Characterization of the Rose Rosette Disease causal agent: potential for biological control and multiflora rose

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Abstract
Rosa multiflora (multiflora rose), introduced to America from Japan for ornamental purposes 200 years ago, was promoted in Iowa during the mid-1930s as a "living fence" that would help to conserve soil and provide cover for wildlife. Multiflora rose has since naturalized, and today some two million acres of Iowa land are infested with this pest, which renders pastures unusable (dense stands exist in counties south of a line from West Pottawattamie through Winneshiek, affecting the southeastern two-thirds of the state). Cattle avoid the prickly stems, and grass dies beneath its thick growth. Although tillage can control the weed, land in permanent pasture or under the Conservation Reserve Program is at risk for the spread of multiflora rose.

Keywords
Plant Pathology and Microbiology, Biocontrol and Integrated Pest Management, Fruit and vegetables

Disciplines
Agricultural Science | Agriculture | Plant Pathology

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Characterization of the Rose Rosette Disease causal agent: potential for biological control of multiflora rose

Background
Rosa multiflora (multiflora rose), introduced to America from Japan for ornamental purposes 200 years ago, was promoted in Iowa during the mid-1930s as a "living fence" that would help to conserve soil and provide cover for wildlife. Multiflora rose has since naturalized, and today some two million acres of Iowa land are infested with this pest, which renders pastures unusable (dense stands exist in counties south of a line from West Pottawattamie through Winneshiek, affecting the southeastern two-thirds of the state). Cattle avoid the prickly stems, and grass dies beneath its thick growth. Although tillage can control the weed, land in permanent pasture or under the Conservation Reserve Program is at risk for the spread of multiflora rose.

In 1980 a state-initiated campaign (replaced in 1982 by a technical committee of the Iowa Department of Agriculture and Land Stewardship, or IDALS) created a cost-sharing program to help landowners eliminate this problem, and some herbicide manufacturers offered price reductions. However, because conventional control (repeated mowing and herbicide applications) was costly compared to the value of infested land, program participation was low. However, interest in finding alternatives for multiflora rose control remains high among Iowa farmers.

Commercial hybrid rose growers are also disadvantaged by the proliferation of multiflora rose because it harbors pathogens and pests that threaten ornamental roses. However, one such pathogen, Rose Rosette Disease (RRD), also offers potential for reducing and controlling multiflora rose. Endemic to midwestern states, RRD was first found in Iowa in 1986. It can now be found in virtually all multiflora rose stands in Iowa. Multiflora rose appears to be the preferred host of RRD. RRD occurs sporadically, however—it has eliminated multiflora rose stands on several tracts of land in southern Iowa while leaving other stands unaffected. The goal of this project was to assess RRD's potential as a biological control agent for multiflora rose.

This work investigated "augmenting" or artificially intensifying RRD in a stand of multiflora rose via grafting or other means to hasten the development of the disease. One year following infection (natural or augmented), infected plants go into the rosette stage (exhibiting distorted leaves and shortened petioles). Once a plant is infected, it becomes highly conducive to build-up of high populations of the eriophyid (wooly) mite Phyllocoptes fructiphilus, the vector for RRD, which is transported primarily via wind. These mites then spread the disease to other plants in the stand. (As the virus spreads, the plants become susceptible to other diseases as well.)

The mites thrive on RRD-infested plants, which become devoid of starch and high in protein as the disease progresses. This protein attracts the mites, which feed on the infested plants. But because the eriophyid mite does not overwinter well in Iowa, RRD has not spread well enough on its own to reliably eliminate multiflora rose.

The objectives of this work included determining the extent of multiflora rose infestation, the distribution of RRD infection in Iowa, the disease mechanisms in the plant, the host range of the disease agent, the character and identity of the causal agent, the disease's epidemiology, means of disseminating the causal agent to achieve practical, field-scale multi-

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Budget
$47,508 for year one
$11,330 for year two
$49,653 for year three
$8,000 extension for field trials
Transmission of RRD via grafting (note bud at center of photo) was highest from the end of May through mid-July; it was also highest on current season growth.

flora rose control, and risks associated with RRD as a biological control.

