# *Minor cultures as hosts for vectors of extensive crop diseases: Does* Salvia sclarea *act as a pathogen and vector reservoir for lavender decline?*

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ORIGINAL PAPER

# Minor cultures as hosts for vectors of extensive crop diseases: Does *Salvia sclarea* act as a pathogen and vector reservoir for lavender decline?

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**Abstract** Stolbur is a phytoplasma disease affecting crops worldwide. The planthopper *Hyalesthes obsoletus* is the main natural vector of '*Candidatus* Phytoplasma solani' responsible of stolbur. In France, lavender (*Lavandula angustifolia*) and lavandin (*Lavandula* × *intermedia*) are strongly affected by this phytoplasma. These plant species are both hosts for the phytoplasma and its insect vector. In 2011, catches of adults were exceptionally sizable on one of the clones of lavandin most tolerant to lavender decline. A high population level of '*Ca.* P. solani' vector was also observed on the adjacent plot of clary sage, *Salvia sclarea*. In order to clarify the potential role of *S. sclarea* as a host plant for *H. obsoletus* and '*Ca*. P. solani,' we conducted field surveys and laboratory experiments. The uprooting of clary sage and root examination showed the presence of nymphs during winter. Harvested nymphs have been reared on *S. sclarea* from seedlings in a greenhouse for many generations. By performing its whole lifecycle on clary sage, we demonstrated for the first time that *S. sclarea* is a host plant of *H. obsoletus* and could be a source of stolbur vector. Nevertheless, status of clary sage as host plant of phytoplasma in the field up to now is not so clear. On 42 Q-PCR runs done on *S. sclarea*, 41 were negative to the phytoplasma, and one positive. Experimental transmission with infected *H. obsoletus* sampled on infected lavender showed that clary sage plant could be infected, expressed symptoms and multiplied '*Ca.* P. solani.'

**Keywords** Bois noir · Lavender decline · Host plant shift · *Hyalesthes obsoletus* rearing · Phytoplasma

## Key message

- The lavender decline is caused by the phytoplasma '*Ca.* P. solani' and transmitted by *Hyalesthes obsoletus.*
- Some cultivars are less susceptible to the disease due to their unsuitability for *H. obsoletus.*
- High disease incidence is observed on less susceptible cultivars.
- We tested if *Salvia sclarea* crops can have a role in the resistance breakdown.
- We found that *S. sclarea* is a reservoir for the vector and the pathogen.
- Our findings are essential in lavender decline management to preserve plant resistance.

## Introduction

Understanding epidemics in crops requires focusing on the direct relationships between the host plant and the pathogen toward different parameters such as pathogen

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virulence/host resistance and propagation among the crops. In the case of vector-borne diseases, a third player, the disease vector, participates in the system allowing the pathogen to disseminate and encounter its host plant. Thus, the biology of insect vectors must be considered and research on insect vectors of plant pathogens should receive more attention (Almeida 2008; Chuche et al. 2017; Purcell and Almeida 2005). The interactions between the crops and how one culture can affect another is not often considered in vector-borne diseases. Some studies, however, show strong interactions between different crops for plant disease epidemiology, a nice example being provided by the planthopper *Pentastiridius leporinus* (Hemiptera: Cixiidae) which is the vector of a recent sugar beet disease called syndrome 'basses richesses' that has been associated with phloem-restricted bacteria (Bressan et al. 2008). This insect species has developed an ecological adaptation to the annual cropping rotation sugar beet–winter cereals in eastern France. The work of Bressan et al. (2009) demonstrated that this cropping system coupled with the faculty of the cixiid to complete its life cycle using both crops allows colonization of newly sown beetroot field from cereal crops and the spread of the disease on beetroot.

Stolbur is a phytoplasma disease affecting crops worldwide in Africa (Bekele et al. 2011), Asia (Chung et al. 2016; Rashidi et al. 2010), Europe (Boudon-Padieu and Cousin 1999; Mitrović et al. 2016) and Middle East (Sharon et al. 2015; Vali Sichani et al. 2011). It is caused by a phytoplasma belonging to the subgroup 16SrXII-A ['*Candidatus* Phytoplasma solani'] (Quaglino et al. 2013). More than 100 plant species, belonging to 40 different families and 22 orders, have been described being infected by the phytoplasma causing stolbur (Table OR1). Many wild (Maixner et al. 1995; Marchi et al. 2015), ornamental (Chung et al. 2016; Rashidi et al. 2010), major (Mitrović et al. 2016; Sharon et al. 2015) and minor crop plants (Carraro et al. 2008; Trkulja et al. 2016) are affected by stolbur. Many different strains of '*Ca*. P. solani' could be identified by molecular characterization, mainly on *tuf*, *secY*, *vmp1* and *stamp* sequencing (Fabre et al. 2011; Fialova et al. 2009; Johannesen et al. 2012; Murolo and Romanazzi 2015; Quaglino et al. 2016). Distribution of these genotypes among the different host plants seems to be specific for most of the stolbur phytoplasmas (Johannesen et al. 2012; Kosovac et al. 2016).

