

Processing Foreign Plant Germ Plasm at the National Plant Germplasm Quarantine Center

Prophylaxis is a primary means of controlling plant diseases and involves protection, eradication, and exclusion. Quarantines are among the exclusionary measures designed to keep pathogens from entering the sphere of the host plant or harvested plant material, and most countries and each of the 50 states of the United States have them. Quarantines are implemented by banning or restricting the movement of plants or their parts across political boundaries by means of inspections, treatment with chemicals or heat, or various tests to detect latent pathogens. This article addresses the U.S. federal regulations for plant material imported for propagation and describes the procedures used to process germ plasm of those genera in the prohibited, but not the postentry, quarantine category (2,7).

The processing of prohibited-category genera through quarantine is a small part of the National Plant Germplasm System, a network of people dedicated to preserving the genetic diversity of Earth's vegetation, especially crop plants (4,9). The system includes collecting, preserving, evaluating, cataloging, and distributing germ plasm from all over the world to breeders and others throughout the world (12,15,16). The system has components located throughout the United States and is coordinated by the Agricultural Research Service (ARS) of the U.S. Department of Agriculture (USDA). Support and funding come from federal appropriations and state contributions, as well as from private industry and special interest groups.

The importation of germ plasm into the United States is an activity even older than the nation (3,19), partly because the

United States was a "have not" area in terms of edible crop species. For example, only a few fruit species originated in the United States (Fig. 1). The situation is the same for vegetable and agronomic crops. Sunflowers, cranberries, pecans, hops, some grapes, and brambles are among our few native edible crops. Early settlers brought seeds and plants, generally without regard for the pathogens they harbored. Consequently, many of the most damaging pathogens of the world became established in the United States long ago. Table 1 lists examples of pathogens of genera that are now processed through the National Plant Germplasm Quarantine Center (NPGQC).

A Continuous Threat

Although thousands of foreign pathogens have become established in the United States (6), many have not yet arrived. The threat that some of these foreign pathogens pose has been assessed in detail (13,18), and some are listed in Table 2. R. C. McGregor, in *The Emigrant Pests*, a 1973 USDA Animal and Plant Health Inspection Service Task Force report (*unpublished*), examined the records and assessed the threat of nearly 2,000 foreign pathogens. Of these, 551 were identified as potential risks to U.S. agriculture, largely on the basis of economic value of the hosts and perceived damage potential of the pathogens. In spite of quarantines, a few of the pathogens ranked potentially most destructive in the 1973 report, such as maize stripe virus and *Cronartium quercuum*, are now in the United States.

Today, most of the 75 or so genera in the prohibited quarantine category are processed at the NPGQC (11). Some of these crops and the reasons for their quarantine are listed in Table 3. The primary objectives of the NPGQC program are: 1) to ensure that imported germ plasm is free of foreign pathogens before it is released from quarantine and 2) to consistently make germ plasm available as rapidly as possible to importers.

The Plant Quarantine Center

Several precautions are taken to ensure that pathogens do not escape. The facility (Fig. 2A), built during the 1980s, was deliberately located at a site remote from agricultural crops, at Beltsville, Maryland. The unit is self-contained, with all laboratories, greenhouses, and screenhouses connected. Special construction features include weather stripping around doors and double entryways to reduce escape of virus-transmitting insects. Greenhouses are divided into small compartments to facilitate isolation of germ plasm (Fig. 2B). Pesticides are applied regularly, and limitations are imposed on visitors. All test plants, samples, and packing and plant debris are autoclaved before being removed from the facility. Construction phases to be completed during the next decade include special treatment of waste water from the laboratories.

The center is managed by the ARS and staffed with three scientists, eight technicians (each of whom focuses on an assigned group of crops), a field manager, a computer-information specialist, and six support personnel. The Animal and Plant Health Inspection Service (APHIS) designed, constructed, and maintains the facility. APHIS determines which genera must be quarantined, which tests for pathogens must be conducted, and when to release the germ plasm from quarantine. APHIS personnel also inspect for insects and pathogens on those several genera, such as rice, conifers, bulb crops, and certain shade and forest tree species, on which tests for pathogens are not required (Fig. 3). This arrangement results in a great deal of interaction between the two agencies and thus attains the dual objective of pest exclusion and germ plasm introduction.

