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COLLEGE OF FOOD, AGRICULTURAL,
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Report Form

Project Title: Improving Chestnut Graft Success Using Biological Controls

Summary

Clonal propagation and the establishment of multi-cultivar clonal orchards are standard in the production systems of domesticated fruit and nut crops around the world. However, mature-phase culinary chestnut trees do not clonally propagate well by any method. The objective of this study was to test commercially available biological control and plant health booster products for their impact on graft success including plant growth promotion and alteration of endophytic communities. None of the biocontrol products tested in this study increased graft success rate, although the fungi and bacteria associated with the products could successfully colonized chestnut buds. In this study, the treatments were only applied once. Additional applications of biocontrol products during graft healing, and monitoring colonization via microscopy may provide additional information as to the role, if any, these microorganisms could have in the healing process. The chestnut industry is keen on identifying sustainable tools to improve graft success and support additional research to achieve a high graft success rate.

What was done?

The goal of this study was to determine if commercially available biological control and plant health booster products could improve the success rate of grafting culinary chestnut. Trees (n=2) were arranged in a randomized complete block design with five replications. Four commercial biological products and one experimental biocontrol were evaluated (Table 1) and the rates were based on the manufacturer recommendations for dipping. Self-grafted, non-treated and non-grafted, non-treated control trees (n=6) were included in the study. Half of the control trees were topped at the time of grafting and half were not topped. Trees were self-grafted using the chip bud technique. One ml of each treatment was applied to the graft wound and the union was immediately wrapped with parafilm. Grafted trees were maintained in the greenhouse (85 F daytime and 75 F nighttime) and monitored weekly. Thirty days after grafting and treatment application samples were collected for diagnostics. Two samples were collected from each grafted tree; one from the graft and one collected from a different branch than where the graft was located with a healthy bud. Samples were stored in liquid nitrogen and DNA for future gene expression studies (beyond the scope of this proposal).

Table 1. Treatment and rates used in this study.

Treatment	**Manufacturer**	**Active Ingredients**	**Lab Rate** (per 100 ml)
8HQC		8-Hydroxyquinoline	500 g
Cease	BioWorks	*Bacillus subtilis*	1.3 ml
Trichoderma asperellum (Experimental)	BioWorks	*Trichoderma asperellum*	0.9 g
DoubleNickel	Certis	*Bacillus amyloliquefaciens strain D747*	18.0 g/
BiOWiSH	BiOWiSH TEchnologies	*Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus licheniformis, Bacillus pumilus*	20.0 g
Mycostop	Verdera	*Streptomyces griseoviridis* Strain K61	0.101g/100ml

What were the results?

All the treated, grafted trees died within 30 days of treatment application. Non-treated and non-grafted trees were living after 30 days. To confirm that the living biocontrol microorganisms colonized the graft union, buds treated with Cease, *Trichoderma asperellum*, DoubleNickel, BiOWiSH, and Mycostop were plated onto potato dextrose agar (PDA). Colonies resembling the active ingredient (See Table 1) were recovered from all the treatments indicating that the treatments contained viable active ingredient. Preliminary gene expression experiments indicate that some tree and plant fungal pathogens are associated with failed grafted union but not healthy non-grafted tissue. For example, *Alternaria alternata*, *Alternaria tenusssima*, *Botrytis cinerea*, *Fusarium avenaceum,* and *Colletotrichum* spp. sequences were associated with failed graft unions but not healthy stem tissue. Among all the fungi species identified using gene expression experiments, *Trichoderma asperellum* (experimental treatment in this study) was most abundant on failed graft unions.

How have the results contributed or will they contribute to sustainable agriculture?

Results of this study indicate that the active ingredients of the biocontrol products tested could colonize chestnut buds but did increase the success rate of grafting. In this study, the treatments were only applied once, and the rates used were based on dipping or drench protocols. Additional applications of biocontrol products during graft healing, and monitoring colonization via microscopy may provide additional information as to the role, if any, these microorganisms could have in the healing process. The collaborating farm in this study assisted with the grafting and advised on treatment application. The chestnut industry is keen on identifying sustainable tools to improve graft success and support additional research to achieve a high graft success rate. Together with the industry, additional experiments have been developed to try to further our understanding of the variables contributing to graft success or failure.