CALIFORNIA DEPARTMENT OF FOOD & AGRICULTURE

California Pest Rating Proposal for

Rhizobium radiobacter (Beijerinck & van Delden 1902) Young et al. 2001

Crown gall

Current Pest Rating: C

Proposed Pest Rating: C

Domain: Bacteria; Phylum: Proteobacteria

Class: Alphaproteobacteria; Order: Rhizobiales

Family: Rhizobiaceae

Comment Period: 7/21/2020 through 9/4/2020

Initiating Event:

On August 9, 2019, USDA-APHIS published a list of "Native and Naturalized Plant Pests Permitted by Regulation". Interstate movement of these plant pests is no longer federally regulated within the 48 contiguous United States. There are 49 plant pathogens (bacteria, fungi, viruses, and nematodes) on this list. California may choose to continue to regulate movement of some or all these pathogens into and within the state. In order to assess the needs and potential requirements to issue a state permit, a formal risk analysis for *Rhizobium radiobacter* is given herein and a permanent pest rating is proposed.

History & Status:

Background:

In the early 1890s, crown gall disease was shown to be caused by a bacterium by USDA plant pathologist Erwin F. Smith. It was thought to be similar or related to cancerous tumors of humans and animals. In the late 1970s and in the 1980s, detailed studies were made to better understand the mechanisms of presumed "plant cancer".

Bacterial infections caused by *R. radiobacter* result in the production of undifferentiated cells in galls (tumors), partially organized teratomas, or hairy roots on plants. Research showed this bacterium, known at the time as *Agrobacterium tumefaciens*, induces tumor formation in plants by transferring a

single-stranded segment of T-DNA into plant cells via the Ti plasmid. The T-DNA becomes incorporated into the plant genome and is transcribed by the infected plant cell. The T-DNA contains several genes related to plant growth regulators, including one that codes for an auxin and another that codes for a cytokinin. When these genes are expressed by the plant cell, the growth regulators they produce lead to uncontrolled enlargement and division of affected plant cells, resulting in the galls. The integrated T-DNA also contains genes that code for substances known as opines. Transformed plant cells produce opines, which are used by the intercellularly growing bacteria as a unique and specific source of food for themselves alone (Agrios, 2005; Cato, 2001).

Although crown gall tumors have certain histological similarities to cancers in humans and animals, many fundamental differences exist, and it is no longer considered to be similar enough to be called plant-cancer. However, the discovery that *R. radiobacter* acts as a type of natural "genetic engineer" of plants led to the use of this bacterium as a tool for the transfer of DNA segments coding for desirable characteristics into plants. This formed the basis of powerful biotechnology for a better understanding of plants and host-pathogen interactions (Agrios, 2005; Cato, 2001).

The genus *Agrobacterium* contained five species of soil-inhabiting bacteria. Four were plant pathogens capable of inducing tumorigenic reactions in a wide range of host plant species. These were named *Agrobacterium tumefaciens*, *A. rhizogenes*, *A. vitis*, and *A. rubi* based on their symptoms and host range. In contrast, the fifth species, *A. radiobacter*, was indistinguishable phenotypically from *A. tumefaciens*, except it was non-pathogenic and lacked the Ti plasmid, which is associated with tumor induction in plants (Sawada et al., 1993). These *Agrobacterium* species, together with *Allorhizobium undicola*, were reclassified into the genus *Rhizobium* based on comparative 16S rRNA gene analyses almost 20 years ago (Young et al., 2001). The new combinations are *Rhizobium radiobacter*, *R. rhizogenes*, *R. vitis*, *R. rubi*, and *R. undicola*. *Rhizobium radiobacter* contains strains formerly assigned to *A. tumefaciens* (with Ti-plasmid) and *A. radiobacter* (without Ti-plasmid). This new taxonomy has not been universally accepted and the names *A. tumefaciens* and *A. radiobacter* are still widely in use.

Hosts: Crown gall affects woody and herbaceous plants belonging to 140 genera of more than 60 families. In California it has been found on pome and stone fruit trees, plus walnuts, brambles, ornamentals, and grapes (CABI-CPC, 2020; French, 1989).

