Phytopathologia Mediterranea (2016) 55, 3, 337–345
DOI: 10.14601/Phytopathol_Mediterr-17682

RESEARCH PAPERS

Xylella fastidiosa from almond in Iran: overwinter recovery and effects of antibiotics

Naser AMANIFAR[1], Mohsen TAGHAVI[2] and Mohammad SALEHI[3]

[1-] Department of Plant Protection Research, Charmahal va Bakhtiary Agricultural and Natural Resources Research and Education Center, Shahrekord, Iran
[2-] Department of Plant Protection, College of Agriculture, Shiraz University, Shiraz, Iran
[3-] Department of Plant Protection Research, Fars Agricultural and Natural Resources Research and Education Center, Shiraz, Iran

Summary. Almond leaf scorch disease (ALSD), caused by *Xylella fastidiosa,* has been reported from some regions of Iran. Biological traits of isolates the pathogen from almond were investigated in pot and orchard conditions. ALSD killed trees of susceptible cultivars during 3 to 4 years outdoors in pots, but overwintered in root tissues in orchards with winter temperatures below -15°C. *Xylella fastidiosa* was not detected by culturing or DAS-enzyme-linked immunosorbent assay (DAS-ELISA) in almond leaves until early summer and peaked in early autumn. However, root samples taken in winter (January and February) and early spring (April) reacted positively in DAS-ELISA, culture media and polymerase chain reaction assays. This demonstrates that *X. fastidiosa* survives in root tissues of almond trees under orchard conditions in very cold (-28°C) winters. Trunk injection of oxytetracycline into leaf scorched almond trees reduced symptoms of the disease, while penicillin applications also reduced symptoms but to a lesser degree.

Key words: leaf scorch, Pierce's disease, biological traits.

Introduction

Xylella fastidiosa Wells *et al.* is a xylem-limited bacterium which causes almond leaf scorch disease (ALSD), Pierce's disease (PD) of grape and numerous other plant diseases (Hopkins and Thompson, 1984). Different strains of *X. fastidiosa* vary in their abilities to cause disease in a wide variety of cultivated and ornamental plants, including almond, grape, alfalfa and oleander (Hopkins and Purcell 2002; Sisterson *et al.*, 2010).

ALSD is a serious disease threatening almond production in several areas of the world (Chen *et al.*, 2007). Sisterson *et al.* (2008) monitored yields of ALSD-affected trees over a 3 year period, and found that ALSD-affected trees had reduced yields, and most did not die during the study period. Extension of yield evaluations from 3 to 5 years demonstrated consistent yield loss due to ALSD over that period. ALSD-affected trees had yields reduced by 20% for the cultivar 'Nonpareil'and 40% for 'Sonora', compared with unaffected trees (Sisterson *et al.*, 2010).

ALSD could be caused by *X. fastidiosa* isolates in two distinct genotype groups: isolates from one group could cause PD and the other could not (Almeida and Purcell, 2003; Chen *et al.*, 2007). Isolates from two genetically and pathologically distinct groups of *X. fastidiosa* strains can co-exist simultaneously in the same infected almond orchard (Hendson *et al.*, 2001; Almeida and Purcell 2003; Chen *et al.*, 2007).

Diseases incited by *X. fastidiosa* occur mainly in tropical/subtropical areas, although leaf scorch diseases also occur in much colder climates, e.g. oak leaf scorch occurs as far north as Canada (Goodwin and Zhang, 1997). *Xylella fastidiosa* is an important pathogen in areas with mild winter climates. A colder envi-

Corresponding author: N. Amanifar
E-mail: sahragardn@yahoo.com

ronment limits the survival of *X. fastidiosa*-induced PD (Purcell, 1980b), and sub-freezing winter conditions have been shown to reduce *X. fastidiosa* populations in potted grapes (Feil and Purcell, 2001). Cold winters have been shown to cure PD in potted grapes, and increased rates of recovery from infection occurred in vines exposed to winter conditions in areas in which temperatures were low (Purcell, 1980b). The concept has been termed 'winter curing', and is among the factors that limit secondary spread of *X. fastidiosa*-induced diseases in regions environmentally sub-optimal to *X. fastidiosa* (Ledbetter and Rogers, 2009).

Xylella fastidiosa was previously reported from symptomatic almond and grape plants in Iran, based on graft transmission, isolation on specific *X. fastidiosa* culture medium, pathogenicity tests, and positive reactions in double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) and polymerase chain reaction (PCR) (Sahragard *et al.*, 2010; Amanifar *et al.*, 2014a).

Almond is economically important and widely grown in Iran, with an estimated crop area of 223,000 ha (reviewed in Amanifar, 2014). ALSD is widespread in southwestern Iran (Amanifar *et al.*, 2014a). Leaf scorching is the most noticeable symptom of the disease during the summer and autumn, although this symptoms can be easily confused with those of other disorders such as salt toxicity. In the present study, we investigated some biological traits of *X. fastidiosa* such as its overwintering survival and seasonal changes in the detectability of the pathogen in various almond tissues in regions with very cold winters (specify region in Iran), and also in a region with warmer winters (southwestern Iran). A preliminary report of this study has been published (Amanifar *et al.*, 2014b).

