CRITICAL ANALYSES OF THE PRINCIPLES OF SEED POTATO CERTIFICATION

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INTRODUCTION

Genotypes of many crop plants including fruit trees, ornamentals, grapes, strawberries, and potatoes are maintained by vegetative propagation. This cultural practice causes certain problems of disease control, which are either nonexistent or of lesser importance in plants that are reproduced from seed. Pathogens already existing in infected propagative materials can continue to develop in the progeny. Cut surfaces of tubers and stem cuttings provide open courts for infection. The high water content of vegetative propagation materials in comparison with true seeds also adds to their vulnerability to infection. Elaborate programs have been devised for many vegetatively propagated plants both to minimize disease incidence in propagative material and to provide relatively disease-free (certified) planting stock to growers. The most extensive and long-lived certification effort has been with potatoes (*Solanum tuberosum* L.) and its success has provided impetus for certification programs in other crops. This article is restricted to an analysis of seed potato certification, and while several of the concepts may also apply to other crops, certain distinctions will be apparent.

HISTORICAL

Potato seed certification was begun in Europe in the early 1900s largely through the efforts of Dr. Otto Appel in Germany (2). During this period, the increase of leafroll and other diseases in North American potatoes stimulated Dr. W. A. Orton of the United States Department of Agriculture to visit Appel (30) and later to present a plan for a certification program patterned after the German program to the First Annual Meeting of the Potato Association of America (1914), the First Official Seed Potato Certification Conference (1914), and the Fifth Annual Meeting of the American Phytopathological Society (1915). In 1914, Appel visited the United States (1)

and collaborated with Orton, Dr. William Stuart of the USDA, and W. J. Morse of the University of Maine in proposing a certification program in several potato producing areas (31). Within five years, 12 states and all Canadian provinces were engaged in seed potato certification (37).

Seed certification programs were commonly begun by Cooperative Extension Service personnel primarily to reduce the incidence of virus disease. Later, separate agencies were created to administer and perform the function of seed certification, and the scope of certification was broadened to include tuber-borne diseases caused by other pathogens.

PRINCIPLES OF CERTIFICATION

The basic objectives of seed certification were presented by Orton in 1914 (32) at the First Official Seed Potato Certification Conference: "It [seed certification] presupposes a movement for the betterment of the potato industry through the improvement of seed potatoes, through the development of specialized growers of seed potatoes, and through an organization created to control diseases, to reduce varietal mixture, to improve varietal types, to be stimulated by the inspecting service organized by the state for the purpose of inspecting seed potatoes and granting certificates to such as may be found worthy of such certification through their varietal purity and freedom from disease." Although techniques of certification have changed, these basic goals and principles are still viable today. Precisely how the principles are implemented has, of course, changed and considerably broadened in scope and now probably represents the most extensive disease-control effort for one commodity.

Administrative Organization of Certification Programs

A necessary first step in seed certification is the creation of an agency with a legal base sufficient to administer and coordinate all facets of the program. This is accomplished in a variety of ways. In several countries, seed certification is under the auspices of an agency of the federal government. In the United States, certification may be the responsibility of a state department of agriculture, a land-grant university, or a grower association (see Table 1) with, in frequent cases, cooperative agreements among the three agencies. In general terms, the individual certification agency is responsible for organizing growers who wish to produce seed, formulating and enforcing "rules and regulations," and for conducting field and storage facility inspections, tagging and labeling operations, and in certain instances shipping point inspections.

Funds for agency operation are partially or totally obtained from certified seed growers who pay to register their fields for certification, and for inspection and tags that certify that the seed has met prescribed tolerances.

In most programs, certification does not constitute a warranty by either the certification agency or the grower of certified seed beyond the express representation that the potatoes were produced, inspected, graded, and packed under the regulations of both the certifying agency and an affiliated state or federal regulatory

Table 1 Seed potato certification in North America

Location	Certification started (37)	Certification acreage in 1973 (45)	Name of agency
	1913-1915		
Canada			
Prince Edward		26,231	Canada Department of Agriculture
New Brunswick		17,566	Canada Department of Agriculture
Nova Scotia		331	Canada Department of Agriculture
Quebec		3,273	Canada Department of Agriculture
Ontario		1,164	Canada Department of Agriculture
Manitoba		2,730	Canada Department of Agriculture
Saskatchewan		162	Canada Department of Agriculture
Alberta		1,660	Canada Department of Agriculture
British Columbia		1,087	Canada Department of Agriculture
United States			
Idaho		31,972	Idaho Crop Improvement Association
Maine		52,159	Maine Department of Agriculture
Vermont		169	Vermont Department of Agriculture
Wisconsin		7,714	University of Wisconsin
	1916-1919		
California		3,481	California Department of Agriculture
Colorado		5,735	Colorado State University
Minnesota		25,363	Minnesota Department of Agriculture
Nebraska		2,380	Potato Certification Association of Nebraska
New Hampshire		6	New Hampshire Department of Agriculture
New York		1,727	New York Seed Improvement Cooperative, Inc.
North Dakota		34,145	North Dakota State Seed Department
Oregon		3,006	Oregon State University
	1920-1922		
Michigan		2,356	Michigan Crop Improvement Association
Montana		4,613	Montana Potato Improvement Association
Pennsylvania		148	Pennsylvania Department of Agriculture
South Dakota		593	South Dakota Potato Growers Association
Utah		91	Utah Crop Improvement Association
Washington		2,327	Washington Department of Agriculture
Wyoming		412	Wyoming Seed Certification Service

agency. In the case of unsatisfactory seed, the liability of the certifying agency usually is limited to the value of the seed when sold.

Disease Control

DISEASE TOLERANCES Profitable "commercial" (i.e. produced for consumption) potato production is possible only when a continued supply of "seed" is available that is totally free from certain diseases and virtually so from most others. It is the responsibility of the individual certification agency to provide the services necessary to assure growers and buyers that the seed in question was produced in compliance with defined standards. The agency must also set minimum necessary standards (rules and regulations) with respect to specific diseases and to a lesser extent other qualifications such as varietal mixtures. The basic principles that are (or should be) followed when regulations are formulated were described by Leach (20): "(1) an accurate knowledge of the extent to which a disease is transmitted in or on seed tubers; (2) the recognition of all other sources of infection; and (3) an accurate evaluation of different sources of infection under various circumstances." For cer-

tain diseases insufficient or inaccurate information has resulted in the formulation of disease tolerances that "growers can live with" rather than what may be biologically justified. An example of this is the blackleg disease caused by *Erwinia atroseptica* (Van Hall) Jennison [and possibly by *E. carotovora* (Jones) Holland]. Tuber lenticels of many North American (A. Kelman, personal communication) and European (33) potato varieties are universally infected, but the conditions necessary for the expression of blackleg symptoms in the field are poorly understood. (33). Present regulations that do not include tests for the presence of the bacterium are, therefore, of questionable value.

As shown in Table 2, disease tolerances presently included in the various certification programs in the United States are similar but not completely standardized. Certified seed produced under one program may harbor greater or lesser amounts of infection by certain organisms than that produced in other programs. Nematodes are considered in the certification programs of only seven states and in only two of these is the species of nematode specified. Some differences in the disease tolerances of individual agencies arise from variation in the distribution of pathogenic organisms. It would be wasteful to conduct exhaustive surveys for diseases that do not exist within a given growing area; also, certification agencies differ in their wherewithal to detect or control certain pathogens.

