e-Xtra*

Safeguarding Fruit Crops in the Age of Agricultural Globalization

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The expansion of fruit production and markets into new geographic areas provides novel opportunities and challenges for the agricultural and marketing industries. Evidence that fruit consumption helps prevent nutrient deficiencies and reduces the risk of cardiovascular disease and cancer (26,42,76,109) has assisted in the expansion of all aspects of the fruit industry. Examples include an increase of more than 85,000 metric tons in U.S. sweet cherry production from 2003 to 2013 (10), the doubling of the blackberry acreage in the southeastern United States within a decade (94), an 83% increase in worldwide blueberry acreage from 2005 to 2010 (6), the almost doubling of harvested strawberries and grape acreage in China from 2002 to 2011, and the tripling of grape acreage in New Zealand from 2000 to 2011 (5). In addition, promotion of mandarins as healthy and easy-to-eat food resulted in acreage doubling in California from 2002 to 2012 (21).

In today's competitive global market environment, producers need access to the best plant material available in terms of genetics and health if they are to maintain a competitive advantage in the market. An ever-increasing amount of plant material in the form of produce, nursery plants, and breeding stock moves vast distances, and this has resulted in an increased risk of pest and disease introductions into new areas. Many crops are now cultivated in regions far from their centers of origin, where they may encounter new pathogens. For example, Chile and Argentina now produce blueberries (14), and plants in nursery and production fields may be exposed to pathogens they have not previously encountered, as blueberry is not endemic to South America (89). When cocoa was first introduced into West Africa in the late 1800s, the plants soon became affected by swollen shoot disease, believed to have been spread to cocoa by mealybugs from indigenous hosts (77). Likewise, flowering cherry trees and sweet cherry production coexisted in British Columbia from 1845 until the introduction of the apple mealybug (*Phenacoccus aceris* Signoret) from Europe in 1929 resulted in the rapid and devastating transmission of little cherry disease from flowering cherry trees and the collapse of production in several areas (40).

One of the primary concerns of the global fruit industry is a group of systemic pathogens for which there are no effective remedies once plants are infected (66). These pathogens include viruses, viroids, phytoplasmas, and systemic bacterial pathogens that cost producers and consumers billions of dollars (24). Additionally, these pathogens and diseases require expensive management and control procedures at nurseries and by producers locally and nationally. For some crops such as grapevine and fruit trees, historical records provide accounts of the disastrous results and great expense of introducing new pathogens into plant propagation materials (22,45,67,70).

The principal means of long-distance spread of these pathogens is through movement of infected propagation material. Once these pathogens are introduced into a new area, they can spread within a field and region by naturally occurring or invasive vectors. Here, we review (i) the characteristics of some of these pathogens and, in particular, features that make them challenging to detect and control, (ii) the history and economic consequences of some notable disease epidemics caused by these pathogens, (iii) the changes in agricultural trade that have exacerbated the risk of pathogen introduction, (iv) the path to production of healthy plants through the U.S. National Clean Plant Network and state certification programs, (v) the economic value of clean stock to nurseries and fruit growers in the United States, and (vi) the current efforts to develop and harmonize effective nursery certification programs within the United States as well as with global trading partners.

The Unique Challenges for the Detection and Control of Systemic Plant Pathogens

Systemic pathogens are spread readily by vegetative propagation. Once a plant is infected, most, if not all, subsequent progeny are also infected. Thus, clonally propagated plants do not benefit from the "pathogen cleansing" which occurs in crops grown from

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seed, which eliminates non-seed-borne pathogens (65). There are no economically feasible therapeutic procedures to cure plants infected with these systemic plant pathogens in nurseries or production fields. Therefore, effective control strategies are directed toward the production of healthy plants and the prevention of reinfection: "start clean, stay clean".

Plants infected with systemic pathogens exhibit phenotypes ranging from asymptomatic to severe decline and death. Symptoms depend on a multitude of factors, including pathogen strain, host genotype, environment, and presence of mixed infections with other pathogens. Symptoms induced by systemic pathogens in crops such as grapevine, apple, pear, stone fruit, citrus, strawberry, blueberry, and caneberries (*Rubus* spp.) are well documented (36,40,57–60,97,104; Fig. 1). Infected hosts may be asymptomatic as plants grown in optimal conditions in nurseries, but when exposed to field stresses, they may establish poorly, decline, or produce lower quantity and quality fruit (58,61,80). Importantly, monitoring of visual symptoms may lead to misdiagnosis, as different virus species or virus complexes often cause identical symptoms (Fig. 2).

Infected, asymptomatic plants may be selected for cultivation inadvertently or deliberately by breeders and nurseries. Whereas these tolerant selections provide a potential means of circumventing the effects of infection, their use is problematic as they may display symptoms during field growth and production, induce a synergistic response when infected with other pathogens, or serve as a reservoir of infection that may be transmitted to other, less tolerant genotypes. It is important to note that mixed infections by two or more viruses that are latent in single infections may lead to severe symptoms or even plant mortality (17,80,96,102,103; Fig. 2).

In addition to transmission via vegetative propagation and grafting, systemic pathogens may also be transmitted by vectors, including arthropods (aphids, mites, thrips, beetles, hoppers, psyllids, mealybugs, and whiteflies), nematodes, and plasmodiophorids. A vector usually transmits a pathogen with a high degree of specific-

