



## Berries Feature Edition

Sweet, juicy, luscious berries—the herald of spring, the assurance of summer. Whether you favor strawberries or raspberries, blackberries or blueberries, the National Clean Plant Network is working hard to make sure you have your fill. Collectively, those four crops had a farm gate value in excess of \$4 Billion in the U.S. in 2017. Species in the genera *Fragaria* (strawberry), *Rubus* (raspberry and blackberry), *Vaccinium* (blueberry and cranberry), *Ribes* (currant and gooseberry) and *Sambucus* (elderberry) are included in the NCPN-Berries charter. Others may follow if there is interest.

### The beginning of NCPN-Berries

The berry industries have been involved with NCPN since the first workshop held in Riverdale, Maryland in May 2007 where an initial strategic plan for NCPN was developed. Several berry representatives participated in NPCN meetings in 2008 and 2009. An organizing meeting for berries was held in the fall of 2009 where industry members, scientists and regulators agreed to move forward. NCPN-Berries was recognized as an official Tier 2 group in 2010.

Since then, the NCPN-Berries network (NCPN-B) has worked to provide clean plants to berry nurseries, improve awareness of the importance of clean plants, educate growers and nurseries on effects of virus diseases and help determine the cause of a devastating outbreak of strawberry viruses in Florida and take measures for future prevention. Efforts are underway to enhance the capacity of NCPN-B and to build in some redundancy, especially for plant cultivars that are in high demand.



Pictured clock-wise from top left; blueberry blossoms on the bush; fresh, harvested raspberries; a row of blackberry bushes; strawberry cluster on the plant. Collectively, these four crops had a farm gate value in excess of \$4 billion in the U.S. in 2017.

## Viruses and the Diseases They Cause in Berries

More than 80 viruses infect berry crops. Some of them have a wide host range, such as tomato ringspot virus, which can infect red raspberry, blackberry, strawberry, blueberry, and cranberry as well as grapes, fruit and nut trees, citrus and other crops. Tomato ringspot virus causes severe crumbly fruit and stunting in red raspberry (Fig. 1A). Other viruses have a very narrow host range, such as blueberry scorch virus, which is only known to infect *Vaccinium* and *Sambucus* species. Blueberry scorch virus can cause nearly 100% flower and leaf necrosis in some blueberry cultivars.

In many cases, virus diseases in berry crops are caused by a complex of two or more viruses (Martin et al., 2012; Martin and Tzanetakis, 2013). Combinations of different viruses can cause similar symptoms, making diagnosis difficult. For example, blackberry yellow vein disease is caused by virus complexes; there are at least ten viruses that can contribute to this disease. Each of the ten viruses is symptomless in single infections in blackberry. Also, strawberry decline disease is caused by a complex of two to seven viruses. If a plant is infected with just one of these viruses, it often does not show symptoms; it is only in combination with other viruses that disease develops. All of these factors make lab testing critical for an accurate diagnosis.



Tomato ringspot virus in raspberries



Blueberry scorch virus



Healthy raspberries



Healthy blueberries

## Disease Disasters: the Economic Impact

For years, berry nurseries and growers in some regions of the country have recognized how important clean plants are, largely due to experience with outbreaks of virus diseases.

In strawberries there have been three notable diseases emerge due to viruses in the last two decades. In California in the 2000s, strawberry decline, caused by a combination of strawberry mottle, strawberry mild yellow edge, strawberry pallidosis, and beet pseudo yellows (as well as others), caused more than \$50 million in losses. During the same time, in Washington state and British Columbia, strawberry crinkle virus emerged which, in combination with other aphid-borne viruses, caused a severe decline. In the Southeastern U.S., strawberry decline devastated the 2012-2013 season because virus-infected plants (from nursery material that was grown improperly) made it into production fields. Infected plants never performed to their full potential and resulted in losses in excess of \$25 million.

**“Plants never performed to their full potential and resulted in losses in excess of \$25 million.”**

In Michigan during the 1980s, blueberry shoestring disease caused by blueberry shoestring virus caused crop losses estimated at \$3 million per year. In the Pacific Northwest, blueberry shock and scorch diseases have caused losses estimated in excess of \$100 million to the industry since 1990.

In the Pacific Northwest during the 1980s and continuing for over three decades, raspberry crumbly fruit disease, caused by a virus complex of raspberry bushy dwarf virus, raspberry leaf mottle virus, raspberry latent virus and/or black raspberry necrosis virus, cost raspberry growers more than \$1000/acre/year.

However, other regions of the country have not learned this difficult lesson yet. With the increase in movement of ‘plants for planting’ across state and country borders, there is an associated increase in the risk of moving plant pathogens. The introduction of pathogens to new areas can result in significant problems for agriculture (Gergerich et al., 2015). To counteract these threats, new diagnostic tools, many of which were developed at NCPN centers, are being applied to detect viruses and improve quarantine and certification programs (Martin et al., 2016).



