Hot-Water Treatments for Control of *Planococcus ficus* (Homoptera: Pseudococcidae) on Dormant Grape Cuttings

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J. Econ. Entomol. 98(4): 1109–1115 (2005)

ABSTRACT Hot-water immersions were tested for control of mealybug *Planococcus ficus* (Signoret), on dormant grape cuttings used for nursery stock. A range of hot-water temperatures (47–58°C) were evaluated at immersion periods of 2, 5, 10, or 20 min, by using a total of 353,720 mealybugs across all treatments. A 5-min immersion at 51°C is effective in killing >99% of *P. ficus*. At or above this immersion period and temperature, there was no difference in mealybug stage mortality. We evaluated a commercial operation, which used a 5-min immersion in each of three water tanks: preheating (30.0 ± 3°C), hot-water (52.8 ± 0.3°C), and cooling (23 ± 3°C). The commercial procedure provided 99.8–100% mealybug control in each of three separate trials.

KEY WORDS *Planococcus ficus*, hot-water treatments, nursery controls, dormant grape cuttings

MEALYBUG *Planococcus ficus* (Signoret) (Homoptera: Pseudococcidae) has quickly become a serious pest of raisin, wine, and table grapes in California. Most vineyard mealybug species lower crop quality by excreting honeydew, which acts as a substrate for sooty mold, and by infesting grape clusters (Flaherty et al. 1992). *P. ficus*, however, has a number of biological characteristics that make it more damaging than species long-resident in California, such as the grape mealybug, *Pseudococcus maritimus* (Ehrhorn), obscure mealybug, *Pseudococcus viburni* (Signoret), and longtailed mealybug, *Pseudococcus longispinus* (Targioni Tozzet). Compared with these *Pseudococcus* species, *P. ficus* has a higher reproductive rate, with >250 eggs per female (Walton 2003), and a faster developmental time, with four to seven generations per year in the San Joaquin Valley (Daane et al. 2004a). This results in a rapid increase in population density and damage. *P. ficus* feeds on all sections of the vine, including the roots (Godfrey et al. 2003), which provide protected areas from insecticides and parasitoids (Daane et al. 2004a). *P. ficus* also has a wider host range, feeding on subtropical and tropical crops (Cox 1989) as well as a number of common weeds (K.M.D., unpublished data), which increases residual populations outside the vineyard. Finally, *P. ficus* can vector viral diseases of grapevines (Engelbrecht and Kasdorf 1990), similar to some *Pseudococcus* spp. (Golino et al. 1999), and therefore can be an economic pest even at low densities.

This invasive pest was accidentally introduced into California in the Coachella Valley, a southern table grape region, in the early 1990s (Gill 1994). It rapidly spread to distant California grape-growing regions, with new infestations found in the San Joaquin Valley (1998), Central Coast (1999), North Coast (2001), Sacramento Valley (2002), and Monterey (2002) regions. Infested nursery stock has been suggested as the most probable cause of the rapid dissemination that occurred from 1999 to 2002 (Daane et al. 2004b). Indirect evidence provides support for this view because new infestations, in previously uninfested regions, have been associated with newly purchased nursery stock from nurseries located in infested regions. Before 2002, there were no effective methods, such as pheromone traps (Millar et al. 2002), to monitor *P. ficus* in nursery operations, or state-mandated regulations for *P. ficus*.

To develop a nursery program, collaborative efforts between commercial, state, and university personnel were initiated in 2002 with studies on chemical and cultural controls for green-growing and dormant cuttings of nursery stock. We report here on hot-water immersion of dormant grape cuttings. The California Department of Food and Agriculture currently allows the use of hot-water immersion as a means for disinfesting dormant grape cuttings and plants from nematodes that do not transmit viruses such as root knot nematodes, *Meloidogyne* spp., and grape phylloxera, *Daktulosphaira vitifoliae* (Fitch). Treatments are composed of a three step process whereby dormant cuttings or plants are immersed for 5 min each into pretreatment, treatment, and posttreatment water baths at 78°C, 125°C, and cool water, respectively. Hot-water treatments also have been used in numerous insect control programs in other crops, including tephritid flies on mangos (Sharp 1986, Sharp et al. 1988, Nasci-

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0022-0493/05/1109–1115$04.00/0

mento et al. 1992) and papayas (Couey and Hayes 1986, Hayes et al. 1987), tortricids infesting sweet cherries (Feng et al. 2004), diaspid scale on ornamentals (Hara et al. 1993, 1994), and two species of mealybugs, *Planococcus citri* Risso and *Pseudococcus odermatti* Miller & Williams, on harvested limes (Gould and McGuire 2000).