Testing Germ Plasm for Viruses and Viruslike Pathogens

The greatest effort at the NPGQC involves the numerous tests required to detect the submicroscopic or fastidious

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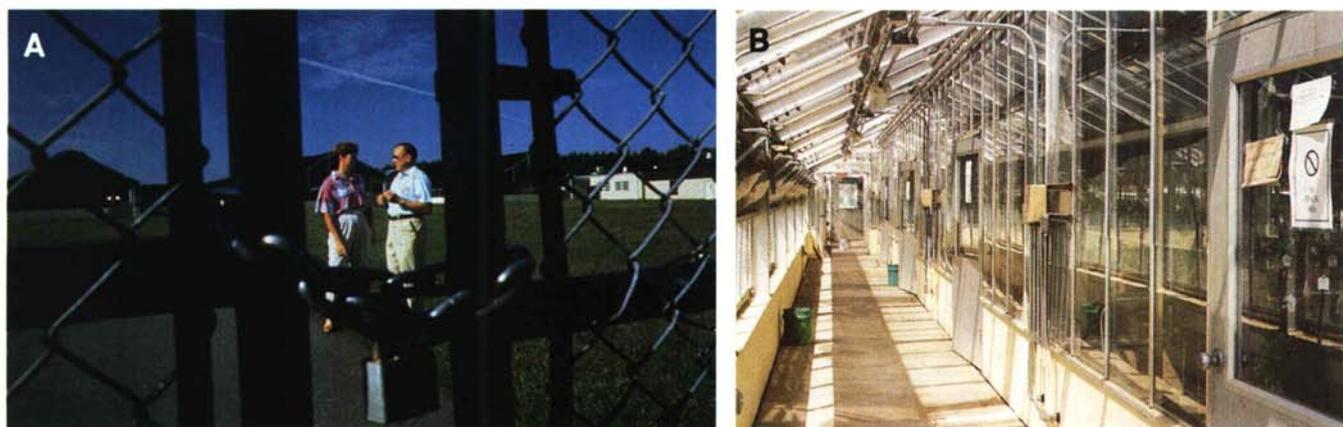


Fig. 2. (A) Federal plant quarantine facility at Beltsville, Maryland, where prohibited genera are examined for pathogens; (B) interior of greenhouse.



Fig. 3. Checking (A) sugarcane accessions for diseases and (B) grasses for scale insects and mites.

Table 3. Some of the crops that are quarantined when imported into the United States

Crop	Countries of origin	Reason for quarantine
Vegetative grasses, grapes, deciduous tree fruits, potatoes, sweetpotatoes	All except Canada	Numerous pathogens
Elms and lilacs	All in Europe	Elm mottle virus
Pines	All in Europe, Japan	Cronartium rusts
Rice seed	All except Mexico	Numerous fungal pathogens
Roses	Australia, Italy, New Zealand	Rose wilt agent
Sugarcane	All	Numerous pathogens

Table 4. Kinds of tests conducted on some of the plant genera in the prohibited quarantine category

Genus	Quarantine duration (yrs)	Tests ^a
<i>Abies</i>	1	O
<i>Acer</i>	3	O, ST, G
<i>Cynodon</i>	2-3	O, EM, ST
<i>Fraxinus</i>	1	O
<i>Gladiolus</i>	1	O
<i>Ipomoea</i>	2-3	O, S, G
<i>Malus</i>	5	O, G
<i>Morus</i>	3	O, ST, G
<i>Pinus</i>	1	O
<i>Ribes</i>	4	O, ST, G
<i>Rubus</i>	2-3	O, S, ST, G
<i>Saccharum</i>	2	O, EM, ST, HT
<i>Theobroma</i>	3	O, G

^a O = observation for pathogens and diseases, ST = sap transmission for viruses, G = grafts to indicator cultivars, EM = electron microscopy, S = serology, HT = heat treatment.

There are no rapid or "high-tech" tests for many of these agents. The result is that germ plasm cannot meet unconditional quarantine release requirements until the most hard-to-detect agent has been sought. This situation applies to some of the small fruit, shade tree, and woody ornamental genera as well the deciduous fruits.

To address this problem, a special conditional quarantine release protocol has been implemented for pome and

stone fruits. This protocol allows for limited distribution of germ plasm before the final 2 years of readings have been taken on field tree indicators. To qualify for early release, accessions must display no symptoms of disease, must have tested negative for pathogens in all laboratory and greenhouse tests, and must have incited no symptoms in field indicators during the first growing season. Requestors must petition their state regulatory office for approval to obtain the germ



Fig. 4. Tests conducted to detect viruses include (A) the graft test for uncharacterized infectious agents and (B) the sap (mechanical) transmission test.