Blackberry yellow vein disease



Strawberry decline, California



Strawberry decline,  
British Columbia Canada

## Adding New Cultivars to NCPN Berry Centers

Clean plant centers may receive plant material from a variety of sources. New cultivars may be introduced from breeding programs or heritage cultivars may come from a repository. In addition, some centers may accept material from outside the U.S. under a Controlled Import Permit. In any case, all submitted material is fully tested for targeted pathogens (viruses, phytoplasma etc.). Plants infected with a targeted pathogen are subjected to thermal therapy prior to excision of meristems.



If tissue culture plants (in vitro) are received, they are grown out in a greenhouse and tested once there are mature leaves on the plants.

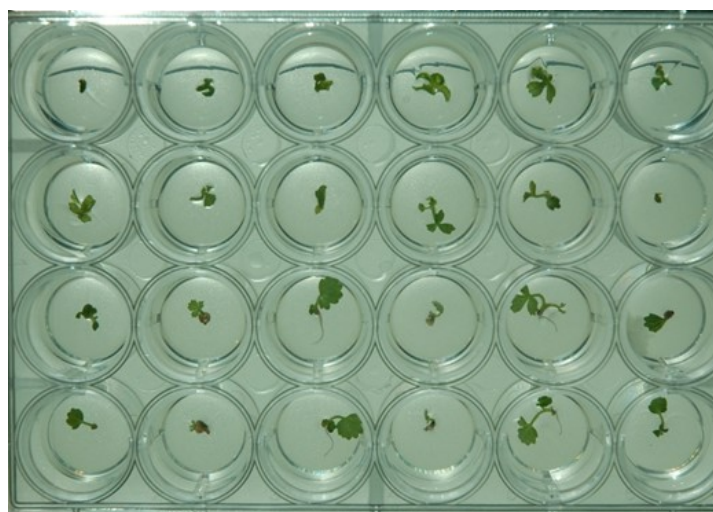


Potted plants are held at elevated temperatures, generally 35-40C, for up to six weeks prior to removing the meristems.

**After initial testing, the meristems are dissected and grown in tissue culture (below), rooted, planted in a greenhouse and then retested.**

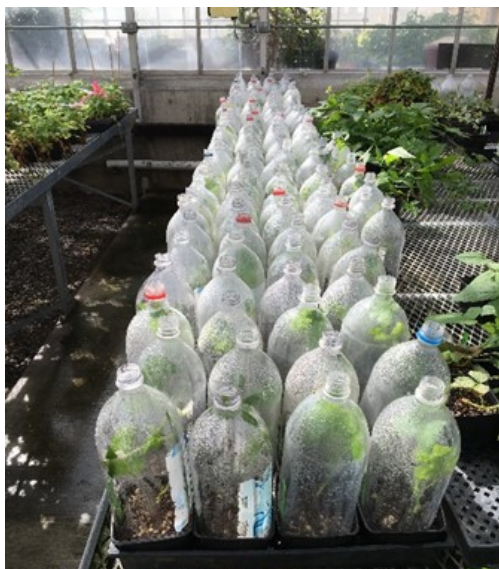


Strawberry meristem ready to transfer to media and eventual regeneration of whole plants.

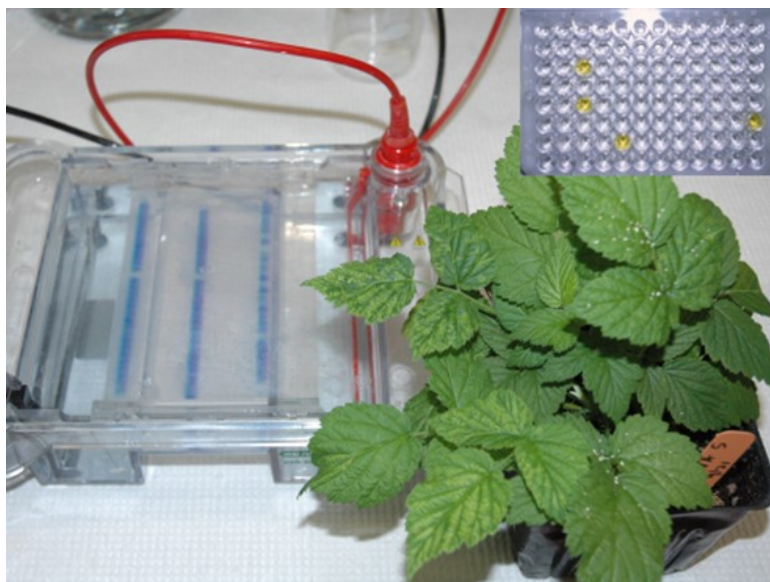


A set of 24 plants regenerated from individual meristems about 3 weeks post transfer.

