Incidence of the tobacco veinal necrotic strain of potato virus Y (PVY^N) in Canada in 1990 and 1991 and scientific basis for eradication of the disease

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Eradication is defined as the application of cultural and biological control measures to cause any significant reduction in inoculum and the demonstration of the absence of pathogen over a period of time, from a representative sample, by using the most sensitive detection procedure available to date. The basis of the Canadian eradication initiative lies in the significant difference between occurrence of PVY^N, the virus spread and aphid flight in European countries in the 1950s, and those of 1990-91 occurring in Canada when the PVY^N outbreak took place. The prospect of PVY^N eradication in Canada is based on the very low virus incidence, low aphid pressure, drastic measures taken to reduce inoculum, the massive number of leaf samples tested, and the application σ highly sensitive detection procedures. The progress of Canadian eradication measures compares well with the PVY^N elimination carried out in New Zealand in 1988-89.

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L'eradication est définie comme etant l'application de pratiques culturales et de moyens de lutte biologique qui causent toute reduction significative de l'inoculum et révèlent l'absence de pathogenes durant une periode de temps, à partir d'un échantillon representatif tout en utilisant la methode de detection la plus sensible disponible presentement. La méthode canadienne en eradication repose sur les differences significatives entre les occurences du PVY^N, l'étendu du virus et les vols de pucerans dans les pays europeens durant les années 1950, et les vols de pucerons au Canada en 1990-1991 lors des premieres manifestations du PVY^N. Les previsions d'eradication du PVY^N au Canada sont basées sur une tres faible incidence du virus, un faible nombre de pucerons, des mesures draconiennes pour reduire l'inoculum, un grand nombre d'echantillons de feuilles examinees, et l'application de procedures de detection extrêmement sensibles. Les progrès qu'ont connus les mesures d'eradication au Canada sont comparables aux progres qui ont été effectues en Nouvelle-Zélande durant les annees 1988-89, en ce qui touche l'élimination du PVY^N.

Introduction

Eradication as originally defined by Whetzel (1929) means the complete elimination or destruction of a pathogen after it is established in a given area. This type of definition implies absoluteness and is not adequate for a holistic approach to crop protection and crop production (Apple, 1977). Experience has shown that absolute control of disease is economically impractical. On the other hand, the National Academy of Sciences (NAS) United States of America (USA) publication (1968) has used the term eradication to connote any significant reduction in inoculum with the application of cultural and biological control measures. The definition adopted by the 1991 Bi-National PVYN meeting based on the North American Plant Protection Organization (NAPPO) recommendation states that eradication of a plant pest has occurred when processes have been applied that lead to the detection of zero levels of the pest in plants; and plant products known to be affected and produced in a defined area (Anon., 1991b). In this paper, the above NAPPO definition is implied for the eradication of PVYN.

Background and history of the eradication proposal. A strain of potato virus Y which induces systemic veinal necrosis of tobacco leaves, known as PVYN (de Bokx and Huttinga, 1981) was discovered in seed potatoes of eastern Canada in 1990 (Anon., 1991a, Coffin et al., 1991). A preliminary survey for the PVY^N strain during the summer of 1990 led to the finding of the virus in three fields each of New Brunswick (NB) and Prince Edward Island (PEI) (Table 1). The seed potatoes planted in NB were traced back to seed sources in PEI, thus indicating only one source of PVY^N. This survey was followed by an extensive leaf test (Florida-grown potato seedlots) and tuber-sprout test during the winner months (Table 1). As a result, in the 1990 crop a total of four cases of PVYN were found in NB and 31 in PEI. This uneven distribution of PVYN from a large number of seedlots and their traceback to one seed source indicated that eradication should be possible, if the main seed sources were fully tested and discarded.

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However, in spite of this type of data, a number of scientists and producers were of the opinion that eradication would not be successful. The main reason presented by scientists and producers alike was that seed potatoes from PEI have gone to several provinces of Canada as well as to several seed-producing States of the USA in preceding years. Thus, PVY^N should be well established all over North America.

A preliminary review of the survey data (Table 1) suggested otherwise. A preliminary proposal was advanced for the eradication of PVY^N from Canada (Singh, 1991). After considerable discussion between scientists from the United States and Canada, along with government and industry personnel, an eradication package was developed mainly for PEI. A progress report on the eradication effort in PEI was made to the annual meeting of the Potato Association of America in Spokane, USA (Coffin *et al.*, 1991).