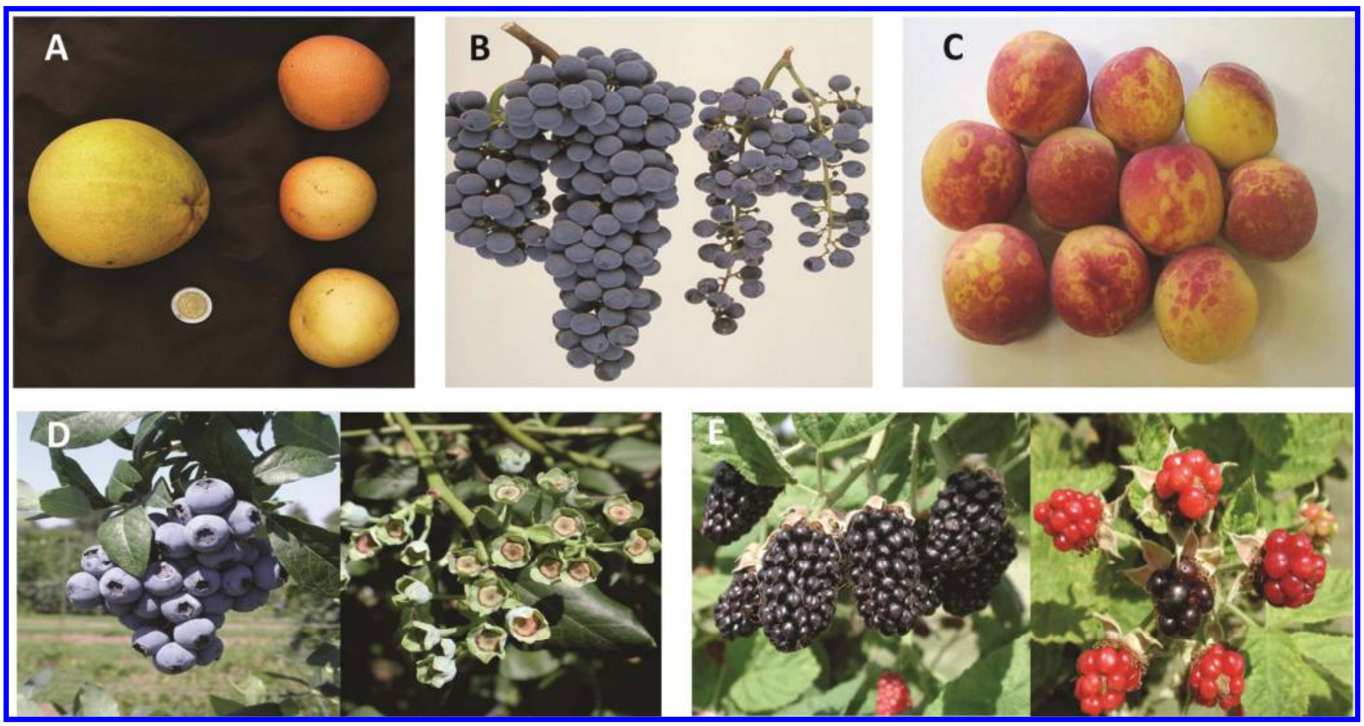


Fig. 1. Symptoms on fruit crops caused by virus infection: **A,** Tristeza stem pitting fruit symptoms. Reduced size fruit (row on right) harvested from tristeza stem pitting infected grapefruit (*Citrus paradisi* Macf.) compared to normal size fruit harvested from a healthy tree (fruit on left); **B,** Grapevine fanleaf symptoms on fruit clusters (right) of Cabernet Sauvignon grape compared to fruit clusters from a healthy vine (left); **C,** Peach fruit with symptoms of plum pox; **D,** Fruit of healthy 'Bluecrop' blueberry (left) and cluster of 'Bluecrop' exhibiting fruit drop associated with the presence of Blueberry fruit drop associated virus (right); **E,** 'Marion' blackberry infected with *Blackberry calico virus*, a virus that causes leaf symptoms but does not affect fruit yield or quality (left) and 'Marion' infected with *Blackberry calico virus* and *Raspberry bushy dwarf virus*, showing severe drupelet abortion.

Fig. 2. Similar symptoms in raspberry caused by different viruses: **A,** *Tomato ringspot virus* in 'Willamette' red raspberry; **B and C,** Mixed infections of *Raspberry latent virus* and *Raspberry leaf mottle virus* in two different advanced selections of red raspberry.

ity, and thus vector control strategies can frequently be implemented as an effective means of disease management. Some epiphytotics have resulted from the introduction of a new vector into a region where the pathogen is already present. For example, *Citrus tristeza virus* (CTV) became an economically important pathogen in South America after the introduction of an efficient aphid vector (81; *Toxoptera citricida* (Kirkaldy)). *Little cherry virus 2* eliminated sweet cherry production from important growing areas of British Columbia after the introduction of the apple mealybug (31; *Phenacoccus aceris* Signoret). Similarly, Pierce's disease, caused by the bacterium *Xylella fastidiosa*, became more prevalent in California vineyards after the introduction of the glassy-winged sharpshooter (18; *Homalodisca vitripennis* (Germar)), whereas grapevine leafroll spread more rapidly in California after the introduction of the vine mealybug (*Planococcus ficus* Signoret) due to its rapid, proliferative reproduction in comparison to native mealybug species (91). Vector exclusion or control on a regional or national level can be an important consideration when managing the spread of vector-transmitted pathogens in nurseries and production fields.

Although relatively rare, some viruses and viroids can be transmitted within pollen to ovaries during pollination and subsequently to the progeny seed and seedlings (23). For those virus– or viroid–host combinations, pollinating insects can thereby aid in plant-to-plant spread and infection of a healthy plant (20,65). Prophylactic blossom removal before flowering in nursery plantings in areas where pollen-transmitted viruses and viroids are common is a recommended management practice to reduce pathogen dispersal by pollen. Many countries have strict phytosanitary regulations to ensure that pathogens are not introduced in pollen that is traded internationally as a source of germplasm by breeders (92).

Accurate detection of pathogens is essential for disease management. Detection and identification of systemic pathogens poses several challenges: (i) false negatives due to low pathogen concentration in plants; (ii) uneven distribution among tissues and organs within a host; (iii) latent or subliminal infections; (iv) lack of pathogen reference collections or data to represent the genetic diversity needed to validate diagnostic tests; (v) lack of validated sampling and testing protocols capable of detecting a broad range of pathogen isolates; (vi) the length of time required to obtain some test results; (vii) the need to adopt and employ newly emerging, rapidly evolving diagnostic methods; and (viii) the lack of robust tests that are easily and economically conducted for large sample sizes (56,82).

Efforts to overcome these challenges have led to the development of diverse detection methods, including biological assays on indicator plants, electron microscopy, serological assays such as enzyme-linked immunosorbent assay (ELISA) and tissue blots, and molecular assays such as nucleic acid hybridization and polymerase chain reaction (PCR) variants (56). The preferred method for detection of a particular pathogen often changes over time as improved methods are developed and validated, and frequently multiple methods are used in order to satisfy phytosanitary requirements. Continuing advancements in detection technologies are probable in the form of macroarrays (101) employed as a single test to detect the most common viruses in a host plant, and "Deep" or "Next generation" sequencing as a diagnostic tool to detect the presence of known or even new pathogens (44,52,95). Reviews of current and promising new methods for detection of systemic pathogens have recently been published (1,28,41,43,56,61,95) and will not be reiterated here.

History and socio-economic consequences of some notable epiphytotics caused by systemic pathogens of fruit crops. Following are descriptions of three diseases of fruit crops (plum pox, citrus tristeza, and huanglongbing) to illustrate the devastating socio-economic consequences that systemic pathogens can inflict on fruit industries. The costs include: (i) direct crop loss realized by producers, and (ii) disease management strategies that may include surveillance, quarantine, eradication and replanting, vector control, production and maintenance of healthy planting stock, and research. Numerous additional examples of epiphytotics caused by systemic pathogens can be found in the literature (3,31,39,58,61, 91,102).

Plum pox. The consequences of the introduction and subsequent eradication of an economically important virus are well illustrated in the response to *Plum pox virus* (PPV), which causes one of the most devastating viral diseases of stone fruits in the world (40). Plum pox disease, also known as "sharka", was limited to Europe for most of the twentieth century, but has since spread to Africa, South America, Asia, and North America, where it causes disease on several species of stone fruits (71). The virus was first reported in North America in Pennsylvania (54) and later in Canada (99). PPV reduces fruit quality and increases fruit drop in certain plum cultivars. Infected trees serve as permanent reservoirs of the virus and the major source of inoculum for local and long-distance spread of the virus by several species of aphid vectors (71). Long-distance dispersal of PPV occurs primarily through host propagation material. Although the geographic route of PPV introduction into the United States has never been determined, it is assumed that the virus was introduced on such material. The virus is primarily transmitted through grafting during nursery production. The virus is also transmitted by several aphid species and by pollen (48), making eradication efforts particularly problematic.

Cambra et al. (22) estimated that the costs associated with sharka management worldwide exceeded 10 billion Euros ($12.3 billion) prior to 2006, and the cost of PPV eradication in Pennsylvania in the decade following its first observation in 1999 has been estimated at $53 million, which included the destruction of 648 hectares of stone fruit orchards (113). The steps taken to eradicate PPV in the United States are well documented (83). The five factors that contributed to the successful eradication in Pennsylvania were: (i) prompt detection after introduction; (ii) ready availability of reliable diagnostic tests; (iii) a rigorous disease eradication plan supported by local growers; (iv) extensive crop surveys conducted over several years to ensure the absence of PPV; and (v) optimal management of resources through coordinated efforts among regulatory agencies with strong support from producers, nurseries, the local land grant universities and extension educators.

