Combating Rose Rosette Disease US National Project

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Abstract:

In the past few decades, Rose Rosette Disease (RRD) has spread from its source in western North America through the Mid-West to the East coast. It now threatens to decimate the US rose industry. Garden roses, which form the cornerstone of the multi-billion dollar landscape industry, annually generate wholesale US domestic bare root and container production valued at around $400 million. RRD is caused by an emaravirus, *Rose rosette virus* (RRV), which is transmitted by wind-blown eriophyid mites (*Phyllocoptes fructiphilus*). Unlike other rose diseases, it can kill a rose within two to three years of infection. In collaboration with scientists from 6 states, private rose breeders, the American Rose Society, AmericanHort, and the rose industry, a project was initiated to develop a multidisciplinary approach to control the disease. In the short term, the project team is working to develop Best Management Practices and educational materials based on host, virus, and vector biology to minimize the effects of RRD. Key to this effort is the development of efficient user-friendly diagnostic tools. In the long term, roses are being assessed for resistance to RRD using both replicated field trials and observational data from collaborators. Marker-trait associations for RRD resistance and consistent flower productivity are being identified to move RRD resistance efficiently into elite rose germplasm. Economic and marketing studies are being done to assess the economic effect of RRD on the rose industry, improve our understanding of consumer preferences, and identify barriers to rose sales.

Keywords: Rose rosette virus, Emaravirus, eriophyid mite*, Phyllocoptes*, disease diagnostics, disease resistance, plant breeding, genetic mapping, neuromarketing,

Although the rose is attacked by a variety of fungal, bacterial, and viral diseases, these pathogens generally decrease the ornamental value of the plants by causing leaf spotting, distortion, discoloration and defoliation but do not kill the plant (Horst and Cloyd, 2007). However,

Rose rosette virus (RRV), is currently killing large numbers of garden roses and threatening the future of the US garden rose industry (Laney et al., 2011; Windham et al., 2016).

This disease has been known since the 1940s (Thomas and Scott, 1953) and is widespread east of the Rocky Mountains. The symptoms for Rose rosette disease (RRD), which vary with the rose cultivar, commonly include proliferation of lateral shoots causing a witches broom symptom, unusual thorniness and reddening of these shoots and distorted flowers leading to stunting, defoliation and eventual death of the plant (Debener and Byrne, 2014; Laney et al., 2011; Olson et al., 2015; Ong et al., 2014; Windham et al., 2016). Although the disease has been known for 70 years, its causal agent was not identified until 2011 when the Tzanetakis laboratory at the University of Arkansas identified the *Rose rosette virus* as the causal agent (Laney et al., 2011). This critical information has accelerated 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 rose (Figure 1). RRV is an *Emaravirus*, a newly described group of viruses which use RNA instead of DNA for its genetic code, has several pieces of RNA, is surrounded by a membrane and is transmitted by the wind transported eriophyid mite (*Phyllocoptes fructiphilus*). There are a few other emaraviruses that have been described which attack corn, cowpea, mountain ash and fig, all transmitted by eriophyid mites (Mielke-Ehret and Mühlbach, 2012). Little is known about how the virus is taken up or transmitted by the mite. It is known that this small mite (140-170 microns) feeds on the tender plant tissues and overwinters on the rose plant. The mite can move via air currents about 100 m per year and has the potential to reproduce very rapidly as it has an 8 day life cycle and can lay an egg a day. Susceptible roses infected by viruliferous *P. fructiphilus* develop symptoms 30 to 146 days after infection (Allington et al. 1968; Amrine, 1996; 2002; 2014; Amrine et al., 1988).

