Journal of Chemical Ecology, Vol. 31, No. 5, May 2005 (©2005)
DOI: 10.1007/s10886-005-4247-z

Vitex agnus-castus IS A PREFERRED HOST PLANT FOR *Hyalesthes obsoletus*

RAKEFET SHARON,[1] VICTORIA SOROKER,[2]
S. DANIEL WESLEY,[2] TIRTZA ZAHAVI,[3]
ALLY HARARI,[2] and PHYLLIS G. WEINTRAUB[4,*]

[1]*Northern Research and Development, Kiryat Sh'mona, Israel*
[2]*Department of Entomology, ARO, Bet Dagan, Israel*
[3]*Extension Service, Ministry of Agriculture, Kiryat Sh'mona, Israel*
[4]*Department of Entomology, ARO, Gilat Research Center, D.N. Negev 85280, Israel*

(Received July 5, 2004; revised December 20, 2005; accepted January 4, 2005)

Abstract—*Hyalesthes obsoletus* Signoret (Homoptera: Cixiidae) is a polyphagous planthopper that transmits stolbur phytoplasma (a causative agent of "yellows" disease) to various weeds, members of the Solanaceae, and wine grapes (*Vitis vinifera* L.) in Europe and the Middle East. Planthoppers were collected by hand vacuuming eight native plant species. *Vitex agnus-castus* L., a shrub in the Verbenaceae, hosted the largest number of *H. obsoletus*, although *Olea europaea* L. also served as a host for adults. Using a Y-olfactometer, we compared the planthoppers relative preference for *V. agnus-castus*, *Convolvulus arvensis*, and *V. vinifera*. *V. agnus-castus* was more attractive to both male and female *H. obsoletus* than the other plants. *H. obsoletus* antennal response was stronger to volatiles collected from *V. agnus-castus* than from Cabernet Sauvignon variety of *V. vinifera*. To determine if *V. agnus-castus* would serve as a reservoir for the pathogen, *H. obsoletus* were collected from leaf and stem samples of native *V. agnus-castus*, and were tested by polymerase chain reaction (PCR) for the presence of phytoplasma DNA. While 14% and 25% (2003 and 2004, respectively) of the insects tested positive for phytoplasma DNA, none of the plant samples tested positive. To determine if *V. agnus-castus* could serve as a host plant for the development of the planthopper, we placed emergence cages beneath field shrubs and enclosed wild-caught *H. obsoletus* in a cage with a potted young shrub. We found adult *H. obsoletus* in the emergence cases and planthopper nymphs in the soil of the potted plant. We concluded that *V. agnus-castus* is attractive to

* To whom correspondence should be addressed. E-mail: phyllisw@volcani.agri.gov.il

H. obsoletus, which seems to be refractory to phytoplasma infections and warrants further testing as a trap plant near vineyards.

Key Words—EAG, "yellows" disease, spread control, management.

INTRODUCTION

The level of infection of a crop by phytoplasma is dependent on the number and abundance of insect vectors and of alternative/reservoir plants harboring the pathogen, provided that the planting material is not the source of infection. In some cases, such as the aster yellows of carrots, lettuce, and celery in the midwestern U.S., the relationship between the vector and native phytoplasma-infected host plants has been elucidated (Hoy et al., 1992). In other cases, such as the stolbur phytoplasma infection in various crop plants in Europe, the role of alternative host plants in disease epidemiology is not completely understood.

