



National Clean Plant Network - Roses

DAVID C. ZLESAK
ASSOCIATE PROFESSOR OF HORTICULTURE
UNIVERSITY OF WI-RIVER FALLS
ZLESAK@ROCKETMAIL.COM

The National Clean Plant Network-Roses (NCPN-R) was recently added to the greater National Clean Plant Network (NCPN), a program administered by the United States Department of Agriculture. The NCPN began with the 2008 Farm Bill. It provides resources to develop, maintain, and distribute virus and other pathogen-indexed plant material (plant material clean of known viruses) to propagators of important clonally propagated cultivars of key plant commodities valuable to US agriculture. Most of the crops included under the NCPN efforts are fruit crops (grapes, raspberries, strawberries, stone fruits, etc.), but root and other specialty crops like sweet potatoes and hops are also included. With the most recent Farm Bill in 2014, roses became the seventh group of plants added to NCPN efforts and represents the first ornamental commodity.

NCPN funding (>\$5 million annually) is distributed to primarily university and governmental agency organizations already involved in clean plant efforts. The money is distributed based on competitive proposals and input of each commodity's advisory board. NCPN funding helps to continue work already occurring with the NCPN supported plant groups at these facilities and allows for new, expanded efforts to be undertaken. Leveraging existing facilities and plant collection resources allows for greater impact with the significant, yet limited NCPN funding.

Ensuring healthy plants are being propagated and used in US agriculture will aid in sustaining and improving yield and quality and ultimately protect our national security. With increased international barriers and stricter quarantines, it is becoming increasingly difficult to get new varieties into and out of the US, and sometimes even across state lines. The primary role of the USDA and associated state agencies has been to test for problematic diseases at strategic check points and destroy plant materials found to be infected. The NCPN is a proactive effort allowing the USDA to participate helping generate and make available clean propagation material of valued cultivars to growers.

Helping fund the facilities that are already set up to do this kind of work and expand their efforts is a great way to help sustain them and ensure their efforts continue and expand as needed. NCPN funding should help buffer uncertain ups and downs of other sources of funding. Many of these facilities have: quarantine resources in place, the capacity to test for known problematic diseases, the ability to do therapy on infected cultivars to clean them up of pathogens, and space to maintain clean stock plant collections that can be used to distribute propagation material to industry stakeholders.

Foundation Plant Services (FPS; University of California-Davis) has supported an extensive clean stock rose program since the 1960's through the pioneering efforts of Dr. George Nyland (<http://fps.ucdavis.edu/Web-SitePDFs/Newsletters&Publications/RoseProgramBrochure.pdf>). FPS likely has the largest clean plant collection for roses in the world with 8 rootstock varieties and over 500 cultivars (roses available for distribution are listed at: <http://fps.ucdavis.edu/roseselections.cfm>). FPS has also recently launched a new resource called The FPS Rose Encyclopedia. They list the roses in their collection with their background information along with a growing number of photographs that can be used freely with photo credit (<http://fps.ucdavis.edu/roses/>). FPS also hosts clean stock programs for grapes, sweet potatoes, and strawberries. They have been a key facility within the NCPN from the program's start working with grapes. Over the years the FPS research team has been conducting valuable rose research characterizing new rose viruses, optimizing tissue culture methods to clean infected roses of virus, and documenting the impact virus infection (a single virus or multiple viruses in combination) has on rose propagation rates for industry and plant performance.

A few years ago Dr. Deborah Golino, director of FPS, started the process to include roses in the NCPN. With her great leadership and understanding of how the NCPN works through her participation in the NCPN as the chair for grapes and her work with other food crops as well, NCPN-R has become a reality. The first of the funding to directly support rose efforts was released fall 2015. In 2013 Dr. Golino reached out to Dr. David Byrne at Texas A&M to establish NCPN-R. He serves as the NCPN-R chair and she serves as vice-chair. So far there have been two NCPN-R meetings with stakeholders. The first was Fall 2014 in Tyler, Texas at the ARS Fall Convention. The goal was to broadly introduce the program. The second meeting was held April 2015 at Foundation Plant Services at UC-Davis. Stakeholder representatives in attendance at FPS included: rose breeders, nursery producers, public garden curators, plant disease clinic directors, university researchers, the USDA, and the American Rose Society.