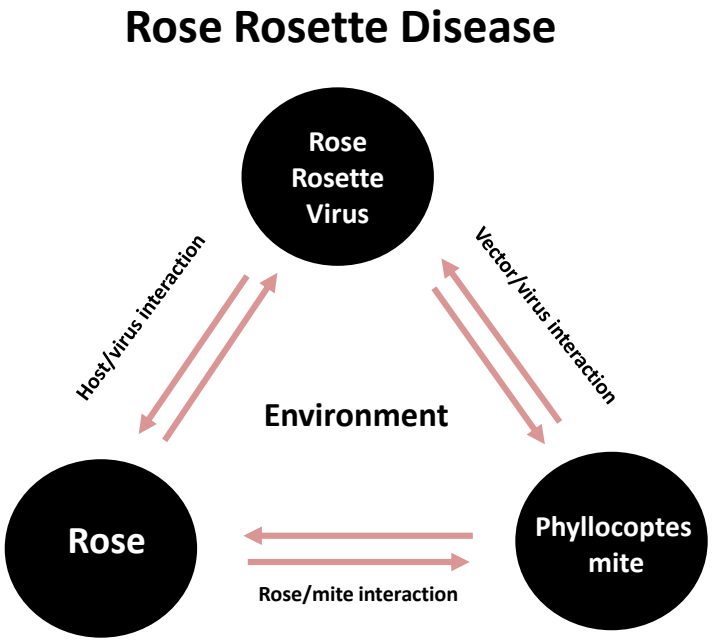

Figure 1. Rose rosette disease complex.

This virus/vector pair originated in the western part of the United States and has spread along with *Rosa multiflora,* a very susceptible rose species introduced and now a widespread host, throughout the eastern seaboard and the Midwest of the country (Amrine et al., 1988; Epstein and Hill, 1999). Thus *Rosa multiflora* serves as the reservoir for the virus 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 rose bushes. Currently, the only control recommendations are to use virus indexed plant material, eliminate infected roses from within 100 m of the planting, eliminate newly infected plants immediately and control the vector by repeated miticide sprays and winter pruning to remove any overwintering mites. Resistance to the virus and/or mite is a necessary tool to manage RRD (Hoy, 2013).

A 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. This conference 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 to develop a plan to direct future research and serve as an outline for the resultant proposal. Over several months, a research and extension team was developed to tackle RRD which involved plant pathologists, rose breeders and geneticists, plant physiologists, 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. The rose industry also committed their resources to the project. The research was also supported by two research grants from the American Rose Society. One went to the University of Tennessee (Mark Windham) to assess the efficiency of various mite control procedures on RRD and the other to Texas A&M University (David Byrne) to develop a new approach to generate molecular markers in rose. These grants were important in developing 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 to manage RRD, to identify additional sources of RRD resistance, to develop the molecular tools to quickly incorporate RRD resistance and other important traits into elite rose germplasm and develop strategies to overcome market barriers to the use of sustainable rose cultivars and increase rose sales. 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 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 RRD resistant and adaptable cultivars. Marketing and educational information obtained in this project will better direct the breeders and nurseries on what product is most highly desired, leading to better products for consumers and increased sales and profits of roses. These effects will be quantified in the course of the project.

Diagnostics

When this project was initiated, the *Rose rosette virus* was only recently described and the diagnostic methods available were reverse transcription polymerase chain reaction (RT-PCR) and

reverse transcription quantitative polymerase chain reaction RT-qPCR based diagnostic methods. RT-PCR was time consuming and somewhat inconsistent in detecting the virus from some symptomatic plants. Real-time RT-qPCR assay is highly sensitive for detection of RRV, but it is expensive and requires well-equipped laboratories. Both the RT-PCR and RT-qPCR cannot be used in a field-based testing for RRV. The objective of this phase of the work was to develop a diagnostic technique that was sensitive, discriminating, easy-to-run and quick for use both in the laboratory and the field. Subsequent work on the virus by our project and others has revealed that the virus is more complex than originally thought and is composed of seven and not four RNA strands (di Bello et al., 2015; Babu et al., 2016a).

