1 (see lines 153-155 of the new version of the manuscript).

Line 155: 'choice test using two-choice olfactometer between shoots of chaste tree vs grapevine'

Provide more information about the type of olfactometer and the method used. In this experiment we indicated the females and males, however no data related to these aspects was included in the results. Indicate if the phytosanitary status of the plants used in this experiment has been evaluated

Answer: We improve the olfactometer description and the phytosanitary status of the plants (see lines 169-171 of the new version of the manuscript). Regarding the females and males data , males and females were considered: "To establish the proportion of males and females that were attracted by one of the two plants was different ..." (see lines 174-175) and the results of these comparisons

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3 were reported at lines 278–281. As reported at lines 160-162 of the old manuscript version males
4 and females were not analysed separately for plant species preference, due to the low number of
5 individuals that chose the plant and at the absence of differences in the choosing between males and
6 females. At this purpose we added a new sentence in M&M at lines 176-179 to explain because the
7 data of the two sexes were pulled.
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10 Line 163: ‘Semi-field conditions’

11 In this experiment, the authors analyzing a very low number of insects. In fact most of these do not
12 choose any plant (See Fig 3). Do you have evaluated the plant’s phytosanitary status? Specify better
13 **Answer:** Concerning the low number of insects analyzed in attractiveness trials in semi-field
14 conditions, it is truth that a low number of insects selected the plants but the results are the average
15 of three replications, representing robust data. Moreover, we indicated the phytosanitary condition
16 of the plants (see lines 182-188 of the new version of the manuscript).
17
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19 Line 176: ‘For each distance, 6 groups of the 3 plants were considered, the distance between each..’
20 From which plants each group was composed? Specify better

21 **Answer:** We modified the manuscript as suggested (see lines 198-199 of the new version of the
22 manuscript).
23
24

25 Line 206: ‘Species recognition was confirmed based on the taxonomic keys by Bertin et al. (2010).
26 Why do you say it only at this point? not needed before

27 **Answer:** The species identification was done collecting randomly specimens throughout all
28 bioassays. We move the citation Bertin et al. (2010) from Transmission trials paragraph to
29 Survival of *Hyalesthes obsoletus* one as requested (see lines 155-156 of the new version of the
30 manuscript).
31
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33 Results

34 Line 248: ‘Attractiveness of chaste tree and grapevine for *Hyalesthes obsoletus* from stinging
35 nettle’

36 How many insects was analyzed in this experiments? How was the analysis done? On the total
37 number of insects, or on males and females separately? Specify.

38 **Answer:** Forty individuals (20 females and 20 males) were tested for each comparison. We
39 changed the Materials and Methods of the manuscript in order to make it clear (see lines 171 of the
40 new version of the manuscript). A G-test of goodness of fit was used putting together males and
41 females (see lines 176 of the new version of the manuscript).
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45 Lines 260-270: ‘The interactions time × plant, plant × distance and time × plant × distance were
46 significant due to the fact that the captures were influenced by time and distance only for stinging
47 nettle and chaste tree (Table 1).

48 From the ANOVA table, this is not clear. It would be appropriate to show the data from which the
49 ANOVA table was obtained.

50 **Answer:** The interpretation of the significance of the interactions is based on the fact that (i) there
51 are significant differences both between dates and between distances, and (ii) these must necessarily
52 be due to the nettle and *Vitex*, being the captures on vines equal to zero in the three samplings
53 (time) and to three distances. To make the interpretation even more explicit, the sentence has been
54 extended (see lines 294-295 of the new version of the manuscript).
55
56

57 Discussion

58 Lines 307-311: ‘Considering the two latter, as no captures occurred in grapevine, chaste tree
59 seemed to be preferred. The higher attractiveness of chaste tree compared to grapevine was showed
60 by semi-field experiments in which even chaste tree was significantly less attractive than stinging

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nettle in only one of the two years. With reference to the two true host plants, i.e. stinging nettle and chaste tree, preference for the former may still be associated with the origin of adults used for the experiments. ‘

This paragraph is very confusing. What you want to explain that the most attractive species is the nettle followed by chaste tree while the less attractive was the grapevine species. This, results could be influenced from the origin of adults used for the experiments all collected from the nettle plants. Simplify.

Answer: We are grateful to the reviewer for these comments allowing the improvement of the manuscript, we rewrote the manuscript (see lines 331-336 of the new version of the manuscript).

For Peer Review

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4 1 **Vitex agnus-castus cannot be used as trap plant for the vector**
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6 2 ***Hyalesthes obsoletus* to prevent infections by '*Candidatus Phytoplasma***
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8 3 ***solani*' in northern Italian vineyards: experimental evidence**
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10 4

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31 16 * These authors contributed equally to the work
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45 24 **Running title:** Possible role of chaste tree in bois noir epidemiology
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Abstract

Bois noir (BN), the more widespread disease of the grapevine yellows complex, is causing a considerable yield loss in vineyards. BN is associated with phytoplasma strains of the species 'Candidatus Phytoplasma solani' (taxonomic subgroup 16SrXII-A). In Europe, BN phytoplasma is transmitted to grapevine mainly by *Hyaalthes obsoletus*, a polyphagous cixiid completing its life cycle on stinging nettle and field bindweed. Due to the complexity of BN epidemiology, no effective control strategies have been developed. In east Mediterranean coast of Israel, chaste tree (*Vitex agnus-castus*), even if found to be the preferred host plant of *H. obsoletus*, did not harbor BN phytoplasma. Thus, a "push and pull" strategy was suggested based on the fact that chaste tree plants located at vineyard borders was an effective trap plant for *H. obsoletus* adults. However, in east Adriatic coast of Montenegro, chaste tree was found to be a key source plant for BN phytoplasma transmission to grapevine. Considering such contradictory data, this study aimed to investigate (i) the interaction between chaste tree and *H. obsoletus* through survival, attractiveness and oviposition experiments conducted comparing the behavior of *H. obsoletus* in chaste tree versus stinging nettle and grapevine, and (ii) the capability of chaste tree to harbor 'Ca. P. solani' in northern Italy through transmission trials. Obtained data showed that (i) *H. obsoletus* adults can survive on chaste tree and grapevine even over a week; (ii) *H. obsoletus* adults prefer chaste tree to grapevine; (iii) *H. obsoletus* can produce eggs and overwinter as nymphs on chaste tree, even if at a lesser extent than on stinging nettle; (iv) *H. obsoletus* originating from nettle is able to transmit 'Ca. P. solani' to chaste tree (two plants out of 16 were found infected by the BN phytoplasma strain St5 identified in *H. obsoletus* specimens). These results increased knowledge about the role of *Vitex agnus-castus* as host plant of *H. obsoletus* and BN phytoplasma in northern Italy and do not allow considering chaste tree as trap plant at vineyard borders.

Key words: insect vectors, Bois noir, trap plant, transmission trials, *stamp gene*

1 INTRODUCTION

Europe is the world leader in grape production with almost half of the global viticulture growing. Italy is the second top producer of grapes after China with about 8.2 million tons (FAO, 2016). Quality and quantity of viticulture production are damaged by a wide-range of pathogens associated with diseases affecting the main cultivated grapevine varieties (Bellée et al., 2018). Among these diseases, the grapevine yellows (GY) complex is one of the most important threats to viticulture in many countries (Magarey, 2017). The GY causal agents are phytoplasmas ('*Candidatus Phytoplasma*'), cell-wall-less obligate parasitic bacteria transmitted by insect vectors to plants, in which they reside in phloem tissues (Angelini et al., 2018). Interestingly, even if undistinguishable based on symptoms, the main diseases within the GY complex are associated with genetically distinct phytoplasmas, belonging to at least six '*Ca. Phytoplasma*' species, characterized by different biological features that reflect on disease epidemiological patterns (Belli et al., 2010; Angelini et al., 2018).


Bois noir (BN) is the most widespread disease of the GY complex in the Euro-Mediterranean area, where it may lead to a total yield loss and even grapevine death (Belli et al., 2010; Pavan et al., 2012). BN is associated with grapevine infection by phytoplasma strains (Bois noir phytoplasma strains, BNp) of the species '*Candidatus Phytoplasma (Ca. P.) solani*' (subgroup 16SrXII-A) (Quaglino et al., 2013). In the Euro-Mediterranean regions the main '*Ca. P. solani*' insect vector is *Hyalesthes obsoletus* Signoret (Homoptera: Cixiidae) (Maixner, 1994; Sforza et al., 1998; Bressan et al., 2007), a polyphagous planthopper living preferentially on stinging nettle (*Urtica dioica* L.), field bindweed (*Convolvulus arvensis* L.), stinking hawk's-beard (*Crepis foetida* L.), and *Artemisia* spp. in and/or around vineyards (Alma et al., 1988; Sforza et al., 1998; Weber & Mainer, 1988; Langer & Maixner, 2004; Mori et al., 2008b, 2013; Cargnus et al., 2012; Kosovac et al., 2013). Recently, *Reptalus panzeri* Low (Homoptera: Cixiidae) has been reported as vector of '*Ca. P. solani*' (CaPsol) in Serbian vineyards (Cvrković et al., 2014) while *Macrosteles quadripunctulatus* (Kirschbaum) (Homoptera: Cicadellidae) was found able to transmit CaPsol to potted grapevine plants (Batlle et al., 2008). In addition *Anaceratagallia ribauti* (Ossiannilsson) (Homoptera: Cicadellidae) and *Reptalus quinquecostatus* (Dufour) (Homoptera: Cixiidae) were reported as vectors even if not to grapevine (Riedle-Bauer et al., 2008; Chuche et al., 2016). Other studies reported that different Cixiidae and Cicadellidae species have been captured within or near BN-diseased vineyards and found to contain CaPsol (Oliveri et al., 2015; Šafářová et al., 2018) but such insects are not currently considered to be involved in CaPsol transmission to grapevine.

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3 92 ~~The sequence~~ analysis of *tufB* gene revealed that two main '*Ca. P. solani*' *tuf*-types are
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5 93 present on grapevines and alternative plant hosts, according to diverse ecological pathosystems: (i)
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7 94 field bindweed - *H. obsoletus* - grapevine *tuf*-type b, (ii) stinging nettle - *H. obsoletus* - grapevine
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9 95 *tuf*-type a (Langer & Maixner, 2004). Recently, in Austria, Aryan et al. (2014) detected a
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11 96 presence of a *tuf*-type b with a distinguished *Hpa*II-restriction profile designed as *tuf*-type b2 that
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13 97 appears to have different ecological features. Interestingly, ~~most~~ recent evidence highlighted the
14
15 98 existence of a new BN epidemiological ~~cases~~ of *tuf*-type b '*Ca. P. solani*' strain in the Balkan
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17 99 region and in east Adriatic coast of Montenegro, ~~spread~~ respectively by *C. foetida* and *Vitex*
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19 100 *agnus-castus* L. transmitted by their associated *H. obsoletus* population (Kosovac et al., 2016,
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21 101 2019). Moreover, several weeds, such as *Chenopodium album* L. and *Malva sylvestris* L., host the
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23 102 '*Ca. P. solani*' in or around infected vineyards and can therefore play a role in BN ~~spreading~~
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25 103 (Marchi et al., 2015; Mori et al., 2015; Oliveri et al., 2015). Molecular epidemiology approaches,
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27 104 using *vmpI*- and *stamp*-based markers ~~al~~ ~~ed~~ knowledge ~~to be increased~~ of the populations of BN
28
29 105 throughout vineyards and their surroundings in the Mediterranean area (Fialová et al., 2009; Fabre
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31 106 et al., 2011; Foissac et al., 2013; Murolo et al., 2014; Landi et al., 2015; Murolo & Romanazzi,
32
33 107 2015; Pierro et al., 2018a, 2018b).

34
35 108 The complexity of BN disease epidemiology renders it difficult to design efficient control
36
37 109 strategies. Insecticides applied to the grapevine canopy influence neither the disease nor the
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39 110 presence of *H. obsoletus* (Maixner, 2007; Mori et al., 2008b). The management of *H. obsoletus* host
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41 111 plants in the vineyards and surrounding areas is therefore considered crucial for BN control
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43 112 (Maixner, 2010; Mori et al., 2012; Panassiti et al., 2017). Thus, preventive measures, such as
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45 113 checking the health status of propagation materials (i.e., mother plants and grafted cuttings) and
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47 114 treating of cuttings through thermotherapy, are applied to limit long distance dissemination and in-
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49 115 field spread of the disease (Mannini et al., 2007). Other strategies for reducing BN spread or
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51 116 incidence are based on (i) preventive removal of the grape suckers on which *H. obsoletus* could
52
53 117 feed ~~after grass mowing~~ (Picciau et al., 2010); (ii) trunk cutting above the ~~cut~~ ~~gement~~ point on
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55 118 symptomatic grapevines (Kast et al., 2008; Riedle-Bauer et al., 2010); (iii) treatments by resistance
56
57 119 inducers (Romanazzi et al., 2009, 2013). ~~In prospective, also plan~~ ~~ing~~ ~~latiles~~ from host plants can be
58
59 120 used for reducing vineyard colonization by *H. obsoletus* (Riolo et al., 2017).


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61 121 In Israel, chaste tree (*Vitex agnus-castus* L.) is a plant where *H. obsoletus* can complete its
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63 122 life cycle (Sharon et al., 2005). In both olfactometric and field studies chaste tree ~~resulted~~ ~~more~~
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65 123 attractive than grapevine for *H. obsoletus* adults (Sharon et al., 2005; Zahavi et al., 2007; Riolo et
66
67 124 al., 2012). Therefore, a "push and pull" strategy based on the use of chaste tree as ~~trap~~ ~~plant~~ at


vineyard borders to reduce the vector population ~~living inside the vineyards~~ was suggested (Zahavi et al., 2007). The ~~validity of this strategy is~~ reinforced by the fact that in Israel chaste tree was never found infected by '*Ca. P. solani*' and thus cannot serve as an inoculation source for grapevine (Sharon et al., 2015).

This study aimed to investigate the ~~possible~~ role of *V. agnus-castus* as host plant of *H. obsoletus* and CaPsol in northern Italy. ~~In detail, the~~ interaction between chaste tree and *H. obsoletus* was examined through survival, attractiveness and oviposition trials, while the capability of chaste tree to harbor CaPsol in northern Italy was studied through transmission trials in controlled conditions. In these studies, *H. obsoletus* adults collected on stinging nettle were used because this plant is the most important external source of infected vectors for Northern Italian vineyards (Mori et al., 2008b, 2015) and therefore  possibility of using chaste tree as trap plant at vineyard borders must be evaluated on this population.

2 MATERIAL AND METHODS

2.1 Survival of *Hyalesthes obsoletus* from stinging nettle on chaste tree and grapevine

Hyalesthes obsoletus adults were collected by using a sweep net and pooter in Veneto region on 4th July 2016 and 27th June 2017 from stinging nettle plants, growing along a ditch bordering a BN infected vineyard (45°23'32.42''N; 11°09'45.62''E), and were maintained for ten days under controlled conditions [25±3 °C, 70±5 RH, 16:8 (L:D) daily light cycle] in ~~insects-proof~~ cages on potted plants of chaste tree, stinging nettle and grapevine. The chaste tree plants were generated by tissue culture in Guagno nursery (Padova, Italy)  stinging nettle plants were taken from field, and grapevine plants were one-year Chardonnay grafted on SO4 rootstock in Vivai Cooperativi Rauscedo (Pordenone, Italy). The plants, grown in 5 L pots, were in good vegetative condition and did not show ~~any~~ symptoms ~~related to~~ biotic and abiotic stresses. The three plant species had similar volume and leaf density (diameter about 0.3 m and ~~high about~~ 0.8 m).

Both years, the *H. obsoletus* individuals, collected from stinging nettle, were randomly confined on 8 singularly caged potted plants ~~per~~ each of the three host species. On average, in 2016 were used 28.5, 14.1, and 25.1 adults on chaste tree, stinging nettle and grapevine  respectively; in 2017, were used 14.6, 15.3, and 17.6 adults on chaste tree, stinging nettle and grapevine, respectively. Species ~~recognition~~ was confirmed based on the taxonomic keys by Bertin et al. (2010).

