

Crown gall on walnuts: Assessing origin of infection, disease management and prevention

Elizabeth J. Fichtner, UCCE Farm Advisor, Tulare and Kings Counties

Crown gall is one of the most common diseases observed in commercial walnut orchards in California. The disease, caused by a plant pathogenic bacterium, is easy to identify based on symptomology alone. *Agrobacterium tumefaciens* causes crown gall (Figure 1), but the disease name is a misnomer because the pathogen also induces galls on roots (Figure 1B) and stems (Figure 2). Another related bacterium, *Agrobacterium rhizogenes*, causes hairy root (Figure 1B) a disease that can easily be identified based on symptomology (root proliferation) alone. Crown gall is more prevalent in commercial walnut orchards than hairy root; however, hairy root incidence may be under-estimated simply because symptoms are below ground. Both pathogens may be established in the same orchard, and occasionally may be observed on the same tree (Figure 1B).

The infection process. *Agrobacterium tumefaciens* is a soilborne pathogen that requires a wound to infect plants. The bacterium survives in soil and is a somewhat ubiquitous soil inhabitant. Although the bacterium may be prevalent in orchard soil, only a fraction of the population is pathogenic. Pathogenic isolates contain a circular piece of extra-chromosomal DNA called a plasmid. The plasmid inserts into plant DNA, thus genetically transforming plant cells to proliferate and form a tumor.

Location of symptoms assists in assessing origin of infection. The most commonly asked question about crown gall is “where did it come from?” Unfortunately, it is often difficult to pinpoint the original source of inoculum in an orchard, particularly if tumors are present on roots or at the crown. The pathogen may be present in nursery or orchard soil and it may take months for symptom development after introduction of the pathogen to a wound. As a consequence, it is difficult to determine the timing of initial infection and whether the pathogen was introduced in the nursery or the field, or perhaps even both.

The location of aboveground (aerial) galls may offer some indication of inoculum source and provide lessons to prevent disease spread. When aerial galls form above or below the graft union, the most probable method of pathogen introduction is on infested tools utilized for pruning or removal of suckers (Figure 2A). Tools may become contaminated with the pathogen upon contact with infested soil or by cutting through infested plant material. The removal of rootstock suckers close to the crown may bring loppers or other pruning tools in contact with infested soil or crown gall tumors. The wounds caused by removal of suckers at the base of the tree may also serve as infection courts for inoculum residing in adjacent soil. Infested soil near the base of the tree may be splashed by rain or microsprinklers to the cut surfaces, resulting in infection and future symptom development.

When galls are observed at the graft union (Figure 2B), the most common thought is that the pathogen was transmitted on a dirty grafting knife. There are other potential sources of inoculum, however, that may be responsible for galls formed at the graft union. Budwood may become contaminated with the pathogen upon collection. If the budwood shoot falls on contaminated soil after cutting from the mother tree, the cut surface may become infested. As a result, an infection may form at the graft union. When walnut rootstocks are field grafted at an older age (ie. two-year old), suckering may be more prevalent at or near the graft union (Figure 3A). The removal of these suckers provides opportunities for infection near the graft union and gall formation long after the graft was made (Figure 3B). Last,

asymptomatic seedlings have been found (experimentally) to contain endophytic populations of *A. tumefaciens*. These endophytic populations have the potential to lead to gall formation at secondary stem wound sites (Yakabe, et al. 2012).

Rootstock selection. Another common question is whether the use of a clonal ‘Paradox’ selection offers some protection from crown gall. ‘Paradox’ rootstock is susceptible to crown gall, regardless of whether the rootstock was produced from a seed or via micropropagation (clonal). Plants produced by micropropagation are less likely to become infested with the pathogen in the nursery than seedlings, simply because the clones are produced in axenic (sterile) culture and plantlets are grown up in pots containing sterilized potting medium. The potted clonal plants could still become infected in the nursery if the pathogen is introduced via contaminated tools/boots, etc. or from the splashing of water from contaminated soil.