Hyalesthes obsoletus Signoret (Homoptera: Cixiidae), is a polyphagous vector of stolbur phytoplasma to some weeds, plants in the Solanaceae (Brcak, 1979), and wine grapes (*Vitis vinifera* L.) (Maixner, 1994; Sforza et al., 1998) in Europe, and is a suspected vector to wine grapes in Israel (Klein et al., 2001). Grape vines are probably not among the preferred plants of *H. obsoletus*, as it survives poorly on them (Maixner, 1994; Sforza et al., 1998). Brcak (1979) reported that *H. obsoletus* does not leave its primary central and east European host, *Convolvulus arvensis* L., as long as the plant remains suitable for feeding. Furthermore, he reported that as long as the planthopper remained on *C. arvensi*s, a natural source of the phytoplasma, there were no observed incidents of stolbur infection in crops. *H. obsoletus* has been found on other weeds in addition to *C. arvensis*, some of which are also potential phytoplasma reservoirs. In vineyards in Germany, Maixner et al. (1995) demonstrated that *C. arvensis* and *Solanum nigrum* L. were infected with stolbur phytoplasma and could serve as an inoculum reservoir. In contrast, Weber et al. (1997) showed that in vineyards where *Ranunculus bulbosus* L. was the dominant weed, there were fewer infected *H. obsoletus*, and *R. bulbosus* was never found to harbor the phytoplasma. In France, *H. obsoletus* was found to complete its life cycle on *Lavendula hybrida* Reverchon (Moreau and Leclant, 1973), *C. arvensis*, *Lavendula angustifolia* Mill., and *Lepidium draba* L. (Sforza et al., 1999), and was shown to be infected by stolbur phytoplasma (Cousin and Moreau, 1991; Sforza et al., 1998).

Yellows disease was first observed in wine grapes in Israel in the 1980s, and has had a devastating effect. To date, over 50 ha of vineyards have been uprooted due to this disease. Vineyards in affected areas have been surveyed for the presence of phloem feeding insects, such as leafhoppers and planthoppers

(Klein et al., 2001; Orenstein et al., 2003), and several candidate species, including *H. obsoletus*, have been identified.

Alternative plant hosts for the phytoplasmas have not been identified in Israel. During the middle of the growing season (June–August), there are few living plants, and virtually no leafhoppers or planthoppers are captured in vineyards (Klein et al., 2001; Orenstein et al., 2003). Because the last precipitation in wine-grape production areas is in mid-April (Orenstein et al., 2003), by May all nonirrigated areas are dry and only deep-rooted plants remain green throughout summer. Two generations of *H. obsoletus* are found annually in Israeli vineyards; there is a small peak population of adults in May and early June, and another larger peak in September and October (Klein et al., 2001; Orenstein et al., 2003). The goal of this research was to determine the native host plant(s) of *H. obsoletus* and their ability to serve as a reservoir for phytoplasmas.

METHODS AND MATERIALS

Native Plant Screening. This study took place in the Golan Heights at an elevation of 350 m in the south to 800 m in the north. We collected insects from native plants that remained green throughout the summer; *Amaranthus retroflexus* L., *C. arvensis*, *Inula viscose* L., *Myrtus communis* L., *Salix alba* L., *Tamarix* sp., and *Vitex agnus-castus* L. We used a modified leaf blower (Echo model No. PB 1000), in which air intake and exhaust ports were switched, and intake was fitted with a fine mesh nylon bag. Vacuum sampling collects all insects indiscriminately and does not rely on their attractiveness to a trap. In the first year (2003), a minimum of three plants of each species were sampled two to four times in May–June and once in September in the central-southern Golan. During the second year (2004), a minimum of three plants of each species were sampled two to four times in May–June and eight times from September to November; *V. agnus-castus* was sampled in central and northern Golan. *H. obsoletus* were found during spring in an olive (*Olea europaea* L.) orchard, and therefore sampled twice in spring and eight times in autumn.

Captured *H. obsoletus* were counted and recorded by sex when collected in large numbers. Live specimens were maintained at room temperature in screen-covered (50 mesh) boxes containing putative feeding plants until they were used in further trials.

Plant Choice Assay. The host plant preference of *H. obsoletus* was determined by olfactometry. The olfactometer consisted of a Y-shaped glass tube, 2 cm diam. The base was 9 cm long, and the two arms of the olfactometer were each 16 cm in length. Each arm was attached to a flow meter and an odor source container (glass sphere, 12 cm diam) into which test plant branches were

placed. In each case, charcoal-filtered air (1 l min^{-1}) was forced through each arm of the apparatus. There were six replicates of each plant pairing: *V. agnus-castus* vs. *C. arvensis* and *V. agnus-castus* vs. *V. vinifera*. *V. agnus-castus* was used in each pair comparison because of the larger number of planthoppers captured in this species. In Israel, the majority of *V. vinifera* are grafted on Richter 110, but three other rootstocks are also used. We tested Richter 110 and Castel 216. In each replicate (six replicates with females and six with males), a group of five to seven planthoppers was placed in the base tube of the Y-olfactometer and allowed to move upwind towards either of the two arms of the olfactometer. Trial periods were 5 min in length, and the location of each test planthopper was recorded at the conclusion of each trial. To compensate for any positional bias, the olfactometer was rotated 180° after every three replicates. In order to avoid contamination by remaining plant volatiles, the olfactometer was washed with acetone and dried after each experiment.