The planthopper *H. obsoletus* (Hemiptera: Cixiidae) is the main natural vector of '*Ca.* P. solani,' even if some other insect species can be involved in '*Ca.* P. solani' transmission (Chuche et al. 2016). This insect is found on a great diversity of plants, mainly wild, and transmits phytoplasma to crops during feeding probing. About 19 species belonging to 10 different plant families are known to harbor both nymphs and adults but adults can be observed on more species (Riolo et al. 2012).

The vector and the pathogen can share the same host plant or not. Nettle (*Urtica dioica*) allows the development of both '*Ca.* P. solani' and *H. obsoletus* while grapevine (*Vitis vinifera*), for example, can be affected by the pathogen but not constitute a host plant of the vector (Johannesen et al. 2008). In this latter situation, the grapevine is a dead-end host for the phytoplasma. *H. obsoletus* becomes infective, mainly as a nymph, by feeding on a wild host plant (e.g., nettle or field bindweed) and transmits the pathogen, as an adult, when probing on grapevine. Contrary to grapevine, lavender (*L. angustifolia*) and lavandin (*Lavandula* × *intermedia*) are both hosts for the phytoplasma '*Ca*. P. solani' and its insect vector (Boudon-Padieu and Cousin 1999). The disease, called lavender decline, is the main threat for lavender production in recent decades, with strong economic consequences. Lavender plants were usually cultivated from 10 to 12 years, but presence of lavender decline constrains the grower to uproot the plants within 4–5 years of planting (Moreau et al. 1970). Symptoms of lavender decline are yellowing and either standing up or rolling down of the leaves, and reduction and abortion of inflorescences (Boudon-Padieu and Cousin 1999). Like in other phytoplasma diseases, symptoms may be located only on some branches or affect the whole plant. After yellowing, the affected branches dry, resulting in plants with mixed dead and still green branches. After several growth cycles, the plants become completely brown and dry (Boudon-Padieu and Cousin 1999).

There is no curative treatment against phytoplasma diseases. The main ways to control the epidemic are to suppress the pathogen transmission from one plant to another by killing the vector (Chuche and Thiéry 2014) or destroying the vector host plant if the susceptible crop is a dead-end host (Kehrli and Delabays 2012). Using insecticides to control adults is impossible in the *H. obsoletus*/stolbur/lavender system because lavender is a very attractive crop to pollinators (honeybees, bumblebees) and bee protection rules prevent the use of insecticides. Nymphs develop on the roots and can be found several tens of centimeters deep (Boudon-Padieu and Cousin 1999) and are thus not affected by insecticide sprays. The main actions undertaken are the use of healthy planting material and selection of less susceptible lavender and lavandin cultivars. But up to now, no cultivar was found resistant to the phytoplasma infection (Gaudin et al. 2011). Others prophylactic methods are currently under investigation such as spraying kaolinite clay or inter-cropping plants. Most of the resistance to lavender decline seems to be linked to the unsuitability and/or poor attractiveness of the plant for *H. obsoletus.* In this way less susceptible cultivars

to disease Diva (lavender) and Grosso (lavandin) host very low numbers of adults and nymphs, while important populations can be found on the very susceptible C15/50 (lavender) and Abrial (lavandin) plant cultivars (Yvin 2013). A surprisingly high level of decline symptoms has been observed in recent years in southeastern France on the less susceptible cultivar Grosso. At the same time, an increasing number of *H. obsoletus* was observed in these fields. Research on insects close to these lavandin fields showed that recently cultivated plots of clary sage, *S. sclarea*, host high population of insects resembling *H. obsoletus*. In the present research, we characterized the insects infesting clary sage fields and investigated their ability to transmit the stolbur phytoplasma.

## Materials and methods

### Collection of insects

Insect were captured during the summer from 2011 to 2016 in the Provence region (43°54′28.00″N; 6°6′5.69″E) in several *S. sclarea* plots. Adults were collected using yellow sticky traps for monitoring flight dynamics and D-Vac vacuum sampling for phytoplasma detection. Nymphs were collected on uprooted *S. sclarea*, on the same plots as adults, during winter time (February 2013). Other populations of *H. obsoletus* were collected in France on field bindweed (*Convolvulus arvensis*; Civrac, Gironde), lavender (*L. angustifolia*; Champlong, Vaucluse) and nettle (*U. dioica*; Kientzheim, Haut-Rhin). Some insects from lavender were used for transmission trials.

Emergence traps were put on *S. sclarea* plot in 2016 in order to determine if clary sage could be a natural host plant of *H. obsoletus*. Ten 10 × 1 m insect mesh tunnels were put in place just after the sage harvest mid-July and left until end of August. Two cylindrical traps made with a 20 × 25 cm yellow glued card attached to an 8-cm-diameter PVC tube were put at ground level under each tunnel. Each week, the number of *H. obsoletus* caught was recorded and glue was renewed.

### Morphological characterization

Collected insects were individually identified according to Holzinger et al. (2003). External morphological features were observed with a stereomicroscope in order to determine family and genus. For species identification, male genitalia (aedeagus, paramere and anal tube) were placed in a 10% potassium hydroxide solution for one day to remove membranous soft tissues and conserve only the chitinous tissues. Then male genitalia were carefully dissected and observed under a stereomicroscope. Adults of *H. obsoletus* sampled on *S. sclarea* and the three other host plants *C. arvensis*, *L. angustifolia* and *U. dioica* (30 females and 30 males per population) were measured under a stereo microscope, length from head to telson. Adult length was compared with a Kruskal–Wallis test, following by post hoc comparisons using Nemenyi's test.