A subset of attendees volunteered to serve on the NCPN-R Advisory Board. I'm grateful for the opportunity to serve on the board representing university researchers and breeders. The goal of the April meeting was to help establish the framework for how we could best move forward setting our specific priorities to seek funding and also help everyone learn how the NCPN is organized and the resources at hand. At this meeting at FPS there were several speakers to help everyone better understand the greater rose industry supply chain.



Virus infection symptoms

The topics included: statistics on sales and changes in the industry over the years, the range of problematic diseases that affect roses, the impact of different rose viruses on plant growth, how roses are cleaned up of viruses, the history of FPS and its rose program, and NCPN history and goals.

After the informational talks and business meeting, we had a wonderful tour of FPS facilities and had the opportunity to enjoy time in the 8 acre Nyland rose clean stock block. In fall of 2015 we had a conference call and are planning the next in person meeting for June 2016 at the ARS headquarters in Shreveport.

WHAT SPECIFIC OUTCOMES WILL BE ABLE TO OCCUR BECAUSE OF NCPN-R?

Repropagate and expand the Nyland block.

The current clean rose stock block is 8 acres and has been funded by ongoing support of the Garden Rose Counsel (GRC). This counsel is made up of key US rose growers that contribute funds to put towards efforts that support the rose industry. The funds needed to maintain the 8 acre clean stock block (about \$125,000 per year) has come from a combination of annual donations from the Garden Rose Counsel and user fees for budsticks and cuttings (between 10,000-70,000 buds /cuttings are prepared and distributed each year).

Due to financial limitations, 8 acres was determined to be the maximum size the GRC could sustain. Initially, 20 plants of each cultivar were planted. As more clean cultivars were ready to include within the 8 acre constraint, less popular cultivars were reduced to ten plants and some now to five.

The current Nyland clean stock block was planted 20 years ago. Many of the plants remain from that initial planting and are in need of rejuvenation. Mike Cunningham, FPS Rose Program Coordinator, initially planted the Nyland block in 1995. He is now semi-retired and has started working proactively this past year to take cuttings of all the cultivars in the Nyland block to get plants ready for the new clean stock rose block. The land for the new block had grapes growing on it and is now being prepared for roses. The NCPN grape effort allowed for a new site for the grapes and has made it possible to open up a larger site at FPS for roses.

Include more rose cultivars into the clean stock plant collection. The current collection of roses was assembled over the years based on a number of factors and priorities.



Nyland clean rose block

The primary goal was to include roses of value to industry. This led to the AARS winners being continually added along with strong selling non-patented roses. Additional, proprietary roses important to specific nurseries were also included with fees for testing and maintenance charged to those nurseries. It takes a minimum of two years for roses to go through all the testing needed to be deemed clean and included in the clean stock plant block. If roses are found to be virus infected, clean up through therapy can take significantly longer. Clean up at FPS is most commonly done using meristem culturing in tissue culture-taking just the very tip of the shoot that virus hasn't moved into yet and regenerating a new plant.



Nyland clean rose block

How long cleanup takes depends on factors like how well the rose responds in tissue culture and how tenacious the virus(es) are that need to be removed. The process of diagnostics and clean up are described more fully below.

NCPN-R funding will help expand the FPS clean rose collection to include many more non-patented roses important to the US rose industry. Mike Shoup from the Antique Rose Emporium has already donated a number of popular old garden roses to FPS for testing/cleanup and there are lists building of other, more recent roses, to include as well (e.g. Earth-Kind® designated roses not already in the collection, key miniatures, etc.). Dr. Malcolm Manners will also provide propagation material from the old garden and other roses he has worked to clean of viruses at Florida Southern College.

Improved diagnostics protocols. There have been several newly characterized rose viruses over the past decade. NCPN-R funding will help pool together this information and develop streamlined, effective, and uniform protocols to test for these viruses for use not only by FPS, but share the information so it can also be used by private and university-based diagnostics laboratories. NCPN-R does not fund efforts to characterize new viruses (scientists can hopefully find other sources of funding for that), but to help with technology transfer so what is already known can be tested for and that information can benefit stakeholders. Dr. Kevin Ong (director of the Texas A&M Plant Disease Clinic) and Dr. Mahar Al Rwahnih and his colleagues at FPS are leading the diagnostics efforts. They are working towards multiplex PCR (polymerase chain reaction) detection. In a single reaction the goal would be to efficiently test for a number of viruses simultaneously. PCR is very sensitive and able to detect even low levels of virus DNA/RNA.