Volatile Collection. Volatiles were collected from blooming *V. agnus-castus* and Cabernet Sauvignon variety of *V. vinifera*. As noted above, samples of *V. vinifera* from both rootstocks were used. Charcoal purified air was forced through a 500-ml glass sampling jar containing a plant branch, then through a 200-mg SuperQ trap at a rate of 0.2 l min^{-1} to collect organic chemicals released from test plants. Traps were washed with pentane to elute the captured volatiles. The resulting extract was concentrated to a volume of 10 μl and stored at −20°C.

Electroantennograms. In order to examine antennal sensitivity to volatiles of various host plants, electroantennograms (EAG) were recorded from detached antennae of male and female *H. obsoletus* exposed to test volatiles. The base of each antenna was inserted into a glass capillary tube, with a silver electrode lining serving as a grounding probe, while the tip of the antennae was inserted into a second capillary tube, with a silver electrode lining serving as a recording probe. Both glass capillaries were filled with 0.1 N KCl. Antennae were positioned by using a micromanipulator (INR-05, Syntech, Hilversum, The Netherlands). Two microliters of concentrated plant volatiles were applied to an approximately 1 cm^2 piece of filter paper (Whatman No. 1). One microliter of pentane was applied to filter paper to serve as a blank. The filter paper was allowed to air dry for 20 sec to allow the solvent to evaporate, and was then placed inside a glass Pasteur pipette (15 cm long). The pipette tip was inserted through a side hole in a glass tube (0.6 cm diam and 15 cm long) through which charcoal-filtered, humidified air flowed at 0.5 ml min^{-1}. Within each stimulus pipette that contained the filter paper with plant volatiles, 3 ml of air were forced into the constant air stream by a mechanical puffing that delivered puffs of 0.4-sec duration. At least 2-min intervals were maintained between each release of plant volatiles. The presentation order of stimuli for one antenna was randomized within the set (two extracts and one blank). EAG recordings were

made by using a serial data acquisition controller (Syntech IDAC-232, The Netherlands).

H. obsoletus Development on V. agnus-castus. To determine if *V. agnus-castus* serves as a host plant upon which *H. obsoletus* can complete its development, two evaluation methods were used: evaluation of *H. obsoletus* emerging from wild *V. agnus-castus* and development of caged *H. obsoletus* on potted *V. agnus-castu*s. Emergence boxes were made of a 50 × 50 × 40 cm wooden frame covered on five sides with 50 mesh screening. One box was placed, open side down, under the canopy of each of three *V. agnus-castus* plants in the center-south of the Golan at the beginning of May 2003 and again at the beginning of September. One yellow sticky trap was placed in each box to catch any emerging adult insects, and traps were replaced once every 2 wk. In May 2004, one box was placed under the canopy of each of 15 *V. agnus-castus* plants. One yellow sticky trap was placed in each box to catch any emerging adult insects, and traps were replaced once every 2 wk until emergence ceased; this was repeated in autumn starting at the beginning of September and continuing until emergence ceased. Additionally, in spring, 50 mesh screening was draped over the bottom branches of two different shrubs and secured to the ground, encompassing a semicircular area 1.5 m from the trunk. One yellow sticky trap was suspended on a branch from each of these shrubs and changed as described.

In September 2003, 40 adult *H. obsoletus* were vacuum-collected and placed in a 1 × 1 × 1 m cage covered with 50 mesh screening and containing one *V. agnus-castus* plant. The shrub was planted in a 10-l pail: the bottom 2/3 of the potting mix was a mixture of 70:30 peat moss:volcanic gravel, and the top 1/3 was a mixture of 1:1 coconut fiber:perlite. The plant was watered with an automatic irrigation system. Since adults are difficult to discern on the plant through the net, in early May of the next year, when the first adults were observed in the field, the pot was removed from the cage and the soil around the roots was carefully examined for presence of *H. obsoletus* nymphs.

