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Crown Gall in Oregon Grapevines:
Biology and Treatment of Planting Stock with Hot Water Dips

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Background and Problem

Oregon is a leader in production of premium wines in the United States and the industry has been expanding rapidly over the past few years. However, continued production and expansion of vineyards are threatened by infestation of phylloxera, *Daktulospaira vitifoliae,* an insect that feeds on roots. Most grapevines in Oregon are growing on their own roots and are susceptible to phylloxera. The use of phylloxera-resistant rootstocks has increased as growers and nurserymen are moving to replace susceptible plants. However, the grafting process provides wounds that can become infected by the crown gall bacterium, *Agrobacterium vitis,* naturally present within the vascular system of some grape rootstocks and/or scion wood.

The goal of our research is to prevent crown gall in phylloxera-resistant grapevines. There are several potential ways to achieve this goal: 1) to eradicate tumorigenic bacteria within the plant material by hot water treatments, 2) to introduce biological control agents at the wound site in grafted plants, and 3) to select rootstocks and scion woods that are resistant to crown gall disease.

Isolation and Characterization

Before any of these methods could be evaluated, it was necessary to isolate and characterize bacteria in grape to assess the diversity and turnorigenicity of agrobacteria throughout Oregon vineyards and nurseries. Isolations for agrobacteria have been made from 56 sample sets which included 24 grafted rootstock/scion wood combinations, four rootstocks, field sap exudates or crown gall tumors from nine grape cultivars, and laboratory pressurized sap extractions from eleven grape cultivars. These materials were collected from eighteen vineyards and two nurseries'across western Oregon. A total of 5,390 bacterial isolates have been recovered. Characterization of approximately half of the collected isolates included a combination of the following: screening for DNA hybridization to three DNA probes unique to the tumor-inducing (Ti) plasmid of pathogenic agrobacteria, turnorigenicity testing on tomato plants, biochemical and physiological tests to determine specific biotype of pathogenic strains, opine utilization, and sensitivity to biological control agents.

Colony Hybridization and Tumorigenicity

DNA from over 2,800 agrobacteria isolates from grape has been probed against labeled-genes encoding for specific tumorigenic and virulence proteins of agrobacteria, and turnorigenic strains have been identified from all types of plant material tested. Tumorigenicity was confirmed by inoculation of

bacteria to wounded tomato plants and observation of gall development over an eight week period. Pathogenic agrobacteria were recovered from eleven of 24 rootstock/scion wood combinations processed. These included rootstocks of 3309C, 101-14Mgt, 5BB, and T5C with scion wood cultivars of Chardonnay, Pinot noir, Pinot gris, and Grenache. Biochemical and physiological characterization identified the agrobacterial isolates as predominantly A. vitis. Some tumorigenic strains of Agrobacterium biovars I and 2 were also identified from these samples. Tumorigenic agrobacteria were also isolated from self-rooted grape varieties of Gewurztraminer, Pinot noir, Muscat, Chardonnay, Muller Thurgau, and Riesling. Additional isolations from both field sap exudates and laboratory pressurized sap extractions of varieties Cabernet franc, Sauvignon blanc, Chenin blanc, Merlot, Pinot blanc, and Symphony also yielded Agrobacterium isolates which are currently being analyzed in our laboratory for tumorigenicity. Of isolates characterized to date, all have been identified as tumorigenic strains of A. vitis except for one isolate identified as Agrobacterium biovar 1.

Opine Utilization

Opines, which are unique compounds synthesized by plant galls upon integration of the bacterial T-DNA into the plant genome, serve as specific substrates for food, influence genetic recombination, and mediate biological control efficacy. We have tested the isolates mentioned above for the utilization of four different opines: octopine, mannopine, nopaline, and succinamopine. Turnorigenic strains of A. vitis analyzed to date have been identified as utilizing nopaline, octopine, and succinamopine, or a combination of these three. None have been identified as utilizing mannopine.

Response to Antagonists

Because turnorigenic agrobacteria require wounded plant cells to initiate crown gall, protection of the graft site with antagonists to A. vitis is a method of preventing crown gall disease. Conversely, tumor formation at unprotected graft unions may severely reduce plant vigor, the economic value of the plant (potential for sale), or result in plant death. Thus, the benefit of removing the opportunity for this disease at the graft site is great to nurserymen throughout Oregon. Our laboratory investigated the use of three non-pathogenic strains of Agrobacterium biovar 2 to inhibit the growth of pathogenic strains of A. vitis isolated from Oregon gape cultivars; and rootstocks. Nonpathogenic bacterial strains, HLB-2 and E26, demonstrated antagonism to many of our pathogens of gape. Of isolates tested in our laboratory, 55% were inhibited by antagonistic strain HLB-2. A second antagonist, E26, inhibited an additional 15% of the tested strains for a total of 70%. Only one of the grape isolates was inhibited by antagonist strain K84. Testing is being initiated on additional strains of A. vitis since the results suggest that these antagonists could potentially protect the graft union site.

