Is it Crown Gall or Leafy Gall?

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Humphrey Gifford, an early English poet said, “I cannot say the crow is white, But needs must call a spade a spade.” To call a thing by its simplest and best understood name is what is meant by calling a spade a spade. We have found confusion around the plant disease typified by leafy galls and shoot proliferation, and we want to call a spade a spade.

Figure 1. Fasciation (flattened growth) of a pumpkin stem, which may be due to disease, a genetic condition, or injury.

The bacterium *Rhodococcus fascians* causes fasciation, leafy galls and shoot proliferation on plants. These symptoms have been attributed variously to crown gall bacteria (*Agrobacterium tumefaciens*), virus infection, herbicide damage, or eriophyid mite infestation. There is also confusion about what to call the symptoms caused by *R. fascians*. Shoot proliferation and leafy galls are sometimes called “fasciation,” a term also used to refer to tissues that grow into a flattened ribbon-like manner (Figure 1). The root for the word fasciation come from the Latin, *fascia*, to fuse, and refers to a joining of tissues. We will reserve the term fasciation for the ribbon like growth of stems and other organs.

Figure 2. Leafy gall of *Verbascum* at the base of the plant

The terms “leafy gall” and “shoot proliferation” are unfamiliar to many people, but are a good description of what is seen on affected plants. A leafy gall is a mass of buds or short shoots tightly packed together and fused at the base. These may appear beneath the soil or near the soil line at the base of the stem (Figure 2). They may also form in leaf axils (Figure 3), and in fewer cases, near leaf veins. Shoot proliferation is a loose collection of shoots that are larger than the shoots in leafy galls, but smaller than uninfected shoots, which may or may not be fused at the base (Figure 4). Leafy galls and shoot proliferations are quite different than the galls caused by the crown gall bacterium, *A. tumefaciens* (Figure 5). Infection with *A. tumefaciens* causes swelling of tissue into tumors or galls on stems or roots, but these galls do not differentiate into buds or stems. In contrast, leafy galls are well differentiated into easily recognized plant parts.

Figure 3. Leafy galls of *Viola* at leaf axils.

Figure 4. Shoot proliferation of *Iberis*.

Figure 5. Crown gall at the base of a hibiscus cutting.

Both *R. fascians* and *A. tumefaciens* are known to infect herbaceous and woody plants. Both bacteria have a wide host range (over 60 species for *R. fascians*, and hundreds for *A. tumefaciens*). In addition, *R. fascians* infects monocots as well as dicots, unlike *A. tumefaciens*, which infects only dicots. There have been field observations which imply that populations of *R. fascians* may persist for one or two years in soil in which diseased plants have been growing. *R. fascians* will also move in water, although this is a passive process as these bacteria have no ability to move on their own. On infected plants, bacteria are primarily limited to the surfaces of the leaves, petioles and stems, although some underlying cells may become infected. There is no evidence that *R. fascians* can systemically infect plants. The disease is primarily spread by taking cuttings from infected plants, and it is difficult to know if the plants you have are clean, because the bacteria can be present on plants for months before symptoms develop.

Plants affected by *R. fascians* often grow with less vigor, have an abnormally short stature, may produce fewer flowers, and may have less root growth, although this varies with the plant species. There appear to be distinct differences in both the ability of various strains to infect various hosts, and in host response. We have found that, within a genus, some plant species are resistant, while others are susceptible. We have also found that some cultivars are susceptible and others apparently are not.

We first started finding *R. fascians* in plants with shoot proliferations and leafy galls that were submitted to the Oregon State University Plant Clinic for diagnosis. The growers asked us to check for crown gall, and we could easily isolate the crown gall bacteria from affected plants: we collected 536 isolates of *Agrobacterium* from dozens of herbaceous perennials. We inoculated 229 of these *Agrobacterium* isolates onto indicator plants to test for

pathogenicity. Only 26 isolates of *Agrobacterium* were found to be pathogenic, and they were all isolated from plants with fleshy tumors typical of crown gall; they produced only tumors upon inoculation. If *Agrobacterium* had been causing these leafy galls, then these inoculations should have produced leafy galls. It was interesting how frequently we recovered *Agrobacterium* from so many plants, yet most of these strains were incapable of causing disease. Table 1 lists the plants submitted to the clinic with leafy galls or shoot proliferation. Obviously, there is a wide range of plants affected by these symptoms.

On most of the plants we received for diagnosis we were making dual isolations, using one method for *Agrobacterium* and a different method for *R. fascians.* Interestingly, we recovered *R. fascians* much less frequently than we did *Agrobacterium*. However, we found that nearly all the *R. fascians* isolates we obtained were pathogenic. This was demonstrated by the ability of the *R. fascians* isolates to cause shoot proliferation when inoculated onto pea seedlings.