### Insect and plant DNA extraction

Insect DNA was individually extracted from collected planthoppers, following the protocol described by Maixner et al. (1995). Briefly, whole insects were ground in 250 µl CTAB-based buffer: 2% w/v cetyl trimethyl ammonium bromide (CTAB); 1.4 M NaCl; 20 mM EDTA pH 8.0; 100 mM Tris-HCl pH 8.0; 0.2% $\beta$-mercaptoethanol. Male specimens were homogenized after removal of the aedeagus. After incubation at 65 °C for 30 min, DNA was extracted with one volume of chloroform/isoamylalcohol (24:1) solution and then precipitated with ice-cold propan-2-ol. DNA pellets were then washed with 70% ethanol and resuspended into 50 µl of sterile water.

For plant DNA extractions, leaf midribs and/or roots (0.5–1 g each) were washed in water then ground in 3 ml of CTAB buffer and DNA was extracted following Maixner et al. (1995) as briefly described above. DNA pellets were resuspended into 100 µl of sterile water. An additional purification step was added for *Salvia sclarea* using the Wizard® genomic DNA purification kit (Promega, Madison, USA).

### Molecular identification of insects

To confirm morphological characterizations, a fragment of about 800 bp of the cytochrome oxidase subunits I and II (COI) mitochondrial gene was amplified using the primers C1-J-2195 (5′-TTGATTTTTTGGTCATCCAGAAGT-3′) and TL2-N-3014 (5′-TCCAATGCACTAATCTGCCATATTA-3′) (Simon et al. 1994). PCR amplifications were performed according to (Bertin et al. 2010) with 1 µl of DNA template in a final volume of 20 µl. Sequencing of PCR products from both 5′ and 3′ end of purified PCR products were performed by Beckman Coulters Genomics (Takeley, UK). The coverage of these sequences was 2X andraw sequence chromatograms were assembled and edited using GAP4 (Bonfield et al. 1995). To identify the species corresponding to the COI nucleotide sequences, BlastN analysis were first performed. Then multiple alignments were performed using ClustalW (Thompson et al. 1994) with the closest homolog sequences. The phylogenetic analyses were carried out with MEGA 4 using maximum parsimony (Tamura et al. 2007).

### Mitochondrial DNA polymorphism

Genomic DNA of individual insects was analyzed after sequencing two mtDNA markers. Polymerase chain reactions (PCR) were performed as described by (Johannesen et al. 2008) using the primers S2792 (5′-ATACCTCGACGTTATTCAGA-3′) and A3661 (5′-CCACAAATTTCTGAACATTGACCA-3′) (Brown et al. 1996) to amplify the cytochrome oxidase subunit I tRNA(Leu) cytochrome oxidase subunit II DNA region (CO I-tRNA(Leu)-CO II). The ribosomal RNA-reduced nicotinamide adenine dinucleotide dehydrogenase subunit I DNA region (16S-tRNA(Leu)-ND1) was amplified using the primers LR-N-1245 (5′-GCACCTCGATGTTGAATTAA-3′) and N1-J-12261 (5′-TCGTAAGAAATTATTTGAGC-3′) (Hedin 1997). Sequencing of PCR products and their subsequent analysis were performed as described above in the molecular identification of insects section. The sequences were deposited in the European Nucleotide Archive (accession number PRJEB20504).

### '*Ca.* P. solani' detection and genotyping

Detection and genotyping of '*Ca*. P. solani' were carried out on the *secY* gene. Fragment of *secY* gene was amplified by nested-PCR using the primers PosecF1 (5′-TCTGCTTTGCCTTTGCCTTT-3′) and PosecR1 (5′-ATTAGTAAACTAGTTCCTCC-3′) and the nested primers PosecN2 (5′-CCATCAAAACTTTTTGGTTTAGGC-34′) and PosecR3 (5′-GCCCCTATAACGGTGATTTTGA-3′) as previously described by Cimerman et al. (2009). Sequencing of nested-PCR products and their subsequent analysis were performed as previously described in this study for molecular identifications of insects.

### Transmission trials of stolbur phytoplasmas to *Salvia*

Transmission trials were conducted with *H. obsoletus* captured in 2014 on lavender and healthy *S. sclarea* grown from seed under insect-proof conditions. *H. obsoletus* were captured on lavender plots and caged by groups of 30 with a clary sage. A total of five cages were made and kept at a constant temperature (23–25 °C) and a photoperiod of 16:8 h. Transmission trials were carried out for as long as insects survived. DNA was then extracted on each individual plant and tested for the presence of '*Ca.* P. solani' using PCR analysis as described above. Plants used in transmission experiments were treated with insecticides (Pyrevert, Valagro, France) and kept in an insect-proof greenhouse until symptoms apparition. Six months after the end of the trial, leaf veins and roots of symptomatic and asymptomatic plants were tested for '*Ca.* P. solani' presence using PCR analysis as described above.