Symptoms: Crown gall first appears as small, round, whitish, soft overgrowths on the stem and roots, particularly just below the soil line. Callus tissue, which is soft and easily wounded, can be a common site of infection and galls can initially resemble normal plant callus tissue. Galls are not limited to the crown and can form on the roots, stems, leaves, ears, tassels, and petioles. The galls appear as irregular swellings and may surround the stem or root or may lie outside, but close to the outer surface, connected by a narrow band of tissue. Some galls are spongy and may crumble or become detached from the plant. As they enlarge, the surface of the gall becomes convoluted, and the outer tissues become dark brown due to the death and decay of the exterior cells, and some become woody and hard, looking knobby or knotty. Crown galls can grow up to 30 cm in diameter. Multiple galls may occur on the same root or stem, continuously or in bunches. Although most often associated with soil or near the soil line, galls have been observed on vines more than a meter above the ground, and on

branches of trees, on petioles, and on leaf veins. Aerial galls are common on grapes and caneberries. Marguerite daisies, chrysanthemums, and grapevines can become infected systemically. Occasionally, galls have been observed on field crops such as cotton, sugar beets, tomatoes, beans, and alfalfa (Cato, 2001). Gall tissue is disorganized growth with an enlarged cambium layer. Vascular bundles in the tumors are ineffective; the water and nourishment that the altered cells are able to obtain can carry them only to a certain developmental point, after which growth stops. Movement of water and nutrients through galls is severely impaired. Affected plants may become stunted, with small, chlorotic leaves. Later, decay sets in, and the necrotic tissues are sloughed off. In some cases, the entire tumor regresses and does not reappear. More often, however, some portion of the tumor remains alive and forms additional tumor tissue during the same or the following season. Plants can become more susceptible to adverse environmental conditions, especially winter injury, and are more prone to breaking at the crown or the spot of a gall.

Galls can form quickly as they are a transformation of normal plant cells to tumor cells; they are not made up of bacterial cells. The transformed cells synthesize opines, which can be utilized only by the pathogen. The genetic change to the host cell redirects the metabolic activities of the cells to no longer produce substances beneficial to the plant. These tumor cells will continue to grow and divide independently of the pathogen, and their organization, rate of growth, and rate of division can no longer be controlled by the host plant.

Transmission: Rhizobium radiobacter enters plants through fresh wounds, often caused during handling in the nursery or when transplanting. The bacteria can also infect newly emerging roots through growth cracks and wounds caused by sucker removal or damage from string trimmers and mowing and disking machines for weed removal in orchards and vineyards. The bacteria do not invade cells but do attach to plant cell walls. Their T-DNA virulence region cuts out a single strand of the T-DNA from the Ti plasmid and transfers it into the plant cell. The T-DNA then becomes integrated into the plant DNA and subsequent gene expression leads to the synthesis of auxins and cytokinins, which transform normal plant cells into tumor cells. The smooth and soft young tumors are easily injured and attacked by insects and saprophytic microbes. Breakdown of these tissues releases crown gall bacteria into the soil, where they can be carried in the water and infect new plants. The bacteria reproduce in galled tissue and may slough off, where they can survive in soil for long periods of time (Agrios, 2005; Cato, 2001).

Damage Potential: Many agricultural areas are already contaminated with *R. radiobacter,* which lives in the soil as a saprotroph, either as an endemic or holding over from previously infested crops. Crown gall disease is most damaging to trees because their galls are perennial and increase in size with growth of the tree. Plants with crown galls or galls on their main roots grow poorly and their yields are reduced. Severely infected plants or vines may die (Schroth et al., 1988). When galls form on the lower stem and main roots of nursery plants, they are unsalable. Susceptible nursery stock should not be planted in fields known to be infested with the pathogen as losses can approach 100% when infected blocks need to be culled. Because the bacterium enters only through relatively fresh wounds, wounding of the crowns and roots during cultivation should be avoided and root-chewing insects in the nursery should be controlled.