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3 157 During the 10-day confinement, the number of dead individuals was counted daily. On the last
4
5 158 sampling day the number of alive individuals was also counted, to know the total number in each
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7 159 cage. Kaplan-Meier analysis was used to estimate the survival curve on the three plants and the
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9 160 comparison between two survival curves was made by the log-rank test.

10 161

11

12 162 **2.2 Attractiveness of chaste tree and grapevine for *Hyalesthes obsoletus* from stinging nettle**

13 163 The attractiveness of chaste tree for *H. obsoletus* collected on stinging nettle was evaluated under
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15 164 laboratory, semi-field and field conditions.

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17 165 ~~(i) Laboratory conditions:~~ the experiment was conducted in 2017 using *H. obsoletus* adults captured
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19 166 on stinging nettle (see survival trials). Before their use in the experiment the adults were left on
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21 167 Petri dishes with water for 12 hours. The planthoppers then underwent a choice test using a custom
22 168 made two-choice olfactometer [following Dicke et al. (1988)] between shoots of chaste tree vs
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24 169 grapevine (cv Chardonnay), chaste tree vs stinging nettle, stinging nettle vs grapevine. The shoots
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26 170 were in good vegetative condition and did not show any symptoms related to biotic and abiotic
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28 171 stresses. Forty individuals (20 females and 20 males) were tested for each comparison. If 10
29 172 minutes after positioning the insect was still at the start on the olfactometer, the test was considered
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31 173 as “No choice”. Data analysis was performed on the individuals that chose one of the two plants
32
33 174 under comparison. To establish if the proportion of males and females that were attracted by one of
34 175 the two plants was different, a Fisher’s exact test was used. To know if one plant was preferred by
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36 176 adults more than the other in comparison, a G-test of goodness of fit was used. Since the
37
38 177 percentages of males and females who have chosen one of the two plants under comparison are
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40 178 always differed for no more than 7%, this last analysis was conducted joining together the adults of
41 179 the two sexes.

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43 180 ~~(ii) Semi-field conditions:~~ in 2016 and 2017, 9 cages (0.5 m × 0.5 m × 1.0 m) containing potted
44
45 181 plants of two species, namely chaste tree and grapevine (n. 3 cages) or chaste tree and nettle (n. 3
46 182 cages) or nettle and grapevine (n. 3) were prepared. The origin and the vegetative status of the
47
48 183 plants were the same of those used in the survival experiment (2.1 §). The plants of the two
49
50 184 species under comparison inside each cage were pruned to similar volume and leaf density
51
52 185 (diameter of about 0.3 m and high of about 0.5 m). In each cage 20 *H. obsoletus* adults (10 females
53 186 and 10 males) (captured on stinging nettle, see survival trials §) were confined. Cages were
54
55 187 maintained under controlled conditions [25±3 °C, 70±5 RH, 16:8 (L:D) daily light cycle].
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57 188 Observation of adult insect’s position was done 1, 4 and 8 hours after caging. If the insect was on
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59 189 the net or on the bottom of the cage, the position was considered as “No choice”. Data analysis was

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3 190 performed on the individuals that chose one of the two plants under comparison using a paired-
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5 191 sample t test.

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7 192 ~~(iii) Field conditions: the trial was conducted in 2017 in a ploughed field (3.7 ha surface,~~
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9 193 ~~(45°23'34.92''N; 11°09'39.10''E) with one side (103 m long) bordered by a ditch covered with~~
10 194 ~~stinging nettle harbouring large *H. obsoletus* populations. At the time of the adults' flight period the~~
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12 195 ~~stinging nettle along the ditch was mowed. Potted chaste tree, grapevine and nettle plants (see~~
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14 196 ~~survival trials §) were placed in the field at 5, 10 and 20 m from the border in the same day of the~~
15 197 ~~stinging nettle mowing. For each distance, 6 groups of the 3 plants were considered, one for each of~~
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17 198 ~~the three species. The distance between each plant group was 15 m and 1.0 m between each plant~~
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19 199 ~~within the group. All potted plants of the three species under comparison were pruned to similar~~
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21 200 ~~volume and leaf density (diameter of about 0.4 m and high of about 0.9 m) and irrigated twice a~~
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23 201 ~~week. The plants were in good vegetative condition and did not show any symptoms related to~~
24 202 ~~biotic and abiotic stresses. The presence of *H. obsoletus* adults on the three potted-plant species was~~
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26 203 ~~monitored after nettle mowing by transparent A5 paper size 148 × 210 mm) positioned~~
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28 204 ~~within their canopy. The number of individuals captured during the first and second week was~~
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30 205 ~~counted. To compare field-trial data (number of *H. obsoletus* adults captured), a three-way ANOVA~~
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32 206 ~~was used, considering as source of variation sampling time (first and second week from stinging~~
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34 207 ~~nettle mowing), host plant (stinging nettle, grapevine and chaste tree) and distance from *H.*~~
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36 208 ~~*obsoletus* source (5 m, 10 m and 20 m). Prior to analysis data normality was tested with the~~
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38 209 ~~Shapiro–Wilk test, homogeneity was tested with Levene's variance test, the presence of outliers~~
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40 210 ~~was assessed, and the data were log(x+1) transformed. For *post hoc* comparisons of means, LSD5%~~
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42 211 ~~(least significant difference between two means at the 5% level) was used.~~

2.3 Egg laying of *Hyalesthes obsoletus* from stinging nettle on chaste tree

43 213 Insects proof cages (0.5 m × 0.5 m × 1.0 m) were arranged on potted plants of chaste tree (grown
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45 214 from tissue culture Guagno nurseries – Padova) and ~~stinging nettle (taken from nature).~~ Four and
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47 215 eight potted plants for each species were considered in 2016 and 2017 respectively. The pots had 50
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49 216 L of capacity and the holes at the bottom were closed with insect-proof net to allow water flow but
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51 217 prevent the hatched-nymphs escaping. ~~The plants size was about 0.4 m in diameter and about 0.9 m~~
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53 218 ~~in height.~~

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55 220 In each cage 100 *H. obsoletus* adults (50 females and 50 males), collected on stinging nettle (see
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57 221 survival trials §) on 21st July 2016 and 14th July 2017, were confined with the plants. The cages
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59 222 were maintained in an open field during winter. In February 2017 and 2018, *H. obsoletus* nymphs

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3 223 were extracted from the soil by Berlese funnel and analysed under stereomicroscope. Nymphs were
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5 224 identified using the dichotomous keys of Cargnus et al. (2012). Data collected in the two years were
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7 225 analysed together using a paired-sample t -test.

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10 227 **2.4. Transmission trials of BN phytoplasmas to chaste tree**

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12 228 In 2017 adults of *H. obsoletus* were collected on stinging nettle in a ditch bordering two BN-
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14 229 affected vineyards in Lombardy (Brescia province: 45°35'37.72''N; 10°09'33.36''E) and Veneto
15 230 (Verona province: 45°23'32.42''N; 11°09'45.62''E) regions. Capturing of adults was done by using
16
17 231 a sweep net and pooter. The captured insects were kept in jars for transport to the laboratory. The
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19 232 transmission trials were conducted with ~~twenty-four~~ chaste tree plants, tested PCR-negative for '*Ca.*
20 233 *P. solani*' in a greenhouse under controlled conditions (25±3 °C, 70±5 RH) located in Verona
21 234 province (45°20'13.72''N; 11°13'03.28''E). The plants were singularly caged and divided into
22 235 three groups: (i) plants TBS1-TBS8, with confined *H. obsoletus* individuals collected in Brescia (30
23 236 adults per plant), (ii) plants TVR1-TVR8, with confined *H. obsoletus* individuals collected in
24 237 Verona (30 adults per plant), and (iii) plants T1-T8, without insects (control plants). Transmission
25 238 trials were ~~left till the~~ end of adult survival. After this period, the plants were kept in an insect-free
26 239 greenhouse.

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29 240 Dead insects (136 in plants TBS1-TBS8; 146 in plants TVR1-TVR8), collected from the end
30 241 of June till mid-July 2017, were stored in absolute ethanol at 4 °C. '*Ca. P. solani*' was ~~detected~~
31 242 by nested PCR-based amplification of *stamp* gene (Fabre et al., 2011) using ~~as templates~~ the total
32 243 nucleic acids extracted from both the individual insect specimens (Marzachi et al., 1998) and the
33 244 leaves of chaste tree plants (Angelini et al., 2001) collected in October 2017 and 2018. The plants
34 245 were kept in an insect-free greenhouse for the ~~whole~~ transmission period. ~~Amplification~~
35 246 products were analyzed by electrophoreses in 1% agarose gel stained with Midori green under a UV
36 247 transilluminator.

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39 248 PCR products (StampF1/StampR1), amplified from insect adults and chaste tree samples,
40 249 were sequenced in both strands (Sanger method, 5X coverage per base position) by a commercial
41 250 service (Eurofins Genomics, Germany). Nucleotide sequences were assembled by the Contig
42 251 Assembling Program and trimmed to the annealing sites of the nested PCR primer pair in the
43 252 software BioEdit, version 7.2.6 (Hall, 1999). Obtained *stamp* gene nucleotide sequences were
44 253 aligned using the ClustalW Multiple Alignment program in the software BioEdit and analysed by
45 254 Sequence Identity Matrix to estimate ~~their~~ genetic diversity. *Stamp* sequence variants, identified in
46 255 the study, were aligned and compared with representative sequences of previously defined sequence

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3 256 variants (Pierro et al., 2018a, 2018b); a nucleotide sequence identity of 100% was necessary for the
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5 257 attribution to such sequence variants.
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10 260 3 RESULTS

11 12 261 13 14 262 3.1 Survival of *Hyalesthes obsoletus* from stinging nettle on chaste tree and grapevine

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16 263 ~~The data gathered in the two years showed that adults~~ of *H. obsoletus* collected from stinging nettle
17 264 can survive on chaste tree and grapevine for some days, but the survival curves were significantly
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19 265 worse than those on stinging nettle (2016: grapevine vs stinging nettle, $X^2 = 251.4$, $p > 0.0001$;
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21 266 chaste tree vs stinging nettle, $X^2 = 104.6$, $p < 0.0001$; 2017: grapevine vs stinging nettle, $X^2 = 151.2$,
22 267 $p > 0.0001$; chaste tree vs stinging nettle, $X^2 = 66.6$, $p < 0.0001$) (Figure 1). Survival on chaste tree
23
24 268 and grapevine fell below 50% after three days in 2016 and after 5 days in 2017. In 2016 the survival
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26 269 curve on chaste tree was significantly better than on grapevine ($X^2 = 4.87$, $p = 0.016$), but in 2017
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28 270 ~~this difference was not confirmed~~ ($X^2 = 1.47$, $p = 0.16$).
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31 272 3.2 Attractiveness of chaste tree and grapevine for *Hyalesthes obsoletus* from stinging nettle

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33 273 In the laboratory experiment with two-choice olfactometer, the proportion of males and females that
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35 274 chose one of the two plants under comparison with “no-choice” individuals was not significantly
36 275 different ($p = 0.10$ for grapevine vs chaste tree, $p = 1$ for chaste tree vs stinging nettle, $p = 1$ for
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38 276 grapevine vs stinging nettle, Fisher’s Exact Test). *Hyalesthes obsoletus* adults did not show any
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40 277 significant preference for grapevine vs chaste tree ($G = 0.081$, $p = 0.78$), chaste tree vs stinging
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42 278 nettle ($G = 0.081$, $p = 0.78$) or grapevine vs stinging nettle ($G = 2.19$, $p = 0.14$) (Figure 2).

43 279 In the semi-field experiment, there were significant differences in the choice of plant species
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45 280 by *H. obsoletus* adults collected on stinging nettle (Figure 3). In particular, chaste tree was
46
47 281 significantly preferred to grapevine in both 2016 ($t = 2.80$, d.f. = 8, $p = 0.02$) and 2017 ($t = 2.80$, d.f.
48 282 = 8, $p = 0.02$); stinging nettle was significantly preferred to grapevine in both 2016 ($t = 3.39$, d.f. =
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50 283 8, $p = 0.0095$) and 2017 ($t = 5.58$, d.f. = 8, $p = 0.0005$); stinging nettle was significantly preferred
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52 284 to chaste tree in 2017 ($t = 2.44$, d.f. = 8, $p = 0.04$), but not in 2016 ($t = 1.42$, d.f. = 8, $p = 0.19$).

53 285 In the open field, captures of *H. obsoletus* from stinging nettle plants along a ditch were
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55 286 significantly influenced by time (i.e., days from nettle mowing), plants and distance from *H.*
56
57 287 *obsoletus* adults’ source (Table 1). In particular, captures were higher the second than the first week
58
59 288 from nettle mowing. On stinging nettle the captures were significantly higher than on the other two
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3 289 plants (Table 2). Although no individual was captured on grapevine, the differences with respect to
4
5 290 chaste tree were not statistically significant based on LSD5% (Table 2). The captures decreased
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7 291 with the increase of distance from the ditch, i.e. from the source of *H. obsoletus* adults, and were
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9 292 significantly higher at 5 m than both 10 m and 20 m (Table 2). The interactions time × plant, plant ×
10 293 distance and time × plant × distance were significant due to the fact that the captures were
11
12 294 influenced by time and distance only for stinging nettle and chaste tree, because in grapevines the
13
14 295 captures were always zero (Table 1).

17 297 3.3 Egg laying of *Hyalesthes obsoletus* from stinging nettle on chaste tree

18
19 298 Based on the nymphs observed in February of the next year, *H. obsoletus* females laid eggs on
20
21 299 potted plants in 10 out of 12 cages. Nymphs were recorded on the roots of both stinging nettle and
22 300 chaste tree, showing indirectly that females had laid eggs on both plants, but a significantly higher
23
24 301 number was observed on the former ($t = 3.36$, d.f. = 9; $p = 0.009$) (Figure 4).

27 303 3.4 Transmission trials

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29 304 The PCR analyses for amplification of the *stamp* gene, performed on the total nucleic acids
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31 305 extracted from the chaste tree plants used in the transmission trials, showed the presence of '*Ca. P.*
32
33 306 *solani*' in two plants (TBS6 and TBS7) out of 16 (12.5%). No amplification was observed in the
34
35 307 other 14 chaste tree plants, on which insects were maintained, and on the eight control plants
36 308 (without insects) (Table 3). The molecular analyses performed on the insect individuals collected
37
38 309 from plants TBS6 and TBS7 revealed that five individuals out of 16 (31%) and six out of 18
39 310 (33%), respectively, were found to be infected by '*Ca. P. solani*'. *H. obsoletus* adults, collected
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41 311 from the 14 chaste tree plants negative to phytoplasma presence, were found to be infected
42
43 312 percentage varying from 0 to 50% (Table 3). Nucleotide sequence analyses of the *stamp* gene
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45 313 showed that chaste tree plants and insect individuals feeding on them harboured the same '*Ca. P.*
46 314 *solani*' strain, characterized by the *stamp* gene sequence variant St5.

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48 315 The PCR analyses performed on chaste tree leaves collected in October 2018 (one year after the
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50 316 transmission trials), showed that all 24 chaste tree plants, including TBS6 and TBS7 (positive in
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52 317 2017), were negative to phytoplasma presence (Table 3).

DISCUSSION

Survival of *H. obsoletus* adults from stinging nettle was better on the plants on which the nymphs developed (i.e. stinging nettle), than on ~~the other plants (i.e. grapevine and chaste tree)~~. This occurrence was previously observed for *H. obsoletus* from stinging nettle or bindweed that had better survival on the origin plant than on the other (Mori et al., 2008; Kessler et al., 2011; Maixner et al., 2014). Survival on chaste tree was significantly better than on grapevine in one of the two study years. However, the differences were not so high as could be expected from the fact that chaste tree, unlike grapevine, is a true host of the planthopper (Sharon et al., 2015). Our study also indirectly confirmed that *H. obsoletus* can complete its life cycle on chaste tree because nymphs were observed in February on the roots of potted chaste tree plants on which planthopper adults had been caged and ~~been~~ able to lay eggs in the previous summer.