**Approach and methods**

Investigators used experimental plots established in 1986 in natural multiflora rose stands in southeast Iowa. Plots in both open and shaded areas consisted of a small group of symptomatic plants to provide an inoculum focus for new infection of 25 nearby healthy plants. The asymptomatic plants were tagged and observed monthly each growing season from 1986 through 1991. Ornamental rose plantings were also observed for RRD symptoms in several Iowa locations.

**Host range studies:** Vegetation in and around the experimental plots was observed for abnormal symptoms. Four additional plots established in 1987 were observed for RRD augmentation studies on rose and effects on related plants. These plots also consisted of one naturally infected multiflora rose plant in the rosette stage as an inoculum source at the center with four each of the test plants planted around the inoculum source. Various rose-related plants (including apple, peach, pear, cherry, strawberry and raspberry) were tested, as were several varieties of rose including multiflora rose. The same plant varieties that were used in the field studies were also tested in the greenhouse. Plants were then observed for development of symptoms for two years.

**Transmission to multiflora rose:** Transmission of RRD to multiflora rose via grafting was studied in both the field and greenhouse. In the field, 20 grafts (two per plant) were made at each of two locations at two-week intervals. Plots were established at Rathbun Reservoir in 1987, in Dallas and Webster counties in 1990, and in Jackson County in 1992 when RRD was found in these areas. Graft transmission was also studied in the greenhouse.

In addition, investigators transported soil from field locations to the greenhouse; they planted a single multiflora rose seedling in each sample to test for RRD transmission via soil. They also examined the potential for transmission via seeds, dodder (a rootless, parasitic vine that establishes itself on a host and draws its food from it), and powdery mildew (which is often severe on infected plants). Investigators also tested whether therapeutic measures such as tetracycline and heat therapy caused remission of RRD symptoms.

Mite populations were monitored May through October of 1990 through 1992; data were collected both from solitary plants and from those growing in large, intermeshed clumps, in pure stands, or in association with other woody plants. Because infected plants are more susceptible than non-symptomatic plants to frost injury, investigators quantitatively determined stored starch and soluble sugars in both. In both field and greenhouse plants, stored starch reserves in the graft-recipient shoots decreased within three weeks after the infected grafts began growing until starch was depleted. Comparison of soluble sugars from multiflora rose plants showed a major decrease in sucrose with corresponding increases in fructose and glucose in symptomatic plants. Electron microscopy revealed that healthy chloroplasts contained large starch granules; those from diseased plants were devoid of starch. Transmission electron microscopy was used to examine diseased plant tissue.

**Field trials:** In 1993 field trials, two transects of ornamental roses were planted in each plot along the lines of prevailing Iowa winds. By recording data on development of mite population density and relative susceptibility of various hybrid roses, investigators could study how augmentation affected the spread of the disease to multiflora rose as well as the danger to hybrid roses.
Findings

Field observations: Investigators characterized three distinct disease stages for RRD by growth pattern, deformity, color, frost susceptibility, and flowering. Numbers of diseased plants increased rapidly for the first three years and then subsided as the supply of symptomless plants was depleted. (Small plants usually die in two to three years; larger ones may survive four or five years.)

Host range: All multiflora rose transplants in the field host-range plots developed symptoms after six weeks. Investigators determined that RRD host range is restricted to rose species; Prairie rose (Rosa setigera) appears to be immune. Several hybrid rose cultivars showed some symptoms. No rose-related plants developed RRD symptoms in the two years of observation, nor were any noted in surrounding vegetation, including the native roses, which are immune. In the greenhouse, no symptoms developed in any of the test plants other than rose, and several rose cultivars appeared either fairly tolerant of or immune to RRD.

Transmission to multiflora rose: Some grafts failed to transmit disease in the field because they lacked buds; others failed due to frost. Viable grafting was highest from the end of May through mid-July; it was also highest on current season growth. Transmission by grafting also failed in plants undergoing drought. Those plants contracting RRD via grafting developed stage-two (rosette) symptoms the following year.

