CROWN GALL TREATMENTS FOR PARADOX WALNUT

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ABSTRACT

Heat and chemical Crown Gall treatments were evaluated on artificially inoculated, above ground stem galls on Paradox walnut rootstock. Bare root Paradox rootstocks were planted into a commercial Chandler orchard in Tehama County. Stems were wounded and a mix of three virulent *A. tumefaciens* bacteria were placed on each wound. The resulting galls were used to test eleven Crown Gall treatments.

Removal of bark tissue immediately surrounding the Gall without chemical treatment resulted in 100% Crown Gall control. Chemical treatments eliminated galls when combined with ring and gall removal surgery but did not perform well without surgery. Heat treatment with a propane blowtorch eliminated all heat treated galls. The torch is a fast, easy method to achieve tissue death around the gall. Heat girdling can be a problem on young trees if heat application is careless.

Galls were rated six and twelve months following treatment. Gall evaluation six months following treatment was not enough time to conclude galls were effectively removed. Most likely 12 months are required to evaluate treatment success.

INTRODUCTION

Crown Gall caused by the bacteria *Agrobacterium tumefaciens* remains a problem for California Walnut growers. Over the past several years an apparent increase in Crown Gall incidence on Paradox rooted English walnut trees has caused concern as to how best to treat the problem both preventative and after galls have developed.

Typical recommendations involve not damaging root or crown tissue, pre plant treatment with *Agrobacterium radiobacter* strain K84 and post plant chemical applications following gall removal. Gall treatment is an expensive and time-consuming process. Galls usually develop at or below the soil line and may remain undetected for several years. Once discovered, galls are often large and difficult to treat. A rapid, inexpensive method to treat Crown Gall on walnut rootstock would be useful.

MATERIALS AND METHODS

During the spring of 1998, 20 Paradox rootstocks were planted into a young Tehama County Chandler orchard with micro sprinkler irrigation. Each Paradox rootstock was planted in between an existing tree and was well irrigated by the micro sprinkler system. A commercial walnut nursery donated the rootstocks. Trees were one-inch caliper and all came from the same Paradox source tree. Using the same source tree was the best available way to make certain host rootstocks were equal and uniform as possible.

Rootstocks were planted on May 8, 1998. On July 7, 1998 five above ground wounds were made on each rootstock. Wounds were 12 inches apart and located radially up the trunk so each gall was 90 degrees offset from the gall below it. Potential Crown Gall sites were inoculated using an artificial wound made with a metal hacksaw blade. At each potential gall site an "X" pattern was cut into the bark about 1/8 inch in depth. After wounding a drop of bacterial suspension was placed on the wound. The wound was wrapped in parafilm and then wrapped with survey flagging tape. The bacterial suspension was a mix of three wild type *Agrobacterium tumefaciens* strains collected in California. Two were agrocin sensitive and one was not. The two agrocin sensitive strains were 2516 and 18W-19C. The resistant strain was 18W-7A. Of the ninety-five wounds artificially infected, ninety-two developed healthy, active baseball-sized galls.

Eighty-eight of the available stem galls were used to evaluate Crown Gall treatments. There were enough relatively similar galls for 11 treatments and 8 replicates. The treatments were randomized to avoid any treatment bias due to gall location on the stem. Six materials were evaluated: Breakthru®, commercial bleach, Gallex®, blowtorch, UN32 and #2 diesel (Table 1).

Galls were treated 7/14/99. For treatments without surgery, materials were simply painted onto undisturbed galls. Galls were completely wetted with the material. To ensure good chemical coverage, galls were retreated on 7/29/99.

The ring/removal technique involved using a bleach-sterilized razor knife to remove a ring of bark around the gall perimeter. The ring technique removed 3/8 to 1/2 inch of bark around the gall. The gall itself was removed with a bleach-sterilized pruning saw. The remaining tissue was either treated or left untreated.

The blowtorch treatment used a propane hand held torch similar to what plumbers use to solder copper pipe. The gall was left intact but the gall and the bark surrounding the gall were charred with the torch.

Two untreated controls were included. One replicate with surgery and one replicate without the ring/removal surgery.

Galls were evaluated and measured for size prior to treatment using a caliper. Gall size was estimated by averaging the top to bottom diameter and the left to right diameter. This technique may not accurately describe gall size but was adequate to determine if galls were increasing in size. Treatment effects were evaluated nearly six months after treatment on 12/29/99 and again one year following treatment on 7/14/00. Galls were visually rated for presence/absence and if present, measured for average diameter.

RESULTS/DISCUSSION

The experimental design was not intended to emulate typical below ground galls. Treatments were applied to man made above ground stem galls, which are very easy to treat compared to naturally occurring below ground galls. Never the less, the design provided a level playing field to accurately compare treatments.

Pay particular attention to the two control treatments, one with ring/removal surgery and one without (Table 1). All eight galls were eliminated by using the ring removal strategy alone. Removing 3/8 to 1/2 inch of bark around the gall was adequate to achieve control on young galls. Bacteria from older galls may invade more of the surrounding tissue and may not be as easily isolated. However, this result is consistent with the observation that the critical tissue to treat is the bark tissue surrounding the gall and not the gall itself.

Another reason for success with the ring removal surgery is the sterilized razor knife. The ring cut did not result in re-infection at the cut surface. In addition, cut surfaces may have healed enough to be non-infectable. This scenario may not apply to below ground surgery. Under natural conditions a bactericidal protectant may be necessary.