In the field, *H. obsoletus* adults from stinging nettle were more attracted by stinging nettle than chaste tree and ~~even~~ not captured on grapevine. Semi-field experiments confirmed both the scarce attractiveness of grapevine and the preference for stinging nettle than chaste tree. With reference to the two true host plants, namely stinging nettle and chaste tree, preference for the former may be associated with the origin of adults used for the experiments, all collected from stinging nettle plants. Based on this result, even the higher attractiveness of chaste tree in comparison with other plants observed in the olfactometer studies by Sharon et al. (2005) may have been influenced by the fact that most of the adults had been collected on chaste tree. The fact that chaste tree resulted significantly more attractive than grapevine would suggest its use as trap plant at vineyard borders. However, since the infected *H. obsoletus* adults that colonize vineyards in northern Italy move mostly from stinging nettle and for this planthopper population, the nettle was more attractive than chaste tree, the use of healthy potted plants of stinging nettle as trap plants would be preferable. Our two-choice olfactometric studies showed no significant preference by *H. obsoletus* for either of the two plants, even if fewer adults were observed on grapevine than stinging nettle.

Results of the transmission trials conducted in the study ~~proved~~ that chaste tree can harbour 'Ca. P. solani' and that infectious *H. obsoletus* adults from stinging nettle can ~~inoculate this phytoplasma in chaste tree~~. This evidence is in agreement with the results obtained by Kosovac et al. (2016), who demonstrated that ~~chaste tree naturally occurring~~ in vineyard agro-ecosystems in Montenegro ~~is~~ infected by 'Ca. P. solani'. The 'Ca. P. solani' strain St5, transmitted with *H. obsoletus* originating from stinging nettle to chaste tree in the present study, is ~~so far~~ known to be associated only with bindweed as ~~source~~ plant, *H. obsoletus* from bindweed as ~~vector~~, and

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3 353 grapevine ~~in wide geographic European areas~~ (Pierro et al., 2018). Moreover, strain St5 groups
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5 354 within the bindweed-related *stamp* phylogenetic Cluster b-II along with strains St1, St2, and St30,
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7 355 previously ~~found~~ associated with chaste tree or transmitted to grapevine by chaste tree associated *H.*
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9 356 *obsoletus* (Kosovac et al., 2016). Thus, this is the first report of strain St5 transmitted to chaste tree
10 357 by *H. obsoletus* from stinging nettle. As chaste tree constitutes an important reservoir for *H.*
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12 358 *obsoletus*-mediated transmission of BN phytoplasma to grapevine (Kosovac et al., 2016), our
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14 359 findings that chaste tree can host the '*Ca. P. solani*' strain St5, largely prevalent in the Franciacorta
15 360 area, open a new intriguing scenario on its possible role in BN epidemiology in north Italy. ~~On the~~
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17 361 ~~contrary, these~~ results are in disagreement with Sharon et al. (2005, 2015), who showed that, even if
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19 362 ~~it is~~ a preferred host plant of *H. obsoletus*, chaste tree did not harbour '*Ca. P. solani*'. Interestingly,
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21 363 ~~even if~~ '*Ca. P. solani*'-infected insect individuals were found on 15 ~~out~~ of 16 chaste tree plants used
22 364 in transmission trials, *H. obsoletus* was only able to transmit the pathogen in two cases. This could
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24 365 be explained considering the short survival of insect adults on chaste tree; in fact, the insect
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26 366 populations decreased dramatically in 4 to 6 days after release. However, adults of *H. obsoletus*
27 367 from stinging nettle survive on grapevine no better than on chaste tree and still are able to inoculate
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29 368 the BN phytoplasma. Moreover, the success of transmission trials can depend on the phytoplasma
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31 369 strain and ~~its~~ titer within the insect adults. For example, it is reasonable to hypothesize that '*Ca. P.*
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33 370 *solani*' strains not transmitted to chaste tree in the present study could be (as expected) those that are
34 371 strictly associated with stinging nettle (*stamp* clusters a1 and a2). The fact that chaste tree plants,
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36 372 found positive for phytoplasma presence in October 2017, were phytoplasma-free in October 2018
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38 373 can be explained by natural recovery from infection, as reported for a broad range of polyannual
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40 374 plants infected by phytoplasmas (Osler et al., 1993; Romanazzi et al., 2009), increased by abiotic
41 375 stresses due to the overgrowth of chaste trees in pots under controlled conditions, which is not
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43 376 convenient in terms of spacing.

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45 377 According to Sharon et al. (2005, 2015), showing that chaste tree is a preferred host plant of
46 378 *H. obsoletus* and does not harbour '*Ca. P. solani*', in Israel a 'push & pull' strategy was suggested
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48 379 to reduce the population of *H. obsoletus* in a vineyard by using chaste tree as a trap plant (Zahavi et
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50 380 al., 2007). On the contrary, based on the findings of this and previous research work (Kosovac et
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52 381 al., 2016), it is doubtful that chaste tree can be used in the containment of the BN spread in Europe
53 382 by using it as an attractant to *H. obsoletus* since it can also act as a reservoir of '*Ca. P. solani*'.
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55 383 However, volatiles from both chaste tree and stinging nettle could be used in the context of 'push &
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57 384 pull strategies' (Riolo et al., 2017).

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3 385 In conclusion, ~~the results obtained increased the~~ knowledge about the role of *V. agnus-*
4 *castus* as host plant of *H. obsoletus* and '*Candidatus* Phytoplasma solani' in north Italy. Further
5 386 studies are needed to determine the actual role of chaste tree in ~~the~~ BN epidemiology.
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11
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14 391 "Methodologies for Bois Noir containment".
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17 393 **CONFLICTS OF INTEREST**

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19 394 The authors declare no potential conflict of interests.
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60**REFERENCES**

- Alma, A., Arnò, C., Arzone, A., & Vidano, C. (1988). New biological reports on Auchenorrhyncha in vineyards. In C. Vidano & A. Arzone (Eds.), *Proceedings of 6th Auchenorrhyncha Meeting*, (pp. 509–516), 7–11 September 1987, Turin, Italy.
- Angelini, E., Constable, F., Duduk, B., Fiore, N., Quaglino, F., & Bertaccini A. (2018). Grapevine phytoplasmas. In G. P. Rao, A. Bertaccini, N. Fiore & L. W. Liefting (Eds.), *Characterisation and Epidemiology of Phytoplasma - Associated Diseases. Phytoplasmas: Plant Pathogenic Bacteria-I*, (pp. 123–152). Springer Nature, Singapore.
- Aryan, A., Brader, G., Mörtel, J., Pastar, M., & Riedle-Bauer, M. (2014). An abundant 'Candidatus Phytoplasma solani' Stolbur *tuf b* phytoplasma strain is associated with grapevine, stinging nettle and *Hyalesthes obsoletus*. *European Journal of Plant Pathology*, **140**, 213–227.
- Atanasova, B., Jakovljević, M., Spasov, D., Jović, J., Mitrović, M., Toševski, I., & Cvrković, T. (2015). The molecular epidemiology of bois noir grapevine yellows caused by 'Candidatus Phytoplasma solani' in the Republic of Macedonia. *European Journal of Plant Pathology*, **142**, 759–770.
- Batlle, A., Altabella, N., Sabaté, J. & Laviña, A. (2008). Study of the transmission of stolbur phytoplasma to different crop species by *Macrostelus quadripunctulatus*. *Annals of Applied Biology*, **152**, 235–242
- Bellée, A., Cluzet, S., Dufour, M.-C., Mérrillon, J. M., & Corio-Costet, M.-F. (2018). Comparison of the impact of two molecules on plant defense and on efficacy against *Botrytis cinerea* in the vineyard: a plant defense inducer (Benzothiadiazole) and a fungicide (Pyrimethanil). *Journal of Agricultural and Food Chemistry*, **66**, 3338–3350.
- Belli, G., Bianco, P. A., & Conti, M. (2010). Grapevine Yellows in Italy: past, present and future. *Journal of Plant Pathology*, **92**, 303–326.
- Bertin, S., Picciau, L., Ács, Z., Alma, A., & Bosco, D. (2010). Molecular identification of the *Hyalesthes obsoletus* (Hemiptera: Cixiidae) occurring in vineyard agroecosystems. *Annals of Applied Biology*, **157**, 435–445.
- Bianco, P. A., Davis, R. E, Prince, J. P., Lee, I. M., Gundersen, D. E., Fortusini, A., & Belli, G. (1993). Double and single infections by aster yellows and elm yellows MLOs in grapevines with symptoms characteristic of flavescente dorée. *Rivista di Patologia Vegetale*, **3**, 69–82.
- Bressan, A., Turata, R., Maixner, M., Spiazzi, S., Boudon-Padieu, E., & Girolami, V. (2007). Vector activity of *Hyalesthes obsoletus* living on nettles and transmitting a stolbur phytoplasma to grapevines: a case study. *Annals of Applied Biology*, **150**, 331–339.

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2
3
4
5
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9
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15
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42
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44
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46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 430 Cargnus, E., Pavan, F., Mori, N., & Martini, M. (2012). Identification and phenology of *Hyalesthes*
431 *obsoletus* (Hemiptera: Auchenorrhyncha: Cixiidae) nymphal instars. *Bulletin of*
432 *Entomological Research*, **102**, 504–514.
- 433 Chuche, J., Danet, J. L., Salar, P., Foissac, X., & Thiéry, D. (2016). Transmission of ‘*Candidatus*
434 *Phytoplasma solani*’ by *Reptalus quinquecostatus* (Hemiptera: Cixiidae). *Annals of Applied*
435 *Biology*, **169**, 214–223.
- 436 Cvrković, T., Jović J., Mitrović, M., Krstić, O., & Toševski, I. (2014). Experimental and molecular
437 evidence of *Reptalus panzeri* as a natural vector of bois noir. *Plant Pathology*, **63**, 42–53.
- 438 Dermastia, M., Bertaccini, A., Constable, F., & Mehele, N. (2017). *Grapevine Yellows Diseases*
439 *and Their Phytoplasma Agents: Biology and Detection*, Springer Nature, Cham,
440 Switzerland.
- 441 Dicke, M., Sabelis, M.W., & De Jong, M. (1988). Analysis of prey preference in phytoseiid mites
442 by using an olfactometer, predation models and electrophoresis. *Experimental & Applied*
443 *Acarology*, **5**, 225–241.
- 444 Đurić, Z., Hrnčić, S., & Delić, D. (2017). Morphological and molecular identification of *Hyalesthes*
445 *obsoletus* Signoret (Auchenorrhyncha: Cixiidae) in Herzegovina vineyards. *Mitteilungen*
446 *Klosterneuburg*, **67**, 177–181.
- 447 Fabre, A., Danet, J. L., & Foissac, X. (2011). The stolbur phytoplasma antigenic membrane protein
448 gene *stamp* is submitted to diversifying positive selection. *Gene*, **472**, 37–41.
- 449 FAO (2016). Comparison between world and Europe total area harvested for grapes.
- 450 Fialová, R., Válková, P., Balakishiyeva, G., Danet, J. L., Sáfárová, D., Foissac, X., & Navrátil, M.
451 (2009). Genetic variability of Stolbur phytoplasma in annual crop and wild plant species in
452 South Moravia. *Journal of Plant Pathology*, **91**, 411–416.
- 453 Foissac, X., Carle, P., Fabre, A., Salar, P., Danet, J. L., & Stolbureuomed Consortium (2013).
454 ‘*Candidatus Phytoplasma solani*’ genome project and genetic diversity in the Euro-
455 Mediterranean basin. In E. Torres, A. Laviña & A. Batlle (Eds.), *Book of Abstracts of 3rd*
456 *European Bois Noir Workshop* (pp. 11–13), 21–22 March 2013, Barcelona, Spain.
- 457 Hall, T. A. BioEdit: a user-friendly biological sequence alignment editor and analysis program for
458 Windows 95/98/NT. Nucleic acids symposium series, 1999. [London]: Information
459 Retrieval Ltd., c1979-c2000., 95–98.
- 460 Hoch, H., & Remane, R. (1865). Evolution und Speziation der Zikaden-Gattung *Hyalesthes*. *Sign.*

- 1
2
3 461 Johannesen, J., Lux, B., Michel, K., Seitz, A., & Maixner, M. (2008). Invasion biology and host
4 specificity of the grapevine yellows disease vector *Hyalesthes obsoletus* in Europe.
5 462
6 463 *Entomologia Experimentalis et Applicata*, **126**, 217–227.
- 8 464 Kast, W. K., Stark-Urnau, M., & Bleyer, K. (2008). Bois noir, a severe outbreak of stolbur type A
9 in Southern Germany - disease abundance and treatments against disease-causing agents and
10 465
11 vectors. *IOBC/WPRS Bulletin*, **36**, 121–125.
- 13 467 Kessler, S., Scherer, S., Delabays, N., Turlings, T. C. J., Trivellone, V., & Kehrl, P. (2011). Host
14 468
15 468 plant preference of *Hyalesthes obsoletus*, the vector of the grapevine yellow disease 'bois
16
17 469 noir', in Switzerland. *Entomologia Experimentalis et Applicata*, **139**, 60–67.
- 18 470 Kosovac, A., Johannesen, J., Krstić, O., Mitrović, M., Cvrković, T., Maixner, M., Toševski, I., &
19 471
20 471 Jović, J. (2013). Microsatellite and mtDNA evidence of genetic differentiation in *Hyalesthes*
21
22 472 *obsoletus* populations associated with a new major host, stinking hawk's-beard (*Crepis*
23
24 473 *foetida*), in southeast Europe. In E. Torres, A. Lavina & A. Batlle (Eds.), *Book of Abstracts*
25
26 474 *of 3rd European Bois Noir Workshop* (pp. 18–19), 21-22 March 2013, Barcelona, Spain.
- 27 475 Kosovac, A., Radonjić, S., Hrnčić, S., Krstić, O., Toševski, I., & Jović, J. (2016). Molecular tracing
28
29 476 of the transmission routes of bois noir in Mediterranean vineyards of Montenegro and
30
31 477 experimental evidence for the epidemiological role of *Vitex agnus-castus* (Lamiaceae) and
32
33 478 associated *Hyalesthes obsoletus* (Cixiidae). *Plant Pathology*, **65**, 285–298.
- 34 479 Kosovac, A., Jakovljević, M., Krstić, O., Cvrković, T., Mitrović, M., Toševski, I., & Jović, J.
35
36 480 (2019). Role of plant-specialized *Hyalesthes obsoletus* associated with *Convolvulus arvensis*
37
38 481 and *Crepis foetida* in the transmission of 'Candidatus Phytoplasma solani'-inflicted bois
39
40 482 noir disease of grapevine in Serbia. *European Journal of Plant Pathology*, **153**, 183–195.
- 41 483 Landi, L., Riolo, P., Murolo, S., Romanazzi, G., Nardi, S., & Isidoro, N. (2015). Genetic variability
42
43 484 of stolbur phytoplasma in *Hyalesthes obsoletus* (Hemiptera: Cixiidae) and its main host
44
45 485 plants in vineyards agroecosystems. *Journal of Economic Entomology*, **108**, 1506–1515.
- 46 486 Langer, M. & Maixner, M. (2004). Molecular characterisation of grapevine yellows associated
47
48 487 phytoplasmas of the stolbur-group based on RFLP-analysis of non ribosomal DNA. *Vitis*,
49
50 488 **43**, 191–199.
- 51 489 Magarey, P. (2017). Grape-vine yellows-aetiology, epidemiology and diagnosis. *South African*
52
53 490 *Journal of Enology and Viticulture*, **7**, 90–100.
- 54 491 Maixner, M. (1994). Transmission of German grapevine yellows (Vergilbungskrankheit) by the
55
56 492 planthopper *Hyalesthes obsoletus* (Auchenorrhyncha: Cixiidae). *Vitis*, **33**, 103–104.
- 58
59
60