Excellent biological control of crown gall can be obtained inoculating plants prior to infection with *R. radiobacter* K84. This strain is antagonistic to most other strains of *R. radiobacter* but is not a plant-pathogen. It establishes itself on the surface of plant tissues, where it produces the bacteriocin, agrocin 84. Because of concerns that the agrocin production genes could be transferred to pathogenic strains, a second biocontrol strain, K-1026, is now used because it lacks the ability to transfer agrocin to pathogenic *Rhizobium* strains (Moore, 1980; Cato, 2001; Jones and Kerr, 1989).

Worldwide Distribution: Africa: *Algeria, Egypt, Ethiopia, Kenya, Libya, Morocco, Mozambique, Seychelles, Somalia, South Africa, Tanzania, Uganda, Zambia, Zimbabwe*; America: *Argentina, Bermuda, Bolivia, Brazil, Canada, Chile, Colombia, Cuba, French Guiana, Guadeloupe, Guyana, Jamaica, Mexico, Peru, Puerto Rico, United States, Uruguay, Venezuela*; Asia: *Afghanistan, China, India, Indonesia, Iran, Israel, Japan, Dem. People's Republic of Korea, Republic of Korea, Lebanon, Malaysia, Saudi Arabia, Sri Lanka, Syria*; Europe: *Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Montenegro, Netherlands, Norway, Poland, Romania, Russia, Serbia, Slovakia, Spain, Sweden, Switzerland, Turkey, Ukraine, United Kingdom;* Oceania: *Australia, New Zealand*. (CABI-CPC, 2020)

Official Control: USDA harmful organism list for Bolivia, Canada, Colombia, Egypt, El Salvador, French Polynesia, Grenada, Guatemala Honduras, Iceland, India, Jordan, Madagascar, Mexico, Morocco, New Caledonia, Nicaragua Norway, Panama, Syrian Arab Republic, Taiwan, Tunisia (USDA PCIT, 2020). It is a regulated non-quarantine pest in Egypt, and on the A2 list for Bahrain and Jordan (EPPO, 2020). Whether official quarantines are in regulation or not, any shipment of plants that has crown gall at the point of destination will probably be rejected (CABI-CPC, 2020).

California Distribution: Widespread statewide, on tree fruit, small fruit, and herbaceous and woody ornamentals (French, 1989; CDFA PDR database)

California Interceptions:

The risk *Rhizobium radiobacter* would pose to California is evaluated below.

Consequences of Introduction:

1) **Climate/Host Interaction:** Climate is not considered a limiting factor as *R. radiobacter* is found closely associated with the rhizosphere soil of its hosts worldwide.

   Evaluate if the pest would have suitable hosts and climate to establish in California.

   Score: **3**

   - Low (1) Not likely to establish in California; or likely to establish in very limited areas.
   - Medium (2) may be able to establish in a larger but limited part of California.
   - **High (3) likely to establish a widespread distribution in California.**

2) **Known Pest Host Range:** The host range of this pathogen is very large, including herbaceous and woody annual and perennial plants and trees in many families.

Evaluate the host range of the pest.

Score: 3

- Low (1) has a very limited host range.
- Medium (2) has a moderate host range.
- **High (3) has a wide host range.**

3) **Pest Reproductive Potential:** *Rhizobium radiobacter* can survive in the soil in nurseries and orchards in the absence of a host. Strict sanitation is necessary to protect plants during propagation as wounded tissues are very susceptible to infection. Irrigation water and contaminated soil and equipment can spread the pathogen. Movement of infected planting material has facilitated the worldwide spread of the disease.

Evaluate the natural and artificial dispersal potential of the pest.

Score: 3

- Low (1) does not have high reproductive or dispersal potential.
- Medium (2) has either high reproductive or dispersal potential.
- **High (3) has both high reproduction and dispersal potential.**

4) **Economic Impact:** This pathogen can cause severe damage to young plants, especially trees. There are no curative treatments once a plant is infected, and once introduced into the soil, it can be difficult to eradicate the bacterium. Nurseries must practice strict sanitation to avoid introduction during propagation such as root pruning or grafting, as wounded tissues are very susceptible to infection.

Evaluate the economic impact of the pest to California using the criteria below.