In greenhouse experiments, grafts lacking buds transmitted RRD as effectively as those with buds. Of 120 plants mechanically inoculated with sap, only two developed symptoms. Transmission of RRD via soil, seed, mildew, or dodder did not occur.

Neither tetracycline treatment nor heat therapy prompted remission of symptoms. Production of new growth in graft-infected plants was strongly inhibited; this finding was consistent with observations of field-dug plants.

Mite populations were low in 1990 and 1991 until mid-July; they peaked at the end of August. Investigators speculated that mite populations increase during extended periods of high temperatures. Eriophyid mites may be transported by other arthropods (as well as by wind), particularly those biologically linked to the mite’s preferred host. The reduced spread of RRD in heavily shaded areas suggests that RRD will not totally eliminate every stand of multiflora rose (see Fig. 1), and that in time, infestations will recur.

Colonization of graft-inoculated plants by mites was earlier and most consistent on symptomatic plants located in groups of large plants with intermeshing branches. Isolated plants had the lowest early-season incidence of, and erratic subsequent colonization by, the mite vector.

The causal agent remains unknown. Investigators eliminated all possible disease-causing agents of RRD in multiflora rose except for viruses. Several ribonucleic acids have been consistently associated with symptomatic tissue, which supports the virus theory.

The risk assessment plots indicate that RRD shows no tendency to move more than about 100 meters from the augmentation site. Although based on only one year of data, this finding may be significant because it suggests minimal likelihood of spread to ornamental rose plantings. Only three ornamental rose

Fig. 1. Rate of occurrence of new infections of RRD in plots located in full sun and in shaded areas.
plants have been documented in Iowa as lost to RRD since 1986. In summary, this project produced the data needed to identify optimal timing and sites to efficiently augment existing RRD infections for effective biological control of multiflora rose.

**Implications**

Multiflora rose constitutes a major pest on noncultivated land. Biological control of this pest can reduce reliance on widespread, expensive pesticides that have potentially significant deleterious effects on non-target plants, wildlife, and groundwater.

These findings strongly suggest that RRD has excellent potential as a biological control for multiflora rose infestation. Although widely distributed, RRD in its natural state is sporadic and thus is unlikely to eliminate multiflora rose on any one parcel of land. *Because the causal agent in RRD that is lethal to multiflora rose can be readily transmitted by grafting in the field, with proper placement it can cause rapid, local intensification of RRD that will eliminate up to 98% of a multiflora rose stand in five to six years.* In addition, because the causal agent is endemic and local flora are either highly resistant or immune, problems resulting from its augmentation are unlikely. Investigators will continue to monitor this aspect as technology is transferred to landowners. The augmentation approach can be applied regardless of terrain; the cost is less than $0.50 for materials plus two hours of labor per stand of multiflora rose.

Because the causal agent is not yet identified and characterized, investigators plan to continue their monitoring surveys as well as risk assessment plots for at least three more years. Ultimately, augmentation of RRD as a biological control may significantly improve the quality, productivity, and value of approximately two million acres of Iowa land.

Education and outreach: Both farmers and commercial rose producers have followed this research closely—landowners are eager to apply this system of control on land infested with multiflora rose; ornamental rose growers, on the other hand, have been concerned that their plantings may be damaged by the 1993 field trials in this project. Investigators have addressed these concerns by following IDALS technical committee recommendations to release RRD on a county-by-county basis, making no releases within one-half mile of known cultivated rose plantings without the owner’s permission. Ultimately, elimination of RRD breeding areas would benefit ornamental roses as well.

Investigators have also produced an informational extension bulletin (ISU Pm-1532) and are organizing an international symposium (supported in part by a grant from a major rose producer). In addition to popular press coverage, presentations, and displays at various conferences and statewide events, a number of technical publications on this work are completed or in progress.

Project investigators cooperated with other ISU plant pathologists, an entomologist, a botanist, a biochemist, IDALS, the Iowa Department of Natural Resources, and private landowners. Commercial rose-producing nurseries have contributed nursery stock (for use in the risk assessment plots) as well as monetary support for the symposium. Communication is ongoing with scientists working on similar projects in other states.