Materials and methods

Pathogen detection, culture and growth characteristics

Isolation

Petioles, midribs, buds, shoots and roots from almond plants exhibiting ALSD symptoms were transferred to sterile plastic bags, surface sterilized by soaking for 3 min in 1% sodium hypochlorite solution and 4 min in 70% ethanol, and rinsed three times each for 3 min in sterile distilled water. Using a sterile scalpel, a longitudinal incision was made in each tissue which was then transferred to a tube containing 10 mL of PD3 broth. After 2 d incubation at 28°C, 50 µL of the liquid was streaked onto culture media PD3 (Davis *et al.*, 1980), PW (Schaad *et al.*, 2001) and nutrient agar (Schaad *et al.*, 2001). The plates were incubated at 28°C for 6 weeks, and examined with a stereomicroscope at weekly intervals for the presence of *Xylella*-like colonies (Wells *et al.*, 1987; Chen *et al.*, 2007). From 2 weeks after plating on PD3 and PW media, small white colonies if found, were serially re-streaked on solid PD3 medium for three subcultures to ensure the purity of the strains. Bacterial growth characteristics, survival in the culture medium, and the appearance of colonies on solid media based on the size and shape of the colonies were recorded. Gram staining, catalase and oxidase tests used standard methods for bacteria as described by Schaad *et al.* (2001).

DAS-ELISA

DAS-ELISA was used to detect *X. fastidiosa* in symptomatic tissues from almond. One gram of petiole, midrib, bud or root tissue from each affected plant was surface sterilized by soaking for 3 min in 70% ethanol, rinsed twice each for 3 min in sterile distilled water, transferred to a plastic bag with 3 mL of extraction buffer (Agdia, Inc., Elkhart, IN, USA), and crushed with a pestle at room temperature. The sap was loaded into strips coated with *X. fastidiosa* -specific antibodies using the *PathoScreen* kit, and processed according to the manufacturer's instructions (Agdia, Inc). Samples with absorbance (at 630 nm) values above the average absorbance values of known negative samples plus three times the standard deviation were considered positive (Sutula *et al.*, 1986).

PCR

PCR reaction was carried out using the TaKaRa TaqTM (Hot Start Version,Takara Bio Inc.) in 25 µL volumes. The components for the PCR included Master Mix (2 × Premix) 12.5 µL, 0.1 µM of each primer (1 µL) and 8 µL of H_2O. Templates consisted of 300 ng extracted bacterial DNA or a small portion of the bacterial colony suspended in distilled water for whole-cell PCR. Two primer sets, RST31/RST33 (Minsavage *et al.* 1994) and Dixon454fa/Dixon1261rg (Chen *et al.* 2007) were used to amplify targeted segments of the *X. fastidiosa* genome, and the resulting products were sequenced. The amplification program consisted of an initial denaturation step at 95°C for 10 min; 40 cycles of 95°C for 45 s, 55°C for 30 s, and 72°C for

30 s; and a final elongation step of 72°C for 10 min. PCR products were separated by electrophoresis on 1% agarose gels run at 5 V cm^{-1} for 1 h, stained with ethidium bromide (10μg L^{-1}) for 10 min, and visualized over UV light. A 100 bp DNA ladder was used as a molecular weight/size calibration in the gels. PCR products were sequenced by Macrogen.

Almond leaf scorch disease progress in the orchard

In August 2011, two almond orchards (local cultivar Mamaee) were selected based on the previously known history of ALSD in two regions. One of these experiences very cold winters, along the Zayanderood River (in 25 km northeast of Shahrekord City in the Chahar Mahal va Bakhtiari province), while the other has relatively mild winters, in near Ardal town (in southwest of Chahar Mahal va Bakhtiari province) in southwestern Iran. In each orchard, seven trees with varying severity of ALSD symptoms were selected and identified (numbered) with oil paint. Information was recorded for each tree, including the percentage of infected branches and severity of symptoms. Five trees without ALSD symptoms were selected as negative controls in each orchard. *Xylella fastidiosa* infection status of the trees was confirmed by DAS-ELISA. Increased severity of ALSD or possible recovery from the disease was investigated on the basis of disease symptoms and DAS-ELISA tests in both orchards during 2011-13. Time of flowering and leaf emergence, severity and type of disease symptoms and dying trees were recorded and compared with those of asymptomatic healthy trees.