DISEASE DETECTION AND DIAGNOSIS Most certification standards relate to diseases, and with the exception of giant hill, they do not include such factors as genetic or horticultural differences within a given variety. Hence, establishing levels of disease within fields to be certified as suitable for seed requires an extensive effort on the part of the certification agencies. Disease detection and diagnoses are accomplished by two or three field inspections performed by agency personnel during a growing season. Except for diseases with a zero tolerance, tolerance differentials between the first and last inspection permit growers to rogue out infected plants between inspections. If tolerances are exceeded at the final reading the crop is not eligible for certification. Following harvest, an additional inspection in storage is conducted primarily for the detection of bacterial ring rot, tuber net necrosis, physiological disorders, and occasionally nematodes.

Apart from "latent" virus detection, which will be dealt with later, viral, bacterial, fungal, and mycoplasmal diseases are nearly always diagnosed on the basis of symptoms visible in the field. Exceptions are found in some European programs where routine testing is performed for potato viruses A, Y, and leafroll. Field diagnoses often are difficult, since symptom expression frequently is influenced by the environment and is subject to differences in varietal response to certain pathogens. Certification personnel need extensive field experience before they can become familiar with the symptoms induced by various pathogens in many potato varieties under differing environmental conditions.

Occasionally, laboratory diagnostic techniques are employed to assist in "judgment calls." Such techniques must be of the type in which results are quickly obtainable, as the time interval between inspection and harvest is usually insufficient

Table 2 Maximum allowable tolerances for various seed potato certification programs in North America[a,b]

	Leaf roll	Mild mosaic	Rugose mosaic	Spindle tuber	Total nonlatent	Haywire	Ring rot	Blackleg	Verticillium	Fusarium wilt	Late blight	*Fusarium eumartii*	Total all other diseases	Giant hill	Varietal mixture	Nematode
California	0.5				1		0	1		5			5		0.25	0.0[c]
Canada					1		0						2			0.1
Colorado	0.5		1.0	0.5	1.5	1	0	excess.			0			0.5	0.25	
Idaho	0.2				1		0	2				0			0.5	0.0
Maine	1.0	2		2	3		0			1			4		0.25	
Michigan	0.5	1	1.0	0.5	1		0			1					0.1	
Minnesota	0.3	1	0.3	0.1	0.5	0.5	0	1		1					0.0	
Montana	0.25				2		0	0.25			0	0		0.25	0.25	0.0[c]
Nebraska	2	2		1	3		0	2.5							0.5	0.0
New York	1	2		1	2		0		5	5					0.25	
North Dakota	0.3	1	0.3	0.1	0.5		0	1		1						0.0
Oregon	0.25	1.5			1.5		0					0.5			0.25	
South Dakota	1	1		1	2	1	0	1		1				1.0	tr	0.0
Washington	0.2				1		0	2							0.1	
Wisconsin	1			1	3		0							0	0.1	0.0
Wyoming	1	1		1	1.5		0							0	0.1	

[a] Data compiled from rules and regulations published by agencies listed.

[b] All numbers expressed as percentage of maximum allowable for the final inspection of certified grade potatoes.

[c] Root knot.

for the completion of Koch's postulates. This may in turn lead to a reliance on marginally accurate confirmatory techniques for some diseases. In the bacterial ring rot disease caused by *Corynebacterium sepedonicum* (Spieck. & Kotth.) Skapt & Burk., there are two confirmatory techniques presently in use. One is the tomato test (42) which relies on the organism producing defined symptoms when inoculated onto tomato. This procedure requires several weeks for definitive results, however, and even then negative results may be misleading because of poor recovery of the bacterium. The second procedure is the Gram stain, (14, 36) since *C. sepedonicum,* unlike other plant pathogenic bacteria outside of the genus *Corynebacterium,* is gram-positive. Unfortunately, diseased or decaying potato tubers often become invaded with a number of other bacteria some of which may also be gram-positive. Either a positive or a negative Gram stain test may, therefore, be suspect. This point was amply illustrated recently in one lot of Montana-certified seed sold out-of-state. In mid-winter, the buyer complained that the seed lot, which was now in his own storage facility was infected with ring rot since he had obtained a positive Gram stain test through regulatory personnel in his state. Inspection of the seed in question indicated that unknown to the grower, moisture had seeped into the storage facility and soft rot had occurred. The problem was resolved by applying a third test (L. E. Claflin and J. F. Shepard, unpublished) in which antiserum prepared against *C. sepedonicum* was used to test extracts from rotted tubers for the presence of *C. sepedonicum.* The absence of a serological reaction with the gram-positive bacteria in the extracts suggested that *C. sepedonicum* was not responsible for the tuber decay. The seed was planted, no ring rot developed, and a law suit was avoided. This points out the need for additional research on diagnostic tests not only for bacterial ring rot but for other diseases such as leafroll and spindle tuber. Inaccurate results from certain of the tests presently in use may not only be expensive but can unfairly damage the reputation of a careful seed producer. There also should be greater involvement, particularly in the United States, of university or state plant pathologists with the implementation of new techniques in seed certification. The staffs of most certification agencies do not include research plant pathologists. Greater cooperation instead of a mutual "hands-off" policy between certification personnel and state or university pathologists will provide mutually beneficial contacts and encourage efficient translation of research discoveries into pragmatic forms of disease control.

ESTABLISHMENT OF QUARANTINES The third quarantine program approved in the United States (1912) involved potatoes and was instrumental in preventing an epidemic of potato wart caused by *Synchytrium endobioticum* (Schilb.) Perc. in North America. Spread of the golden cyst nematode, *Heterodera rostochiensis* Wr., from eastern United States to other potato growing regions also was prevented through strict quarantine regulations. At a more local level, barriers have been established by individual certification agencies in growing areas containing both commercial and certified potatoes. As discussed later, certification programs involved in the control of latent virus spread have relied heavily upon isolation and/or quarantine to reduce reinfection rates.

FOUNDATION PLANTINGS A great deal more is required of seed certification programs than simply the obtention of disease readings. If this were the case, it is probable that there would be no potatoes capable of passing certification standards. Thus, an integral part of disease control includes a number of additional approaches, one of which includes the supervision of or actual provision for foundation plantings.

Certified seed producers must each year have stock available that when replanted conforms to prescribed disease tolerances. This is frequently accomplished by the replanting of certified seedstock. The limitations of this approach become apparent, however, when the basic seed source becomes too heavily infected and the grower must look elsewhere for planting material. As an alternative, foundation blocks may be maintained by the grower that are isolated from certified seedstocks, are of relatively small size, and have more stringent disease tolerances. Foundation blocks because of their small size, may be rogued more intensively to minimize disease problems, and resultant seed that complies with appropriate tolerances may then be entered the following year for certification.

Despite continued vigilance, infections may occur in foundation blocks sufficiently late in the growing season to preclude detection. Hence, an acceptable disease incidence reading may be inaccurate and plantings the following year would be "over-tolerance" at the outset. This, for example, is an all-too-frequent occurrence with infection by potato leafroll virus. It is, therefore, a requirement of all United States certification programs that a "winter index" be performed on seed entered as foundation stock. Soon after harvest, tubers from foundation blocks are sent to a central location and treated with ethylene chlorohydrin (or rindite) or gibberellic acid to break dormancy. The potatoes are then shipped to such southern states as Florida or California for planting in test plots, and during January, February, or March plants are evaluated for disease incidence. A similar approach has been used by other programs that utilize greenhouses for the off-season tests. Limitations on space and disease expression frequently restrict the efficacy of the greenhouse approach, however. As a consequence of the winter index, results of the previous year's field evaluations may be either corroborated or invalidated before spring planting begins, and recommendations are provided for the foundation stock not only with respect to disease incidence but also varietal purity and vigor. The importance of winter indexing is apparent from the fact that all United States programs require it for the foundation seed tag.