ELISA is an older technology that uses antibodies specific to protein coats of viruses and results in color changes when the virus protein adheres to the specific antibody (comparable to how a typical pregnancy test is conducted). There may be instances where ELISA tests may still be useful in these ongoing efforts due to their ease. Improved diagnostics tests will not only help FPS test their collection for the more recently described viruses and roses in the process of entering the collection and be of benefit to growers or anyone wishing to have their roses screened by a plant disease clinic.

THE ROSE VIRUS SCREENING PROTOCOL AT FPS

Before a new rose can be entered into the clean stock block, the rose needs to pass three kinds of testing. First roses undergo molecular tests (PCR/ELISA) to determine if they are positive for the rose viruses that have been well characterized and detection protocols are readily available. If they pass that test, then there are two biological tests they will also need to pass. One is being bud grafted onto a very virus sensitive rootstock called *R. multiflora* 'Burr's Multiflora' that shows very strong symptoms when infected. The rootstock is checked for symptoms over two years.

The other biological test is bud grafting the rose in question onto 'Shirofugen' cherry, another virus sensitive indicator plant. The biological tests are very helpful, but do not tell us which virus(es) are present, just that there appears to be a virus or virus-like pathogen of some kind. In addition, there may be a virus present that the indicator plant may not show symptoms for. That is partly why two different indicator plants are used. Passing all three tests offers strong peace of mind that the rose is practically clean and they can feel comfortable including it in the clean stock collection.

Typically five plants are provided when industry members submit a new cultivar to FPS for inclusion into the clean plant collection. Each of the plants are independently screened using PCR and/or ELISA methods for the customary rose viruses (e.g. PNRV: Prunus Necrotic Ringspot Virus; ApMV: Apple Mosaic Virus; Arabis Mosaic Virus; etc.). Up to three plants that test clean for all key viruses are retained to move forward for biological indexing. If only one or two plants test clean, only those plants move forward. If all test positive for one or more viruses, the cultivar will need to undergo meristem culture to try to generate a plant of the cultivar clean of those virus(es). When the plants that are regenerated from meristem culture are large enough, they are retested using PCR and/or ELISA and the process continues again.

For biological testing, both the bud grafting of 'Burr's Multiflora' and 'Shirofugen' cherry can happen the same summer. Judy Lee does a great job managing the biological indexing of roses at FPS. Clean plants of 'Burr's Multiflora' are planted out in the field, typically as hardwood cuttings the fall before bud grafting. There are at least two rootstock plants budded from each of the up to three putatively clean plants retained of each cultivar after the molecular tests. Rootstock shoot growth is not removed after grafts take because the goal is to observe the leaves and stems of the rootstock for virus symptoms. If the grafted cultivar is clean, the rootstock looks normal and vigorously overshadows the budded growth. For virused plants, the rootstock is often severely stunted and one can easily see the growth of the rose cultivar originating from the graft. Throughout the screening field, rootstock plants are bud grafted with cultivars known to be positive for particular viruses for use as controls/comparisons.

The plants are rated for virus symptoms on the rootstock the first and second spring after grafting. Signs of stunting, mottling, streaking, watermarks, etc. are noted on the new growth. By the spring after grafting, virus has typically replicated and transferred to the rootstock in abundance. Spring is the best time to notice symptoms for most viruses. As things heat up virus concentration typically declines. Newer growth in the heat of summer may still show some stunting, but yellow patterns in the foliage are often less obvious.

The 'Shirofugen' cherry biological assay is a much faster test than 'Burr's Multiflora' and is rated between 30-35 days after bud grafting. Long current season stems of the cherry are bud grafted with often several buds from various roses in tandem. At least two buds originating back to each starting cultivar plant are budded to have replication and greater confidence in the results, like for the 'Burr's Multiflora' biological assay. After 30-35 days after bud grafting, the stems are harvested and each bud is assessed. Virused grafts lead to a gummy, sticky, discolored reaction around the bud. By removing the grafted stems within about a month, virus should not have moved into the rest of the cherry tree. After removal of the grafted stems, the cherry tree should be clean and can be grafted onto again in subsequent years. These trees as they age eventually have stout bases and in some ways have some similarities in appearance to bonsai. An established cherry tree can support many long and sturdy stems for serial bud grafting.

