Occurrence of alfalfa mosaic virus in Prince Edward Island[1]

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Alfalfa mosaic virus (AMV), although variable in occurrence was found at relatively low levels in forage legumes in Prince Edward Island. On the basis of testing 90 samples per field by enzyme-linked immunosorbent assay, four fields of 2-year old alfalfa, ***Medicago*** sativa L., averaged 5% infection and three 3-year old fields averaged 7%. AMV was not, however, detected in plantings of red clover, ***Trifolium pratense*** L., even though 180 selected 3-year old clones were sampled. Testing of several fields of birdsfoot trefoil, Lotus ***corniculatus*** L., revealed, for the first time, the natural susceptibility of this species. Two of the most common wild legumes in hedgerows, ***Trefolium repens*** L. and ***Vicia craca*** L., were found to harbour significant levels of the virus. This reservoir does not appear to constitute a threat to the potato crop, ***Solanum tuberosum*** L., as calico disease of potato, incited by AMV, is only rarely encountered.

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Le virus de la mosaique de la luzerne (AMV), quoique distribué inégalement, a été detect6 à un niveau assez bas dans les légumineuses fourragères de l'Ile-du-Prince-Edouard. En se basant sur 90 échantillons par champ, testes par la méthode ELISA, on peut dire que quatre champs de luzerne ***(Medicago sativa*** L.) de deux ans étaient en moyenne infecté à 5% et que trois champs de trois ans l'étaient à 7%. Toutefois, AMV n'a pas été détecté dans les plantations de trèfle rouge ***(Trifolium pratense*** L.) même si 180 clones sélectionnés de trois ans furent échantillonnés. L'échantillonnage de plusieurs champs de lotier corniculé (Lotus ***corniculatus*** L.) a permis de révéler pour la premiere fois la susceptibilité naturelle de cette espèce. Deux des légumineuses sauvages les plus communes dans les bordures de haies, ***Trifolium repens*** L. et ***Vicia craca*** L., recèlent des niveaux significatifs de virus. Ce reservoir viral ne semble pas constituer une menace pour la culture de la pomme de terre Btant donné le peu d'importance du calicot de la pomme de terre, cause par AMV.

Introduction

Alfalfa mosaic virus (AMV) is worldwide in distribution and has a wide natural host range (3,6). It is particularlyprevalent in certain legume species, amonst the most important and studied of which is alfalfa, ***Medicago sativa*** L. In this species, AMV is seed transmitted and this fact, combined with its efficient spread by a number of aphid species has resulted in very high levels of infection (approaching 100%) occurring in old alfalfa stands (2). Although the symptoms of AMV in alfalfa are not severe, a number of studies have shown that a reduction in fresh weight from 5-30% can occur that is associated with a general impairment of host physiology, decreased capacity of nodulation, and an increased susceptibilityto winterkill (11).

Systematic efforts in Canada to determine the incidence of AMV in forage and wild host plants have been limited to Ontario and western Quebec (4,9). In Prince Edward Island (P.E.I.), AMV has not been recorded from alfalfa, but in a survey of clover viruses in eastern Canada in 1967, was reported from red and white clover in the province (10). This study was therefore undertaken to determine the extent of AMV infection in forage and wild legumes in P.E.I. and to assess the relative importanceof this virus in crop production.

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Materials and Methods

Plant samples. Unless otherwise stated, sampling was done from June to August, 1982, from various locations in P.E.I. Ninety leaf samples per field were collected at random from 13 fields of alfalfa, ***Medicago safiva*** L., one field of red clover, ***Trifolium pratense*** L., and three fields of birdsfoot trefoil, ***Lotus corniculatus*** L. Similar sampling was done of ***vicia craca*** L. and ***Melitotus alba*** L. growing on the borders of three and two fields, respectively. In addition, three year old clones of red clover and some plots of alfalfa, being grown at the Research Station (Agriculture Canada, Charlottetown), were tested. The spread of AMV in a research plot of alfalfa planted in 1982 was monitored by taking 180 samples at random three times during the growing season.

Extraction procedures. Leaf extracts for ELISA were prepared by wrapping leaf samples inside a single layer of distilled water-moistened cheese-cloth and expressing the sap with pliers. Two drops per sample were collected in 1 ml sample vials (Flow Lab) containing about 200 μl of sample buffer (see below). After addition of the leaf sap, vials were filled to 1 ml with buffer.

Virus isolates. Isolates of AMV from alfalfa were tested on ***Phaseolus vulgaris*** L. cv. Green Provided, ***Vigna unguiculata*** L. cv. Early Ramshorn and ***Solanum tuberosum*** L. cvs. Superior, Kennebec, and Russet Burbank (Netted Gem). One of the isolates was used as a standard in enzyme-linked immunosorbent assay (ELISA)(1) and was maintained in ***Nicotiana tobacum*** L. cv. Samsun NN.