We report here on research to determine the effectiveness of different hot-water immersion periods and temperatures on *P. ficus* mortality for use as a nursery control of *P. ficus* on grape cuttings. Our goal was to develop data that support the use of existing 5-min hot-water immersion programs for control of this new pest. We also investigated shorter and longer immersion periods in case data from 5-min immersion periods did not lead to a commercially viable treatment program.

Materials and Methods

Insect and Plant Materials. Laboratory colonies of *P. ficus* were established from field-collected material in vineyards located near Del Rey, Fresno County, California. Mealybugs were reared on butternut squash, *Cucurbita moschata* L., that had been washed in a 0.5% bleach solution to reduce mold growth and then triple rinsed. Each squash was inoculated with five to 10 gravid female mealybugs or their ovisacs, and all squash were held at 22 ± 2°C, with a photoperiod of 12:12 (L:D) h until mealybug generations overlapped such that all stages of *P. ficus* were present on each squash.

Green and dormant cuttings of *Vitis vinifera* L. ('Thomson Seedless') were collected from untreated vineyards located near Bakersfield, Kern County, California. Cuttings were 30–45 cm in length and 0.75–1.0 cm in diameter. To infest green cuttings, the collected material was reduced to 15 cm in length, with two nodes on each cutting. On one end of each cutting, a razor blade was used to peel back 1.0 cm of bark to simulate the natural cracks present in dormant wood, where the vineyard mealybugs commonly overwinter (Geiger and Daane 2001). The other end of the cutting was inserted into an 8-ml Aquapic Aquatube (Syndicate Sales, Inc., Kokomo, IN), which is commonly used to provide a water source for cut ornamental flowers. The green cuttings were then placed on top of an infested butternut squash for 3 d. To infest dormant cuttings, full-length sections were placed directly on the *P. ficus* colony without any alterations. For both green and dormant cuttings, the tested cuttings were each infested with >200 *P. ficus*, and typically with representation of all stages of nymphs and adult females, although crawlers were most common. Eggs were not included in the study due to the high unlikelihood that they would be present on dormant grape cuttings under natural conditions in the field.

Laboratory Development of Dose–Response Curves. Laboratory experiments were conducted in a 28-liter stainless steel open bath (Fisher, Pittsburgh, PA) to determine dose–response curves for *P. ficus* on dormant cuttings. In each trial, infested green cuttings were placed, individually, into nylon sleeves that were water permeable but prevented movement of mealybugs through the nylon sleeve (759.5 squares per cm^2). Treatments compared *P. ficus* mortality among combinations of immersion periods and hot-water temperatures. Immersion periods tested were 2, 5, 10, or 20 min. Hot-water temperatures tested were as follows for each immersion period: 2 min at 30, 47, 48, 49, 50, 51, 52, 54, 56, and 58°C; 5 min at 30, 47, 48, 49, 50, 51, 52, 54, and 56°C; 10 min at 30, 47, 48, 49, 50, and 51°C; and 20 min at 30, 43, 44, 45, 46, 47, 48, 49, 50, and 51°C. Water temperatures were regulated (±0.1°C) by using an Isotemp immersion circulator (Fisher), with temperature verified by a waterproof thermometer (Control Company, Friendswood, TX). There were six replications of each immersion period and temperature combination tested; with only a single water bath, replication was completed through randomly ordered sequential trials that were conducted from July to October 2003.

Treatment impact was determined by recording the number of dead and live *P. ficus*, categorized by developmental stage, on the green cuttings and caught within the nylon sleeves. The cuttings were placed, individually, on the sticky surface of an open-faced pheromone trap (Pherocon AM Trap, Trécé, Inc., Salinas, CA) to catch live mealybugs leaving the cuttings. After 24 h, the numbers of mealybugs on the cuttings and pheromone traps were recorded. To collect mealybugs on the nylon sleeves, the sleeves were inverted and rinsed with water through a porous wire sieve (to remove plant material), and the collected water was poured through filter paper under very light suction (to catch mealybugs). The filter paper was transferred to petri dish and air-dried for 2 h, and the numbers of mealybugs were recorded. Mealybugs were considered dead if they were desiccated or if there was no leg movement when lightly prodded with a teasing needle.