Foundation seedstock is produced by many growers as an integral part of their individual seed programs. In other cases, it is made available to growers in certain countries, provinces, or states from isolated foundation seed farms that operate under the auspices of a cooperating certification agency. Foundation farms are responsible for providing an annual source of seedstock for many of their certified seed growers. This service provides assistance to growers whose seedstock is no longer suitable for replanting or who otherwise may wish to change seedstock.

EXTENSION Participation in seed certification programs is voluntary, and the responsibility to observe and carry out both recommendations and regulations rests

with the grower. Hence, all seed growers must continually be apprised of current disease-control recommendations and program regulations. While certain of these functions may be and are performed by cooperative extension service personnel apart from the certification agency, it, nevertheless, is the certification personnel who have the greatest amount of contact with seed growers and hence, maximal opportunity to keep growers informed. In certain regions, for example, potato vines (haulms) must be killed by spraying when populations of the green peach aphid (*Myzus persicae* Sulz.) have attained a critical level (15). This practice effectively reduces late-season leafroll infections. Notice is promptly given to all growers by the certification agency of the final date by which vines must be sprayed.

Varietal Improvement

In addition to the elimination of tuber-transmitted diseases, seed potato quality is enhanced through improvements in the yield and physical appearance of the tubers. Factors generally considered include the grade, type, and maturity of the tubers and organisms that directly affect the outward appearance of tubers. The primary means by which a given variety is improved is through an intensive selection program where such factors as vigor, tuber type, earliness of maturity, and varietal purity are considered. This approach is accomplished through the following procedures and is performed both by individual growers and at certification agency foundation farms.

TUBER INDEXING Tubers are selected from storage that are uniform in type (i.e. conformation and other physical characteristics), free from detectable diseases, and within an acceptable size range. Each tuber is coded for identification, and an eye, preferably from the stolon end, is removed and planted in the greenhouse. Resultant plants are evaluated for vigor and diseases and those, along with the mother tuber that have undesirable characteristics are destroyed.

TUBER UNIT PLANTING Tubers kept after indexing are cut into four pieces of nearly equal size and planted in sequence 20 to 25 cm apart to complete the unit. Additional space is provided between tuber units to make each clearly identifiable. There should be four replications in each unit, permiting a more accurate means for selection. Progeny of selected tuber units are placed in coded containers at harvest, and a sample from each is indexed and evaluated during the winter months. Increase from selected units may be used as progeny lines or mixed with other progeny for foundation planting.

HILL SELECTION This method consists of selecting and labeling promising plants during the growing season. The plants are removed during harvest and the tubers are appropriately evaluated and stored in coded containers. Tubers are then winter indexed, and tubers from desirable hills are increased through tuber unit planting.

MASS SELECTION This procedure is similar to that of hill selection except that tubers from desirable plants are stored in bulk rather than separately.

FIELD ROGUING By this procedure, the quality of a given seedstock is maintained by removing all diseased and off-type plants during the growing season. Tubers, root systems (as completely as is practical), and tops are removed from the field and destroyed. Fields are generally subjected to roguing three times during a growing season.

NEW SEED SOURCES Improved seed sources are commonly made available to growers either in the form of improved varietal selections or new varieties from foundation seed farms of the certification agency. In certain regions where a large number of different varieties are grown and there is an active breeding program, the evaluation and release of improved seedstocks may require an extensive effort on the part of seed-certification personnel.

THE VIRUS-FREE AFFAIR

Viruses known to infect potatoes may conveniently be separated into two general categories: (*a*) those that normally cause distinct foliar, or in certain instances, tuber symptoms, for example, potato leafroll virus, potato virus Y, etc, and (*b*) those that frequently do not produce consistent foliar or tuber symptoms, for example, potato virus X, potato virus S, and potato virus M. This second category is often referred to as the latent virus group although the designation is often an inaccurate one depending upon variety, virus strain, and plant-growth conditions.

Certified seed potatoes determined to be completely free of infection by all known viruses of both groups are designated "virus-free." However, concern over the accuracy of the term has led to the emergence of such qualified epithets as "virus-tested," "X-tested," and "X-free" potatoes. These terms also suffer from a lack of precision and have not received wide acceptance. All disease certification programs require that infection by nonlatent viruses be maintained within narrow tolerances (see Table 2). Similar standardization has not been achieved for "latent" virus infections. In many countries including the United States, most seed potatoes are infected with both potato virus X (PVX) and potato virus S (PVS), while potato virus M occurs with considerably less frequency in most varieties.

The first successful virus-free seed potato programs were developed in the Netherlands and in Scotland more than 20 years ago. This stimulated similar interests in the United States. At first, it was anticipated that an improvement in US seed potato quality similar to that achieved in Europe would result from the implementation of European virus technology. Indeed, presentations on the subject of virus-free potatoes are common features of seed potato seminars in many states and "virus-free potato programs" receive annual billing at the certification section meeting of the Potato Association of America. After two decades, however, the concept of virus-free potatoes in North America may still be described in the words of Carl Sagan (40) as one "of muddy surmise, unfettered speculation, stodgy conservatism, and unimaginative disinterest." Technically, there are no latent virus-free potato certification programs in the United States and at presently only three states (chronologically—Nebraska, North Dakota, and Montana) are engaged in large-scale

certification for PVX. Certain other states and Canadian provinces are in developmental phases of PVX-free programs or perform limited testing for PVX in foundation seedstocks. The only serious attempt being made in North America to produce PVX- and PVS-free certified seed at the grower level is in British Columbia, Canada.

Why No Virus-Free Potatoes?

In view of the long-term success of European virus-free seed potato programs, it is surprising that none have been established in North America. Cause for this incongruity is at the very least multifaceted and includes a variety of potentialities any one of which may be responsible for precluding the establishment of a latent-virus testing program within a particular growing region.

YIELD INCREASES Probably the most persistent question that looms over all latent-virus–free attempts in North America is whether increased yields are realized when seed potatoes are freed from all latent virus infection. In this context, unresolved areas include the question of the relative effects on yield of PVX and PVS infection both singly and in combination, and the potential effects of PVX and PVS on plant uniformity, tuber quality, and storage characteristics. It is well established that depending upon the variety and virus strain, each of these points deserves consideration in certain European potato programs, but none has been fully resolved for North American varieties.