Citrus tristeza. CTV causes the most important virus disease of citrus. There are many strains which cause different symptoms depending on the cultivar and the scion/rootstock combination (67). This virus has killed more than 100 million trees propagated on the widely used sour orange rootstock during the last 80 years in South America, the United States, Spain, and Israel (67). In other citrus crops, some virus strains do not kill but cause significant economic losses because of tree decline and yield reductions (32). Long-distance spread occurs by the movement of infected propagation material or movement of viruliferous aphids. Several aphid species are responsible for local spread and transmit CTV with varying efficiency. The brown citrus aphid (BrCA), *Toxoptera citricida* (Kirkaldy), is the most efficient vector, and its introduction into areas where CTV is present has accelerated virus spread (67). Devastating epidemics of citrus tristeza have occurred in association with BrCA presence in Brazil and Venezuela (64), and the introduction of BrCA into regions where CTV is present, such as the central valley of California, is likely to accelerate virus dispersal.

The economic impact of the virus on the citrus industry worldwide results from the cost of: (i) loss of trees through death and decline; (ii) maintenance of quarantines in CTV-free areas; (iii) destruction of infected trees; and (iv) implementation of virus, vector, and disease management strategies. The latter may include quarantine and certification programs to avoid importation and propagation of severe strains, the use of disease-tolerant rootstocks, nursery stock production in aphid-free screenhouses to minimize infection during propagation, use of certified virus-tested budwood, and preinoculation of trees with mild strains to protect them from infection by severe strains (67,84).

Huanglongbing. Huanglongbing (HLB), also known as citrus greening, has severely affected citrus production in East Asia for more than a century (19) and now threatens the citrus industry in the United States (110). The disease is associated with a phloem-limited bacterium, '*Candidatus* Liberobacter asiaticus', which is vectored by the Asian citrus psyllid, *Diaphorina citri* Kuwayama. Economic losses are due to tree decline, premature fruit drop, and the production of small misshapen fruit that produces bitter juice (35). HLB was first detected in the United States in Florida in 2005, and has since spread throughout the commercial production areas of the state (45). The disease has impacted Florida's citrus industry and threatens citrus production in the other citrus-producing states where it has been reported (110). Since 2006, HLB has cost the citrus industry in Florida an estimated $3.63 billion in lost revenue and more than 6,600 jobs (45). The present and anticipated impact of HLB on the U.S. citrus industry is reflected in the $24 million appropriated to fight HLB in the 2014 Fiscal Year Federal Omnibus Spending Bill (8). Management strategies for HLB include the use of certified, pathogen-free nursery stock, rogueing of infected trees, and chemical or biological suppression of vectors (37). In California, where the psyllid vector is not endemic, local occurrences of psyllid populations have triggered regional quarantines and aggressive pesticidal treatments to prevent the establishment of the vector (38).

Changes in agricultural trade that have exacerbated the risk of pathogen introduction. Many elements are responsible for the increase in global trade in plants and plant products. Whereas the merits of these changes can be argued, the fact remains that global trade continues to increase. Free trade agreements such as the North American Free Trade Agreement (NAFTA), the Central American Free Trade Agreement (CAFTA), and the European Union Free Trade Agreements (EUFTA) and bilateral trade agreements between countries (U.S.–Chile, U.S.–Australia, etc.) have been established with the goal of reducing tariffs and other barriers to international trade. It is estimated that, on average, a bilateral free trade agreement approximately doubles trade within a decade (13). Improvements in transportation systems for perishable products, container shipping (29), internet trade (47), and the increase in multinational companies that have a presence in both exporting and importing countries have improved the prospects for international trade of perishable fruit and plants for propagation.

The importance of plant introduction as a driver for emerging infectious diseases (EIDs) is illustrated by a 1996–2002 analysis reported in ProMED-mail (3). This analysis indicated that viruses and phytoplasmas accounted for over 50% of the reports, and that plant introduction was the most important driver of plant EIDs. For plant viruses, plant introduction and changes in vector populations accounted for 71 and 16% of EIDs, respectively. The impact of vector introduction is illustrated by the emergence of Pierce's disease, grapevine leafroll, little cherry disease, and citrus tristeza/stem-pitting as important agricultural problems following the introduction of efficient vectors (3,31,91).

Current Safeguards

Plant quarantine regulations and eradication, the PPV paradigm. The first line of defense against exotic pathogens is quarantine. Quarantine is imposed to prevent the introduction or limit the spread of designated pests/pathogens into or out of a specified area. The definition for a quarantine pest/pathogen (North American Plant Protection Organization [NAPPO]) is "a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled." Quarantines may be federal, restricting import from all foreign countries or restricting importation into designated parts of the country, or state/province, restricting movement into a state from other states or restricting movement within a state. In the U.S. Department of Agriculture (USDA), the APHIS (Animal and Plant Health Inspection Service) Plant Protection and Quarantine (PPQ) program regulates the importation of plants and plant products into the United States under the authority of the Plant Protection Act (4). Commercial importations of nursery plants into the United States must originate from sources approved by APHIS and authorized by an import permit. Alternatively, all nursery stock of specified *Prunus* species from designated countries must be tested and found to be free of quarantine pests before they are released for commercial use. Despite this long-standing quarantine, PPV was inadvertently introduced in Pennsylvania, Michigan, and New York (53). It is largely because of the introduction of PPV and other invasive pests that Quarantine-37 has recently undergone a substantial revision. Many plant genera formerly admissible to the United States without specific restrictions or entering the post-entry quarantine program are now required to undergo a pest risk assessment to determine whether additional safeguards are needed before plants can be imported (9).

Following the discovery of PPV in the United States, the second line of defense against sharka was the imposition of federal domestic quarantines in New York, Michigan, and Pennsylvania. Intensive surveys (46) of cultivated and wild hosts were conducted to clearly define the affected areas within each state, and this information was used to set the geographic quarantine boundaries within each state. Interstate movement of stone fruit trees, seed, budwood, and nursery stock from these areas was officially prohibited to prevent the spread to areas of the United States that were PPV-free.

The third line of defense was an intensive survey and eradication program to detect and destroy plants infected with the virus. In 2009, Pennsylvania was declared PPV-free, and currently New York is the only U.S. state with an active PPV eradication program. Once eradication was confirmed by survey for 3 years in Pennsylvania (2012), nursery stock production in the original quarantined areas was allowed to resume (112). The PPV example illustrates that quarantine can be an effective deterrent to the introduction of a pest or pathogen into a country. The USDA National Plant Quarantine Center successfully intercepted seven PPV-infected foreign *Prunus* accessions (out of 480 total accessions) between 1991 and 1993 (111). Unfortunately, despite the PPV quarantine, illegal movement of germplasm does occur. In 2004, germplasm from the Ukraine was hand-carried into the United States without a permit, and when the material was turned over to regulatory officials and analyzed at the USDA-ARS (Agricultural Research Service) quarantine facility (Beltsville, MD), it was found that 4 of the 19 accessions were infected with PPV (62). This illustrates the importance of educating the stakeholders, e.g., producers, nurseries, plant distributors, consultants, the scientific community, extension educators, and homeowners about the risk and potential economic consequences of illegally imported plant material entering the United States from another country.