The Oklahoma group explored several detection methods such as RT-PCR, the current standard, loop mediated amplification of DNA (LAMP), helicase dependent amplification with self quenched primers (HDA-SqP) and a genus specific discriminatory Emaravirus RT-PCR coupled to high resolution melting analysis (RT-PCR+HRM). The group developed and validated an improved real-time PCR which is reliable and provides results in as little as 3 hours reducing the time for analysis by 50% (Dohal et al., 2016). Subsequent work has emphasized isothermal DNA amplification technologies. Of these, the LAMP approach showed high potential as it uses isothermal amplification carried out at a constant temperature (60–65 °C) which avoids the use of a thermal-cycler and the results can be measured by turbidity, change of color, or by fluorescence using intercalating dyes that allows visualization of the reaction by the naked eye. The detection limit of a diagnostic segment in the P4 gene was 1 pg/µL with plasmid carrying the targeted sequence. Products were visualized by electrophoresis and the visual detection limit using RRV-plasmid in colorimetric reactions with hydroxynaphthol blue (HNB) (120 µM) without BSA and PVP was 0.01 ng/µL. No cross-reactions with cDNA from ten frequently co-infecting viruses (HPV, MSV, INSV, TSWV, GRSV, ApMV, ArMV, PNRSV, TRSV and TMV) was detected. Healthy tissue and non-template controls were included in all reactions.

A second set of LAMP-primers designed to amplify a RRV P3 diagnostic segment were also developed. Positive amplification was obtained with a LAMP master mix from Optigene to a limit of detection of 1fg. This reaction can be done in either thermocycler or dry bath and is substantially faster. The disadvantages of LAMP are that primer design may be laborious because two or three sets of primers are required and that LAMP DNA amplification uses a polymerase with high strand displacement activity. This may make the reaction prone to contaminations that can lead to false positives. Thus the method is friendly to lab trained end users and continues to be optimized for this use, but challenging for non-skilled operators (Salazar-Aguirre et al., 2016).

Helicase dependent amplification (HDA) is an isothermal DNA amplification that does not require temperature cycling. A study of primers sharing similar thermodynamics, but different GC content in their targeted amplification products, was made to assess fluorescence using self-quenched primers (SqP) reacting in HDA standard temperature and chemistry. *Rose rosette virus* (RRV) and another Emaravirus, *High plains wheat mosaic virus* (HPWMV), were virus models. RT-PCR was used as the reference method. Detection limits using a plasmid DNA carrying the target sequences for RRV and HPWMV are 0.0001 ng. This study explored primer design criteria for HDA with SqP for sensitive detection of plant viruses. The use of SqP reduces the cost of qPCR and qtHDA and has acceptable levels of

sensitivity. HDA with SqP bring new possibilities for field deployment since the reaction is conducted at a single temperature (Molina Cardenas et al., 2016).

Another unique diagnostic approach called EDNA (Electronic probe Diagnostic Nucleic acid Analysis) is a relatively rapid diagnostic method when next generation sequencing is available and affordable. This technology is based on the fact that unique DNA signatures of plant viruses can be detected in the sequences of rose tissue samples. Thus specific electronic probes (e-probes) for the detection of 24 viruses infecting rose worldwide were developed. These were tested with a mock positive control database created *in silico* using pathogen sequences retrieved from GenBank to mimic single and multiple infections. The *in silico* study indicated that this would be an effective diagnostic tool.

To further facilitate the virus testing of roses, Oklahoma State University researchers developed an artificial positive control incorporating sequences from RRV as well as multiple other common rose viruses. In addition, a test to broadly detect viruses in the genus *Emaravirus* was developed by designing the EMARA F&R7/8 primer set from the conserved domains of the RNA dependent RNA polymerase of the six species of *Emaravirus* (Olmedo-Velarde et al., 2016).

The Florida group developed a novel probe based, isothermal reverse transcription-recombinase polymerase amplification (RT-exoRPA) assay, using a primer/probe design based on the nucleocapsid gene of the RRV. The assay is highly specific and did not give a positive reaction to other viruses infecting roses belonging to both inclusive and exclusive genera. Dilution assays using the *in vitro* transcript showed that the primer/probe set is highly sensitive, with a detection limit of 1 fg. In addition, a rapid technique for the extraction of viral RNA (< 5 min) has been standardized with RRV infected tissue sources, using PBS-T buffer (pH 7.4), which facilitates the virus adsorption onto the PCR tubes at 4°C for 2 min, followed by denaturation to release the RNA. Different incubation times of the sap at 4°C indicated that the virus can be efficiently adsorbed onto the PCR tubes at shorter times of incubation (15 s to 3 min), while the higher incubation times produced a negative effect on virus adsorption and subsequent RT-exoRPA analysis. RT-exoRPA analysis of the infected plants using the primer/probe indicated that the virus could be detected from leaves, stems, petals, pollen, primary roots and secondary roots. In addition, the assay was efficiently used in the diagnosis of RRV from different rose varieties, collected from different states in the U.S. The entire process, including the extraction can be completed in 25 min (Babu et al., 2016a; 2016b; 2017).

