Research Updates

Hot-water Dipping Eradicates Phylloxera from Grape Nursery Stock

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ADDITIONAL INDEX WORDS.

Daktulosphaira vitifoliae, rootstock

SUMMARY. All life stages of grape phylloxera [*Daktulosphaira vitifoliae* (Fitch) (Homoptera: Phylloxeridae)] were eradicated with a hot-water treatment (dip) of 5 minutes at 43 °C (110 °F) to warm roots, followed by a 5-minute dip at 52 °C (125 °F). Neither grafted nor nongrafted dormant grape plants were damaged by the hot-water treatment.

Grape phylloxera is the most serious insect pest of vineyards worldwide (Flaherty, 1992).

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This pest first was discovered in commercial vineyards in Oregon in 1990 (Connelly and Strik, 1991). There is no control for this pest other than replanting to vines grafted to resistant rootstocks. The easiest way to introduce grape phylloxera into a vineyard is by planting infested grafted or self-rooted nursery stock. If nurseries could eradicate phylloxera effectively without damaging planting stock, introducing phylloxera to new areas would be eliminated (Strik and Stonerod, 1995). Hot-water dipping has been used to control nematodes (Lear and Lider, 1959), crown gall (*Agrobacterium tumefaciens*) (Ophel et al., 1990), and certain life stages of phylloxera (Davidson and Nougaret, 1921; Flaherty, 1992) from nursery stock. However, depending on water temperature and length of treatment, there has been damage to grapevine cuttings (Wample et al., 1991). Our objectives were to determine methods of dipping young nongrafted and grafted grapevines (nursery stock) to eradicate existing phylloxera populations without causing plant damage.

Materials and methods

HOT WATER DIPPING OF PHYLLOXERA.

In Jan. 1994 and 1995, phylloxera life stages (eggs, nymphs, nymph-adults, adults, and hibernants) (Flaherty, 1992) were dipped in hot water (52 °C; 125 °F) to determine percent survival. Phylloxera populations were maintained on 'Pinot Noir' (*Vitis vinifera* L.) root pieces in petri dishes in laboratory incubators (20 °C) before conducting hot-water dips. The root pieces were 3 to 5 mm in diameter and 5.0 to 7.5 cm long. All life stages were present in the populations living on the root pieces used for dipping. To

obtain hibernants, young nymphs were forced into hibernation through depletion of food (no new root pieces) and by reduction of temperature (16 °C).

When populations of all life stages of phylloxera increased sufficiently, root pieces with populations were selected for hot-water dipping. A precount of each life stage was noted for each root piece. There were seven time lengths for dipping with three replicates each (total of 21 root pieces). The roots plus associated phylloxera populations were dipped for 0, 3, 5, 7, 9, 11, and 13 min at 52 °C in a hot-water bath.

Each root piece was placed on a large piece of filter paper (Whatman no. 1), folded and stapled in a teabag fashion. This package was covered by a second piece of filter paper to prevent tearing. The root piece was confined loosely within the filter paper so that the insects were not harmed, yet the they also could not escape when dipped in water. Three root pieces individually wrapped in filter paper then were bundled in cheesecloth with a piece of thread tied around the package to provide a handle for dipping.

After hot-water dipping, the outside covering of filter paper was removed and the paper inside was unfolded to expose the root piece. Root pieces and associated filter paper were placed in individual petri dishes in an incubator (20 °C). Every 7 d, the number of dead and live phylloxera in each life stage were counted. Percent survival was calculated for each treatment. Populations were maintained and checked for survival for 42 d.

In 1995, phylloxera populations were prepared and maintained as in 1994. However, based on results in 1994, a control, in which root pieces and associated phylloxera populations were dipped in water at room tempera-

Table 1. Number of nodes and percent budbreak of nongrafted vines dipped in 20 °C (68 °F) water for 10 min or hot water for 10 min [43 °C (110 °F) water for 5 min + 52 °C (125 °F) for 5 min]. The treatment effect was nonsignificant; means were averaged to show the significant rootstock effect.