Initial attempts at establishing whether or not PVX depressed the yields of North American potato varieties received little acceptance because the PVX-free clones used for comparison were not also reinoculated with the same strain of virus, subsequently increased, and then the effect on yield determined. Instead, progeny of infected and either selected or meristem-derived PVX-free plants were compared. Since clonal variation is the rule in potatoes, results of these studies were difficult to assess. Nevertheless, numerous investigators have reported increased yields when PVX-free stock of several varieties were compared with infected seed sources (16, 29, 43). Probably the most definitive work was recently published by Wright (53) who conducted a systematic study on the effects of PVX- and PVS-simultaneous infections on both total yield and tuber numbers for the Russet Burbank and White Rose varieties. Three US and one Canadian growing region were selected for test plots and results analyzed for each. With one exception, all areas recorded yield increases for latent virus-free stock as compared to infected material of the same clones. Yield increases ranged from 14 to 37%. The one exception was in the plot located in the state of Washington where yield differences were not significant. Subsequently, Kunkel et al (18), in the state of Washington, compared the yields of certified PVX-free Russet Burbank seed and PVX-infected Russet Burbank seed from several growers. In correspondence accompanying the yet unpublished results of further studies, Dr. Kunkel concluded: "When the data for 1972 and 1973 are considered as a whole, the yields of check (PVX- and PVS-infected) tubers . . . were equal to or better than the paired virus tested seed in 8 out of 10 comparisons." The significance of his results may be questioned for the same reasons as were prior studies with different clones, fertility levels, and nonstandardized virus strains.

However, in view of Wright's results (53) in the same region, it is possible that PVX-free Russet Burbank, and perhaps other potato varieties, have a greater yield potential only when grown under certain climatic conditions. If this is true, variations in environmental conditions may contribute to the variation encountered when attempting to establish the depressive effect of PVX and/or PVS infection on yields.

There is a firm scientific basis for believing that environmental conditions may significantly influence the severity of virus disease in plants, and there is little reason to suspect that potatoes are an exception. When potatoes infected with PVX are grown under suboptimal conditions of cool temperatures and low light intensity, many varieties are more severely affected. In the Russet Burbank variety, for example, many PVX strains elicit a pronounced mottle and crinkling of leaves when plants are grown under these conditions. When these varieties are grown at high light intensity with temperatures above 21–27°C neither of these symptoms is readily apparent except for unusually virulent strains of the virus. No studies have been conducted to establish whether environmentally enhanced foliar symptoms in PVX-infected American varieties are accompanied by a corresponding decrease in tuber yields and/or quality.

In conclusion, it has been difficult to establish experimentally that elimination of PVX from North American seedstocks invariably will result in a significant increase in yield. On the part of individual growers, however, far greater concern will continue to be placed on the probable effects of PVX and PVS on average yields over a period of years. Reliable evidence on such long-term effects will be even more difficult to obtain.

Experience with latent-virus–free potatoes in a strict certification program suggests that additional yield increases may occur indirectly. For example, Russet Burbank is a relatively indeterminant variety characterized by luxuriant top growth; if not properly spaced in the row, this variety produces exceptionally large and often knobby tubers. The PVX- and PVS-free Russet Burbank clone presently grown in Montana (originally supplied by R. Stace-Smith, Canada Department of Agriculture) is more vigorous than the normal latent-virus–infected one in terms of plant height, apparent rate of top growth, and average tuber size. When in-the-row spacings were decreased from a maximum of 28 cm to a maximum of 22 cm, a decrease in the number of oversize tubers was observed along with an overall yield increase (our unpublished observations). Thus, an increased plant population effectively enhanced both tuber quality and overall yield. With more determinant varieties such as the Norgold Russet, or under suboptimal growth conditions, a similar effect may occur to only a lesser extent, or perhaps not at all. This should be investigated. In this context, it is important that differences in growth habit between virus-free and latent-virus–infected potatoes should be appreciated fully especially within a variety such as the Russet Burbank. Changes in routine planting and cultivation practices may be required to accommodate increased vigor without a simultaneous reduction in tuber quality.

There is only suggestive evidence that PVX and PVS infection may influence either the susceptibility or resistance of potatoes to other types of diseases (13, 17). There is firm evidence, however, that the stringent sanitation requirements of a

PVX-free program may indirectly decrease the incidence of other types of diseases. A case in point is the blackleg disease caused by *Erwinia atroseptica,* a perennial problem in potatoes. It has recently become established that the causal organism is universally present within the lenticels of potato tubers (33) unless proper steps are taken to eliminate it. One means of eliminatin
to make vegetative cuttings from the stems of potato plants and produce nuclear stock (source material) from them. Meristem tip culture would also be effective. Thereafter, care must be taken through strict sanitation to prevent the recontamination of tubers with *E. atroseptica.* In our experience, the sanitary procedures used in Montana to prevent PVX contamination have also been effective in preventing *E. atroseptica* recontamination. Tests conducted in 1972 and 1973, by A. Kelman of the University of Wisconsin (personal communication) indicated that none of the PVX-free tubers supplied to him by selected growers in Montana contained the blackleg organism even though the seedstock had been in the grower's hands for four years. Also, blackleg has not been observed within the respective PVX-free fields throughout this period. Those growers in Montana who have experienced the blackleg disease in PVX-free stock were also those whose seedstock became recontaminated with PVX during the same time period. Failure to adhere to sanitary procedures is clearly indicated in these examples. Similar logic should pertain to ring rot, a tuber-borne disease caused by *Corynebacterium sepedonicum.*

No convincing evidence has been published regarding the potential effects of PVS infection on North American potato varieties. Studies conducted thus far (N. S. Wright, personal communication) suggest that PVS had no demonstrable effect on the varieties he studied. Detailed investigations with European varieties (39) indicates that the virus depresses yield and quality to greater or lesser degrees depending upon variety and PVS strain.

THE PROBLEM OF VIRUS SPREAD Potato varieties rendered free of PVX and/or PVS by either selection or meristem-tip culture remain fully susceptible to reinfection by both viruses. In the field, reinfection may occur rapidly if no attempts are made to minimize spread of the virus. And even when serious steps are taken to prevent PVX and PVS reinfection, the numbers of infected plants may nonetheless increase at an alarming rate. The apparent ease by which latent virus-free seedstocks become recontaminated has discouraged in significant measure the extension of latent-virus–free seedstock programs to the grower level.

Potato virus X may be transmitted by natural biological vectors including grasshoppers (*Melanoplus differentialis* Thos.) (52) and the chytrid fungus, *Synchytrium endobioticum* (28). The primary method by which PVX is introduced into virus-free fields is by contaminated equipment, field personnel, and animals (50). The virus may also be introduced into clean seedstock in storage through sprout-to-sprout contact (4, 25) and to some extent by tuber cutting knives (23). Once in a seedstock, PVX may spread by plant-to-plant contact both above (21) and below ground (38). The rate of virus X reinfection within a seedstock protected from external sources of inoculum has been reported to be relatively low (8). However, amounts of infection within a field may increase because of cultural practices in addition

to entry of PVX from external sources. Roguing, hilling, and cultivation all are effective, inadvertent means of PVX transmission. In Montana, the incidence of PVX reinfection has been observed to increase from as little as 0.5 to 40% in one year.

A two-step approach is commonly used to control PVX infection. Seedstocks should be produced in growing areas isolated by several miles from sources of PVX inoculum, and strict practices of sanitation must be followed during the production and storage of PVX-free stocks. Decontamination of all implements prior to entry into the field, has been accomplished through the use of appropriate chemicals. Formaldehyde has traditionally been used for this purpose, but in Montana it has been replaced by a 1% solution of pyrrolidine. Pyrrolidine is a highly basic compound which at low concentrations is far less unpleasant to handle than formaldehyde. Potato storage facilities are routinely disinfected with this compound. All persons entering PVX-free fields should wear freshly laundered clothes, disinfect footwear, and avoid contact with plants wherever possible. These two procedures in combination with a genealogical increase (see Table 4) and virus indexing program have generally proven to be successful (26) in the control of PVX reinfection of uninfected seedstocks.