The Maryland group has focused on the serological approach. Thus far polyclonal antibody and McAb 8D2F4 were developed and are being evaluated (primarily in TAS-ELISA) against known healthy and RRV-infected rose samples from a variety of geographical locations. These tests will include evaluation of specificity, sensitivity and reproducibility using different extraction buffers and treatments. The epitope specificity of these antibodies will also be studied. *In silico* consensus alignment of the NP proteins from the 25 RRV isolates was used to identify four to five conserved regions predicted to contain 'good' epitopes for analysis of the epitope-site specificity of the current set of antibodies. To that end, twelve 25-mer synthetic peptides have been produced for analysis, and four key regions of the NP (77-97aa each) have been sub-cloned for expression and serological analysis.

The antibodies will subsequently be evaluated for usage in antibody-based lateral flow devices in collaboration with Agdia, Inc., based on their proprietary Immunostrip platform. Another

potential usage to be examined in collaboration with other grant locations will be to determine if trapping antibodies can increase sensitivity and decrease sample preparation steps of Immunocapture-RT-PCR (IC-RT-PCR).

All three groups are assembling diagnostic reagent kits to be laboratory tested by Jen Olson at the Plant Disease and Insect Diagnostic Laboratory (Oklahoma State University, Stillwater, OK) and Kevin Ong at the Texas Plant Disease Diagnostic Laboratory (Texas A&M University, College Station, TX) for the validation phase of this project. As the validation process develops, the interaction between the developers of the diagnostics and the validators will lead to modifications to improve the robustness of the techniques.

Achieving the stated goals should result in the availability of sensitive, accurate, reliable, and inexpensive molecular, serological, or in the case of IC-RT-PCR, combined serological/molecular tests for the occurrence of RRV suitable for use by high-volume commercial producers of roses, and in the case of Immunostrip lateral flow devices, even for plant collectors and gardeners.

Best management practices

Epidemiology. The first information developed in this project was to show that the frequent approach of controlling RRD in a garden, which was to eliminate the infected branch was ineffective as these plants frequently remained infected. More effective was the rapid bagging and rogueing of symptomatic plants and the use of nonhost barriers, such as *Miscanthus sinensis,* which served to reduce the appearance of symptoms by more than 50% presumably by blocking the spread of the vector mite (Windham et al., 2014; 2016).

As there is a need to count the mites on plants and in the air, the mite count methodology was optimized. Initially, the methodology to count mites on plants was optimized using standard laboratory equipment which may cost more than $US 9,000. Since this is too expensive for nursery and landscape professionals and homeowners that want to monitor mite levels to determine when to apply miticides as a part of their management protocol, a counting protocol/kit and handbook was developed with materials that were readily available that costs less than $100. The kit allows the user to use a cellphone to photograph the eriophyid mites they are counting. Currently these kits are being field-tested by nursery owners, professionals who maintain rose gardens, and private garden owners.

To sample the mites moving via wind currents, a prototype of a mite sampler has been developed. This allows automatic sampling of mites floating in the air to gauge when the mites begin to disperse to new plants and make decisions on the timing of actions to reduce mite populations such as the applications of miticides or the release of predators.

Epidemiology data continues to be collected to determine how the spread of rose rosette disease occurs in different environments. To determine why RRD seems to affect some areas and not others, rose plots have been established in commercial beds of roses in Georgia, Alabama, Mississippi and Louisiana both above and below the lattitude where RRD is either found or not found. These plots are being monitored for the virus, the mite and the environmental conditions. In parallel to the field studies, environmental chambers are used to study eriophyid mite survivability at different temperature/relative humidity ranges and the effects of light intensity on ballooning

behavior of the mite populations. From these data, a model is being developed to predict where RRD will be severe and what areas of the country may escape this disease.

