Identification and characterization of the Rose Rosette disease causal agent

Abstract: *Rose rosette disease is lethal to multiflora rose, a noxious weed occurring in pastureland in most of Iowa. The potential use of rose rosette disease as a biocontrol agent can be enhanced by grafting infected shoots onto plants in established stands (i.e., augmentation). However, questions arose about whether the disease could be spread to ornamental roses. This study probes the identity of the causal agent for the disease in hopes of determining whether fears of transmission to ornamental roses were valid.*

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$35,145 for one year

Background

Multiflora rose is a vigorous, thorny shrub native to Japan that has been widely planted as an aid to soil conservation, wildlife cover, and as a "living fence." The plant has been more aggressive than first recognized and has become particularly troublesome in the southern half of the U.S. Corn Belt on non-cultivated sites such as pastureland, woodlands, conservation reserve acres, recreational areas, utility rights of way, and wildlife areas. In several states, multiflora rose has been designated as a noxious weed. In Iowa, it is estimated that more than one million acres of pastureland and wildlife areas have sustained significant degradation as a result of colonization by the multiflora rose. The principal areas of infestation are 61 counties (nearly two-thirds of the counties in Iowa).

Costs of conventional control methods are high ($50 to $200 an acre) and there are concerns about water contamination effects of repeated application of herbicides to pasturelands. Biological control methods would be less environmentally costly because they are better targeted and run no risk of added pollution to groundwater. The difficulty with biological control lies in identifying pathogens and pests with minimal or no risk to non-target organisms.

Several pathogens and insects that might be potential biocontrol agents have been reported to infect or infest multiflora rose in the United States. The seed chalcid (insect) attacks and destroys the embryo of the seed but requires too much time (20 years) to be effective. The rose rosette disease (RRD) has proved fatal to infected multiflora rose within two to four years after first observation, depending on the size and complexity of the bush. Electron microscopy of thin sections from the diseased leaf tissue has not presented convincing evidence for the identity of the causal agent, which remains unidentified.

Graft transmission of RRD has been successful in the greenhouse and in the field. Tests for soil and seed transmission of the causal agent of RRD have been negative. In the field, the RRD agent can be transmitted to multiflora rose by the eriophyid mite. Populations of the mite are generally higher (by a factor as great as 17) on diseased multiflora rose plants than on nonsymptomatic plants.

Earliest symptoms of RRD: purple blotches on bottom side of leaf blades and veins

Heavy infestation of RRD; hillside nearly 50 percent covered

The host range of RRD is limited. Symptoms occur on the wood rose and Macartney rose as well as on the four ornamental hybrid rose cultivars tested. A wide range of rose-related species (representatives of the deciduous fruits and ornamentals hardy in Iowa) such as apple, apricot, cherry, peach, pear, plum, black raspberry, strawberry, and potentilla are resistant to the agent. Thus, RRD seems to be restricted to the genus Rosa and not all members of the genus are equally susceptible to the infection.

In the field, RRD occurs naturally, but sporadically in Iowa. Natural spread either remains static or increases slowly with infection rates that are approximately five times slower than rates characteristic of plant viruses. Biological control of multiflora rose can be achieved by augmentation of existing RRD infection. One way to achieve transmission of the disease agent is by the grafting of buds obtained from naturally infected plants to healthy multiflora rose. At the end of the third year following augmentation, nearly two-thirds of the multiflora rose plants show new RRD infection symptoms.

Conditions for the effective use of a biocontrol agent should be based on the demonstration of biosafety and effectiveness. In Iowa, effectiveness has been shown with pasturelands that can be reclaimed from multiflora rose infestations within five to six years after augmentation. As for safety, it appears that the risk of infection for ornamental roses as a result of augmentation is low. Disease gradients from local sources are very steep, with no spread beyond 100 meters. Four to six years after augmentation, no symptoms are observed on ornamental rose plants planted at 20-meter intervals from the inoculation source. Surveys taken between 1992 and 1997 showed that the annual natural RRD infection rate in ornamental rose plantings is one infection per 1,200 plants.

Efforts to confirm the identity of the RRD agent have been stymied. Researchers focused on the identification and characterization of the RRD agent. Pinpointing the causal agent would encourage development of rapid methods for screening hybrid rose and rootstock for disease. The objectives of this project were as follows:

- Characterize the infectious agent of rose rosette disease,
- Clone and sequence disease-associated nucleic acids, and
- Use molecular probes to study disease transmission.

Approach and methods

Several novel approaches were used in attempts to achieve project objectives.

Analysis of protein from infected and healthy multiflora rose tissue. For comparison of protein profiles from healthy and ailing tissue and to identify specific disease-related proteins, rose tissue was extracted with various protein denaturants and examined.