Seasonal infection changes and overwinter survival of *Xylella fastidiosa* in almond

The seasonal changes of the presence of *X. fastidiosa* in shoots (leaves and buds) and roots, and overwinter survival in almond seedlings in pots and in the orchard, were studied during 2012–13 in southwestern Iran, in the region with very cold winters (along the Zayanderood River). Shoot and root tissues were sampled on a monthly basis, with sampling intervals ranging from 25 to 31 d, from March 27, 2012 to December 29, 2013.

Disease in pot conditions

Sixteen almond seedlings of cv. Mamaee were grown under greenhouse conditions in pots containing 8 L volume of soil (at 27 ± 8°C) at the Agricultural and Natural Resources Research Center of Chahar Mahal va Bakhtiari in southwestern Iran. In late September 2011, fourteen seedlings were inoculated with an isolate of *X. fastidiosa* from ALSD-affected almond. Suspensions of bacteria were prepared with estimated cell concentrations of 10^6 CFU mL^{-1} (OD600 = 0.4). A 5 μL drop of the suspension was placed on a each inoculated young stem of the test plants, and the tissue was pricked through the drop five times with a no. 0 entomological pin, on five sites per plant. The inoculated plants were then kept under greenhouse conditions (Chang and Donaldson, 2009). Symptoms on seedlings were recorded periodically every 2 weeks from the time of inoculation. At 3 months after inoculation, *X. fastidiosa* was confirmed by DAS-ELISA, culture on PD3, and PCR. Two seedlings were injected with distilled water as a experimental controls. On 5 March 2012, 12 inoculated potted almond trees were moved from the greenhouse to outdoor conditions. Two inoculated seedlings and two control seedlings remained in greenhouse. The symptom progression was recorded in both series of seedlings. Before transferring, DAS-ELISA and culture on PD3 results confirmed infection in each seedling. At the end of each month from March 2012 to December 2013, some shoots or leaves (if present) and the roots of all seedlings were sampled to confirm the status of infection with *X. fastidiosa* using DAS-ELISA and culture on PD3. The identity of bacterial colonies as *X. fastidiosa* was confirmed by PCR with primers RST31/RST33 and Dixon454fa/Dixon1261rg.

Disease in the colder region orchard

The methods of Hopkins and Thompson (1984) and Chang and Walker (1988) (with slight modifications, by dilution plating on PD3 and DAS-ELISA with sampling of roots and leaves if present, or shoots during the year) were used to study the seasonal infection changes of *X. fastidiosa* and the effects of winter on remission of symptoms of ALSD or the elimination of *X. fastidiosa*, under orchard conditions. One almond orchard, with 8-year-old trees (cv. Mamaee) that were confirmed previously to have ALSD, was selected along the Zayanderood River. In September 2011 ten almond trees were selected as having ALSD symptoms and also had *X. fastidiosa* infection confirmed by DAS-ELISA, culture on PD3 and PCR assays using RST31/RST33 and Dix-

on454fa/Dixon1261rg primer pairs. Detection and seasonal population changes of *X. fastidiosa* were studied as described above.

Trunk injection with antibiotics

A 10 to 12 year-old almond orchard with scorch symptoms was selected along Zayanderood River in July, 2011. Twenty four trees were marked and divided into three groups: group 1 was treated with oxytetracycline (20% w/v, = 200 mg mL^{-1} oxytetracycline in distilled water), group 2 with penicillin (10%, = 100 mg mL^{-1} penicillin in distilled water). Both antibiotics were acquired from a local veterinary drugstore. The third group trees as controls and was injected with distilled water. Trees were selected based on scorch symptoms and being positive in DAS-ELISA. Antibiotics were prepared in human serum soluble pouches (1 L capacity), with 15 mL from oxytetracycline or 30 ml penicillin per 1 L of distilled water. For every tree, 3 g of pure antibiotic was injected (Hartman *et al.*, 2010) into the trunk at a height of 20 cm from the soil surface, into a 1 cm diam. hole drilled to a depth of 7 cm. A nozzle head was wedged into the hole and the delivery rate of the antibiotic solution was adjusted to 8 h. ALSD symptoms were rated during September 2012. In addition, five trees were selected randomly from each group, and some leaves from the trees were collected randomly from different directions. A sample of 120 leaves was selected, and percentage of infection (percentage of leaves with symptoms) was determined. Severity of disease was calculated using the severity scores outlined below (Madden and Nutter, 1995). Disease incidence severity and disease indices were also calculated (see below). Data were analyzed using the SAS statistical software (SAS 2004).

Percent leaf area affected	0	1-10	11-20	21-30	31-45	46-70	>70
Severity score	0	1	2	3	4	5	6

Percent leaf infection = (number of leaves with scorched symptoms)/ 120 × 100

Disease severity = ∑(number of leaves in each class × scale)/120

Disease index = (Disease severity) × (1/6)

Weather data

Weather data were obtained from the agricultural meteorological research station of Shahrekord 10 km from the selected orchards. Air and 50 cm depth soil minimum temperature measurements every month were collected using mercury thermometers during the 22 month period of the trials. Pot soil daily temperatures were recorded each day from March 2012 to December 2013. For these measurements, a thermometer was placed each morning into a 25 cm deep hole in a pot.