Rootstock	Nodes (no.)	Budbreak (%)
5C	6.7	75
101-14	5.4	79
3309C	6.3	96
Freedom	6.1	78
Pinot noir	6.2	79
LSD _{0.05}	0.55	15.0

ture (20 °C) for 5 min, was compared to a hot-water dip for 5 min at 52 °C. There were three root pieces per replicate and 10 replicates per treatment. Percent survival for each life stage was calculated every 7 d for 42 d.

HOT-WATER DIPPING OF PLANTS.

In Winter 1994, 2-year-old dormant, self-rooted plants ('Pinot Noir' and the rootstocks 3309C, 5C, 101-14, and Freedom) growing in 1-gallon containers were used for the hot-water dipping study.

Just before dipping, plants were removed from pots, and roots were washed free of all soil. Roots were pruned to ≈15 cm long and shoots to six to seven buds. One plant of each cultivar was tied into a bundle (replicate) with 10 replicates per treatment.

Hot-water treated plants were dipped for 5 min at 43 °C to preheat roots then in 52 °C water for 5 min. Preheating roots aides in maintaining a constant temperature (Goussard, 1977). The control plants were dipped in water at 20 °C for 10 min. All plants then were dipped in cold water (14 °C) for 2 min. To evaluate damage, plants were repotted and placed in a greenhouse. After budbreak, the numbers of nodes and primary, secondary, and tertiary shoots were counted to evaluate treatment effects.

In 1995, the response of bare-root grafted nursery vines to the hot-water dipping was evaluated using the same procedure as described for 1994. The rootstock-scion combinations used were 101-14-'Pinot Noir' clone Pommard, 3309C-'Chardonnay' clone Draper, and *Riparia gloire*-'Chardonnay' clone 76. One plant of each was bundled together; there were 10 replicates per dipping treatment.

After dipping, plants were set in flats of sawdust and placed in a greenhouse. To evaluate treatment effects, nodes and shoots were counted on each vine. The graft union of each plant then was sliced longitudinally and was evaluated for discoloration of pith and cambium tissues using a rating system from 1 to 4 (pith, 1 = cream or light green, 2 = tan, 3 = light brown, and 4 = dark brown or black; cambium, 1 = bright green, 2 = olive green, 3 = light brown, and 4 = cream. Data were subjected to analysis of variance, and means were compared using a protected least significant difference (SAS Institute, Cary, N.C.).

Results and discussion

GRAPE PHYLLOXERA. Data collected in 1994 on percent survival and amount of time to kill each life stage indicated that all life stages of phyllox-

era were killed with a 5-min dip in 52 °C water (data not shown). The 3-min dip was not totally effective at killing all individuals. About 21% of the eggs, 60% of the nymphs, and 15% of the adults survived for ≤7 d after dipping. At 14 d after dipping, insects subjected to a 3-min hot-water dip appeared to be dead. Davidson and Nougaret (1921) found a 1-min dip at 52 °C killed hibernants and eggs. Evaluation from 7 to 42 d after dipping for the 5-, 7-, 9-, 11-, and 13-min dips indicated all insects were dead. All stages in the control group were alive; eggs were hatching, nymphs and nymph-adults were molting, adults were laying eggs, and hibernants were becoming active.

The 1995 data confirmed that all life stages of phylloxera were killed when dipped in hot water (5 min at 43 °C + 5 min at 52 °C) and survived when dipped in 20 °C water for 10 min (data not shown). From 7 to 42 d after dipping, insects dipped in hot water were dead; insects in the control group were hatching, molting, reproducing, and breaking dormancy.

PLANTS. In 1994, data on percent budbreak of primary, secondary, and tertiary shoots showed that no plants displayed adverse effect from hot-water dipping ($P > 0.05$). However, there was a significant difference in percent budbreak among rootstocks (Table 1).

In 1995, there were no adverse effects of hot-water dipping on the graft union (based on a visual browning rating) or percent budbreak (Table 2). However, rootstock-scion plants did differ in overall ratings and percent budbreak (Table 2).