Clean Plant Centers and Certification Programs

Production of plants free of targeted pathogens through the U.S. National Clean Plant Network and state certification programs (SCP). Rather than describing the various governmental programs and organizations involved in the production and regulation of healthy nursery stock, we will "follow the plant" in the next sections to describe how these programs ideally work together to facilitate the production of plants free from targeted pathogens for use by commercial growers and homeowners. A targeted pathogen is a disease agent for which control measures are implemented. The inclusion of an organism on a list of targeted pathogens for a crop depends on the goals of the regulatory agency. For example, the most inclusive lists of targeted pathogens are employed by (i) import and quarantine facilities that are concerned with the potential presence of pathogens with a worldwide distribution, and (ii) clean plant centers that are charged with supplying the cleanest possible plants to the nursery industry. The least inclusive lists of targeted pathogens are concerned with locally occurring pathogens that may spread into nursery plants during propagation. Ideally, an organism is identified as a targeted pathogen based on published evidence for its role as a disease agent (either alone or in concert with other organisms), and not until validated diagnostic tests are available.

There are usually several cycles of plant propagation ending with certified nursery stock for planting, and at each stage of multiplication, progeny plants are moved to the next certification level (Fig. 3). Unfortunately, a confusing assortment of terms is used by different countries to identify the plants in these successive cycles of propagation (Fig. 4). A consistent terminology based on the propagation steps or "generations" has been strongly recommended by NAPPO (69) and has been adopted in Canada (25) and considered for use in other countries (Fig. 4). In the United States, this simplified terminology has been adopted in State Model Regulatory Standards for the certification of fruit tree nursery stock (114), and efforts are being made to move toward similar terminology in certification programs for blueberry, grapevine, *Rubus*, and strawberry nursery stocks.

National Clean Plant Network (NCPN). Many countries have clean plant centers (CPCs) that are responsible for screening plants for economically important pathogens, performing therapy, and maintaining collections of pathogen tested (G1) material (40). Increasingly, private companies are maintaining and testing their own G1 materials. In the United States, the USDA NCPN was established in the 2008 Farm Bill "…to provide high quality asexually propagated plant material free of targeted pathogens and pests that cause economic loss to protect the environment and ensure the global competitiveness of specialty crop producers." NCPN is a collaborative effort among three USDA agencies: APHIS for quarantine and regulatory programs, USDA-ARS for technology and germplasm issues, and the National Institute for Food and Agriculture (NIFA) for outreach and partnership initiatives (http://nationalcleanplantnetwork.org/). NCPN brings together and funds diverse programs that historically have been involved in the production of virus-tested or clean plants with the goal of advancing collaboration and communication among CPCs, maximizing coverage of stakeholder needs, fostering efficiencies and synergies, and establishing rigorous standards for clean plant programs. Currently, NCPN focuses on five specialty groups of crops: berries, citrus, fruit and nut trees, grapevine, and hops, with several additional crops including rose and sweet potato being considered for entry (Fig. 5). The NCPN center directors are scientists who are authorities on the pathogens affecting the crops handled by the center. NCPN is administered nationally by a governing board composed of eight members representing broad federal and state governmental interests in the clean plant concept. Additionally, stakeholders seeking support under the NCPN banner are requested to form specialty crop-focused governing bodies to advance and harmonize governance and networking of the program both within and among the members of the network. The governing bodies of each of the NCPN crops are composed of representatives from industry, state regulatory agencies, scientists, and extension educators who work together to identify program needs, set priorities and funding, and help address opportunities and challenges as they emerge. Although the scientists who manage the centers work with different crops and their pathogens, they use similar protocols to produce, test, maintain, and distribute clean propagating material to consumers.

When a candidate plant enters an NCPN CPC, it undergoes a series of diagnostic tests and, if needed, appropriate treatment before it is deemed ready for distribution (Fig. 6). Candidate plants of interest are selections or cultivars developed by public and private breeding programs, as well as currently used cultivars and "heritage" cultivars whose health status is unknown or suspect. Ideally, plant breeders submit advanced selections to a CPC for testing and virus elimination prior to cultivar release, so that plants tested for targeted viruses are available at the time of release. Some NCPN CPCs also serve as quarantine facilities to expedite the import of

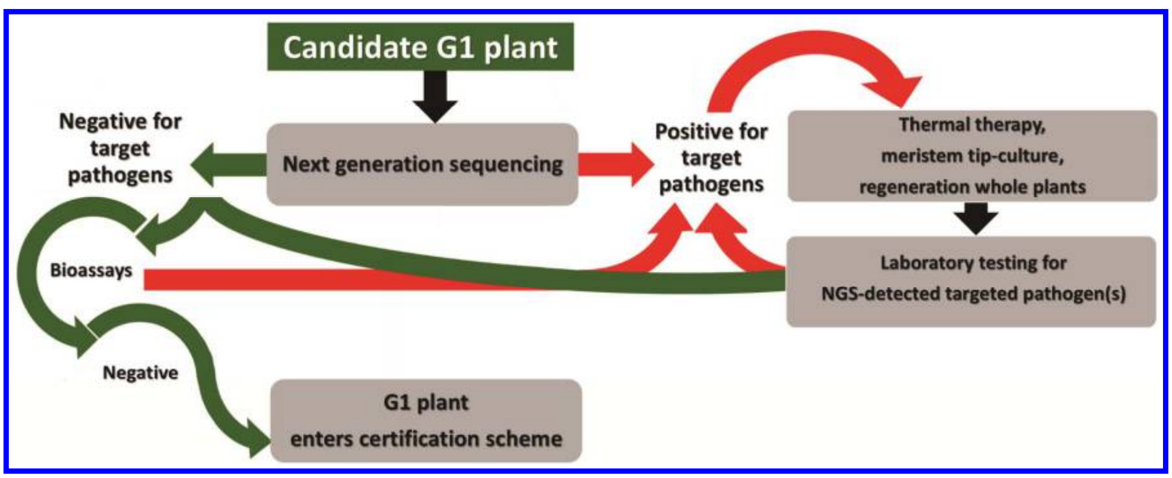


Fig. 3. Idealized journey of a candidate plant from the state of advanced breeding selection through a National Clean Plant Network Clean Plant Center for distribution to nurseries for maintenance, propagation, and eventual sale to producers.

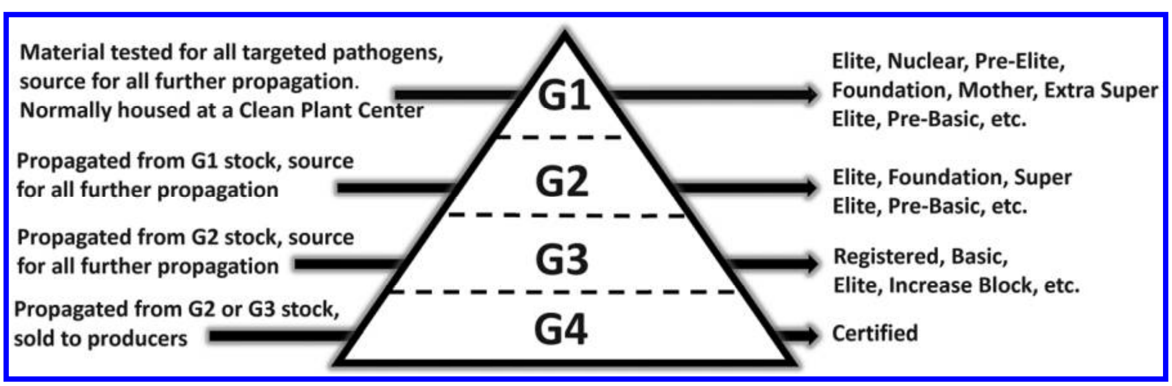
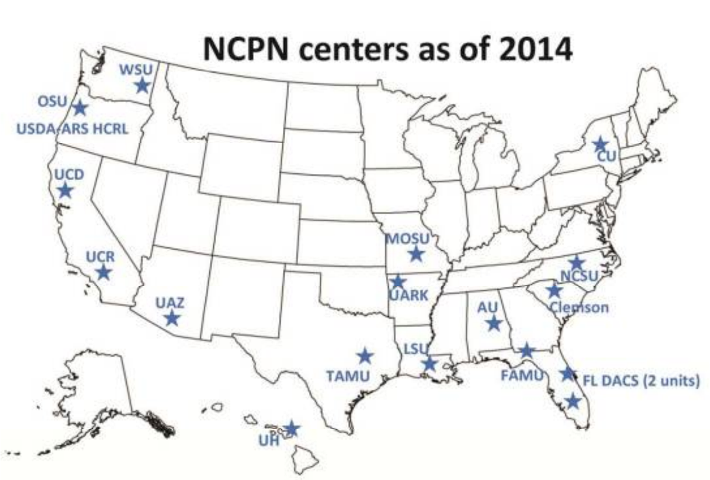


