Figure 1. Multiple buds and partly expanded shoots are typical of a *R. fascians* infection. Shown here is *Veronica spicata* 'Royal Candles', which is particularly susceptible.

Demystifying *Rhodococcus fascians*

Prevention is the grower's best defense against this tenacious pathogen

By Melodie Putnam

Disease caused by the bacterium *Rhodococcus fascians* continues to slip under the radar of some growers. This may be because the symptoms, although identifiable once you become familiar with them, may be mistaken for other things. It is difficult to combat a problem if you do not recognize it for what it is.

We at the OSU Plant Clinic have been working for more than a decade with *R. fascians*. This article will address disease-related questions we have received from growers over the years.

What are the symptoms of disease?

The primary response of plants to infection by the bacterium *R. fascians* is the production of numerous buds that expand only partially (Figure 1), ▶

An ongoing series provided by Oregon State University in collaboration with the United States Department of Agriculture and in partnership with OAN

Figure 2 (left). Leafy gall disease on a *Leucanthemum* plug. Figure 3 (right). A massive crown gall due to *Agrobacterium tumefaciens* at the base of a *Gaillardia* cutting. These galls are quite different from leafy galls due to *Rhodococcus fascians*.

resulting in shoot proliferation. When the buds are even more numerous, a structure called a leafy gall is formed (Figure 2).

Disease from *R. fascians* is often mistaken for that caused by the crown gall bacterium, *Agrobacterium tumefaciens* (also known as *Rhizobium radiobacter*). However, the symptoms are significantly different. Crown gall bacteria cause growth of largely undifferentiated tissue (galls), which are roughly round and cream-colored when fresh (Figure 3).

In contrast, *R. fascians* produces well-differentiated tissues into buds and leaves in tight clusters. This extra growth usually occurs at the base of the plant, but may also be present in leaf axils, and may be mistaken for the outcome of hard or repeated pinching, the effects of growth regulators, or abnormalities that sometimes arise from tissue culture (Figure 4, see Page 36).

Once a plant is infected, there is no cure and the plant must be discarded to prevent spread of disease.

How does the disease get started?

Leafy galls don't occur in a vacuum. In nearly all instances, the disease has been brought into a nursery on propagation material.

In general, material derived from seed is clean, but *R. fascians* can be seed-borne in some crops such as nasturtium and possibly petunia. Once present, the bacteria grow on the surface of plants without causing symptoms, sometimes for an extended period. At some point the bacteria move into the interior, where chemical signaling between the pathogen and plant takes place, triggering symptom development.

Unfortunately for propagators, that "extended period" during which the bacteria are present but not causing disease is variable, and can be as long as several months. This means the bacteria can be more widespread in a greenhouse or field than may be apparent from symptoms alone.

We have detected *R. fascians* in plants still in tissue culture boxes, so tissue culture by itself is no guarantee plants will be clean. Growers interested in obtaining tissue culture material should inquire whether the company producing the plantlets uses safeguards to prevent bacteria from moving with the material they are propagating.

How does *R. fascians* spread?

The primary means of transmission is by clonal propagation of infected material. Once present in a nursery, *R.*

fascians will move via water splash and in flood irrigation systems.

Anecdotal information from growers suggests the bacteria will persist long enough on pruning tools to be transmitted to plants during cutting operations. Growers who use mowers to trim plants have experienced increased problems with disease, especially when the mower blades were not cleaned and disinfected between groups of plants. The bacteria are not known to be wind-borne (for example, there is no aerial phase), nor does it appear to be moved by insects.

Can cuttings be taken from an infected mother plant?

It's not a good idea to do that. Although the bacteria do not become systemic within a plant, we have detected bacteria on stems many inches away from leafy galls.

In greenhouse experiments we were able to recover the bacteria 28 days after they were sprayed onto plants, and the plants looked perfectly healthy the entire time. Taking cuttings from diseased plants is too great a risk. Growers should cut their losses and discard infected plants before they shed bacteria onto surrounding plants.

Which plants are susceptible?

R. fascians has a wide host range and is capable of infecting plants in nearly 50 families, most of which are herbaceous perennials, although a few woody plants are also susceptible.

Certain genera seem particularly prone to infection. These include *Leucanthemum*, *Viola*, *Veronica*, *Lavatera*, *Phlox*, *Petunia*, *Hosta*, *Campanula*, *Iberis* and *Aster*. For a comprehensive list, including cultivars, see http://plant-clinic.bpp.oregonstate.edu/rhodococcus.

How is the disease controlled?

