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The *Canadian Plant Disease Survey* is a periodical of information and record on the occurrence and severity of plant diseases in Canada and on the assessment of losses from disease. Other original information such as the development of methods of investigation and control, including the evaluation of new materials, will also be accepted. Review papers and compilations of practical value to plant pathologists will be included from time to time.

L'Inventaire des maladies des plantes au Canada est un périodique d'information sur la fréquence des maladies des plantes au Canada, leur gravité, et les pertes qu'elles occasionnent. La rédaction accepte d'autres communications originales notamment sur la mise au point de nouvelles méthodes d'enquête et de lutte ainsi que sur l'évaluation des nouveaux produits. De temps à autre, il inclut des revues et des synthèses de rapports d'intérêt immediat pour les phytopathologistes.

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Observations on Monilinia Twig and Blossom Blight of the Lowbush Blueberry in the Maritime Provinces[1]

C. L. Lockhart[2], R. W. Delbridge[3] and D. McIsaac[4]

Lowbush blueberry fields in Nova Scotia, Prince Edward Island and New Brunswick were surveyed to determine the incidence of Monilinia twig and blossom blight. The disease was more serious in fields having wet soil throughout early May and in localities with extended wet periods. It was considered a serious threat in 40% of the lowbush blueberry fields, and a potential threat to an additional 43%.

Can. Plant Dis. Surv. 63:2, 31-34, 1983.

Les bleuetières de Nouvelle-Ecosse, de l'Ile-du-Prince-Edouard et du Nouveau-Brunswick ont été inventoriées afin de déterminer l'incidence de pourriture sclérotique. La maladie était plus sévère dans les champs au sol mouillé durant le début du mois de mai et dans les localités subissant des périodes de pluies prolongées. Elle est considérée comme une menace sérieuse dans 40% des bleuetières et comme une menace potentielle pour un autre 43%.

Introduction

Twig and blossom blight caused by *Monilinia vaccinii-corymbosi* (Reade) Honey is an economic disease of the lowbush blueberry (*Vaccinium myrtilloides* Michx., *V. angustifolium* Ait. *f. angustifolium* Aalders and Hall and *V. angustifolium* f. *nigrum* (Wood) Boivin) and highbush blueberry (*V. corymbosum* L.) (2,5,8). The fungus and disease cycle have been described (1,2). The disease was first reported on lowbush blueberry in Charlotte County, New Brunswick in 1952 when 24 fields were surveyed, four had over 50% of the blossoms and twigs infected (2). In 1956 13 fields in New Brunswick and two in Cumberland County of Nova Scotia were severely infected (2).

In Nova Scotia the disease has become a major concern to lowbush blueberry growers, especially during springs with above normal rainfall. This paper reports on surveys of Monilinia twig and blossom blight in Nova Scotia for 1980-82, Prince Edward Island in 1980 and 1982 and New Brunswick in 1982, with observations on factors influencing disease development.

Materials and Methods

Lowbush blueberry fields were observed at 7-10 day intervals from early May until mid June of each year, primarily in Cumberland County, Nova Scotia in order to follow the development of blossom and twig blight. During the first 3 weeks of June 1980-82 a general survey of Nova Scotia fields was conducted in the counties of Cumberland, Colchester, Halifax and Hants with Antigonish and Pictou added to the survey in 1981-82. Yarmouth was only included in the 1981 survey. In 1980 and 1982 surveys were carried out in lowbush blueberry fields in Prince Edward Island, and in Elgin County, New Brunswick in 1982. Ten sites in a field were selected at random in a semi-circle and the percent infection determined by counting the number of infected twigs and blossoms per 100 shoots in each site. Field sizes varied from one to 40 ha.

During the first three weeks of May observations were made periodically on the apothecial development in lowbush blueberry fields in Cumberland County where the disease was known to be a recurring problem.

Precipitation records collected at Kentville, Nappan, Parrsboro, Truro and Yarmouth were obtained from the local weather stations or the Scientific Services Section of Atmospheric Environment Services, Regional Office, Bedford, Nova Scotia. The Parrsboro weather station is located in a lowbush blueberry growing area in Cumberland County with Kentville, Nappan and Truro being on the outskirts of the main lowbush blueberry growing area. Leaf wetness information was obtained from a CR21 micrologger and probe installed on May 6, 1982 in the Westchester Station field located in Cumberland county (P. Dzikowski, personal communication).

