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Quarantine Testing of Prohibited Germplasm by the USDA at Beltsville, Maryland

JOHN S. HARTUNG[1]

Abstract

Quarantine testing of imported plant germplasm is essential to protect U.S. agriculture against the inadvertent introduction of foreign pests and pathogens and is required by law. The testing of pome and stone fruits, brambles, potatoes, sweet potatoes, sugarcane, rice, grasses and some other crops is carried out jointly by two agencies within the United States Department of Agriculture at Beltsville, MD. Policy, budget, horticultural and technological questions as well as the perceptions of the user community have a direct impact on the operation of the testing program. A policy question that is of paramount concern to the operation of the testing program is whether or not to release foreign accessions if they are known to be infected with strains of a pathogen that is already found in the U.S. Low funding levels have hampered the program in the past, but additional funds have recently been made available for the program. The implementation of advanced technology has improved the testing program and will continue to do so in the future. A customer panel composed of people with technical knowledge in horticulture, plant pathology and advanced diagnostic methods is essential. Because the composition of this group will necessarily reflect the diverse crop responsibilities of the PGQO, communications among the 'customer' communities will be facilitated which should lead to a better understanding among the user groups of the complexities of the PGQO operations.

Introduction

The importation of foreign germplasm is necessary to maintain the competitiveness of domestic fruit and vegetable industries for the simple reason that nearly all commercially produced crops have originated outside of the United States and therefore the genetic diversity for these crops is found elsewhere. In addition, other countries also produce horticultural varieties that may be of interest to the U. S. industry. The international movement of plant germplasm has gained importance with the advent of international trade in fresh produce. The risk of inadvertent introduction of pathogens along with foreign germplasm is well understood, since the center of origin for pathogens adapted to a particular plant can be expected to be the same as the center of origin for the plant itself. Bos (2) provides a discussion of the Andes as the center of origin for potato viruses, as well as the role of humans in transporting viruses along with plant germplasm. Further, in nature, viruses are presumably closely adapted to their hosts and may cause no obvious symptoms (6). However, when introduced into different genotypes either experimentally or through horticultural practices, severe disease may occur (2) . This risk can only be mitigated by thorough testing, which is why plant germplasm is held in quarantine between the time of importation and distribution.

In the United States, testing of stone and pome fruit germplasm is done at Beltsville, Maryland by the Plant Germplasm Quarantine Office (PGQO). This unit is also responsible for the quarantine testing of other major crops including rice, potatoes, sweet potatoes, sugarcane, roses, small fruits (including brambles, currants and gooseberries), as well as cassava and even maize from certain countries. Responsibility for testing is shared by two agencies within the United States Department of Agriculture. The Agricultural Research Service (ARS) performs all testing and provides land, as well as all funding and personnel for plant testing. The Animal and Plant Health Inspection Service (APHIS) provides and maintains the laboratory and

[1]USDA-ARS, Plant Germplasm Quarantine Office-Fruit Laboratory, Building 010-A, BARC-West, Beltsville, MD 20705. e-mail: jhartung@asrr.arsusda.gov

greenhouse space, approves all testing protocols prior to implementation, and certifies the results of testing and releases germplasm for distribution. There have been difficulties in the implementation of the plant quarantine and testing program, many of long-standing (7). The unit was administratively moved into the Fruit Laboratory at Beltsville in July, 1997 in an effort to address a complex of inter-related issues. Both the systemic issues relating to the quarantine testing program and changes that are being made in the local management of this testing program are the subjects of this report. This report is intended to initiate dialog among the members of the germplasm community and contribute towards the resolution of at least some of the serious problems that make this essential activity so difficult to implement. The pome fruit testing program is the subject of an accompanying report (4).