Additionally, clonally propagated plants that are sold bare-root may become infected if grown out in contaminated soil. ‘Paradox’ seedlings may become infested with the pathogen if the seed contacts contaminated soil upon collection, or if the nursery block is planted in contaminated soil. To mitigate potential for contamination of seedling trees, nurseries tend to shake rootstock seed source trees onto tarps, disinfest the seed, and plant seed in ground with no prior history of infestation. Regardless of rootstock source (seed vs. clone), a low level of crown gall incidence may be anticipated in new plantings simply due to the endemic nature of the pathogen and ease of transmission, despite the vigilance in sanitation at the nursery level.

Clonal selections of ‘Paradox’ are available in the nursery trade. These include ‘Vlach,’ ‘VX211,’ and ‘RX1,’ which are regarded as vigorous, highly vigorous, and moderately vigorous, respectively. All are susceptible to crown gall, but ‘RX1’ may have low to moderate resistance, making it a potential choice rootstock for replant holes contaminated with *A. tumefaciens*.

For information on rootstock terminology utilized in the walnut nursery trade, please visit the following article posted on the UC Fruit and Nut Information Center website: <https://ucanr.edu/datastoreFiles/391-536.pdf>

Influence of crown gall on tree health and productivity. Many walnut trees live to maturity even with crown gall infection; however, infections that girdle the tree may cause early mortality. Crown gall is associated with reduction in tree size and yield; the higher the severity of the disease (ie. percent of circumference of the tree affected), the smaller the tree diameter and yield (Yaghmour, et al. 2016; Olson and Buchner, 2001). Crown gall may also predispose trees to future damage by pests and diseases (Fichtner, 2011; Yaghmour, et al. 2016).

Treatment of crown gall in the field. Removal of galls from infected trees is time-consuming and expensive. A decision on whether to rogue infected trees and replant or remove the tumors is determined by the extent of galling and age of the tree. The decision can be aided by exposing the gall with compressed air to better judge the extent of galling (Figure 4A). If a decision is made to remove the gall it can be surgically removed (Figure 4B), and surrounding tissue can be disinfected. On trees with galls colonizing three-fourths the perimeter of the tree, heat treatment has been found to provide better control than surgery followed by chemical treatment (Olson and Buchner, 2001). Unfortunately, the exact amount of heat required to kill the pathogen while preserving cambium tissue is not known. Excess heat may damage the tree and inhibit recovery (Figure 4C). Guidelines for assessing the value of replanting vs. treating affected trees, as well as the efficacy of various methodologies implemented for gall removal, can be found in the following article: <http://ceglenn.ucanr.edu/files/185675.pdf>.

Chemical and biological treatments for managing crown gall. First and foremost, tools (ie. pruning tools, grafting knives, etc.) should be sanitized between trees to prevent transmission of the pathogen. Sodium hypochlorite solution (bleach) is an inexpensive disinfectant with an LD₁₀₀ of 0.5 ppm for *A. tumefaciens*; however, it is corrosive to tools, may be phytotoxic, and exhibits reduced efficacy in the presence of dissolved and suspended solids. In order to maintain the efficacy of sodium hypochlorite solution for tool sanitization, fresh solution would have to be continually replenished in

the container to prevent the buildup of solids. Cationic surfactants, such as quarternary ammonium compounds, disrupt cell membranes of the pathogen. In a USDA ARS research study, a commercially available cationic surfactant, Physan 20® (Maril Products Incorporated, Tustin, CA), exhibited an LD₁₀₀ of 2 ppm. In this study, the presence of solids in solution had less impact on the efficacy of the cationic surfactants than on sodium hypochlorite, another benefit that these products have over bleach.

Strains of *A. tumefaciens* (Strain K84) (ie. Galltrol A®, AgBioChem, Los Molinos, CA) are sold as biological control agents for protection of plants from pathogenic strains of *A. tumefaciens*. The product is sprayed on the roots prior to planting to ensure colonization of wounds by the biocontrol agent prior to exposure to the pathogen. Research studies have demonstrated the efficacy of Strain K84 for preventing crown gall; however, efficacy of the product may vary based on pathogen population dynamics and environmental conditions.

Another registered product, composed of a mixture of two phenols (ie. Gallex®, AgBioChem, Los Molinos, CA), can be utilized as a post-plant treatment of galls. The product may be applied directly to small galls, or as a disinfectant on exposed areas after gall excision.