Antiserum. Antiserum to AMV was kindly supplied by R. I. Hamilton, Agriculture Canada Research Station, Vancouver.

Table 1. Alfalfa mosaic virus (AMV) infections detected by enzyme-linked immunosorbent assay in samples of forage legumes from various locations in Prince Edward Island.

Species and cultivar	Seeding Year	Field Location	Infected plants* No.	%
Alfalfa				
Saranac	1981	Milton	1	1
	1981	Valleyfield	0	0
	1980	Valleyfield	3	3
	1979	Winsloe	16	17
	1977	Winsloe	77	86
Iroquois	1981	Mt. Herbert	6	7
	1981	Heatherdale	9	10
	1976	Heatherdale	12	13
Vernal	1979	Clinton	1	1
	1977	Clinton	11	12
Algonquin	1980	Winsloe	8	9
Roamer	1980	Winsloe	7	8
Narragansett	1978	Kensington	76	84
Birdsfoot trefoil				
Leo	1981	Charlettetown	0	0
	1979	Charlettetown	1	1
	1979	O'Leary	16	18
Red Clover				
Prosper	1981	Charlettetown	0	0

*Based on 90 samples per test for AMV, from July to August, 1982.

ELISA procedure. Immunoglobulin (Ig) was purified according to the published procedure (1). Ig at 1 mg of protein per millilitre (A_{280} = 1.4) was conjugated with alkaline phosphatase (Grade 1, Boehringer Mannheim, Dorval, Quebec) at an enzyme/Ig ratio of 3:2 (w/w) by extensive dialysis into 50 mM potassium phosphate, 0.8% NaCl, 0.1% NaN_3, pH 7.5 (PBS). Glutaraldehyde was added to 0.05% final concentration and allowed to incubate overnight at 4°C. Glutaraldehyde was removed by dialysis into PBS and the bovine serum albumin was added to 5 mg/ml, for storage.

Methods for ELISA were essentially as described by Clark and Adams (1). Wells in polystyrene microtitration plates (76-301-05; Flow Labs Inc., Mississauga, Ont.) were coated by incubating 200 µl of unlabelled Ig diluted in 0.05 M sodium carbonate, pH 9.6 at 30°C for 5 hr and overnight at 4°C. Plates were covered and put in plastic containers (T195C; Tri-State Molded Plastics Inc., Worthington, Ohio) containing moist cheese-cloth. After incubation, they were rinsed four times with distilled water.

Antigen preparations in PBS containing 0.05% Tween-20 and 2% polyvinylpyrrolidone (PVP, M.W. 40,000; BDH, Toronto, Ont.) (sample buffer) were then incubated similarly overnight at 4°C in the coated rinsed wells to react with the bound Ig. After further rinsing, diluted enzyme-conjugated Ig was added to react with bound antigen during a further incubation of 5 hr at 30°C.

Finally, unreacted conjugate was rinsed away, and specific antibody-antigen reactions were assessed by adding 200 µl p-nitrophenyl phosphate at 0.5 mg/ml in 10% diethanolamine buffer, pH 9.8. Assay was by visual observation of the yellow nitrophenolate based on a "plus" and "minus" classification. Those reaction giving obvious yellow colour were regarded as positive. Based on results of preliminary tests, coating and conjugate Igs were used at 2 µg/ml.

Results and Discussion

AMV was found to be prevalent in alfalfa field in P.E.I. (Table 1) and the level of infection tended to increase with the age of the planting. This was particularly so within plantings of the same cultivar. Four fields in their second year averaged 5% infection and three in their third year averaged 7%; the highest levels detected were 84 and 86% in fields in their fifth and sixth year, respectively. The apparent variability in infection levels between plantings of the same age might be partly due to differences in rates of seed transmission in the seed lots (5.9). and in cultivar susceptibility to prevalent virus strains (2).

These infection levels, particularly for second and third year fields are considerably lower than have been reported in other locations (3). In Ontario and western Quebec, 21 fields in their second year of growth surveyed by Paliwal in 1980-81 (9) had an average infection level of 27%. In southwestern Ontario in 1970-73, Gates and Bronskill (4) detected 11% in first year fields and 44% in second and later yields.

The lower levels of AMV infection of alfalfa in P.E.I. may be related to aphid populations. Monitoring of aphid populations in P.E.I. potato fields, using yellow pan traps (unpublisheddata of J.G.M.), has indicated that aphid populations (including *Myzus persicae,* an important vector of AMV) occur at lower levels than in adjacent more continental areas (e.g. upper valley of the St. John river). This is probably due to the relatively cool, wet, and windy conditions that typify summer weather in P.E.I. Therefore, the relatively unfavorable conditions for aphid transmission of AMV in P.E.I. is a factor that could contribute to the low levels of this virus in alfalfa.

