Reduction of *Xylella fastidiosa* Transmission Through Pecan Scion Wood by Hot-Water Treatment

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ABSTRACT

Sanderlin, R. S., and Melanson, R. A. 2008. Reduction of *Xylella fastidiosa* transmission through pecan scion wood by hot-water treatment. Plant Dis. 92:1124-1126.

Pecan (*Carya illinoensis*) bacterial leaf scorch disease, caused by the bacterium *Xylella fastidiosa*, causes leaf loss and reduction in yield of pecans. One of the ways that the pathogen infects newly developing trees is by graft-transmission through infected scion wood. Submersion of pecan scion wood in 46°C water for 30 min greatly reduced transmission of *X. fastidiosa* following grafting. During a 2-year study with potted rootstock grafted to either hot-water-treated or nontreated scion wood collected from limbs of 'Cape Fear' pecan infected with *X. fastidiosa*, the pathogen was detected in 21% of the trees that developed from the nontreated scion wood and 0.7% of the trees from the hot-water-treated scions. The hot-water treatment of 46°C for 30 min did not affect graft success. Likewise, scion diameter had no effect on success of grafting or on the efficacy of hot-water treatment. Similar hot-water treatments have been efficacious in elimination of *X. fastidiosa* and some other pathogens from grapevine cuttings. The use of the hot-water treatment demonstrated in this report could be helpful to individual pecan growers and nurseries that use scion wood that may be infected with *X. fastidiosa* to reduce the occurrence of pecan bacterial leaf scorch disease in new trees.

Pecan (*Carya illinoensis* (Wangenheim) K. Koch) bacterial leaf scorch (PBLs) disease, caused by *Xylella fastidiosa* Wells et al., can cause significant defoliation in susceptible cultivars and significantly reduce nut yields (10). PBLs is chronic; once a pecan tree is infected, the disease tends to occur every year. Visible symptoms include leaflets turning tan to reddish brown in color beginning at the tips and edges of the leaflets followed shortly with abscission of the individual affected leaflets. Bare rachises usually remain attached to terminals for a few days then also abscise. Symptoms can begin in late spring and increase in intensity through summer. As with other leaf scorch diseases of hardwood trees caused by *X. fastidiosa*, PBLs may occur on one limb of a tree or throughout the tree (5).

Over a 3-year study, the average kernel weight loss of nuts from infected terminals of severely diseased 'Cape Fear' trees was 16% (10). The crop production lifetime of pecan trees can be several decades, and cumulative loss of crop from trees that are infected early in their life may total hundreds of kilograms of yield loss.

Graft-transmission is one means that the pathogen can be introduced into orchards and may represent a primary means of infection of young trees because pecan cultivars are clonally propagated (9,10). A simple method for eliminating *X. fastidiosa* from scion wood could be a useful technique to reduce the rate of introduction of the pathogen into young trees.

Hot-water treatment of infected scions has been used to eliminate *X. fastidiosa*, fungal pathogens, insects, and nematodes from grape cuttings (1,2,4,6,7,13). The objective of this study was to evaluate the efficacy of hot-water treatment for elimination of *X. fastidiosa* from pecan scion wood prior to grafting. The possible effect of scion diameter on graft success and efficacy of hot-water treatment was also investigated.

MATERIALS AND METHODS

Scion wood collection and storage. In 2004, 17-year-old 'Cape Fear' pecan trees that had been identified as infected with *X. fastidiosa* by symptoms and enzyme-linked immunosorbent assay (ELISA) (Agdia, Inc., Elkhart, IN) were used as sources of scion wood. To induce growth that would be suitable for scion wood, limbs on these trees were pruned at least 1 m from their terminals in spring 2004 and again in 2005. Scion wood was cut from these trees in January 2005 and 2006. Only limbs that had been identified with PBLs during the previous summer were cut to stimulate growth that could be used for scion wood. Scions were prepared from

either 1- or 2-year-old limb growth. Limb sections without lateral bud growth were cut into approximately 15-cm lengths. Each scion contained two or more primary or secondary lateral buds. The cut ends of the scions were sealed with a beeswax and rosin mixture to reduce dehydration. The scions were grouped into bundles of 50 pieces, wrapped in moist paper towels, and stored at 4 to 7°C in plastic bags until use.

