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The IR-2 Program for Obtaining Virus-free Fruit Trees

Awareness of deciduous fruit tree virus and viruslike diseases in North America began shortly after peach culture started in New England. There, where the pathogen is endemic, peach yellows was recognized possibly as early as 1630 and definitely by 1790 near Philadelphia (6). By May 1796, peach yellows had become so serious that the American Philosophical Society offered a reward "for the best method, verified by experiment, of preventing the premature decay of peach trees, a premium of $60" (6). The premium was eventually awarded on the basis of insect injury.

It was not until 1888 that Erwin F. Smith demonstrated the infectious nature of peach yellows by graft transmission from diseased to healthy trees (6). These were the first carefully controlled experiments of their kind, although Smith mentioned that 10 other persons had made similar graft transmissions, some as early as 1828. From his experiments, Smith reasoned that peach yellows was an infectious disease caused by an unknown pathogen he considered to be similar to the previously discovered infectious variegation. Thus, the symptoms, effects, and transmission were reported long before the true nature of virus and viruslike diseases was known.

Interest Lags, then Revives

Smith's work failed to inspire continued research on virus diseases of fruit trees. By 1930 only five virus diseases of stone fruits (*Prunus*) were recognized, and all affected peach. During the late 1930s and early 1940s, interest in these kinds of diseases reawakened. By 1951, between 40 and 50 distinct virus diseases of stone fruits were recognized. Meanwhile, as North American interest in stone fruit diseases intensified, European interests were directed more toward the pome fruits (*Malus*, *Pyrus*, and *Cydonia*).

Fruit tree virologists recently suffered the same embarrassment as virologists working with other plants. Some of the classic "virus" diseases were found not to be incited by viruses at all, but by other kinds of pathogens. The first elucidations occurred when mycoplasmalike agents were shown to incite the classic "virus-yellows" symptoms. Later, some rickettsia-like bacteria and viroids were shown to cause viruslike diseases. The original misidentifications evolved from the assumption that any pathogen was a virus if it could be graft-transmitted and could not be observed with a light microscope or cultured easily. As all these kinds of pathogens are still considered to be within the realm of fruit tree virus research, the term "virus," when generally applied herein, includes true viruses, viroids, and organisms that incite viruslike diseases.

Symptoms, Frequencies, Vectors

Most of the typical virus disease symptoms of herbaceous plants also occur in woody fruit trees. These include necrotic and chlorotic spots and rings, line and oak-leaf patterns, leaf vein necrosis, leaf mottles and mosaics, premature defoliation and fall coloration, blemished or insipid fruit, late or early ripening of fruit, stem pitting, hypersensitivity at graft unions, and death of the host. Sometimes the viruses are latent. Symptom occurrence and disease severity depend on the particular cultivar/pathogen combination.

The natural frequencies of specific virus diseases vary considerably because of diversities in pathogens, hosts, and vectoring. Only a single tree naturally infected with Tulare apple mosaic and extremely few peach trees with peach wart have been found. Examples like these suggest that the inciting viruses probably occur naturally in other plants and are vectored to fruit trees during some unique accident. In contrast, the pollen-vectored Prunus ringspot and prune dwarf viruses are almost omnipresent in older cherry trees and are found in many peach, plum, apricot, and almond trees. The nematode-vectored strains of tomato ringspot and the eriophyid mite-vectored peach mosaic can be epiphytotic among fruit trees in local areas. Some potentially dangerous insect-vectored pathogens that appear to spread rapidly, such as the plum pox virus (Sarka) and the apple proliferation mycoplasmalike agent, have not yet occurred in North America.

Relatively few vectors have been identified in North America, and most are homopteran insects that transmit the organisms causing viruslike diseases. The few proven vectors of true viruses are pollen, eriophyid mites, and nematodes. In Europe a number of common aphid species transmit the plum pox virus. Nonvectored spread through natural root grafting in orchards and nursery rows is well known. Many viruses, particularly most pome fruit viruses, appear not to be vectored naturally, as authentic spread has never been documented.

Sometimes disease occurrence is cyclical, as with the peach yellows epiphytotics of 1791, 1806–1807, 1817–1821, 1845–1858, 1874–1878, 1886–1888, and 1920; during the intervening periods, the disease was relatively quiescent. Such cycles are related to vector population dynamics rather than to the pathogen.

