



RESEARCH FOCUS

Clean Plants for the Future of the Eastern Wine and Grape Industry

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Following winter injury in 2004, these Cabernet franc replacement vines were planted in 2005-2006, and soon showed classic symptoms of grapevine leafroll disease, resulting in an additional two years of lost production. New York State's revitalized nursery inspection and certification program will offer growers additional options for purchasing certified, virus-tested vines.

Photos by Tim Martinson

How the National Clean Plant Network, new testing protocols and a revitalized New York certification program will reduce the risk of nursery-transmitted viral pathogens.

Clean, virus-tested vines are now making their way to mother blocks in New York nurseries – and New York-certified vines will find their way to the marketplace in the next three to five years. They will offer growers a clean start – and dramatically reduce losses from grapevine leafroll disease, tomato ringspot virus, grapevine red blotch disease and other viral pathogens.

Their availability is the result of a pipeline that started with establishment of the National Clean Plant Network (NCPN). Established by the 2008 Farm Bill, the NCPN has provided support that has enabled University-based clean plant centers, state government agencies, and the USDA to work together to produce and distribute virus-tested planting material to nurseries and producers.

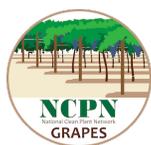
KEY CONCEPTS

- Clean, virus-tested budwood from newly-established 'Protocol 2010' foundation blocks is arriving at New York nurseries.
- New York nurseries are establishing new plantings to produce certified scion and rootstock material in the near future.
- New York State Department of Agriculture and Markets is renewing a dormant vine certification program with new, rigorous testing and inspecting standards.
- As certified mother blocks come into production in three to five years, producers will be able to purchase a wide range of varieties and clones of New York-certified, virus-tested vines.
- Certified vines will provide growers with a clean start and limit the spread and economic impact of grapevine leafroll, tomato and tobacco ringspot, and grapevine red blotch disease.

In turn, Cornell virologist Marc Fuchs, the three NY-based grapevine nurseries, and the NYS Dept of Ag and Markets have worked together to revise and resurrect New York's grapevine certification program, which will provide perhaps the most rigorous standards for testing and certifying grapevine mother blocks in the US. The nurseries are investing hundreds of thousands of dollars in new 'increase blocks' and new procedures to produce certified vines for the industry.

Background. Grapevines are host to over 60 different viral pathogens. These viruses have a variety of effects, but many are associated with reduced yield and quality. Once infected, vines in commercial vineyards remain infected – there are no treatments that can be applied in the field to ‘cure’ the infection. Viruses spread to infect additional vines in two ways: The first is through propagation of infected budwood, which spreads virus to new vineyards. Once infected vines are present, insect and nematode vectors can transmit the viruses to uninfected vines within vineyards.

Propagating clean vines tested for viral infections is the key to preventing the spread of viruses to newly-planted vineyards. This requires a concerted effort starting with foundation blocks at clean plant centers and continuing through nursery ‘increase blocks’ to final delivery of finished vines to growers. The keys to this process are traceability and auditing. *Traceability* means that the final product (commercial vines) can be traced directly back to the source material. *Auditing* means that vines, from foundation to final product, are tested to insure that they haven’t been reinfected in the field.



The National Clean Plant Network. Producing and disseminating traceable and audited planting material requires a national infrastructure. The National Clean Plant Network (NCPN) was established by Congress in 2008 to bring together existing clean plant centers into a coordinated national network focused on providing healthy planting stock to specialty crops, including grapes, to nurseries and growers. Through five clean plant centers in California, Washington, New York, Missouri, and Florida, the NCPN-Grapes has focused on

supporting virus elimination and testing to produce clean foundation material to nurseries. Now, eight years later, material resulting from this investment is making its way to commercial nurseries in New York.

Diseases of importance to the East. Testing for several pathogens is part of the [2010 Protocol](#), but three diseases associated with viral pathogen groups are most economically important in the East:



Figure 1: *Tomato Ringspot Virus.* Stunted Dechaunac shoots (L), Leaf mottling on Vidal blanc (middle) and poor fruit set on Chelois (R).