- 1
2
3 493 Maixner, M. (2010). Phytoplasmas epidemiological systems with multiple plant hosts. In P.G.
4 Weintraub & P. Jones (Eds.), *Phytoplasmas: Genomes, Plant Hosts and Vectors* (pp. 213–
5 494 232), CABI Publishing, Wallingford, UK.
6
7 495
8 496 Maixner, M., Albert, A., & Johannesen, J. (2014). Survival relative to new and ancestral host plants,
9 phytoplasma infection, and genetic constitution in host races of a polyphagous insect disease
10 497 vector. *Ecology and Evolution*, **4**, 3082–3092.
11
12 498
13 499 Mannini, F. (2007). Relazione sulla termoterapia, applicata presso alcuni vivai Piemontesi.
14 *L'Informatore Agrario*, **63** (24), 87–90.
15 500
16
17 501 Marchi, G., Cinelli, T., Rizzo, D., Stefani, L., Goti, E., Della Bartola, M., Luvisi, A., Panattoni, A.,
18 & Materazzi, A. (2015). Occurrence of different phytoplasma infections in wild herbaceous
19 502 dicots growing in vineyards affected by bois noir in Tuscany (Italy). *Phytopathologia*
20 503 *Mediterranea*, **54**, 504–515.
21
22 504
23
24 505 Mori, N., Pavan, F., Bacchiavini, M., Reggiani, N., Bonomi, F., & Bertaccini, A. (2008a).
25 Fenologia di *Hyalesthes obsoletus* Signoret su convolvolo e ortica. In *Atti "4° Incontro*
26 506 *Nazionale sulle Malattie da Fitoplasmii"*, 28-30 maggio 2008 Roma, Italia. *Petria*, **18**, 229–
27 507 231.
28
29 508
30
31 509 Mori, N., Pavan, F., Bondavalli, R., Reggiani, N., Paltrinieri, S., & Bertaccini, A. (2008b). Factors
32 affecting the spread of “Bois Noir” disease in north Italy vineyards. *Vitis*, **47**, 65–72.
33 510
34 511 Mori, N., Pavan, F., Reggiani, N., Bacchiavini, M., Mazzon, L., Paltrinieri, S., & Bertaccini, A.
35 (2012). Correlation of bois noir disease with nettle and vector abundance in northern Italy
36 512 vineyards. *Journal of Pest Science*, **85**, 23–28.
37
38 513
39 514 Mori, N., Mitrović, J., Smiljković, M., Duduk, N., Paltrinieri, S., Bertaccini, A., & Duduk, B.
40 (2013). *Hyalesthes obsoletus* in Serbia and its role in the epidemiology of corn reddening.
41 515 *Bulletin of Insectology*, **66**, 245–250.
42
43 516
44 517 Mori, N., Quaglino, F., Tessari, F., Pozzebon, A., Bulgari, D., Casati, P., & Bianco, P. A. (2015).
45 Investigation on ‘bois noir’ epidemiology in north-eastern Italian vineyards through a
46 518 multidisciplinary approach. *Annals of Applied Biology*, **166**, 75–89.
47
48 519
49 520 Murolo, S., Mancini, V., & Romanazzi, G. (2014). Spatial and temporal stolbur population structure
50 521 in a cv Chardonnay vineyard according to *vmp1* gene characterisation. *Plant Pathology*, **63**,
51 522 700–707.
52
53 523
54 524 Murolo, S., & Romanazzi, G. (2015). In-vineyard population structure of '*Candidatus* Phytoplasma
55 525 *solani*' using multilocus sequence typing analysis. *Infection, Genetics and Evolution*, **31**,
56 221–230.
57
58
59
60

- 1
2
3 526 Oliveri, C., Pacifico, D., D'Urso, V., La Rosa, R., Marzachi, C., & Tessitori, M. (2015). Bois noir
4
5 527 phytoplasma variability in a Mediterranean vineyard system: new plant host and putative
6
7 528 vectors. *Australasian Plant Pathology*, **44**, 235–244.
- 8
9 529 Osler R., Carraro L., Loi N., & Refatti, E. (1993). Symptom expression and disease occurrence of a
10 530 yellows disease of grapevine in northeastern Italy. *Plant Disease*, **77**, 496–498.
- 11
12 531 Panassiti, B., Hartig, F., Fahrentrapp, J., Breuer, M., & Biedermann, R. (2017). Identifying local
13
14 532 drivers of a vector-pathogen disease system using Bayesian modeling. *Basic and Applied
15 533 Ecology*, **18**, 75–85.
- 16
17 534 Paolacci, A. R., Catarcione, G., Ederli, L., Zadra, C., Pasqualini, S., Badiani, M., Musetti, R., Santi,
18
19 535 S., & Ciaffi, M. (2017). Jasmonate-mediated defence responses, unlike salicylate-mediated
20
21 536 responses, are involved in the recovery of grapevine from bois noir disease. *BMC Plant
22 537 Biology*, **17**, 118.
- 23
24 538 Pavan, F., Mori, N., Bressan, S., & Mutton, P. (2012). Control strategies for grapevine phytoplasma
25
26 539 diseases: factors influencing the profitability of replacing symptomatic plants.
27 540 *Phytopathologia Mediterranea*, **51**, 11–22.
- 28
29 541 Picciau, L., Lavezzaro, S., Morando, A., Cesano, A., Cuttini, D., Saladini, M. A., & Alma, A.
30
31 542 (2010). Spollonatura e pulizia sottofila limitano il legno nero della vite. *L'Informatore
32 543 Agrario*, **66** (25), 57–59.
- 34 544 Pierro, R., Passera, A., Panattoni, A., Casati, P., Luvisi, A., Rizzo, D., Bianco, P.A., Quaglino, F., &
35
36 545 Materazzi, A. (2018a). Molecular typing of 'bois noir' phytoplasma strains in the *Chianti
37
38 546 Classico* area (Tuscany, central Italy) and their association with symptom severity in *Vitis
39 547 vinifera* L. cv. Sangiovese. *Phytopathology*, **108**, 362–373.
- 41 548 Pierro, R., Passera, A., Panattoni, A., Rizzo, D., Stefani, L., Bartolini, L., Casati, P., Luvisi, A.,
42
43 549 Quaglino, F., & Materazzi, A. (2018b). Prevalence of a '*Candidatus* Phytoplasma solani'
44
45 550 strain, so far associated only with other hosts, in Bois noir-affected grapevines within
46 551 Tuscan vineyards. *Annals of Applied Biology*, **173**, 202–212.
- 47
48 552 Riedle-Bauer, M., Sára, A., & Regner, F. (2008). Transmission of a stolbur phytoplasma by the
49
50 553 agalliinae leafhopper *Anaceratagallia ribauti* (Hemiptera, Auchenorrhyncha, Cicadellidae).
51 554 *Journal of Phytopathology*, **156**, 687–690.
- 53 555 Riedle-Bauer, M., Hanak, K., Regner, F., & Tiefenbrunner, W. (2010). Influence of pruning
54
55 556 measures on recovery of Bois noir-infected grapevines. *Journal of Phytopathology*, **158**,
56
57 557 628–632.
- 58
59
60

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8
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51
52
53
54
55
56
57
58
59
60

- 558 Riolo, P., Minuz, R. L., Anfora, G., Stacconi, M. V. R., Carlin, S., Isidoro, N., & Romani, R.
559 (2012). Perception of host plant volatiles in *Hyalesthes obsoletus*: behavior, morphology,
560 and electrophysiology. *Journal of Chemical Ecology*, **38**, 1017-1030.
- 561 Riolo, P., Minuz, R.L., Peri, E., & Isidoro, N. (2017). Behavioral responses of *Hyalesthes obsoletus*
562 to host-plant volatiles cues. *Arthropod-Plant Interactions*, **11**, 71–78
- 563 Romanazzi, G., D'Ascenzo, D., & Murolo, S. (2009). Field treatment with resistance inducers for
564 the control of grapevine Bois noir. *Journal of Plant Pathology*, **91**, 677–682.
- 565 Romanazzi, G., Murolo, S., & Feliziani, E. (2013). Effects of an innovative strategy to contain Bois
566 noir: field treatment with resistance inducers. *Phytopathology*, **103**, 785–791.
- 567 Quaglino, F., Zhao, Y., Casati, P., Bulgari, D., Bianco, P. A., Wei, W., & Davis, R. E. (2013).
568 ‘*Candidatus Phytoplasma solani*’, a novel taxon associated with stolbur-and bois noir-
569 related diseases of plants. *International Journal of Systematic and Evolutionary*
570 *Microbiology*, **63**, 2879–2894.
- 571 Šafářová, D., Lauterer, P., Starý, M., Válková, P., & Navrátil, M. (2018). Insight into
572 epidemiological importance of phytoplasma vectors in vineyards in South Moravia, Czech
573 Republic. *Plant Protection Science*, **54**, 234–239.
- 574 Sharon, R., Soroker, V., Wesley, S. D., Zahavi, T., Harari, A., & Weintraub, P. G. (2005). *Vitex*
575 *agnus-castus* is a preferred host plant for *Hyalesthes obsoletus*. *Journal of Chemical*
576 *Ecology*, **31**, 1051–1063.
- 577 Sharon, R., Harari, A. R., Zahavi, T., Raz, R., Dafny-Yelin, M., Tomer, M., Sofer-Arad, C.,
578 Weintraub, P. G., & Naor, V. (2015). A yellows disease system with differing principal host
579 plants for the obligatory pathogen and its vector. *Plant Pathology*, **64**, 785–791.
- 580 Sforza, R., Clair, D., Daire, X., Larrue, J., & Boudon-Padieu, E. (1988). The role of *Hyalesthes*
581 *obsoletus* (Hemiptera: Cixiidae) in the occurrence of bois noir of grapevines in France.
582 *Journal of Phytopathology*, **146**, 549–556.
- 583 Weber, A., & Maixner, M. (1998). Habitat requirements of *Hyalesthes obsoletus* Signoret
584 (Auchenorrhyncha: Cixiidae) and approaches to control this planthopper in vineyards.
585 *IOBC/WPRS Bulletin*, **21** (2), 77–78.
- 586 Zahavi, T., Peles, S., Harari, A. R., Soroker, V., & Sharon, R. (2007). Push and pull strategy to
587 reduce *Hyalesthes obsoletus* population in vineyards by *Vitex agnus castus* as trap plant.
588 *Bulletin of Insectology*, **60**, 297.

TABLE 1 Results of ANOVA on the captures of *H. obsoletus* recorded in the field on three potted plants (i.e., grapevine, chaste tree and stinging nettle) at two different times after stinging nettle mowing (1st and 2nd week) and at three different distances from the ditch source of the *H. obsoletus* adults (5 m, 10 m, 20 m).

Source of variation	F	df	P
Time	19.88	1, 90	< 0.0001
Plant	59.50	2, 90	< 0.0001
Distance	50.04	2, 90	< 0.0001
Time × plant	9.11	2, 90	< 0.0001
Time × distance	2.28	4, 90	0.10
Plant × distance	19.00	4, 90	< 0.0001
Time × plant × distance	3.55	4, 90	0.010

TABLE 2 Average capture recorded on the three plants and at the three different distances from the ditch where stinging nettle was mowed. SED, standard error of the differences between two means; LSD 5%, least significant difference between two means at $P = 0.05$; d.f., degrees of freedom associated with LSDs and SEDs.

Plant	Mean	Mean [log (x+1)]	Distance	Mean	Mean [log (x+1)]
Stinging nettle	5.72	(0.50)	5 m	5.64	(0.48)
Chaste tree	1.00	(0.13)	10 m	0.94	(0.12)
Grapevine	0.00	(0.00)	20 m	0.14	(0.03)
Stinging nettle vs chaste tree			5 m vs 10m		
SED		(0.15)			(0.15)
LSD 5%		(0.30)			(0.31)
d.f.		34			34
Chaste tree vs grapevine			10 m vs 20 m		
SED		(0.07)			(0.08)
LSD 5%		(0.15)			(0.16)
d.f.		34			34

TABLE 3 Results of transmission trials conducted using *H. obsoletus*, collected on sting nettle bordering BN-infected vineyards, and PCR-negative for '*Ca. P. solani*' chaste tree plants.

Origin	#	Plants		Insects			BNp strain
		BNp-infected (strain)		Number		BNp-infected	
		Oct 17	Oct 18	Released	Collected		
Verona	TVR1	-	-	30	27	10 (37%)	
	TVR2	-	-	30	13	3 (23%)	
	TVR3	-	-	30	21	10 (48%)	
	TVR4	-	-	30	20	4 (20%)	
	TVR5	-	-	30	15	4 (27%)	
	TVR6	-	-	30	19	3 (16%)	
	TVR7	-	-	30	16	4 (25%)	
	TVR8	-	-	30	15	1 (7%)	
Brescia	TBS1	-	-	30	17	4 (23%)	
	TBS2	-	-	30	18	3 (17%)	
	TBS3	-	-	30	17	8 (47%)	
	TBS4	-	-	30	20	2 (10%)	
	TBS5	-	-	30	10	0	
	TBS6	+ (St5)	-	30	16	5 (31%)	St5
	TBS7	+ (St5)	-	30	18	6 (33%)	St5
	TBS8	-	-	30	20	10 (50%)	
Control	T1	-	-				
	T2	-	-				
	T3	-	-				
	T4	-	-				
	T5	-	-				
	T6	-	-				
	T7	-	-				
	T8	-	-				

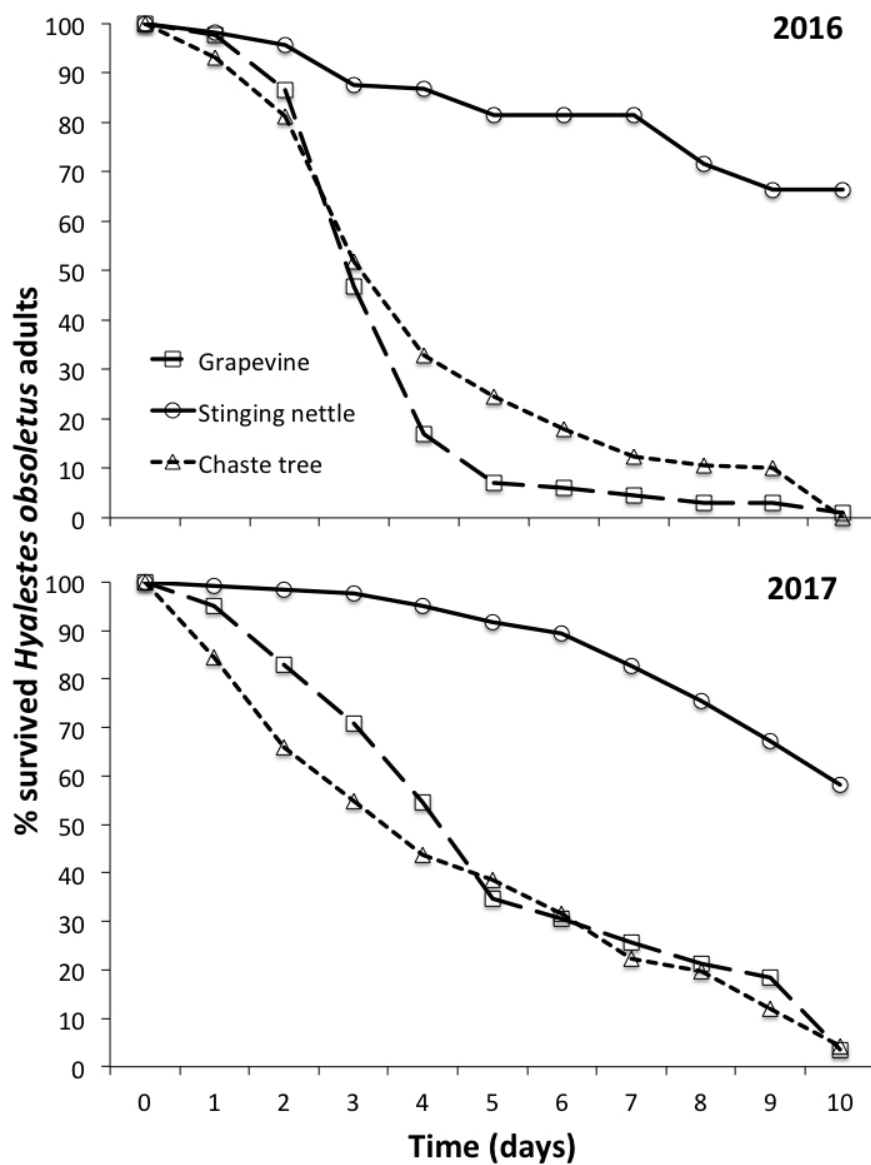


FIGURE 1 Survival of *H. obsolete* from stinging nettle recorded in 2016 and 2017 on three different plant species.

