Combating Rose Rosette Disease: Are there resistant roses?

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HOW DID THIS SPECIALTY CROP INITIATIVE PROPOSAL EVOLVE?

The proposal was developed in collaboration with the rose industry beginning with the Rose Rosette Conference organized by *Star Roses and Plants* and the Garden Rose Council in April of 2013. At this conference, which brought together trade associations, growers, breeders, landscape management firms, botanical gardens, federal regulatory agencies, biocontrol corporations, consultants, state plant disease diagnostic laboratories and researchers from both the state and federal levels, a plan was developed to direct future research and serve as an outline for the resultant proposal. Over a period of months, a research and extension team was developed to tackle RRD which involved plant pathologists, rose breeders and geneticists, molecular geneticists, an entomologist, agricultural economists, marketing experts and extension personnel. This team is from state, federal and private organizations from Texas, Oklahoma, California, Florida, Tennessee, Delaware, Pennsylvania, Wisconsin and Connecticut (Table 1). The rose industry also committed their resources to the project. We have also been supported by two research grants from the American Rose Society. One went to Dr. Mark Windham of the University of Tennessee, who examined the efficiency of various mite control procedures on RRD, and one went to David Byrne of Texas A&M University to develop a new approach to generate molecular markers in roses. These grants were important in producing preliminary information essential for the development of the proposal.

The goals of this project are to develop and promote the use of sustainable Best Management Practices (BMPs) to manage RRD; identify additional sources of RRD resistance; develop the molecular tools to quickly incorporate RRD resistance and other important traits into elite rose germplasm; and to develop strategies to increase rose sales and overcome market barriers to the use of sustainable rose cultivars. This will lead to well-adapted, long-lived landscape roses which need little care and minimal agricultural chemicals for their production and use in the garden. Producers and breeders benefit from such long market-life cultivars through increased returns for product investment. The breeding tools and approaches developed in this project will benefit breeders and producers by allowing quicker development of adaptable and RRD resistant cultivars. Marketing information obtained in this project will direct the efforts of breeders and nurseries towards the production of roses with highly desired traits, leading to better products for consumers and increased sales and profits of roses. These effects will be quantified in the course of the project.

ROSE ROSETTE DISEASE: WHAT CAUSES IT & HOW IT AFFECTS YOU

The rose is attacked by a plethora of fungal, bacterial and viral diseases which generally cause leaf spotting, distortion, discoloration, and defoliation, reducing the ornamental value of these plants but generally not killing them. Rose Rosette Virus (RRV), however, is currently killing large numbers of garden roses and threatening the future of the garden rose industry.

This disease has been known since the 1940s and is widespread east of the Rocky Mountains. The symptoms for RRD, which may vary with the rose cultivar, commonly include proliferation of lateral shoots causing a witches' broom appearance, unusual thorniness and reddening of these shoots and distorted flowers. Eventually, this leads to stunting, defoliation and death of the plant. If Rose Rosette Virus is suspected, you can consult with your local county extension office for confirmation or send a sample for diagnosis to the Plant Disease and Insect Diagnostic Laboratory at Oklahoma State University ($35.00 per sample).

Although the disease has been known for 70 years, it was

Table 1: Investigators and key collaborators working on the Specialty Crop Initiative Project "Combating Rose Rosette: Short Term and Long Term Approaches"