Since *C. arvensis* is a known host in Europe, in May 2003 and 2004, we dug up >50 plants in areas where *V. agnus-castus* were also found, and searched the roots for *H. obsoletus* nymphs.

Phytoplasma Infection in V. agnus-castus. Samples of *H. obsoletus* adult males and females were analyzed individually by polymerase chain reaction (PCR) to determine if they were positive for phytoplasma. Plants on which were they were captured were observed for typical signs and symptoms of phytoplasma infection (proliferation of small leaves, "witches' broom," or yellow leaves) through the duration of the study, and they were analyzed by PCR for presence of phytoplasma at the end of the season (October) in 2003 and 2004.

DNA Extraction and Polymerase Chain Reaction. DNA extracted from phytoplasma-infected *Vinca minor* L. (periwinkle) was used as a positive

control, and DNA extracted from asymptomatic *V. minor* served as a negative control. Insects were homogenized individually in 150 μl extraction buffer, and 1 g of plant tissue was homogenized in a total of 3 ml of extraction buffer (2% cetyl trimethyl ammonium bromide (CTAB), 100 mM Tris–HCl pH 8.0, 1.4 M NaCl, 40 mM EDTA, 0.2% β-mercaptoethanol), then put in a 65°C water bath for 30 min with periodic vortexing. Both insect and plant samples were centrifuged (1500 × *g* for 5 min), the supernatant was mixed with an equal volume of chloroform:isoamyl alcohol (24:1 v/v), and recentrifuged (20,000 × *g* for 5 min). The supernatant was mixed with 0.6 volume of isopropanol for 15 min then centrifuged (20,000 × *g* for 20 min) to pellet the DNA. The extracted DNA was washed twice with 70% ethanol, then resuspended in 60 μl distilled H_2O, and stored at −20°C.

PCR amplification of DNA was performed by using a Tgradient thermocycler (Tamar Laboratory Supplies, Israel). The purified DNA from plant and insect samples was passed through one 30-cycle PCR reaction in the presence of universal primers defined as rU3 and fU5 (Lorenz et al., 1995). Amplification was carried out in a total of 20 μl; 0.1 μl (2.5 units μl^{-1}) *Taq* polymerase (Promega), 16.5 μl amplification buffer (supplied with *Taq* polymerase, and dNTPS), and 5–10 ng test DNA. The PCR reaction began a 5-min 94°C heat shock step followed by a 2-min step at 50°C, a 2-min step at 72°C, 35 cycles of 92°C (30 sec), 55°C (30 sec), 72°C (45 sec). In the last cycle, the 72°C step was extended for 7 min as an elongation step. The amplified PCR products were analyzed by electrophoresis of 12 μl of reaction mixture in 1.5% agarose gel (40 mM Tris–HCl, pH 7.5; 20 mM acetic acid; 1 mM EDTA), stained with ethidium bromide, and visualized with a UV transilluminator. The size standard 100-bp DNA ladder used in gels was obtained from MBI Fermentas.

Statistical Analysis. Comparisons between the numbers of planthoppers caught from the different plant species were analyzed by one-way analysis of variance (ANOVA), and means were separated by the Tukey HSD multiple range test using JMP 5.1 software (SAS software). Results of the olfactometer tests were analyzed by the log-likelihood ratio (*G*-statistic). Kruskal–Wallis test was used to compare the EAG responses to the headspace preparations and the control. Medians were separated by the Median-Notch method. All tests were conducted at $\alpha = 0.05$ level.

RESULTS

Native Host Plants. In the first year, more specimens of *H. obsoletus* were present on *V. agnus-castus* than or any other plant species (df 7, 118, $F = 153.86$, $P < 0.001$) (Table 1). *H. obsoletus* were found on *M. communis* only in autumn.