254x338mm (72 x 72 DPI)

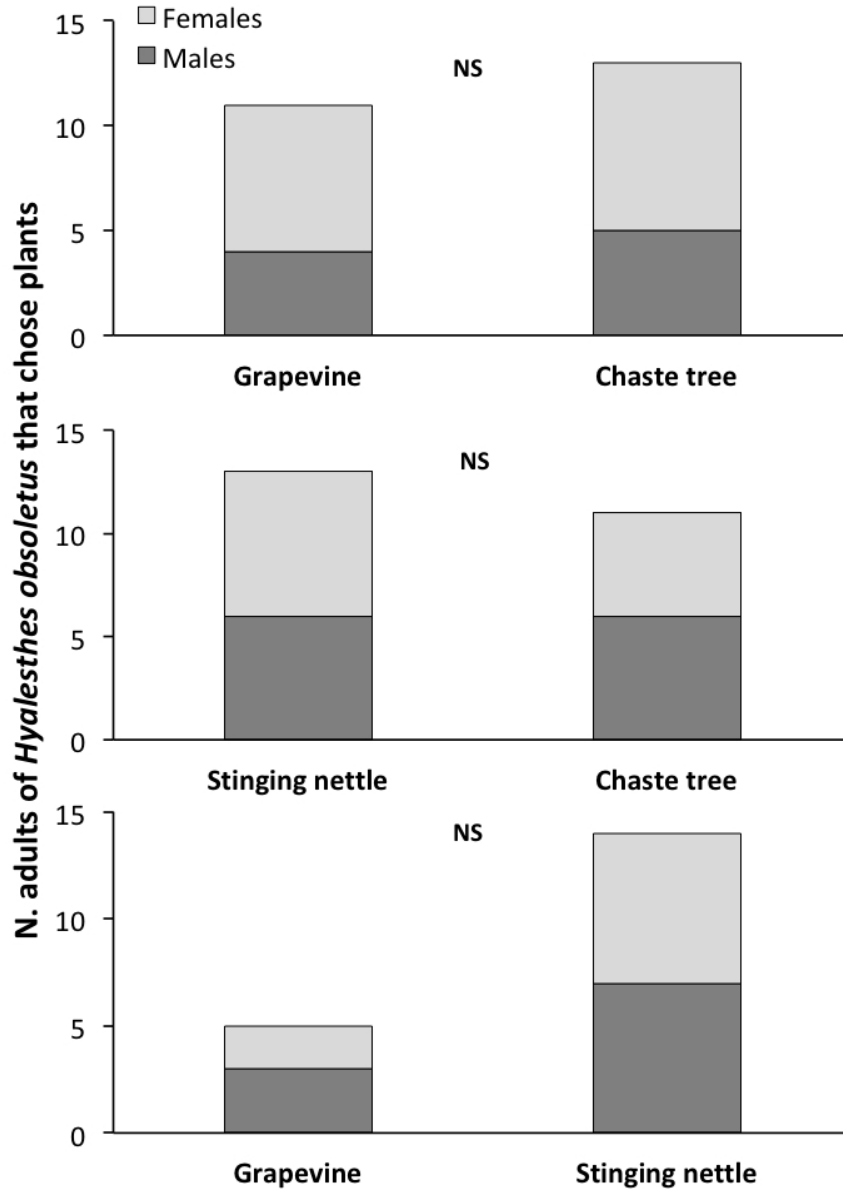


FIGURE 2 Number of *H. obsoletus* individuals (males and females) out of 40 that moved towards the two plants under comparison in two-choice olfactometer tests. NS indicates not significant differences ($\alpha = 0.05$) with G-test of goodness of fit.

254x338mm (72 x 72 DPI)

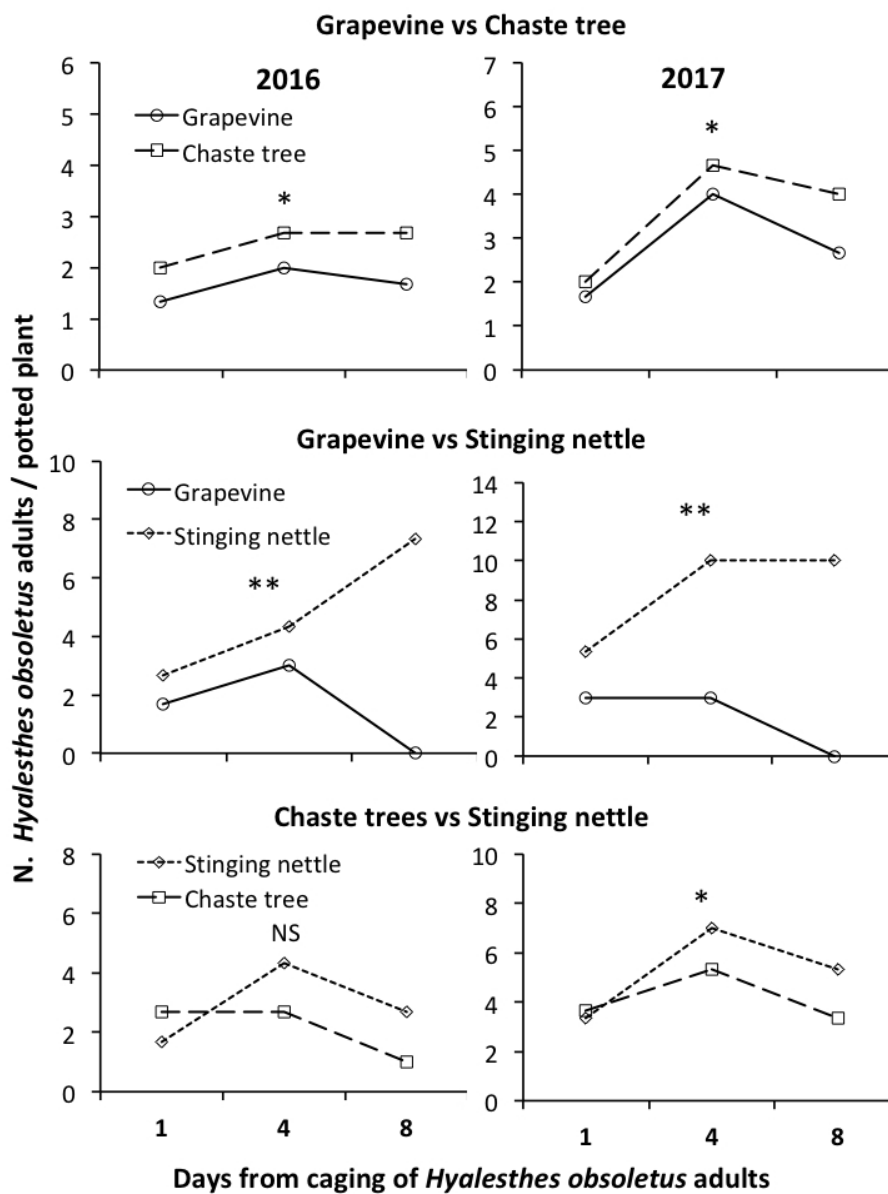


FIGURE 3 Number of *H. obsoletus* individuals out of 60 choosing the different plants in two-choice test with potted plants. NS, * and ** indicate, respectively, not significant and significant differences according to a paired-sample t test ($\alpha = 0.05$ and 0.01).

254x338mm (72 x 72 DPI)

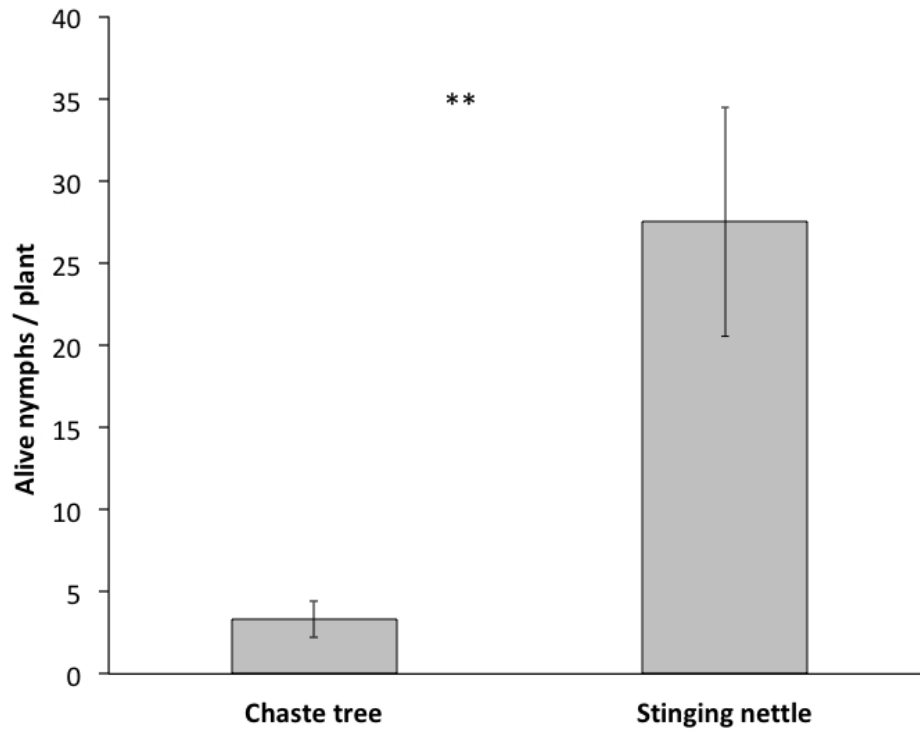


FIGURE 4 Alive nymphs of *H. obsoletus* observed on the roots of the two plant species in the February following the oviposition period in late summer of the previous year. ** = significant differences according to a paired-sample t test ($\alpha = 0.01$).

254x190mm (72 x 72 DPI)

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4 1 **Vitex agnus-castus cannot be used as trap plant for the vector**
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6 2 ***Hyalesthes obsoletus* to prevent infections by 'Candidatus Phytoplasma**
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8 3 ***solani*' in northern Italian vineyards: experimental evidence Can**
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10 4 ~~***Vitex agnus-castus* to be used as trap plant for the vector *Hyalesthes***~~
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12 5 ~~***obsoletus* to prevent infections by 'Candidatus Phytoplasma solani' in**~~
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14 6 ~~**Northern Italy vineyards?**~~
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51 27 **Running title:** Possible role of chaste tree in bois noir epidemiology
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Abstract

Bois noir (BN), the more widespread disease of the grapevine yellows complex, is causing a considerable yield loss in vineyards. BN is associated with phytoplasma strains of the species '*Candidatus Phytoplasma solani*' (taxonomic subgroup 16SrXII-A). In Europe, BN phytoplasma is transmitted to grapevine mainly by *Hyaalsthes obsoletus*, a polyphagous cixiid completing its life cycle on stinging nettle and field bindweed. Due to the complexity of BN epidemiology, no effective control strategies have been developed. In ~~east Mediterranean coast of Israel~~^{Israel}, chaste tree (*Vitex agnus-castus*), even if found to be the preferred host plant of *H. obsoletus*, did not harbor BN phytoplasma. Thus, a "push and pull" strategy was suggested based on the fact that chaste tree plants located at vineyard borders was an effective trap plant for *H. obsoletus* adults. However, in ~~east Adriatic coast of Montenegro~~^{Eastern Europe}, chaste tree was found to be a key source plant for BN phytoplasma transmission to grapevine. Considering such contradictory data, this study aimed to investigate (i) the interaction between chaste tree and *H. obsoletus* through survival, attractiveness and oviposition experiments conducted comparing the behavior of *H. obsoletus* in chaste tree versus stinging nettle and grapevine, and (ii) the capability of chaste tree to harbor '*Ca. P. solani*' in ~~n~~ⁿorthern Italy through transmission trials. Obtained data showed that (i) *H. obsoletus* adults can survive on chaste tree and grapevine even over a week; (ii) *H. obsoletus* adults prefer chaste tree to grapevine; (iii) *H. obsoletus* can produce eggs and overwinter as nymphs on chaste tree, even if at a lesser extent than on stinging nettle; (iv) *H. obsoletus* ~~originating from nettle~~ is able to transmit '*Ca. P. solani*' ~~from nettle~~ to chaste tree (two plants out of 16 were found infected by the BN phytoplasma strain St5 identified in *H. obsoletus* specimens). These results increased knowledge about the role of *Vitex agnus-castus* as host plant of *H. obsoletus* and BN phytoplasma in ~~n~~ⁿorthern Italy and do not allow considering chaste tree as trap plant at vineyard borders.

Key words: insect vectors, Bois noir, trap plant, transmission trials, *stamp gene*

1 INTRODUCTION

Europe is the world leader in grape production with almost half of the global vine-growing. Italy is the second top producer of grapes after China with about 8.2 million tons (FAO, 2016). Quality and quantity of viticulture production are damaged by a wide range of pathogens associated with diseases affecting the main cultivated grapevine varieties (Bellée et al., 2018). Among these diseases, the grapevine yellows (GY) complex is one of the most important threats to viticulture in many countries (Magarey, 2017). The GY causal agents are phytoplasmas ('*Candidatus Phytoplasma*'), cell-wall less obligate parasitic bacteria transmitted by insect vectors to plants, in which they reside in phloem tissues (Angelini et al., 2018). Interestingly, even if undistinguishable based on symptoms, the main diseases within the GY complex are associated with genetically distinct phytoplasmas, belonging to at least six '*Ca. Phytoplasma*' species, characterized by different biological features that reflect on disease epidemiological patterns (Belli et al., 2010; Angelini et al., 2018).

Bois noir (BN) is the most widespread disease of the GY complex in the Euro-Mediterranean area, where it may lead to a total yield loss and even grapevine death (Belli et al., 2010; Pavan et al., 2012). BN is associated with grapevine infection by phytoplasma strains (Bois noir phytoplasma strains, BNp) of the species '*Candidatus Phytoplasma (Ca. P.) solani*' (subgroup 16SrXII-A) (Quaglino et al., 2013). In the Euro-Mediterranean regions the main '*Ca. P. solani*' insect vector is *Hyalesthes obsoletus* Signoret (Homoptera: Cixiidae) (Maixner, 1994; Sforza et al., 1998; Bressan et al., 2007), a polyphagous planthopper living preferentially on stinging nettle (*Urtica dioica* L.), field bindweed (*Convolvulus arvensis* L.), stinking hawk's-beard (*Crepis foetida* L.), and *Artemisia* spp. in and/or around vineyards (Alma et al., 1988; Sforza et al., 1998; Weber & Mainer, 1988; Langer & Maixner, 2004; Mori et al., 2008b, 2013; Cargnus et al., 2012; Kosovac et al., 2013). Recently, *Reptalus panzeri* (Low) (Homoptera: Cixiidae) has been reported as vector of '*Ca. P. solani*' (CaPsol) in Serbian vineyards (Cvrković et al., 2014) while *Macrosteles quadripunctulatus* (Kirschbaum) (Homoptera: Cicadellidae) was found able to transmit CaPsol to potted grapevine plants (Batlle et al., 2008). In addition *Anaceratagallia ribauti* (Ossiannilsson) (Homoptera: Cicadellidae) and *Reptalus quinquecostatus* (Dufour) (Homoptera: Cixiidae) were reported as vectors even if not to grapevine (Riedle-Bauer et al., 2008; Chuche et al., 2016). Other studies reported that different Cixiidae and Cicadellidae species have been captured within or near BN-diseased vineyards and found to contain CaPsol (Oliveri et al., 2015; Šafařová et al., 2018) but such insects are not currently considered to be involved in CaPsol transmission to grapevine.

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2
3 95 The sequence analysis of *tufB* gene revealed that two main '*Ca. P. solani*' *tuf*-types are
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5 96 present on grapevines and alternative plant hosts, according to diverse ecological pathosystems: (i)
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7 97 field bindweed - *H. obsoletus* - grapevine *tuf*-type b, (ii) stinging nettle - *H. obsoletus* - grapevine
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9 98 *tuf*-type a (Langer & Maixner, 2004). Recently, in Austria, Aryan et al. (2014) detected a large
10 99 presence of a *tuf*-type b with a distinguished *Hpa*II-restriction profile designed as *tuf*-type b2 that
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12 100 appears to have different ecological features. Interestingly, most recent evidence highlighted the
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14 101 existence of a new BN epidemiological cycles of *tuf*-type b '*Ca. P. solani*' strain in the Balkan
15 102 region and in east Adriatic coast of Montenegro, sourced respectively by *C. foetida* and *Vitex*
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17 103 *agnus-castus* L. transmitted by ~~its~~ their associated *H. obsoletus* population (Kosovac et al., 2016,
18
19 104 2019). Moreover, several weeds, such as *Chenopodium album* L. and *Malva sylvestris* L., host the
20 105 '*Ca. P. solani*' in or around infected vineyards and can therefore play a role in BN spreading
21 106 (Marchi et al., 2015; Mori et al., 2015; Oliveri et al., 2015). Molecular epidemiology approaches,
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24 107 using *vmpI*- and *stamp*-based markers allowed knowledge to be increased of the populations of BN
25 108 throughout vineyards and their surroundings in the Mediterranean area (Fialová et al., 2009; Fabre
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27 109 et al., 2011; Foissac et al., 2013; Murolo et al., 2014; Landi et al., 2015; Murolo & Romanazzi,
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29 110 2015; Pierro et al., 2018a, 2018b).