Photos by Marc Fuchs

Tomato ringspot virus (ToRSV) and *Tobacco ringspot virus (TRSV)* are classified as nepoviruses, which means that they are transmitted by nematodes that feed on roots. The nematodes that transmit these viruses, a complex of species known collectively as *Xiphinema americanum*, feed on many host plants and are common throughout the East. ToRSV reduces shoot growth and fruit set, and leads to severe vine decline. ToRSV has led to the removal of many ‘French Hybrid’ varieties, such as Chelois, Cascade, Dechaunac, and Baco noir that were widely planted on an estimated 1000 acres in the Finger Lakes in the late 1970s (*Sidebar: How Tomato Ringspot Virus Affected Hybrids in the Finger Lakes*). Grapevine fanleaf degeneration, important in western U.S. production areas, has not been detected in the East, because the vector *X. index* isn’t present in the East.

HOW TOMATO RINGSPOT VIRUS AFFECTED HYBRIDS IN THE FINGER LAKES

The 1970s saw widespread planting of several so-called ‘French-American hybrids’ in the Finger Lakes and Ontario. Originally known as numbered selections (eg. Seibel 5278), Finger Lakes wineries gave them varietal names for marketing. By the mid 1980s, ‘French Hybrids’ comprised over 25% of Finger Lakes wine grape acreage. Unfortunately, many proved susceptible to ToRSV – and growers saw vineyard blocks decline within 10-15 years.

Dechaunac (originally Seibel 9549) was a widely planted red hybrid, at its peak in 1985 comprising over 600 acres (~6% of total plantings). Between 1985 and 1990, its acreage dropped by 50% – in part due to changing markets, but also due to TRSV infection. From 1995 to 2006, its acreage further declined by 72% and production by 85%. The productive vineyard lifespan declined from an expected 25 years to 15 or less – due in large part to ToRSV. Similar trends occurred with once-common varieties such as Cascade and Chelois – which largely disappeared within a decade of their introduction. Own rooted Vidal blanc and Baco noir are also affected by ToRSV.

Acreage and production of Dechaunac grown in New York, 1985-2006.

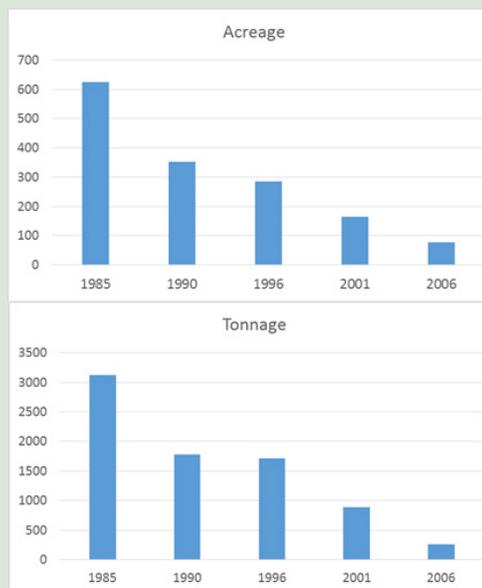




Figure 2: Grapevine Leafroll Disease. Leaf and fruit symptoms on Cabernet franc (L); Leaf symptoms on Chardonnay (middle), and grapevine mealybug *Pseudococcus maritimus*, insect vector (R).
Photos by Marc Fuchs

Grapevine leafroll disease. This disease, caused by a complex of five different viruses known as Grapevine leafroll-associated viruses (GLRaV) is common worldwide and the most common nursery-transmitted viral disease in Michigan, Virginia, and New York (**Table 1**). It is associated with up to 30% yield reduction, and also significantly delays sugar accumulation in infected vines. It is vectored in the East by the grape mealybug and also by Gill’s mealybug (*Ferrisia gilli*) in Virginia.