Analysis of RNA/DNA from infected and healthy multiflora rose tissue. Some difficul-

ties were experienced initially until the proper solution could be found to amplify nucleic acid products unique to infected rose tissue. A highly sensitive procedure, representational difference analysis, was then used to search for nucleic acid species that might be specific to multiflora rose symptomatic tissue.

Fatty acid analysis of healthy and infected multiflora rose tissue. Because some infections can lead to altered fatty acid composition (or unusually modified fatty acids) of the host, comparative fatty acid analysis was conducted for healthy and diseased RRD tissue.

Host range analysis. Previous attempts to mechanically transmit the RRD agent have been largely unsuccessful. Infected leaves and buds generally have been used for preparation of the inoculum, but these parts of the plant may contain substances that inhibit transmission. Instead, inoculum was generated from the roots of infected plants, injected into host plants, and thin sections of the inoculated tissue were prepared for light and electron microscopy.

Results and discussion

Several unique and exhaustive approaches have failed to identify the RRD agent.

Results of peptide profiles from protein preparations extracted from healthy and diseased multiflora rose showed quantitative but not qualitative differences. Initially symptomatic tissue was believed to contain a unique peptide or perhaps novel amino acids. However their presence was inconsistent and was later indicated as being present in healthy tissue.

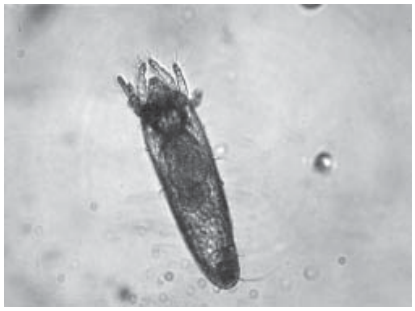
A small nucleic acid species was believed to be associated with diseased tissue. Denaturing gels suggested that it had considerable secondary structure and efforts at end-labeling were not successful. Further attempts to clone the species were not successful because of its very small size, and perhaps because of the secondary structure. No foreign nucleic acid species were detected in multiple tests with RRD-infected multiflora rose tissue.

Previous evidence for presence of phytoplasma-like organisms as the causal agent has been consistently negative. Fatty acid tests were conducted on both sick and healthy tissue and results showed quantitative, but not qualitative, differences between the samples.

In mechanical inoculation experiments involving root extracts from infected rose tissue, pale green spots were produced along the major leaf veins of tobacco plants of various species. Four weeks after inoculation electron microscopy revealed crystalline arrays of virus-like particles, reminiscent of those commonly seen in plant cells infected with polyhedral viruses. Although these particles could be propagated by serial transmission, they have not yet been proven to be the causal agent of RRD. However, this finding may provide a means for characterization of the RRD agent in the future.

Conclusions

The identity of the RRD agent still remains elusive (and has not yet been successfully identified or characterized). There is a possibility that further research on the virus-like particles found in tobacco plants inoculated with RRD disease may lead to discovery of the RRD causal agent.



***Phylocoptes fructiphyllus* vector of RRD agent**

The failure to definitely establish the causal agent does not preclude the safe, effective use of RRD for biocontrol of multiflora rose. Peer-reviewed scientific research has determined that:

- No exotic or novel pathogen or vector is introduced.
- The disease agent has a narrow host range with little potential risk to ornamental roses.
- The RRD agent has not been explicitly identified, but the epidemiology and management of the agent are now well-established.
- The agent is lethal to multiflora rose, and will return infested lands to their original quality and productivity in five to six years without using pesticides or compromising environmental quality.
- Experience with field training sessions in this method of biocontrol has demonstrated the individual landowners can become sufficiently adept at the method with modest (two to four hour) training.
- Unlike with some other biocontrol agents, there are no problems with product stability.

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Impact of results

The RRD agent has been shown to be effective for the biocontrol of multiflora rose and the grafting technique for augmentation of the RRD agent has been easily mastered by land managers. Conditions for biosafety and efficiency as a biocontrol have been demonstrated for the RRD agent.

Although transmission by grafting remains somewhat laborious and time-consuming, it is effective. Successful manipulation of the mite vectors may provide an easier method for inoculating multiflora rose with RRD. In addition, development of disease-specific probes that result from identification and characterization of the RRD agent will provide an efficient means to screen ornamental rose germplasm for disease resistance.

Education and outreach

Four reports about this research have been published in a book of proceedings from an international RRD symposium. Four peer-reviewed publications have appeared in scholarly journals such as *Weed Science* and *Plant Disease*. Articles have been written for Cooperative Extension publications and the *American Rose* magazine.

In addition, tours of research plots were given and five presentations were made on the progress of the research. Education and outreach activities were severely restricted by the ban on dissemination of information through ISU Extension regarding augmentation of RRD for biocontrol of multiflora rose.