Apparently man causes the greatest increase of virus diseases by propagating with diseased scions and rootstocks, grafting existing trees with diseased pollenizers and/or more desirable cultivars, and grafting diseased trees with new clones or cultivars for preservation or observation. Similarly, man is an important agent in geographical dissemination of many viruses by transporting them in infected plant parts. As early as 1888, Smith (6) noted that man was the principal cause of introducing peach yellows into previously noninfested areas with diseased nursery stock. Local epiphytotics resulted from these introductions. Other tree-destroying epiphytotics have occurred because man introduced a pathogen into new areas, eg, little cherry in the Kootenay region of British Columbia, tomato ringspot in

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some nurseries and orchards in the eastern and western United States, and plum pox in northern Europe.

Procedures for protecting orchards from viruses vary and include roguing infected trees, eliminating nearby wild hosts, and imposing quarantines against importing replacement trees from areas where specific diseases occur. Chemotherapy of orchard trees mitigates symptoms of some viruslike diseases incited by organisms but does not control true viruses. Thermotherapy of a diseased orchard tree is not possible. Vector suppression to control virus disease is normally inefficient, except perhaps with preplanting fumigations for nematode control. The most successful control method is exclusion of virus diseases from new orchards, and exclusion is best accomplished by planting virus-free nursery stock produced under a supervised program.

Variable and Insidious Losses

Losses from virus diseases vary from nil, or nearly so, to unmarketable fruit or death of orchard trees. Often, a specific virus is latent in a particular cultivar and the resulting losses can be demonstrated only with complex techniques. Some viruses cause fruit deformities or blemishes without other discernible symptoms. These diseases are particularly insidious not only because the tree must be replaced but also because the tree's worthlessness is not detectable until after considerable time and money are wasted bringing it to bearing age. Other viruses may reduce winter hardiness or perhaps kill trees outright from hypersensitive reactions.

Erroneous research results obtained by using virus-infected tree parts are seldom mentioned losses. For example, measurements of assumed horticultural incompatibilities may be measurements of the hypersensitivity of one graft partner to a pathogen naturally infecting the other. Similarly, apparently superior rootstocks within a seedling population may be an expression of the tolerance of some individuals to a virus infecting the scion cultivar. Obviously, a heterogeneous population of healthy and virus-infected trees is not very good for making comparisons among different treatments. Thus, researchers should use virus-free plant parts for research to avoid making experimental errors and publishing misinformation.

In hindsight, a research project based on early fruit tree virus disease information and oriented toward solving problems of research and industry caused by these viruses seemed inevitable. Thus, the Interregional Research Project (IR-2) was initiated.

Organization of IR-2

During the decade 1936–1945, plant pathologists and virologists studying virus diseases of fruit trees realized that virus infection and variant clones of fruit trees had confounded their research. Accordingly, most of these scientists cooperatively devised plans for making virus-free plant parts available for research through regional repositories. After considerable study, a single national repository to provide standard virus-free materials was deemed advisable for several reasons: It would be more economical, it would avoid the inevitable duplications occurring among regional repositories, and its operations would be more accurate, more efficient, and performed on a higher scientific level. Commercial needs for the materials were assessed and incorporated into the plans. The repository was designed to be a practical working one instead of one purely for clonal preservation of potential genetic resources.

The project was ultimately supported by regional research funds of the Hatch Act, and IR-2 was officially activated in July 1955 (1,2). Since then, its general progress has been guided by an Interregional Technical Committee composed of research personnel, administrative advisors, and consultants representing all regions of the United States.