TABLE 1. SURVEY OF THE NUMBER OF *Hyalesthes obsoletus* FOUND ON NATIVE PLANTS IN 2003

Species	Samplings	Total *H. obsoletus*
Amaranthus retroflexus	13	4 b*
Convolvulus arvensis	18	2 b
Inula viscose	12	0 b
Myrtus communis	15	15 b
Salix alba	12	0 b
Tamarix sp.	12	0 b
Vitex agnus-castus	18	549 a
Vitis vinifera	26	13 b

*Data followed by different letters are significantly different at $\alpha = 0.05$.

Repeated sampling of four wild *V. agnus-castus* shrubs in June yielded 218 *H. obsoletus* individuals with a male:female ratio of 58:42. Vacuuming the same four shrubs in October yielded 564 specimens, with a male:female ratio of 46:54. In spring of the second year, more specimens of *H. obsoletus* were present on *V. agnus-castus*, followed by olives, than for any other plant species ($df = 9, 51$, $F = 87.47$, $P < 0.001$) (Table 2). In autumn, significantly more specimens of *H. obsoletus* were present on *V. agnus-castus* than on all other plants ($df = 10, 210$, $F = 34.04$, $P < 0.001$) (Table 2). *H. obsoletus* were found on olive trees in spring, and only until mid-October in autumn.

TABLE 2. SURVEY OF THE NUMBER OF *Hyalesthes obsoletus* FOUND ON NATIVE PLANTS IN 2004

	Spring		Autumn	
Plant species	Samplings	Total *H. obsoletus**	Samplings	Total *H. obsoletus*
Amaranthus retroflexus	6	2 c	24	1 C
Convolvulus arvensis	6	1 c	18	5 C
Inula viscose	6	0 c	24	0 C
Myrtus communis	6	0 c	24	13 C
Olea europaea 1 yr	4	39 b	3	2 C
Olea europaea 3 yr	3	29 b	15	56 C
Salix alba	6	0 c	24	0 C
Tamarix sp.	6	0 c	24	0 C
Vitex agnus-castus South	9	294 a	22	728 A
Vitex agnus-castus North			21	156 B
Vitis vinifera	9	5 c	18	4 C

*Data followed by different letters are significantly different at $\alpha = 0.05$.

TABLE 3. OLFACTOMETER RESULTS FOR MALE AND FEMALE *Hyalesthes obsoletus* GIVEN A CHOICE BETWEEN *Vitex agnus-castus* (*V. A.-C.*) AND *Convolvulus arvensis* (*C. A.*) OR *Vitex agnus-castus* AND *Vitis vinifera* (*V. V.*)

Sex	Choice	*df*	G-statistic	*P*
Male	*V. a.-c.* × *C. a.*	2	10.66	<0.005
Female	*V. a.-c.* × *C. a.*	2	8.46	<0.025
Male	*V. a.-c.* × *V. v.*	2	22.32	<0.001
Female	*V. a.-c.* × *V. v.*	2	61.76	<0.001

Since *H. obsoletus* was present in large numbers on *V. agnus-castus*, further analysis was possible. Significantly fewer *H. obsoletus* were found on *V. agnus-castus* in the northern Golan (El Rom), and only until the end of October, whereas in the south-center of the Golan, they were abundant until the end of November. In October 2004, the monthly average low temperature in the north was 14.0°C, whereas in the center-south it was 16.5°C. By November, the average low temperature was 7.5°C in the north and 11.3°C in the center-south.

Olfactometer and Electroantennogram Trials. In an olfactometer assay, both sexes of *H. obsoletus* significantly preferred *V. agnus-castus* over grape vines (*V. vinifera*) and *C. arvensis* (Table 3), although the number of females choosing *V. agnus-castus* over grape vines was higher than that of males. Conversely, more males chose *V. agnus-castus* over *C. arvensis*. There was no rootstock effect.

Antennal response of adults (Table 4) was significantly higher to *V. agnus-castus* volatiles than to grape vines (Cabernet Sauvignon) or the blank control ($df = 3, 20$, $F = 9.58$, $P < 0.001$). The antennal response to the grape vine was not different from the response to the blank control. There was no rootstock effect.