Results are presented herein as means per treatment (±SEM). Temperature–mortality regressions were calculated to determine the impact of temperature and immersion periods on *P. ficus* mortality. Lethal temperatures (LT_{99}), 95% confidence intervals (CI), and regression slopes were calculated using the probit option of POLO-PC (LeOra Software 1987). Differences in the LT_{99} of different life stages at each immersion period were considered significant if the 95% CI did not overlap. Calculated LT_{99} values were fit to immersion periods by using a simple three-parameter model describing an exponential decay (SAS Institute 1999):

$$y = ae^{\left(\frac{b}{x+c}\right)} \quad [1]$$

Natural mortality in the water-dip control (30°C) was corrected with Abbott (1925) methods for graphic presentation.

Commercial Hot-Water Treatment. Based on our laboratory results, recommendations for a commercial hot-water treatment were developed using immersion

periods and temperatures that provided >99% *P. ficus* mortality. We evaluated the effectiveness of this program with collaborators at the Sunridge Nursery (Bakersfield, Kern County, California). The commercial operation used three 7,571-liter (2,000-gallon) gondolas as water tanks. The gondolas were set in a single line and underneath a steel frame, which had a center I-beam with an electronic crane to lift and move a steel pallet of nursery stock between the gondolas. Each pallet could carry 100 bundles of dormant grape cuttings, with each bundle containing 50–100 individual cuttings. Water in the first gondola, the "preheating tank," was maintained at 30.0°C, water in the second gondola or "hot-water tank" was at 52.8°C, whereas water in the third gondola or "cooling tank" was at 23°C. A gasoline-powered, thermostatically controlled water heater maintained these temperatures, with water circulated at 946 liter/min (250 gal/min). Temperatures were monitored with digital, waterproof thermometers placed in each water tank and an in-flow thermometer permanently positioned within the water circulation system between the hot-water tank and furnace. The commercial operation used a 5-min immersion period for each of the three tank treatments.

To test the effectiveness of a commercial operation, dormant cuttings were inoculated with mealybugs and placed in nylon sleeves, as described previously, and then placed in a commercially sized bundle of 50–100 dormant cuttings. In each of four replicates, two artificially infested bundles were placed, in randomly assigned locations, in the pallet along with other commercially treated bundles. After treatment, the artificially infested dormant cuttings and nylon sleeves were processed for live and dead mealybugs, as described previously. Initial tests were conducted on 24 January and 22 April 2003 and compared the commercial hot-water immersion procedure with a 5-min immersion in the cooling tank, which was used as a control treatment. The test was repeated on 11 January 2004, during which the commercial hot-water procedure (eight replicates) was compared with individual 5-min immersions in the preheating, hot-water and cooling tanks (four replicates each) to determine whether the hot-water tank alone was responsible for the observed mortality. Results are presented herein as means per treatment (±SEM). Within each experiment, treatment impacts are compared using analysis of variance (ANOVA), with treatment means separated with Fisher's protected least significance difference test (SAS Institute 1999).

Results and Discussion

Laboratory Development of Dose–Response Curves. A total of 353,720 *P. ficus* were evaluated, comprised of 198,846 crawlers (56.2% of the total), 118,729 first instars (33.6%), 22,848 second instars (6.5%), 8,985 third instars (2.5%), and 4,312 adult females (1.2%). *P. ficus* percentage of mortality, summarized across all mealybug developmental stages, increased with increasing temperatures and immersion periods (Fig. 1). In the preheating tank (30°C), which was used as a relative control treatment, mealybug percentage of mortality at 2, 5, 10, and 20 min was 26.4 ± 3.5, 29.3 ± 3.9, 24.4 ± 4.3, and 29.5 ± 5.2, respectively. There was no significant change in mortality until temperatures were increased to 45, 49, 50, and 51°C for the 20-, 10-, 5-, and 2-min immersion periods, respectively (Fig. 1). Above these temperatures, there was a rapid increase in mealybug mortality with increasing temperatures for the 20-, 10-, and 5-min immersion periods, with LT_{99} levels surpassed at 50°C for the 20- and 10-min immersion periods, and 51°C for the 5-min immersion period. In the 2-min immersion period, the LT_{99} level was not surpassed until water temperatures were at 58°C. The calculated LT_{99} values were negatively related with immersion periods (Fig. 2).

