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Pathogen-Tested Planting Material

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Glossary

Buffer zone An area surrounding or adjacent to an area for production of plants in a certification scheme designed to minimize the probability of spread of the target pathogens, pollen, or seed into or out of the block, to meet phytosanitary or other control measures as defined in a certification standard.

Certification standard Comprehensive process established and authorized by a regulatory entity for the production of plants free of regulated pathogens, with predefined trueness-to-type or genetic purity. These regulations also define plant production, plant identification and labeling, and quality assurance requirements.

Generation or G-level It indicates the relationship of plants in a certification scheme to the original pathogen-tested plant material at the top of the scheme. Regulations developed by certifying agencies specify the conditions under which each Generation (G) level must be maintained in order to meet the standard.

Heat therapy A method used to eliminate viruses in which plants are grown at 37–40 °C for 4–6 weeks. After this meristem tips (0.3–1.0 mm) are removed and used to regenerate plants. This results in the production of plants that are often free of targeted pathogens. Once grown in tissue culture, rooted and planted in soil, the plants are retested for the targeted pathogens to determine their health status.

Index Procedure to determine whether a given plant is infected by a targeted pathogen. It involves the transfer of a bud, scion, sap, etc. from one plant to one or more indicator plants sensitive to the virus.

International Standard for Phytosanitary Measures It is an international standard adopted by the Conference of the Food & Agriculture Organization(FAO) of the United Nations, the Interim Commission on Phytosanitary Measures, or the Commission on Phytosanitary Measures, established under the International Plant Protection Convention.

Meristem-tip (tissue) culture A pathogen-elimination technique whereby tissue pieces (cells) are separated from an organism and cultured in a sterile growth media apart from that organism. One of the preferred pathogen-elimination methods for plants is 'microshoot tip culture,' which is effective for eliminating most viral, bacterial, and fungal contaminants. In this technique a meristem tip of less than 0.3–1.0 mm is extracted from the shoot and placed in sterile tissue culture growth media; the meristem then grows to a complete plant.

Phytosanitary measure Any legislation, regulation, or official procedure with the purpose to prevent the introduction or spread of pests or diseases.

Quarantine Official confinement of regulated articles for observation and research or for further inspection, testing, and treatment.

Vegetative propagation Plants are reproduced using the following methods: cuttings, layering, division, grafting, budding, and tissue culture.

Introduction

Large-scale production and globalization of agriculture has resulted in a narrow germplasm base for all major food crops being grown over vast areas, which increases the risk of epidemics that could impact food security on a broad scale (Strange and Scott, 2005). In addition, the majority of the production of these crops is in areas far from their centers of origin and subjected to many plant pathogens that they did not coevolve with. Cassava is an excellent example of this, as its center of origin is in South America, but African cassava mosaic virus, which causes a devastating disease in Africa, is not present in South America (Fauquet and Fargette, 1990; Thresh *et al.*, 1998). Most of the production of these crops is isolated from new strains of pathogens as they evolve at centers of origin and are at risk of severe disease outbreaks should new strains of a pathogen be distributed with planting material. This is also true for most of the fruit, vegetable, and

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ornamental species that are grown commercially. Concomitant with the globalization of crop plants is an increased risk of globalization of plant pathogens that can lead to serious epidemics. The classic example of such movement of a pathogen is *Phytophthora infestans*, the causal agent of potato late blight in the mid-nineteenth century, where its impact on human suffering and migration is well documented (Schumann, 1991). Yet, 150 years later, new strains of this pathogen have emerged and continue to cause serious plant disease problems (Vleeshouwers *et al.*, 2011). There are examples of outbreaks of all types of pathogens (fungi, bacteria, nematodes, and viruses) that have resulted from movement of plant pathogens with the movement of plant propagation material.

To protect agricultural production from introduced pathogens, two types of phytosanitary systems have been employed. Plant quarantine is generally used at the national level to restrict introduction of plant pathogens that are not present in a country or have limited distribution and active programs to eradicate or delimit the pathogen. There are also domestic quarantines that restrict the movement of plant material within a country to address pests and pathogens that have limited distribution or may have been deregulated as a federal quarantine pest. Plum pox virus (PPV), Golden nematode, Light brown apple moth, *Ralstonia solanacearum* race 3 biovar 2, and *Phytophthora ramorum* are examples of quarantine pathogens and pests in the United States. Certification programs are national, state, or provincial programs used to produce planting materials, vegetative or seed, that are free of or have less than some predetermined threshold of various pathogens (Waterworth, 1998). These programs primarily target domestic pathogens for plants at the G1–G4 (**Figure 1**) level of certification programs. These programs aim to ensure that the planted crop has a pathogen(s) level low enough to minimize the risk of severe crop loss, increasing the chance of harvesting an acceptable crop. The pathogen tolerances allowed in certified plants depends on the pathogen, crop, and agroecosystem where the crop is grown. The primary goal of quarantine and certification programs is to facilitate movement of plants without increasing trade of plant pathogens. The objective of all plant certification programs is to provide a supply of healthy plants, which is accomplished through adherence to Best Management Practices (BMPs) that are science based together with a defined level of testing to monitor the effectiveness of the program. The application of BMPs to the production system combined with audit testing has proven to be a very useful means of producing plants of high health status. Certification programs should be reevaluated periodically to take into account new pathogens or vectors that have been reported in the region, or other changes that affect the pathogen, vector, host, or environment that may impact the integrity of a certification scheme.