We were interested to see if we could reproduce the original symptoms of leafy galls on plants used in the trade, so we inoculated our *R. fascians* isolates onto 16 different ornamental plants. Pathogenic *Agrobacterium* isolates were also inoculated onto seven plant species for a comparison of symptoms. The *Agrobacterium* isolates produced crown gall-like tumors on the inoculated plants in all cases (Figure

Figure 6. Tumor at the base of an *Achillea* inoculated with *Agrobacterium tumefaciens*.

Table 1. Plants submitted to the Oregon State University Plant Clinic showing symptoms of shoot proliferation or leafy galls

Acanthus	*Leucanthemum*
Aruncus	*Linaria*
Aster	*Monarda*
Asclepias	*Nemesia*
Baptisia	*Nierembergia*
Boltonia	*Oenethera*
Campanula	*Penstemon*
Coreopsis	*Petunia*
Cosmos	*Phlox*
Dianthus	*Polemonium*
Dicentra	*Primula*
Echinacea	*Salvia*
Erodium	*Santolina*
Erysimum	*Scabiosa*
Euphorbia	*Sedum*
Fuchsia	*Stachys*
Gaura	*Stokesia*
Geranium	*Symphyotricum*
Heliopsis	*Thymus*
Heuchera	*Tiarella*
Heucherella	*Tradescantia*
Hosta	*Verbascum*
Iberis	*Verbena*
Lavatera	*Veronica*
Lavendula	*Viola*

6) except for *Echinacea*, which produced multiple shoots arising from crown gall-like tumors. In no case did *Agrobacterium* inoculation result in leafy gall production. In contrast, inoculation with *R. fascians* isolates produced only leafy galls (Figure 7) or shoot proliferation. The division of symptoms was clear: *Agrobacterium* caused crown gall-like tumors and *R. fascians* produced leafy galls and shoot proliferations.

Figure 7. Leafy gall at the base of *Erysimum* inoculated with *Rhodococcus fascians*.

Disease Management

What can be done about leafy gall (due to *R. fascians*) and crown gall (due to *Agrobacterium*)? For one thing, it is important to get an accurate diagnosis. Leafy gall production from *R. fascians* can be mistakenly attributed to other causes, and the control for a bacterial infection will be different than for an eriophyid mite infestation, for example.

Unfortunately, there is no treatment for either *R. fascians* or *Agrobacterium* infection at this time, therefore steps must be taken to **prevent** disease. There is a biological control product for prevention of crown gall called NoGall. When woody cuttings are dipped into this before planting, the biocontrol bacteria present in the formulation prevent tumor formation by preemptively colonizing the cutting, and by producing an antibiotic that is toxic to *Agrobacterium tumefaciens*.

We wanted to know if NoGall would work to prevent crown gall in herbaceous perennials. In research funded through the OAN/ODA, we treated plants of *Bryophyllum daigremontianum* with NoGall, then applied various strains of *Agrobacterium tumefaciens*. We also tested BlightBan, another biocontrol product that produces a toxin effective against Gram negative bacteria. Neither product was effective in reducing the incidence of crown gall tumors in our test plants. We have found that the predominant strains of *Agrobacterium* recovered from herbaceous plants to be different than those recovered from woody plants. This may explain why the NoGall was less effective in herbaceous perennials than it was in woody plants. We also tested NoGall and BlightBan against *R. fascians* infection, but again neither product offered any control.

We have not tested other products for control of *R. fascians*. *R. fascians* colonizes mainly plant surfaces, but it can also penetrate several cell layers below, which means surface treatment will not kill all the bacteria. An effective product would have to have systemic activity. We hope to try other control products in the future. Although there are no good chemical options at this time, good old-fashioned sanitation measures are still effective in preventing leafy gall and crown gall. Control measures for both diseases are listed in the box below.

Control measures for the crown gall (*Agrobacterium*) and leafy gall (*Rhodococcus fascians*) bacteria:

Sanitation

1. Make every effort to start with clean plants. Do not take cuttings from symptomatic plants or plants in close proximity to diseased plants. *Agrobacterium* infection can be systemic in some plants, and plants can also harbor *R. fascians* without showing symptoms. There are also varietal differences in susceptibility to both bacteria; use indexed tissue culture derived plants for those cultivars that appear particularly susceptible.

2. Start with clean planting trays, preferably new. Used ones must be washed free of all organic debris before treating with a disinfectant such as Greenshield, household bleach, or Physan 20.

3. Potting mix or field soil should be pasteurized (60 minutes at 160 F aerated steam) before use.

4. Knives or razor blades should be changed or sterilized between plants during propagation.

5. Keep plants off the greenhouse floor and solid surfaces. Runoff water can disperse the bacteria.

6. Immediately remove and destroy any diseased plants plus any neighboring plants or trays. Plants can be infected with *R. fascians* for up to several weeks prior to symptom development, so even though the plants may look healthy, they could be infected. It's best to toss them out. Clean up and discard all old leaves and other plant debris. Soil can harbor both *Agrobacterium* and *R. fascians*.

Water management

Bacteria need water for movement, infection, and multiplication. Minimize the length of time leaves are wet; apply irrigation under conditions where leaves can dry in 1-2 hours. Good ventilation will help.

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