Policies that Affect Quarantine Testing

The split responsibility between ARS and APHIS for the management of the plant germplasm quarantine program has not created major problems. However, the agencies do have different responsibilities and interests which stretch the program in different directions. The interest of APHIS in plant quarantine is to protect U. S. agriculture against the introduction of foreign pathogens. The interests of ARS are broader. The ARS has long had a policy of not recommending germplasm for release from quarantine unless the germplasm is shown to be free of all known pathogens, not just the exotics. This policy requires testing beyond what is required by APHIS and has been justified on the grounds of preventing the further spread of endemic pathogens or the introduction of novel strains of endemic pathogens. The ARS policy reduces the amount of pathogen testing and or therapy that would otherwise be done by the various crop repositories or state agencies and makes unrestricted releases of germplasm within the entire U.S. possible. In the absence of such pathogen testing of germplasm at the Federal level, each state would have to regulate the testing and distribution of germplasm. This would inevitably greatly restrict the exchange of germplasm within the U. S., to the detriment of agricultural industries. As such, the implications of changing the 'pathogen free' policy of ARS would be large. The desire for expeditious and economical processing of germplasm through quarantine must be balanced against both the risks of spreading existing pathogens and the additional burden placed on the states to regulate the interstate transfer of germplasm.

The PGQO also maintains a substantial number of apple and pear accessions that have been pathogen-tested and found to be infected with viruses or viroids. These have neither been released nor destroyed, but instead have accumulated to accommodate the wishes of the importers. This material represents a tremendous drain on the limited resources of the PGQO. Limited attempts have been made to produce, by thermal therapy, clean propagants for release, often without success. In any case, funds have not been available to conduct a routine thermal therapy program for pome fruit germplasm at the PGQO.

A change in policy by ARS that would allow conditional release of material infected with strains of endemic pathogens to states that would accept such accessions would contribute to the resolution of this problem. However, in some instances, the importer will have to accept that the material can not be maintained indefinitely in hopes of a technological breakthrough that would allow it to be freed of the viruses or viroids. The orchard at Glenn Dale, Maryland where this material was maintained was terminated at the end of 1997 and all orchard operations have been consolidated in Beltsville at the Building 580 site. However, the virus and viroid positive accessions have been repropagated and planted in the Building 580 orchard. These decisions were made in the interest of operational efficiency.

User Perceptions that Affect Plant Germplasm Quarantine and Testing

Another major issue is the failure on the part of some members of industry and the plant germplasm community to acknowledge the essential role of quarantine in the germplasm acquisition process. In a recent study of the entire plant germplasm program of the USDA, the Crop Germplasm Committees (CGCs) were asked to rank National Plant Germplasm System (NPGS) activities as to the priority to be given the activities should additional funding become available from Congress. The CGCs ranked the "acquisition of new germplasm" as the number one priority, and "quarantine" as the fourteenth and lowest priority (1). This is an excellent illustration of the disconnect that exists in the minds of at least some members of the CGCs: They want to acquire more and more germplasm but do not acknowledge that this can be done only with quarantine testing. The two activities are necessarily and inextricably linked; they can not properly be viewed as the first and last in priority. It would be counter-productive and benefit no one to spend additional funds on germplasm acquisition without a proportional increase in funding for quarantine testing.

A related problem is that there has been no clear process for the prioritization of accessions for quarantine testing. In an environment where there have been inadequate resources to adequately test all accessions accepted into the program, the PGQO needs the CGCs to establish the priorities to be given to testing individual accessions. Indeed, since the quarantine testing for all crops is carried out with a single funding source and by the same limited staff, some way to balance the competing demands of the 20 CGCs with an interest in the operation of the PGQO needs to be developed. The PGQO is involved in the planning of germplasm exploration trips so that resources will be available when the accessions arrive for quarantine testing. A corollary of this is that the PGQO must be able to refuse to accept accessions that can not be tested with present resources. A simple approach would be to require that all importers get their importation permits from PGQO rather than from others who have no responsibility for post entry testing of germplasm. This would assure that resources would be available for post entry testing of the imported germplasm.