BMPs require that a Hazard Analysis and Critical Control Points evaluation of the entire certification system be completed. This process is used to: (1) identify weak points and greatest risk factors to the system in terms of introduction of pathogens or pests, and (2) establish inspection, testing and mitigation procedures, and record-keeping requirements (Parke and Grünwald, 2012). Once the weak points are identified, management practices are designed to prevent intrusion of pests and pathogens. Mesh size on a screenhouse might be adjusted to account for the primary virus vectors in the region to protect a G1 block. For example, if pathogens in a region are vectored by aphids the screen size might be larger than in another region with pathogens vectored by thrips. Vector management in field blocks of certified plants will be adjusted based on the vectors and pathogens present in the region. Knowledge of the phenology of vectors is also important, because times of peak vector movement present the greatest risk of pathogen intrusion into the system; thus, vector control measures may well change during the year to take into account the risk of vector migration into a nursery. Knowing which of the targeted pathogens are most prevalent in a region and how they are vectored are important considerations when developing BMPs for a nursery (Martin and Tzanetakis, 2013).

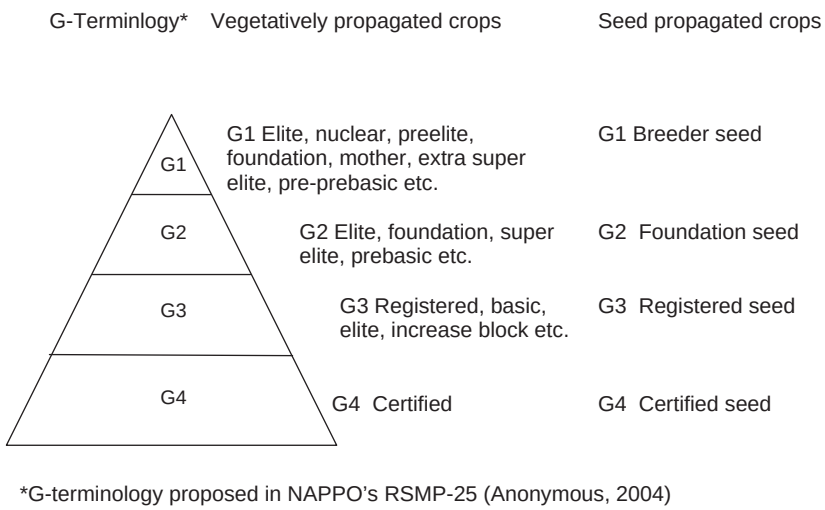

Figure 1 Simplicity of the G-terminology and the concept of G-levels for identifying the stages of plant propagation in certification programs for phytosanitary purposes.

Quarantine

In addition to certification programs that are designed for mass production of planting materials of high health status for plant, food, and fiber production, there are extensive quarantine programs that have been developed in many countries to reduce the risk of introduction of exotic pests. Though not the focus of this article, quarantine goes hand-in-hand with certification to provide an integrated system to protect agriculture and environment from plant pests and pathogens, thus its relation to certification will be touched on briefly. The rationale for a brief overview of quarantine is that many certification programs focus on domestic or endemic pathogens, with the assumption that quarantine pathogens are detected and eliminated at the stage where plants enter the country and need not be included in the domestic certification program. Quarantine programs are operated primarily at the national level and deal with pathogens that are not known to occur in the country or are present at a very restricted level and have active programs targeted to eradicate or prevent further spread of the pest or pathogen (Foster and Hadidi, 1998; Reed and Foster, 2011; Anonymous, 2006). As examples, plant material of berry crops, citrus, grape, potato, sweet potato, pome, and stone fruit, etc. coming into the United States under quarantine are tested for exotic pathogens before they are released for propagation, but they could be released if found to be infected only with viruses already endemic in the country. In some cases plants may be held in quarantine for 3–4 years while graft indexing on indicators is carried out.