Figure 3: Red Blotch. Leaf symptoms on Cabernet Franc (L) and Chardonnay (left middle). Pinot noir clusters on infected (right middle) and healthy vines (R).
Photos by Marc Fuchs

Red blotch disease. Grapevine red blotch disease is caused by a recently identified virus called Grapevine red blotch-associated virus (GRBaV). On red varieties, leaf symptoms are similar to grapevine leafroll disease – and some researchers believe that it has been present for decades but misidentified as leafroll. It is also hypothesized that woody indexing used to screen new accessions for foundation plantings has had the side-effect of limiting its entry into foundation blocks. In the East, it was first identified in new plantings in New York and Virginia in 2008. The three-cornered alfalfa treehopper was recently reported to be a potential vector in California but there is no evidence of spread in the East, so we suspect that all of

the infected vines identified in the East originated in nursery stock. Unlike leafroll, GRBaV infection seems to have a limited detrimental effect on yield, but it significantly reduces soluble solids accumulation in infected vines.

Virus infections are common in New York, Virginia, and Michigan vineyards. Vineyard surveys from New York, Virginia, and Michigan tell a consistent story. Grapevine leafroll-associated viruses were detected in at least one vine in two-thirds (65-68%) of the vineyard blocks sampled (**Table 1**), and overall 26 to 33% of the samples tested positive for GLRaV, most commonly GLRaV-3 which is vectored by grape mealybug.

In Michigan, Schilder (personal communication) also surveyed for Tobacco ringspot virus (TRSV) and found positive samples in 18% of the 47 vineyards surveyed.

A second Virginia survey (Fuchs, Schilder & Nita 2016) detected 166/722 grapevines (23%) infected with GLRaV-3, 372/722 (51%) with rupestris stem-pitting virus, and, remarkably, 125/574 (22%) with detectable grapevine red blotch-associated virus.

In each state, around 25% of the samples had multiple infections.

How did they get there? Viral infections are spread from vineyard to vineyard largely through infected planting stock. Once present in the vineyard, they can be transmitted to uninfected vines by insect or nematode vectors. Over several years, an initially small infection can spread within a vineyard to affect progressively more of the vineyard. For this reason, establishing vineyards with planting material derived from certified, virus-tested vines is the key to limiting economic impact of these viruses.

The pipeline: Producing clean plants. Clean plant centers use virus elimination (*Sidebar: Virus Elimination Through Meristem Shoot-tip Culture*) and a battery of testing methods (*Sidebar: Testing Methods to Detect Pathogens*) to detect pathogens and ‘clean up’ accessions. This process takes time (*Sidebar: The Pipeline: from Tissue Culture to Vineyard*). By the time an accession is ready to be planted to a certified foundation block, it’s been through a battery of indexing and testing (2-3 years), and possibly tissue culture therapy (2-3 years), a process that ends up producing

Table 1. Grapevine leafroll incidence in New York, Virginia, and Michigan surveys.

| State | Vineyards sampled | Number of varieties tested | % of vineyards with at least one infected sample | Number of samples | % of samples testing positive |
|-----------------------|-------------------|----------------------------|--|-------------------|-------------------------------|
| New York ¹ | 95 | 16 | 66% | 1900 | 26% |
| Virginia ² | 77 | 17 | 65% | 1300 | 33% |
| Michigan ³ | 47 | - | 68% | 394 | 28% |

¹ Includes GLRaV-1, GLRaV-2, and GLRaV-3. 20 samples collected per vineyard. (Martinson et al. 2007)

² Includes GLRaV-2, GLRaV-3, and Grapevine fleck virus (GFkV) (Jones, Naidu, and Nita 2015).

³ Includes GLRaV-1, GLRaV-2, GLRaV-3, and GLRaV-4 through GLRaV 9. (Schilder, pers. comm.)

VIRUS ELIMINATION THROUGH MERISTEM SHOOT TIP CULTURE

Clean plant centers use micro shoot-tip culture to eliminate virus infections from new accessions (*Left*). The growing shoot tip is removed from an infected accession, and a small layer of cells is cut from the apical meristem. Because the apical meristem has no vascular tissue, virus particles that are transported through the xylem or phloem are not present there. This small group of cells is then placed in a growth medium to regenerate virus-free plants. This process takes about 6-12 months.

After plants are generated, they are transferred to the greenhouse (*Right*), and grown out in pots and tested to confirm successful virus elimination, before being planted in a foundation block. This process takes about a year.