Microscopic examination of 52 rose varieties and *R. multiflora* indicate that these are good hosts for *P. fructiphilus* and that this species is the predominant eriophyid mite on rose. Additional studies are needed on the other wild rose species as field work in Tennessee indicates large differences among species in mite suitability. Low temperature Scanning Electron Microscopy (LT SEM) has shown that the preferred environment of *P. fructiphilus* are the protected regions within the sepals of dissected floral buds, and within the stipules of vegetative buds, where they may be protected from larger predatory mites and other predators. As dense populations of the mites and large numbers of eggs were observed close to the base of glandular hairs, these appear to provide a protective environment for *P. fructiphilus* which presumably act as a physical and chemical barrier to the larger predatory mites and insect predators.

In some leaf areas, mite feeding sites were obvious, with some cells showing multiple puncture wounds consistent with eriophyid mite probe feeding. Some epidermal cells with obvious feeding holes showed apparent signs of deflation, suggestive of significant fluid loss either from direct feeding or subsequent dehydration. The presence of multiple feeding sites in individual cells suggests the possibility that the mites' stylets may become clogged (perhaps as a result of virus-induced changes in the cell – a potential host response to infection). This may cause the mite vector to probe repeatedly to obtain sufficient nutrition, and potentially increase the uptake of virus particles.

*Chemical/biological field control options***.** The application of miticides (bifenthrin, fenpyroximate, spiromesifen and spirotetramat) was found to be effective (one-week spray intervals) in preventing rose rosette symptom development. Ongoing work is determining the optimal spray interval (2, 4, 6 week intervals with 4 previously mentioned miticides), and the effectiveness of additional miticides (abamectin and bifenazate on 2 week schedule), and a novel antiviral compound SP 7788 (SePRO Corp. Carmel, IN 46032) on mite populations and symptom development.

At the USDA, various predator mites (*Neoseiulus spp*. and a smaller mite *Tydeus spp*.) were observed on roses. At the University of Delaware two species of predatory mites (*Neoseiulus californicus* and *Amblyseius andersoni*) were observed under a microscope interacting with *P. fructiphilus* on infected rose shoots with all of the leaves removed. Both mites readily consumed *P. fructiphilus*, indicating their potential as biocontrol agents. Future work will involve quantifying consumption of the eriophyids using a limited number of predators on rose shoots with the leaves intact, which will also indicate if the predators are of a suitable size to enter the refuges of *P. fructiphilus*.

*Search for resistance to RRD***.** When we began this project, only a few species and no cultivated roses were reported resistant to RRD. We took two approaches to find sources of resistance. First was to collect observational data on which roses show symptoms and which do not when growing in gardens with RRD infections. Five hundred and fifty roses were identified as susceptible to RRD and another 50 that have not yet shown symptoms and need to be tested further (Byrne et al., 2015). Formal disease resistance screening was initiated in replicated trials in Tennessee (Mark Windham) and Delaware (Tom Evans, Danielle Novick) and has recently been extended to Oklahoma (Jen Olson) and Texas (Kevin Ong, Maddi Shires). Three sites in Oklahoma

were planted in replicated trials: Perkins with 41 rose cultivars and 44 species hybrids, Tulsa with 140 rose cultivars and 250 seedlings, and Oklahoma City with 6 rose cultivars. Five sites in Texas (Wichita Falls, Dallas, Farmer's Branch, Denton and Cleburne) have been planted over the last two years with rose cultivars, species and species hybrids for screening for RRD resistance. Currently we have about 250 rose cultivars, 50 species accessions, and 250 seedlings from genetic studies in these trials. These trials are actively infested with infected mites to augment the disease pressure that the plants experience. Thus far, 130 of the roses being tested have been confirmed susceptible in either the Tennessee or Delaware trial. As some known susceptible plants have not yet shown RRD symptoms, the remaining plants continue to be infested and observed. In parallel to this, these materials are also being evaluated for resistance to black spot and cercospora, tolerance to heat, and landscape suitability in two trials in Texas. Of the roses being evaluated, 19% show good field resistance to black spot, 69% show good field resistance to cercospora and 9% show good resistance to both diseases.