Hot Water Dips

Although hot water dips of plant material have been used previously to eradicate nematodes and to decrease the incidence of crown gall disease in grapevines, little work had been done to ascertain the efficiency of this method for the eradication of phylloxera. Dr. Bernadine Strik demonstrated that all life stages of phylloxera can be eradicated from grape root pieces using a 5 minute dip at 52°C combined with a pre-dip treatment at 43°C for 5 minutes. These time/temperature parameters were then applied to grafted vines (Pinot noir/101-14Mgt, Chardonnay/3309C, and Chardonnay/Riparia gloire) to ascertain any effect on bud break and outgrowth of treated canes. Time intervals for bud break on grafted and treated rootstocks were no different from non-treated controls (2). Since then, control and heat-treated canes of grafted plants were analyzed by our laboratory to determine if agrobacteria could be recovered. Scion wood was removed from the rootstock above the graft union site. Rootstock and scion wood samples were prepared separately. Naturally-occurring agrobacteria were recovered from both heat-treated and control samples of Chardonnay scion wood which had been grafted onto rootstock 3309C

and from heat-treated and control samples of all three rootstocks tested. When subjected to DNA hybridization against labeled probes, 17 isolates were identified as positive for all three probes. Therefore, we concluded that a heat treatment of 52C for 5 minutes was inadequate for eliminating agrobacteria.

Hot water treatments of naturally-infested dormant gape cuttings tested the time/temperature parameters of 50'C for 30 minutes (1). While a decrease in the incidence of crown gall was observed, these parameters were not sufficient to eradicate agrobacteria from these canes (1). Additionally, maximum heat tolerance of dormant cuttings was reported (3) to be greater than previously expected and suggested that dormant cuttings could be subjected to more severe hot water dips for the eradication of agrobacteria. Preliminary trials in our laboratory were conducted to test the time/temperature parameters of 54C for 30 minutes on eleven cultivars of grape that had been infiltrated with an antibiotic-resistant, tumorigenic strain of A. vitis, T60/94, previously isolated from an Oregon vineyard and characterized by our laboratory. The cultivars included in this initial study were Cabernet franc, Chardonnay, Chenin blanc, Gewurztraminer, Merlot, Muller Thurgau, Pinot blanc, Pinot noir, Riesling, Sauvignon blanc, and Symphony.

Cane sections were infiltrated with a known volume of bacterial suspension (ca. 108 cfu/ml) to a population level in extreme excess of that found naturally-occurring within canes. The infiltrated canes were then subjected to a hot water dip for 30 minutes at 54'C. Vascular tissues of half of the treated canes were immediately flushed with sterile water, and the recovered samples were plated to medium amended with antibiotic to assess survival. The remaining test canes were incubated at 28'C for 2 days then flushed with sterile water and plated as described above. Control cane sections for each cultivar were infiltrated with the marked strain of A. vitis and then flushed with sterile water to confirm the recovery of the introduced bacteria. Results demonstrated that our marked strain could be infiltrated and recovered from non-heated control canes at comparable levels. Bacteria were recovered from only three cultivars which had been hot-water dipped then immediately flushed with sterile water. The bacterial population in these cane sections was reduced from 10 million *GCO* colony forming units to between 10' to 105 cfu. When infiltrated canes were incubated for two days at 28'C following hot water dips, bacteria were not recovered from ten varieties and from one variety, the infiltrated population was reduced to 10' cfu. We suspected that these low survival rates were due to some plant effect since these grape canes were collected during the late winter and early spring months of 1995 and stored at 4'C for several months until processed for treatment. The number of chilling units acquired by each cultivar during this storage period may affect their level of dormancy and could influence responsiveness to heat treatment.

A second hot water treatment was conducted on five rootstocks and four scion wood cultivars which were collected together in December, 1995. Sample cane sections were divided into at least two experimental groups, processed, and treated within one month to reduce any effect storage length may have on efficacy of treatment. The grape cultivars included in this study were Chardonnay, Pinot gris, Pinot noir, and Riesling. Rootstocks included 101-14, 3309C, S04, *Riparia gloire,* and T5C. In two independent experimental trials, no bacteria were recovered from any of the infiltrated cane sections which had been hot water dipped for 30 minutes at 540C. Two additional greenhouse trials were added to this experimental design to ascertain any effect this hot water treatment may have on bud break and subsequent outgrowth of treated cane sections. Cane sections were hot water dipped and incubated for 2 days at 28'C. Following treatment, these canes were planted out and observed for bud break. Control cane sections were planted out without heat treatment. To date, no time interval difference for bud break has been observed between treated and control cane sections.

An additional study further supports the heat sensitivity of A. *vitis* and the involvement of plant factors, i.e. time of collection and storage length, in efficacy of hot water treatment. A variety of strains of A.

vitis were selected for *in vitro* trials to determine their thermal sensitivity to 54'C for 30 minutes at two different concentrations, 1W and 1 07 cfu- These isolates were recovered from several Oregon nursery and field tumor sites and were characterized in our laboratory as tumorigenic. Plant material included 10 different rootstock/scion wood combinations and four different cultivars of self-rooted vines. Recovered strains were overwhelmingly A. *vitis;* however, two isolates were pathogenic A. *turnefaciens* biovar 2. To date, none of the 97 strains survived when subjected to 54'C for 30 minutes in the absence of plant material.

Results presented here are very encouraging for reduction of crown gall disease and eradication of agrobacteria in dormant grape vines. 'Hot water dips of 540C for 30 minutes were successful in killing a diverse collection of turnorigenic strains of A. *vitis* in the absence of plant material and, when infiltrated cane sections were similarly treated, no bacteria were recovered.

References

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