### Rearing trials of *H. obsoletus* on *Salvia*

Nymphs collected on uprooted *S. sclarea* from the field were transferred to healthy *S. sclarea* grown from seed under insect-proof conditions in polyglass cages at a constant temperature (23–25 °C) and a photoperiod of 16:8 h in greenhouse. Plants were weekly watered by pouring water in a plant saucer. Emerging adults were counted and transferred on a new *S. sclarea* plant to check if they are able to reproduce and lay eggs on this plant.

## Results

### *Salvia sclarea* as a host plant of *H. obsoletus* in the field

During spring of 2011, *H. obsoletus* was found for the first time to infest a plot of *S. sclarea* on the plateau of Valensole (43°52′23.46″N, 6° 3′29.45″E). During the following years, other clary sage plots were found to be infested with this insect (localities of Brunet, Entrevennes, Puimoisson and Saint-Jurs). Indeed, above 500 adults could be caught in 2 h using a D-Vac. Insects were identified based on their aedeagus morphology and bar coding study. The overall morphology of insects and the anatomy of the aedeagus showed that these insects sampled on *S. sclarea* belonged to the species *H. obsoletus*. The nucleotide sequence of a fragment of COI mitochondrial gene was identical to the sequence of *H. obsoletus* (Fig. 1).

Most of the uprooted clary sage (80%) harbored nymphs and up to 51 nymphs per plant were found, with a mean of 12 individuals (Fig. 2). Most of the *H. obsoletus* were in their fourth nymphal instar (75%), followed by third (23%) and fifth (2%). To observe the emergence of adults from these nymphs, emergence yellow traps were placed on clary sage plants after the flower harvest. Although there was a lot of variation in the level of capture between traps, up to 615 individuals could be caught on one yellow trap (10 $m^2$) in 1 month (Table 1). Based on trap monitoring, the flight period extended from end of June to mid-August with slight annual fluctuations (Fig. 3).

### *Salvia sclarea* as a host plant of *H. obsoletus* in greenhouse and rearing

From the 240 nymphs collected on the field in 2012 in Valensole and put on two clary sages for our rearing trial, 55 became adult (28 males and 27 females) after 17 weeks.

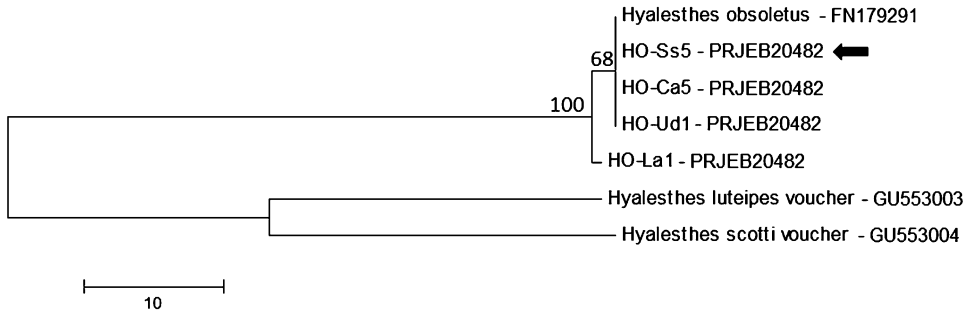


**Fig. 1** Phylogenetic position of clary sage *Hyalesthes obsoletus*. Maximum parsimony tree was constructed from COI mitochondrial gene sequences of three different species of *Hyalesthes*, and *H. obsoletus* sampled on clary sage (HO-Ss, *arrow*), field bindweed (HO-Ca), nettle (HO-Ud) and lavender (HO-La). Accession numbers are indicated after the name of *Hyalesthes* species

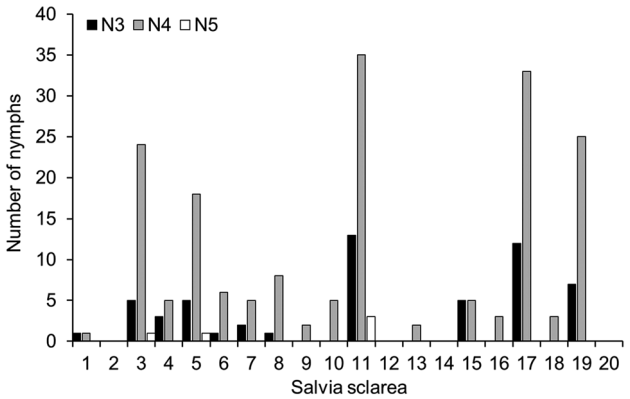


**Fig. 2** Presence of *H. obsoletus* nymph on *S. sclarea* roots. Nymphs of *H. obsoletus* were counted from 20 uprooted *S. sclarea* plants during the sixth week of 2013 where the third (*black*), fourth (*gray*) and fifth (*white*) nymphal instar were observed