It is important to note that our objectives were to find treatments requiring the least amount of time in hot water required to kill all life stages of phylloxera and, thus, subject plants to the minimum possible contact with hot water. A hot-water dip of 5 min at 43 + 52 °C was sufficient to kill all stages of phylloxera and did not injure dormant plants.

Other experiments have indicated that bud injury from hot-water treatment is minimal at 52 and 54 °C with time lengths of 10, 20, or 30 min. Critical injury from dipping nonrooted cuttings appears to occur at 56 °C for 30 min. (Wample, 1993). In an experiment on using hot-water dips to eliminate crown gall, Wample (1991) found that percent budbreak after hot-

Table 2. Effect of hot-water dipping of grafted vines on scion budbreak and graft union damage in 1995.²

Scion-rootstock ²	Dipping treatment ³	Visual rating		Shoot no./plant	Budbreak (%) ⁴
		Pith ⁵	Cambium ⁵		
PN-101-14	C	2.3	1.8	4.6	126
	T	<u>2.1</u>	<u>1.7</u>	<u>4.9</u>	<u>133</u>
CH-3309C	C	1.8	1.6	5.1	139
	T	2.7	2.4	2.3	79
CH- <i>R. gloire</i>	C	<u>2.6</u>	<u>2.3</u>	<u>2.8</u>	<u>95</u>
	T	2.4	2.2	3.3	111
	C	1.4	1.2	5.2	101
	T	<u>1.4</u>	<u>1.3</u>	<u>4.9</u>	<u>99</u>
LSD _{0.05} ^u		0.51	0.42	0.91	26.2
ANOVA ¹					
Block		NS	NS	NS	NS
Dipping		NS	NS	NS	NS
Rootstock		***	***	***	**
D × P		NS	NS	NS	NS

²Means for the main effect of rootstock-scion are underlined.

³PN = Pinot noir; CH = Chardonnay.

⁴C = control, dipped 10 min at 20 °C; T = treated, dipped 5 min at 43 °C + 5 min at 52 °C.

⁵Percentages >100 indicate presence of more than one shoot per node.

⁶Visual rating: pith, 1 = cream or green, 2 = tan, 3 = light brown, 4 = dark brown or black; cambium, 1 = bright green, 2 = olive green, 3 = light brown, 4 = cream.

⁷Least significant difference value is for main effect rootstock-scion combination as there was no dipping treatment effect.

⁸ANOVA = analysis of variance.

NS, **, *** Nonsignificant or significant at $P < 0.01$ or 0.001 , respectively.

water treatment at $>54^{\circ}\text{C}$ depended on cultivar. Budbreak also depended on chilling requirements and storage of vines after dipping (Wample, 1993). Our results indicate that there was no critical plant injury at 52°C , and a 5-min dip was successful for eradicating grape phylloxera.

Literature Cited

Connelly, A. and B. Strik. 1991. Phylloxera—An update, Ask OSU. The Oregon Grapevine 9:9–10.

Davidson, W.M. and R.L. Nougaret. 1921. The grape phylloxera in California. USDA Bul. 903.

Flaherty, D.L. (ed.). 1992. Grape pest management guide. 2nd ed. Univ. of California–Davis.

Goussard, P.G. 1977. Effect of hot-water treatments on vine cuttings and one-year-old grafts. *Vitis* 16:272–278.

Lear, B. and L.A. Lider. 1959. Eradication of root-knot nematodes from grapevine rootings by hot water. *Plant Dis. Rpt.* 43:314–317.

Ophel, K., P.R. Nicholas, P.A. Magarey, and A.W. Bass. 1990. Hot water treatment of dormant grape cuttings reduces crown gall incidence in a field nursery. *Amer. J. Enol. and Viticult.* 41:325–329.

Strik, B. and P. Stonerod. 1995. Chapter 8. In: Buying winegrape plants. In: Phylloxera: Strategies for management in Oregon's vineyards. Oregon State Univ. Extension Pub. no. EC1463.

Wample, R.L. 1993. Influence of pre- and post-treatment storage on budbreak of hot water treated cuttings of Cabernet Sauvignon. *Amer. J. Enol. and Viticult.* 44:153–158.