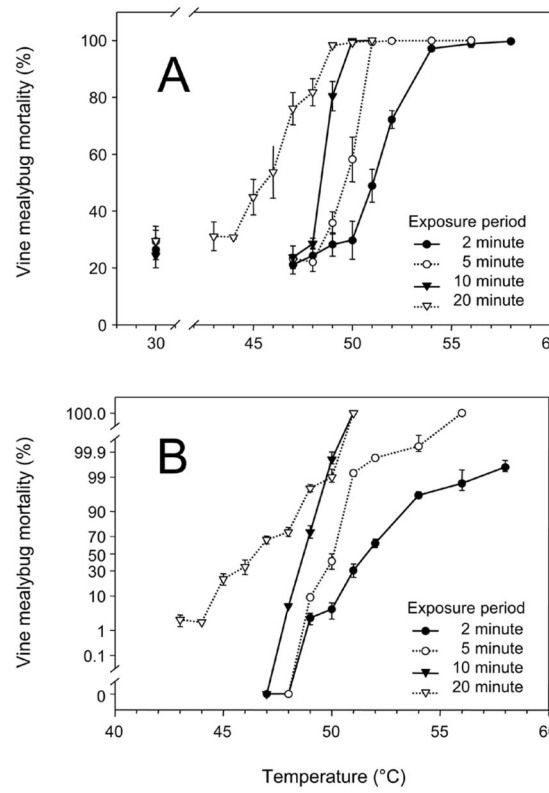

Fig. 1. *P. ficus* percentage of mortality (±SEM) under varying immersion periods and hot-water temperatures presented as (A) unadjusted data and (B) percentage of mortality corrected for mortality at 30°C and plotted (*y*-axis) on a probability scale.

There were few differences among mealybug developmental stages in calculated LT_{99} values within each immersion period tested (Table 1). LT_{99} values in the 2-min immersion period were considerably higher, compared with the other immersion periods, across all developmental stages, and ranged from 56.8 to 57.7°C. At this immersion period, there was 100% mortality of only adult females, whereas some indi-

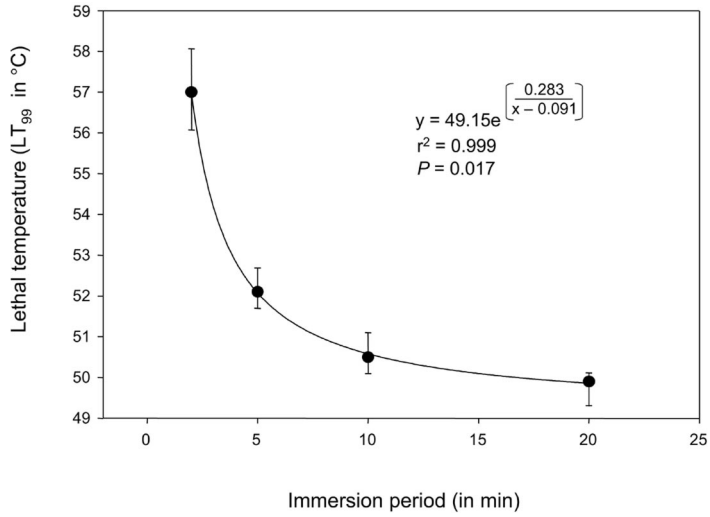

Fig. 2. Calculated LT_{99} values (with 95% CI) for *P. ficus* immersed in hot-water treatments for 2, 5, 10, and 20 min.

viduals survived from each of the other life stages evaluated (Table 1). Those mealybugs found alive were found buried deep within cracks on the dormant cuttings, where they would have been most likely to evade treatment effects. It is likely that the smaller mealybug stages are able to penetrate deeper into cracks of the dormant cuttings, and find better shelter. The greatest range of LT_{99} values among mealybug developmental stages was in the 5-min immersion period, from 51.9°C (crawlers) to 53.3°C (adults), although these differences were not significant based on overlap of the 95% CI. At the 10- and 20-min immersion periods, the LT_{99} varied little among mealybug developmental stages, ranging from 50.2 to 51.9°C and 49.9 to 50.9°C, respectively. With the exception of first instars at 10 min, there were no significant differences in mortality among life stages at either immersion period. With the exception of crawlers and first instars at 5 min, no *P. ficus* were found alive in any replication of any treatment above the calculated LT_{99} values for immersion periods of 5, 10, or 20 min.