Horticultural Issues that Affect Plant Germplasm Quarantine Testing

All testing of germplasm at Beltsville has recently been consolidated at facilities provided by APHIS at the Beltsville Agricultural Research Center. Although this provides significant modern laboratory, greenhouse and screenhouse facilities for germplasm quarantine testing, the space available for testing is still not sufficient. Federal funding to build additional laboratories, greenhouses and screenhouses has been obtained by APHIS and construction is underway. When construction has been completed, the physical facilities and space will be excellent. Experimental orchards for necessary field indexing are also located at this site. Although the soils at this site are extremely variable, the site is, on balance, adequate for the needs of the PGQO. Soils are being improved by crop rotation prior to planting the orchards. An irrigation system is in place and fertilization and other practices are based on standard orchard practices (8). The most difficult long term problems will likely be radiative frosts in the spring season since the site is flat, fire blight, and browse damage by deer and groundhogs. The latter will be limited through fencing and permitted hunting. The testing protocols have also been changed to reduce the number of years of orchard growth required to test accessions (4).

Accessions must survive the testing process, and the recipient deserves to know the status of their accessions during the testing process. These are essential aspects of the testing protocols, particularly for crops that require several years to complete routine testing. Accessions have died during testing and the germ-

plasm has been lost. This can occur due to inadequate care during testing, or during severe weather events. However, if budwood is not received by PGQO in good condition, the propagation of the accessions can fail for this reason, through no fault of the PGQO. This has also occurred and minimal standards for budwood selection, shipping and propagation need to be established and followed by all parties. Such standards have been proposed for tropical germplasm (9). Tissue culture propagation of *Solanum, Ipomoea, Rubus,* and *Ribes* germplasm has been established as a part of the testing protocol for these genera. This prevents the loss of germplasm of these genera during testing, and facilitates thermal therapy to produce healthy propagants for distribution from infected accessions.

Accession importers currently can contact the PGQO regarding the status of their accessions via telephone and electronic mail, etc. In addition to this, we plan to post PGQO accession inventories and summaries of quarantine test results on the World Wide Web. This is a cost-effective way to allow recipients to inform themselves regularly about the status of their material as it moves through the testing protocols.

Budget Issues that Affect Plant Germplasm Quarantine Testing

The PGQO has had serious funding problems. The ARS provides all funds for the quarantine testing program, and does not have statutory authority to charge a user "fee for service," as does for example the National Research Support Program-5 (NRSP-5). This program is available for the quarantine testing of stone and pome fruit varieties intended for immediate commercial testing or introduction. NRSP-5, at Prosser, Washington charges $1000 per accession, payable upon receipt of the accession, as well as various miscellaneous fees for specific testing services. In contrast, after salaries and indirect research costs were paid, the original budget plan for the PGQO for FY-98 provided $25,000 to operate quarantine testing programs for stone and pome fruit crop germplasm, as well as quarantine testing of germplasm of *Rubus, Ribes, Solanum, Ipomoea, Oryza* and various Rosaceous species, turf grass and occasional accessions of *Hibiscus,* manioc and maize. These funds were intended to cover laboratory and greenhouse supplies and equipment for pathogen testing, tissue culture materials, rootstocks, orchard maintenance and equipment, weekend greenhouse watering, postage and telephone as well as travel costs necessary to attend CGC or professional meetings, and were consistent with funding levels of previous years.

However, in January of 1998 an additional $110,000 was made available for quarantine testing. These funds will be used primarily in the pome fruit program to hire new staff to conduct pome fruit testing and therapy of virus- and viroid-infected germplasm. The addition of these staff members plus a new technician to implement molecular methods for pathogen detection will improve the capabilities and efficiency of the PGQO. In spite of this increased level of funding, resources to conduct the testing program will continue to be a challenge. One approach to alleviate this problem would be to include an overhead charge written into germplasm collection grant proposals. This overhead charge could be applied to post-entry quarantine processing of germplasm and would help match work load to resources available.