Results

Biochemical and morphological characteristics of *Xylella fastidiosa* isolates

Colonies on media were "translucent-white", convex, smooth and with distinct margins. *Xylella fastidiosa* cultures survived on solid media PW and PD3 for 3 weeks after colonies appeared. After this period, subculturing of colonies did not produce bacterial growth.

Disease progress

Progression of bacterial populations, as estimated by DAS-ELISA results, and symptom severity differed according to the region and the severity of symptoms first recorded. Symptom severity decreased from year to year in some trees. Along the Zayanderood River, where the winter is very cold, ALSD symptoms decreased in three of seven trees from 2012 to 2013, but in the milder winter region, symptom severity continued to increase during the same time (Table 1). In the colder region, two trees had severe leaf scorch and leaf stunting symptoms, while in the moderate climate region one tree was affected in this manner. Fewer flowers appeared in these trees than on the other trees, and the severel affected trees died in August 2013.

Survival of *Xylella fastidiosa* in almond trees during different seasons in orchard or pot conditions

In the colder region orchard

Of the ten trees selected in September 2011 along the Zayanderood River that were positive for *X. fa-*

Table 1. Almond leaf scorch disease progressing in seven trees (cv. Mamaee), under orchard conditions along the Zayanderood River and Ardal region in southwestern Iran during 2011-13. DAS ELISA assay results for sampled plant tissues are also presented.

No. of tree	Disease symptoms in August			DAS-ELISA (+/-)		
	2011	2012	2013	2011	2012	2013
1	A slight leaf scorch on a branch	Some branches with scorch symptoms	Leaf scorch on all branches of the tree	+	+	+
	Severe leaf scorch	Severe leaf scorch	Severe leaf scorch	+	+	+
2	Leaf scorch on all branches of the tree	Symptoms decrease in comparison with 2011	Symptomless	+	-	-
	Severe leaf scorch	Severe leaf scorch and leaf stunting	Severe leaf stunting and death tree	+	+	+
3	A slight leaf scorch on a branch	Some branches with scorch symptoms	Severe leaf scorch	+	+	+
	Leaf scorch on all branches of the tree	Leaf stunting and leaf scorch	Severe leaf scorch and leaf stunting	+	+	+
4	Severe leaf scorch	Severe leaf scorch	Severe leaf scorch and leaf stunting	+	+	+
	A slight leaf scorch on a branch	Some branches with scorch symptoms	Severe leaf scorch	+	+	+
5	Leaf scorch on all branches of the tree	Symptoms decrease in comparison with 2011	Symptomless	+	-	-
	Leaf scorch on all branches of the tree	Severe leaf scorch	Severe leaf scorch and leaf stunting	+	+	+
6	Severe leaf scorch	Severe leaf scorch and leaf stunting	Severe leaf stunting and death tree	+	+	+
	A slight leaf scorch on a branch	Some branches with scorch symptoms	A faint leaf scorch	+	-	+
7	Leaf scorch on all branches of the tree	Symptoms decrease in comparison with 2011	Symptomless	+	-	-
	Leaf scorch on all branches of the tree	Severe leaf scorch and leaf stunting	Severe leaf stunting and death tree	+	+	-

stidiosa by DAS-ELISA, only three trees tested positive in sampled leaves in May 2012 (Figure 1).

The percentages of trees that tested positive increased in both years, with maximum frequencies of isolation on PD3 and detection by DAS-ELISA from August until September (Figure 1). Severe symptoms of ALSD were observed in November in both years. In 2013, disease symptoms decreased in three trees compared to 2012, and the number of trees that tested positive for *X. fastidiosa* by DAS-ELISA also decreased.

The detection of *X. fastidiosa* in roots were consistently less than from leaves or shoots from the same trees. Sampling from roots of trees that were previously positive detected *X. fastidiosa* in only about half of the trees sampled in January and February 2012 and December 2013 (Figure 2). In both years in summer, very few of the samples of roots revealed infection by *X. fastidiosa*. The minimum frequency of isolation and detection by DAS-ELISA from roots was from samples taken in July and August, while samples of leaf had the most infections at this time.