To be effective, quarantine programs are dependent on the public understanding the risks associated with introducing exotic pathogens into natural and agricultural ecosystems. Public in this sense includes anyone who may wish to transport plant material from a foreign country to their homeland, including hobbyist interested in a new ornamental or food crop to add to their garden or plant collection, plant breeders interested in new potential germplasm, plant pathologists interested in adding to their pathogen collection for scientific study, germplasm curators attempting to broaden the diversity of their collections, or growers interested in getting a head start on a new cultivar developed in a foreign country. Also, an effective quarantine program needs to have an effective and efficient mechanism to introduce plant material into the country. This combination of an educated public that understands the risks of introducing foreign pathogens and pests, together with an efficient system to bring plant material through approved quarantine and testing facilities will reduce the temptation by individuals to do 'suitcase' imports that could threaten local environments and agriculture. The introduction of PPV into Pennsylvania highlights the benefits of quarantine systems to exclude exotic pathogens. Plum pox is a devastating disease first identified in the early 1900s in Bulgaria that has since spread through much of Europe and the Mediterranean countries, where it is the most serious disease of stone fruits. It was detected for the first time in the Western Hemisphere in Chile in 1994 (Herrera *et al.*, 1998) and is now considered to be widespread there and has since been detected in Argentina. PPV was detected in a localized area of Pennsylvania in the United States in 1998 (Levy *et al.*, 2000) and an eradication program was implemented immediately. After 14 years, the eradication effort was deemed successful, but the cost was in excess of $55 million US dollars to eliminate the pathogen from a relatively small geographic area (Welliver, 2012). Thus, quarantine programs can be an effective and economical way to reduce international movement of plant pathogens and pests to protect local agriculture and native flora.

The International Plant Protection Convention (IPPC) is an international agreement on plant health to which 181 countries are signatories. The Secretariat of the IPPC is provided by the Food and Agricultural Organization of the United Nations. The IPPC has the mission to protect cultivated and wild plants by preventing the introduction and spread of pests and pathogens. They develop standards (International Standards for Phytosanitary Measures (ISPMs)) that are recognized by participating countries and provide for science-based standards for the safe movement of plants and plant products. As an example, ISMP No. 28 (Anonymous, 2007b) provides recognized standard treatments to eliminate plant pathogens and pests, including fumigation, cold treatment, heat treatment, and irradiation; and ISMP No. 31 (Anonymous, 2008) provides agreed on sampling levels that participating countries use when shipping plants or plant products internationally to protect against movement of quarantine pests and pathogens. The IPPC develops standards for range of issues related to plant protection. Once the standards are approved by the IPPC member countries, they become an ISMP.

Certification

Certification programs have been developed for many vegetatively propagated food crops over the past 50 years (Hadidi *et al.*, 1998; Hadidi *et al.*, 2011). The first certification programs were developed in The Netherlands and Germany in the early 1900s for potato production when they realized that leaf crinkling, rolling, and mosaics were transmitted through the tubers to the next generation and that by selecting the most vigorous and healthy-looking plants for propagation, tuber production was improved greatly. They developed a program for plant inspection and production, which was adopted in the United States and other countries long before it was known that many of the diseases were caused by viruses. In the United States and Canada the first seed potato production programs were established in 1913 (Slack and Singh, 1998). Visual inspections still play a major part in seed potato certification programs with inspections carried out early and midlate in the growing season to observe foliar symptoms, at harvest for observing tubers symptoms, and a postharvest inspection of plants grown from tubers to look for late-season infections (Frost *et al.*, 2013). Visual inspections are part of all plant certification programs. Trained inspectors also watch for other problems, such as herbicide damage, cultivar mixtures or trueness-to-type issues, physiological disorders, etc. During visual inspections, thousands of plants can be observed in a relatively short time. In most programs there is also laboratory testing that is carried out on each tuber or 'seed' lot to identify viruses that may be present and to monitor for symptomless pathogens. As laboratory-based diagnostics are improved in terms of sensitivity, specificity, and costs, more programs are

incorporating their use to improve the quality of the program. Certification programs have been developed for a wide range of crops (Hadidi *et al.*, 1998). Testing methods including mechanical transmission to herbaceous hosts, immunological and molecular techniques (Enzyme-linked immunocapture assay (ELISA), Polymerase chain reaction (PCR), etc.), isolation of fungi or bacteria, etc. that are used for detection of various pathogens are similar in all programs. With woody crops there is often the need to do graft transmission onto indicator plants to test for uncharacterized graft-transmissible agents (Converse, 1987; Hadidi *et al.*, 2011).