Fig. 5. Orchard test plots for detecting pathogens requiring several years to incite symptoms: (A) Golden Delicious apple indicators for detecting the apple proliferation MLO and (B) Bing cherry indicators for detecting several foreign pathogens that cause bark or fruit symptoms. Indicator trees are identified and inventoried with a bar code scanner and portable computer.

plasm and must comply with growing and use conditions specified by their state. This new protocol reduces waiting time by about 2 years.

Another means of obtaining foreign germ plasm of most genera faster is to import true seed rather than vegetative propagules, because seed is less apt to contain pathogens. Except for potato, peanut, mango, elm, cotton, barberry, and some stone fruits and cereals, quarantine is not required. Furthermore, none of the tests required on seedlings takes longer than 2 years.

Virus and viruslike pathogens have been detected in nearly all vegetatively propagated genera (Table 6). Since the virus indexing program began with stone fruits in 1958, data have been obtained on frequency of infections (10; H. Waterworth, unpublished). Frequencies ranged from less than 1% in several hundred cacao accessions to 65% of the imported citrus germ plasm (Table 6). Multiple infections in some crops, especially apple and potato, are not uncommon. Usually the combination of detection methods and experienced personnel results in identification of the pathogens. Only limited attempts are made to identify unusual pathogens. In any case, the

policy of the National Plant Germplasm System is not to distribute infected germ plasm even if the pathogen is already widespread in the United States, because of the concern over importing new strains.

When Germ Plasm Is Infected

Several courses of action are available after an accession is found to be infected. The first is to test clones of the accession, as described above. If all are infected, the importing scientist is informed and decides to: 1) attempt reimportation of the item, 2) treat the accession in an effort to produce disease-free tissue, 3) destroy the accession, or 4) obtain pollen or seed if possible from the infected plant. The decision rests with the importer to the degree that all of these options are available.

The NPGQC has therapy and tissue culture programs for producing disease-free germ plasm (Fig. 7). Some items, especially *Prunus* spp., have been heat-treated by colleagues at the IR-2 project in Prosser, Washington. After tissues are treated and regenerated, tests are repeated until one or more plants are identified as pathogen-free. The original plant is then destroyed.

Establishing New Accessions

Accessions of genera in the prohibited category from overseas enter the NPGQC via the APHIS Inspection Station in Beltsville. APHIS inspects the germ plasm for insects and obvious pathogens and may treat the germ plasm as required by federal regulations, depending on the genus, country of origin, and what is found during the inspection.

Table 5. Virus and viruslike pathogens and diseases tested for in stone fruits held in quarantine

Category	Pathogen or disease
Viruses	Apple chlorotic leafspot, apple mosaic, apricot ring pox, cherry leafroll, cherry little cherry, cherry mottle leaf, cherry rasp leaf, cherry twisted leaf, green ring mottle, Myrobalan latent ringspot, necrotic ringspot, four nepoviruses, peach mosaic, plum line pattern, plum pox ^a , prune dwarf
Mycoplasmalike organisms	Apricot chlorotic leafroll ^a , cherry blossom anomaly, peach yellows, peach X
Viroids	Apple scar skin, peach latent mosaic ^a , plum dapple fruit ^a
Bacteria	Almond leaf scorch, phony peach
Uncharacterized agents	Almond bud failure, apricot chlorotic leaf mottle ^a , apricot mosaic, apricot pucker leaf, apricot stone pitting ^a , cherry albino, cherry black canker, cherry detrimental canker ^a , cherry freckle fruit, cherry necrotic rusty mottle, cherry rosette, cherry rough fruit, cherry rusty mottle, cherry rusty spot ^a , cherry short stem, cherry spur cherry, cherry xylem aberration ^a , European rusty mottle ^a , peach asteroid spot, peach blotch, peach calico, peach chlorotic spot ^a , peach enation ^a , peach mottle, peach oil blotch ^a , peach purple mosaic ^a , peach rosette, peach seedling chlorosis ^a , peach star mosaic ^a , peach stubby twig, peach wart, peach willow leaf rosette ^a , peach yellow mottle ^a , weak peach

^a Not known to occur in the United States.



Fig. 6. Typical symptoms incited by plum pox virus in plum fruits.