Treatments 1-4 (bleach and Breakthru®) represent a new idea for Crown Gall control. Research by Steve Lindow at UC Berkeley showed silicone-based spray adjuvants improved copper penetration into walnut buds. The idea was to capitalize on the penetrating ability of Breakthru® to deliver bleach to the target bacteria.

Table 1. Technique and material evaluation for Crown Gall control. Galls were initially treated 7/14/99. Breakthru®, Gallex®, Bleach and Diesel were reapplied 7/29/99 to ensure good material coverage. Galls were rated 12/29/1999 and again 7/14/2000.

Treatment	Surgery Ring/Removal	Live Galls[1] 12/29/99	Live Galls 7/14/00
1. Breakthru® 1%	Yes	0/8	2/8
2. Bleach 10%	Yes	1/8	6/8
3. Breakthru® 1% + Bleach 10%	Yes	0/8	1/8
4. Breakthru® 1% + Bleach 10%	No	7/8	8/8
5. Gallex®	Yes	0/8	0/8
6. Gallex®	No	8/8	8/8
7. Blowtorch	Burned	0/8	0/8
8. UN32	Yes	0/8	0/8
9. #2 Diesel	Yes	0/8	1/8
10. None/Control	Yes	0/8	0/8
11. None/Control	No	8/8	8/8

[1]Numbers represent the number of live galls compared to the total number of galls treated.

For the Breakthru®/Bleach treatments, the ring removal strategy may have been the reason for some success. 1% Breakthu® + 10% bleach treatments resulted in negative gall control when galls were treated without ring and removal surgery. Treatments including ring removal surgery performed better (Table 1). Bleach treatment alone resulted in six live galls out of eight treated. The addition of Breakthru® improved gall control to one live gall out of eight treated. The new silicone-based penetrants may be useful additives to current gall treatments. One problem may be flooding the surgical wound and spreading Crown Gall bacterial to cut surfaces.

Treatments 5 and 6 evaluated Gallex®. Gallex® combined with the ring removal surgery resulted in no live galls out of eight originally treated. Gallex® without ring removal surgery did not eradicate Paradox stem galls. Consistent with the Gallex® label, surgery is an important component of using Gallex® successfully.

Treatments 8 and 9 involved the nitrogen fertilizer UN32 and #2 Diesel plus the ring removal surgery. Both treatments eliminated their respective galls. The ring removal surgery may have been responsible for the success of these two products. In fact, the ring removal surgery may have been the reason for success with the chemical treatments in general.

Treatment number 7 was the heat treatment using the propane blowtorch. Burning the gall without removal and burning the bark tissue surrounding the gall resulted in 100% Crown Gall control. The torch technique represents a fast, relatively easy, safe and economical way to treat Crown Gall on Paradox rootstock. Additional research and experience is necessary to evaluate any long-term treatment effects on walnut tree performance. In this experiment, the heat treatment did not appear to affect tree vigor or performance. However, it is very easy to girdle young trees using a propane torch to burn galls and surrounding tissues. Extreme care is required for successful use of this technique on small trees.

Gall control (Table 1) was initially rated on 12/29/99 and again on 7/14/2000. The number of galls growing on 7/14/2000 increased compared to the 12/29/99 evaluation. This suggests more than six months and perhaps one year following treatment is required to accurately evaluate treatment success.

Measuring average gall diameter at the start and end of the experiment made it possible to estimate annual gall growth potential. Between 7/7/99 and 7/14/00, average gall diameter increase varied between 3.23 cm/year and 13.14 cm/year (Table 2). Average gall diameter increase was 6.15 cm/year. These galls represent the effect of a single site infection. Natural galls may be multiple site infections and different strains of bacteria could grow differently.

Table 2. Gall growth rate for untreated Crown Galls. Values represent the average gall diameter in centimeters. Gall diameter is the average of the top to bottom gall diameter plus the left to right gall diameter.

Untreated Gall #	Gall dia. (cm) 7/7/99	Gall dia. (cm) 12/29/99	Gall dia. (cm) 7/14/00	Gall Annual increase
1	3.89	6.05	9.15	5.26
2	3.07	6.20	7.48	4.41
3	5.47	8.50	10.21	4.74
4	1.94	5.73	10.43	8.49
5	7.31	11.90	20.45	13.14
6	7.52	9.28	10.75	3.23
7	6.86	7.78	10.78	3.92
8	7.06	8.45	13.12	6.06
				Average = 6.15 cm

SUMMARY

For above ground manmade stem galls on Paradox walnut, ring removal surgery eliminated Crown Galls. Treating the margin of the gall was critical for successful eradication. These were above ground stem galls relatively easy to treat. Ring and removal surgery or complete burning around the gall may not be possible for below ground infections. Crown Galls that develop underneath the crown are very difficult to isolate and treat. Early detection and treatment of small galls is essential.

The blowtorch heat treatment was very effective. Essentially the torch is a fast, easy way to destroy live tissue around the gall. Its long-term effect on walnut trees is unknown and young trees can be easily girdled if heat application is careless. Effectiveness on well-established, below ground galls has not been researched. These galls are more challenging to treat because gall margins are difficult to identify.

Average gall increase in diameter was 6.15 cm per year for untreated stem galls.