

## Biological control of bacterial fasciation in flowers

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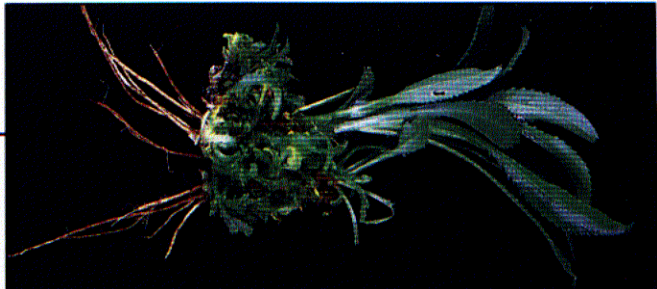
Bacterial fasciation disease, caused by the bacterium *Rhodococcus fascians*, affects a wide range of plant hosts, including many ornamental and landscape plants. At present, the disease is economically important in California on chrysanthemum, impatiens, and hebe, although it can occur at low levels on many other plants and could conceivably become economically important on some of these.

There are presently no effective control measures once the disease has become established on ornamental or landscape plants. Control efforts are therefore directed at prevention by use of pathogen-free propagative materials and by disinfestation of plant materials with antibiotic substances. However, since this pathogen survives well in soil, its elimination on propagative materials does not necessarily prevent the disease. Development of a biological control would offer an economical and environmentally sound alternative for prevention of bacterial fasciation. Control would benefit nursery growers involved in ornamental plant production, as well as homeowners and managers of public landscapes who use plants such as daisies, chrysanthemums, and hebe, which are highly susceptible to this disease.

Biological control methods have been used successfully for other important bacterial diseases of plants, such as crown gall disease and frost damage caused by ice-nucleating bacteria. In these systems, nonpathogenic bacteria are applied to plants in high enough numbers to exclude the growth of pathogenic bacteria. The nonpathogenic bacteria can be natural isolates from soils or plants, or they can be constructed from pathogenic strains by the use of recombinant DNA technology. We have taken both approaches in attempting to develop biological control agents for bacterial fasciation disease.

### Greenhouse tests

Naturally occurring nonpathogenic bacteria were isolated from plants and soil and tested for biological control of bacterial fasciation. We developed a greenhouse test for this pur-



Bacterial fasciation, once established on plants such as Shasta daisy (above) or other ornamentals, cannot be easily controlled. Nonpathogenic mutants developed by genetic manipulation offer some hope of biological control.

pose, in which seedlings were inoculated with potential antagonists and then planted into soil infested with the bacterial fasciation pathogen. This assay closely approximated the situation a grower would encounter when planting propagative materials into a contaminated soil.

None of the natural isolates that we tested provided protection against the fasciation disease. This result was consistent with many other attempts in other systems to use saprophytic organisms for biological control; such organisms often lack the ability to colonize plant surfaces sufficiently to compete with the pathogen. We therefore concentrated on the second approach to the isolation of nonpathogenic bacteria by genetic manipulation of pathogenic strains. Since the bacterial fasciation pathogen has an excellent ability to colonize plant surfaces saprophytically, we should be able to obtain mutants that are no longer pathogenic but still colonize plants.

The best way to obtain such mutants is to identify and mutate specific genes that are essential for pathogenicity. We have successfully identified and cloned a gene that is probably the major pathogenicity gene in *R. fascians*, and we are presently attempting to mutate this gene specifically. Pathogenicity in this bacterium has been associated with the production of a plant hormone, called cytokinin, that causes the shoot proliferation characteristic of the fasciation symptom. We took advantage of research by other workers on the genes that produce cytokinins in another plant pathogen, *Agrobacterium tumefaciens*. We found that the cloned cytokinin gene from *A. tumefaciens* was homologous to a gene in *R. fascians*. Using recombinant DNA methods, we cloned this gene from *R. fascians*, and we are presently characterizing it to confirm its role in cytokinin production.

### Progress in developing mutants

The genetic manipulations necessary to develop nonpathogenic mutants altered in cytokinin production are now in progress, and we hope to test such mutants for biological control in the near future. The use of genetically altered (attenuated) plant pathogenic bacteria for biological control is a logical and promising application of recombinant DNA technology. Although the use of such organisms has met with some public opposition, we believe that this approach will be accepted in the near future as an environmentally sound and economical alternative for disease control.

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