There are many definitions for certification programs. North American Plant Protection Organization (NAPPO) defines a certification program as: "A domestic program consisting of maintenance, multiplication, distribution, and production of plant materials intended for release either domestically or for export, under an officially sponsored certificate attesting to the status of the material" (Lanterman *et al.*, 1996). Many certification programs are based on a published standard that defines site selection and preparation, isolation distances from plants of the same species and other vegetation, number of inspections, record keeping on plant traceability so that tracebacks or traceforwards can be done if a problem should arise, a pest and disease management plan, records of all pest management activities, the conditions and protocols to be followed during plant or seed production, and types and amount of testing that needs to be done at each level in the propagation cycle. The selection of plant material that is true-to-type is an essential first step. The selected plant(s) is then subjected to pathogen testing using a range of laboratory and biological indexing. If infected with any of the targeted pathogens listed in the standard, the plants are subjected to 'cleanup.' After 'cleanup' the plants are retested to ensure the pathogen(s) have been eliminated. Once determined to be free of targeted pathogens and true-to-type, this plant can be designated as a G1 (**Figure 1**) plant that enters into the certification program. In many cases these plants are maintained in protected culture (screenhouse) and become the source plants that are propagated in certification programs.

There is an effort in the United States to develop audit-based certification programs that rely on BMPs outlined in the Certification Standard (Thompson, 2011). An audit-based program relies on compliance monitoring and requires a reasonable level of trust between the regulatory agency (inspectors) and nursery managers for the certification systems to function. The audit or inspection assesses the degree of compliance of the nursery to the certification standard, so that plants can be certified to meet that standard. An auditor or inspector designated by the certifying agency is responsible for the audit process. All records of the nursery can be reviewed by the auditor (Thompson, 2011). With such a system in place, record keeping by the nursery becomes even more critical as the inspector's decision will be based on the records reviewed, visual inspection of the nursery, and some limited testing. The goal is to have a systems approach that uses science BMPs for nursery production of plant materials (Parke and Grünwald, 2012).

The use of pathogen-tested planting material is the first and arguably the most important step for the control of many systemic plant pathogens. Most effort in this area has focused on viruses, viroids, systemic bacteria, and phytoplasmas because there are no postinfection treatments that can be used in plant or food production systems to rid plants of these pathogens. Many important systemic pathogens are not transmitted through the seed or transmitted to less than 100% of the seedlings, or transmitted as seed coat contaminants that can be controlled with various seed treatment methods (Ling, 2010; Liu *et al.*, 2014). In this case, seedlings free of the targeted pathogen(s) can be identified and then grown in isolation to produce seed free of these pathogens or with an incidence below some predetermined threshold. With seed coat contaminants the seed can be treated to inactivate pathogens on the seed surface (Ling, 2010). In the case of vegetatively propagated crops it is often necessary to eliminate a specific pathogen, usually through thermal-, cryo-, or chemotherapy combined with meristem-tip culture to produce plants free of the targeted pathogens (Mink *et al.*, 1998; Laimer and Barba, 2011). The plants regenerated from such meristems are then thoroughly tested, and a single plant free of pathogens of interest is the starting point for massive vegetative propagation. In the case of seed or vegetatively propagated plants, the plants are propagated under a defined set of conditions described in a certification standard.

Certification standards often have tolerance levels of some small percentage of infection, defined requirements for the site where the crop (seed or vegetative) is grown, required field inspection(s) during the production cycle that include cleanliness in terms of weeds, other crops that may serve as contaminants in seed lots or as hosts for targeted pathogens and freedom from pathogen vectors. Tolerances for pathogens depend on the rate of pathogen spread for annual crops, but tolerances are much lower for perennial crops. Crops that are only grown for a single year or a few years in production fields, such as most seed crops and potatoes, strawberries, sweet potatoes etc., may have higher tolerances than most of the fruit and nut crops, such as citrus, tree fruits, grapevines, or berries, which are expected to be productive for many years or decades in the same field. To set tolerances for various pathogens requires sampling and testing, and a major concern is how many samples to test. The number of samples that need to be tested per lot to achieve a confidence level (i.e., 95% or 99%) that a pathogen is not present is outlined in ISPM No. 31 (Anonymous, 2008). In some countries, certification schemes are managed at the national level and in others at the state or provincial level.