The problem of PVS reinfection is substantially different from that of PVX. Strict sanitation must also be observed to avoid reinfection but in addition, a biological vector also appears to be involved. Despite earlier reports to the contrary (39), at least some strains of PVS can be transmitted by the aphid, *Myzus persicae* Sulz. (6, 22). Aphid transmission is inefficient at best, however, and has been discounted as the primary means of PVS spread in Holland (12). It has been our experience as well as that of others (22) that PVS reinfection has occurred in seedstocks maintained in sufficient isolation from sources of infection to prevent reintroduction of PVX. In some instances, many kilometers have separated PVX- and PVS-free fields from infected ones. The rate of reinfection by PVS in a single year is usually not as high as for PVX, but under certain conditions may be more of a problem than previously believed (12). Aphids and/or any as yet unidentified biological vectors necessitate production of PVS-free potatoes in areas isolated by at least 10 km from any sources of PVS (including home-garden potatoes) if reinfection is to be avoided. Unfortunately, this is a difficult qualification for most North American growing areas, so it may be necessary to concede PVS infection in tolerant potato varieties.

PRIORITIES The basic question of whether or not to attempt to eliminate latent viruses from seed potatoes can be answered only after consideration of certain crucial questions. These questions relate to the present status and effectiveness of ongoing programs within each state or potato-growing region.

The most successful seed-certification programs require coordinated efforts in extension, research, and disease diagnostic service. They also consider agronomic and horticultural as well as pathological aspects of potato production. When all phases function successfully, optimal production is possible for a given variety grown in a particular region. If this goal has been attained, the time would seem

to be right for developing a program to eliminate latent viruses with the aim of further improving seed quality. If on the other hand, production levels are far lower than they should be, improvements within the existing program are necessary before additional features should be added to it. For example, a number of years ago, we visited a growing region in central United States where Russet Burbank potatoes were being grown on sandy soil with essentially no irrigation. Yields were very low in periods when rainfall was inadequate or poorly distributed within the growing season. To compound the problem, the Russet Burbank variety is prone to going "off type" when soil moisture levels fluctuate significantly. Heavy rains falling on fields of this variety resulted in excessive nobbiness of tubers, etc, which in turn reduced the quality of the seed crop. A latent-virus–free program would have little meaning under these conditions. Instead, emphasis here should be placed on improved grower education, including varietal selection and irrigation.

Grower education through extension is very crucial in all seed potato certification programs. Failures in extension usually result in continuing problems in all phases of the program. The challenge of grower education becomes even more acute when attempting to institute a latent-virus–free program. It is first necessary to convince *all* growers that latent-virus–free seed is superior to virus-infected stock. Then their enthusiasm must continually be maintained to ensure that they will practice the necessarily strict sanitary procedures. This task is never easy, but is absolutely necessary if a program is to succeed.

RESOLUTION OF OLD WIVES' TALES AND UNSUBSTANTIATED CLAIMS

Efforts to develop PVX-free potatoes in North America have often been discouraged by many unsubstantiated and otherwise disquieting comments commonly heard within the seed potato industry.

One such claim is that reinfection of PVX-free potatoes by PVX causes "shock symptoms" and a significantly greater decrease in yield than would be experienced if the plants had suffered from tuber-borne PVX infection. Despite repeated attempts, we have been wholly unsuccessful in demonstrating this purported phenomenon (J. F. Shepard and G. A. Secor, unpublished). In our experiments both a mild mottle strain and a more severe strain of PVX were used as inoculum. Much more pronounced foliar symptoms did appear when PVX-free potatoes were inoculated with the more severe strain. This observation has led us to believe that so-called shock symptoms are simply the result of reinfection of PVX-free potato plants with a single relatively severe strain of the virus. In such cases the protective effect of milder PVX strains within the preinfected plant was eliminated.

In this context, another suggestion is sometimes advanced—that it is preferable to continue to utilize the mild strains of PVX already present in most North American varieties to cross-protect against severe strains rather than risk reinfection of PVX-free stock with more virulent strains. This argument has a number of significant shortcomings both practically and theoretically. One factor relates to varietal improvement, selection, and breeding programs. The phenomenon called "potato divergence" was recognized in Europe over 30 years ago. PVX, and in some varieties PVS, may cause a continual divergence in the growth habit of tubers, and

foliage from that previously bred or selected in a given variety (39). Hence, intravarietal selection must continually be practiced to minimize virus-induced plant-to-plant variation (i.e. enhance uniformity). Within breeding programs, PVX and PVS may cause even more confusing results because the reaction of F_1 individuals to either virus is unknown. Neither PVX nor PVS are seed transmitted in potato, but because F_1 seedlings are only rarely grown in complete isolation from virus-infected plants reinfection often occurs. Since the effects of the virus(es) on the horticultural characteristics of the selections have not been described, a distinction between genetic and virus-induced effects is difficult to make.

Naturally infected potatoes represent a massive reservoir of PVX and PVS, and this constant source of inoculum results in severe problems when attempts are made to produce PVX-free stock. More virulent strains of the virus may emerge that increase yield losses or negate cross-protection (19, 27). Moreover, PVX is not limited in its host range to potatoes but also infects other commercial crops such as tobacco and tomato as well as solanaceous weed hosts. For this reason, in certain growing areas, it is not advisable to grow potatoes in close proximity to tobacco or tomato fields.

An even more significant deterrent to development of PVX-free programs relates to the amount of PVX in seedstocks certified and sold as "PVX-free." Certain certification agencies in North America test for PVX only in foundation plantings. This may be two or more generations before the certified seed is sold for commercial production. Unfortunately, potatoes often become heavily infected with PVX and PVS when released from agency foundation farms to certified growers. As a result commercial buyers of resultant certified seed receive no data on PVS and PVX infection. Such programs are not only ineffective but also give well-conceived ones a "black eye." In Montana PVX testing is conducted on all seedstocks through the final year of increase and sometimes also in winter indexes. In this way, as much information about PVX infection is provided to the buyer of the seed as for any of the other diseases included within the certification scheme. While current-season infection may increase the amount of PVX in the following year's planting, the problem is not unique to latent virus diseases; this is a basic shortcoming of disease certification in general. When claims of the occurrence of PVX in indexed stock are made by buyers it is often difficult to determine whether contamination occurred prior or subsequent to sale. Insufficient attention to sanitation sometimes applies to certification agencies which may test incoming seed for virus infection. PVX spreads very rapidly when potatoes are grown in greenhouse benches, etc, prior to testing, and this may lead to inflated estimates of infection. Although somewhat less sensitive, tests of tuber sprouts (46) may provide more accurate information in this regard.