Table 1. Indicators, temperatures, and retention periods for efficient detection of most North American deciduous fruit tree viruses[a]

Cultivars used as greenhouse indicators for:	Viruses	Best temperature (C)	Weeks needed for complete symptom expression equivalent to field indicator retention period of: 1 year	2 years or more[b]
Malus viruses				
R 12740-7A	Chlorotic leaf spot	18–22	4	
Radiant or Sparkler[c]	Stem pitting	22–26	4	
Virginia crab[d]	Stem grooving	22–26		3–4
Pyrus viruses				
Nouveau Poiteau or Passe Crassane[e]	Vein yellows	22		10
Prunus viruses				
Shirofugen (*P. serrulata*)[f]	Latents	18–26	4	
Kwanzan (*P. serrulata*)	Green ring mottle	18	4	
Bing (*P. avium*)	Cherry	18		10
Sam (*P. avium*)	Necrotic rusty mottle	18		10
	Little Cherry	26+		6–8[g]
Shiro plum	North American line pattern	18–	8	
Elberta (*P. persica*)	Peach	22		10
Tilton (*P. armeniaca*)	Apricot ring pox	26		4
P. tomentosa seedlings	Latents and others	18–22	3[h]	

[a] IR-2 field indicators are *Malus* 'Golden Delicious,' 'Lord Lambourne,' and 'Spartan' and *Pyrus* 'Beurre Bosc' and 'Bartlett.' Field indicators must be observed for transmitted fruit and bark deformities for at least 2 years after inoculation.
[b] Indicator must be cut back and defoliated if more than 4 weeks are required to detect all strains.
[c] Replaces Spy 227; does not indicate pear vein yellows.
[d] Expressed as yellow flecks in some leaves with leaf margin distortion.
[e] Also indicate apple stem pitting virus.
[f] Easier done in field if climate permits; double budding in greenhouse is good method.
[g] Indicators must not be cut back and defoliated but should be exposed with 24-hour photoperiod for 3 months, then placed outside in shade for symptom development.
[h] All Prunus ringspot and prune dwarf virus strains cause symptoms within 23 days at 22 C. Defoliation and refoliation are necessary for other virus symptoms, such as those of the Montmorency bark splitting virus.

Canada has been an active cooperator from the onset and has maintained close ties through an officially appointed consultant to the committee.

IR-2's general objectives are to: 1) obtain apparently virus-free valuable cultivars and clones of deciduous fruit trees, verify their freedom from viruses, maintain them in isolated repositories, and distribute small amounts of propagating materials to research or regulatory scientists for research or release to industry; 2) develop virus-free individuals by any method from cultivars and clones with no known virus-free individuals from which horticultural propagations can be made; and 3) do research on techniques, viruses, and host plants with emphasis on improving repository performance. The nature of these objectives mandates a strong orientation toward plant pathology.

Procedures of IR-2

IR-2 normally receives requests to acquire specific clones from identified sources. Requests come from interested scientists, state regulatory personnel, and leading members of industry and sometimes from IR-2 personnel in anticipation of future requirements. The needs for each clone are evaluated, and propagation materials of the desirable ones are obtained. In this way, IR-2 acquires clones with immediate scientific, commercial, and/or practical interest.

All candidate clones are indexed to verify freedom from specific viruses universally infecting that species or infecting a certain species from particular area. A candidate clone found to be infected during this preliminary indexing is usually discarded, and an attempt is made to find a different, virus-free source. Sometimes the candidate is saved for thermotherapy.

Current IR-2 indexing procedures are based on graft-inoculated woody indicators. Herbaceous indicators, with one exception, have not been as accurate or conveniently handled as woody plant indicators. The exception is *Chenopodium quinoa* Willd., which will detect a few extremely mild strains of the apple chlorotic leaf spot and apple stem grooving viruses that cannot be detected

Fig. 1. Evolution of indexing for the apple chlorotic leaf spot virus using the apple virus indicator R 12740-7A: (Top) Original indexing methods required large, field-grown indicator trees for bud inoculations. Production was expensive and labor-intensive, and completed results usually required 2 years. (Middle) With the double-budding technique of field indexing, three inoculated indicators and one control per clone tested were used. Completed results required about 9 months. (Bottom) Current indexing methods use a greenhouse space 30.5 × 30.5 cm for 20 indicators. Completed results require less than 4 weeks.

with woody indicators. Several serologic techniques have excellent potential for mass indexing but need further refinement before being adopted by IR-2. Additionally, most fruit tree viruses have never been isolated or purified, so antisera are not available. Electron microscopy, unless combined with serologic techniques, is not diagnostic. Chemical tests, except in the case of phony peach caused by a rickettsialike bacterium, are not diagnostic. Therefore, the methods used by IR-2 appear to be the best available at this time. The woody plant indicators used are listed in Table 1.