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31 111 The complexity of BN disease epidemiology renders it difficult to design efficient control
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33 112 strategies. Insecticides applied to the grapevine canopy influence neither the disease nor the
34 113 presence of *H. obsoletus* (Maixner, 2007; Mori et al., 2008b). The management of *H. obsoletus* host
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36 114 plants in the vineyards and surrounding areas is therefore considered crucial for BN control
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38 115 (Maixner, 2010; Mori et al., 2012; Panassiti et al., 2017). Thus, preventive measures, such as
39 116 checking the health status of propagation materials (i.e., mother plants and grafted cuttings) and
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41 117 treating of cuttings through thermotherapy, are applied to limit long distance dissemination and in-
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43 118 field spread of the disease (Mannini et al., 2007). Other strategies for reducing BN spread or
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45 119 incidence are based on (i) preventive removal of the grape suckers on which *H. obsoletus* could
46 120 feed after grass mowing (Picciau et al., 2010); (ii) trunk cutting above the engagement point on
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48 121 symptomatic grapevines (Kast et al., 2008; Riedle-Bauer et al., 2010); (iii) treatments by resistance
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50 122 inducers (Romanazzi et al., 2009, 2013). In prospective, also plant volatiles from host plants can be
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52 123 used for reducing vineyard colonization by *H. obsoletus* (Riolo et al., 2017).

53 124 In Israel, chaste tree (*Vitex agnus-castus* L.) is a plant where *H. obsoletus* can complete its
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55 125 life cycle (Sharon et al., 2005). In both olfactometric and field studies chaste tree resulted more
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57 126 attractive than grapevine for *H. obsoletus* adults (Sharon et al., 2005; Zahavi et al., 2007; Riolo et
58 127 al., 2012). Therefore, a "push and pull" strategy based on the use of chaste tree as trap plant at
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vineyard borders to reduce the vector population living inside the vineyards was suggested (Zahavi et al., 2007). The validity of this strategy is reinforced by the fact that in Israel chaste tree was never found to be infected by '*Ca. P. solani*' and thus cannot serve as an inoculation source for grapevine (Sharon et al., 2015). ~~However, a study conducted in east Adriatic coast of Montenegro Eastern Europe reported the direct epidemiological role of *V. agnus-castus* as '*Ca. P. solani*' source in the *H. obsoletus* mediated transmission to grapevine (Kosovae et al., 2016)~~

~~Considering such contradictory data,~~ This study aimed to investigate the possible role of *Vitex-V. agnus-castus* as host plant of *H. obsoletus* and '*Ca. P. solani*' CaPsol in Northern Italy. In detail, the interaction between chaste tree and *H. obsoletus* was examined through survival, attractiveness and oviposition trials, while the capability of chaste tree to harbor CaPsol '*Ca. P. solani*' in Northern Italy was studied through transmission trials in controlled conditions. In these studies, *H. obsoletus* adults collected on stinging nettle were used because this plant is the most important external source of infected vectors for Northern Italian vineyards (Mori et al., 2008b, 2015) and therefore the possibility of using chaste tree as trap plant at vineyard borders must be evaluated on this population.

2 MATERIAL AND METHODS

2.1 Survival of *Hyalesthes obsoletus* from stinging nettle on chaste tree and grapevine

Hyalesthes obsoletus adults were collected by using a sweep net and pooter in Veneto region on 4th July 2016 and 27th June 2017 from stinging nettle plants, growing along a ditch bordering a BN infected vineyard (45°23'32.42''N; 11°09'45.62''E), and were maintained for ten days under controlled conditions [25±3 °C, 70±5 RH, 16:8 (L:D) daily light cycle] in insects proof cages on potted plants of chaste tree, stinging nettle and grapevine ~~(ev Chardonnay)~~. The chaste tree plants were generated by tissue culture in Guagno nursery (Padova, Italy), stinging nettle plants were taken from field, and grapevine plants were one-year Chardonnay grafted on SO4 rootstock in Vivai Cooperativi Rauscedo (Pordenone, Italy). The plants, grown in 5 L pots, were in good vegetative condition and did not show any symptoms related to biotic and abiotic stresses. The three plant species had similar volume and leaf density (diameter about 0.3 m and high about 0.8 m).

Both years, the *H. obsoletus* individuals, collected from stinging nettle, were randomly confined on 8 singularly caged potted plants per each of the three host species. On average, in 2016 were used 28.5, 14.1, and 25.1 adults on chaste tree, stinging nettle and grapevine, respectively; in 2017, were

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3 151 used 14.6, 15.3, and 17.6 adults on chaste tree, stinging nettle and grapevine, respectively. Species
4 recognition was confirmed based on the taxonomic keys by Bertin et al. (2010).
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7 163 During the 10-day confinement, the number of dead individuals was counted daily. On the last
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9 164 sampling day the number of alive individuals was also counted, to know the total number in each
10 165 cage. Kaplan-Meier analysis was used to estimate the survival curve on the three plants and the
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12 166 comparison between two survival curves was made by the log-rank test.

13 167 14 15 168 **2.2 Attractiveness of chaste tree and grapevine for *Hyalesthes obsoletus* from stinging nettle**

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17 169 The attractiveness of chaste tree for *H. obsoletus* collected on stinging nettle was evaluated under
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19 170 laboratory, semi-field and field conditions.

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21 171 (i) Laboratory conditions: the experiment was conducted in 2017 ~~on 40 using~~ *H. obsoletus* adults
22 172 ~~(20 females and 20 males)~~, captured on stinging nettle (see survival trials §). Before their use in the
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24 173 experiment the ~~*H. obsoletus*~~ adults were left on Petri dishes with water for 12 hours. The
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26 174 planthoppers then underwent a choice test using a ~~custom made~~ two-choice olfactometer
27 175 ~~(following Dicke et al., (1988))~~ between shoots of chaste tree vs grapevine (cv Chardonnay),
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29 176 chaste tree vs stinging nettle, stinging nettle vs ~~grapevine~~ ~~chaste tree~~. The shoots were in good
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31 177 vegetative condition and did not show any symptoms related to biotic and abiotic stresses. Forty
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33 178 individuals (20 females and 20 males) were tested for each comparison. If 10 minutes after
34 179 positioning the insect was still at the start on the olfactometer, the test was considered as “No
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36 180 choice”. Data analysis was performed on the individuals that chose one of the two plants under
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38 181 comparison. To establish if the proportion of males and females that were attracted by one of the
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40 182 two plants was different, a Fisher’s exact test was used. To know if one plant was ~~chosen~~
41 183 by preferred by adults ~~adults (males plus females)~~ more than the other ~~under in~~ comparison, a G-test
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43 184 of goodness of fit was used. Since the percentages of males and females who have chosen one of
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45 185 the two plants under comparison are always differed for no more than 7%, this last analysis was
46 186 conducted pulling together the adults of the two sexes.

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48 187 (ii) Semi-field conditions: in 2016 and 2017, 9 cages (0.5 m × 0.5 m × 1.0 m) containing potted
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50 188 plants of two species ~~20 *H. obsoletus* adults (10 females and 10 males) (captured on stinging nettle,~~
51 189 ~~see survival trials §) were confined in cages (0.5 m × 0.5 m × 1.0 m) containing potted plants of two~~
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53 190 species, namely chaste tree and grapevine (n. 3 cages) or chaste tree and nettle (n. 3 cages) or nettle
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55 191 and ~~chaste tree~~ grapevine (n. 3) were prepared. The origin and the vegetative status of the plants
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57 192 were the same of those used in the survival experiment (see 2.1 §). The plants of the two species
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59 193 under comparison inside each cage were pruned to similar volume and leaf density (diameter of

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3 194 about 0.3 m and high of about 0.5 m). In each cage 20 *H. obsoletus* adults (10 females and 10
4 males) (captured on stinging nettle, see survival trials §) were confined. Cages were maintained
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6 under controlled conditions [25±3 °C, 70±5 RH, 16:8 (L:D) daily light cycle]. Observation of adult
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8 insect's position was done 1, 4 and 8 hours after caging. If the insect was on the net or on the
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10 198 bottom of the cage, the position was considered as "No choice". ~~Three cages for each pair and for~~
11 ~~each year were used.~~ Data analysis was performed on the individuals that chose one of the two
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13 plants under comparison using a paired-sample *t* test.
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15 201 (iii) Field conditions: the trial was conducted in 2017 in a ploughed field (3.7 ha surface,
16 202 (45°23'34.92"N; 11°09'39.10"E) with one side (103 m long) bordered by a ditch covered with
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18 stinging nettle harbouring large *H. obsoletus* populations. At the time of the adults' flight period-
19 203
20 the stinging nettle along the ditch was mowed. Potted chaste tree, grapevine (see survival trials §)
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22 and nettle plants (taken from nature and then potted in 5L pot) - (see survival trials §) were placed
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24 in the field at 5, 10 and 20 m from the border in the same day of the stinging nettle mowing. For
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26 each distance, 6 groups of the 3 plants were considered, one for each of the three species. ~~T~~
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28 the distance between each plant group was 15 m and 1.0 m between each plant within the group. All
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30 potted plants of the three species under comparison were pruned to similar volume and leaf density
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32 (diameter of about 0.4 m and high of about 0.9 m) and irrigated twice a week. The plants were in
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34 good vegetative condition and did not show any symptoms related to biotic and abiotic stresses.
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36 ~~Coinciding with the plants positioning, the stinging nettle along the ditch was mowed.~~ The presence
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38 of *H. obsoletus* adults on the three potted-plant species was monitored after nettle mowing by
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40 transparent sticky traps (A5 paper size 148 × 210 mm) positioned within their canopy. The number
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42 of individuals captured during the first and second week was counted. To compare field-trial data
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44 (number of *H. obsoletus* adults captured), a three-way ANOVA was used, considering as source of
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46 variation sampling time (first and second week from stinging nettle mowing), host plant (stinging
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48 nettle, grapevine and chaste tree) and distance from *H. obsoletus* source (5 m, 10 m and 20 m). Prior
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50 to analysis data normality was tested with the Shapiro–Wilk test, homogeneity was tested with
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52 Levene's variance test, the presence of outliers was assessed, and the data were log(x+1)
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54 transformed. For *post hoc* comparisons of means, LSD5% (least significant difference between two
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56 means at the 5% level) was used.
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55 224 **2.3 Egg laying of *Hyalesthes obsoletus* from stinging nettle on chaste tree**

56 225 Insects proof cages (0.5 m × 0.5 m × 1.0 m) were arranged on potted plants of chaste tree (grown
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58 from tissue culture Guagno nurseries – Padova) and stinging nettle (taken from nature). Four and
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3 227 eight potted plants for each species were considered in 2016 and 2017 respectively. The pots had 50
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5 228 L of capacity and the holes at the bottom were closed with insect-proof net to allow water flow but
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7 229 prevent the hatched-nymphs escaping. The plants size was about 0.4 m in diameter and about 0.9 m
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9 230 in height.

10 231 In each cage 100 *H. obsoletus* adults (50 females and 50 males), collected on stinging nettle (see
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12 232 survival trials §) on 21st July 2016 and 14th July 2017, were confined with the plants. The cages
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14 233 were maintained in an open field during winter. In February 2017 and 2018, *H. obsoletus* nymphs
15 234 were extracted from the soil by Berlese funnel and analysed under stereomicroscope. Nymphs were
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17 235 identified using the dichotomous keys of Cargnus et al. (2012). Data collected in the two years were
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19 236 analysed together using a paired-sample *t* test.

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22 238 **2.4. Transmission trials of BN phytoplasmas to chaste tree**

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24 239 In 2017 adults of *H. obsoletus* were collected on stinging nettle in a ditch bordering two BN-
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26 240 affected vineyards in Lombardy (Brescia province: 45°35'37.72''N; 10°09'33.36''E) and Veneto
27 241 (Verona province: 45°23'32.42''N; 11°09'45.62''E) regions. ~~Species recognition was confirmed~~
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29 242 ~~based on the taxonomic keys by Bertin et al. (2010).~~ Capturing of adults was done by using a sweep
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31 243 net and pooter. The captured insects were kept in jars for transport to the laboratory. The
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33 244 transmission trials were conducted with twenty four chaste tree plants, tested PCR-negative for '*Ca.*
34 245 *P. solani*' in a greenhouse under controlled conditions (25±3 °C, 70±5 RH) located in Verona
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36 246 province (45°20'13.72''N; 11°13'03.28''E). The plants were singularly caged and divided into
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38 247 three groups: (i) plants TBS1-TBS8, with confined *H. obsoletus* individuals collected in Brescia (30
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40 248 adults per plant), (ii) plants TVR1-TVR8, with confined *H. obsoletus* individuals collected in
41 249 Verona (30 adults per plant), and (iii) plants T1-T8, without insects (control plants). Transmission
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43 250 trials were left till the end of adult survival. After this period, the plants were kept in an insect-free
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45 251 greenhouse.

46 252 Dead insects (~~127-136~~ in plants TBS1-TBS8; 146 in plants TVR1-TVR8), collected from
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48 253 the end of June till mid-July 2017, were stored in absolute ethanol at 4 °C. '*Ca. P. solani*' was
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50 254 detected by nested PCR-based amplification of *stamp* gene (Fabre et al., 2011) using as templates
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52 255 the total nucleic acids extracted from both the individual insect specimens (Marzachi et al., 1998)
53 256 and the leaves of chaste tree plants (Angelini et al., 2001) collected in October 2017 and 2018. The
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55 257 plants were kept in an insect-free greenhouse for the whole transmission period. Amplification
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57 258 products were analysed by electrophoreses in 1% agarose gel stained with Midori green under a
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59 259 UV transilluminator.

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3 260 PCR products (StampF1/StampR1), amplified from insect adults and chaste tree samples,
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5 261 were sequenced in both strands (Sanger method, 5X coverage per base position) by a commercial
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7 262 service (Eurofins Genomics, Germany). Nucleotide sequences were assembled by the Contig
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9 263 Assembling Program and trimmed to the annealing sites of the nested PCR primer pair in the
10 264 software BioEdit, version 7.2.6 (Hall, 1999). Obtained *stamp* gene nucleotide sequences were
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12 265 aligned using the ClustalW Multiple Alignment program in the software BioEdit and analysed by
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14 266 Sequence Identity Matrix to estimate their genetic diversity. *Stamp* sequence variants, identified in
15 267 the study, were aligned and compared with representative sequences of previously defined sequence
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17 268 variants (Pierro et al., 2018a, 2018b); a nucleotide sequence identity of 100% was necessary for the
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19 269 attribution to such sequence variants.

20 270 21 22 271 23 24 272 **3 RESULTS**

25 26 273 27 28 274 **3.1 Survival of *Hyalesthes obsoletus* from stinging nettle on chaste tree and grapevine**

29 275 The data gathered in the two years showed that adults of *H. obsoletus* collected from stinging nettle
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31 276 can survive on chaste tree and grapevine for some days, but the survival curves were significantly
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33 277 worse than those on stinging nettle (2016: grapevine vs stinging nettle, $X^2 = 251.4$, $p > 0.0001$;
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35 278 chaste tree vs stinging nettle, $X^2 = 104.6$, $p < 0.0001$; 2017: grapevine vs stinging nettle, $X^2 = 151.2$,
36 279 $p > 0.0001$; chaste tree vs stinging nettle, $X^2 = 66.6$, $p < 0.0001$) (Figure 1). Survival on chaste tree
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38 280 and grapevine fell below 50% after three days in 2016 and after 5 days in 2017. In 2016 the survival
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40 281 curve on chaste tree was significantly better than on grapevine ($X^2 = 4.87$, $p = 0.016$), but in 2017
41 282 this difference was not confirmed ($X^2 = 1.47$, $p = 0.16$).