During the summer of 1991, the opinions of internationally recognized PVY^N specialists were sought. Potato and tobacco virologists from Germany, Peru, the United Kingdom and the United States, held two days of discussions with virologists and entomologists from Canada. The objective was to assess the feasibility of eradication of PVY^N from Canada. The outcome of this discussion was that eradication was a possibility, however, for the foreseeable future management of seed potato production within affected areas would be required to avoid infection by the virus (Anon., 1991c). Since then, there have been many questions from virologists, potato inspectors and potato producers regarding PVYN eradication. From these discussions it is apparent that there is not a good understanding of the situation in Canada when compared to countries where eradication of PVY^N was not successful.

The eradication proposal (Singh, 1991) identified a limited number of infection sources, limited virus spread and most of the spread through seed movement of the potato cultivar Atlantic. In this paper, an attempt is made to demonstrate that a contrast between the situations of Canada and Europe existed at the time of the PVY^N outbreak, thus the Canadian eradication proposal is scientifically based, and it compares favorably with an outbreak and control of PVY^N in New Zealand (Fletcher, 1989).

Occurrence of PVY^N in European countries. The diagnosis of PVY^N in different countries has been based on the distinctive property of this strain to induce severe veinal necrosis on healthy tobacco plants 7 to 15 days after inoculation. The symptoms in tobacco at first are confined to the smaller veinlets (Fig. 1A), but later the necrosis spreads to petiole (Fig. 1B), and stems (Fig. 1C) with collapse of lower leaves (Fig. 1C,D). However, the necrotic symptoms have to be viewed in absence of other viruses infecting tobacco.

PVYN first appeared in European seed potato growing areas in the 1950s causing a severe epidemic with high losses (Weidemann, 1988). It was supposed to have originated in South America (Smith and Dennis, 1940; Nobrega and Silberschmidt, 1944; Bawden and Kassanis, 1951) from where it was introduced to Europe. In Europe, it was first detected at the Commonwealth Potato Collection from a naturally infected potato plant collected in South America and was possibly identical with Nobrega and Silberschmidt's isolate (Bawden and Kassanis, 1951). The rapid spread of PVY^N in Europe was noted from its reports from several countries within a short period. For example, PVYN was identified from Bulgaria (Kavachevsky, 1950); Switzerland (Bovey, 1955); The Netherlands in 1957 (de Bokx, 1961); England (Richardson, 1958); Germany (Schmelzer and Klinkowski, 1958); Hungary (Szirmai, 1958); Italy (Marceli, 1960); Scotland (Todd, 1961); and Poland (Jankowski and Florczak, 1962). PVYN became economically important first in Germany, where certain commercial cultivars became almost wholly infected (Weidemann, 1988), and in 1958-1960 potato crops suffered seriously due to PVY^N in The Netherlands (de Bokx, 1964).

High incidence of virus in the 1950s. From some studies published during the 1950s, it appears that the European tobacco crop was severely infected with PVYN. For example, in one study dealing with the economics of tobacco production, it was stated that the tobacco quality started deteriorating when PVYN incidence reached 10-20%, and at 60-70% harvesting of tobacco became unprofitable. In some areas with optimum climate and soil conditions a high incidence of PVYN (100%) was observed in 1957 (Seehofer *et al.*, 1958). During a tour of the tobacco-growing areas of Baden in 1958, a clear correlation was observed between the development of viral symptoms in tobacco and the proximity of diseased potato fields (Steiner, 1959).

High incidence of PVY^N in Europe was not limited to the tobacco crop only. In a study dealing with PVY^N incidence in potato in Switzerland, the virus incidence was 40-100%, with the seed potatoes received from Northern Germany, The Netherlands, Poland, Austria and Denmark (Keller and Munster, 1961).

Research work from Brunswick, Germany during the 1950s showed that the extent and rapidity of PVY^N infection in the tobacco crop depended on the annual situation of aphid vectors. Commencement of aphid activity before June resulted in mass infection by PVYN at an early stage of tobacco growth (Volk, 1960a). In 1954, 1957, and 1959 there were early and high aphid activity resulting in over 50% infection by mid July. In 1955, flights were late and only a few aphids were present, and 50% infection was delayed until early August. *Myzus persicae* was found in the greatest numbers throughout most of the 1950s, except the year 1956, *M. persicae* was the main vector in the fields (Volk, 1960b).