Once the plants are fully tested and found free of targeted pathogens, the resulting plants are designated as G1 (first generation) and grown in protected environments and designated as G1 blocks. NCPN targets only a defined list of viruses for a given crop, since there may be a larger number of viruses that are not currently of concern. The viruses not on the targeted list, known as *cryptic viruses*, may not cause any symptoms and/or there are no known ways to eliminate them from a cultivar.



Grafting plants are held in humidity chamber for 10 days.



Gel shows separation of marker dyes after electrophoresis; this is used to separate PCR products. Upper right, ELISA plate shows positive samples (yellow). Lower right, symptoms on indicator plant shows mottling of leaves; this was from a plant that tested negative in all laboratory assays at the time.

**Further testing includes bioassays (above), ELISA and PCR assays, and High Throughput Sequencing (HTS).  
More information on HTS at [http://nationalcleanplantnetwork.org/NCPN\\_Factsheets\\_/](http://nationalcleanplantnetwork.org/NCPN_Factsheets_/)**

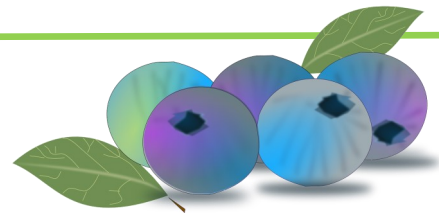


G1 blackberry tissue culture plants in gas permeable plastic bags held at 4C in Corvallis, Oregon. Picture is about one year after transfer; these plants can survive up to three years without any maintenance.



Potted G1 blackberry plants in a screenhouse at North Carolina State University.

## The NCPN Berries Centers



### Corvallis, Oregon

The Corvallis clean plant center has been part of the USDA-ARS berry virology program since the mid-60s as. The program initially focused on developing clean strawberries, raspberries and blackberries, and in the 1990s began developing virus-tested blueberries. Today the program works closely with breeders to develop plants free of targeted pathogens before they are released for commercial production. The program uses thermal and chemotherapy in combination with meristem-tip culture to eliminate pathogens. Diagnostics used in the program range from bioassays on indicator plants, to serology, nucleic acid-based detection and high throughput sequencing (HTS). The program maintains several APHIS permits in support of NCPN activities. Clean plant material at Corvallis is maintained in tissue culture at 4C, which requires very little maintenance and only needs to be transferred about every two years. This is the material requested most frequently from the program. The named cultivars are also maintained in a screenhouse. Material that is not requested for 10 years is removed from the program and the clean stock is offered to the clonal germplasm repository.

### Fayetteville, Arkansas

The Arkansas program at the University of Arkansas focuses on the development and optimization of diagnostic assays to improve testing for the NCPN programs, certification and quarantine (Ho and Tzanetakis, 2014; Villamor et al., 2019). This is done through the development of vertical pipelines starting from wet lab protocols to custom-designed bioinformatics tools for detection and discovery of pathogens using high throughput sequencing. The goal is to provide laboratory-based tests for all the virus and virus-like agents that infect strawberry, raspberry, blackberry and blueberry. For novel viruses identified through HTS, PCR-based assays are developed for detection in certification programs.

### Raleigh, North Carolina

The Micropropagation and Repository Unit (MPRU) at North Carolina State University Center conducts testing for targeted pathogens and therapy for pathogen elimination (heat treatment and meristem-tip culture) for cultivars of strawberry, blackberry, raspberry and blueberry. Once fully tested, the materials are added to established G1 blocks that serve as a source of plants for distribution to nurseries in the production of certified plants. The center also has a repository mission and G1 blocks are maintained in vitro, in greenhouses and in screenhouses. The NC program focuses on new cultivars developed in domestic breeding programs and uses detection procedures for viruses developed by the Oregon and Arkansas Centers. The Center is committed to expanding current testing and therapy activities to meet the needs of the berry industries in the U.S.

### Davis, California

The program at Foundation Plant Services (FPS), University of California Davis works exclusively with UC Patented strawberry cultivars. Strawberry foundation plants are maintained in a greenhouse and annually tested for targeted pathogens by bioassay and laboratory assays, including qPCR and HTS. Clean, state-certified foundation plants that have been through microshoot tip tissue culture are distributed to U.S. and foreign nurseries annually. A video showing the microshoot tip process under the microscope is available at <http://fps.ucdavis.edu/strawberry.cfm>. FPS has worked closely with the UC strawberry breeding program, one of the largest in the U.S., since the 1970s. Advanced breeder selections are tested for targeted pathogens and treated using heat therapy and microshoot tip culture before they are released as a new cultivar. The FPS strawberry program is funded by the University of California. FPS is an active member of NCPN-B with a great interest in developments in testing for strawberry pathogens, virus therapy and other relevant issues.