The identity of which stock plant the buds used for bud grafting both the 'Burr's Multiflora' and 'Shirofugen' cherry came from is retained. The original stock plants are maintained in large pots in the greenhouse or shadehouse throughout the biological indexing.

At the end of the study, one can go back to the stock plants that passed the molecular and biological indexing and propagate

plants to go to the clean stock field. If there are no plants of a cultivar that passed all three molecular and biological tests, the cultivar is meristem cultured and the process starts again.



Rosa multiflora 'Burr Multiflora' plants for budding

FPS MERISTEM CULTURING

Tissue culture is a highly controlled environment where nutrition, temperature, light, and moisture can be optimized and microbes excluded. Most viruses move from cell to cell using channels called plasmodesmata. The very youngest cells in the growing points have not developed plasmodesmata yet and, therefore, are typically not yet infected. These small groups of dividing cells can be carefully excised and placed onto nutrient rich medium with the hope of them continuing to divide and develop stem and leaf tissue. Months later hopefully whole plants with roots are regenerated and can be taken out of culture and grown on in the greenhouse. It takes significant skill and dexterity to effectively meristem culture and ultimately clean plants of virus. Dissecting microscopes are used to better see and isolate the desired cells from growing points, and one needs to work relatively fast so the cells do not dry out between isolation and being put on moist culture medium. The larger the clump of cells the greater the chance of survival, but also the greater the chance of still being infected. Rose cultivars respond differently to plant tissue culture. Some are forgiving and have a wider range of nutrition and other conditions that they are able to grow well, while others struggle and are difficult to have survive and grow to a suitable size to take out of culture and transition to the greenhouse. One can work to optimize conditions for problematic roses, but that becomes time consuming.

Susan Sim coordinates activities in the FPS Plant Tissue Culture Lab and Waclawa Pudlo is the primary researcher that conducts the meristem culturing. It was a special treat when Waclawa took the time to walk me through her techniques and kindly invited me to spend some time practicing in the lab. I learned a lot and recently was able to secure a teaching grant to purchase a higher quality dissecting microscope so students and I can do a better job at virus clean up in our plant tissue culture teaching laboratory I manage at UW-River Falls.



David Zlesak practicing rose meristem isolation

CONCLUDING THOUGHTS:

NCPN-R is great news for the rose industry and rose lovers. NCPN-R will support:

1. Technology transfer to optimize and make available diagnostics for especially the more recently characterized rose viruses
2. Rejuvenation of the Nyland clean rose collection
3. Inclusion of additional older and newer cultivars important to the greater rose community

The key to ensure positive impact is for rose industry members to take advantage of NCPN-R resources and use clean stock for propagation. Fortunately, FPS is not only here for industry, but for all of us to take advantage of their vast collection of cultivars (minimum orders of budsticks or cuttings is \$25). If a rose in the collection has an active patent or trademark we would need to secure written permission from the owner before FPS could send the material. Fortunately, most of the >500 cultivars are not patented. Some nurseries already heavily support and rely on FPS. One nursery even has their cultivars on a rotating schedule to secure new propagation stock from FPS every few years in case virus is inadvertently reintroduced through unintentionally using infected rootstock, etc.

Unfortunately, some nurseries do not routinely take advantage of clean stock and/or managing it well when they get it to help keep it clean once at their facility. Hopefully the multiple benefits of having clean stock will someday persuade all nurseries to care about clean stock in order to benefit themselves, the greater industry, and ultimately consumers. At the April 2015 NCPN-R meeting, Dr. Golino encouraged breeders in the audience to submit their new cultivars as soon as possible to FPS for testing and inclusion because earlier in the process it is more likely their cultivars would be clean. It is much more affordable and faster to get new cultivars tested and into the collection if they are clean coming in than if they need therapy.

NCPN has fortunately become a permanent part of the Farm Bill with bipartisan support. We have a lot to look forward to regarding NCPN-R activities and impacts in the years ahead.