Photos and graphics: Regents of the University of California, Foundation Plant Services

two to six vines in a foundation block at Foundation Plant Services (UC Davis, Davis, CA), the Clean Plant Center of the Northwest (Prosser, Washington) or the Missouri Clean Plant Center (Missouri State University, Mountain Grove, MO). These vines are the source of certified budwood distributed to nurseries and growers throughout the U.S.

At the nurseries. Nurseries then establish their own mother blocks, with budwood sourced from and traceable to, certified foundation blocks. These blocks are audited through visual inspections, and in some cases through testing by state departments of agriculture. In three to four years, mother blocks are then able to supply cuttings to produce commercial vines. For own-rooted 2-3-bud cuttings, it is estimated that each vine in the mother block can produce 50-75 cuttings. For grafted vines, both the scion and rootstock must be traceable to be certified, but the scion vines can produce more budwood (100-150 cuttings) for grafting.

Protocol 2010 material from the Russell Ranch. As a result of NCPN funding, Foundation Plant Services established a new foundation block at the Russell Ranch, that serves as a national industry resource (**Figure 4**). First planted in 2011, all foundation vines represent material that has gone through tissue culture, and has satisfied the [2010 Protocol](#) for testing. As of 2015, 3215 vines representing 1425 accessions (2-12 plants per accession) have been planted. Along with other foundation blocks in Washington and Missouri, these plantings will provide the nursery industry with a new source of elite propagation material.

From two vines to commercial quantities. Five-bud cuttings and mist-propagated plants from these foundation vineyards are being released to nurseries. Initial quantities are small, but will increase to 50-100 cuttings per foundation vine. An additional three to four years after nurseries plant their increase block, each mother vine should have produced 50-80 commercial vines. One NY nursery envisions 150 mother vines for each variety or clone, capable of producing 7,500 to 12,000 finished vines – with multiple rows for high-demand varieties.

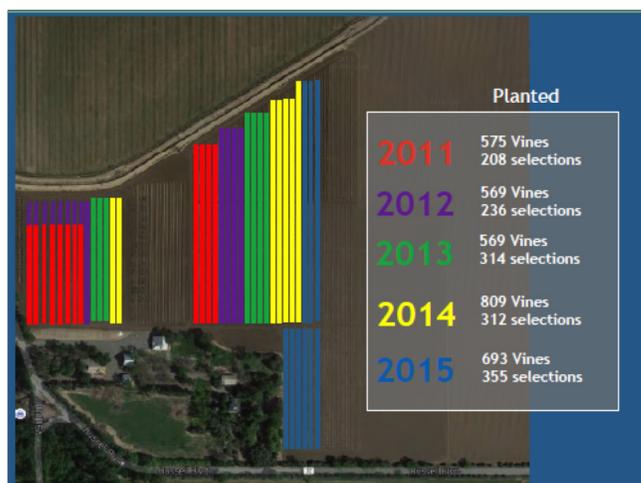


Figure 4: Foundation plantings and selections at the Russell Ranch, Foundation Plant Services, UC Davis.

Photo by Regents of the University of California, Foundation Plant Services

TESTING METHODS TO DETECT PATHOGENS

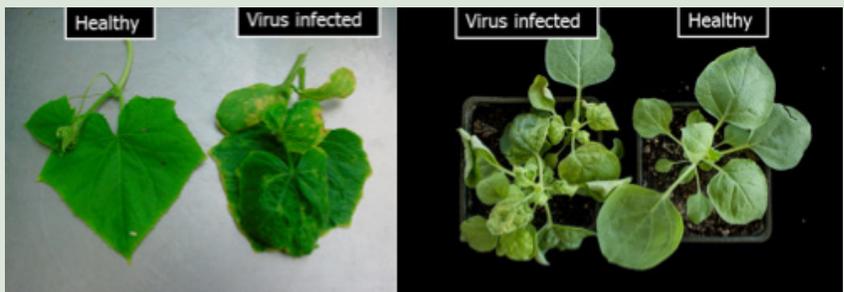
Technology for testing for viral pathogens, which began with herbaceous and woody host indexing, has expanded to use increasingly reliable laboratory tests with labeled antibodies (ELISA), PCR testing of DNA, to high-throughput DNA sequencing.