Vegetatively Propagated Crops

Plant pathogens are recognized as major constraints to agriculture production worldwide. Most fruit and nut crops; major food crops such as potato, cassava, sweet potatoes; and many ornamentals are vegetatively propagated, using cuttings, tubers, or rhizomes. More recently, tissue culture propagation has become a major component of mass propagation for many of these crops. Elaborate protocols are used to eliminate targeted pathogens from one or a few infected plants to provide a source of plant material free of these pathogens (Mink *et al.*, 1998; Laimer and Barba, 2011). Plants entering a certification program are often advanced selections, cultivars, or varieties

developed in breeding programs. In some cases breeders work closely with the cleanup programs and get plants into the testing and cleanup phase before cultivar release, in an effort to have certified plants available at the time of release. Another source of material entering certification programs are 'heritage' cultivars – cultivars that have not been used for many years but are maintained at germplasm repositories. As requests for these cultivars have increased, there is a need to confirm that they meet current certification standards before commercial nurseries are willing to add them to their production systems. In the United States, there is an effort to have new cultivars coming into the country, as well as those developed in-country go through one of the 'clean plant centers' that have been developed over the past 50 years. Increased funding for these programs at the federal level since 2009 has provided for capacity building and the ability to process materials in a more timely manner to meet the needs for food production. These plants are tested for target pathogens before they enter into a G1 block, then a certification program and made available for production. In many cases, the G1 block is maintained by federal, provincial, or state government agencies, or some type of government/private entity, though recently private companies are maintaining their own proprietary G1 germplasm. These plants are tested on a predetermined schedule to monitor for reinfection, in addition, these blocks are retested if a new virus is discovered in the crop that may have been missed with the testing procedures used previously.

Producing G1 Plants

1. Candidate plant (advanced selections, heritage, new, or imported cultivars) arrive at clean plant center, (time = 0, T = 0).
2. Testing program to determine the health status of the plants with respect to targeted pathogens listed in the certification standard, grafting for many fruit and nut crops. This process can take up to three years for crops, such as grapevines or fruit trees, but less time for other crops (T = 0.2–3 years). If no grafting is required this can be completed in several weeks; this is the case for some crops, such as potatoes.
3. If negative for all targeted pathogens, the plant enters the G1 block. If infected, the plants are put through therapy treatment (2–6 months), meristem-tip cultured and whole plants regenerated (3–12 months).
4. These plants are then retested and, if 'clean,' enter the G1 block; but if still positive they go through the therapy again (T = 0.1–3 years). This is repeated until 'clean' plants are obtained. Each cycle of testing can take up to 3–4years for crops like grapevines or fruit trees, 1–2 years for strawberry or *Rubus*. In most cases protocols have been developed such that there is a reasonable likelihood of having 'clean' plants after the initial cycle of therapy. However, if three cycles of cleanup were needed to get plants free of targeted pathogens this can easily require 6–12 years, depending on the crop.

There is a misconception among many growers and researchers that 'tissue culture' propagated is synonymous with virus free. This is not the case. To eliminate viruses it is necessary to regenerate plants from meristematic tip, generally less than 0.5 mm and often in combination with some type of therapy (thermal, cryo, or chemo) before removing meristems (Mink *et al.*, 1998). Some viruses move into the meristematic tissue quite effectively, and a combination of thermal therapy or chemotherapy are required to get meristematic tissue free of the virus (Chen and Sherwood, 1991). In the past these were referred to as 'heat stable' viruses and were 'difficult' to eliminate using thermal therapy and meristem-tip culture, whereas 'heat labile' viruses were much easier to eliminate. One now know that many of the 'heat labile' viruses are phloem-associated viruses, such as luteoviruses and closteroviruses, and the reason they are 'heat labile' is that the phloem tissue is not differentiated in the meristematic dome. Thus, the virus does not move readily into the meristem. This is the case for the Grapevine leafroll-associated viruses, which are in the *Closteroviridae* family. In raspberry, Raspberry leaf mottle and Raspberry leaf spot were considered 'heat labile' as they were relatively easy to eliminate. These two diseases are caused by strains of Raspberry leaf mottle virus, which is a Closterovirus and phloem limited. Raspberry bushy dwarf virus (RBDV) was considered a 'heat stable' virus because it is difficult to eliminate using thermal therapy and meristem-tip culture. RBDV infects most cell types and is not restricted to phloem tissue, thus, it likely moves into the meristematic dome much sooner than phloem-restricted viruses. The ease of obtaining meristems free of a virus in combination with heat therapy is not related to the heat stability of the virus, but rather how rapidly or effectively it can invade the meristematic tissues.

The 'cleanedup' G1 plants are maintained under clearly defined conditions to minimize the risk of reinfection and should be free of all targeted pathogens outlined in the certification standard. In many cases the G1 plants are maintained in protected culture, such as in a screenhouse. The G1 plants are then sold to private nurseries for mass propagation under a set of conditions outlined in a certification program that is managed by a regulatory agency. The goal is to have a systems-based approach that addresses risks of reinfection and pathogen spread during plant propagation.

