A search for leaf roll virus that does not cause diagnostic symptoms in potato foliage

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Potatoes from various parts of New Brunswick, Canada, were tested for leaf roll virus by means of aphid vectors and the indicator plant ***P. floridana.*** Only moderate strains of the virus were recovered, always from plants that developed clear symptoms of leaf roll. There was no conclusive evidence that any of the potatoes were infected by mild strains that do not cause leaf rolling in potatoes. When Kennebec plants from various sources were inoculated with a moderate strain of the virus, all of them became infected showing that they were not already infected by mild strains that protect against stronger ones. Mild strains of leaf roll were not recovered from supposedly infected tubers obtained from western Canada. Nor was the virus recovered from these same clones during concurrent tests made in western Canada by one of those who made the original diagnosis of infection some years ago. Possible reasons from these unexpected results are mentioned. In any event there is no reason to believe that mild strains of leaf roll are common in New Brunswick.

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La reaction au virus de l'enroulement des pommes de terre provenant de diverses regions du Nouveau-Brunswick a ete mesuree en utilisant les pucerons comme agents de transmission et ***P. floridana*** comme plante indicateur. On n'a pu trouver que des souches de virulence moyenne et toujours sur des plants qui presentaient des symptômes nets d'enroulement. Il ne semble pas que les plants aient été infectés par des souches benignes qui ne provoquent pas l'enroulement chez la pomme de terre. Quand on a inoculé des plants de la variete Kennebec provenant de diverses sources avec une souche modérée du virus, tous les plants ont par la suite ete virosé ce qui montre qu'ils n'étaient pas déjà infectes d'une souche benigne les protegeant des souches plus virulentes. On n'a pas isolé de souches bénignes dans des tubercules pretendument infectes provenant de l'ouest du Canada, pas plus qu'on en a retrouvé dans ces mêmes clones au cours d'essais simultanes effectues dans l'ouest du pays par l'un des chercheurs qui avait établi le premier diagnostic d'infection, il y a quelques annees. Plusieurs raisons plausibles sont proposées pour expliquer ces resultats imprevus. Quoi qu'il en soit, rien ne permet de croire que les souches faibles de l'enroulement soient courantes au Nouveau-Brunswick.

Introduction

Wright and MacCarthy (10) reported that they recovered leaf roll virus (LRV) from potatoes that did not develop diagnostic symptoms of leaf roll in the field or greenhouse. Their first recoveries were made from Kennebec potatoes grown near the west coast of Canada. Apparently these isolates were designated as LRV because: (a) they were obtained from potato, (b) transmissible via aphids, and (c) in the host plant ***Physalis*** floridana Rybd. they caused symptoms like those that Webb ***et al.*** (9) had described for mild strains of LRV. But these three characteristics in common are not sufficient to establish a strain relationship. Moreover, the Kennebec isolates differed from previously described strains of LRV (2, 9) in that they did not cause leaf roll symptoms in potato plants nor protect them from virulent LRV (11). So it may be premature to designate these isolates as LRV. Assuming that they are viruses, I shall refer to them simply as mild aphid-borne potato virus (MAPV). The term mild LRV will be used here for isolates having the characteristics of the mild strains described by Webb ***et al.*** (9).

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Later Wright et ***al.*** (11) recovered MAPV from 25% of plants of 16 potato varieties obtained from various sources in Canada and the United States, recoveries being made from all the varieties except 3 of which only a total of 10 plants were tested. From this it appeared that MAPV may be widespread in North America. If so, and it does not cause diagnostic foliar symptoms in other areas, presumably it will spread undetected and eventually become endemic. An endemic potato virus is potentially troublesome even though it does not cause obvious disease symptoms. Especially vulnerable would be an area like eastern Canada where most of the country's seed potatoes are grown and large quantities are exported to many countries.

Inevitably the work by Wright and colleagues caused concern in other potato growing areas. Perhaps nowhere was the impact greater than in the Eastern Canadian province of New Brunswick, because Kennebec is widely grown there and appears to be very tolerant to MAPV (12). To determine if MAPV was already endemic in New Brunswick, MacKinnon (5) tested various potatoes that were at hand but found none infected. That was in 1970 and shortly thereafter the incidence of leaf roll in New Brunswick increased to the point where there was widespread fear of an impending epidemic. In trying to account for this unexpected increase, some people

postulated on the basis of the work by Wright *et al.* (10, 11, 12) that the New Brunswick clones of Kennebec may be infected by LRV that does not cause symptoms in that variety but causes leaf roll when transmitted via aphids to other locally grown varieties. The previous tests by MacKinnon had not been extensive enough to preclude this possibility, and since he no longer worked in the province I was asked to check New Brunswick potatoes further for strains of LRV that do not cause diagnostic foliar symptoms.