Name	Specialty	Responsibility	Location
David H. Byrne	Rose Breeding and Genetics	Rose Breeding and Genetics	Department of Horticultural Sciences, Texas A&M University, College Station, TX
Mark Windham	Plant Pathology	Screening for resistance, BMP	Entomology and Plant Pathology Department, University of Tennessee, Knoxville, TN
Brent Pemberton	Plant physiology, horticulturist	Outreach, rose evaluation trials	Texas AgriLife Research and Extension Center, Texas A&M University, Overton, TX
Frank Hale	Entomologist	BMP	Soil, Plant, and Pest Center, The University of Tennessee, Nashville, TN
Ronald Ochoa	Entomologist	Mite-plant interactions	Systematic Entomology, USDA, ARS, Beltsville, MD
Mathews Paret	Plant Pathologist	Diagnostic techniques	North Florida Research and Education Center, Quincy, FL
Francisco Ochoa Corona	Plant Pathologist	Diagnostic techniques	Deprtment of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK
John Hammond	Plant Pathologist	Diagnostic techniques	Floral and Nursery Plants Research Unit, USDA, ARS, Beltsville, MD
Ramon Jordan	Plant Pathologist	Diagnostic techniques	Floral and Nursery Plants Research Unit, USDA, ARS, Beltsville, MD
Patricia Klein	Molecular Biologist	Molecular genetics, marker technology	Department of Horticultural Sciences, Texas A&M University, College Station, TX
Tom Evans	Plant Pathology, Genetics	Screening for resistance	Department of Plant and Soil Sciences, University of Delaware, Newark, DE
Jennifer Olson	Plant Pathologist	Outreach, Diagnostics validation, Screening for resistance	Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK
Kevin Ong	Plant Pathologist	Outreach, Monitoring Network, Diagnostics validation	The Texas Plant Disease Diagnostic Laboratory, Texas AgriLife Extension, College Station, TX

Table 1 continued

Gary Knox	Extension Horticulturist	Outreach	North Florida Research and Education Center, Quincy, FL
Alan Windham	Extension Plant Pathologist	Outreach, Social Media	Soil, Plant, and Pest Center, The University of Tennessee, Nashville, TN
Marco Palma	Extension Economist	Marketing and Economics	Department of Agricultural Economics, Texas A&M University, College Station, TX
Charles Hall	Extension Specialist	Marketing and Economics	Department of Horticultural Sciences, Texas A&M University, College Station, TX
Luis Ribera	Economist-Management	Marketing and Economics	Department of Agricultural Economics, Texas AgriLife Research and Extension Center, Weslaco, TX
Christian Bedard	Rose Breeding	Population creation	Weeks Roses, Pomona, CA
Ping Lim	Rose Breeding	Population creation	Altman Plants, Vista, CA
Jim Sproul	Rose Breeding	Population creation	Roses by Design, Bakersfield, CA
Michele Schreiber	Rose Breeding	Population creation	NovaFlora, West Grove, PA
David Zlesak	Rose Breeding	Population creation	Department of Plant and Earth Sciences, Univeristy of Wisconsin-River Falls. WI
Don Holeman	Rose Breeding	Population creation	Enfield, CT
Marco Bink	Bioinformatics	Genetic analysis	Plant Research Institute, Wageningen, The Netherlands
Eric van de Weg	Bioinformatics	Genetic analysis	Plant Research Institute, Wageningen, The Netherlands

not until 2011 that the causal agent was determined by the Tzanetakis laboratory at the University of Arkansas to be a virus (see Laney et al., 2011). This critical information is accelerating our ability to study and eventually tame this potentially devastating disease.

The disease complex has three important biological components: the Rose Rosette Virus (RRV), the eriophyid mite (*Phyllocoptes fructiphilus*) and the large expanses of naturalized *Rosa multiflora* east of the Rocky Mountains.

RRV is an emaravirus, which is a newly described group of viruses that use RNA instead of DNA for its genetic code. RRV has several pieces of RNA instead of one, is surrounded by a membrane and is transmitted by an airborne eriophyid mite (*Phyllocoptes fructiphilus*). There are a few other emaraviruses that attack corn, fig and mountain ash, all transmitted by eriophyid mites that have been described. Little is known about how the virus is taken up or transmitted by the mite. It is known, however, that this small mite (140-170 microns) feeds on the tender plant tissues and overwinters on the rose plant. The mite can move about 100 meters per year via air currents and has the potential to reproduce very rapidly due to its eight day life cycle and its ability to lay an egg a day. Susceptible roses infected by *viruliferous P. fructiphilus* develop symptoms 30 to 146 days after infection.

This virus/vector pair originated in the western part of the United States and has spread along with *Rosa multiflora*, a very susceptible introduced rose species and now a widespread host of RRD. Thus, *Rosa multiflora* serves as the reservoir of inoculum and vector. In recent years, the disease has spread onto garden roses via the mite vector throughout the central and eastern USA resulting in the death of countless rosebushes. This has led to a reduction in the use of roses in the landscape.