There are multiple cycles of plant increase for vegetatively propagated crops and unfortunately a wide range of terms have been used to identify each cycle (**Figure 1**). In 2004, the NAPPO (RSMP no. 25; Anonymous, 2004) suggested the use of a simple terminology for the cycles of vegetative propagation. G1 plants are the plants that have tested negative for all targeted pathogens outlined in the certification scheme. G1 plants propagated by tissue culture or by traditional vegetative propagation methods become G2 plants, and multiple cycles of tissue culture can be carried out and still retain G2 status. The use of tissue-culture propagation and the number of propagation cycles allowed in tissue culture should be defined in the certification standard and will vary depending on the crop and certifying agency. G3 plants are derived from G1 or G2 plants. G4 plants are grown as potted plants that are propagated from G1, G2, or G3 plants and grown at another location. Most existing programs use the various terminologies shown in **Figure 1**, but follow this basic scheme of scale up in stages 2–4 to produce plants that are sold to growers.

With the application of tissue culture in some propagation schemes, nurseries are able to sell G2 or G3 plants to growers, which should translate into higher health-status plants being

used by the industry. However, for many crops conventional vegetative propagation is still the primary means of plant multiplication that is used. There are several reasons for this: (1) costs for conventional propagation of crops, such as strawberry, where a 300–500 fold increase can be obtained in field production each year, is relatively inexpensive to produce millions of plants; (2) the agency that regulates the certification program may prohibit or limit the number of cycles of tissue culture as a means of propagation due to concerns about somatic mutations; (3) grafted plants may use a rootstock that is generated from seed, which is inexpensive. In such cases, the rootstock may be seed-propagated and the scions by tissue culture or by conventional vegetative propagation methods. During propagation it is important that care is taken to prevent contamination of tools with viruses that are readily mechanically transmitted. Tools are sterilized during tissue-culture propagation to maintain sterile conditions during transfers of tissue-culture plants. It is also necessary in conventional propagation to prevent spread of viruses during the cutting of tubers, rhizomes, taking cuttings, etc. (Lewandowski *et al.*, 2010). Contamination using cutting tools is much more likely in herbaceous crops, such as potatoes, than in woody crops like grapevines, tree fruits, nut crops, etc.

There are efforts among regional plant protection organizations (RPPOs) to harmonize quarantine standards between the member countries. There are nine RPPOs (Asian and Pacific PPO; Caribbean Plant Protection Commission; Comite de Sanidad Vegetal del Cono Sur; Comunidad Andina; European and Mediterranean PPO (EPPO); InterAfrican Phytosanitary Council; Near East PPO; North American PPO; Organismo Internacional Regional de Sanidad Agropecuaria and Pacific PPO (Roy, 2011)). There are also efforts at harmonizing certification standards across some of the RPPOs, such as for fruit trees and grapevines in NAPPO countries, and the EPPO countries have adopted certification schemes for 20 crops (Roy, 2011; EPPO website). These RPPO-developed standards are often a minimum standard that is required, but member countries can require a more stringent standard internally. Although this is happening at the international level, there are many cases where the harmonization of certification programs within countries has not happened. In countries where these programs are regulated at the province or state level, there are often significant differences between the certification standards. For example, in the United States some states require that the G1 plants for some crops be maintained within protected culture (screenhouse) to minimize vector transmission of pathogens, whereas other states do not require that same level of protection. Also, the type and level of testing required at each level in the certification scheme can vary between states. There is an effort underway to harmonize certification standards for some of the fruit crops across the United States. As these programs are developed, they are looking at certification schemes in other countries and RPPOs with the goal of harmonizing standards on a broader scale if possible.

Seed Propagated Crops

For seed propagated crops, seed is often increased in a country other than where the seed will be planted for food or fiber production. To speed up increase of seed for commercial production many companies grow seed in the northern and southern hemisphere to get two cycles of increase in a single year. As a result, seed is moved between countries on a frequent basis and are potentially exposed to quarantine or certification pests of concern in the country where the final crop is grown. Also, because seed has a long shelf life, it may be moved to several countries before it is planted, or a seed lot may be subdivided and shipped to multiple countries. Many countries have phytosanitary requirements for the movement of seed though requirements for field inspections, sampling, and testing can vary and cause problems if trying to move the seed internationally. The IPPC's ISPM No. 28 provides information on agreed on treatments for a range of regulated plants pathogens and pests (Anonymous, 2007a). The preparation of a phytosanitary certificate is done by the exporting country but must meet the requirements of the importing country.Thus, if a seed lot is being shipped to multiple countries the documentation can become very complicated. IPPC has also developed ISPM No. 12, which covers the reexport of the seed to a third country, where the phytosanitary requirements would have to be met by the original exporting country (Anonymous, 2011). For these reasons, harmonized standards could greatly facilitate seed trade to meet the needs of the increasing globalization of agriculture.