Materials and methods

The potatoes for testing were grown in a screened greenhouse, singly in pots of fertile soil. Unless otherwise stated they were from tubers of the 1973 New Brunswick potato crop, the various sources being given later. Most of the testing was carried on during the winter, spring, and summer of 1974 and 1975. After each tuber was marked with an identification number, a small piece was removed from the seed end for planting and the rest stored so that additional plants could be grown from it for retesting if necessary. Except where otherwise stated each plant was tested for LRV and MAPV soon after emergence and before any foliar symptoms had developed. An attempt was made to transmit virus from each plant by means of aphids of *Myzus persicae* (Sulz.) to seedlings of *P. floridana* in the cotyledon stage.

The method of testing was essentially the same as that described originally by MacCarthy (3) and also used by Wright *et al.* (10, 12). Like others who have used this method, I modified it slightly from time to time to better suit local conditions; but each change was first checked to be sure that it did not interfere with the test by visibly affecting the test seedlings. Initially the test seedlings were grown individually in small pots, but mostly they were grown in 9 inch diameter bulb pots, about 12 evenly spaced seedlings per pot. The *P. floridana* seed used was derived from the same line as that used by Wright *et al.* (10, 12).

Experience showed that with aphids from my culture of *M. persicae,* which was reared on rape, the nymphs remained and fed on the test seedlings much better than the apterous adults used by others (3, 10, 12). So for the most part my tests were made with nymphs. To avoid transferring them twice by hand, each potato was infested with 2 or 3 vigorously reproducing apterous adults, which would give birth to 30 or more nymphs within about 3 days. About a week after the potato was infested, from 4 to 6 of the largest nymphs were transferred to each of 4 test seedlings. Following this the potato was freed of aphids by spraying it with a 1:500 aqueous solution of Black Leaf 40 (nicotine sulphate) and returned to the glasshouse for the periodic observation of disease symptoms.

Once infested each seedling was covered with a small transparent cylinder to confine the aphids, which were allowed 2 days to feed at about 20°C. After this the seedlings were freed of aphids and maintained about a month in a growth cabinet at 27°C, 84% R.H., and 1200 ft-c of fluorescent light for 16 hr each day (Cabinet Model 60 made by Controlled Environments Ltd., Winnipeg, Manitoba). When aphids were removed from each seedling by means of a small brush as recommended by MacCarthy (3), some of them were invariably missed and reproduced before being detected. This led to the growth cabinet becoming infested and there were a few accidental transmissions of LRV to healthy controls at the start of the work. To prevent this from happening all seedlings were subsequently freed of aphids by spraying them with a 1:500 solution of Black Leaf 40, which did nor visibly affect the test seedlings; and there was no further evidence of accidental transmissions of virus.

Two sets of controls were included in all tests: one was healthy seedlings to check for accidental transmissions; the other was to show whether the aphids being used and the method would result in a high level of virus transmission. About one seedling out of 5 was left as a healthy control. Initially every second seedling of this group was infested with about 5 nymphs from the aphid culture on rape, and the remaining healthy controls were left uninfested. After some weeks of testing when no differences were observed between the infested and uninfested controls, subsequently all of them were left uninfested. For the second set of controls, several seedlings were infested each week with nymphs from potatoes known to be infected by LRV. The number of nymphs per seedling, the time they spent on the source of virus and test seedlings, and the test method were the same as those described above.

Results

Growth and appearance of the P. floridana test seedlings

When young seedlings of *P. floridana* are infected by mild LRV (9) or by MAPV (10), the symptoms that have been described are very mild consisting mainly of a slight upward rolling of the leaves, which may also show a mild chlorosis and epinasty and there may also be slight twisting of the petioles. Apparently these symptoms are so mild that they are difficult to diagnose unless the test seedlings grow uniformly and well and otherwise appear healthy. During my tests more than 5000 test seedlings were used and for the most part their growth was excellent and uniform. Occasionally a seedling died for no apparent reason while it was infested by aphids or within a few days of being placed in the growth cabinet; and now and then a seedling developed a slight chlorosis and was slightly stunted. But unless otherwise stated both of these anomalies occurred as often in healthy controls as in the test seedlings and could not therefore safely be attributed to virus infection. Whenever more than one of a set of four test seedlings did not grow well that test was repeated in its entirety, either by using the same potato plant while it was still young or by growing another plant from the original tuber. Also for some unknown reason sometimes an entire pot of seedlings

including the controls became slightly chlorotic. Since these seedlings were unsuitable for showing the symptoms expected of virus infection, these tests also had to be repeated.