The current best management practices focus on either excluding the virus or preventing its spread by controlling the movement/populations of the mite vector.

Approaches to exclude the virus in your planting are the following.

- Before planting your rose garden, eliminate RRD infected roses (cultivated and wild) from within 100 meters of your garden, as is possible.
- Only plant roses that are free of RRD.
- Monitor your garden on a weekly basis and eliminate any symptomatic plant as soon as it is identified. This lowers the virus level in your garden. Experience in Tennessee in an area with high RRD pressure, the disease can be managed with the replacement of two to four percent of the roses per year.
- If you find RRD in your garden, continue to scout the area for source plants. It is likely that a nearby source will continue to contaminate your landscape if diseased plants are not removed.

Approaches to limit the spread and population levels of the mite are as follows.

- Do not plant roses too close together as this increases the chances that the mites will crawl from one plant to another and spread the virus. Mixed plantings of non-*Rosa* spp. are useful. Both the mites and virus are specific to *Rosa* spp.
- When infected plants or debris is removed from the garden, bag it to prevent the mites from spreading. Do not use a blower to clean the debris out of a rose garden as this will likely spread mites well. Remember, these mites are small and spread by floating in the air.
- Mites can also travel on your clothing so do not go from a highly infested garden to another garden as it is likely you are carrying mites and thus spreading the disease. Mites are thought to survive only about eight hours without a host. Therefore, if equipment, gloves and tools are free of rose debris, they can be reused the next day.
- Prune your roses heavily in late winter to remove the overwintering mites. The prunings should be removed safely so mites do not spread. Apply dormant oil to reduce the numbers of mites still on the plants. Summer oil can be applied throughout the season as needed.

The development of BMPs is divided into three components: Diagnostics, Epidemiology and Breeding. In this update, the search and work towards breeding for RRD resistance will be discussed.

BREEDING ROSES FOR RRD RESISTANCE

Are there cultivated roses that are resistant to RRD? We do not know. Thus, answering this question is a major focus of the next several years.

It was reported decades ago that various North American rose species such as *Rosa palustris* and *Rosa setigera* are resistant to viral infection and that the Asian species *Rosa bracteata* is resistant to the eriophyid mite vector but susceptible to the virus. Unfortunately, we do not have the specific plants used in those studies and it is not wise to assume that all members of any species will be the same. Therefore, we have set up trials in Tennessee with Dr. Mark Windham and in Delaware with Dr. Tom Evans to test roses for resistance to RRD. RRD is common in both locations. Three actions will be done to ensure good infection.

- The plants will be planted close together to encourage mite movement among plants.
- Rose plants already infected by RRD will be planted within the evaluation plot to serve as a source of virus and vector.
- Rose plants for evaluation will be inoculated by placing mite infested shoots from plants showing symptoms of RRD on them.

The plants will be infested several times and monitored for symptom development over three years. However, as documented by Dr. Olson of OSU and others, specific symptoms displayed vary with cultivars. She has been examining RRD symptom development on a wide range of rose cultivars and has observed that the symptoms vary from the typical reddening of shoots, rosette formation, thickened and enhanced prickle development of new shoots followed by decline and plant death, to a slight shoot/leaf distortion. Any plants that do not show clear symptoms will be further studied to determine if the lack of symptoms is due to resistance to the virus, resistance to the mite, and/or a tolerance to the virus.

Our goal is to evaluate resis-

Table 2: Sources of resistance to the Rose Rosette Virus

Diploid species	Tetraploid species
Carolinae *Rosa palustris*	Carolinae *Rosa palustris* *Rosa carolina*
Cinnamomeae *Rosa blanda* *Rosa californica* *Rosa pisocarpa*	Cinnamomeae *Rosa acicularis (4x, 8x)* *Rosa arkansana*
Synstylae *Rosa setigera*	Pimpinellifoliae *Rosa spinosissima*

tance in about 400 roses but given that there are thousands of roses in commerce and in collections, how do we approach selecting the roses for testing? We took a couple of approaches.