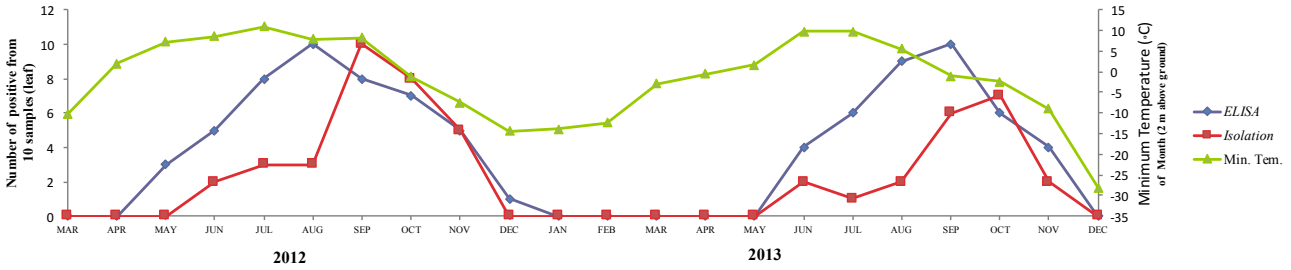

Figure 1. Detection of *Xylella fastidiosa* in leaves and shoots of almond trees in orchards during 2012-13.

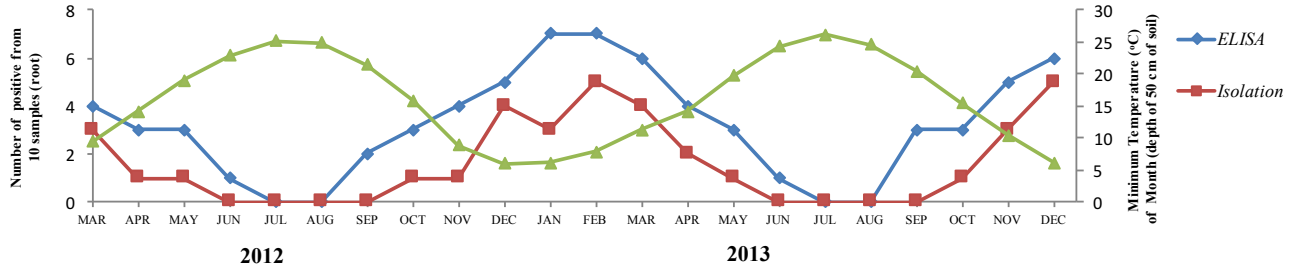

Figure 2. Detection of *Xylella fastidiosa* in roots of almond trees in orchards during 2012-13.

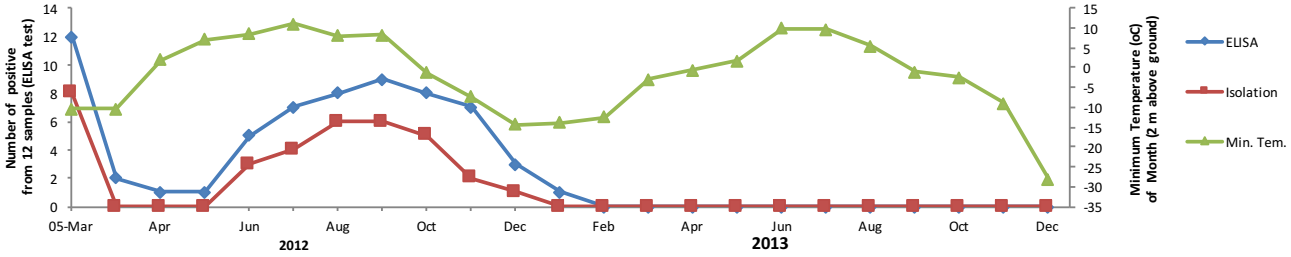

Figure 3. Detection of *Xylella fastidiosa* in leaves and shoots of almond trees in outdoor pots during 2012-13.

In potted plants

All 12 plants kept under greenhouse conditions and that had been inoculated about 3 months previously were positive by DAS-ELISA. About a month after the transfer of pots to the outside of the greenhouse in early spring, only two seedlings had detectable *X. fastidiosa* by DAS-ELISA on 27 March 2012 (Figure 3). Soil temperatures within the pots during the coldest nights were -20°C**.** In March 2012, about a week after transferring potted plants from the greenhouse, air temperatures dropped to -10°C for two nights, causing new leaves to drop. New leaves grew back. On 27 March, *X. fastidiosa* was detected from 33% of root samples (Figure 4), but not from any samples of leaves and buds (Figure 3). After the winter of 2012, *X. fastidiosa* were not detectable by DAS-ELISA or culture on PD3 from any leaves or roots. The lack of ALSD symptoms in these almond seedlings also

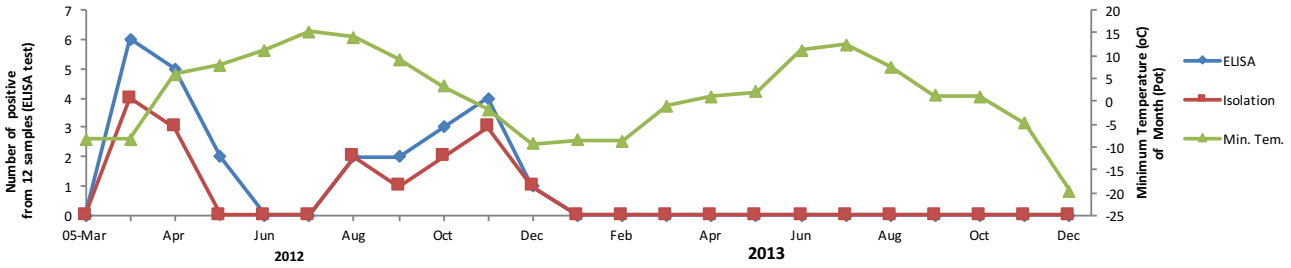

Figure 4. Detection of *Xylella fastidiosa* in roots of almond trees in outdoor pots during 2012-13.

confirmed that the plants had completely recovered during the winter. *Xylella fastidiosa* continued to be detected in both positive control plants kept in the greenhouse during winter 2012.

