Summary 42





Title: "Hot-water treatment for control of *Planococcus ficus* (Homoptera: Pseudococcidae) on dormant grape cuttings"

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The authors optimize the duration and temperature of hot-water immersions of grapevine cuttings as a means to control the spread of the grape mealybug (*Planococcus ficus*).

- The vine mealybug is a serious problem in California vineyards because it excretes a sticky substance –honeydew- which attracts sooty mold, infesting whole clusters and thus lowering quality. The vine mealybug (*Planococcus ficus*) is more damaging than other species of mealybugs with very similar names, such as the grape mealybug (*Pseudococcus maritimus*), the obscure mealybug (*Pseudococcus viburni*), and the long-tailed mealybug (*Pseudococcus longispinus*). This is because the vine mealybug has 1) higher reproductive rates, 2) faster developmental times (4-7 generations per year), and 3) a wider host range. Additionally, the vine mealybug, as well as other mealybugs, can vector viral diseases.
- Lab hot-water treatments. The authors isolated mealybugs from Fresno County and reared them on butternut squash. Then they collected green and dormant grape vine cuttings, peeled back their bark, and placed them on top of the infested squash to allow the cuttings to become infested. The individual infested cuttings were then wrapped in nylon sleeves (permeable to water but able to retain the insects) to ready them for submersion in a hot water bath. The treatments consisted of combinations of different immersion times (2, 5, 10, and 20 minutes) and different temperatures (ranging from 30 to 58°C) (86 to 136°F). The authors recorded treatment impact by determining the number of dead and live mealybugs, categorized by developmental stage (crawler, first instar, second instar, third instar, and adults).
- The authors found that, in the lab, mealybug mortality increased with increasing temperature and increasing immersion time. Predictably, the amount of time needed to destroy 99% of mealybugs, called lethal dose₉₉, was inversely proportional to the temperature of the water bath. For example, the authors found that with 5, 10 or 20 minute immersion, the lethal dose₉₉ was reached with at least 50°C, but at the 2 minute immersion temperatures had to be above 58°C to have the same efficacy. In general, the authors found no differences in mortality among life stages with the different treatments. This is important as it means that effective control can happen regardless of the mealybug stage present.
- Nursery hot-water treatments. The authors also evaluated the effectiveness of the water-treatment at the commercial scale with a collaborating nursery (Sunridge Nursery). The nursery used 3 gondolas in parallel as water tanks, and a crane to lift pallets of nursery stock between gondolas. The treatment evaluated consisted of successive 5 minute immersions each into a first gondola kept at 30°C

(preheating tank), followed by a second gondola kept at 53°C (hot-water tank), and a final gondola kept at 23°C (cooling tank). As before, dormant cuttings were inoculated with mealybugs, placed in bundles of 50-100 cuttings at random locations within the pallet, and mortality was assessed by counting the living and the dead mealybugs. Results were compared with a single immersion in the cooling tank (23°C), which was the Control.

• Results showed that the commercial system of **5-minute hot water treatments provided excellent mealybug control**. As results showed some mortality in the Control alone (cool water-dip), the authors tested whether the dip in the hot-water tank could be causing the high mortality observed. And the answer was yes: they found 100% mortality in the hot water alone. Still, the nursery uses the preheating and cooling tanks to maintain the health of the dormant cuttings (some crops are sensitive to temperature, even though grapevines don't seem to be among them).

In summary, the authors showed that a 5 minute immersion at 51°C is effective in killing 99% of all mealybug developmental stages present in grapevine dormant cuttings. Lower temperatures also worked, provided that the immersion time was extended. Because of the large volumes of cuttings that nurseries must process, and the preference for shorter immersion times, the authors recommend to set the thermostat between 51 and 53°C and go for the 5-minute immersion time. The commercial use of this hot-water program for mealybug control is now in place. The authors expect that proper use of this program will reduce or eliminate the spread of *P. ficus* from dormant grapevine cuttings in nursery stock. As a bonus, the hot-water treatment has beneficial effects in reducing other grapevine pathogens, such as root-knot nematode (Meloidogyne spp.), Pierce's disease (*Xylella fastidiosa*), and crown gall (*Agrobacterium spp.*).

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