Although the incidence of the above growth anomalies was low, it increased the difficulty of diagnosing infections by both mild LRV and MAPV. Therefore, early in the work I decided that if there was any suggestion whatsoever that a test might be positive it was repeated over and over until there was no doubt about the result.

Potatoes from the New Brunswick elite potato seed farm at Bonaccord

Tubers from the provincial seed farm were tested first because at the time it seemed important to determine if mild LRV or MAPV was endemic in the main source of Elite seed for the province. Since the lowest grade of seed grown there is Elite II, it was tested initially on the assumption that the highest level of virus infection ought to occur in the lowest grade of seed. Single plants were grown from each of 850 tubers -- 400 of Kennebec and 450 of Netted Gem (Russet Burbank). Soon after emergence 135 of the Kennebec plants and 50 of the Netted Gem were selected at random for aphid transmission tests to P. *floridana.* The results were as follows: 2 potatoes proved to be infected by a moderate strain of LRV, both being of the variety Kennebec; and there was no conclusive evidence that any of the remaining 183 potatoes were infected by an aphid-borne virus that causes symptoms in P. *floridana.* Several of the initial tests were repeated for various reasons given in the preceding section. In one of the initial tests all 4 seedlings became slightly chlorotic with mild rolling of the lower leaves, rather like the symptoms expected of either mild LRV or MAPV. Furthermore, none of the nearby controls developed these symptoms. Thus it appeared that the test was positive. As soon as this was observed, attempts were made to transmit virus via aphids from each of the 4 test seedlings showing symptoms to healthy test seedlings, the methods and numbers of nymphs used being the same as that for the tests from potato. Also, the original potato plant was retested twice before it was 40 cm high, and a second plant grown from the original tuber for retesting at emergence. Despite repeated attempts to transmit virus via aphids from the original 4 test seedlings and from the original potato and another from the same tuber, none of the P. *floridana* seedlings used developed any virus-like symptoms. Nor did the potato plants themselves develop any disease symptoms. Thus it could not be confirmed that this potato, which was Kennebec, was infected by a mild aphid-borne virus.

All 400 plants of Kennebec and 450 of Netted Gem were observed periodically for symptoms of leaf roll until they were mature and dying. The readings were as follows.

Leaf Roll Symptoms	*Kennebec*	*Netted Gem*
Typical	5	3
Atypical	10	7
None	385	440

Of the potatoes that developed typical leaf roll, 2 of the Kennebec had been tested by means of aphids and P. *floridana* at emergence and both had given an unmistakable positive test. The remaining 6 potatoes that developed typical leaf roll were tested when about 20 cm high and each also gave a positive test, the symptoms on P. *floridana* corresponding to those that Webb *et al.* (9) described for moderate strains of LRV.

The 17 potatoes that were diagnosed as showing atypical leaf roll developed a variety of symptoms. These ranged from very weak plants with all leaves rolled upwards to unstunted plants with slight upward rolling of the lower leaves. As soon as any symptoms suggestive of leaf roll were observed on these potatoes, each was tested for virus by means of aphids and P. *floridana.* Also the 10 potatoes that developed the least severe symptoms and therefore the ones judged to be the most likely to be infected by mild LRV or MAPV, were given additional tests. For these a second plant was grown from the original tuber, an aphid transmission test to P. *floridana* carried out at emergence, and this test repeated from one to 4 times during the first month of growth. None of the 10 additional potatoes grown from the original tubers developed any leaf rolling as the first plants had done. And none of the aphid transmission tests from the original potatoes showing symptoms or the second plants without symptoms gave any evidence that the tubers were infected by LRV or MAPV.

Throughout the tests of Elite II seed from the provincial farm, the method used gave excellent transmission of LRV from the potatoes found to be infected and from known sources of LRV used as controls. In all 68 P. *floridana* seedlings were infested with nymphs from potatoes known or later found to be infected by LRV, and all but 3 of these seedlings developed unmistakable symptoms of leaf roll.