## NCPN is a link between Scientists, Certification Programs, Nurseries, and Fruit Producers

NCPN-B provides a link between all parties interested in the health, welfare and financial success of the U.S. berry industry. This includes university and private scientists, breeders, state regulators responsible for certification programs, nurseries and fruit producers. Communication between these groups is essential to address disease issues, plant movement and appropriate technological advances. The existence of NCPN-B has greatly enhanced timely and efficient communication.

The primary mission of NCPN-B programs is to provide pathogen-tested planting material to the U.S. berry industry. The programs at Corvallis, Davis and Raleigh maintain collections of virus-tested foundation (G1) material and distribute propagation material from these G1 sources to nurseries. The program at Fayetteville leads the development of diagnostics to continually improve the quality of the plants. The plant materials often are distributed in the form of small tissue culture plants on sterile media, or plug plants grown in a screenhouse.

The NCPN-B members have been active in working towards developing harmonized certification standards for each of the major berry crops (blueberry, strawberry, raspberry and blackberry) with the goal of having a single program that would be used by all states. Essentially, the result would be a National Certification Standard that would ease plant movement between states. For more information on the harmonized certification standards, or berry viruses see the NCPN-B website. (<http://www.ncpnberries.org>)

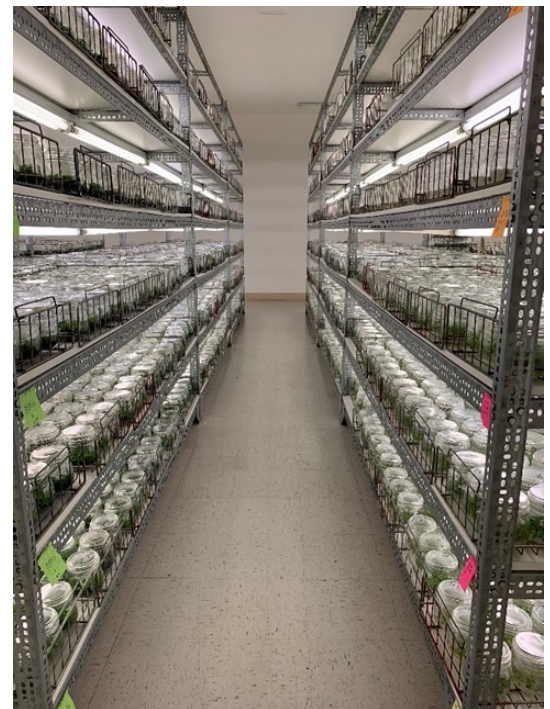
The Network educates and encourages nurseries to *start clean* using plants from a G1 source. It is then the nurseries' responsibility to apply appropriate control measures to keep the plants clean during propagation. Plants are usually increased at the nursery for several years during which they may be self-monitored or, depending on the location, monitored by a state or provincial certification program. Monitoring usually takes the form of audits for best management practices and virus testing. NCPN-B is involved in some of this testing and works closely with nurseries and state departments of agriculture to provide test results that are used in preparing phytosanitary certificates and the support exports of berry plants.

Nurseries are responsible for controlling viruses from spreading into their G2, G3 and G4 blocks.

The Network educates and encourages fruit growers to request and use clean plants. The main goal for a fruit grower is to control disease. Because many major berry virus diseases are caused by virus complexes, controlling one virus in the complex may be enough to control a disease. Therefore, growers benefit by knowing which of the



Blueberry plug plants from tissue culture rooted in small plug trays at a nursery.



Blackberry plants propagated in tissue culture as first increase at a nursery.

Continued next page.

### NCPN is a link, continued

critical viruses is easiest to control in the field, rather than trying to control all viruses.

NCPN-B centers develop, maintain and distribute the G1 materials that feed into certification programs for the benefit of the berry industries in the U.S. The desired outcome is that the berry industries in the U.S. remain competitive globally, and that viruses are not introduced into the U.S. that may threaten agricultural and natural environments.



[www.ncpnberries.org](http://www.ncpnberries.org)

Visit the NCPN Berries website for research articles on all the major berry crops with a focus on virus diseases. Also available: proposed certification programs for the major berry crops, news, events, industry and organization contacts, and additional resources for growers.

### NCPN-Berries Tier 2

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### A Look Ahead: *What's Happening in the Network*

- **June 5-6, 2019** NCPN Communications Initiative, Sacramento, CA
- **June 11-13, 2019** WERA-20 Meeting, Cornell University, Geneva, NY
- **June 14, 2019** NCPN Governing Board Mid-Year Business Meeting
- **July 15, 2019** NCPN Hop Tier 2 Meeting, Boise, ID
- **August 3-7, 2019** [American Phyto. Society Annual Meeting](#), Cleveland, OH
- **August 8-10, 2019** [Texas Nursery & Landscape Expo](#), San Antonio TX

### National Clean Plant Network

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