Table 6. Percentage of quarantined accessions infected with virus or viruslike agents

Crop	Infected (%)
Citrus	65
Apples	60
Potatoes	56
Grapes	50
Sweetpotatoes	48
Pears	35
Small fruits	20
Stone fruits	15
Woody ornamentals	5
Vegetative grasses	5
Sugarcane	1
Cacao	<1

Nearly all accessions arrive as a small sample of several propagules, e.g., a packet of seed, a bundle of small plants, or a few bud sticks, rooted cuttings, tubers, cane pieces, or bulbs. Grasses arrive as seed or small clumps with roots

or rhizomes. Regardless of the germ plasm form, every propagule within a sample is handled as if it came from a different source plant—because some may be infected and others may be pathogen-free. Likewise, every bud on a cutting is labeled and processed separately, because some pathogens are distributed erratically within a shoot. Some buds may be infected while adjacent buds are not.

Several plants (clones) of each accession are established, and each label is unique, usually “A” through “n.” The series of tests for pathogens is performed on a single plant, and when all tests are completed, usually 2–5 years later, only this plant and its progeny are released from quarantine and distributed. In the case of plants grown from seed accessions, several plants are tested simultaneously in an effort to maintain the genetic diversity within the original sample.

Keeping Track of Accessions and Pathogen Test Results

Keeping track of several thousand transient plants of many genera, including clones, subpropagations, tissue-cultured plantlets, and heat-treated items, is challenging. These plants and field indicator trees are identified with computer-generated bar code labels produced on site. Hardware is available and software has been developed that permits accurate inventories to be maintained by location, label, and condition of the plant; the status or result of each pathogen test; the quarantine release date; the



Fig. 7. Tissue culture is employed to produce pathogen-free germ plasm from infected accessions and to propagate accessions received as cultured plantlets.

distribution and recipient(s) of the germ plasm, etc. This program is linked with the Germplasm Resources Information Network (GRIN), which provides scientists throughout the country with information about any accession in quarantine. Technicians receive training in dBASE IV to enable them to conduct inventories with a bar code scanner and portable computer (Fig. 5B), to enter disease observation notes on original accession plants, and to generate data by year from pathogen tests in the laboratory, the greenhouses, and the field. The software is regularly updated to accommodate unique situations and new tests and to eventually generate form letters to inform importers as to the status of their germ plasm, i.e., received, tests begun, released from quarantine, etc.

Release and Distribution of Tested Germ Plasm

The protocols for distributing germ plasm after tests are completed differ considerably among the genera. The only common element is that each accession is sent to the importing scientist at no cost to the recipient. Most nonpatented accessions are also offered, with the concurrence of the importer, to the appropriate germ plasm repository. Pears and small fruits are sent to Corvallis, Oregon; apples to Geneva, New York; stone fruits to Davis, California; grasses and sweetpotatoes to Griffin, Georgia; ornamentals to the National Arboretum in Washington, D.C.; sugarcane to Miami, Florida, and to an international repository in India; rice to Aberdeen, Idaho; potatoes to Sturgeon Bay, Wis-

consin; and certain tropical crops to Mayaguez, Puerto Rico, or Miami.

Except for restricted or patented items, requests from other scientists are usually honored. Most of the distribution is within the United States, with a few requests from Canada or overseas. At the time of distribution, a plant inventory (PI) number may be assigned, depending largely on the desires of the importer or repository. For most genera, distribution is in the form of seed, tubers, budwood, or cane pieces. Entire plants are available only for grasses, mangoes, and some ornamental and shade tree genera. When applicable, the source plant is maintained for another year at the NPGQC or until the germ plasm is established by the recipient. The NPGQC does not maintain an ongoing repository for any genus.

The unique distribution procedure for stone fruits evolved over the years. A list of new releases with relevant background information is sent to many U.S. stone fruit specialists, who may place orders. Budwood is shipped for spring or fall budding according to the desires of the requestors. Each accession is offered in two consecutive years. Under the provisional early-release program, stone fruit germ plasm is shipped when written approval is received from the appropriate state regulatory office.

In-House Research Program

Protocols employed for detecting the various kinds of pathogens are updated as new technologies become available. New pathogen detection procedures that involve less labor, are more reliable, or provide quicker results are evaluated. The ultimate objective is to rapidly process as much germ plasm as possible, consistent with absence of pathogens. Potentially useful techniques become available from at least two sources: published reports and an in-house research program that focuses on developing or adapting pathogen detection technologies for use in the quarantine program. Current research objectives include modifying nucleic acid probes to detect tomato ringspot virus, developing a polymerase chain reaction (PCR) procedure to detect the plum pox virus in *Prunus* spp., and developing cold therapy as a means to produce potato viroid-free germ plasm.