Eriophyid mite populations were estimated on 18 *Rosa* species every two weeks from May to September in 2016 by destructive sampling of shoots, from which mites were extracted via sieving and then counted using a stereomicroscope. Mite counts were expressed as number of mites per gram of leaf tissue. Five species (*R. arkansana, R. palustris* EB*, R. clinophylla, R. nitida* and *R. wichuriana* Basye ARE) maintained low mite populations, whereas other accessions of *R. wichuriana* and *R. palustris* maintained high mite populations. This indicates intraspecific variability in the resistance to mite reproduction.

Mapping and marker assisted breeding. The genotyping by sequencing protocol has been optimized and used to sequence/identify single nucleotide polymorphisms (SNPs) on about 600 rose genotypes using the strawberry genome sequence as the reference genome. A consensus map with 3,500 SNPs, (~0.25 cM/marker) was constructed by combining the individual genetic maps for five diploid mapping populations. This is the best diploid genetic map reported thus far for the rose. The next steps would be to genotype the rose germplasm for an association study and the diploid/tetraploid mapping populations to create additional consensus maps. With the tetraploid population, it is likely that the rose SNP chip (Koning-Boucoiran et al., 2014) will be used as currently it is not possible to determine the SNP dosage using GBS.

The consensus map, the pedigrees, the genotypic data and the phenotypic information was combined using FlexQTL software, a pedigree based analysis program (Bink et al., 2008; Bink and van Eeumijk, 2009; van de Weg, 2017). This analysis approach is being tested on phenotypic traits such as black spot resistance, cercospora resistance and various flower/architectural traits. As FlexQTL requires high quality genotypic data, this data was further cleaned to remove any inconsistencies which resulted in ~800 markers. The black spot analysis has identified a strong QTL on linkage group 3. As this is the first time FlexQTL has been used with GBS data, we are assessing its usefulness as compared to a SNP chip. As we collect data on the RRD populations, we will use FlexQTL to discover important QTLs associated with RRD resistance.

In the years 2015-2017, TAMU with commercial rose breeding partners (Weeks Roses, Star Roses and Plants, Ball Horticulture, Roses by Design, Roses by Ping, David Zlesak, Don Holeman) have made more than 15,000 hand pollinations and stratified more than 20,000 hybrid seed for this project. The germination of the crosses, as is common with roses, was highly variable. In particular, the crosses with the resistant diploid species (*Rosa palustris, R. setigera* and *R. blanda*) was quite

poor. This suggests that incorporating RRD resistance from these species into the cultivated rose is a long term project for which good markers would be extremely useful. The first set of seedlings from the RRD populations were propagated and planted in replicated trials in Tennessee, Oklahoma and Texas. The second set of seedlings is being propagated and will planted in replicated trials in the spring of 2018. The third set, which we are making now, will be germinated/propagated in 2018 and planted in replicated trials in the spring of 2019. Once the hybrids from the first set flower, backcrosses to the cultivated germplasm will begin.

Consumer preferences and industry barriers to selling roses

The preferences of U.S. consumers are being assessed by both consumer willingness-to-pay experiments using eye tracking and other biometrics to establish consumer valuations for different rose attributes and via rose surveys to consumers, growers and rose professionals. Initial work has indicated that critical traits desired by the consumer and professionals are disease resistance, fragrance, flower color and flower productivity (Byrne, 2015; Waliczek et al., 2015).

To set the stage for identifying market barriers inhibiting the sale of new roses and determining the socioeconomic impacts of RRD research, the team has been analyzing the chronological value chain for nursery growers who produce roses and other important ornamental shrubs. During the first year of the project, the key data collection points were identified and data management protocols established to ensure that the appropriate data are captured during each phase of the project that will be utilized for the economic analyses. Meetings were held with all research team members to (a) identify the appropriate *ex ante* economic cost parameters to assess and (b) develop data collection forms to ensure consistent and compatible data is acquired during the activities of each objective. In year 2, an economic risk-based simulation model was developed to estimate the initial capital investment, production costs, and product prices for the baseline and alternative nursery pest and disease management models. The models are being simulated with representative characteristics of nursery operations and proper (BMP) disease and pest management equipment and protocols for the rose crops being studied.