We showed that at 5-, 10-, and 20-min immersion periods, the LT_{99s} for *P. ficus* were reached at or above 50°C, whereas at the 2-min immersion period temperatures must be at or above 58°C. Generally, immersion period is positively related with percentage of mortality (Hara et al. 1994, Lester and Greenwood 1997), and most studies suggest immersion periods be >10 min for 100% control (Sharp 1986, Gould and McGuire 2000). We also showed that there was little

Table 1. Calculated LT_{99}, 95% CI, and regression slopes for each of five *P. ficus* development stages and four hot-water immersion periods

Immersion (min)	Mealybug development stage	LT_{99} (°C)	95% CI (°C)	Regression slope ± SEM	Mealybugs counted (*n*)
2	Crawler	56.8	55.9–57.9	0.373 ± 0.003	47,075
	First	58.0	56.8–59.6	0.335 ± 0.004	21,746
	Second	57.9	56.8–59.4	0.356 ± 0.009	5,087
	Third	57.8	56.9–59.1	0.399 ± 0.016	2,387
	Adult	57.7	56.3–60.2	0.431 ± 0.027	1,137
5	Crawler	51.9	51.4–52.5	0.848 ± 0.007	36,981
	First	52.0	51.5–52.7	0.772 ± 0.008	22,600
	Second	52.0	51.4–52.8	0.812 ± 0.019	4,563
	Third	52.6	51.9–53.8	0.771 ± 0.031	1,769
	Adult	53.3	52.4–55.3	0.710 ± 0.046	988
10	Crawler	50.2	49.8–50.7	1.181 ± 0.012	29,018
	First	51.9	51.1–53.1	0.601 ± 0.008	18,532
	Second	51.6	50.9–52.7	0.675 ± 0.018	3,825
	Third	50.9	50.3–52.0	0.920 ± 0.033	1,844
	Adult	50.9	50.2–52.1	0.903 ± 0.053	715
20	Crawler	50.2	49.6–51.0	0.455 ± 0.004	85,772
	First	50.3	49.6–51.2	0.457 ± 0.004	55,851
	Second	50.9	50.0–52.2	0.443 ± 0.010	9,373
	Third	50.2	49.6–51.1	0.605 ± 0.019	2,985
	Adult	49.8	49.4–50.5	0.662 ± 0.030	1,472

LT_{99}, confidence intervals, and regression slopes were calculated using the probit option of POLO, LeOra Software (1987). POLO software forces the regression line through zero on the *x*-axis.

Table 2. *P. ficus* percentage of mortality on dormant grape cuttings, separated by developmental stage, after a 5-min immersion per tank immersion in preheating, hot-water, and cooling tank during commercial operations in a grape nursery

Trial	Treatment[a]	Mortality (%) by mealybug developmental stage					Mortality (%) for all stages[b]	*n*
		Crawler	First	Second	Third	Adult		
24 Jan. 2003	Cooling	9.6 ± 3.1	—[c]	31.8 ± 31.8	—	25.0 ± 10.3	12.5 ± 1.4a	234
	All tanks	99.7 ± 0.3	100	100	100	100	99.8 ± 0.2b	1,573
22 April 2003	Cooling	25.9 ± 6.6	15.5 ± 3.6	9.0 ± 1.9	7.5 ± 4.0	18.3 ± 6.9	15.0 ± 3.5a	1,258
	All tanks	100	100	100	100	100	100b	1,561
11 Jan. 2004	Pre-heating	51.2 ± 10.6	52.1 ± 8.4	36.9 ± 2.7	31.5 ± 5.3	17.5 ± 6.9	50.9 ± 8.6a	3,680
	Hot-water	100	100	100	100	100	100b	2,507
	Cooling	43.5 ± 0.9	39.5 ± 3.5	31.1 ± 8.7	24.1 ± 5.3	25.0 ± 5.9	40.4 ± 2.0a	2,776
	All tanks	100	100	100	100	100	100b	3,871

[a] Temperatures in the preheating, hot-water, and cooling tanks were maintained at 30 ± 3, 52.8 ± 0.3, and 23 ± 3°C, respectively.
[b] On 11 Jan. 2004, for all mealybug combined, different letters among treatment averages are significantly different (Fisher's protected least significance difference test, $P < 0.05$).
[c] No first or third instars were found.

difference in LT_{99s} among mealybug developmental stages, suggesting that treatment impact will be acceptable regardless of the *P. ficus* stage present. Hara et al. (1994) found that green scale, *Coccus viridis* (Green) adults, nymphs, and crawlers, respectively, were the more difficult developmental stage to control at 49°C, although 100% mortality was achieved for all stages at or above 7-min immersion periods. In contrast to our results, Jang (1986) reported developmental stage can impact hot-water treatments; however, these results were reported for tephritid flies.