For more information on historic research conducted on crown gall, visit the Walnut Research Reports, which can be searched by topic, author, or year on the UC Fruit and Nut Research and Information Center website: <https://ucanr.edu/sites/cawalnut/>. Always read the label of the product being used, and note that all registered pesticides are not necessarily listed on the UC IPM Online website (<http://www.ipm.ucdavis.edu>) or in this newsletter. Always check with certifier to determine which products are organically acceptable.

Select References

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- Olson, B. and Buchner, R. 2001. Field treatment of crown gall on walnut: Different options' effects on growth and productivity. Pacific Nut Producer. July/August 2001.
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- Yakabe, L. E., Parker, S. R., and Kluepfel, D. A. 2012. Cationic surfactants: Potential surface disinfectants to manage *Agrobacterium tumefaciens* biovar 1 contamination of grafting tools. Plant Dis. 96:409-415.
- Yakabe, L.E., Parker, S.R., Kluepfel, D.A. 2012. Role of systemic *Agrobacterium tumefaciens* populations in crown gall incidence on the walnut hybrid rootstock 'Paradox.' Plant Disease 96: 1415-1421.



Figure 1. **A)** Crown gall at the crown of a 'Paradox' walnut rootstock; **B)** Tree affected by both crown gall and hairy root.



Figure 2. Crown gall above the graft union (**A**) and at the graft union (**B**). Red arrows indicate location of graft union.



Figure 3. These clonal ‘Paradox’ rootstocks were planted in an orchard formerly containing crown gall-infected cherry. The trees were field grafted in the second leaf and exhibited excess suckering (**A**) on the rootstock. As these suckers were cut, loppers occasionally touched infested soil, suggesting that inoculum causing galls at the graft union (**B**) may have originated within the orchard.

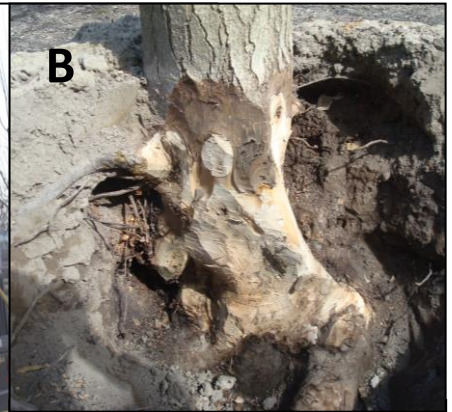


Figure 4. An air spade can be used to expose the crown of the tree prior to gall excision (**A**). The gall can be cut off (**B**) and the resulting wound may be cauterized by flaming with a propane torch. Excessive heat may be both unsightly, leaving charred tissue and resulting in damage to the cambium (**C**).

2019 Statewide Pistachio Day

Visalia Convention Center

Wednesday, January 16

Register at:

<https://ucanr.edu/survey/survey.cfm?surveynumber=25680>

Agenda

- 8:00 am **Welcome and Announcements**
Elizabeth Fichtner, UCCE Farm Advisor, Tulare County, Pistachio Day Chair
Moderator: Phoebe Gordon, Farm Advisor, Madera County
- 8:10 **Industry Update** – Bob Klein, Research Director, California Pistachio Research Board

SESSION 1

Moderator: Elizabeth Fichtner, UC ANR Cooperative Extension Advisor, Tulare County

- 8:30 **Managing Groundwater Quality in Pistachios** - Thomas Harter, UC Cooperative Extension Specialist, Department of Land Air and Water Resources, UC Davis
- 9:00 **25 Years of Salinity Research: What We Know** - Louise Ferguson, UC Cooperative Extension Specialist, Department of Plant Sciences, UC Davis
- 9:20 **A New Technology for Determining Salinity** - Blake Sanden, Farm Advisor Emeritus, Kern County
- 9:30 **Choosing Reclamation Amendments and Rates for Effective Salinity Management**
Mae Culumber, Farm Advisor, Fresno, County
- 10:00 **Break**

SESSION 2

Moderator: Bruce Lampinen, UC Cooperative Extension Specialist, Department of Plant Sciences, UC Davis