Detection procedures used in the 1950s. PVY^N was detected serologically using polyclonal antisera and precipitin tests of eyesprouts or inoculating extracts to A6 (S. *demissum* x S. *tuberosum* 'Aquila') test plant (Arenz and Hunnis, 1961). In other laboratories, dormant tuber extracts were used for the inoculation of A6 or *Solanum demissum*Y (SdY) test plants (de Bokx, 1961).

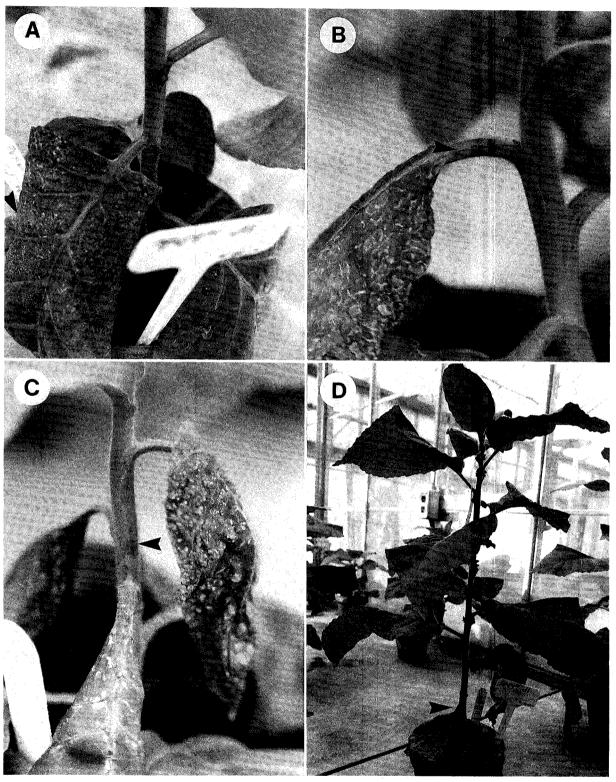


Fig. 1. Samsun tobacco showing necrosis of veinlets (A), petiole (B), stem (C), and collapse of leawes (C, D), infected with PVY^N. Arrow heads indicate the location of symptoms.

Situation in Canada at the time of PVY^N discovery

New Brunswick and Prince Edward Island. The survey results for 1990-91 carried out in Agriculture Canada Seed Certification and Plant Quarantine laboratories are presented in Table 1. It showed that 1.57% of the seedlots from PEI and 0.43% from NB contained PVY^N. Detailed analysis of postharvest tuber testing from PEI provided further insight into the distribution of PVY^N. The PVY^N infection was pre-

dominantly in the cultivar Atlantic (Table 2). Infection of PVY^N in highly PVY^O susceptible cultivars Russet Burbank, Shepody and Superior was not detected without infected Atlantic fields being present on the same farm and even then the incidence was limited to one or two fielcls.

As far as the tobacco crop is concerned, there has not been any record of necrotic symptoms in PEI. In 1991, tobacco plants were planted as bait-plants in several potato fields.

Table 1. Detection of PVY^N in potatoes during summer and winter of 1990-91 in the Atlantic Provinces, Canada'

Type of Survey	Sample Size	New Brunswick	Nova Scotia ²	Prince Edward Island
Field-Summer	100 leaves	31163	0125	31299
Winter-Florida Test	100 leaves	0/512	NT ³	01438
Winter-Tuber Test	100 leaves	1/239	NT	2811237
	Total	41914 (0.43%)	0125 (0%)	31/1974 (1.57%)

1 Data from seed certification laboratories, Food Production and Inspection Branch, Agriculture Canada.

2 Tested at Research Branch, Research Station, Fredericton, New Brunswick.

3 Not tested.

Table 2. Incidence of PVYN in Prince Edward Island potato fields in 1990-91 winter test'

		Potato Cultivars with PVYN Positives				
Field#	Atlantic	Russet Burbank	Shepody	Superior		
Farm 1	1/12	016	NT ³	0/1		
2	214	NT	NT	NT		
3	1/1	NT	NT	NT		
4	511 1	313	NT	1/2		
5	213	013	1/4	012		
6	1/1	012	012	0/1		
7	1/2	011	012	0/1		
8	1/1	012	NT	0/1		
9	1/1	014	NT	0/1		
10	1/2	012	NT	0/1		
11	1/1	014	012	015		
12	1/1	1/4	NT	NT		
13	212	0/1	0/1	012		
14	1/5	NT	NT	NT		
Total	21/36	4/32	1/1 1	1/17		

1 Data was obtained from Seed Certification Laboratory, Charlottetown, PEI, Food Production and Inspection Branch, Agriculture Canada. One additional PVY^N positive was in cultivar Bellisle at a different farm, where no other cultivars were tested.