TABLE 4. RESULTS OF DETACHED *Hyalesthes obsoletus* ANTENNAE STIMULATED BY VOLATILES ISOLATED FROM *Vitex agnus-castus* AND CABERNET SAUVIGNON VARIETY OF *Vitis vinifera* ON TWO DIFFERENT ROOT STOCKS (CASTEL 216 AND RICHTER 110)

Sample	Number of replicates	Mean (mV) ± standard deviation
Blank	6	390 ± 122 b*
Vitex agnus-castus	6	695 ± 125 a
Vitis vinifera (Richter)	6	455 ± 71 b
Vitis vinifera (Castel)	6	498 ± 89 b

*Data followed by different letters are significantly different at $\alpha = 0.05$.

TABLE 5. STICKY TRAP CATCHES OF *Hyalesthes obsoletus* IN EMERGENCE CAGES, 2004

No. *Vitex agnus-castus*	Capture technique	No. *Hyalesthes obsoletus*												
		May 14	May 31	June 11	June 20	September 1	September 8	September 23	October 1	October 12	October 24	November 5	November 8	November 15
15	Cage	14	13	2	0	4	3	7	3	0	16	8	0	1
2	Drape	37	0	0	0									

H. obsoletus Development on V. agnus-castus. H. obsoletus were caught on sticky traps in emergence boxes placed under *V. agnus-castus* in the field. In the first year (2003) a total of eight adults were caught in May; in autumn, 10 adults were caught from the beginning of September until the middle of October. In the second year (2004), more cages were set up and emergence was monitored in spring and autumn (Table 5). In spring, adults emerged for a maximum of 6 wk, whereas in autumn they emerged over a period of almost 3 mo. In the spring, approximately half the area under each of two shrubs was covered with net; because the entire emergence area was not covered, few planthoppers were caught. Assuming a nonclustered distribution for this species, an estimated mean number of 37 (2 × number captured from 1/2 of each shrub) adult *H. obsoletus* emerged from each shrub. In May, we found 25 third to fifth instar *H. obsoletus* nymphs on the caged *V. agnus-castus* that were exposed to mated females in September. No *H. obsoletus* nymphs were found on any of the 50 + *C. arvensis* plants that were uprooted in spring of 2003 and 2004.

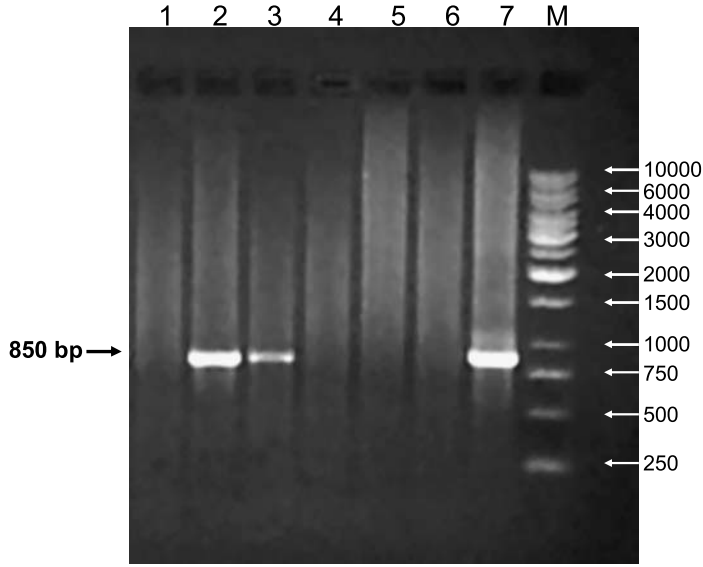

FIG. 1. Photograph of 1.5% agarose gel stained with ethidium bromide, showing amplified products from universal primers rU3 and fU5 for carrots and leafhoppers. Lane: M. molecular markers (in addition to those indicated, 8000, 5000, 4000, 3500, 2500 bp are also present), 1–3 *Hyalesthes obsoletus*, 4–5 *Vitex agnus-castus*, 6 healthy *Vinca minor*, 7 Stolbur-infected *Vinca minor*.