There are a number of agencies for seed testing and accreditation of certification schemes. The testing and accreditation by these agencies are recognized by various countries or groups of countries and provide a mechanism to harmonize seed certification standards for international movement of seed among a group of countries. The Association of Official Seed Analysts, Association of Official Seed Certifying Agencies, International Seed Trade Association (ISTA), National Seed Health System, OECD Seed Schemes (US), Society of Commercial Seed Technologists are all involved in seed testing and are recognized by multiple countries. ISTA testing and accreditation is recognized by more than 70 countries. NAPPO regional standards for phytosanitary measures No. 36 provides information on the movement of seed between countries in North America (Anonymous, 2013).

For seed production, the emphasis is on eliminating pathogens from the elite germplasm that can be transmitted through seed or contaminate the seed surface, including viruses, bacteria, and fungi. In many cases, elite germplasm of seed crops is produced and maintained by private companies. With seed certification, genetic purity is often as great a concern as pathogen contamination. There are multiple levels in certification programs for seed crops, similar to those used for vegetatively propagated crops. The seed crops would clearly fit under the G terminology shown in **Figure 1**, and in this case G would stand for generation in the true sense of the word. The four common levels in seed certification (AOSCA) include: Breeder Seed (seed controlled by the plant breeder, or the institution or company where the breeder works), Foundation Seed (propagated from Breeder Seed under conditions that retain genetic purity, identity, and health status), Registered Seed (propagated from Breeder or Foundation Seed under conditions that maintain genetic purity, identity, and health status), and Certified Seed (progeny of Breeder, Foundation or Registered Seed and grown under conditions that maintain

genetic purity, identity, and health status). In addition to genetic purity, identity, and health status, seed certification also requires information on percentage germination, date of germination test, and percentage contamination with other seed.

For pathogens that are transmitted internally in the seed, it is often possible to grow out seed and identify plants free of the pathogen because this type of transmission is rarely 100% efficient (Liu *et al.*, 2014; Mink, 1993). The exceptions to this are the cryptic viruses, which are seed transmitted at 100%. These viruses are not known to cause disease and are not considered in quarantine or certification programs. Heat treatments for eradication of embryo infection by virus have not been successful without a loss in seed viability (Maury *et al.*, 1998). For seed contaminants, various types of seed treatment have been used to eliminate or reduce pathogen level below set tolerances. Dry heat at 35°C for 24 h, followed by 50°C for 24 h, and finally 75°C for 72 h was very effective at controlling very stable viruses, such as tobamoviruses (Tobacco mosaic virus, Tomato mosaic virus, and Cucumber green mottle mosaic virus, Lee, 2004) and potexviruses (Pepino mosaic virus, Ling, 2010) as well as for a wide range of fungal and bacterial pathogens with little or no effect on seed germination (Lee, 2004). Soaking tomato seed for 30 min in a 1% sodium hypochlorite solution (dilution of commercial bleach, depends on the percentage active ingredient in the bleach product) containing 0.1% Triton-X-100 as a wetting agent completely inactivates potexviruses (Pepino mosaic virus; Ling, 2010) on the seed surface with little or no impact on seed germination. Hydrochloric acid or trisodium phosphate were not as effective as bleach in eliminating virus from seed coats (Ling, 2010).

Impact of Improvements in Pathogen Detection on Certification Programs

With woody plants that involve graft transmission assays for detection of viruses, grafted plants may need to be observed for two or more years for symptoms before the plant is determined to be free of the pathogen. These potentially long intervals for detection using biological indexing has prompted most quarantine facilities to adopt laboratory-based testing where possible (Rowhani *et al.*, 2005; Martin *et al.*, 2012; Martin *et al.*, 2013), including: electron microscopy, ELISA, immunospecific electron microscopy, nucleic-acid-based techniques such as PCR or reverse transcription –PCR (RT-PCR) combined with gel electrophoresis and sequencing of the any amplicons obtained, double-stranded ribonucleic acid (dsRNA) detection; and mechanical transmission to herbaceous hosts (Reed and Foster, 2011; Miller *et al.*, 2009). However, with widely used tests currently available, there is still a need for biological indexing onto woody indicators for some exotic pathogens. For many of the crops there are various laboratory tests available for the major pathogens of concern. Thus, in some cases, once all laboratory tests have been completed and found negative, plants are released to the importer on a provisional basis while the biological indexing is completed. This is done with an agreement that in case the tests are positive, the plant will be destroyed. This process allows the importer to begin multiplication of a new cultivar, which in some cases can reduce the time to get the materials to production fields by 2–3 years. The plants must be maintained and propagated under quarantine-approved conditions until all testing is completed.