Rootstocks. The rootstocks used in this test were 'Moore' seedlings purchased from a commercial nursery as bare-rooted trees with approximately 0.6 to 1 m of central stem growth. The rootstocks were transplanted into Metro-Mix 702 plant medium (SunGro Horticulture Distribution, Inc., Bellevue, WA) in 20.9-liter pots. Trees used in 2005 were maintained in a greenhouse; those used in 2006 were maintained outside through the duration of the test.

Hot-water treatment and grafting. Scion wood used in the test was grafted onto the rootstocks in May 2005 and 2006. The scions were grafted immediately after removal from storage or subjected to the hot-water treatment procedure then immediately used for grafting. The hot-water treatment consisted of submerging the scion wood in a water bath at 46°C for 30 min. Scions were submerged by bundling five pieces with a metal rod (18 g). Ten or 20 scions were treated each time. The minimum and maximum temperature variations of the water were recorded throughout each treatment period. After 30 min, the scions were removed and immediately submerged in room temperature water (about 22°C) for 1 min to remove the heat.

The four-flap technique was used for all grafts (12). Following grafting, the trees were arranged in a completely randomized design with rootstocks grafted to nontreated and hot-water-treated scions intermixed within rows of pots. The trees used in the 2005 test were maintained in an area of 2.9 m² and the 2006 trees in an area of 20.6 m². The diameter of the scions used in the test was measured immediately following grafting.

PBLs and *X. fastidiosa* infection. The trees were observed for graft success and symptoms of PBLs throughout the foliation period in 2005 and 2006. When PBLs-like symptoms were found on individual trees, the trees were assayed for infection by ELISA using the rachises

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Accepted for publication 26 March 2008.

from three symptomatic leaves per tree. An absorbance at 640 nm at least three times greater than the absorbance of noninfected 'Cape Fear' foliage was rated as a positive reaction for *X. fastidiosa*. All trees that had not developed symptoms and assayed positive for *X. fastidiosa* during the summer were tested for infection in October by ELISA. The 2005 test trees were assayed again in October 2006, and the 2006 test trees were assayed again in October 2007.

Data analysis. *t* tests of parameter values were used to evaluate the effects of the hot-water treatment. Correlation analysis was used to compare scion diameters for the treated and nontreated scions.

RESULTS

Hot-water treatment and grafting.

The temperature of the water bath generally did not vary more than 0.5°C from 46°C during the 30 min hot-water treatment. In 2005, 62.5% of the scions subjected to the hot-water treatment prior to grafting produced successful grafts (Table 1). Eighty percent of the scions that were not treated produced successful grafts (Table 1). Graft success in 2006 was 92 and 87% for the hot-water-treated and nontreated scions, respectively (Table 1). There was no significant difference between treatments for graft success (Table 2).

There were no significant differences in the mean scion diameter used for each treatment in either year (Tables 1 and 3). Similarly, there was no correlation between scion diameter and graft success in either year (Table 3). In addition, there was no correlation between the diameter of the scions used in this test and the scion-transmission of the pathogen or the apparent elimination of the pathogen by hot-water treatment and scion size (Table 3).

PBLS and *X. fastidiosa* infection.

None of the 25 trees that grew from the hot-water-treated scions in 2005 developed symptoms of PBLS, and they all tested negative for infection by *X. fastidiosa* when assayed by ELISA in both 2005 and 2006. Two of the 24 trees produced from the nontreated scions in 2005 were symptomatic. These two and one other were positive for *X. fastidiosa* infection in 2005;

the same three trees were symptomatic and ELISA positive in 2006 (Table 1).

One of the trees that grew from the hot-water-treated scions grafted in 2006 developed PBLS and was positive for *X. fastidiosa* infection (Table 1). Another tree from a treated scion was rated as having possible symptoms but was negative in the ELISA test (Table 1). This tree did not develop symptoms in 2007 and was negative for *X. fastidiosa* in two subsequent assays. Sixteen trees from the nontreated scions were infected (Table 1).

When the 2006 test trees were assayed in October 2007, 15 of the nontreated and 17 of the hot-water-treated trees had died, apparently from maintenance neglect. Included in this group were seven of the 16 nontreated trees that were infected in 2006, and the one infected hot-water-treated tree. The remaining nine infected trees from 2006 gave a positive reaction for *X. fastidiosa* in the 2007 ELISA, and one additional nontreated tree that was negative in 2006 was positive for infection in 2007 (data not shown). Of the remaining 98 hot-water-treated trees from 2006, none assayed positive for infection.