Potato leafroll virus is a continual problem in both certified and commercial potato plantings (see Table 3). One of the most recent claims is that PVX-free potato plants are more susceptible to potato leafroll virus infection than PVX-infected ones. To date, this contention has not been scientifically substantiated. It is commonly accepted that PVX-free potatoes are more vigorous in their growth habit than are their PVX-infected counterparts, and it is probably in this that the confusion has

Table 3 Area of potatoes rejected by certification programs in the United States and Canada (1968–1972)[a]

Reason	Number of agencies reporting	Percentage of total rejections	Total area rejected per year (ha)	Total area rejected per year (acres)	Percentage of total entered for certification
Ring rot	12	59.9	6,536	16,140	5.64 (2.2–13.8)
Leaf roll	10	9.0	985	2,432	.85 (0.0– 3.1)
Varietal mixture	9	7.2	788	1,946	.68 (0.0– 2.7)
Mosaic	9	5.4	591	1,459	.51 (0.0– 3.7)
Blackleg	5	4.5	487	1,202	.42 (0.0– 2.1)
Verticillium wilt	7	3.0	324	801	.28 (0.0– 2.0)
Chemical damage	3	2.8	301	744	.26 (0.0– 2.67)
Isolation	4	2.0	220	544	.19 (0.0– 1.1)
Spindle tuber	7	1.8	197	486	.17 (0.0– 0.9)
Withdrawn	2	1.6	174	429	.15 (0.0– 0.31)
Poor risk	2	1.4	151	372	.13 (0.0– 1.44)
Other	12	1.4	151	372	.13 (0.0– 2.4)

[a]Data compiled from reference 34.

arisen. More vigorous plant growth may reduce the intensity of leafroll virus symptoms at least for a time. Current season infections or infections by mild leafroll strains (55) may thus be more difficult to diagnose visually, and this would result in an apparent increase in the amount of disease during the following season.

THE PROBLEM OF MASS INDEXING Wherever programs to free seed potatoes of latent viruses have been successful, there has been a concerted team effort between certification personnel and state or university affiliated virologists. The breadth of expertise required to maintain certified seed free of latent viruses is simply too great for a single discipline to handle competently. Improved serological techniques have largely obviated the need for indicator plants as a means of mass indexing potatoes for PVX or PVS infection. Serological testing requires expertise not only in the performance of routine tests but also in virus purification and preparation of antiserum. Qualified personnel must be engaged to evaluate new procedures and incorporate them into the testing programs. Certain of these functions could be accomplished in national laboratories as it is in the Netherlands. Similarly, antisera could be produced at a single location and made available to all testing programs in North America. Annual workshops could be established in which recently developed or improved techniques would be demonstrated to those involved in virus (or other disease) diagnosis. Such a program could also assist certification agencies that do not have affiliated virologists. As an initial step toward this objective, in 1974 the Department of Plant Pathology at Montana State University made available standardized antisera to PVX, PVS, and their respective protein subunits. The former antisera are suitable for microprecipitin or other "whole virus" diagnostic techniques whereas "subunit" antisera are used in immunodiffusion techniques for diagnosis of PVX and PVS (47).

Reliable techniques for large-scale testing are essential to accurately determine the amounts of virus in all categories of seed potatoes. Sensitivity for small amounts of virus is an important consideration with regard to technique reliability, and much emphasis has been placed on the relative sensitivity of different diagnostic techniques for both PVX (41, 48) and PVS (11, 41). Inoculation of *Gomphrena globosa* is probably the most sensitive routine test for PVX. This is true, however, only when plants are grown under the proper environment, several leaves are inoculated, and the strain of PVX present produces optimal numbers of lesions. Some PVX strains produce far fewer lesions per μg virus than do others (J. F. Shepard, unpublished). Growing sufficient numbers of Gomphrena plants under ideal environmental conditions for virus indexing is a formidable and unnecessary task. Serological techniques of far lower sensitivity have been more useful for eliminating PVX infection from seedstocks simply because they can be conducted quickly and in numbers several orders of magnitude greater than Gomphrena tests. One thousand tests per acre with a technique of a slightly lesser sensitivity will provide more information concerning PVX infection rates than 10 idealized Gomphrena tests per acre. Indeed, the relatively insensitive chloroplast agglutination test and the microprecipitin technique have been responsible for the elimination of PVX and PVS from potatoes in Europe (51). Reliable immunodiffusion techniques have also been developed for PVX and PVS diagnosis on a mass scale (47) and have been successfully employed for several years in Montana. In 1973, over 250,000 serological tests were conducted for the two viruses. No PVX-free seed potato program with a long history of success is presently using only the Gomphrena test.

Detection of PVS with indicator hosts is presently not reliable (5). PVS strains vary greatly in the extent of lesion production on *Chenopodium* sp. (11), and *Nicotiana debneyii* in our experience has varied as a systemic host depending upon virus strain and growth conditions. More consistent results have been obtained through properly conducted serological tests of both the microprecipitin and radial-diffusion types.

Whatever the diagnostic technique, successful detection of potato latent viruses is influenced by the pattern of virus distribution in the plant. For PVX, tuber-borne infection commonly, but not always (54), leads to detectable concentrations of the virus throughout most of the foliage. Current-season infection of PVX-free stock, however, may or may not be detectable depending upon the stage of plant development and the virus strain.

In detecting PVS, the problem of virus distribution in chronically infected plants is far more acute than for PVX. Ordinarily, field infections of potato plants with PVS will not lead to detectable amounts of virus in the foliage (10) even though PVS will be detectable in plants grown from tubers of the same plants. Furthermore, the concentration and distribution of PVS in the foliage of systemically infected plants is not consistent throughout the growing season (3). Young as well as very old plants are particularly difficult to index. The general consensus is to sample plants for viruses just before they begin to flower. Because present techniques will only detect PVS infections that developed in the previous year, if in-field spread has occurred, it is difficult to eradicate the virus by roguing.

The Basic Requirements of a Latent-Virus–Free Program

It is beyond the scope of this article to provide a compendium of all past and present approaches to the development of programs for virus-free potatoes. Each has differed in detail and will probably continue to do so. The following list of primary requirements common to several successful programs may be helpful, however.

VIRUS-FREE STARTING MATERIAL Most commercially important potato seed-stocks are universally infected with PVX and PVS. For this reason, relatively few individuals in a very few varieties can be used to obtain noninfected plants by selection. Usually, meristem tip culture (35) or heat treatment followed by axillary meristem culture (49) is used to eliminate viruses from infected plants. Resultant plants may be rapidly increased in number to provide primary material for further selection. An efficient means of initially increasing meristem-derived or other virus-free starting material is the use of vegetative cuttings (9). This not only permits rapid increase of the plants, but also serves to eliminate other tuber-borne pathogens such as *Erwinia atroseptica*. Many meristem-derived plants are screened initially to eliminate genetic aberrants and to select for improved horticultural characteristics. All phases of increase of virus-free starting material must be conducted in strict isolation from other potatoes or hosts of potato pathogens whether in the greenhouse or in the field. Examples of reinfection by latent viruses or other pathogens, such as the spindle tuber viroid, are far too numerous to assume the contrary. In certain instances, virus-free programs have been set back several years as a result of contamination during the initial greenhouse increase.

SERODIAGNOSTIC CAPABILITY The diagnosis of latent-virus infections requires laboratory techniques. Test-plant or electron-microscope techniques may be quite efficient for screening initial plants for diseases. However, serological diagnostic methods must be employed during later phases of the program. Commonly, they are conducted at a central or regional laboratory properly equipped to handle a large number of samples. In a few countries, such as Germany, individual growers perform their own tests. Both approaches have been successful over a number of years when a continued supply of antiserum has been assured and sampling procedures are rigidly enforced.