- 10:30 **Pistachio Potassium Needs, Application and Availability** - Phoebe Gordon, Farm Advisor, Madera County
- 11:00 **Understanding the Pistachio Tree's Response to Mechanical and Hand Pruning**
Bob Beede, Farm Advisor Emeritus, Kings County
- 11:30 **Growing and Producing Golden Hills Pistachios** - Craig Kallsen, Farm Advisor, Kern County
- 12:00 pm Lunch

SESSION 3: Integrated Pest Management (IPM)

Moderator: Houston Wilson, Extension Entomologist, Kearney Agricultural Research and Extension Center, Parlier

- 1:00 **Pest Management in Young Orchards: Ants, Mealy Bugs, Aphids, Pacific Mite, Darkling Ground Beetle** - Kris Tollerup, Area Entomology Farm Advisor, Kearney Agriculture Research and Extension Center, Parlier
- 1:30 **AF 36** - Themis Michailides, Professor, Department of Plant Pathology, UC Davis and Kearney Agriculture Research and Extension Center, Parlier
- 2:00 Break
- 2:30 **Insect Management Update: Gill's Mealybug, BMSB and Mating Disruption for NOW**
David Haviland, Entomology Farm Advisor, Kern County
- 3:00 **Navel Orangeworm Management: Nut Susceptibility, Insecticides and Sanitation**
Bradley S. Higbee, Field Research and Development Manager, Trece Inc.
- 3:30 **Evaluating Performance of Irradiated Navel Orangeworm for Sterile Insect Program**
Houston Wilson, Extension Entomologist, Kearney Agriculture Research and Extension Center, Parlier
- 4:00 Adjourn

**50th TRI-COUNTY WALNUT DAY - Wyndham Visalia
Thursday, February 7, 2019**

REGISTRATION

- 7:00 a.m. **REGISTRATION**
Coffee and Danish Courtesy of California Walnut Commission/Walnut Board
Moderator: Elizabeth Fichtner, UCCE Farm Advisor, Tulare and Kings Counties
- 8:00 **Welcome Walnut Growers, PCAs, and Members of Allied Industries**
Elizabeth Fichtner, UCCE Farm Advisor
- 8:05 **Working for the Future**
California Walnut Commission
- 8:30 **The Call of the Wild: Taming the Sleeping Dragon (Botryosphaeria and Phomopsis)**
Themis Michailides, Professor, Dept. of Plant Pathology, UC Davis
- 9:00 **Biology and management of walnut husk fly**
R.A. Van Steenwyk, Research Entomologist and emeritus, Dept. E.S.P.M. UC Berkeley
- 9:30 **Update on training walnut during the canopy development phase**
Bruce Lampinen, CE Specialist, Dept. of Plant Sciences, UC Davis
- 10:00 **Break**
Moderator: Mohammad Yaghmour, UCCE Farm Advisor, Kern County
- 10:30 **Whole orchard recycling and the effect on second generation tree growth, yield, fertility, and replant disease**
Brent Holtz, UCCE Farm Advisor and County Director, San Joaquin County
- 11:00 **Water Management in Walnuts: Spotlight on Early Season**
Allan Fulton, UCCE Farm Advisor, Tehama, Glenn, Colusa, Shasta Counties
- 11:30 **Applying crown gall research-based knowledge to orchard management**
Elizabeth Fichtner, UCCE Farm Advisor Tulare and Kings Counties.
- 12:00 p.m. **Lunch graciously provided by our sponsors**
Continuing Education Credit Requested
1.0 hours of PCA (Other)
3.5 hours of CCA

**LUNCHEON SPACE IS LIMITED
TO FIRST 240 REGISTRANTS**

Option 1 (\$15): Register Online by 2/6/2019
<http://ucanr.edu/tcwd2019>

Option 2 (\$15): Register by mail by 2/4/2019

Please detach and mail this form with a check made payable to **UC REGENTS**

Mail to: UC Cooperative Extension
TCWD
4437B S LASPINA ST
TULARE CA 93274-9537

Name: _____

Number of attendees in party: _____

Amount Enclosed (\$15 per person) _____

Company: _____

Address: _____

City/State/Zip: _____

Phone: _____

Option 3 (\$20): Register at the door; checks and cash accepted. (Lunch not guaranteed when paying at the door)

In a Nutshell

December 2018

Elizabeth Fichtner, Mohammad Yaghmour, Phoebe Gordon, Mae Culumber
Farm Advisors

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