The basic indexing method called "double budding" is not new, but its application has been refined. Two inoculum buds are budded low on a healthy rootstock, and a bud of a specific indicator, depending on the virus to be detected, is budded immediately above the two inoculum buds. The healthy rootstock is cut back, forcing the indicator bud to grow. Characteristic symptoms occur in the resulting foliage of the indicator. Thus, any infecting virus is graft-transmitted from the inoculum buds to the healthy rootstock and in turn to the indicator bud. Indicator cultivars produce strong symptoms after infection with a specific virus even though that virus may be latent in some cultivars and other viruses may be latent in the indicator.

Field indexing has been nearly discontinued because of superior techniques developed by IR-2 for greenhouse use. The change from field to greenhouse indexing became possible because manipulations of environment and hosts yielded more rapid and accurate results (3–5). Virus indicators were also developed for greenhouse use that detect strains not normally disclosed by the previously used indicators, again with more rapid and accurate results. Additionally, a few foliage indicators were developed to replace field indicators that normally produce symptoms only on fruits. These modifications reduced the time for most indexing results from 1–5 years in the field to 3–10 weeks in the greenhouse (Fig. 1).

After preliminary indexing, the procedures for processing each candidate are governed by genus. Different procedures are necessary because of the frequency and kinds of viruses found among genera. No procedure or index host of IR-2 remains static; the methods are changed in response to each discovery.

Because IR-2 initially accepted only candidate clones of stone fruits (*Prunus*), the original processing procedures were developed specifically for this genus. Three trees of each candidate are propagated by budding on healthy, containerized seedlings simultaneously with the preliminary indexing; three trees are needed to avoid loss of the candidate in case of mechanical accident or natural infection. These trees are grown under screen until they attain adequate size to provide sufficient budwood for indexing on the standard indicators. Meanwhile, a candidate shown by preliminary indexing to be virus-infected is usually discarded and attempts are made to locate a source elsewhere that is virus-free.

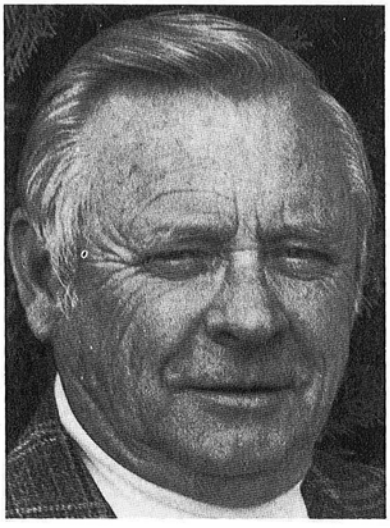
When the three trees of a candidate reach sufficient size for intensive indexing, one is selected to be indexed on eight stone fruit virus indicators. If the tree appears to be virus-free after this indexing, it is designated the nucleus mother tree of that clone and is maintained permanently in a screenhouse (Fig. 2). The two unindexed trees are then discarded. If the first tree is found to be virus-infected, the second or third tree is indexed intensively to determine if it was propagated from a virus-escaped bud. The second and third trees are indexed, however, only when virus-escaped buds are known to occur in that particular host/virus combination. A candidate clone that appears completely virus-infected at this stage is discarded, and a further search is made for a healthy individual. In some instances, when a healthy individual appears unavailable, the infected trees of the candidate are given thermotherapy.

Fig. 2. Virus-free nucleus mother trees are containerized and maintained permanently in screenhouses.

Fig. 3. Daughter trees propagated from nucleus mother trees are maintained in isolated desert repositories and usually produce the budwood that is distributed.

Two daughter trees are propagated from each nucleus mother tree and planted in isolated field repositories (Fig. 3). When possible, these propagations are made by rooting soft wood cuttings under mist to avoid chance contamination from virus-infected rootstock. These daughter trees usually produce the budwood that is distributed.

The nucleus mother trees and the daughter repository trees are reindexed



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periodically to detect chance natural infections. To date, no natural infections have been detected even among trees 20 or more years old. The absence of natural infections indicates that isolation has been adequate.