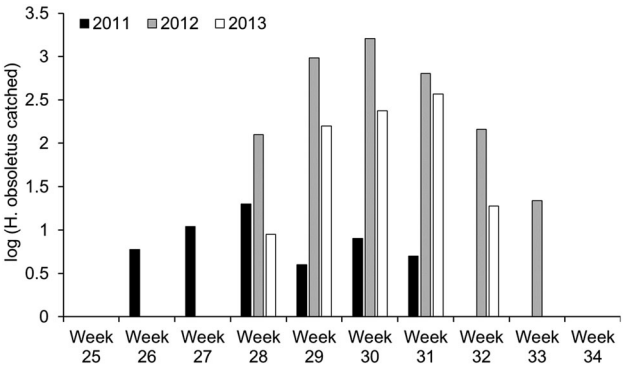


**Fig. 3** Flight period of *H. obsoletus* on *S. sclarea* plots. Adult *H. obsoletus* were counted on yellow traps every week from mid-June to end August in 2011 (*black*), 2012 (*gray*) and 2013 (*white*). Each value is the sum of five yellow sticky traps

Those adults were able to produce offspring that developed on clary sage. More than ten subsequent generations were obtained on *S. sclarea* during 3 years. Even though the plants were never placed in cold room to mimic winter, the nymphs developed. Thus, all the results show that *H. obsoletus* can complete its life cycle on *S. sclarea*.

### *Hyalesthes obsoletus* population characterization

As *H. obsoletus* completed its life cycle on different host plants, we compared *H. obsoletus* from *S. sclarea* to insects collected on 3 other host plants found in France: *C. arvensis*, *L. angustifolia* and *U. dioica*. We first compared their morphology by measuring the length from head to end

**Table 1** Number of *H. obsoletus* adults caught under emergence traps in 2016

| Emergence trap | 25-July | 01-August | 09-August | 17-August | 23-August | Total |
|---|---|---|---|---|---|---|
| 1 | 20 | 9 | 1 | 0 | 0 | 30 |
| 2 | 55 | 27 | 3 | 3 | 0 | 88 |
| 3 | 86 | 15 | 2 | 0 | 0 | 103 |
| 4 | 85 | 16 | 1 | 0 | 0 | 102 |
| 5 | 77 | 21 | 1 | 0 | 0 | 99 |
| 6 | 150 | 62 | 9 | 1 | 2 | 224 |
| 7 | 220 | 95 | 11 | 1 | 0 | 327 |
| 8 | 74 | 35 | 7 | 0 | 0 | 116 |
| 9 | 74 | 36 | 4 | 0 | 0 | 114 |
| 10 | 408 | 167 | 35 | 3 | 2 | 615 |
| Total | 1249 | 483 | 74 | 8 | 4 | 1818 |

Each value is the sum of two yellow sticky traps

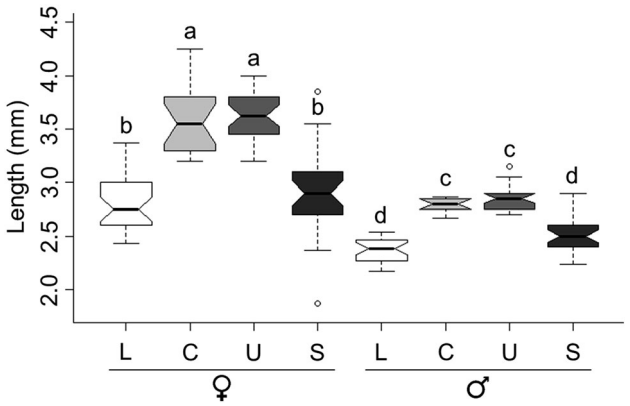


**Fig. 4** Length of *H. obsoletus* females and males sampled on *S. sclarea* (S), *L. angustifolia* (L), *U. dioica* (U) and *C. arvensis* (C). Boxplots with *different letters* are significantly different under the Kruskal–Wallis and Nemenyi's post hoc tests at 1% threshold

of their telson. Whatever the plant host, females were systematically longer than males (Fig. 4). Both males and females from clary sage and lavender were significantly smaller than individuals from field bindweed and nettle. Females sampled on *L. angustifolia* and *S. sclarea* were about 20% smaller than ones from *C. arvensis* and *U. dioica* ones, and males were about 15% smaller. It should be noted that *H. obsoletus* males from clary sage were significantly bigger than males from lavender.

To correlate morphological observations with genotypes of *H. obsoletus*, we analyzed genetic diversity of the four French populations using mitochondrial markers. The mitochondrial haplotypes are shown in Table 2. Three main haplotypes aa, ab and bb were found, but contrary to *Convolvulus* and *Urtica Hyalesthes* populations, insects sampled from *Salvia* or *Lavandula* showed a unique mtDNA haplotype per plant host species. Haplotypes of *Salvia H. obsoletus* (bb) were the same as those found in half of the *Convolvulus H. obsoletus* tested, and haplotypes of *Lavandula H. obsoletus* (ab) were unique in France. The highest diversity was observed within the COI region of the mitochondrial DNA of the *H. obsoletus* sampled on *C. arvensis*. Thus, no strong correlation appeared between haplotypes and host plants but genetic diversity was observed between insects collected on lavender and clary sage. When morphological and genetic studies were combined, it appears that *H. obsoletus* sampled on clary sage are different from those sampled on field bindweed, lavender and nettle.