Woody Indexing. Buds are chip-grafted on to indicator plants (St. George, Cabernet franc, LN-33) and planted in the field. Vines are then observed for symptoms associated with virus infection.

This process takes one to two years.



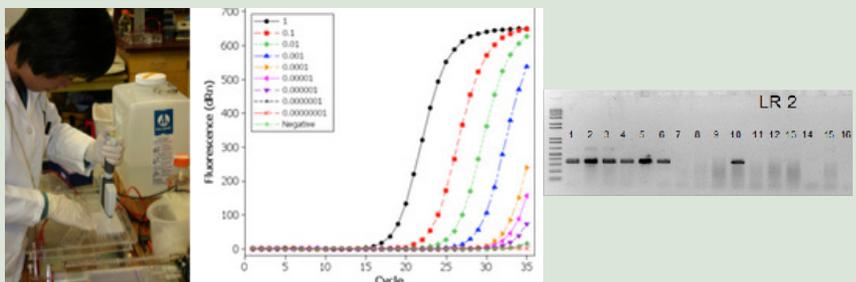
Herbaceous Indexing. Several indicator plants (eg. *Chenopodium amaranticolor*, *Chenopodium quinoa*, *Nicotiana clevelandii*, Cucumber 'National Pickling') are inoculated with ground tissue and observed for symptoms 7-21 days after inoculation.



ELISA testing. Virus particles are exposed to antibodies that bind to them, producing visible color change when positive.



Real-time PCR. DNA sequences associated with virus are extracted and amplified using the polymerization chain reaction (PCR), producing bands associated with viral infection.



Photos and graphics: Regents of the University of California, Foundation Plant Services

High-throughput DNA Sequencing. Also known as 'next-gen' sequencing, automated DNA sequencing has dropped dramatically in cost, and allows simultaneous detection of all viruses. While still being fine-tuned, it can replace plant-based indexing and reduce turnaround time from 2-3 years (woody indexing) to a few weeks.



NYS Certification Program.

The New York State Department of Agriculture and Markets (NYSDAM), Division of Plant Industry has been working with Cornell University and New York nurseries to reinstate a grapevine certification program under "Part 150. Voluntary Program For the Production of Virus-Tested Plant Materials". Certification of grapevines in New York was initiated in 1973, reduced in the 1980s, and

completely eliminated in the 1990s. Responding to industry demand, virus certification of grapevines is being reinstated in New York. Following several years of planning, the program expects to be operational in 2016.

Under this certification program, NYSDAM will screen nursery increase blocks for viruses that cause economic losses, and authorize certification tags on nursery stock that meets standards. Program elements will include:

- **Source material.** Plant material for inclusion in nursery mother blocks will be sourced through NCPN Clean Plant Centers in California (FPS) and Washington (Clean Plant Center for the Northwest at Prosser, Washington), or from material from other sources that has been cleaned via tissue culture.

- **Site selection for mother blocks.** New mother blocks must be isolated at least 100 feet from existing grape plantings, and tested before planting for dagger nematodes (the standard is less than 50 nematodes/250 cc of soil). Production blocks for certified vines will be subject to a buffer zone of 30 feet from existing blocks.

- **Inspection.** Mother blocks will be inspected by NYS-DAM Horticultural Inspectors. They will sample 1 of 4 vines (25%) in the mother blocks annually, and each vine will be tested every four years. Testing by NYSDAM inspectors will establish a chain of custody, with sample test results linked to individual vines.

- **Testing methods.** Each sample will be tested for the presence of tomato ringspot virus, tobacco ringspot virus, grapevine fanleaf virus, five grapevine leafroll-associated viruses, and grapevine red blotch-associated virus. Laboratory tests will be a combination of ELISA and DNA testing, as appropriate, under supervision of the virologist at the NYS Agricultural Experiment Station.

- **Removal in the event of positive tests.** If a mother block vine tests positive, the protocol calls for removal of all vines within five meters for a mealybug vectored virus, and ten meters for a nematode vectored virus.