Fig. 4. Diagram illustrating the G-terminology proposed for the stages of plant propagation from clean plant centers (G1) through the propagation steps in nurseries enrolled in certification programs (68,69).

advanced clonal selections, commercial cultivars or materials for germplasm improvement, and materials that have the potential to improve the competitiveness of the U.S. industries.

The first step in the production of clean nursery stock is the determination of its initial pathogen profile. In some programs, the material is prophylactically subjected to elimination of all targeted pathogens from one or a few candidate plants to become a source of clean (pathogen-tested) material, designated here as G1 plants. The successful production and maintenance of G1 plants at CPCs requires a thorough understanding of the plant and its pathogens, the availability of validated diagnostic tests for targeted pathogens, effective therapeutic protocols for pathogen elimination, and secure facilities for maintenance of clean plants. The detailed protocols for these activities have been covered elsewhere (40,43,49,56,61, 85,105–107) and will not be considered here. Instead, the following sections will highlight NCPN activities at CPCs to improve and standardize diagnostic and therapeutic protocols, develop state-of-the-art facilities and methods to maintain clean plants, and design effective outreach and education programs to increase stakeholder awareness of the economic benefits of clean plant material.

Diagnostic assessments are performed at CPCs to determine the health status of candidate plants and to verify that plants maintained at the CPCs are free of targeted pathogens (Fig. 6). The diagnostic tests utilize biological indicators and/or laboratory assays based on serology or nucleic acid analysis, including next



Cooperator Institution	Crops
Auburn University, Alabama (AU)	Citrus
Clemson University, Clemson, SC (Clemson)	Fruit trees
Cornell University, Geneva, NY (CU)	Grapevine
FL Dept. of Agric. & Consumer Svc. Gainesville, FL (FL DACS)	Citrus
FL Dept. of Agric. & Consumer Svc. Winter Haven, FL (FL DACS)	Citrus
Florida A&M University Tallahassee, FL (FAMU)	Grapevine
Louisiana State University, Baton Rouge, LA (LSU)	Citrus
Missouri State University, Mountain Grove, MO, (MOSU)	Grapevine
North Carolina State University, Raleigh, NC (NCSU)	Berries
Texas A&M University Kingsville, TX (TAMU)	Citrus
University of Arizona Yuma, AZ (UAZ)	Citrus
University of Arkansas Fayetteville, AR (UARK)	Berries
University of California Davis, CA (UC-D)	Fruit and nut trees, grapevine and strawberry
University of California Riverside, CA (UC-R)	Citrus
University of Hawaii, Honolulu, HI (UH)	Citrus
USDA/ARS, Corvallis, OR (USDA/ARS HCRL and OSU)	Berries
Washington State University, Prosser, WA WSU	Fruit trees, grapevine and hops

Fig. 5. Map indicating the location and identity of the 19 National Clean Plant Network (NCPN) centers.

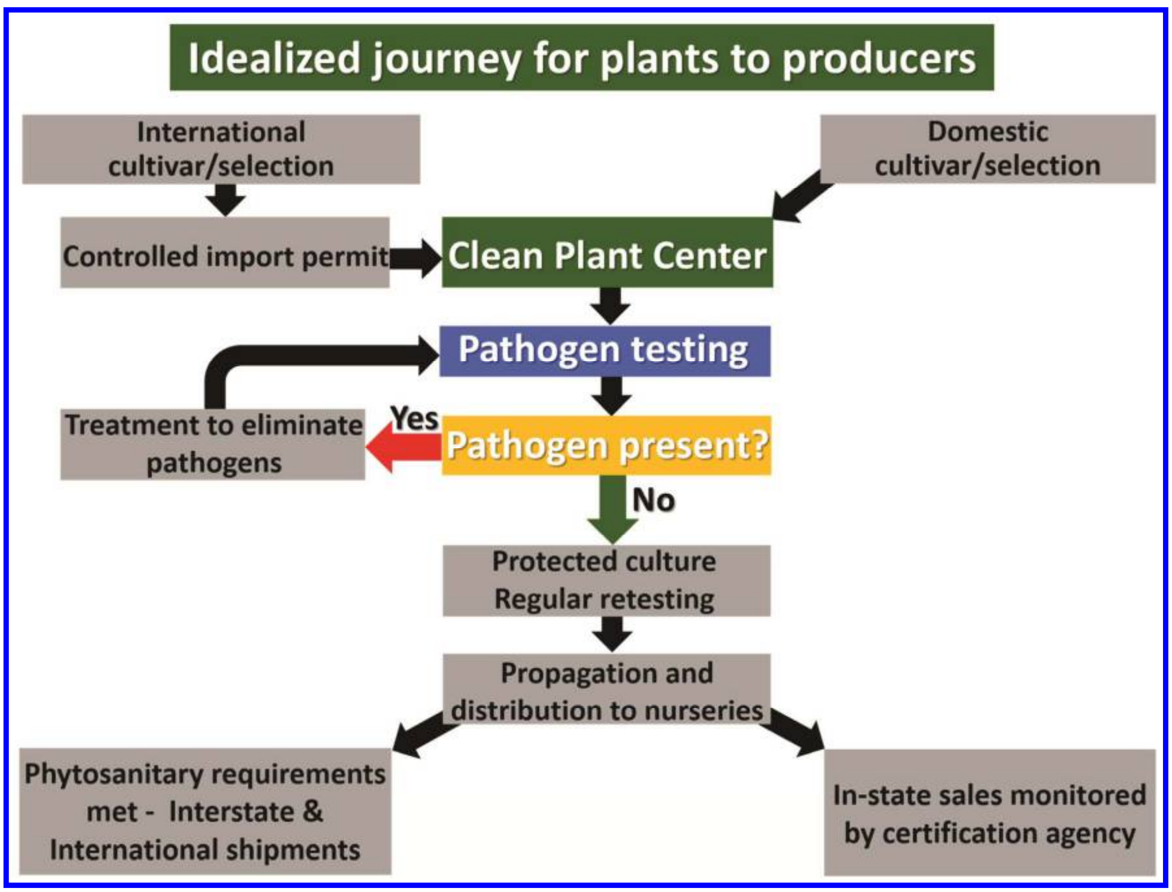


Fig. 6. Scheme for laboratory testing, bioassays, therapy, and meristem tip culture at a Clean Plant Center.