## Role of *S. sclarea* in stolbur epidemiology

Stolbur phytoplasma was found in the plant hosts of *H. obsoletus* field bindweed, lavender and nettle. To know if clary sage could also be a plant host for this phytoplasma, *H. obsoletus* collected on clary sage and several plants of *S. sclarea* from the field were analyzed by *secY* PCR after DNA extraction. One out of 48 insects was found positive to stolbur phytoplasma with *secY* PCR. Sequence analysis shows that *secY* sequence belongs to the group S4 (Fig. 5). Forty-two clary sages were analyzed for detection of '*Ca*. P. solani.' None of the plants in the field showed symptoms. Only one was found positive using *secY* PCR. In this case, the *secY* sequence belonged to the group S1 (Fig. 5).

Experimental transmission trials were also carried on to show that clary sage could be a host plant for the phytoplasma. As clary sage plots are very close to lavender and lavandin fields where phytoplasmas are responsible for decline lavender, transmission tests of '*Ca*. P. solani' to *S. sclarea* were conducted with *H. obsoletus* sampled on lavender. From five plants caged with *H. obsoletus*, one clary sage presented symptoms of stolbur as stunted and very small leaves. Detection of '*Ca*. P. solani' with *secY* PCR showed positive result only with the clary sage with

**Table 2** Genetic polymorphism of *H. obsoletus* sampled on *C. arvensis*, *L. angustifolia*, *S. sclarea* and *U. dioica* using mitochondrial DNA markers

| Locality | Host plant | Year | Number of insects tested | Number of mtDNA haplotypes (CO I-ND1)[a] | | | |
|---|---|---|---|---|---|---|---|
| | | | | Aa | Ab | Bb | New |
| Civrac (Gironde) | *Convolvulus* | 2012 | 10 | | | 5 | 2 ($\delta-b$) |
| | | | | | | | 2 ($\varepsilon-b$) |
| | | | | | | | 1 ($\zeta-b$) |
| Champlong (Vaucluse) | *Lavandula* | 2012 | 9 | | 9 | | |
| Valensole | *Salvia* | 2012 | 14 | | | 14 | |
| (Alpes Hautes Provence) | | 2013 | 4 | | | 4 | |
| Kientzheim (Haut-Rhin) | *Urtica* | 2012 | 9 | 8 | | 1 | |

[a] CO I: CO I-tRNA(Leu)-CO II DNA region, ND1: ribosomal RNA (16S)-tRNA(Leu)-ND1 DNA region

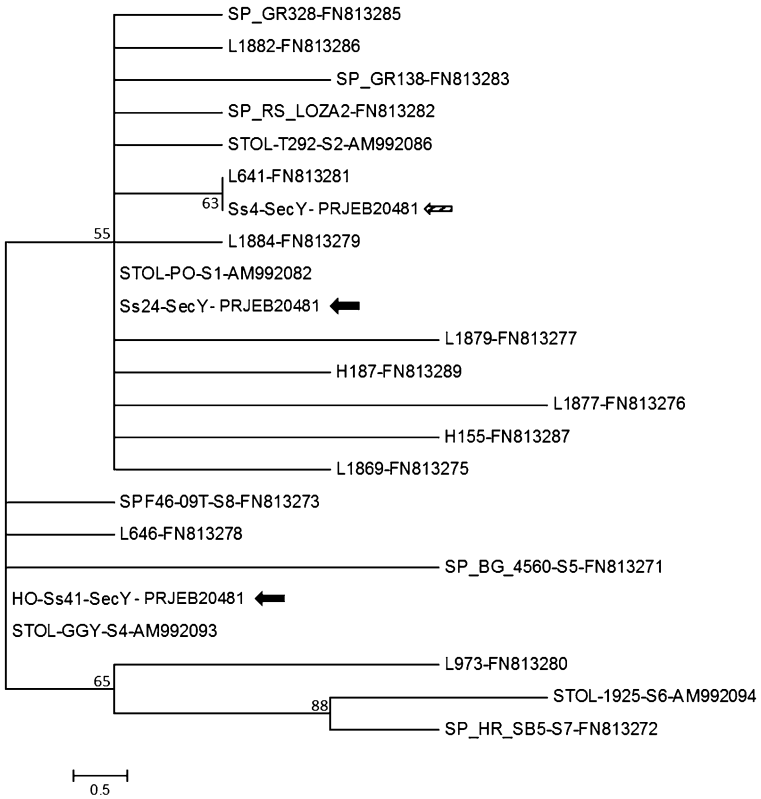


**Fig. 5** Phylogenetic analysis using maximum of parsimony of *secY* sequences of phytoplasmas contained in *H. obsoletus* sampled on clary sage (HO-Ss41, *gray arrow*), clary sage harvested in the field (Ss24-SecY, *black arrow*) and clary sage from experimental transmission of '*Ca.* P. solani' with lavender *H. obsoletus* (Ss4-SecY, *hatched arrow*). Accession numbers are indicated after the name of phytoplasma strains. The secY genotype numbering is according to the nomenclature of the network SEE-ERANET Phytoplasma epidemiology in Southeastern Europe (Foissac et al. 2013). The origin (Country, host) of '*Ca.* P. solani' strains are: SP-GR328 (FN813285), Greece, *Capsium annum*; L1882 (FN813286), France, *L. angustifolia*; SP_GR138 (FN813283), Greece, *V. vinifera*; SP_RS_LOZA2 (FN813282), Serbia and Montenegro, *V. vinifera*; STOL-T292-S2 (AM992086), Yugoslavia, Red-pepper; L641 (FN813281) France, *L. angustifolia*; L1884 (FN813279), France, *L. angustifolia*; STOL-PO-S1 (AM992082), France; *H. obsoletus*; L1879 (FN813277), France, *L. angustifolia*; H155 (FN813287), France, *L. angustifolia*; L1877 (FN813276), France, *L. angustifolia* x *Lavandula latifolia*; H155 (FN813287), France, *L. angustifolia*; L1869 (FN813275), France, *L. angustifolia* × *L. latifolia*; SP F46-09T-S8 (FN813273), France, *Nicotiana tabacum*; L646 (FN813278), France, *L. angustifolia*; SP_BG4560-S5 (FN813271), Bulgaria; *V. vinifera*; STOL-GGY-S4 (AM992093), Yugoslavia, Red-pepper; L973 (FN813280), France, *L. angustifolia* × *L. latifolia*; STOL-1925-S6 (AM992094), Germany, *V. vinifera*; SP_HR_SB5-S7 (FN813272), Croatia, *V. vinifera*