When no evidence was obtained that tubers from the 1973 crop of the provincial seed farm were infected by mild LRV or MAPV, somewhat different samples of tubers were tested the following two years. Some potatoes are rejected every year at the farm because of mild to moderate rolling of the leaves, this being recorded as 'suspicious leaf roll'. If mild LRV or MAPV occurred on the farm, it seemed more likely to be found in such plants once no evidence of either virus had been found in random tuber samples. So tubers were obtained for testing from plants diagnosed as 'suspicious leaf roll' either during the growing season in New Brunswick or during the checking of these stocks each winter in Florida. Of the tubers tested, 15 were of Kennebec, 5 were of Netted Gem, and there was one each of Red Pontiac, Fundy, and Belleisle. When plants were grown from these tubers in the greenhouse at Fredericton, all of them appeared healthy and showed no symptoms of leaf roll whatsoever. And when the plants were tested by means of aphids and P. *floridana,* none of the test seedlings developed any symptoms suggestive of leaf roll. Thus there was no reason to

believe that any of these rejected potatoes were infected by mild LRV or MAPV.

Kennebec seed potatoes from commercial growers

Samples of Kennebec tubers were obtained from each of 18 seed growers scattered throughout the main potato growing area. The numbers of samples that were of Class Elite III, Foundation, and Certified were 4, 9 and 5 respectively. From 20 to 40 tubers were selected at random from each sample for testing, the total being 460. When single plants were grown from each tuber and tested at emergence by using P. ***floridana,*** 3 of the potatoes were found to be infected by a moderate strain of LRV judging from the symptoms in ***P. floridana*** (9). No conclusive evidence was obtained that any of the remaining 457 potatoes were infected by mild LRV or MAPV. As the potato plants grew and were observed for foliar symptoms, 3 developed typical leaf roll symptoms, these being the same plants that had been found infected by the P. ***floridana*** test. Of the remaining potato plants, 429 showed no symptoms of leaf roll whatsoever, and 28 were diagnosed as atypical leaf roll. These latter plants developed symptoms ranging from slight rolling of lower leaves to severely stunted plants whose leaves developed various degrees of rolling. Although these 28 plants had been tested at emergence and none found to be infected, each one was retested when about 20 cm high using ***P. floridana*** and again no evidence was obtained that any was infected by an aphid-borne virus that causes symptoms in P. ***floridana.*** Furthermore a single tuber was saved from each of the 28 atypical leaf roll plants and a plant grown from each tuber for retesting the following year. Not only was no evidence obtained that any of these second generation plants was infected by mild LRV or MAPV, but all of the tubers except 2 produced healthy appearing plants. The 2 exceptions developed atypical leaf roll, but further tests of these for aphid-borne virus gave only negative results.

Although there was no evidence that any of the potatoes tested thus far were infected by either mild LRV or MAPV, it is possible that they were infected by strains that are so mild they do not cause symptoms in either potato or the indicator plant P. ***floridana.*** One means of testing for such strains would be to determine if the potatoes are protected from infection by known strains of LRV. Protection would be evidence of mild LRV; but lack of it would not prove the absence of symptomless strains of LRV or of MAPV (10). A test for protection was made by growing a second plant from each of the first 5 tubers selected from each of the 16 samples of Kennebec seed from commercial growers. Soon after emergence each plant was infested for 2 days with from 5 to 10 nymphs that had been reared on potatoes infected by moderate LRV. Nearly all of the 80 plants infested this way developed clear symptoms of leaf roll as they matured. Furthermore, when 2 tubers were saved from each of the 80 plants and replanted the following year, all 160 of the tubers gave rise to plants that developed typical leaf roll. Thus there was no evidence that any of the potatoes were protected from infection by LRV.

Progeny of potatoes from which MAPV had been recovered

As the testing progressed and MAPV was not recovered, one could not ignore the possibility that the method being used might not be suitable under my conditions. With the intention of checking this point, I requested MAPV infected tubers from Dr. H.R. MacCarthy of the Vancouver Research Station where the original work on this virus was done. He kindly supplied one tuber from each of 6 clones of Netted Gem that had been found to be infected some years previously. Since the original diagnosis, these clones had been maintained in the field and were tested for MAPV from time to time. The virus had been recovered during each of these periodic tests except the final one made a few months prior to my request. In view of this unexpected result, Dr. MacCarthy decided to make further tests using tubers of the same clones that were forwarded to me. Later he informed me that despite repeated tests he had failed to recover MAPV from any of the clones sent to me and from other supposedly infected clones as well. In the meantime I too had tried repeatedly and failed to recover MAPV from any of the Vancouver clones. Initially 2 plants were grown from each of the 6 tubers, one being kept for the observation of disease symptoms and the other tested for MAPV by means of aphids and P. ***floridana*** seedlings. One plant from each clone was tested at emergence, again when about 20 cm high, and again at flowering. Furthermore tubers were saved from each of the plants observed for symptoms and plants grown from these for testing at various stages of growth the following year. More than 100 *P. floridana* seedlings were infested with aphids from plants derived from the Vancouver clones of Netted Gem without obtaining any conclusive evidence of MAPV or any other aphid-borne virus that causes symptoms in P. ***floridana.*** The 6 potato plants observed for disease symptoms did not appear as vigorous as did those of New Brunswick clones of Netted Gem and at first this was looked upon as possibly being mild symptoms caused by MAPV or another virus. But other than this no confirmation of a virus in the Vancouver clones was obtained.