generation sequencing (49,55,56,61). Experts at NCPN centers are charged with developing and validating diagnostic tests for the full spectrum of systemic pathogens that may infect candidate plants. An essential feature of these tests is their capacity to potentially detect all known variants of a pathogen. Often when a diagnostic procedure is first published and developed, it is based on reactivity to one or a few isolates from a limited geographic area. Further research is then required to accumulate a geographically and genetically diverse collection of the pathogen, evaluate existing diagnostic tests to determine whether an array of isolates are reactive, and if not, redesign the tests to detect the full spectrum of variants. For example, detection of *Blackberry yellow vein associated virus* (BYVaV) was based on the use of several primers in reverse transcription (RT)-PCR, as each set could only detect a subset of the available isolates. Based on the population structure of the virus, improved protocols were developed for the detection of all available isolates (78,79). CPCs also build and share their reference collections of properly validated positive controls which are needed for routine use in diagnostic procedures (16). Positive controls for diagnostic tests can be stored and shipped as nucleic acids, preserved plant tissue, or blotted sap extracts, depending upon the regulatory requirements of the receiving state or country (83). The use of these materials as positive controls reduces the risk of moving infected plant material to other geographic regions (83), and makes it easier for CPCs to share materials and establish diagnostic capability for exotic pathogens.

The methods of choice for pathogen detection are continually revised when more sensitive and economical tests are developed and become available (49,56,61,73–75,88). Researchers at CPCs are investigating the application of new techniques such as next-generation sequencing (15,44,52,88,95) and arrays (41,100,101) to plant pathogen diagnosis. For example, next-generation sequencing has been used to identify viruses associated with several diseases of unknown etiology in fruit crops (15,63,86,87,98,108) that currently require costly and time-consuming grafting onto indicator plants for diagnosis. Once these newly described viruses are confirmed as the causal agents of diseases, the development of nucleic acid-based diagnostic tests may reduce or eventually replace the costly and time-consuming dependence on biological indexing for diagnosis.

If diagnostic tests show that a candidate plant at a CPC is infected with a targeted pathogen(s), the plant undergoes one or more rounds of thermal treatment, chemotherapy, and/or meristem culture (40; Fig. 6). Depending on the initial health status of the candidate plant and the type of crop, the time needed to produce a G1 plant can range from a few months to several years (61). G1 plants are maintained at CPCs as whole plants and/or in tissue culture under defined, stringent conditions to prevent infection. All G1 plants are retested for target pathogens on a specified schedule, and when new pathogens of concern are discovered in that crop, all G1 plants are tested for those new pathogens. Once a candidate G1 plant has been tested and found to be free of targeted pathogens for two consecutive years, it is designated as a G1 plant (Fig. 6) and becomes the source for further propagation and distribution to nurseries.

A recent proof of concept involved a new grapevine pathogen: NCPN supported a new Foundation Vineyard, Russell Ranch, at Foundation Plant Services, University of California-Davis. Russell Ranch sets a new standard as plants are tested for more than 30 viruses and virus-like organisms. To qualify for planting in Russell Ranch, vines must be treated with microshoot tip culture to eliminate viruses. In 2012, *Grapevine red blotch-associated virus* (GRBaV, red blotch) was discovered using technologies supported by NCPN (51,88) and later found to be distributed throughout the United States (50). Because of the foresight of NCPN and the willingness of nursery industry stakeholders to move forward with this new G1 planting stock, at least a large part of the solution to this crisis was already at hand. None of the 1,823 vines planted at Russell Ranch were positive when tested for GRBaV, even though the virus had not yet been discovered when the vineyard was first propagated. Grape nurseries across the country are planting new G2 blocks from the G1 vines at Russell Ranch in sites isolated from any commercial vineyards.

Certification Programs

To qualify as certified, all propagative material produced by nurseries should be derived from G1 stock (or its equivalent in other schemes) and grown under conditions that minimize the risk of infection. G1 plants from NCPN centers, or equivalent plants from equivalent facilities, are provided to nursery production systems for mass propagation under the certification standards designed to safeguard the plant.

Certification schemes vary widely among different crops and countries. This is because of the differences in the objectives of programs, facilities, financial and human resources, and the magnitude of disease problems to be managed. Despite these differences, the core components of these programs are similar and include the following essential elements (66):

- Published standards that define the purpose, terminology, and protocols
- Sources of material tested for targeted pathogens
- Availability of reliable indexing methods for the targeted pathogens
- A system to monitor established standards
- A means to finance the certification program

The central tenet of all certification schemes is the application of a systems-based approach that addresses risks of infection and pathogen spread during propagation (30). Various independent components, such as production facility and planting site compliance, pathogen testing, scheduled inspections of greenhouse and field grown plants, isolation distances, and vector control, contribute to minimize the presence and spread of targeted pathogens. Verification of the effectiveness of a systems-based approach to certification is further supported by the auditing of the system at established and agreed critical control points. The clean status of plants as they are maintained, multiplied, and matured for sale at nurseries is monitored through official government certification programs that have the authority to enforce regulatory measures and grant phytosanitary documentation.

In the United States, state departments of agriculture have the legal authority to develop certification programs and provide official documentation attesting that the plants have been maintained, propagated, and tested according to the guidelines outlined in the state's certification standard. Many states that have extensive nursery stock production and/or large commercial fruit production for a commodity have developed crop-specific certification programs, including blueberry, citrus, fruit and nut trees, grapevine, *Rubus,* and strawberry. These crop-specific programs address pathogens that the standard nursery regulatory program of visual inspection cannot effectively detect and manage. Whereas state standards for crop-specific nursery certification programs vary in complexity and requirements, they often include specifications for some or all of the following: site selection and preparation, isolation distances from plants of the same species or genus and other vegetation, conditions and protocols to be followed for plant production and maintenance, a management plan for pests and diseases, a schedule for inspections, and types, extent, and frequency of testing to be done at each propagation level. Certification standards also require nurseries to keep records of pest management activities and plant lineage so that trace-backs and trace-forwards can be done if a problem should arise.

Harmonization of state-level certification standards. A perusal of the published guidelines for several state certification programs for fruit crops (Supplementary Table 1) illustrates the variation in format and the requirements among programs. This variation is also present among state certification programs for a single nursery crop such as strawberry. Crop-specific nursery certification programs have generally developed as a result of pressure from producers who experienced production losses due to diseased planting material. A classic example is the potato seed certification program initiated in five states in the United States more than 100 years ago in response to observations of "potato degeneration" long before viruses were shown as the causal agents (33,93). Surveys of the stone fruit industry in Washington State revealed that 5.5% of all sweet cherry trees were afflicted with serious, debilitating diseases caused by virus-like agents, and many additional trees were infected with other virus diseases (27). This led to the creation of the progenitor of the Clean Plant Center Northwest for fruit trees at Washington State University in 1955, and the accompanying state certification program. Similarly, the grape program at Foundation Plant Services, housed at UC Davis, and the California Grapevine Registration and Certification Program, had its origins in the 1930s with the realization by growers and researchers that California vineyards were threatened by virus and virus-like diseases (2). In contrast, some state certification programs were initiated to protect nascent fruit industries in their states based on the experiences of growers in established production regions. For example, the blueberry nursery certification program in Arkansas was initiated in the early 1980s to protect "a new and growing industry in Arkansas" from diseases such as red ringspot, necrotic ringspot, stunt, and Phytophthora root rot that posed serious threats to the state's developing blueberry industry (11). Over the last century, individual states independently developed crop-specific nursery certification programs as growers, researchers, and regulatory personnel identified the need for them. The independent development of these programs within each state's regulatory framework, and the diversity of factors leading to the development of these regulations, helps explain the dissimilarities among state nursery certification guidelines.