As with other bacterial diseases, prevention is the best cure. No specific products have been found to be effective in curing a diseased plant or preventing disease when bacteria were ▶




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sprayed onto plants. A program of training, sanitation, scouting and roguing will allow quick detection and action.

Growers should familiarize themselves with the symptoms and train their workers to also recognize and report suspect plants. A regular scouting program is advisable, especially when new material is brought in. Once the disease has been confirmed, it is best to:

1. Discard the affected plants;
2. Discard any plants that had contact with the infected plants; and
3. Sanitize the surface on which the plants had been sitting and any other surfaces which had contact with the plants (for example, pruners and hose ends).

Follow up with regular scouting, and repeat steps 1–3, if needed.

Are there any bright spots in the picture?

One big question has been whether *R. fascians* is widely distributed in the environment, including soil. Although *R. fascians* has been recovered from surfaces as diverse as the rind of some brick cheeses, ice cores, and the backs of sheep with fly-induced sores, there has been no clear indication of whether isolates that infect plants are common in the environment.

Is it possible the bacteria are present on a variety of surfaces and can move to plants when the opportunity arises?

To check this, we collected samples from a variety of substrates and hard surfaces to see if we could recover the bacteria. We isolated some nutrient-rich sources, including animal manure and mud, and also from what we thought should be nutrient-poor sources, such as hard surfaces of buildings and equipment. We also collected samples from more conventional places, such as the soil beneath a variety of trees and exterior planting beds.

We collected nearly 100 samples and evaluated more than 1,000 bacteria. Not a single one was *R. fascians*. This was very curious, and we decided to back up and see if the bacteria are even capable of surviving in soil.

Figure 4. A petunia plantlet with abnormal growth as a result of tissue culture propagation. No *Rhodococcus fascians* was detected with either molecular assays or culturing.

The genus *Rhodococcus* is considered an excellent soil resident. *R. fascians* has been reported from soils under northern forests to a desert with furnace-like temperatures. Given that range, it would appear that the bacteria are pretty hardy and would persist in soil for quite a while.

We set out to determine if we could isolate *R. fascians* from soil spiked with the bacteria. We looked at a sandy loam field soil and a common commercial peat-based soilless potting mix. We added live bacteria then tried to recover them after three days.

What we found was a complete surprise — the bacterial numbers started to decline in those three days. When we air-dried the inoculated soil and tried to recover the bacteria, the population had plunged to 1–14 percent of what we had started with.

These experiments were performed in bare soil, and it may be the bacteria can survive only as long as living plant matter is present. We are currently looking at whether the bacteria will survive longer in soils if plant tissue is present.

What does this mean? It means that the bacteria do not appear to be widely distributed in the environment, and that if a grower can exclude infected material from the nursery, there is no reason to be concerned about *R. fascians*.

If the disease does show up, the bacteria are not likely to persist for extended periods in soil free of plant material.

What research is currently under way?

Molecular methods are being used to enlighten investigators about the strategies and mechanisms of biological processes.

Just as examining human DNA allows researchers to gain insights into human disease processes, determining the genetic structure of *R. fascians* is allowing us to determine how the bacterium is able to cause disease. This, in turn, will lead us to find weaknesses in the pro-

cess, which can then provide insights into effective management strategies.

We have been working with Dr. Jeff Chang of OSU's Department of Botany and Plant Pathology to demystify *R. fascians*.

The entire genetic structure of 20 isolates of *R. fascians* was determined. So far we have found that "*Rhodococcus fascians*" is not a single organism, but at least two very different species of bacteria, which possess more genetic variability than was expected. On a practical basis, this genetic mutability can influence whether the bacteria can be detected using molecular methods. Dependence on a single test can result in a misdiagnosis.

Our lab has created three different molecular assays, which target different genes. Over 11 years, we have tested nearly 200 plants using these molecular assays. Some infected plants reacted with only one of the assays and not the other two. Dr. Chang's group is learning the genetic basis for this response. To minimize the possibility of missing an infection, the OSU Plant Clinic uses a combination of molecular and traditional microbiological methods to diagnose plants suspected of having leafy gall disease.

Used together, a molecular approach and an applied approach will allow us to gain an understanding of how *R. fascians* is put together. Eventually, we hope to provide growers with more tools for managing leafy gall disease.

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Resources

For more photos of plants infected with *R. fascians*, see http://plant-clinic.bpp.oregonstate.edu/rhodococcus-hosts.

For photos of plants with crown gall, see http://plant-clinic.bpp.oregonstate.edu/crown-gall.




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