The identities and/or gross mechanical errors among the clones can usually be verified or detected directly in the isolated repository trees. However, many clones are self-sterile, so no fruit develops on the nucleus mother trees grown in the insectproof screenhouses. Additionally, fruit that develops in instances of self-fertility may not be typical because it was produced under screen. Therefore, propagations of the nucleus mother trees are made in an experimental field to verify their identities. Horticultural evaluations of clones are not made because such appraisals might be valid only for the repository site where they were made.

Accumulation of apple (*Malus*) candidate clones began several years later than that of stone fruit clones. The procedures used for stone fruits proved to be inappropriate because of the high incidence of naturally occurring viruses in apple, so new procedures were developed. Now all apple candidate clones are immediately given thermotherapy and indexed, regardless of virus content. Consequently, the preliminary indexing becomes merely advisory. Thermotherapy-produced trees are indexed annually for about 3 years before candidate clone trees are selected for further processing. This delay is necessary to ensure that thermotherapy of known infecting viruses succeeded and did not merely depress virus populations to a temporarily undetectable level.

Trees of apple candidate clones are then indexed intensively on four greenhouse and three field indicators using methods similar to those for stone fruits. Some field indicators are required because substitute foliage indicators have not been developed for a few viruses that deform fruit and bark. A nucleus mother tree approved after this indexing is maintained permanently under screen as a containerized tree. Propagations from nucleus mother trees are also planted in an isolated repository. Extreme isolation is not as necessary for apple repositories as for stone fruit repositories because of differences in methods of virus spread.

Candidate clones of pear (*Pyrus*) and quince (*Cydonia*) require the same procedures as those of apple for the same reasons. However, pear and quince indexing is done on three greenhouse and two field indicators. Interestingly, all three genera appear to host genus-related strains of the same viruses.

Accomplishments of IR-2

If use is the proof of value or acceptance, then IR-2 is successful. To date, propagation materials from IR-2 have been distributed to 40 states, 5 Canadian provinces, and 40 other nations, the latter via the USDA in response to requests from USDA-AID, FAO of the UN, and other agencies.

During the first 15 years of budwood releases, distributions consistently exceeded the previous year's record by every measurement, including numbers of clones, buds, and scientists requesting materials. Obviously, a distribution plateau will be reached when saturation with the currently available clones occurs. The continuous addition of new clones, however, will ensure that IR-2 remains a viable program, although perhaps not as aggressive with distributions.

According to available information, the virus-free materials released have been used for the intended purposes. Field and greenhouse experimental uses predominate among releases made for research, with other releases being used for chemical analysis, electron microscopy, and aseptic tissue culture. Many new state programs for virus-free nursery stock are based entirely on IR-2 releases, whereas other states, including those with older programs, supplement their inventories from IR-2. Accordingly, IR-2 is a very important source for materials used in the production of virus-free fruit tree nursery stock in the United States. All foreign distributions are made via the USDA Plant Germplasm Quarantine Center and may be used as exchange materials for desirable foreign germ plasm. Distribution of new and current virus indicators to researchers in other nations has helped bring uniformity to the indicators used worldwide, which is extremely valuable for scientific comparisons. In some instances, releases to emerging nations have provided a basis for initiating increased food production.

The development of economical thermotherapy facilities plus modifications and refinements of techniques helped make IR-2 a worldwide leader in eliminating viruses from deciduous fruit trees. Through 1979, heat treatments were used for 702 fruit tree clones, and nearly all were successful. The few unsuccessful treatments were due to extreme intolerance of cultivars to heat and to the heat-refractory apple stem grooving virus. For these reasons, a small, aseptic, tissue culture laboratory was developed with the immediate objective of obtaining virus freedom through substitute methods of meristem tip isolation.

Research always has been an important part of IR-2. Studies on virus distribution, graft transmission, seed transmission, symptom production, host ranges, inoculation techniques, indicator development, and environmental effects on symptoms are among the contributions toward improving repository performance. Combining the results permitted development of the accurate, rapid, and efficient indexing techniques currently used by IR-2 and being adopted rapidly by others.

From the lessons of the past, the need for a program like IR-2 to assist with control of virus diseases in the future is easy to anticipate. The dangers lie in becoming complacent because of the current successes in attaining freedom from viruses. Virus problems will return rapidly if controls are not used continuously. The future obviously will bring new methods of propagation, detection, and control, but until then, the current procedures will persist.

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