symptoms. Both leaves and roots were positive. The *secY* genotype of '*Ca.* P. solani' present in this clary sage was identical to those of a phytoplasma sampled from a lavender (Fig. 5). Thus, *H. obsoletus* from lavender are able to transmit '*Ca.* P. solani' to *S. sclarea* in which it could multiply. All together, these results show that *S. sclarea* can be infected by '*Ca.* P. solani.'

## Discussion

This article describes the results of various experiments performed over the last 4 years. We conducted our study both on wild specimens of *H. obsoletus* naturally infected with '*Ca.* P. solani' and collected in production lavender and clary sage fields, and with laboratory experiments. Our studies demonstrate that *H. obsoletus* can successfully live and reproduce on *S. sclarea*, and transmit the pathogen to uninfected clary sage.

Unlike lavender/lavandin, clary sage is a semi-perennial crop with commonly a 3 years' lifespan. The sowing is done the first year and the stems are harvested during years 2 and 3. Then a new crop, usually wheat or lavandin, is grown in place of clary sage. This short-term presence means that *H. obsoletus* can quickly colonize and adapt to *S. sclarea*. Nevertheless, the origin of the population of *H. obsoletus* on the clary sage is unknown. Our genetic polymorphism comparisons between different host plant populations revealed that *H. obsoletus* from *S. sclarea* are closer to individuals from field bindweed and nettle that were collected from distant areas, rather than insects from lavender of the same region. Indeed, mtDNA

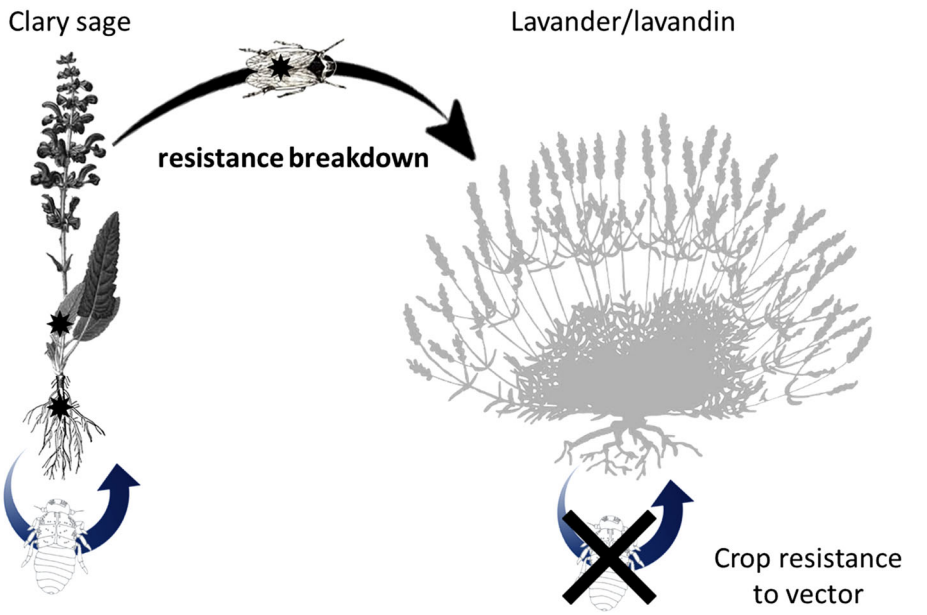


**Fig. 6** Role of *S. sclarea* on the breakdown of lavandin resistance to *H. obsoletus* and lavender decline. *Star* represents phytoplasmas