Table 2. Alfalfa mosaic virus (AMV) infections detected by enzyme-linked immunosorbent assay in research plantings of Iroquois alfalfa.

Seeding year	Test date	Infected plants No.	Infected plants %
1982	July 7"	3	2
	September 1"	17	9
	October 5"	27	15
1980	July 7[a]	44	40
1981	July 7[a]	75	83

* Based on 180 samples per test for AMV.
[a] Based on 90 samples per test for AMV.

Higher levels of infection, however, were detected in the Research Station farm (Table 2) where alfalfa (cv. Iroquois) and been planted successively in strip-plots in each of the previous four years (1978-82). Three and four year old plots, for example, had 40% and 83% infection, respectively. To examine the rate of AMV spread in these plots, the 1982 planting was monitored. On July 7, when the seedlings were about 8-15 cm tall, and just before the adjacent (older) plots were cut, 3% were infected. By September 1, just before cutting, 9% were infected, and by October 5, this had increased to 15%. This high rate of spread is presumably due to the abundance of inoculum in the adjacent (older) plantings and the migration of viruliferous aphids that would be stimulated by hay cutting (6). Although the plots were harvested only twice a year, the pathways and borders of these plots were frequently trimmed, and this would also tend to stimulate aphid movement.

AMV was detected in two older plantings of birdsfoot trefoil (Table 1). The susceptibility and natural occurrence of AMV in this species have not been reported previously (3,6). Testing of a two year old field of red clover, however, failed to reveal the presence of this virus (Table 1). To maximize the chances of detecting AMV in red clover, a collection of three year old clones maintained at the Station were tested. Sixty clones of each of the cultilvars Prosper, Tristan, and Florex were included, but the results were all negative. This is in contrast to the report by Pratt (10) who estimated a frequency of AMV in red clover at one location in P.E.I. at 1-5% on the basis of plant symptoms. AMV has, though, been found to occur at low frequency in red clover in Pennsylvania (7) and southwestern Ontario (4). Evidently, this virus cannot be considered a significant factor in the production of red clover in P.E.I.

The predominant wild legumes in hedgerows and field borders are T. *repens* and *V. craca.* The finding of AMV in *T. repens* (Table 3) confirms the previous report by Pratt (10). *V. craca* has been recognized only recently as a natural host of AMV (9) and our findings (Table 3) indicate that it is also a significant reservoir in P.E.I.

Calico disease of potato, incited by AMV. is known to occur sporadically in P.E.I. (R. Longmoore, [Agriculture Canada, Food Production and Inspection, Charlottetown] - personal communication). Typically it is at very low incidence and is easily controlled by roguing. It was of interest, however, to determine whether isolates of AMV from alfalfa were infectious to potato, as Paliwal had reported (9) that none of the 22 isolates that he had tested from Ontario and western Quebec infected the cultivars, Russet Burbank and Kennebec.

Therefore, four isolates from alfalfa were selected, two that produced primary chlorotic lesions on P. *vulgaris* and *V. unguiculata* and moved systemically, and two that were restricted to primary nectrotic lesions in those hosts. Inoculation of these isolates to the cultivars, Russet Burbank, Kennebec, and Superior, and back-inoculation to P. *vulgaris* indicated that the three cultivars were susceptible to the four isolates. If this was a representative sample, it would appear that AMV isolates in P.E.I. are more likely to be infectious to potato than those in Ontario and western Quebec. One might also conclude that the low incidence of AMV in potato in P.E.I. is not due to a lack of susceptibility to predominant AMV isolates.

Table 3. Alfalfa mosaic virus (AMV) infections detected by enzyme-linked immunosorbent assay in samples of wild legumes from borders of fields at various locations in Prince Edward Island.

Species	Location	Adjacent crop species	Infected plants" No.	Infected plants" %
White clover (*Trifolium rupens*)	Charlottetown	Red Clover	1	1
	Charlottetown	Alfalfa	8	9
Tufted vetch (*Vicia* craca)	Charlottetown	Mixed grain	14	16
	York Point	Potato	8	9
	Winsloe	Mixed	6	7

* Based on 90 samples per test for AMV, from July to August, 1982.

With *T. repens* and *V. craca* being so common in hedgerows and field borders, it is surprising that this reservoir of AMV does not appear to significantly threaten the potato crop. The explanation for this anomaly might lie in the biology of the

aphid vectors. A study in California (8) would suggest that spread of AMV from alfalfa to adjacent crops of potato is mainly caused by forcing the viruliferous aphids to migrate at hay cutting. Presumably, so long as the viruliferous aphids in hedgerows are not unduly disturbed they will not be a serious hazard.

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