2 Number of PVY^N positives/number of fields tested on each farm.

3 Not tested.

No PVY^N infection was detected in any of them (unpublished observation, R. Coffin, PEI Department of Agriculture). Although aphids can be detected in PEI prior to June, potato colonizing aphids are not observed until July and even then only in very low numbers. *M. persicae* flights are recorded in late July or early August (unpublished data, J. Diamond, PEI Department of Agriculture).

Surveys of seed potatoes supplied by PEI to several Provinces and States have not yielded PVYN-infected plants except a few seedlots in New Brunswick, Nova Scotia, and Ontario. PEI potato seed grown in tobacco-producing areas of the USA has not been observed contributing to an infection due to PVYN.

Ontario. In 1990, symptoms of PVY^N in tobacco were observed in some fields planted in close proximity to the infected potatoes. However, in Ontario, PVY^N has been detected several times on the basis of tobacco symptoms as well as by tobacco bioassay from potato and tobacco crops (Singh, 1969; McDonald *et al.*, 1991). Infection in individual tobacco fields had ranged from 20 to 50% (unpublished observation, OMAF, Delhi). However, most of the infections in tobacco crop have been associated with the plantings of illegally imported foreign potato cultivars and infections in tobacco have dropped drastically in subsequent years following PVY^N discovery. Volunteer plants and weed hosts may be perpetuating the virus. Compared to the Atlantic Provinces, there are early as well as an abundance of aphid species in Ontario.

Comparative evaluation of situations in Europe and Canada. From the foregoing description of European countries in the 1950s and the Canadian situation in 1990-91, the following comparison can be made. PVY^N in Europe was widespread, with high infection levels in both tobacco and potato crops. Aphids were available early in the season and testing for PVY^N was carried out on a limited scale with less sensitive procedures. On the other hand, in PEI, the PVYN infection of tobacco had not been observed; PVYN infection of potato was less than 2%; aphid pressure was negligible; testing had been done on a large scale to determine the distribution of the virus; and the test procedures used were more sensitive than those available in the 1950s in Europe. In the case of Ontario, the incidence of PVY^N in tobacco fluctuates depending on the source of potato seed planted and the aphid pressure has been higher than PEI. Thus, in comparison with Europe, the amount of PVYN infection in Canada is "slight" and factors contributing to the spread of the virus are different.

In Europe, there is no evidence indicating that any attempt was made to eradicate PVYN. Most probably, it could have been because infection with PVY^N reached epidemic proportions before its importance was realized. However, in Canada, PVYN was detected in the early stages of virus spread, when the incidence was very low. In Europe, there was no evidence of an extensive leaf or tuber testing for the eradication of PVY^N. In Canada, a high proportion of seedlots intended for planting were tested and those found infected were not used for subsequent planting, thus minimizing the amount of inoculum sources in the field.

The seed certification system used in Canada requires that the seedstocks must be derived from virus-free tissue culture material. They are grown for a limited generation in a flush through system. This practice ensures freedom of the nuclear stock from known viruses and viroids. Since there was no emphasis placed on testing for PVYN in Canada in earlier years, the present PVY^N problem could have occurred a few years earlier and remained unnoticed. In the absence of visible symptoms in potato due to PVY^N, virus spread in the plants for several generations could have taken place. Since PVY^N testing of planting material would be carried out routinely, PVY^N has less chance of building up in the potato crop. This was not the situation in Europe, because there certain commercial potato crops were almost wholly infected with PVY^N in the 1950s (Weidemann, 1988).

Spread of PVYN in eastern Canada. The spread of the virus in eastern Canada was not as rapid as it was in Europe. From Table 1 it is clear that potato cv. Atlantic had 58% of samples with PVY^N whereas other cultivars had only 5 to 13% samples with PVYN. These other cultivars (Russet Burbank and Shepody) are highly susceptible to PVY^O by aphid inoculation under field conditions and possess high virus titres (Bagnall and Tai, 1986; Singh and Somerville, 1987). They should have been infected with PVY^N more than cv. Atlantic if its spread was taking place by aphids (Bagnall and Tai, 1986). The infection of these cultivars is limited to only two farms where PVY^N infection in cv. Atlantic samples was also high. This could be due to smallscale aphid transmission of PVYN to nearby fields from cv. Atlantic. Absence of infection of other cultivars on several farms, where PVYN was present in cv. Atlantic, may indicate that other cultivars were farther away from cv. Atlantic fields and aphids did not transmit the virus. Under eastern Canadian conditions a 600 m isolation band has been shown to prevent PVY^O spread (Bagnall and Tai, 1986). The high incidence of PVYN infection in cv. Atlantic could be explained by multiplication and accumulation of infected seed over a few years and its movement to other parts of PEI. Atlantic seed potatoes were distributed in PEI from a limited source of four elite growers in 1989-90; any one of them could have had PVYN in their seed for a few years. As the seed potatoes were sold to other farmers, PVY^N was introduced to other areas of the Island.