Because of the volume of cuttings that must be processed, the shorter (5-min) immersion period is preferred by commercial operations and, for this reason, we have suggested hot-water temperatures be set between 51 and 53°C. Some crops are temperature sensitive and cannot withstand prolonged periods of hot-water treatments or short periods of high temperatures (Feng et al. 2004). Short immersion periods and relatively low temperatures have been effective for some pests; for example, mites were controlled on strawberries with an immersion period of 6.5 min at 46°C (Hellqvist 2002). However, we believe that for effective control of *P. ficus* the temperatures must be >50°C if a short (5-min) immersion period is used. This temperature range is higher than that commonly reported (46–49°C) for most tortricids (Jones et al. 1995, Lester and Greenwood 1997), tephritids (Sharp et al. 1988, Nascimento et al. 1992), and homopterans (Hara et al. 1993, Hara et al. 1994, Gould and McGuire 2000). However, most of these studies developed quarantine guidelines for postharvest treatments of tropical or subtropical fruit; in contrast, dormant grape cuttings can be processed at these higher temperatures with no plant damage (Goussard 1977, Wample et al. 1991, Wample 1993). Furthermore, the 1–2°C temperature increase, above the commonly used 49°C, provides improved control for *P. ficus* (Fig. 1) at the shorter immersion periods (Fig. 2) that are preferred by the nursery industry.

Commercial Hot-Water Treatment. Temperatures in the preheating, hot-water and cooling tanks were maintained at 30 ± 3, 52.8 ± 0.3, and 23 ± 3°C, respectively. The heating and circulation systems were sufficient to maintain even temperature throughout the hot-water tank. Our research showed the commercial system, by using three separate tanks and a 5-min immersion period in each tank, provided excellent *P. ficus* control (Table 2). In the 24 January 2003 trial, there was an overall 99.8% mortality. In this trial, most of the mealybugs were crawlers, and only three of 1,033 (99.7%) were found alive, buried deep within a crack of a single dormant cutting. In the 22 April 2003 and 11 January 2004 trials, there was 100% mortality across all mealybug developmental stages.

In each trial, we used the cooling tank as limited control to determine the impact of a water immersion, without adverse temperatures. Results show some mortality from the water-dip alone (Table 2). For this reason, in the 11 January 2004 trial we compared all three tanks individually and against the three tank combination to determine whether the hot-water tank alone was responsible for the observed mortality. Across all mealybug stages, the 100% mortality found in the hot-water tank only and the full three-tank commercial process was significantly higher than the 50.9 ± 8.6 and 40.4 ± 2.0% mortality in the preheating and cooling tanks, respectively ($F = 51.28$; df = 3, 12; $P < 0.001$; Table 2). This pattern was similar across all mealybug developmental stages (Table 2). The nursery uses the preheating and cooling tanks to maintain health of the dormant cuttings, and whereas some mortality was found in each tank, the hot-water tank was solely responsible for required levels of mealybug mortality.

The commercial use of this hot-water program for mealybug control is now in place. Existing California regulations recommend the three step process for hot-water treatments of dormant grape plants and cuttings, as described previously. The recommended program has additional benefits as a treatment for other pests, such as root knot nematodes, *Meloidogyne* spp. (Lear and Lider 1959, Barbercheck 1986) and grape phylloxera, *Daktulosphaira vitifoliae* (Fitch) (Stonerod and Strik 1996). Moreover, hot-water treatments to dormant grape cuttings provide partial to complete control of Pierce's disease (*Xylella fastidiosa*) (Goheen et al. 1973), *Phytopthora cinnamomi* (Von Broembsen and Marais 1978), *Flavescence dorée* (Caudwell et al. 1997) and *Agrobacterium* spp. (Burr

and Katz 1989, Ophel et al. 1990, Burr et al. 1996). Proper use of this program will significantly to completely reduce the spread of *P. ficus* from dormant grape cuttings in nursery stock.

Acknowledgments

We are extremely grateful to Steve Maniaci of Sunridge Nurseries for providing nursery materials and access to hot-water treatment facilities; and to Jed DuBose, Lee Martin, and Susan Mallek for assistance with data collection and analysis. We thank the Fruit Tree, Nut Tree, and Grapevine Improvement Advisory Board (IAB) and the University of California Integrated Pest Management Program for funding in support of this work.

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Received 10 August 2004; accepted 1 April 2005.