Trunk injection of almond trees with antibiotics

Treatment of almond trees reduced subsequent ALSD symptoms. Oxytetracycline-treated trees averaged 73% less disease severity ratings than the untreated control trees ($P \leq 0.01$: Table 2). On three of eight trees treated with oxytetracycline, symptoms of ALSD in 2012 were more severe than the previous year, which may have been caused by poor uptake of the antibiotic in the ALSD affected trees. Penicillin-treated trees had a 19.5% reduction in average symptom severity rating ($P \leq 0.01$).

Discussion

Biological traits and overwintering habit of *X. fastidiosa* have been considered in similar studies of grape, almond, sweet orange, red oak and sycamore infected with this bacterium (Chang and Walker, 1988; Almeida and Purcell, 2003; Henneberger *et al.*, 2004). In the present study, we investigated some biological traits of *X. fastidiosa* on almond under orchard and pot conditions in southwestern Iran. *Xylella fastidiosa* killed almond trees both under field and greenhouse conditions. Curing and recovery of ALSD in regions with very cold winters has sometimes been observed, especially in orchards that have been appropriately managed with fertilizing and watering. The incidence and rate of spread of ALSD varies, and is possibly related to almond cultivar and management of irrigation and nutrition. In orchards with poor nutrition and water stress, symptom severity increased rapidly, causing tree death in less than 4 years (Amanifar, unpublished data).

Cold winters and temperatures less than -5°C were hypothesized as important factors limiting the geographical spread of *X. fastidiosa* in sycamore and grape (Henneberger *et al.*, 2004). Our results suggest that the temperatures of the shoots rather the roots may be most relevant to curing plants of infection by *X. fastidiosa*. Based on weather data, minimum temperatures recorded at a height of 2 m above the ground in the winters of 2012 and 2013

Table 2. Mean leaf infectivity percent, disease severity, disease index and amount of *Xylella fastidiosa* bacteria (based on absorbance at 630 nm in DAS-ELISA) on almond trees with natural infections to the pathogen treated with antibiotics.

Treatment	Infectivity percent	Disease severity	Disease index	DAS-ELISA
Oxytetracycline	26.8 b	1.1 c	0.19 c	0.41 b
Penicillin	58.5 a	3.3 b	0.56 b	0.81 a
Control	63.5 a	4.1 a	0.70 a	1.25 a

* Means followed by the same letter are not significantly different ($P \leq 0.01$), Tukey test.

were much less than tolerable temperatures for *X. fastidiosa* cells, but the soil temperature at a depth of 50 cm the coldest day was at least 6°C, about 34°C warmer than at 2 m above the ground. The survival of *X. fastidiosa* in roots in the above freezing temperatures in soil may explain the lack of recovery in some almond trees we monitored in this study. Reducing potential root pressure in winter may facilitate the movement of *X. fastidiosa* cells to root xylem (Chatterjee *et al*., 2008). Soil temperature is also higher than the minimum threshold required for survival of *X. fastidiosa* cells.

Temperature fluctuations in soil were less than air temperatures. For example, on 8 January 2012 maximum and minimum air temperatures were 15.7 and -13.8°C, while the equivalent soil temperatures were, respectively, 9.3 and 6.1°C. The minimum temperature every 2 years from December until February and was about 6°C, a temperature which can be tolerated by *X. fastidiosa* (Chanway, 1997, Feil and Purcell, 2001, Henneberger *et al.*, 2004). In January of 2012 and 2013, air temperatures were colder: -13.8°C to -18.2°C. The temperature difference between air temperature at height of 2 m and at soil depth of 50 cm on the coldest nights was about 34°C.

During this study, death of almond trees infected by *X. fastidiosa* was observed both under field and greenhouse conditions. In California, the pathogen did not kill many almond trees (Sisterson *et al.*, 2008). This difference from our results could be due to the pathogen strain, susceptibility of cultivar, climate, or other unknown factors.