During the course of pathogen detection research, "new" pathogens may be found in foreign germ plasm accessions, as, for example, the recent detection of a viroid in pear accessions (8). The importing scientist who is awaiting release of germ plasm from quarantine can perceive this as a downside of the research support program, since the germ plasm may be held until information about the pathogen becomes available. Is it already in the country, possibly via previously introduced germ plasm? What is known about its damage potential? Release of

the accession is contingent on an informed decision regarding the hazard posed by the "new" pathogen.

The Value of Communication

Frequent interaction with a broad spectrum of persons at various institutions is an integral component of the program. Effective communication improves compliance with an array of state and federal regulations, enhances program efficiency, reduces errors and duplication of effort, and promotes patience and understanding.

Regular interaction occurs with those 12 of the 40 crop advisory committees whose germ plasm is quarantined upon arrival. The committees coordinate with NPGQC program staff on impending large importations of germ plasm, advise on virus testing priorities, and serve as a sounding board for ideas and as an excellent source of general information. Quarantine program personnel also work with the several U.S. germ plasm repositories that employ the crop specialists, to exchange working materials and to ensure the establishment of released germ plasm before original quarantined plants are destroyed at the NPGQC.

Another beneficial exchange is with the plant quarantine station operated by Agriculture Canada on Vancouver Island, British Columbia. The U.S. and Canadian quarantine stations process many of the same genera, so it is not unusual for the same foreign cultivars to be imported into quarantine in both countries. And germ plasm of most genera processed through the Canadian quarantine station is enterable into the United States. Regular exchange of inventories and of virus indexing status reports has reduced duplication and expedited availability of germ plasm to U.S. breeders.

Communication with state regulatory offices has benefited U.S. scientists and promoted understanding and confidence in federal quarantine procedures. As a result, permits have been granted to scientists in their respective states to receive germ plasm that is conditionally released or that contains certain viruses already widespread in the United States.

Benefits to U.S. Breeders

The germ plasm quarantine program serves as an important support for current and future U.S. crop improvement programs. The broadest spectrum of genetic diversity for most of the prohibited genera resides in their centers of origin, which usually are not in the United States. This program enables scientists, hobbyists, and others to import germ plasm from any country at any time of the year with the reasonable assurance that, after quarantine, the germ plasm will be free of pests. Because

much of the germ plasm is automatically deposited in U.S. repositories, it will be available to meet future crop improvement needs.

The federal quarantine program serves as a source of techniques for detecting pathogens and of expert information on regulations and foreign pathogens. Information on all items processed through quarantine is maintained in the national GRIN database, accessible to scientists at sites throughout the country. Also, case-by-case arrangements can be made with NPGQC to collect certain kinds of data on germ plasm or to obtain pollen or seed of some genera still in quarantine.

The program appears to be successful in keeping foreign pests from becoming established in the United States via germ plasm processed at the NPGQC. Quarantine personnel are very much aware of the spectrum of views held by germ plasm importers. The most common complaint concerns the length of time accessions are held in quarantine. There is ongoing emphasis at the NPGQC to



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Dr. Waterworth, a research plant pathologist (virologist), has been with the USDA-ARS since he joined the New Crops Research Branch in 1964. His graduate work, under the direction of Robert Fulton at the University of Wisconsin, dealt with viruses of stone fruit crops. Except for 7 years spent in ARS administration, he has been closely associated with plant quarantine and viruses of fruit crops. He has published on matters related to quarantine and on a broad spectrum of viruses associated with imported germ plasm of fruits, vegetables, ornamentals, and tropical crops. He became location leader of the ARS Glenn Dale Plant Introduction/Quarantine Station in 1974 and served as chief of the New Crops Research Branch from 1980 to 1982. He is currently at the National Germplasm Resources Laboratory Plant Science Institute.

move germ plasm through quarantine as rapidly as possible by adopting new technologies, eliminating unnecessary tests, updating regulations, and increasing staff. The risks associated with circumventing the legal system are real, and importers should be aware that Congress has implemented a system of penalties for violators, that laws are being enforced, and that adverse actions have been taken against individuals and companies.

Information on obtaining federal permits to import plant germ plasm is available from the Animal and Plant Health Inspection Service, Permit Unit, 6505 Belcrest Road, Hyattsville, MD 20782. Information about GRIN, about arranging for importation of plants, and about foreign explorations is available from the National Plant Germplasm Laboratory, USDA-ARS, Building 003, 10300 Baltimore Avenue, Beltsville, MD 20705.

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