Wample, R., A. Bary, and T.J. Burr. 1991. Heat tolerance of dormant *Vitis vinifera* cuttings. *Amer. J. Enol. and Viticult.* 42:67–72.

Fertilization Rate and Growth of 'Hamlin' Orange Trees Related to Preplant Leaf Nitrogen Levels in the Nursery

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ADDITIONAL INDEX WORDS. citrus, nitrate, nutrition, soil

SUMMARY. Our objectives were to determine the effects of leaf N concentration in citrus nursery trees on subsequent growth responses to fertilization for the first 2 years after planting and the impact of N fertilizer rate on soil $\text{NO}_3\text{-N}$ concentration. 'Hamlin' orange [*Citrus sinensis* (L.) Osb.] trees on 'Swingle' citrumelo rootstock [*C. paradisi* Macf. x *P. trifoliata* (L.) Raf.] were purchased from commercial nurseries in Apr. 1992 (Expt. 1) and Jan. 1993 (Expt. 2) and were grown in the greenhouse at differing N rates. Five months later, trees for each experiment were separated into three groups (low, medium, and high) based on leaf N concentration and were planted in the field in Oct. 1992 (Expt. 1) or Apr. 1993 (Expt. 2). Trees were fertilized with granular material (8N–2.6P–6.6K–2Mg–0.2Mn–0.12Cu–0.27Zn–1.78Fe) with N at 0, 0.11, 0.17, 0.23, 0.28, or 0.34 kg/tree per year. Soil $\text{NO}_3\text{-N}$ levels were determined at 0- to 15- and 16- to 30-cm depths for the 0.11-, 0.23-, and 0.34-kg rates over the first two seasons in Expt. 2. Preplant leaf N concentration in the nursery varied from 1.4% (Expt. 1) to 4.1% (Expt. 2) but had no effect on trunk diameter, height, shoot growth and number, or dry weight in year 1 (Expt. 1) or years 1 and 2 (Expt. 2) in the field. Similarly, fertilizer rate in

the field had no effect on growth during year 1 in the field. However, trunk diameter increased with increasing N rate in year 2 and reached a maximum with N at 0.17 kg/tree per year but decreased at higher rates. Shoot number during the second growth flush in year 2 was much lower for nonfertilized vs. fertilized trees at all rates, which had similar shoot numbers. Nevertheless, leaf N concentrations increased during the season for trees with initially low levels, even for trees receiving low fertilizer rates. This suggests translocation of N from other organs to leaves. Soil $\text{NO}_3\text{-N}$ levels were highest for the 0.34-kg rate and lowest at the 0.11-kg rate. Within 2 to 3 weeks of fertilizing, $\text{NO}_3\text{-N}$ levels decreased rapidly in the root zone.

Several young-tree fertilizer studies have been conducted on citrus in Florida. Optimum N rates per tree for the first year that trees are in the field in Florida were reported to be 56 (Rasmussen and Smith, 1961), 70 (Marler et al., 1987), 108 (Obreza and Rouse, 1993), and 230 g (Willis et al., 1990). Moreover, in several studies (Obreza and Rouse, 1993; Rasmussen and Smith, 1961), fertilization rate had no effect on tree growth during the first year in the field. Furthermore, the same lack of response to fertilizer rate occurred during the second year in studies by Rasmussen and Smith (1961) and Calvert (1969). In contrast, Obreza and Rouse (1993) found tree growth increased with increasing fertilization rate.

Differences in responses to fertilization by young citrus trees may result from several factors, including soil type (Obreza and Rouse, 1993), tree age and size (Calvert, 1969), rootstock (Wutscher, 1989), amount of stored reserves (Legaz et al., 1995), or type of nursery trees (bareroot or container grown) (Davies, unpublished). Some or all of these factors may account for the variation in fertilizer responses observed in field experiments. In our previous studies (Marler et al., 1987; Willis et al., 1990), leaf nutrient levels, particularly N, varied considerably among nursery trees before planting and appeared to be a possible reason for differences in fertilizer responses.

Concern over high soil $\text{NO}_3\text{-N}$ in some areas of Florida also have caused growers to re-evaluate fertilization rates

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