- The first step was to obtain plants of the species that have been reported resistant (Table 2). All these are North American species except for *Rosa spinosissima*. We are testing plants from multiple sources for each species and are still looking for more specimens. Thus, if anyone has the ability to collect seed or the plants of various North American or other potentially RRD resistant species, your help would be much appreciated.
- Observational data was collected from plant pathologists, horticulturists and rosarians. In this way we collected more than 600 observations. Those cultivars that were observed with symptoms (300 cultivars) were not considered further and those without clear symptoms or asymptomatic were/are being obtained to test in the project's evaluation trial (Table 3). This group contains about 100 cultivars representing most major rose classes. It should be noted that these cannot be deemed resistant without further testing. In fact, all could be just escapes
- The last criterion was to select a range of cultivars to represent the diversity of the cultivated rose. This group included those with RRD resistant species in their background and representatives from all major rose classes and major breeding programs.

This year we planted about 250 distinct roses in Tennessee and Delaware for evaluation for RRD resistance and in two sites in Texas (College Station and Overton) for evaluation of foliage disease resistance, heat tolerance and horticultural traits. The plan is to plant another 150 rose accessions for evaluation next year.

Concurrently, we are working towards developing the tools to create RRD resistant roses for our gardens. This process involves the following activities:

- Make crosses among susceptible and resistant roses to create the appropriate populations to study the inheritance of resistance and identify genetic markers, unique regions of DNA used to identify and locate genes linked to resistance. In this activity we are working with six other breeders: Don Holeman (Connecticut), David Zlesak (Wisconsin), Michele Scheiber (Pennsylvania, NovaFlora), Ping Lim (Roses by Ping and Altman Plants, California), Jim Sproul (Roses by Design, California) and Christian Bedard (Weeks Roses, California). We will harvest the first set of seed from these crosses this fall.
- Develop a molecular technique called digital genotyping or genotyping by sequencing to generate markers along the length of all the chromosomes. This technique can improve our ability to generate these markers by 100 fold over older techniques. It is amazing how quickly our ability to sequence DNA has improved over the last decade—it reminds me of the speed in which our computing power has improved. Muqing Yan, a doctoral student studying rose breeding and genetics, has developed the methodology to extract high quality DNA for the rose for sequencing. She is currently analyzing the sequence data from four families to construct a genetic map with several thousand markers.
- Use these markers to accelerate our breeding process. This is where the previously created populations come into the picture again. These populations of plants will be assessed for their resistance to RRD as well as characterized for the markers along their chromosomes. What we want to find

is the markers that are in the section of DNA that condition resistance to RRD. To do this we are working with two scientists (Drs. Bink and van de Weg) in the Netherlands at the Plant Research Institute in Wageningen. Their computer program, FlexQTL, combines the field and the lab data with pedigree records to allow us to identify the markers associated with RRD resistance.

So how do these molecular markers help us develop RRD resistant rose cultivars? It tells us if the gene for RRD resistance is in the plant. This saves time and money!

- The marker will tell us if the resistant gene is in the plant. This information can be obtained when the plant is a small seedling in the greenhouse. The alternative to determine if a plant is resistant is a replicated trial in which the plants are inoculated with the virus/mite. This process takes 2-3 years to complete versus 2 months of germinating the seed! That saves a tremendous amount of time and effort. This allows the breeder to look at more seedlings, and in plant breeding the more seedlings you can examine, the greater the chance of success.
- The marker can be used to identify which parents have which resistance genes. This will allow a crossing strategy to be effectively planned to optimize the breeder's chances of getting all the useful resistance genes combined in the seedlings produced.

There is much to be done, but given the coordinated approach that is now in place under the Specialty Crop Research Initiative grant, Combating Rose Rosette Disease: Short Term and Long Term Approaches we should make rapid progress in understanding how best to manage this devastating rose disease.

Table 3: Summary of observational data on rose cultivars and RRD symptoms.

Rose Class	Symptomatic	Suspect	Asymptomatic
China/Tea	18	0	1
Floribunda	28	13	12
Grandiflora	18	2	2
Hybrid multiflora	7	0	0
Hybrid rugosa	5	0	3
Hybrid tea	71	14	10
Hybrid wichuriana	4	0	0
Mini/miniflora	15	0	4
Shrub	59	5	8
Species	7	0	13
All others	68	5	12
Total	300	39	65

SUGGESTED READINGS:

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