In 2005, there was a marginal difference ($P = 0.07$) between scion wood treatment prior to grafting and the number of infected trees produced from the grafts (Table 2). No infected trees developed from the treated scions. In 2006, there was a highly significant difference ($P < 0.001$) between treatment and the number of infected trees (Table 2). Over the period of the test, 21.3% (19/89) of the trees from the nontreated scions were infected. A 21% graft-transmission rate would have pro-

duced 29 infected trees from the 140 trees produced from the hot-water-treated scions. Only one of 140 trees (0.7%) that developed from the hot-water-treated scions was infected. Thus, the hot water treatment had an apparent efficacy rate of nearly 97% in this test for elimination of the pathogen from scion wood.

DISCUSSION

Submerging scions in 46°C water for 30 min prior to grafting was effective in near complete elimination of *X. fastidiosa* from infected scion wood. During the course of the test, only one tree of the 140 trees produced from hot-water-treated scions was infected with *X. fastidiosa*. Grafting with nontreated scions resulted in infection of 21.3% of the resulting trees. The test trees were monitored for two growing seasons following grafting. There were no changes in the 2005 test trees during 2006. In 2007, only one additional nontreated 2006 tree became infected in the second year of growth, and no additional trees in the hot-water-treated group were infected. These results indicate that hot-water treatment is very effective in reducing transmission of *X. fastidiosa* through pecan scion wood.

Scion wood pieces used in this test were of typical diameter sizes used for standard pecan grafting procedures (whip, four-flap, and bark grafts). Scion diameter had no effect on graft success, the rate of graft-transmission of *X. fastidiosa*, or on the efficacy of the hot-water treatment for killing the pathogen.

Because the test trees were not isolated from insects, it is possible that the one hot-water-treated scion that produced an in-

Table 2. Treatment effects on hot-water test parameters^a

Year	Graft success	PBLS symptoms		<i>Xylella fastidiosa</i> -infected	
		2005 ^b	2006	2005	2006
2005	0.1172	0.1465	0.0705	0.0705	0.0705
		2006 ^b	2007	2006	2007
2006	0.2256	<0.0001	<0.0001	<0.0001	<0.0001

^a Probability values from *t* test comparison of trees produced from scions which received hot-water treatment and scions that were not treated. PBLS = pecan bacterial leaf scorch.

^b The trees used in 2005 were monitored through 2006, and the trees used in 2006 were monitored through 2007.

Table 1. Hot-water treatment test parameters

	No. scions used		Graft success		Mean scion diameter ^a		Trees with PBLS symptoms		No. <i>X. fastidiosa</i> -infected trees ^b	
	2005	2006	2005	2006	2005	2006	2005 ^c	2006 ^d	2005	2006
Hot-water-treated scions ^e	40	125	25	115	11.5	11.6	0	2	0	1
Nontreated scions	30	75	24	65	11.3	11.6	2	16	3	16

^a Scion diameter measured in millimeters. Diameter range in 2005, Min. = 8.0, Max. = 15.3, SD = 1.8; in 2006, Min. = 7.4, Max. = 21.6, SD = 2.2.

^b Infection was determined by enzyme-linked immunosorbent assay (ELISA).

^c The trees used in 2005 were monitored through 2006; the infected tree that did not develop symptoms in 2005 did have symptoms in 2006. There were no other changes in the 2005 trees.

^d The trees used in 2006 were monitored through 2007. One additional tree in the nontreated group was recorded as infected in 2007; no other trees in the treated group became infected and the tree that had possible pecan bacterial leaf scorch (PBLS) symptoms in 2006 did not develop symptoms in 2007 and assayed negative for *Xylella fastidiosa*.

^e Hot-water-treated scion wood was submerged in water at 46°C for 30 min prior to grafting.