In Montana, radial-immunodiffusion techniques (47) are used to index plants for PVX and PVS. Antiserum prepared against dissociated (and denatured) viral protein is incorporated into buffered agar and used to test extracts from plants to which a final concentration of 2.5% pyrrolidine has been added. Pyrrolidine serves to dissociate virions into diffusible protein oligomers capable of reaction with homologous antibody preparations. Leaf samples are delivered to the testing laboratory with care being taken to keep the leaves dry and cool. Excessive moisture or humidity leads to rapid deterioration of potato leaves. Juice is expressed from individual leaves with a hydraulic press using a multisample press plate–plunger system. A drop of juice is collected for each sample and placed in a separate 6 X 25 mm test tube. At this point, the juice may either be frozen for later testing

or be treated with pyrrolidine and placed in a radial diffusion depot. Extracts to be assayed may not be frozen subsequent to treatment with pyrrolidine. Using these procedures, a laboratory with ten people may test 6000 to 7000 samples per day for PVX and PVS.

GENEALOGICAL PROGENY INCREASE The process of increasing virus-free starting material through the certified seed stage must continually follow a genealogical selection scheme. This involves the continued entry into the program of recently derived virus-free starting material which will be increased over 4 or 5 growing seasons before marketing. It is well established that replanting of virus-free stock year after year will result in reinfection.

The basic scheme used by all virus-free programs which is slightly modified from Rozendaal & Brust (39) is similar to that shown in Table 4. Nearly all programs differ in one or more specific areas of the scheme, however, particularly within the indexing category. The type and intensity of virus testing is highly variable, and each program must be evaluated separately. Other programs may either add steps to or delete steps from the basic scheme. As indicated in Table 4, the terms used to designate classes of seed potatoes are not uniform. Commonly, the first two or three sequential steps are performed on foundation farms maintained by the state or grower association. Such farms are far from commercial potato plantings, thus minimizing the potential for reinfection by viruses. Foundation farms are not always utilized, however, and in a few programs, for example Montana, growers perform the full sequence of steps.

EFFECTIVENESS OF SEED CERTIFICATION PROGRAMS AND PROSPECTS FOR THE FUTURE

Each seed certification program has merit because seedstocks will be improved if any of the principles of certification are implemented. It is doubtful, however, whether any program has developed to the point where disease control is complete

Table 4 Genealogical scheme for increase of virus-free potato increase[a]

Class	Other common descriptions	Indexing
Mother plant	Pre-elite I, nuclear I	Serologically tested
First year family	Elite I, nuclear II	100% serologically tested plus field inspection
Second year family	Elite II, foundation I	10% serologically tested plus field inspection
Third year family	Elite III, foundation II	2% serologically tested plus field inspection
Fourth year family	Foundation, certified virus-free	30–50 plants/acre tested plus field inspection

[a] After Rozendaal & Brust (39).

or where all existing knowledge has been utilized fully. The value of seed certification in the United States is shown in Figure 1 in which the potato acreage entered for certification was calculated against the number of acres that met prescribed tolerances. During the formative years of 1919–1929, a relatively low percentage of the acreage was found acceptable. A dramatic upswing soon occurred, but was followed by only modest increases in the percentage of acres certified. While these figures have certain limitations, they nevertheless indicate that little overall improvement in the control of the diseases subject to certification standards has occurred during the past several years. Seed certification has been and will continue to be an effective means of improving the quality of seed potatoes, but new approaches and techniques probably will be required before additional improvements are realized.

The science of plant breeding would seem to offer the greatest promise for improving the quality of seed potatoes in North America. Bacterial ring rot, for example, is responsible for more seed rejections in the United States and Canada than all of the other diseases combined (see Table 3). Thus, improved sources of resistance to ring rot would be particularly valuable. Unfortunately, research gains in this area have been limited. Even when increased resistance was obtained, new varieties generally have failed to achieve acceptance by industry. Indeed, the oldest potato variety grown in the United States (i.e. Russet Burbank) commands an ever increasing percentage of the total seed acreage and in 1973 represented 32% of the seed potatoes grown (45). Nevertheless, continued emphasis in potato breeding is necessary, at the very least, to broaden the genetic base of potatoes in North America.

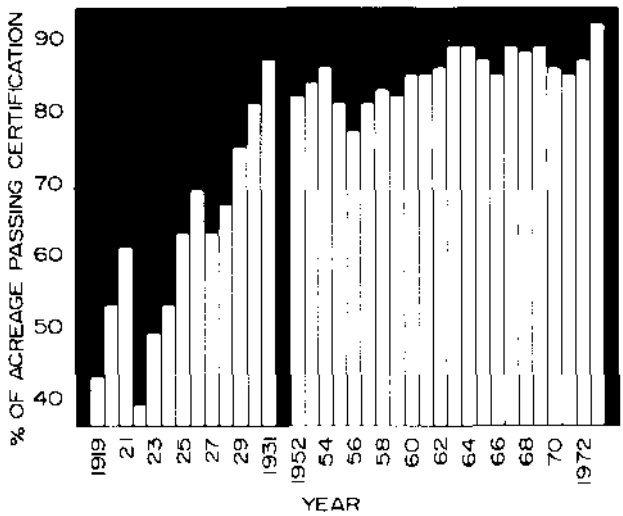

Figure 1 The percentage of seed potato acreage entered for certification that passed certification standards from 1919–1932 (24) and 1952–1973 (44). Data from 1932–1951 could not be obtained.

Another, and as yet untested, approach may be to improve certain varietal characteristics including resistance to diseases through somatic cell selection techniques. Recent advances in the isolation and regeneration of mesophyll cell protoplasts suggest that resistance to disease may be enhanced by these procedures (7) while essential varietal characteristics would be maintained.

Pending new developments in the areas of breeding or cell selection, a great deal may still be done in the control of diseases within existing varieties. Certification programs may be expanded to include the control of latent virus infections, and when maintained under regulations of strict sanitation, may be integrated with programs for blackleg and ring-rot control. Rapid diagnostic techniques for bacterial diseases would be of great value. Serological diagnostic techniques such as immunofluorescence offer great potential for the detection and identification of even small numbers of bacteria in infected tissues. Greater coordination among research agencies and improved methods of diagnosis and disease assessment will be required, however, before these potentialities can be realized.