V. agnus-castus Infection with Phytoplasma. V. agnus-castus plants in the field were examined for signs and symptoms of phytoplasma infection. Although no symptoms were observed throughout the entire season, leaf and stem samples were collected from each plant in October and were analyzed by PCR for presence of phytoplasma DNA. Results of these analyses were negative for all 40 *V. agnus-castus* samples and healthy periwinkle, and were positive for phytoplasma-infected periwinkle (Figure 1). Of the *H. obsoletus* collected from these plants, 12.24% of the females (6 of 49 individuals) and 1.92% of the males (2 of 52 individuals) tested positive in 2003, and 17.78% of the females (8 of 45 individuals) and 7.5% of the males (3 of 40 individuals) tested positive in 2004.

DISCUSSION

This is the first study identifying *V. agnus-castus* as the preferred developmental host plant of adult *H. obsoletus* as observed, by vacuum sampling, on *A. retroflexus*, *C. arvensis*, *M. communis*, *O. europaea*, and *V. agnus-castus*. Other than *C. arvensis*, these are native plants that remain green throughout the year. This is consistent with findings of other researchers in Europe (Hoch and Remane, 1986; Maixner et al., 1995; Sforza et al., 1998). In central and eastern Europe, *C. arvensis* is the primary host for the planthopper (Brcak, 1979); however, in Israel aerial portions of this plant are completely dried by May when the first generation of *H. obsoletus* appears, except in irrigated areas where it is usually controlled along with other weeds.

C. arvensis does not appear in the field until after the first rains, usually in November–December, when *H. obsoletus* are usually no longer found (Orenstein et al., 2003). Since the weed and the planthopper are temporally different, it was not surprising that examination of the roots of more than 100 *C. arvensis* yielded no *H. obsoletus* nymphs. Furthermore, based on olfactometer and electroantennagram bioassays with *C. arvensis* and *V. agnus-castus*, the planthopper responded differently to host plant volatiles; response was higher to *V. agnus-castus*. Our results indicate that *V. agnus-castus* is a viable developmental host for the planthopper. When adult *H. obsoletus* were contained in a cage with young potted *V. agnus-castus*, nymphs developed in the same length of time as planthopper development in the field. Differences in the numbers of *H. obsoletus* captured on the shrub in the northern (fewer individuals) vs. central-southern Golan are consistent with previous findings on the distribution of the planthopper in vineyards (Orenstein et al., 2003) and with the distribution of phytoplasma (less incidence in the north) (Orenstein et al., 2001).

Having determined that *V. agnus-castus* is a preferred host plant and supports the complete development of *H. obsoletus*, it was necessary to determine

if the shrub would serve as a reservoir for the pathogen. To date, no signs or symptoms of yellows disease have been observed in *V. agnus-castus* or other wild plants examined in this study. Phytoplasmas can be detected in plants that do not exhibit symptoms of infection, and the part of the plant assayed can be critical to detection (Constable et al., 2003). Asymptomatic plants may be resistant or tolerant to phytoplasma infection. Fourteen percent to 25% of the *H. obsoletus* adults tested by PCR were positive for phytoplasma. Sforza et al. (1998) found between ~28% and 39% of *H. obsoletus* in France were infected with phytoplasma. Since there is a latent period between insect feeding on an infected plant and its capability to transmit the phytoplasma, a positive PCR result does not necessarily mean that the specific insect is capable of transmitting the phytoplasma. However, since these insects were collected in October, at the end of the second peak of *H. obsoletus* activity (Klein et al., 2001; Orenstein et al., 2003), it is reasonable to assume that some were capable of transmitting phytoplasma.

The attraction of *H. obsoletus* to *V. agnus-castus*, as well as the apparent lack of phytoplasma infection in this plant species, suggests that there is potential for using *V. agnus-castus* as a trap plant near vineyards to direct them away from grapevines. Identifying the volatile plant attractants and incorporating them in traps may be effective in protecting crops from inoculation by *H. obsoletus*. We are currently working on identifying the dominant components of the attractant compounds.

Acknowledgments—The authors thank LeAnn Beanland for critical comments on this manuscript. This research was supported in part by a grant from the Chief Scientist, Ministry of Agriculture, State of Israel and the Wine-Grape Growers Board.

REFERENCES

BRCAK, J. 1979. Leafhopper and planthopper vectors of plant disease agents in central and southern Europe, pp. 97–154, *in* K. Maramorosch and K. F. Harris (eds.). Leafhopper Vectors and Plant Disease Agents. Academic Press, USA.