42 43 283 44 45 284 **3.2 Attractiveness of chaste tree and grapevine for *Hyalesthes obsoletus* from stinging nettle**

46 285 In the laboratory experiment with two-choice olfactometer, the proportion of males and females that
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48 286 chose one of the two plants under comparison with “no-choice” individuals was not significantly
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50 287 different ($p = 0.10$ for grapevine vs chaste tree, $p = 1$ for chaste tree vs stinging nettle, $p = 1$ for
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52 288 grapevine vs stinging nettle, Fisher’s Exact Test). *Hyalesthes obsoletus* adults did not show any
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54 289 significant preference for grapevine vs chaste tree ($G = 0.081$, $p = 0.78$), chaste tree vs stinging
55 290 nettle ($G = 0.081$, $p = 0.78$) or grapevine vs stinging nettle ($G = 2.19$, $p = 0.14$) (Figure 2).

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57 291 In the semi-field experiment, there were significant differences in the choice of plant species
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59 292 by *H. obsoletus* adults collected on stinging nettle (Figure 3). In particular, chaste tree was

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3 293 significantly preferred to grapevine in both 2016 ($t = 2.80$, d.f. = 8, $p = 0.02$) and 2017 ($t = 2.80$, d.f.
4 = 8, $p = 0.02$); stinging nettle was significantly preferred to grapevine in both 2016 ($t = 3.39$, d.f. =
5 294 = 8, $p = 0.0095$) and 2017 ($t = 5.58$, d.f. = 8, $p = 0.0005$); stinging nettle was significantly preferred
6 295 to chaste tree in 2017 ($t = 2.44$, df = 8, $p = 0.04$), but not in 2016 ($t = 1.42$, d.f. = 8, $p = 0.19$).
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9 298
10 297 In the open field, captures of *H. obsoletus* from stinging nettle plants along a ditch were
11 significantly influenced by time (i.e., days from nettle mowing), plants and distance from *H.*
12 298 *obsoletus* adults' source (Table 1). In particular, captures were higher the second than the first week
13 299 from nettle mowing. On stinging nettle the captures were significantly higher than on the other two
14 300 plants (Table 2). Although no individual was captured on grapevine, the differences with respect to
15 301 chaste tree were not statistically significant based on LSD5% (Table 2). The captures decreased
16 302 with the increase of distance from the ditch, i.e. from the source of *H. obsoletus* adults, and were
17 303 significantly higher at 5 m than both 10 m and 20 m (Table 2). The interactions time \times plant, plant \times
18 304 distance and time \times plant \times distance were significant due to the fact that the captures were
19 305 influenced by time and distance only for stinging nettle and chaste tree, because on grapevines the
20 306 captures were always zero (Table 1).
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31 309 **3.3 Egg laying of *Hyalesthes obsoletus* from stinging nettle on chaste tree**

32 310 Based on the nymphs observed in February of the next year, *H. obsoletus* females laid eggs on
33 311 potted plants in 10 out of 12 cages. Nymphs were recorded on the roots of both stinging nettle and
34 312 chaste tree, showing indirectly that females had laid eggs on both plants, but a significantly higher
35 313 number was observed on the former ($t = 3.36$, d.f. = 9; $p = 0.009$) (Figure 4).
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41 315 **3.4 Transmission trials**

42 316 The PCR analyses for amplification of the *stamp* gene, performed on the total nucleic acids
43 317 extracted from the chaste tree plants used in the transmission trials, showed the presence of '*Ca. P.*
44 318 *solani*' in two plants (TBS6 and TBS7) out of 16 (12.5%). No amplification was observed in the
45 319 other 14 chaste tree plants, on which insects were maintained, and on the eight control plants
46 320 (without insects) (Table 3). The molecular analyses performed on the insect individuals collected
47 321 from plants TBS6 and TBS7 revealed that five individuals out of 16 (31.25%) and six out of 18
48 322 (33.33%), respectively, were found to be infected by '*Ca. P. solani*'. *H. obsoletus* adults, collected
49 323 from the 14 chaste tree plants negative to phytoplasma presence, were found to be infected at a
50 324 percentage varying from 7-0 to 50% (Table 3). Nucleotide sequence analyses of the *stamp* gene
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3 325 showed that chaste tree plants and insect individuals feeding on them harboured the same 'Ca. P.
4 solani' strain, characterized by the *stamp* gene sequence variant St5.

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6 327 The PCR analyses performed on chaste tree leaves collected in October 2018 (one year after the
7 transmission trials), showed that all 24 chaste tree plants, including TBS6 and TBS7 (positive in
8 328 2017), were negative to phytoplasma presence (Table 3).
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15 332 DISCUSSION

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17 333 Survival of *H. obsoletus* adults from stinging nettle was better on the plants on which the nymphs
18 developed (i.e. stinging nettle), than on the other plants (i.e. grapevine and chaste tree). This
19 334 occurrence was previously observed for *H. obsoletus* from stinging nettle or bindweed that had
20 335 better survival on the origin plant than on the other (Mori et al., 2008; Kessler et al., 2011; Maixner
21 et al., 2014). Survival on chaste tree was significantly better than on grapevine in one of the two
22 336 study years. However, the differences were not so high as could be expected from the fact that
23 337 chaste tree, unlike grapevine, is a true host of the planthopper (Sharon et al., 2015). Our study also
24 338 indirectly confirmed that *H. obsoletus* can complete its life cycle on chaste tree because nymphs
25 339 were observed in February on the roots of potted chaste tree plants on which planthopper adults had
26 340 been caged and been able to lay eggs in the previous summer.
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29 343 In the field, *H. obsoletus* adults from stinging nettle were more attracted by ~~potted plants of~~
30 344 stinging nettle than ~~either grapevine or chaste tree. Considering the two latter, as and even not~~
31 345 ~~captureds occurred in on~~ grapevine, ~~chaste tree seemed to be preferred. The higher attractiveness of~~
32 346 ~~chaste tree compared to grapevine was showed by s~~Semi-field experiments confirmed both the
33 347 scarce attractiveness of grapevine and the preference for stinging nettle than chaste tree in which
34 348 even chaste tree was significantly less attractive than stinging nettle in only one of the two years.
35 349 With reference to the two true host plants, i.e. namely stinging nettle and chaste tree, preference for
36 350 the former may still be associated with the origin of adults used for the experiments, all collected
37 351 from stinging nettle plants. Based on this result, even the higher attractiveness of chaste tree in
38 352 comparison with other plants observed in the olfactometer studies by Sharon et al. (2005) may have
39 353 been influenced by the fact that most of the adults had been collected on chaste tree. The fact that
40 354 chaste tree resulted significantly more attractive than grapevine would suggest its use as trap plant
41 355 at vineyard borders. However, since the infected *H. obsoletus* adults that colonize vineyards in
42 356 nNorthern Italy move mostly from stinging nettle and for this planthopper population the nettle was
43 357 more attractive than chaste tree, the use of healthy potted plants of stinging nettle as trap plants
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3 358 would be preferable. Our two-choice olfactometric studies showed no significant preference by *H.*
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5 359 *obsoletus* for either of the two plants, even if fewer adults were observed on grapevine than stinging
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7 360 nettle.

8 361 Results of the transmission trials conducted in the study proved that chaste tree can harbour
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10 362 '*Ca. P. solani*' and that infectious *H. obsoletus* adults from stinging nettle can inoculate this
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12 363 phytoplasma in chaste tree. This evidence is in agreement with the results obtained by Kosovac et
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14 364 al. (2016), who demonstrated that chaste tree naturally occurring in vineyard agro-ecosystems in
15 365 Montenegro Serbia is infected by '*Ca. P. solani*'. The '*Ca. P. solani*' strain St5, transmitted with *H.*
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17 366 *obsoletus* originating from stinging nettle to chaste tree in the present study, is so far known to be
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19 367 associated only with bindweed as source plant, *H. obsoletus* from bindweed as vector, and
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21 368 grapevine in wide geographic European areas (Pierro et al., 2018). Moreover, strain St5 groups
22 369 within the bindweed-related *stamp* phylogenetic Cluster b-II along with strains St1, St2, and St30,
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24 370 previously found associated with chaste tree or transmitted to grapevine by chaste tree associated *H.*
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26 371 *obsoletus* (Kosovac et al., 2016). Thus, this is the first report of strain St5 transmitted to chaste tree
27 372 by *H. obsoletus* from stinging nettle. Moreover, as chaste tree constitutes an important reservoir
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29 373 for *H. obsoletus*-mediated transmission of BN phytoplasma to grapevine (Kosovac et al., 2016), our
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31 374 findings that chaste tree can host the '*Ca. P. solani*' strain St5, largely prevalent in the Franciacorta
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33 375 area, open a new intriguing scenario on its possible role in BN epidemiology in north Italy. On the
34 376 contrary, these results are in disagreement with Sharon et al. (2005, 2015), who showed that, even if
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36 377 it is a preferred host plant of *H. obsoletus*, chaste tree did not harbour '*Ca. P. solani*'. Interestingly,
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38 378 even if '*Ca. P. solani*'-infected insect individuals were found on 15 out of 16 chaste tree plants used
39 379 in transmission trials, *H. obsoletus* was only able to transmit the pathogen in two cases. This could
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41 380 be explained considering the short survival of insect adults on chaste tree; in fact, the insect
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43 381 populations decreased dramatically in 4 to 6 days after release. However, adults of *H. obsoletus*
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45 382 from stinging nettle survive on grapevine no better than on chaste tree and still are able to inoculate
46 383 the BN phytoplasma. Moreover, the success of transmission trials can depend on the phytoplasma
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48 384 strain and its titer within the insect adults. For example, it is reasonable to hypothesize that '*Ca. P.*
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50 385 *solani*' strains not transmitted to chaste tree in the present study could be (as expected) those that are
51 386 strictly associated with stinging nettle (*stamp* clusters a1 and a2). -The fact that chaste tree plants,
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53 387 found positive for phytoplasma presence in October 2017, were phytoplasma-free in October 2018
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55 388 can be explained by natural recovery from infection, as reported for a broad range of polyannual
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57 389 plants infected by phytoplasmas (Osler et al., 1993; Romanazzi et al., 2009), increased by abiotic
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3 390 stresses due to the overgrowth of chaste trees in pots under controlled conditions, which is not
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5 391 convenient in terms of spacing.

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7 392 According to Sharon et al. (2005, 2015), showing that chaste tree is a preferred host plant of
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9 393 *H. obsoletus* and does not harbour '*Ca. P. solani*', in Israel a 'push & pull' strategy was suggested
10 394 to reduce the population of *H. obsoletus* in a vineyard by using chaste tree as a trap plant (Zahavi et
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12 395 al., 2007). On the contrary, based on the findings of this and previous research work (Kosovac et
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14 396 al., 2016), it is doubtful that chaste tree can be used in the containment of the BN spread in Europe
15 397 by using it as an attractant to *H. obsoletus* since it can also act as a reservoir of '*Ca. P. solani*'.
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17 398 However, volatiles from both chaste tree and stinging nettle could be used in the context of 'push &
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19 399 pull strategies' (Riolo et al., 2017).

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21 400 In conclusion, the results obtained increased the knowledge about the role of *Vitex-V. agnus-*
22 401 *castus* as host plant of *H. obsoletus* and '*Candidatus Phytoplasma solani*' in nNorth Italy. Further
23
24 402 studies are needed to determine the actual role of chaste tree in the BN epidemiology.
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31 406 "Methodologies for Bois Noir containment".
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34 408 **CONFLICTS OF INTEREST**

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36 409 The authors declare no potential conflict of interests.
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46
47
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60**REFERENCES**

- Alma, A., Arnò, C., Arzone, A., & Vidano, C. (1988). New biological reports on Auchenorrhyncha in vineyards. In C. Vidano & A. Arzone (Eds.), *Proceedings of 6th Auchenorrhyncha Meeting*, (pp. 509–516), 7–11 September 1987, Turin, Italy.
- Angelini, E., Constable, F., Duduk, B., Fiore, N., Quaglino, F., & Bertaccini A. (2018). Grapevine phytoplasmas. In G. P. Rao, A. Bertaccini, N. Fiore & L. W. Liefting (Eds.), *Characterisation and Epidemiology of Phytoplasma - Associated Diseases. Phytoplasmas: Plant Pathogenic Bacteria-I*, (pp. 123–152). Springer Nature, Singapore.
- Aryan, A., Brader, G., Mörtel, J., Pastar, M., & Riedle-Bauer, M. (2014). An abundant 'Candidatus Phytoplasma solani' Stolbur *tuf b* phytoplasma strain is associated with grapevine, stinging nettle and *Hyalesthes obsoletus*. *European Journal of Plant Pathology*, **140**, 213–227.
- Atanasova, B., Jakovljević, M., Spasov, D., Jović, J., Mitrović, M., Toševski, I., & Cvrković, T. (2015). The molecular epidemiology of bois noir grapevine yellows caused by 'Candidatus Phytoplasma solani' in the Republic of Macedonia. *European Journal of Plant Pathology*, **142**, 759–770.
- Batlle, A., Altabella, N., Sabaté, J. & Laviña, A. (2008). Study of the transmission of stolbur phytoplasma to different crop species by *Macrosteles quadripunctulatus*. *Annals of Applied Biology*, **152**, 235–242
- Bellée, A., Cluzet, S., Dufour, M.-C., Mérrillon, J. M., & Corio-Costet, M.-F. (2018). Comparison of the impact of two molecules on plant defense and on efficacy against *Botrytis cinerea* in the vineyard: a plant defense inducer (Benzothiadiazole) and a fungicide (Pyrimethanil). *Journal of Agricultural and Food Chemistry*, **66**, 3338–3350.
- Belli, G., Bianco, P. A., & Conti, M. (2010). Grapevine Yellows in Italy: past, present and future. *Journal of Plant Pathology*, **92**, 303–326.
- Bertin, S., Picciau, L., Ács, Z., Alma, A., & Bosco, D. (2010). Molecular identification of the *Hyalesthes obsoletus* (Hemiptera: Cixiidae) occurring in vineyard agroecosystems. *Annals of Applied Biology*, **157**, 435–445.
- Bianco, P. A., Davis, R. E, Prince, J. P., Lee, I. M., Gundersen, D. E., Fortusini, A., & Belli, G. (1993). Double and single infections by aster yellows and elm yellows MLOs in grapevines with symptoms characteristic of flavescente dorée. *Rivista di Patologia Vegetale*, **3**, 69–82.
- Bressan, A., Turata, R., Maixner, M., Spiazzi, S., Boudon-Padieu, E., & Girolami, V. (2007). Vector activity of *Hyalesthes obsoletus* living on nettles and transmitting a stolbur phytoplasma to grapevines: a case study. *Annals of Applied Biology*, **150**, 331–339.