Miscellaneous tests

The breeding of new varieties of potato in Canada is mainly carried out at the Fredericton Research Station with the seedlings being grown in isolation at a substation. At the time of my tests for MAPV and mild LRV, there was a minor outbreak of leaf roll in the potato seedlings being grown at the substation, and there was concern that this might stem from mild strains of LRV like those reported by Wright et ***al.*** (10, 12). In view of this I was asked to test samples of the seedlings from LRV that does not cause diagnostic symptoms in potato foliage. Accordingly 10 tubers were tested from seedling lines that had been maintained at the substation for some years on the assumption that these lines were the

most likely to be infected. A single test of plants derived from each tuber gave no indication whatsoever that they were infected by MAPV or mild LRV. At about the same time as these 10 tubers were tested, another 30 tubers were sent for similar testing to **Dr.** N.S. Wright of the Vancouver Research Station; 10 of these tubers were from the older seedlings lines like those tested by me and 20 were of the cultivar Kennebec that had been propogated for some years at the potato seedling substation. Subsequently **Dr.** Wright reported that his tests gave no evidence that any of the 30 tubers were infected by mild LRV.

Discussion

Had MAPV been recovered during this work as there was every reason to expect (10, 12), it could have been characterized further until it was shown whether it is related to LRV or not. The failure to recover it from supposedly infected western clones in itself shows a need for further study, especially since similar results were obtained during concurrent tests in western Canada where the original diagnosis of infection was made. Why the virus could not be detected in the western clones cannot be explained, but there are several possibilities based on what has been reported for other plant viruses. For example, maintaining the virus for some years in potatoes reproduced vegetatively may have caused it to become further attenuated until it no longer causes visible symptoms in the indicator plant *P. floridana;* or the virus may have ceased to be transmissible via its vector as has been reported for some other aphid-borne viruses that have been maintained a long time without being transmitted by their vectors (e.g. 1, 7, 8).

Had MAPV been recovered from the western clones, my failure to recover it from New Brunswick potatoes would have been convincing evidence that it is not endemic in New Brunswick. But without any positive tests to confirm the test method that was used, one could postulate that MAPV had been endemic in New Brunswick for some time and that it cannot now be detected by the *P. floridana* test just as was found with the supposedly infected western clones. If such mild strains do occur, some way will have to be found to detect them before one can determine if they are important enough to justify control measures; and presumably they would be widespread in potatoes of most countries just as was found with other latent potato viruses such as X and S.

A more likely interpretation of my results is that neither MAPV nor mild LRV are common in New Brunswick. All of the isolates made during my tests caused clear symptoms of leaf roll in potatoes, and similar results were reported some years ago by Webb *et al.* (9), who tested 36 isolates of LRV from eastern Canada. If a mild strain of LRV that does not cause leaf roll symptoms in potato did occur in New Brunswick, possibly it would be controlled under our conditions. Unless our varieties were completely tolerant to such strains, they would affect plants enough that they would appear abnormal, and such plants would be removed during roguing of seed potatoes where the practice is 'if in doubt pull it out'. In a major seed growing area like New Brunswick where aphid-borne viruses spread slowly, the roguing of infected plants from the highest grades of seed usually suffices to give practical control throughout the crop. Certainly plants showing atypical leaf roll symptoms would be removed during roguing, though on the basis of my tests these are rarely infected by LRV.

Finally, my results are similar to those reported by Manzer *et al.* (6), who carried out a similar research at about the same time in the adjoining state of Maine. They found that aphid transmission tests to *P. floridana* gave a few more positive readings than did field readings of leaf roll symptoms in potato foliage. They concluded that the higher readings with *P. floridana* probably resulted from mild LRV like that reported by Wright *et al.* (10, 12). But the symptoms that they obtained in *P. floridana* were often questionable, so they cautioned that their tests with *P. floridana* probably overestimated the incidence of LRV. Nor did they confirm that the *P. floridana* showing questionable symptoms were in fact infected by an aphid-borne virus by transmitting it via aphids to other *P. floridana* seedlings. In any event, the searches in both Maine and New Brunswick gave no reason to believe that mild strains of LRV are common in these important seed growing areas.

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