- **New York-certified labeling.** Vines propagated from certified mother block accessions will be allowed to carry a special label authorized by the New York State Department of Agriculture and Markets.

This testing and inspection program, with each mother vine tested every 4 years will start in 2016, and when implemented, will be among the most rigorous in the United States.

Nursery participation. Three NY nurseries have started establishing mother blocks for producing certified scion and rootstock material (Table 2). While plans vary, all

have started establishing new blocks (starting in 2014 and 2015) and they anticipate eventual plantings of 20-40 acres, producing both certified rootstock and scion material. Finished vines are expected in limited quantities starting in 2018, with production kicking in around 2020, when the new mother blocks are mature enough to produce a significant amount of budwood.

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How much value do certified vines add? Many economic analyses (Yeh et al 2014) propose an economic lifespan of 25 years for new vineyards. Viral infections can reduce yield and/or quality, and limit the productivity of the vineyard. Over the life of the vineyard, what are the consequences of not using certified vines on the net revenue of the vineyard? A study of grapevine leafroll virus in the Finger Lakes (Gomez et al 2010) used Net Present Value (NPV) analysis to estimate a range of \$9,693 to \$16,014 per acre of lower revenue, based on assumptions about what percentage of vines were infected at planting and how fast the virus spread.

Costs and Benefits. Nurseries will incur added expenses associated with the testing and certification program – and will likely have to charge a premium for New York-certified vines. How much of a price premium could nurseries charge and still provide net value to growers?

Economists Shady Atallah and Miguel Gomez (personal communication) used NPV analysis to compare the risk of using propagation material collected from commercial

Table 2. Nursery plans for certified mother blocks in New York.

| Nursery | Site for Certified Mother blocks | Anticipated size | Year of planting | Accessions/clones and rootstock | Anticipated availability |
|--------------------|--|---------------------------------------|------------------|--|--|
| Grafted Grapevine | 4 sites out of production for 20-50 yr | 40 acres 20 scion, 20 rootstock | 2017 - 2019 | 130 scion varieties and clones, 12 rootstocks | 2019-2020 |
| Double A Vineyards | 80 Acre farm, former tree nursery | 40 acres | 2015-2019 | 150 selections, <i>vinifera</i> , Cornell, UMN, and hybrids. 4 rootstocks. | 2018 (limited), 2019 and on, increasing availability |
| Hermann J. Wiemer | 20 acre farm, never in grapes | 20 acres -3-5 years | 2014 - 2017 | <i>vinifera</i> varieties and clones; 4 rootstocks. | 2018 -2020 |

Source: Clean Plants for the future webinar #4.

THE PIPELINE: FROM TISSUE CULTURE TO YOUR VINEYARD

Step 1: Accessions from domestic or foreign sources with unknown virus status arrive at Clean Plant Centers.

Step 2: Accessions go through a battery of diagnostic tests for viruses, including woody and herbaceous indexing and ELISA/DNA laboratory testing.

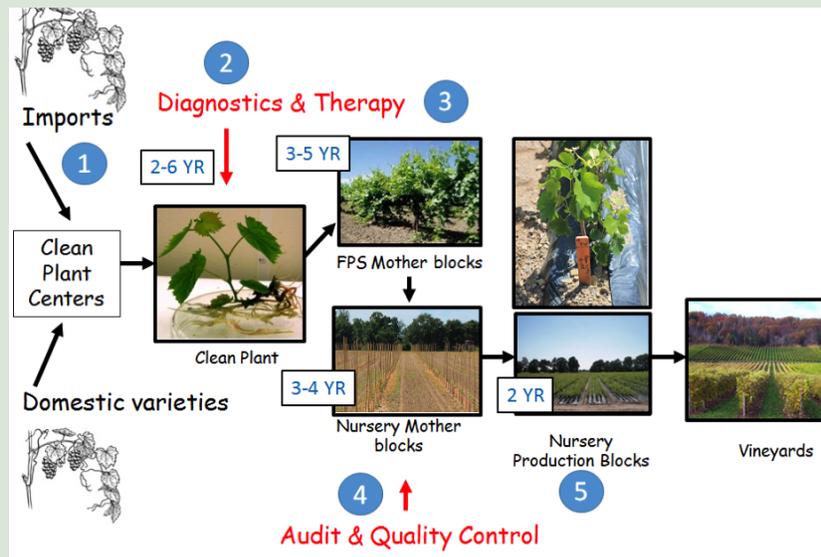
If indexing (2-3 years) and other tests are negative, vines are released to Foundation Block