Technical Issues that Affect Plant Germplasm Quarantine Testing

Modern molecular-based detection methods have great promise in quarantine testing protocols, and could substantially reduce the testing time for some crops. New PCR- or hybridization probe-based tests for various pathogens of interest are published frequently. The utilization of these modern methods to expedite the quarantine testing process is now a priority, particularly when the incorporation of a single molecular test will drastically shorten the length of time accessions

spend in quarantine. Because of this, an additional $25,000 has been provided to strengthen a research program to develop molecular diagnostic tests for plant pathogens of quarantine significance. The development of improved detection methods is essential, but another problem remains: Novel pathogen detection methods must be demonstrated to work reliably in the hands of the PGQO staff before APHIS will consider them in alternative, approved protocols. It is not sufficient to cite a publication in the scientific literature and then begin to use it in testing. This is obviously the correct approach but because of the sheer volume of testing required to meet current demands on the quarantine testing system, validation tests of alternative methods can only be done as time becomes available after the regular quarantine testing is completed. In spite of these obstacles, PCR-based assays for phytoplasmas have been incorporated into the standard testing protocol for *Prunus, Malus* and *Pyrus* and will reduce the time required prior to provisional release of germplasm from four years to one year (4). A PCR-based test for the 'reversion virus' (5) of *Ribes* is also in the validation stage of testing and will reduce time spent by *Ribes* in quarantine from three years to one year.

Molecular tests have specific limitations. Molecular tests should not be expected to completely replace graft-based whole plant testing for infectious agents, since molecular methods by definition can only be used to detect known and thoroughly characterized agents. They will not detect novel or poorly characterized agents with which foreign accessions may occasionally be infected. For these reasons we have proposed to APHIS that PCR-based assays for viruses be substituted for one, but not both replications of the current graft indexing experiments for the detection of pome fruit viruses. This change will reduce the time required for quarantine testing of pome fruit germplasm. A similar strategy could be used for other crops if funds become available.

Once a virus or viroid is detected in an accession, a decision must be made as to whether the isolate is a potential threat to agriculture, or simply another strain of a virus that is already widely disseminated in the U.S. Because of the wide dissemination of plant viruses both in cultivated crops and in wild plant material (2) this situation occurs continually, particularly with pome fruits and potatoes. PCR-based amplification of pathogen genome segments followed by endonuclease fingerprinting of the amplicons can readily differentiate among closely related strains of a given virus, as has been demonstrated with plum pox virus (3). Alternatively, the nucleotide sequences of the amplicons can be determined. The development of an adequate database of such fingerprints or sequences for the commonly encountered viruses is an urgent, unfunded research priority. This would allow quarantine officials to make a 'science based' decision as to whether a virus-infected accession should be destroyed as a hazard to agriculture or released based on the determination that the particular virus strain involved represented a minimal risk to agriculture. At present, all infected material is presumed to present a hazard to agriculture.

The Essential Role of a 'Customer Panel'

Quarantine testing of imported germplasm for pathogens remains essential to protect American agriculture. The management of plant germplasm quarantine testing is an extremely complex and challenging task, constrained by budget and staffing realities, the conflicting requirements of very diverse crop species, and by an incomplete understanding of these constraints by the user community. Progress on these issues can only be made cooperatively. We propose the establishment of a 'customer panel' composed of both technical subject matter experts and representatives from industry. This group will assist the PGQO on all aspects of its quarantine testing program including appropriate horticultural practices and the development or implementation of faster,

better and cheaper testing protocols. Because the composition of this group will necessarily reflect the diverse crop responsibilities of the PGQO, communications among the 'customer' communities will be facilitated which should lead to a better understanding among the user groups of the complexities of the PGQO operations. More importantly, the expertise of the board members will be used to develop the best plant germplasm quarantine center in the world.

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Temperature for Optimum Anthocyanin Accumulation

Pre-climateric fruit developed more anthocyanin than did tissue from climateric fruit. Each cultivar had a distinct temperature optimum for anthocyanin accumulation: 'Delicious' and 'Fuji' (25° C); 'Gala' (23° C); 'Braeburn' (21° C). Interestingly 'Gala' which is harvested early (warm part of season) has a low temperature optimum. Pie cooling before exposing to the temperature increased the amount of pigment. 'Scarlet Spur' had a higher accumulation rate than 'Oregon Spur' but a similar temperature optimum. From Curry. 1997. J. Hort Sci. 72(5):723-729.

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