CONSTABLE, F. E., GIBB, K. S., and SYMONS, R. H. 2003. Seasonal distribution of phytoplasmas in Australian grapevines. *Plant Pathol.* 52:267–276.

COUSIN, M. T. and MOREAU, J. P. 1991. Yellow decline of Lavandin (Hybrid *L. Officinalis* × *L. Latifolia*): A MLO (mycoplasma-like organism) disease. Symptoms, experimental transmission, ultrastuctural studies, role of leafhoppers, methods of control, pp. 59–62, *in* S. P. Raychaudhuri (ed). Recent Advances in Medicinal, Aromatic and Spice Crops, Vol. 1. Today and Tomorrow's Printers and Publishers, New Delhi, India.

HOCH, H. and REMANE, R. 1986. Evolution und speziation der zikaden-gattung *Hyalesthes* Signoret, 1865 (Homoptera Auchenorrhyncha Fulgoroidea Cixiidae). *Marbg. Entomol. Publ.* 2:1–427.

HOY, C. W., HEADY, S. E., and KOCH, T. A. 1992. Species composition, phenology and possible origins of leafhoppers (Cicadellidae) in Ohio vegetable crops. *J. Econ. Entomol.* 85:2336–2343.

KLEIN, M., WEINTRAUB, P. G., DAVIDOVICH, M., KUZNETSOVA, L., ZAHAVI, T., ASHANOVA, A., ORENSTEIN, S., and TANNE, E. 2001. Monitoring phytoplasma-bearing leafhoppers/planthoppers in vineyards in the Golan Heights, Israel. *J. Appl. Entomol.* 125:19–23.

LORENZ, K. H., SCHNEIDER, B., AHRENS, U., and SEEMÜLLER, E. 1995. Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and non-ribosomal DNA. *Phytopathology* 85:771–776.

MAIXNER, M. 1994. Transmission of German grapevine yellows (Vergilbungskrankheit) by the planthopper *Hyalesthes obsoletus* (Auchenorrhyncha: Cixiidae). *Vitis* 33:103–104.

MAIXNER, M., AHRENS, U., and SEEMULLER, E. 1995. Detection of the German grapevine yellows (Vergilbungskrankheit) MLO in grapevine, alternative hosts and a vector by a specific PCR procedure. *Eur. J. Plant Pathol.* 101:241–250.

MOREAU, J. P. and LECLANT, F. 1973. Contribution to the study of two lavandin insects *Hyalesthes obsoletus* Sign. and *Cechenotettix martini* Leth. (Homoptera Auchenorrhyncha). *Ann. Zool. Ecol. Ani.* 5:361–364.

ORENSTEIN, S., ZAHAVI, T., and WEINTRAUB, P. G. 2001. Distribution of phytoplasma in grapevines in the Golan Heights, Israel, and development of a new universal primer. *Vitis* 40:219–223.

ORENSTEIN, S., ZAHAVI, T., NESTEL, D., SHARON, R., BARKALIFA, M., and WEINTRAUB, P. G. 2003. Spatial dispersion patterns of potential leafhopper and planthopper (Homoptera) vectors of phytoplasma, and their associated phytoplasmas, in wine vineyards. *Ann. Appl. Biol.* 142:341–348.

SFORZA, R., CLAIR, D., DAIRE, X., LARRUE, J., and BOUDON-PADIEU, E. 1998. The role of *Hyalesthes obsoletus* (Hemiptera: Cixiidae) in the occurrence of Bois Noir of grapevines in France. *J. Phytopathol.* 146:549–556.

SFORZA, R., BOURGOIN, T., WILSON, S. W., and BOUDON-PADIEW, E. 1999. Field observations, laboratory rearing and descriptions of immatures of the planthopper *Hyalesthes obsoletus* (Hemiptera: Cixiidae). *Eur. J. Entomol.* 96:409–418.

WEBER, A., MAIXNER, and M., REINERT, W. 1997. Monitoring of field populations of the vector *Hyalesthes obsoletus* for infestation with 'Vergilbungskrankheit'. *Proc. of the 12th ICVG Meeting*, pp. 67–68.