If positive for one or more of the viruses, vines are propagated through meristem tissue culture therapy to eliminate viruses, then retested to verify virus-free status (2-3 years)

Step 3: After foundation block vines established (3-4 years), certified material released as budwood to nurseries. Mist-propagation (1 year) can shorten this time and increase availability of material.

Step 4: Nurseries establish mother blocks. When mature (3-4 years), each vine can produce approximately 50 3-bud cuttings (own rooted). Mother blocks are audited and tested after planting to determine their virus status. Vines reinfected in the field, and neighboring vines in a buffer zone are removed.

Step 5: Cuttings planted into nursery production blocks, and sold commercially to growers (1-2 years).



vineyards (a formerly common practice in NY) to the practice of planting with certified, virus-tested material. Using information on leafroll prevalence in commercial blocks in the Finger Lakes (60% of vineyard blocks with some leafroll, 5% of vines infected with leafroll on average), Atallah estimated that the value of certified material supported a price premium of \$1.68 per vine over the \$3.50 base vine price. In other words, if uncertified vines cost \$3.50, growers can afford to pay up to \$5.18 per vine and have higher net returns over the life of the vineyard.

Clean plants and the future. Viral infections impose hidden costs on grape producers in terms of yield and quality reductions. Past propagation practices, such as collecting budwood from a variety of commercial sites, has resulted in widespread dissemination of infected vines – and subsequent spread by vectors can, over time, greatly increase the number of vines infected in commercial blocks. Increasing the availability and use of certified, virus-tested vines will

greatly reduce the spread and impact of these diseases.

This will be particularly important as grape production continues to expand to new production areas, and new cultivars, such as the cold-hardy varieties, are released by breeding programs. Traceability and auditing associated with certification will allow growers to ‘start clean’ and avoid losses associated with propagation practices of the past.

Like the interstate highway system, conceived in the 1950s but not fully realized until the late 1970s, the NCPN funding and New York certification program are investments in infrastructure. This infrastructure – put in place starting in 2008 – is producing products that will, over time, enter the marketplace and provide economic benefits for decades to come. As certified vines make their way into commercial vineyards, growers will see lower risks of production and quality losses – and lower risk of premature removal of vineyards due to viral infections.

WHAT ABOUT CROWN GALL?



Photo by Tim Martinson

Crown gall, caused by *Agrobacterium vitis*, is a bacterial disease also disseminated through previously infected propagation material. It causes economic losses to vineyards, particularly in cool and cold-climate regions subject to periodic winter injury. Why is it not a part of the certification program?

To date, studies have shown that crown gall elimination through micro shoot tip culture is possible, but there have been mixed success in results in tissue-cultured material produced for foundation plantings.

A fundamental problem is that unlike viral diseases that live only in infected material, *A. vitis* is able to survive outside of plants in a variety of places – therefore the opportunities for reinfection during the production cycle are many.

Using a new, very sensitive diagnostic test called *Magnetic capture hybridization*, the Burr laboratory has some new insights into *A. vitis* biology and distribution in nature.

- *A. vitis* is present in wild vines, and was detected in collections made in New York (33% of 90 samples in 2013 and 2014 and California (25 of 87 samples collected in Napa County, 2015).
- *A. vitis* was also detected for the first time in dormant grape buds and on surfaces of leaves and shoot tips.
- *A. vitis* has been known to survive in soils for several years, and potentially can be redistributed through water movement.

For these reasons, it is not yet possible to certify that planting material is crown-gall free.

For more information, see: [I have galls in my vineyard. Should I call my my nursery?](#)

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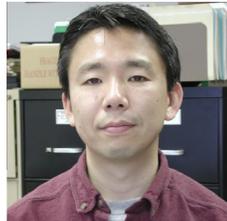
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