haplotypes of insects from *S. sclarea* are all bb, like half of the *H. obsoletus* from *C. arvensis*, while cixiids from *L. angustifolia* are all ab. Either *H. obsoletus* from field bindweed colonized clary sage, or the haplotype diversity in the lavender population is wider than that shown by our sample. According to Johannesen et al. (2008), south-eastern France, where this study took place, is in the area of the ancestral haplotypes ab where the haplotype diversity is the highest. The homogeneity of *H. obsoletus* mtDNA haplotype from clary sage suggests that a small number of insects initiated the clary sage population and/or that the bb haplotype is the only one that can develop on this plant. Our morphological study showed that individuals from lavender and clary sage are of similar size and are very small compared to insects from nettle and field bindweed. This could be influenced by the similarity of metabolites produced by these plants, which belong to the same family, the Lamiaceae. Host plant quality can greatly affect phytophagous insects (Awmack and Leather 2002; Bernays 2001), and such an effect was reported on the wing length of *H. obsoletus* living sympatrically on *U. dioica* and *C. arvensis* (Johannesen et al. 2008). Our results do not reveal whether the *H. obsoletus* populations and phytoplasma strains found in *S. sclarea* plots have the same plant origin. We found an S1 stolbur strain in clary sage from the field, while a *H. obsoletus* collected on sage harbored the S4 strain. The S1 and S4 strains are frequently found in field bindweed and in young lavender plots (X. Foissac unpublished data). Thus, the origin of clary sage *H. obsoletus* is yet unresolved and is under study.

For years, resistant lavandin cultivars such as Grosso were not really affected by lavender decline due to the impossibility of *H. obsoletus* to complete its life cycle on it. Although some adults could be collected on these cultivars, very few nymphs were found on the roots. So, the key point in the resistance of this plant to the vector was the impossibility of the nymphs to develop on it (Fig. 6). Because lavandin and lavender are rarely cultivated on the same area, the absence of sustainable *H. obsoletus* populations on resistant lavandin allowed meant that phytoplasma propagation in the crop was avoided. Most of the few decline symptoms observed were probably due to inoculation by infected vectors from wild plants that are quite rare in lavandin plots. The increase in *S. sclarea* cultivation in which high populations of *H. obsoletus* were found in areas where resistant lavandin cultivars are grown most probably allowed the breakdown of the lavender decline resistance. *S. sclarea* plots can constitute a reservoir for the pathogen and its vector close to the lavandin plots (Fig. 6). Indeed *S. sclarea* was experimentally infected using *H. obsoletus* naturally infected with '*Ca*. P. solani.' The role of *S. sclarea* as a reservoir of '*Ca.* P. solani' for lavender crop is also supported by the numerous stolbur-diseased lavandin in the neighboring plot of our clary sage site study. Many Grosso lavandin cultivar plants expressed symptoms, while this cultivar does not allow the development of *H. obsoletus*. Thus, infective adults from

clary sage could transmit the pathogen to Grosso cultivar that would be a dead-end-host for the phytoplasma. This could be a way for the disease to overcome the plant cultivar resistance to it, based on the very low ability of the vector to develop on it. The clary sage harvesting method, consisting of harvesting all the plants in summer during the *H. obsoletus* flight period, probably forces the cixiids to temporarily desert the plots. This will increase the dispersal of the adult carrying phytoplasma, and an increase in the inoculation probability to lavandin can be expected.

Because infected *S. sclarea* do not show obvious symptoms, it is difficult to estimate the proportion of plants harboring the stolbur phytoplasma. Moreover, there is no way for the lavender/lavandin growers to estimate the risk due to the presence of clary sages close to their fields. The high commercial demand of sclareol, the main fragrant chemical compound extracted from *S. sclarea*, and its high commercial value have led to an increasing area where clary sage is cultivated in southeastern France. With such crop expansion, an increase in the '*Ca.* P. solani' vectorial capacity could be expected and less susceptible cultivars of lavender/lavandin could decline in the near future. Moreover, the fact that '*Ca.* P. solani' experimentally infected clary sage that developed stolbur symptoms shows that a new disease could emerge on clary sage in southeastern France. As previously shown, plants of the genus *Salvia* are also described as susceptible to aster yellow phytoplasma (Olivier et al. 2009).

Our results suggest that the role of minor crops in stolbur epidemiology should be better investigated. Most of studies focus on wild plants and neglect minor crops. Due to the ability of both stolbur phytoplasma and *H. obsoletus* to develop on a wide range of host plants, there are many candidates, including clary sage, which could be involved in stolbur epidemic globally. It is essential to take into account the local context. A host plant of *H. obsoletus* and stolbur phytoplasma in one country can have no role in another. For example, while *Vitex agnus castus* is a host plant of *H. obsoletus* in Israel and known as a '*Ca.* P. solani' reservoir in Montenegro (Kosovac et al. 2016), no link was made between *V. agnus castus* and grapevine for the presence of '*Ca.* P. solani' in that country (Sharon et al. 2015).

This is the first description of *S. sclarea* as a host plant for '*Ca.* P. solani' and its vector *H. obsoletus*. Our results also showed that *H. obsoletus* from lavender are able to transmit phytoplasma to clary sage. These results support the claim that *S. sclarea* could be responsible for the increasing incidence of lavender decline on less susceptible cultivars. The mechanism of the colonization of its plant is under investigation and will lead to a better understanding of the ability of *H. obsoletus* to settle on a new host plant. Further work is needed to fully determine how clary sage could constitute an epidemic reservoir of '*Ca.* P. solani' for lavender crops. The stolbur epidemiology involving *S. sclarea* is a good example showing how a minor culture can indirectly impact a major one by modifying the parameters of disease epidemiology.

## Author contributions

JC, JLD and JBR conceived and designed the experiments. JC, JLD and JBR performed the experiments. JC, JLD, JBR and NAB analyzed the data. JC, NAB and DT wrote the paper. All authors read and approved the manuscript.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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