The currently used assays for virus detection (ELISA, PCR, and q-PCR) are great for detecting a virus in a large number of samples, such as in surveys or ecological or epidemiology studies. However, for quarantine and certification programs a method that was capable of detecting all viruses in a single test would be ideal, rather than doing individual tests for each virus known to infect the host. In some crops, this means 40–60 tests per plant (berries or grapevines). Recent work with macroarrays has shown great promise for detecting the most common viruses in grapevine, with 38 viruses detected in a single test (Thompson *et al.*, 2014).

Potentially, within the next five years as 'Deep' or 'NexGen' sequencing becomes more widely adapted (Studholme *et al.*, 2011; Kreuze *et al.*, 2009), and universal plant microarrays are perfected (Hammond, 2011) they could replace indexing. This would require extensive validation of these technologies to ensure they are as good or better than current methods. The advantage of these technologies is that they have the potential to provide information on any virus in a plant without any *a priori* knowledge of the virus, in contrast to other laboratory techniques that detect known viruses (Kreuze *et al.*, 2009; Al Rwahnih *et al.*, 2009; Kashif *et al.*, 2012; Thekke-Veetil *et al.*, 2013; Seguin *et al.*, 2014; Hammond, 2011; Esteban *et al.*, 2010). Wang *et al.* (2002) demonstrated the feasibility and utility of the microarray technology to identify and characterize new viruses. They developed microarrays containing oligonucleotides that represented conserved sequences of all fully sequenced human respiratory viruses, which at the time represented a few hundred viruses. Using this array they identified a novel Coronavirus and showed that it caused severe acute respiratory syndrome, a newly emerging disease at the time (Wang *et al.*, 2003). NexGen sequencing has a huge impact on many aspects of biology and is used in virus discovery and is being investigated for virus diagnostics. Sequencing of total nucleic acids in plants has led to the identification of multiple viruses and viroids in single plants (Adams *et al.*, 2009; Al Rwahnih *et al.*, 2009, 2011; Kreuze *et al.*, 2009; Sequin *et al.*, 2014) and offers the potential to certify plants as virus-free rather than virus-tested. Obtaining the 'virome' of a plant provides much information on the range of viruses and their diversity within a single plant. However, work with grapevines has shown that many of the viruses were related to mycoviruses rather than plant viruses (Al Rwahnih *et al.*, 2011; Coetzee *et al.*, 2010). This leads to questions on the significance of these viruses in plant health. The new technology will allow for rapid discovery of many new viruses; unfortunately, characterizing the biological significance of these viruses will take much longer. Mycoviruses in grapevines may actually be modifying endophytes and indirectly impacting the health status of grapevines. Thus, eventually how viruses and other microorganisms impact the whole plant (understanding the microbiome) may be important in certification and quarantine, but we are not there yet. Although it is good to know what is in the plant, this technology is resulting in the identification of many new

viruses for which there is no biological information. For the next decade it is likely that the biological significance will lag behind the discovery of microorganisms in plants, and it is important that for certification and quarantine programs, any changes are made in response to the biology of these organisms rather than their presence. It seems reasonable that new plant viruses related to known plant pathogenic viruses should be considered as the highest priority for evaluating their biological significance and determining if they should be part of quarantine and certification regulations.

Certification standards vary widely among crops and regulatory authorities. The viruses included in a certification program can vary from country to country or state to state. An excellent example is grapevines where certification in Italy includes more viruses than programs in Germany or France; or grapevine certification in Washington State is different from California. Some of these differences are due to environmental considerations, where infection by a pathogen may cause severe disease in one setting, such as crown gall in grapevine in New York State, but be latent or symptomless under different environmental conditions, such as crown gall in grapevine in California. Attempts to harmonize certification schemes across boundaries to facilitate trade of plants without increasing trade in plant pathogens will require certification programs to account for disease expression by pathogens in areas where the certified plants may be sold, rather than only where the nursery stock is produced. In the United States, where certification programs are regulated by individual states, there are efforts underway in multiple crops (blueberry, grapevines, hops, *Rubus*, strawberry, and fruit trees) to develop a single certification standard that all states with programs for that crop would adopt. If successful, this in essence would provide a national program for certification of these crops. As this process is developing, there is an ongoing communication with trading partners to harmonize these standards as much as possible with their certification programs to facilitate international trade.

See also: Climate Change and Plant Disease. Emerging Plant Diseases. International Trade. Quarantine and Biosecurity. Regulatory Conventions and Institutions that Govern Global Agricultural Trade

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