The severity of winter climate limits the range of *X. fastidiosa*-caused diseases such as PD, which is not reported from very cold winter climate regions and is most severe where winters are mild (Purcell, 1980a). A similar geographic distribution of the pathogen suggest that the same is true for the pathogen in sycamore (Henneberger *et al.*, 2004). Our data show that overwinter curing also occurs for ALSD in almond. Experimental freezing treatments and experiments (Purcell, 1980b) with overwintering PD vines in a variety of locations confirm that freezing temperatures can be therapeutic for PD in grape. Analysis of these data can provide estimates of how much recovery from disease occurs as a function of winter temperatures (reviewed in Purcell, 2013). Previous researchers have studied the threshold of tolerable temperature for *X. fastidiosa* in pot conditions (Feil and Purcell, 2001; Henneberger *et al*., 2004). However, the volume of soil in pots does not provide as much thermal insulation to roots as soil in field situations. Soil temperatures in pots decrease much more rapidly and fluctuate over a narrower range than in orchard soil. In other words, greater temperature fluctuations occur in pots than in field soil. We propose that these differences enable overwintering *X. fastidiosa* to survive in regions with very cold winters. This hypothesis is supported by other studies of temperature-dependent growth and survival of *X. fastidiosa,* both *in vitro* and in orchards (Feil and Purcell, 2001; Henneberger *et al*., 2004).

The treatments of trunk injection of almond trees with antibiotics applied in this study gave decreased ALSD symptom ratings of 73% in trees treated with oxytetracycline, but only about 20% from penicillin. Some *X. fastidiosa* strains from almond are known to be resistant to penicillin (Schaad *et al*., 2004).

Successful isolation on culture medium, in some cases, and detection by DAS-ELISA during the coldest times of the year in both years of our study provide evidence that *X. fastidiosa* may be able to overwinter within the root xylem of infected almond trees. This suggests that cells of the pathogen may move to distal limbs in spring via the translocation streams (Chatterjee *et al*., 2008).

Antibiotics such as streptomycin sulfate and oxytetracycline are active against gram negative bacteria such as *X. fastidiosa*. These antibiotics are already used for management of bacterial diseases of many agricultural crops (Hartman *et al*., 2010). Oxytetracycline has been shown to delay bacterial leaf scorch symptoms in elm (Kostka *et al*., 1985). Therapeutic treatments do not provide a cure for trees infected with bacterial leaf scorch, but may prolong tree life (Hartman *et al*., 2010). This treatment of temporary effectiveness should be repeated annually (Hartman *et al*., 2010). In addition, human health and environmental concerns related to the widespread use of antibiotics are increasing.

Acknowledgements

We thank Dr. Alexander H. Purcell, University of California, Berkeley for his suggestions for the manuscript of this paper. This work was supported by the grant No. 42-16-87019, from Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran

Literature cited

Almeida R. P. P. and A. H. Purcell, 2003. Biological traits of *Xylella fastidiosa* strains from grapes and almonds. *Applied and Environmental Microbiology* 69, 7447–7452.

Amanifar N., 2014. Molecular and biological characterization of *Xylella fastidiosa* in Iran. PhD thesis, Shiraz University, Shiraz, Iran, 214 pp. (in Persian).

Amanifar N., M. Taghavi, K. Izadpanah and Gh. Babaei, 2014a. Isolation and Pathogenicity of *Xylella fastidiosa* from Grapevine and Almond in Iran. *Phytopathologia Mediterranea* 53, 318–327.

Amanifar N., M. Taghavi and M. Salehi, 2014b. Some of biological traits of *Xylella fastidiosa* in Iran. In: Proceedings 21th Iranian Plant Protection Congress, Uromia University, Uromia, Iran, p. 216.

Chang C.J. and J.T. Walker, 1988. Bacterial leaf scorch of northern red oak: Isolation, cultivation, and pathogenicity of a xylem-limited bacterium. *Plant Disease* 72, 730–733.

Chang C.J. and R. Donaldson, 2009. Bacterial leaf scorch, a new blueberry disease caused by *Xylella fastidiosa*. *HortScience* 44, 413–417.

Chanway C.P. 1997. Inoculation of tree roots with plant growth promoting soil bacteria: an emerging technology for reforestation. *Forest Science* 43, 99–112.

Chatterjee S., K.L. Newman and S.E. Lindow, 2008. Cell-to-cell signaling in *Xylella fastidiosa* suppresses movement and xylem vessel colonization in grape. *Molecular Plant-Microbe Interactions* 21, 1309–1315.

Chen J., R. Groves, Y. Zheng, E.L. Civerolo, M. Viveros and M. Freeman, 2007. Colony morphology of *Xylella fastidiosa* almond leaf scorch strains. *Canadian Journal of Plant Pathology* 29, 225–231.

Davis M.J., A.H. Purcell and S.V. Thomson, 1980. Isolation media for the Pierce's disease bacterium. *Phytopathology* 70, 425–429.

Feil H. and A.H. Purcell, 2001. Temperature-dependent growth and survival of *Xylella fastidiosa* in vitro and in potted grapevines. *Plant Disease* 85, 1230–1234.