Information pipeline

Develop a RRD Monitoring Network. Kevin Ong has worked with the University of Georgia Center for Invasive Species to develop a reporting system for RRV using EDDmapS (https://www.eddmaps.org/) and regional verifiers. The E-learning module to train our collaborators is completed and he is currently finishing PSA (public service announcement) for this website (RoseRosette.com) to direct users to utilize the e-learning modules.

Develop national RRD BMP training materials. Jen Olson has developed a Pictorial Guide to Rose Rosette Disease Symptoms (additional images on bugwood.org), a power point presentation with a voice over, and is in the process of developing a series of 5-6 videos on RRD. These will be about 5 minutes in length and will include segments on an overview of RRD, symptoms, management of RRD in the landscape, management of RRD in the nursery/retail outlet, biology of the mite and virus, and possibly an additional segment on preparing samples for testing. All these will be available through the clearing house web site (RoseRosette.com) being developed by Kevin Ong.

The "Combating Rose Rosette Disease" team uses the social media platform, Facebook and Twitter, for outreach. It currently has about 650 (up from 200 in 2015) Facebook followers. Over 2200 unique Facebook users have engaged with the page by interacting with posts by the CRR Team. Content of the CRR Facebook page reached the pages of over 23,000 Facebook users since October 2016.

CONCLUSIONS

Rose rosette disease, caused by *Rose rosette virus* (RRV; genus *Emaravirus*) is a major threat to the rose industry in the U.S. The strategy currently available for disease management is early detection and eradication of the infected plants, thereby limiting its potential spread. Thus, diagnostics for early detection are critical not only for disease management but also for research in viral epidemiology. The project has developed more efficient, sensitive and quicker diagnostics appropriate for laboratory as well as field use. These new diagnostics are now being further validated in the Plant Disease Diagnostic laboratories in Oklahoma and Texas. Achieving the stated goals should result in the availability of sensitive, accurate, reliable, and inexpensive diagnostic tests for the occurrence of RRV suitable for use by high-volume commercial producers of roses, and in the case of lateral flow devices, even for plant collectors and home-owner gardeners.

Epidemiology studies have clearly shown that pruning out infected branches is not an effective approach. This has led to the updating of the Best Management Practice recommendation to include the early rogueing of infected plants. Furthermore, as the suppression of the mite vector via miticides appears to control the disease, mite population monitoring tools that are inexpensive and easy-to-use have been developed so the professional horticulturist to gardeners can monitor mite populations to best time chemical/biological control measures. Over 500 rose accessions have been confirmed to be susceptible to RRD in the field. Nevertheless a few rose species and some cultivated rose cultivars have not yet succumbed to the disease and thus appear resistant to the disease. Some of these have been used to create families for the genetic analysis of the source of resistance. These will be planted in replicated trials at multiple sites for thorough phenotyping of their resistance to RRD but also their resistance to other diseases and key horticultural traits to fully evaluate their use in breeding by others.

Great progress has been made in the development of genotyping by sequencing for the diploid rose. Thus far a high density diploid rose consensus map with 3500 SNP markers has been developed and is currently being used in conjunction with the FlexQTL program to identify important QTLs for black spot resistance and other traits. Although this approach appears to work with diploid rose, we may have to use the more expensive SNP chip when working with tetraploid germplasm. As the assessment for RRD resistance can take 2-3 years, the discovery of useful markers associated with resistance could reduce our breeding cycle by at least 50% and the number of seedlings that need to be grown in the field substantially as it would allow greenhouse screening.

The marketing group has gathered much of the economic data needed, is finalizing the surveys to collect additional information on the industry practices and consumer preferences, and are beginning to do consumer preference experiments using a neuromarketing approach. These studies will guide breeders, producers and marketers by identifying consumer preferences and market barriers to the introduction of new cultivars and increasing sales.

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