The unfortunate consequence of disparity among state-level, virus-tested certification programs is that nurseries producing virus-tested certified plants in different states operate under different guidelines, and therefore plants purchased from nurseries in different states may not be of uniform quality. For example, some states have virus-tested certification programs that include mandatory laboratory testing and nursery inspection, whereas others rely on visual inspections alone, which as mentioned previously do not warranty virus absence.

Currently, any country or state wishing to import virus-tested certified plants from a state in the United States must evaluate each individual program before allowing an importation. Such a system is not adapted to safe and facile exchange of plant material for propagation. A single, uniform, U.S. national standard for virus certification of nursery stock for fruit crops would allow for more transparent discussions between national and international trading partners, and would allow for the movement of the highest quality propagation material to all areas where these crops are grown. While it is not in the purview of the NCPN groups to develop state certification programs, they do assist in discussions to coordinate and harmonize state certification programs for their respective crops. The goal is to develop national certification minimum standards that will qualify for interstate distribution and export to foreign markets.

The pioneering effort to establish a national standard for nursery stock production in the United States was initiated by the fruit tree group within NCPN in 2009. In a joint effort by state regulatory officials, industry representatives, and scientists, an audit-based virus certification program was designed by incorporating elements from existing state certification programs as well as additional guidelines to ensure plant health. The format for the national standard was patterned after the NAPPO Regulatory Standard for Phytosanitary Measure 25 and 35 (RSMP) "Guidelines for the Movement of Stone and Pome Fruit Trees and Grapevines into a NAPPO Member Country" (68,69). This resulted in the draft "State Level Model Regulatory Standard: Virus-Tested Certification Program for *Prunus*, *Malus*, *Pyrus*, *Chaenomeles,* and *Cydonia* Nursery Stock Production Systems" (114). Pilot studies in nurseries were conducted in Michigan, Pennsylvania, and Oregon to evaluate the efficacy of the guidelines to mitigate the risk of spreading viruses of regulatory concern through fruit tree nursery stock. As a result of those pilot studies, the State Level Model Regulatory Standard was finalized (114).

National nursery certification standards are being developed for other fruit crops, including blueberry, grapevine*, Rubus,* and strawberry. The initial focus for harmonization of these certification standards is to establish a national standard for each commodity group that will facilitate interstate movement and export of nursery stock. The working groups for the development of these standards include NCPN members for each commodity, state regulatory officials, industry representatives, and scientists. Members from each of the national nursery certification standards working groups met to establish a standard format and content for the national certification standards for all specialty crops. This allowed for a unifying language and format to maximize ease-of-use for nurseries, scientists, and regulatory personnel at the state, national, and international levels. The following elements are considered mandatory for the State Level Model Regulations: (i) a standard glossary (7) developed by the group that uses published definitions from international, national, and state agencies; (ii) use of the G-level terminology, strongly recommended by NAPPO (69); (iii) a framework for the standards that has parts and subparts similar to those that are present in the Draft State Level Model Regulatory Standard for Fruit Trees as adapted from NAPPO RSPM 35 (114); and (iv) publication of draft National Certification Standards on the NCPN webpage for a given crop.

The national standards focus on production processes of nurseries in order to provide an alternative to the current approach that relies on end-product inspection for phytosanitary certification. A critical need identified is a harmonized system for constructing and maintaining databases for the pathogens covered by the standards. It was proposed that scientists within each NCPN commodity group would develop and update data sheets for all the pathogens included in their National Certification Standards for use by national and international regulators, nurseries, scientists, and growers. These regularly updated, comprehensive data sheets would be available online and include essential information such as symptoms, host range, distribution, means of spread, acceptable tests, sampling requirements for each pathogen, and representative photographs of symptomatic plants. In addition to the crafting and maintenance of this harmonized national database to convey updated and reliable information, the ongoing participation of NCPN experts who are aware of new and emerging disease issues domestically and internationally would also facilitate early detection of exotic pests and minimize their potential spread by heightening awareness and front-line monitoring of plant material.

The national certification guidelines are being harmonized with the understanding that details within these guidelines vary among crops and regions. For example, guidelines for production of G2-G4 blackberry nursery stock are tailored to reflect the endemic blackberry viruses at the location of the nursery. BYVaV has been found in the southern and southeastern United States, but not in other major U.S. blackberry production areas (79). The certification guidelines for blackberry in these southern states will include testing for BYVaV in G2-G4 nursery blocks since it is endemic to the region, but certification programs in states outside of this region will not. Similarly, the required isolation distance from uncertified plants is larger when pollen-borne viruses of the crop are known to occur in the region. Within the certification guidelines for a particular crop, the stringency of production requirements decreases as the G number increases. G1 plants have the most restrictive requirements for testing and maintenance since they are the source for propagation of G2, G3, and G4 plants. G4 nursery plants (those ready for sale) have the least stringent requirements since they are not used for propagation and are usually grown for short periods in the nursery prior to sale.

A parallel effort to harmonize the certification standards in the EU by the European Plant Protection Organization (EPPO) has been ongoing for more than 20 years. EPPO certification standards have been published for each fruit crop in NCPN (72). A review of the EPPO certification standards for various fruit crops revealed a common format, but also differences due to dissimilarities between crop propagation practices and the pathogens targeted by the standards. The EPPO certification standards are concerned with the same types of systemic pathogens being considered for berries, fruit trees, and grapevines in the United States.

The development of effective model certification guidelines in the United States that can be implemented in nursery production systems is the result of deliberations among scientists who specialize in the pathogens and diseases of crops under consideration, representatives from nurseries who understand the business of plant propagation and maintenance, including related financial constraints, and the state regulators who are responsible for developing certification standards and monitoring compliance. There are several requirements for the successful development of protocols, their acceptance by states, and implementation by nurseries of model certification guidelines including, but not limited to: (i) science-based evidence to fill in the gaps in knowledge that prevent informed decisions regarding requirements; (ii) a comprehensive and up-to-date database on targeted pathogens that is available to regulators, nurseries, and growers; (iii) outreach efforts to educate nurseries and growers about the benefits of using pathogen-tested plant material; (iv) incorporation of current nursery industry practices in the development of guidelines; (v) consideration of modern diagnostic technologies to expedite the production, release, and exchange of clean propagation material; and (vi) ongoing pilot studies of draft guidelines to identify problems with implementation by state agencies and nurseries followed by discussions on how to overcome these difficulties and still maintain the effectiveness of the program.

NCPN education and outreach. One of the goals of NCPN is to design effective media for outreach and education and the programs to increase stakeholder awareness of the economic benefits of pathogen-tested plant material. An important step in this process is a careful evaluation of the cost to nurseries, producers, and consumers for a specific disease and the benefits of pathogen testing and certification programs for a crop. Several economic studies have attempted to estimate the impact of viruses and the value of using pathogen-tested plant material. An analysis of the costs and benefits of testing and a certification program for *Grapevine leafroll virus 3* in the north coastal region of California concluded that the benefits exceed $60 million per year for the region, and economic returns outweigh the cost (34). A study on the impact of grapevine leafroll disease in the Finger Lake region of New York estimated that losses from this disease ranged from $25,000 to $40,000 per hectare over 25 years in the absence of any control measures (12), but in this study, the losses were not compared to the cost of a clean stock program. Cembali et al. (24) estimated the economic benefit of the clean plant center in Prosser, WA to nurseries, producers, and consumers based on projected yield loss and quality decline in apples, sweet cherries, and clingstone peaches. It was determined that the total gross annual benefit was approximately $227 million, or 420 times the cost of the clean plant center. The value of plant certification programs to the tree fruit growers in the U.S. Pacific Northwest estimated under different production scenarios ranged from $828 million to $4.7 billion (90).