Goodwin P.H. and S. Zhang, 1997. Distribution of *Xylella fastidiosa* in southern Ontario as determined by the polymerase chain reaction. *Canadian Journal of Plant Pathology* 19, 13–18.

Hartman J., E. Dixon and S. Bernick, 2010. Evaluation of therapeutic treatments to manage oak bacterial leaf scorch. *Arboriculture and Urban Forestry* 36, 140–146.

Hendson M., A.H. Pucell, D. Chen, C. Smart, M. Guilhabert and B. Kirkpatrick, 2001. Genetic diversity of Pierce's disease strains and other pathotypes of *Xylella fastidiosa*. *Applied and Environmental Microbiology* 67, 895–903.

Henneberger T.S.M., K.L. Stevenson, K.O. Britton and C.J. Chang, 2004. Distribution of *Xylella fastidiosa* in sycamore associated with low temperature and host resistance. *Plant Disease* 88, 951–958.

Hopkins D. L. and C.M. Thompson, 1984. Seasonal concentration of the Pierce's disease bacterium in cultivars Carlos and Welder muscadine grapes *Vitis rotundifolia* compared with cultivar Schuyler bunch grape *Vitis labrusca*. *Hortscience* 19, 419–420.

Hopkins D.L. and A.H. Purcell, 2002. *Xylella fastidiosa*: Cause of Pierce's disease of grapevine and other emergent diseases. *Plant Disease* 86, 1056–1066.

Kostka S.J., T.A. Tattar and J.L. Sherald. 1985. Suppression of bacterial leaf scorch in American elm through oxytetracycline microinjection. *Journal of Arboriculture* 11: 54–58.

Ledbetter C.A. and E.E. Rogers, 2009. Differential susceptibility of *Prunus* germplasm (subgenus *Amygdalus*) to a California isolate of *Xylella fastidiosa*. *HortScience* 44, 1928–1931.

Madden L.V. and F.V. Jr. Nutter, 1995. Modeling crop losses at the field scale. *Canadian Journal of Plant Pathology* 17, 124–137.

Minsavage G.V., C.M. Thompson, D.L. Hopkins, R.M. Leite and R.E. Stall, 1994. Development of a polymerase chain reaction protocol for detection of *Xylella fastidiosa* in plant tissue. *Phytopathology* 84, 456–461.

Purcell A.H. 1980a. Almond leaf scorch: leafhopper and spittlebug vectors. *Journal of Economic Entomology* 73, 834–838.

Purcell A.H. 1980b. Environmental therapy for Pierce's disease of grapevines. *Plant Disease* 64, 388–390.

Purcell A.H. 2013. Paradigms: examples from the Bacterium *Xylella fastidiosa*. *Annual Review of Phytopathology* 51: 339–356.

Sahragard N., G.H. Babaei, S. Fatahi, K. Izadpanah, M. Taghavi and M. Salehi, 2010. Etiology of almond leaf scorch in Iran. In: Proceedings 19th Iran. Plant Protection Congress, Tehran, Iran. Volume 2, p. 518.

SAS, 2004. *The SAS System for Windows 9.1*. SAS Institute Inc, Cary, NC.

Schaad N.W., J.B. Jones and W. Chun, 2001. *Laboratory Guide for Identification of Plant Pathogenic Bacteria*. APS Press, St. Paul, MN, USA.

Schaad N.W., E. Postnikova, G. Lacy, M. Fatmi and C.J. Chang, 2004. *Xylella fastidiosa* subspecies: *X. fastidiosa* subsp. *piercei*, subsp. nov., *X. fastidiosa* subsp. *multiplex* subsp. nov., and *X. fastidiosa* subsp. *pauca* subsp. nov. *Systematic and Applied Microbiology* 27, 290–300.

Sisterson M.S., J. Chen, M.A. Viveros, E.L. Civerolo, C. Ledbetter and R. L. Groves, 2008. Effects of almond leaf scorch disease on almond yield: Implications for management. *Plant Disease* 92, 409–414.

Sisterson M.S., S.R. Thammiraju, K. Lynn-Patterson, R.L. Groves and K.M. Daane, 2010. Epidemiology of diseases caused by *Xylella fastidiosa* in California: Evaluation of alfalfa as a source of vectors and inoculation. *Plant Disease* 94, 827–834.

Sutula C.L., J.M. Gillett, S.M. Morrissey and D.E. Ramsdell, 1986. Interpreting DAS-ELISA data and establishing thepositive-negative threshold. *Plant Disease* 70, 722–726.

Wells J.M., B.C. Raju, H.Y. Hung, W.G. Weisberg, L. Mandelco-Paul and D. J. Brenner, 1987. *Xylella fastidiosa* new-genus new-species Gram-negative xylem limited fastidious plant bacteria related to *Xanthomonas* spp. *International Journal of Systematic Bacteriology* 37, 136–143.

Accepted for publication: June 27, 2016
Published online: July 28, 2016