Literature Cited

1. Appel, O. 1915. Leaf roll diseases of the potato. *Phytopathology* 5:139–48
2. Appel, O. 1934. Vitality and vitality determination in potatoes. *Phytopathology* 24:482–94
3. Arenz, B., Vulic, M., Hunnius, W. 1964. Die Nachweisbarkeit des S-Virus in den Verschiedenen Pflanzenteilen sekundar infizierter Kartoffelpflanzen. *Bayer. Landwirtsch. Jahrb.* 6:683–90
4. Bawden, F. C., Kassanis, B., Roberts, F. M. 1948. Studies on the importance and control of potato virus X. *Ann. Appl. Biol.* 35:250–65
5. Beemster, A. B. R., Rozendaal, A. 1972. Potato viruses: properties and symptoms. In *Viruses of Potatoes and Seed Potato Production,* ed. J. A. de Bokx, 115–43. Wageningen: Centre Agric. Publ. Doc. 233 pp.
6. Bode, O., Weidemann, H. L. 1971. Untersuchungen zur Blattausübertragbarkeit von Kartoffel-M-und-S-Virus. *Potato Res.* 14:119–29
7. Carlson, P. S. 1973. Methionine sulfoximine-resistant mutants of tobacco. *Science* 180:1366–68
8. Cockerham, G. 1958. Observations on the spread of virus X. *Conf. Potato Virus Dis.* 3:144–48
9. Cole, E. F., Wright, N. S. 1967. Propagation of potato by stem cuttings. *Am. Potato J.* 44:301–4
10. de Bokx, J. A. 1968. The translocation of various isolates of potato virus S in potato plants with primary infection. *Meded. Rijksfac. Landbouwwetensch. Gent* 33:1179–85
11. de Bokx, J. A. 1970. Reactions of various plant species to inoculation with potato virus S. *Neth. J. Plant Pathol.* 76:70–78
12. de Bokx, J. A. 1972. Spread of potato virus S. *Potato Res.* 15:67–70
13. Dowley, L. J. 1973. Effects of primary and secondary infection with potato virus X (PVX) on yield, size, chemical composition, blight resistance, and cooking quality of potato variety Kerr's Pink. *Potato Res.* 16:3–9
14. Glick, D. P., Ark, P. A., Racicot, H. N. 1944. Outline of procedure for the diagnosis of bacterial ring rot of potatoes. *Am. Potato J.* 21:311–14
15. Hille Ris Lambers, D. 1972. Aphids: Their life cycles and their role as virus vectors. See Ref. 5, 36–56
16. Hoyman, W. G. 1964. Red Pontiac vine and tuber yields as affected by virus X. *Am. Potato J.* 41:208–11
17. Jones, E. D., Mullen, J. M. 1974. The effect of potato virus X on susceptibility of potato tubers to *Fusarium roseum 'Avenaceum'*. *Am. Potato J.* 51:209–15
18. Kunkel, R., Holstad, N., Butala, H., Thornton, R. E. 1973. Influence of potato viruses X and S on yield of Russet Burbank potatoes. *Am. Potato J.* 50:385 (Abstr.)
19. Ladeburg, R. C., Larson, R. H.,

Walker, J. C. 1950. Origin, interrelation and properties of ringspot strains of virus X in American potato varieties. *Univ. Wis. Res. Bull.* 165. 47 pp.
20. Leach, J. G. 1938. The biological basis for certification of seed potatoes. *Am. Potato J.* 15:117–30
21. Loughnane, J. B., Murphy, P. A. 1938. Dissemination of virus X and F by leaf contact. *Sci. Proc. R. Dublin Soc., NS* 22:1–15
22. MacKinnon, J. P. 1974. Detection, spread, and aphid transmission of potato virus S. *Can. J. Bot.* 52:461–65
23. Mai, W. F. 1947. Virus X in the newer potato varieties and the transmission of the virus by the cutting knife. *Am. Potato J.* 24:341–51
24. Martin, W. H. 1931. Report of the seed potato certification committee. *Proc. 18th Ann. Meet. Potato Assoc. Am.* 115–24
25. McIntosh, T. P. 1944. Potato troubles. *Gard. Chron.* 116:87–88
26. Munro, J. 1954. Maintenance of virus X-free potatoes. *Am. Potato J.* 31:73–82
27. Munro, J. 1961. The importance of potato virus X. *Am. Potato J.* 38:440–47
28. Nienhaus, F., Stille, B. 1965. Übertragung des Kartoffel-X-Virus durch Zoosporen von *Synchytrium endobioticum*. *Phytopathol. Z.* 54:335–37
29. Ohms, R. et al 1973. Comparison of PVX-free Russet Burbank Canadian source seedstock with regular PVX infected Idaho seedstock. *Am. Potato J.* 50:385–86 (Abstr.)
30. Orton, W. A. 1914. Inspection and certification of potato seedstock. *Phytopathology* 4:39–40 (Abstr.)
31. Orton, W. A. 1914. The potato study trip of 1914. *Phytopathology* 4:412–13 (Abstr.)
32. Orton, W. A. 1914. Improvement of potato seed stocks through official inspection and certification. *Proc. 1st Ann. Meet. Nat. Potato Assoc. Am., Philadelphia* 37–43
33. Pérombelon, M. C. M. 1972. The extent and survival of contamination of potato stocks in Scotland by *Erwinia carotovora* var. *caratovora* and *E. carotovora* var. *atroseptica*. *Ann. Appl. Biol.* 71:111–17
34. *Proceedings, Certification Sect., Potato Assoc. Am. Meet. Omaha, 1973*
35. Quak, F. 1972. Therapy. See Ref. 5, 158–66
36. Racicot, H. N., Savile, D. B. O., Conners, I. L. 1938. Bacterial wilt and rot of potatoes—some suggestions for its detection, verification and control. *Am. Potato J.* 15:312–18
37. Rieman, G. H. 1956. Early history of potato seed certification in North America, 1913–1922. *Potato Handbook.* New Brunswick: Potato Assoc. Am. 1:6–10
38. Roberts, F. M. 1946. Underground spread of potato virus X. *Nature London* 158:663
39. Rozendaal, A., Brust, J. H. 1955. The significance of potato virus S in seed potato culture. *Conf. Potato Virus Dis.* 2:120–33
40. Sagan, C. 1973. *The Cosmic Connection: an Extraterrestrial Perspective.* Garden City, NY: Anchor. 274 pp.
41. Sampson, P. J., Taylor, R. H. 1968. A comparison of the electron microscope, microprecipitin tests, and indicator plants for the detection of potato viruses S, X, and Y. *Phytopathology* 58:489–93
42. Savile, D. B. O., Racicot, H. N. 1937. Bacterial wilt and rot of potatoes. *Sci. Agric.* 17:518–22
43. Schultz, E. S., Bonde, R. 1944. The effect of latent mosaic (virus X) on yield of potatoes in Maine. *Am. Potato J.* 21:278–83
44. Seed reports. 1952–1973. *Spudlight.* Certified Seed Editions. Washington: United Fresh Fruit Veg. Assoc.
45. See Ref. 44, 1973 edition
46. Shepard, J. F. 1969. Serodiagnosis of PVX in potato tuber sprouts. *Plant Dis. Reptr.* 53:845–48
47. Shepard, J. F. 1972. Gel-diffusion methods for the serological detection of potato viruses X, S, and M. *Montana Agric. Exp. Stn. Bull.* 662. 72 pp.
48. Shepard, J. F., Secor, G. A. 1969. Detection of potato virus X in infected plant tissue by radial and double-diffusion tests in agar. *Phytopathology* 59:1838–44
49. Stace-Smith, R., Mellor, F. C. 1968. Eradication of potato viruses X and S by thermotherapy and axillary bud culture. *Phytopathology* 58:199–203
50. Todd, J. M. 1958. Spread of potato virus X over a distance. *Conf. Potato Virus Dis.* 3:132–43
51. van Slogteren, E., van Slogteren, D. H. M. 1957. Serological identification of plant viruses and serological diagnosis of virus diseases of plants. *Ann. Rev. Microbiol.* 11:149–64
52. Walters, H. J. 1952. Some relationships of three plant viruses to the differential

grasshopper, *Melanoplus differentialis* (Thos.). *Phytopathology* 42:355–62
53. Wright, N. S. 1970. Combined effects of potato viruses X and S on yield of Netted Gem and White Rose potatoes. *Am. Potato J.* 47:475–78
54. Wright, N. S. 1974. Potato virus X infection of Netted Gem potato. *Am. Potato J.* 51:202–5
55. Wright, N. S., MacCarthy, H. R., Cole, E. F. 1967. Detection and control of mild strains of potato leafroll virus. *Am. Potato J.* 44:245–48