Basis of PVYN eradication recommendations. An eradication strategy must aim to reduce the amount of inoculum from which the disease starts or reduce the rate at which the disease increases in a plant population or both (Van der Planck, 1972). When applying this principle to a tuber-borne disease such as PVY^N, the basis of eradication was to pre-

vent the multiplication of virus- infected seed potatoes and its spread to other parts of the country. As a result all potato seedlots found positive in any test (winter or summer), their sister-lots and those seedlots in close proximity (buffer lots) were banned from planting. This resulted in drastically reduced inoculum potential. In addition, only PVY^N-free laboratory tested material was allowed for planting. In order to reduce the inoculum further, steps were taken to destroy the volunteer potatoes from PVYN positive fields, and the growing of potatoes in private gardens was prohibited in PEI.

The second basic premise for disease eradication is to generate data, which would allow proper timing and application of control measures, particularly for diseases which have sporadic occurrences. In the case of PVY^N, infected tubers provide the initial foci. However, in the case of PEI, such foci are in trace amounts; therefore, massive leaf testing steps were recommended with 1,000 to 5,000 leaves per field to detect the seedlots containing PVY^N. As shown (Table 3), 4.7 million leaves encompassing about 4,000 fields were tested in the 1991 growing season. Although the survey was large, only 0.58% of the samples were found to be positive for PVY^N. This shows a trend for successful reduction of the inoculum source compared to 1990 potato crop (Table 1).

Comparable situation in a PVYN outbreak in New Zealand. A situation similar to the one in PEI was encountered in 1985 in New Zealand (Fletcher, 1989). PVYN was detected in 9% of potato samples in 1985. Three years later no PVYN was detected in any sample. The test procedure in New Zealand was similar to that used in Canada, where samples were bulked in groups of 100 leaves and assayed for PVYN using ELISA. *M. persicae* is a common aphid on potato plants in New Zealand, and in laboratory tests it was shown to be an efficient vector of PVYN (Fletcher, 1989). The occurrence of PVY^N does not mean it is going to be the dominant PVY strain in an area. For example, PVY^N is an important problem in the Netherlands and Northern Germany, and less a problem in Czechoslovakia, Great Britain and France (Weidemann, 1988), although PVY^N occurs in most European countries. Climatic conditions may be responsible for PVY^N establishment. Thus, if PVYN in North America has not yet been established, it may be because of some unknown factors or niches, and thus justifying eradication efforts.

Future prospects. From the comparative situation between Europe and Canada, it is clear that eradication of PVY^N in Canada is scientifically feasible. The experience in New Zealand supports this optimism. It is possible that seed producing areas of Canada may be free of PVY^N within two years, but table-stock or processing potato fields in Ontario may require additional years. In order to declare an area free from a pathogen, regular monitoring of a large number of samples would be needed for four to five years. In the case of the potato in Canada, this period of five years represents the complete cycle of elite material subjected to field growing conditions. Failure to detect PVY^N in the samples during this period should be considered as complete eradication. Since PVY^N testing should be a part of pre-elite seed production, future crops should remain free of this strain of PVY.

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Province		# Fields		<u># PVYN Po</u> sitives	
	# Leaves Tested	Potato	Tobacco	Potato	Tobacco
Alberta	200	2	0	0	0
Manitoba	300	3	0	0	0
New Brunswick	450,000	297	0	1	0
Nova Scotia	34,000	21	0	1	0
Ontario	277,000	140	2	10	2
Prince Edward Island	3,900,000	3,495	4	10	0
Quebec	40,000	8	0	1	0
Total	4,701,500	3,966	6	23	2

Table 3. Survey of PVYN in potato in various provinces of Canada during summer 1991.

1 Data from Seed Certification Laboratories, Food Production and Inspection Branch, Agriculture Canada.

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