All CPCs are charged with the task of informing breeders, regulatory agencies, nurseries, producers, extension educators, homeowners, and the public at large of the value of clean plants and clean plant programs. This is accomplished through verbal presentations at local, regional, and national producer meetings by experts working at CPCs, development and dissemination of fact sheets on targeted pathogens, development and publication of best management practices to prevent infection of crops by targeted pathogens, and funding of economic studies that gather and analyze data for use by nurseries, producers, and others, on the value of using clean plants. Additionally, a well-informed public is less likely to smuggle propagation material if they understand the danger of exotic pathogen introduction to economies and environments. A well-informed public is also less likely to smuggle in germplasm if they are aware that clean material is available within the United States.

The goal of NCPN through the work of CPCs is to produce, maintain, and distribute to the nursery industry plants that are free of targeted pathogens. These activities, in concert with carefully designed nursery certification guidelines for the acquisition, propagation, and maintenance of healthy nursery stock, provide a system to ensure that producers have access to the best propagation material possible. Furthermore, because NCPN provides avenues for communication among scientists, USDA, state certification personnel, nurseries, producers, and extension educators, it is uniquely suited to identify and combat new pathogen outbreaks. The ultimate goal is to create a process that minimizes the risk of unintended introduction of pests while encouraging the safe trade of healthy plants. It is all encompassed in the NCPN motto "Start Clean, Stay Clean".

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Dr. Gergerich is professor emeritus in the Department of Plant Pathology at the University of Arkansas. She earned her B.A. and M.S. degrees from the University of Wisconsin in Milwaukee and her Ph.D. in plant virology from Michigan State University. During her research career at the University of Arkansas as a plant virologist, she studied the mechanism of virus transmission by beetle and nematode vectors, and characterized plant viruses in horticultural and agronomic crops. She served as the Chair of Studies for the Interdepartmental Program in Plant Sciences. She currently serves as Administrative Officer for the National Clean Plant Network for Berries.

Dr. Welliver is Plant Pathology Program Manager at the Pennsylvania Department of Agriculture (PDA). She received a B.S. in microbiology at the Pennsylvania State University. She then completed a Ph.D. in the cellular and molecular biology program at the University of Wisconsin-Madison. Her work at PDA has primarily revolved around diagnosis of virus diseases in fruit trees and greenhouse crops. She serves on committees within the National Plant Board and the National Clean Plant Network.

Sarah Gettys works in the virus-tested fruit certification programs of the Pennsylvania Department of Agriculture (PDA), spending much of her time running the day-to-day operations of the Pennsylvania Fruit Tree Improvement Program, as well as contributing to several projects of the National Clean Plant Network–Fruit Trees. She received her B.A. in anthropology at Bryn Mawr College, then earned career changing degrees in environmental science and geographic information systems. She began working at the PDA in the plum pox virus eradication program in 2009, and remained to contribute to the working group that produced the harmonized draft of the state level model regulatory standard.

Dr. Osterbauer is Plant Health Program Manager with the Oregon Department of Agriculture in Salem. She received her B.A. in biology from the College of St. Scholastica, her M.S. in plant pathology from the University of Minnesota, and her Ph.D. in botany and plant pathology from Oregon State University. Much of her research is focused on developing or improving diagnostic methods for pathogens of regulatory concern and on applying the systems approach to mitigate the risk of spreading pathogens through the shipment of plants for planting. She currently serves on the National Clean Plant Network–Fruit Trees Tier II Commodity Committee and is developing the draft national standard to harmonize state regulations for the certification of strawberry nursery stock as pathogen-tested.

Dr. Kamenidou is a staff research associate in the Department of Plant Pathology and Microbiology at the University of California, Riverside. She earned her M.S. in horticulture and her Ph.D. in plant science from Oklahoma State University. Her graduate research focused on mineral nutrition and plant diseases of greenhouse ornamental crops. She currently serves as administrative officer for the National Clean Plant Network for Citrus.

Dr. Martin is a research plant pathologist working on viruses and virus diseases of berry crops at the USDA-ARS Horticultural Crops Research Unit in Corvallis, OR. He received his B.S. in forestry and Ph.D. in plant pathology from the University of Wisconsin–Madison. He then joined the staff at Agriculture and Agri-Food Canada. He returned to the USDA-ARS in 1995 and continues working on the characterization, detection, and management of viruses of berry crops and grapes. Recent efforts have focused on virus complexes. He has been involved with the National Clean Plant Network since its inception in 2007 and serves as chair of the NCPN for berries.

Dr. Golino has been the director of Foundation Plant Services (FPS) since 1994. FPS is a unit of the College of Agriculture and Environmental Sciences at University of California, Davis, and is dedicated to the distribution of disease-tested, true-to-identity plant materials produced or improved by UC researchers. She is a cooperative extension specialist in the Department of Plant Pathology. Her research program is directed at controlling the virus and virus-like diseases of grapevines with a focus on improved methods of pathogen detection and streamlining virus elimination techniques for clean stock and quarantine programs. She currently serves as the Chair for the National Clean Plant Network for Grapes.

Dr. Eastwell received a doctorate in plant biochemistry from the University of Alberta, Canada. As research scientist at Agriculture and Agri-Food Canada in Summerland, British Columbia, he characterized viruses and directed disease management programs for fruit trees, particularly little cherry disease. Eastwell joined the Department of Plant Pathology at Washington State University in 1997 to direct the program that distributes virus-tested fruit tree clones internationally. He advanced the operation of the National Clean Plant Network for virus control captured in the 2008 Farm Bill. He maintains an active research program investigating virus-like agents of perennial specialty crops.

Dr. Fuchs is an associate professor in the Department of Plant Pathology and Plant Microbe Biology at the Cornell University New York State Agricultural Experiment Station. He received his B.S., M.S., and Ph.D. in life sciences and molecular biology from the University Louis Pasteur in Strasbourg, France. For the past 7 years, he has worked closely with USDA-APHIS and the New York State Department of Agriculture and Markets to contribute to the eradication program of *Plum pox virus* in stone fruits in New York. He currently serves as vice-chair of the National Clean Plant Network for grapes and director of the Northeast Plant Diagnostic Network.

Dr. Vidalakis became the director of CCPP in the summer of 2005, a year after earning his Ph.D. from the Department of Plant Pathology at the University of California, Riverside. In 1998, he completed his studies (B.S. and M.Sc.) in the Agricultural University of Athens, Greece, with concentration in plant protection and environment and specialization in plant virology. Dr. Vidalakis represents UC Riverside and the California citrus industry in national and international programs and organizations such as the National Clean Plant Network while he serves as the secretary of the International Organization of Citrus Virologists.

Dr. Tzanetakis is working on the epidemiology and molecular biology of specialty crops viruses in the Department of Plant Pathology, University of Arkansas System. He participates in the National Clean Plant Network for berries and education and outreach groups, as well as the groups involved in the development of guidelines for a national certification schemes for berry crops.