Economic Impact: A, B, C, D, G

A. The pest could lower crop yield.
B. The pest could lower crop value (includes increasing crop production costs).
C. The pest could trigger the loss of markets (includes quarantines).
D. The pest could negatively change normal cultural practices.
E. The pest can vector, or is vectored, by another pestiferous organism.
F. The organism is injurious or poisonous to agriculturally important animals.
G. The organism can interfere with the delivery or supply of water for agricultural uses.

Economic Impact Score: **3**

- Low (1) causes 0 or 1 of these impacts.
- Medium (2) causes 2 of these impacts.
- **High (3) causes 3 or more of these impacts.**

5) **Environmental Impact:** *Rhizobium radiobacter* is a ubiquitous inhabitant of rhizosphere soils and plant roots. *Rhizobium* spp. can be recovered from most soils, but pathogenic strains with the Ti plasmid are

rarely isolated from soil or healthy plants. Although native plants can be hosts, reports of damage are not in the literature.

Environmental Impact:

A. The pest could have a significant environmental impact such as lowering biodiversity, disrupting natural communities, or changing ecosystem processes.
B. The pest could directly affect threatened or endangered species.
C. The pest could impact threatened or endangered species by disrupting critical habitats.
D. The pest could trigger additional official or private treatment programs.
E. The pest significantly impacts cultural practices, home/urban gardening or ornamental plantings.

Environmental Impact Score: 1
- Low (1) causes none of the above to occur.
- Medium (2) causes one of the above to occur.
- High (3) causes two or more of the above to occur.

Consequences of Introduction to California for *Rhizobium radiobacter:* High

Add up the total score and include it here. **#**
-Low = 5-8 points
-Medium = 9-12 points
-High = 13-15 points

6) **Post Entry Distribution and Survey Information**: Evaluate the known distribution in California. Only official records identified by a taxonomic expert and supported by voucher specimens deposited in natural history collections should be considered. Pest incursions that have been eradicated, are under eradication, or have been delimited with no further detections should not be included.

Evaluation is 'High'. This pathogen is already widespread in California in diverse cropping and ornamental systems.

Score: -3
-Not established (0) Pest never detected in California or known only from incursions.
-Low (-1) Pest has a localized distribution in California or is established in one suitable climate/host area (region).
-Medium (-2) Pest is widespread in California but not fully established in the endangered area, or pest established in two contiguous suitable climate/host areas.
-High (-3) Pest has fully established in the endangered area, or pest is reported in more than two contiguous or non-contiguous suitable climate/host areas.

7) **The final score is** the consequences of introduction score minus the post entry distribution and survey information score: (Score)

Final Score: *Score of Consequences of Introduction – Score of Post Entry Distribution and Survey Information* **= 10**

Uncertainty:

None.

Conclusion and Rating Justification:

Based on the evidence provided above the proposed rating for *Rhizobium radiobacter* is C.

References:

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USDA Phytosanitary Certificate Issuance and Tracking System, Phytosanitary Export Database (PExD) Harmful Organisms Database Report. *Agrobacterium tumefasciens*. Accessed 7/2/2020

Responsible Party:

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***Comment Period: 7/21/2020 through 9/4/2020**

***NOTE:**

You must be registered and logged in to post a comment. If you have registered and have not received the registration confirmation, please contact us at permits[@]cdfa.ca.gov.

Comment Format:

- Comments should refer to the appropriate California Pest Rating Proposal Form subsection(s) being commented on, as shown below.

  Example Comment:

  Consequences of Introduction: 1. Climate/Host Interaction: [Your comment that relates to "Climate/Host Interaction" here.]

- Posted comments will not be able to be viewed immediately.
- Comments may not be posted if they:

  Contain inappropriate language which is not germane to the pest rating proposal;

Contains defamatory, false, inaccurate, abusive, obscene, pornographic, sexually oriented, threatening, racially offensive, discriminatory or illegal material;

Violates agency regulations prohibiting sexual harassment or other forms of discrimination;

Violates agency regulations prohibiting workplace violence, including threats.

- Comments may be edited prior to posting to ensure they are entirely germane.
- Posted comments shall be those which have been approved in content and posted to the website to be viewed, not just submitted.

Proposed Pest Rating: C