Table 3. Lack of correlation of hot-water test parameters with scion diameter^a

Year	Correlation analysis	Treatment ^b	Graft success	PBLS symptoms ^c		<i>Xylella fastidiosa</i> -infected	
				2005 ^d	2006	2005	2006
2005	Correlation coefficient	0.0589	0.0590	0.0173	0.1125	0.1125	0.1125
	P value	0.6283	0.6274	0.9062	0.4415	0.4415	0.4415
	No. comparisons	70	70	49	49	49	49
2006	Correlation coefficient	0.0090	0.0829	0.0347	0.0040	0.0123	0.0187
	P value	0.8997	0.2430	0.6438	0.9620	0.8699	0.8212
	No. comparisons	200	200	180	148	180	148

^a Scion diameter was measured immediately after grafting.

^b Treatments were scion wood submerged in 46°C water for 30 min prior to grafting or no treatment of scion wood.

^c PBLS = pecan bacterial leaf scorch.

^d The trees used in 2005 were monitored through 2006, and the trees used in 2006 were monitored through 2007.

fectured tree could be the result of insect-transmission. Likewise, some of the infected trees in the nontreated scion group could also be the result of insect-transmission of the pathogen rather than scion-transmission. If insect-transmission occurred in this test, it appears to have been inconsequential relative to the interpretation of the results because only one of the trees from the treated scions was infected. The lack of additional infected trees in the treated group the following year suggests that no insect transmission occurred.

The rootstocks were not assayed for infection prior to grafting and the possibility of some pathogen transmission through the rootstock cannot be eliminated (11). Nonetheless, a survey of the nursery from which the rootstocks were purchased during the summer did not reveal rootstock with symptoms of PBLS (personal observation). As with the possibility of insect-transmission, any rootstock-transmission that occurred in this test must have been low because only one infected tree developed in the hot-water-treated scion group. In addition, none of the seedlings that grew from the failed grafts developed PBLS symptoms. ELISA screening of some of the rootstocks that grew following failed grafts did not detect any infected rootstocks.

For this study, only one temperature and exposure time was tested. This regime was chosen based on the positive results obtained with elimination of the Pierce's disease pathogen from grape cuttings (4). It may be that other temperature and time regimes would work as well for the elimination of the bacterium. However, the combination of 46°C for 30 min did not cause detectable harm to the scion wood and did not affect the success rate of the graft attempts.

A preliminary evaluation of the response of scion wood exposed to higher water temperatures for 30 min was evaluated visually. Scion wood of four cultivars (Cape Fear, Desirable, Schley, and Pointe Coupee) was treated at 46, 50, and 52°C. Heat damage was determined from longi-

tudinal cuts made beneath the outer bark up to 72 h after treatment. There were visible differences in sensitivity to hot water among the cultivars used in this test. None of the cultivars showed visible damage to the wood or cambium when submerged at 46°C for 30 min. Only minimal damage was observed on one cultivar after treatment at 50°C, but damage was common in three of the four cultivars when treated at 52°C. The scions used in these temperature exposures were not grafted to rootstocks, but it is assumed that the use of scion wood with visible discoloration to the cambium would reduce the graft success rate. Thus the use of 46°C allows for a margin of error in temperature overrun without damaging the scion wood.

Heat shock treatment of dormant grape cuttings has been shown to increase the heat tolerance of the cuttings during heat therapy (8). If dormant pecan scion wood responds in the same way to heat shock treatment, it may be possible to use a higher temperature to eradicate *X. fastidiosa* from scion wood without damage to the woody tissue.

Because of the simplicity and high level of effectiveness of the hot-water treatment procedure tested in this study for reduction of the pathogen in pecan scion wood, this technique could be very useful for growers who collect their own scion wood. It could also be a useful method for commercial nurseries whose only source of scion wood for certain cultivars is from *X. fastidiosa*-infected trees.

Heat therapy using hot water has long been recognized as a practical and effective means of freeing infected grape (*Vitis vinifera*) cuttings of *X. fastidiosa* (4), and is sometimes recommended as a means to reduce the probability of introducing exotic strains of the pathogen through grapevine movement (3). However, hot-water treatment apparently has not been tested with other hosts of *X. fastidiosa* as a potential management tool. The work reported here using pecan scion wood appears to be the first demonstration of using hot water to reduce *X. fastidiosa* transmission from a host other than grape. The use of this tech-

nique may prove efficacious in some other commercial hardwood hosts of *X. fastidiosa*, such as almonds, walnuts, peaches, and plums, that can be propagated by grafting.

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