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60

- 445 Cargnus, E., Pavan, F., Mori, N., & Martini, M. (2012). Identification and phenology of *Hyalesthes*
446 *obsoletus* (Hemiptera: Auchenorrhyncha: Cixiidae) nymphal instars. *Bulletin of*
447 *Entomological Research*, **102**, 504–514.
- 448 Chuche, J., Danet, J. L., Salar, P., Foissac, X., & Thiéry, D. (2016). Transmission of ‘*Candidatus*
449 *Phytoplasma solani*’ by *Reptalus quinquecostatus* (Hemiptera: Cixiidae). *Annals of Applied*
450 *Biology*, **169**, 214–223.
- 451 Cvrković, T., Jović J., Mitrović, M., Krstić, O., & Toševski, I. (2014). Experimental and molecular
452 evidence of *Reptalus panzeri* as a natural vector of bois noir. *Plant Pathology*, **63**, 42–53.
- 453 Dermastia, M., Bertaccini, A., Constable, F., & Mehele, N. (2017). *Grapevine Yellows Diseases*
454 *and Their Phytoplasma Agents: Biology and Detection*, Springer Nature, Cham,
455 Switzerland.
- 456 [Dicke, M., Sabelis, M.W., & De Jong, M. \(1988\). Analysis of prey preference in phytoseiid mites](#)
457 [by using an olfactometer, predation models and electrophoresis." *Experimental & Applied*](#)
458 [Acarology](#), **5:3-4**, 225-241.
- 459 Đurić, Z., Hrnčić, S., & Delić, D. (2017). Morphological and molecular identification of *Hyalesthes*
460 *obsoletus* Signoret (Auchenorrhyncha: Cixiidae) in Herzegovina vineyards. *Mitteilungen*
461 *Klosterneuburg*, **67**, 177–181.
- 462 Fabre, A., Danet, J. L., & Foissac, X. (2011). The stolbur phytoplasma antigenic membrane protein
463 gene *stamp* is submitted to diversifying positive selection. *Gene*, **472**, 37–41.
- 464 FAO (2016). Comparison between world and Europe total area harvested for grapes.
- 465 Fialová, R., Válková, P., Balakishiyeva, G., Danet, J. L., Sáfárová, D., Foissac, X., & Navrátil, M.
466 (2009). Genetic variability of Stolbur phytoplasma in annual crop and wild plant species in
467 South Moravia. *Journal of Plant Pathology*, **91**, 411–416.
- 468 Foissac, X., Carle, P., Fabre, A., Salar, P., Danet, J. L., & Stolbureuomed Consortium (2013).
469 ‘*Candidatus Phytoplasma solani*’ genome project and genetic diversity in the Euro-
470 Mediterranean basin. In E. Torres, A. Laviña & A. Batlle (Eds.), *Book of Abstracts of 3rd*
471 *European Bois Noir Workshop* (pp. 11–13), 21–22 March 2013, Barcelona, Spain.
- 472 Hall, T. A. BioEdit: a user-friendly biological sequence alignment editor and analysis program for
473 Windows 95/98/NT. Nucleic acids symposium series, 1999. [London]: Information
474 Retrieval Ltd., c1979-c2000., 95–98.
- 475 Hoch, H., & Remane, R. (1865). Evolution und Speziation der Zikaden-Gattung *Hyalesthes*. *Sign.*

- 1
2
3 476 Johannesen, J., Lux, B., Michel, K., Seitz, A., & Maixner, M. (2008). Invasion biology and host
4 specificity of the grapevine yellows disease vector *Hyalesthes obsoletus* in Europe.
5 477
6 478 *Entomologia Experimentalis et Applicata*, **126**, 217–227.
- 8 479 Kast, W. K., Stark-Urnau, M., & Bleyer, K. (2008). Bois noir, a severe outbreak of stolbur type A
9 in Southern Germany - disease abundance and treatments against disease-causing agents and
10 480
11 vectors. *IOBC/WPRS Bulletin*, **36**, 121–125.
- 13 482 Kessler, S., Scherer, S., Delabays, N., Turlings, T. C. J., Trivellone, V., & Kehrl, P. (2011). Host
14 483
15 484 plant preference of *Hyalesthes obsoletus*, the vector of the grapevine yellow disease 'bois
16 485
17 486 noir', in Switzerland. *Entomologia Experimentalis et Applicata*, **139**, 60–67.
- 19 485 Kosovac, A., Johannesen, J., Krstić, O., Mitrović, M., Cvrković, T., Maixner, M., Toševski, I., &
20 486
21 487 Jović, J. (2013). Microsatellite and mtDNA evidence of genetic differentiation in *Hyalesthes*
22 488
23 489 *obsoletus* populations associated with a new major host, stinking hawk's-beard (*Crepis*
24 490
25 491 *foetida*), in southeast Europe. In E. Torres, A. Lavina & A. Batlle (Eds.), *Book of Abstracts*
26 492
27 493 *of 3rd European Bois Noir Workshop* (pp. 18–19), 21–22 March 2013, Barcelona, Spain.
- 27 490 Kosovac, A., Radonjić, S., Hrnčić, S., Krstić, O., Toševski, I., & Jović, J. (2016). Molecular tracing
28 491
29 492 of the transmission routes of bois noir in Mediterranean vineyards of Montenegro and
30 493
31 494 experimental evidence for the epidemiological role of *Vitex agnus-castus* (Lamiaceae) and
32 495
33 496 associated *Hyalesthes obsoletus* (Cixiidae). *Plant Pathology*, **65**, 285–298.
- 34 494 Kosovac, A., Jakovljević, M., Krstić, O., Cvrković, T., Mitrović, M., Toševski, I., & Jović, J.
35 495
36 496 (2019). Role of plant-specialized *Hyalesthes obsoletus* associated with *Convolvulus arvensis*
37 497
38 498 and *Crepis foetida* in the transmission of 'Candidatus Phytoplasma solani'-inflicted bois
39 499
40 500 noir disease of grapevine in Serbia. *European Journal of Plant Pathology*, **153**, 183–195.
- 41 498 Landi, L., Riolo, P., Murolo, S., Romanazzi, G., Nardi, S., & Isidoro, N. (2015). Genetic variability
42 499
43 500 of stolbur phytoplasma in *Hyalesthes obsoletus* (Hemiptera: Cixiidae) and its main host
44 501
45 502 plants in vineyards agroecosystems. *Journal of Economic Entomology*, **108**, 1506–1515.
- 46 501 Langer, M. & Maixner, M. (2004). Molecular characterisation of grapevine yellows associated
47 502
48 503 phytoplasmas of the stolbur-group based on RFLP-analysis of non ribosomal DNA. *Vitis*,
49 504
50 505 **43**, 191–199.
- 51 504 Magarey, P. (2017). Grape-vine yellows-aetiology, epidemiology and diagnosis. *South African*
52 505
53 506 *Journal of Enology and Viticulture*, **7**, 90–100.
- 55 506 Maixner, M. (1994). Transmission of German grapevine yellows (Vergilbungskrankheit) by the
56 507
57 508 planthopper *Hyalesthes obsoletus* (Auchenorrhyncha: Cixiidae). *Vitis*, **33**, 103–104.
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52
53
54
55
56
57
58
59
60

- 508 Maixner, M. (2010). Phytoplasmas epidemiological systems with multiple plant hosts. In P.G.
509 Weintraub & P. Jones (Eds.), *Phytoplasmas: Genomes, Plant Hosts and Vectors* (pp. 213–
510 232), CABI Publishing, Wallingford, UK.
- 511 Maixner, M., Albert, A., & Johannesen, J. (2014). Survival relative to new and ancestral host plants,
512 phytoplasma infection, and genetic constitution in host races of a polyphagous insect disease
513 vector. *Ecology and Evolution*, **4**, 3082–3092.
- 514 Mannini, F. (2007). Relazione sulla termoterapia, applicata presso alcuni vivai Piemontesi.
515 *L'Informatore Agrario*, **63** (24), 87–90.
- 516 Marchi, G., Cinelli, T., Rizzo, D., Stefani, L., Goti, E., Della Bartola, M., Luvisi, A., Panattoni, A.,
517 & Materazzi, A. (2015). Occurrence of different phytoplasma infections in wild herbaceous
518 dicots growing in vineyards affected by bois noir in Tuscany (Italy). *Phytopathologia
519 Mediterranea*, **54**, 504–515.
- 520 Mori, N., Pavan, F., Bacchiavini, M., Reggiani, N., Bonomi, F., & Bertaccini, A. (2008a).
521 Fenologia di *Hyalesthes obsoletus* Signoret su convolvolo e ortica. In *Atti "4° Incontro
522 Nazionale sulle Malattie da Fitoplasmii"*, 28-30 maggio 2008 Roma, Italia. *Petria*, **18**, 229–
523 231.
- 524 Mori, N., Pavan, F., Bondavalli, R., Reggiani, N., Paltrinieri, S., & Bertaccini, A. (2008b). Factors
525 affecting the spread of “Bois Noir” disease in north Italy vineyards. *Vitis*, **47**, 65–72.
- 526 Mori, N., Pavan, F., Reggiani, N., Bacchiavini, M., Mazzon, L., Paltrinieri, S., & Bertaccini, A.
527 (2012). Correlation of bois noir disease with nettle and vector abundance in northern Italy
528 vineyards. *Journal of Pest Science*, **85**, 23–28.
- 529 Mori, N., Mitrović, J., Smiljković, M., Duduk, N., Paltrinieri, S., Bertaccini, A., & Duduk, B.
530 (2013). *Hyalesthes obsoletus* in Serbia and its role in the epidemiology of corn reddening.
531 *Bulletin of Insectology*, **66**, 245–250.
- 532 Mori, N., Quaglino, F., Tessari, F., Pozzebon, A., Bulgari, D., Casati, P., & Bianco, P. A. (2015).
533 Investigation on ‘bois noir’ epidemiology in north-eastern Italian vineyards through a
534 multidisciplinary approach. *Annals of Applied Biology*, **166**, 75–89.
- 535 Murolo, S., Mancini, V., & Romanazzi, G. (2014). Spatial and temporal stolbur population structure
536 in a cv Chardonnay vineyard according to *vmp1* gene characterisation. *Plant Pathology*, **63**,
537 700–707.
- 538 Murolo, S., & Romanazzi, G. (2015). In-vineyard population structure of '*Candidatus* Phytoplasma
539 solani' using multilocus sequence typing analysis. *Infection, Genetics and Evolution*, **31**,
540 221–230.

- 1
2
3 541 Oliveri, C., Pacifico, D., D'Urso, V., La Rosa, R., Marzachi, C., & Tessitori, M. (2015). Bois noir
4
5 542 phytoplasma variability in a Mediterranean vineyard system: new plant host and putative
6
7 543 vectors. *Australasian Plant Pathology*, **44**, 235–244.
- 8
9 544 Osler R., Carraro L., Loi N., & Refatti, E. (1993). Symptom expression and disease occurrence of a
10 545 yellows disease of grapevine in northeastern Italy. *Plant Disease*, **77**, 496–498.
- 11
12 546 Panassiti, B., Hartig, F., Fahrentrapp, J., Breuer, M., & Biedermann, R. (2017). Identifying local
13
14 547 drivers of a vector-pathogen disease system using Bayesian modeling. *Basic and Applied
15 548 Ecology*, **18**, 75–85.
- 16
17 549 Paolacci, A. R., Catarcione, G., Ederli, L., Zadra, C., Pasqualini, S., Badiani, M., Musetti, R., Santi,
18
19 550 S., & Ciaffi, M. (2017). Jasmonate-mediated defence responses, unlike salicylate-mediated
20
21 551 responses, are involved in the recovery of grapevine from bois noir disease. *BMC Plant
22 552 Biology*, **17**, 118.
- 23
24 553 Pavan, F., Mori, N., Bressan, S., & Mutton, P. (2012). Control strategies for grapevine phytoplasma
25
26 554 diseases: factors influencing the profitability of replacing symptomatic plants.
27
28 555 *Phytopathologia Mediterranea*, **51**, 11–22.
- 29 556 Picciau, L., Lavezzaro, S., Morando, A., Cesano, A., Cuttini, D., Saladini, M. A., & Alma, A.
30
31 557 (2010). Spollonatura e pulizia sottofila limitano il legno nero della vite. *L'Informatore
32 558 Agrario*, **66** (25), 57–59.
- 33
34 559 Pierro, R., Passera, A., Panattoni, A., Casati, P., Luvisi, A., Rizzo, D., Bianco, P.A., Quaglino, F., &
35
36 560 Materazzi, A. (2018a). Molecular typing of 'bois noir' phytoplasma strains in the *Chianti
37
38 561 Classico* area (Tuscany, central Italy) and their association with symptom severity in *Vitis
39 562 vinifera* L. cv. Sangiovese. *Phytopathology*, **108**, 362–373.
- 40
41 563 Pierro, R., Passera, A., Panattoni, A., Rizzo, D., Stefani, L., Bartolini, L., Casati, P., Luvisi, A.,
42
43 564 Quaglino, F., & Materazzi, A. (2018b). Prevalence of a 'Candidatus Phytoplasma solani'
44
45 565 strain, so far associated only with other hosts, in Bois noir-affected grapevines within
46 566 Tuscan vineyards. *Annals of Applied Biology*, **173**, 202–212.
- 47
48 567 Riedle-Bauer, M., Sára, A., & Regner, F. (2008). Transmission of a stolbur phytoplasma by the
49
50 568 agalliinae leafhopper *Anaceratagallia ribauti* (Hemiptera, Auchenorrhyncha, Cicadellidae).
51
52 569 *Journal of Phytopathology*, **156**, 687–690.
- 53 570 Riedle-Bauer, M., Hanak, K., Regner, F., & Tiefenbrunner, W. (2010). Influence of pruning
54
55 571 measures on recovery of Bois noir-infected grapevines. *Journal of Phytopathology*, **158**,
56
57 572 628–632.
- 58
59
60

- 1
2
3 573 Riolo, P., Minuz, R. L., Anfora, G., Stacconi, M. V. R., Carlin, S., Isidoro, N., & Romani, R.
4
5 574 (2012). Perception of host plant volatiles in *Hyalesthes obsoletus*: behavior, morphology,
6
7 575 and electrophysiology. *Journal of Chemical Ecology*, **38**(8), 1017-1030.
- 8 576 Riolo, P., Minuz, R.L., Peri, E., & Isidoro, N. (2017). Behavioral responses of *Hyalesthes obsoletus*
9
10 577 to host-plant volatiles cues. *Arthropod-Plant Interactions*, **11**, 71–78
- 11
12 578 Romanazzi, G., D'Ascenzo, D., & Murolo, S. (2009). Field treatment with resistance inducers for
13
14 579 the control of grapevine Bois noir. *Journal of Plant Pathology*, **91**, 677–682.
- 15 580 Romanazzi, G., Murolo, S., & Feliziani, E. (2013). Effects of an innovative strategy to contain Bois
16
17 581 noir: field treatment with resistance inducers. *Phytopathology*, **103**, 785–791.
- 18
19 582 Quaglino, F., Zhao, Y., Casati, P., Bulgari, D., Bianco, P. A., Wei, W., & Davis, R. E. (2013).
20
21 583 ‘*Candidatus Phytoplasma solani*’, a novel taxon associated with stolbur-and bois noir-
22 584 related diseases of plants. *International Journal of Systematic and Evolutionary*
23
24 585 *Microbiology*, **63**, 2879–2894.
- 25
26 586 Šafářová, D., Lauterer, P., Starý, M., Válová, P., & Navrátil, M. (2018). Insight into
27 587 epidemiological importance of phytoplasma vectors in vineyards in South Moravia, Czech
28
29 588 Republic. *Plant Protection Science*, <https://doi.org/10.17221/8/2018-PPS54>, 234–239.
- 30
31 589 Sharon, R., Soroker, V., Wesley, S. D., Zahavi, T., Harari, A., & Weintraub, P. G. (2005). *Vitex*
32
33 590 *agnus-castus* is a preferred host plant for *Hyalesthes obsoletus*. *Journal of Chemical*
34 591 *Ecology*, **31**, 1051–1063.
- 35
36 592 Sharon, R., Harari, A. R., Zahavi, T., Raz, R., Dafny-Yelin, M., Tomer, M., Sofer-Arad, C.,
37
38 593 Weintraub, P. G., & Naor, V. (2015). A yellows disease system with differing principal host
39 594 plants for the obligatory pathogen and its vector. *Plant Pathology*, **64**, 785–791.
- 40
41 595 Sforza, R., Clair, D., Daire, X., Larrue, J., & Boudon-Padieu, E. (1988). The role of *Hyalesthes*
42
43 596 *obsoletus* (Hemiptera: Cixiidae) in the occurrence of bois noir of grapevines in France.
44
45 597 *Journal of Phytopathology*, **146**, 549–556.
- 46 598 Weber, A., & Maixner, M. (1998). Habitat requirements of *Hyalesthes obsoletus* Signoret
47
48 599 (Auchenorrhyncha: Cixiidae) and approaches to control this planthopper in vineyards.
49
50 600 *IOBC/WPRS Bulletin*, **21** (2), 77–78.
- 51 601 Zahavi, T., Peles, S., Harari, A. R., Soroker, V., & Sharon, R. (2007). Push and pull strategy to
52
53 602 reduce *Hyalesthes obsoletus* population in vineyards by *Vitex agnus castus* as trap plant.
54
55 603 *Bulletin of Insectology*, **60**, 297.
- 56
57 604
58
59
60