Results

Mature apothecia of *M. vaccinni-corymbosi* were first observed in lowbush blueberry fields on May 4 and 18 in Cumberland County in 1982, on May 13, 1980 but none were seen in 1981 (Table 1). Vegetative and flower buds began to break during the first week of May, while immature apothecia were observed as early as April 29 in 1981.

Infections of lowbush blueberries, caused by ascospores of *M. vaccinni-corymbosi* were observed on May 28, 1980, May 21,

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1981 and May 20, 1982 (Table 1). Twig blight infections were found to increase in intensity during late May and early June, usually peaking between the first and second week of June with the highest amount of blossom infections attained by the middle of June. The earlier infections were more severe on *V. angustifolium* f. *nigrum* than on *V. angustifolium* f. *angustifolium* or *V. myrtilloides*.

Twig and blossom blight infections are often found scattered uniformly throughout lowbush blueberry fields. It is not uncommon, however, to find areas or patches where the disease is more abundant in the same general location each crop year. In 1981 under ideal conditions for infections a complete loss of crop occurred in an 8 ha field in Antigonish county. Typical, severe, Monilinia blight infections of leaves and flowers are shown in Figure 1.

In 1980 twig and blossom blight infections were more abundant in lowbush blueberry fields having wet soil throughout the month of May where mummified fruits were exposed to bare soil. Mummyberries lying on fallen leaves or other debris did not develop apothecia but were occasionally observed with apothecial initials. In sandy fields or well drained soils infections were generally very light with the greatest intensity noted along the borders or headlands where blueberries were not burned for pruning purposes and often there was a shading effect from weeds or trees favoring the retention of moisture.

Table 2 shows the results of the survey for 1980-82. During this period 29 to 43% of the lowbush blueberry fields surveyed in Nova Scotia had 5% or more twig and blossom blight infections (Table 2). The disease was less severe in Prince Edward Island in 1980 than it was in 1982. It was present in the three New Brunswick fields examined in 1982.

Data on rainfall for the months of May and June for five locations are shown as weekly precipitation in Table 3 and days of wetness in Figure 2.

We were unable to detect any marked differences between the amount of twig and blossom blight infections in fields treated and not treated with herbicides and fertilizer.

Discussion

Apothecia of *M. vaccinii-corymbosi* develop in close relationship with the initiation of growth of the lowbush blueberry in the spring in Nova Scotia and ascospores are mature at the time buds swell and begin to open. Normally the ascospores are mature and buds break in the first week of May however apothecia have matured as early as April 21 (2). Pepin (5) in British Columbia and Ramsdell *et al.* (6) in Michigan reported that ascospores were mature when the buds begin to swell in the highbush blueberry.

Figure 1. Healthy lowbush blueberry shoot on (left) and *M. vaccinii-corymbosi* infected twig and blossoms (right).

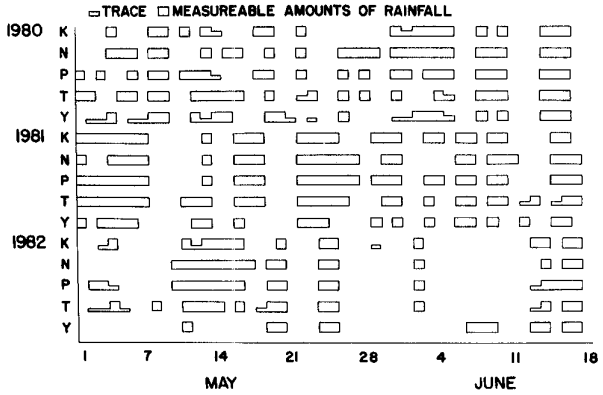

Figure 2. Days of wetness during May and June for Kentville (K), Nappan (N), Parrsboro (P), Truro (T) and Yarmouth (Y).

Table 1. Field observations on *M. vaccinii-corymbosi* apothecia and initial infections of the lowbush blueberry in Cumberland County, N.S.

Year	Location	Dates apothecia observed		Twig blight infection
		Immature	Mature	
1980	Fox Point	May 7	–	–
	Glasgow Mountain	May 12	–	–
	West Brook	May 13	–	–
	Pigeon Hill	–	May 13	–
	Westchester Station	–	May 13	May 28
1981	Westchester Station	April 29	–	May 21
1982	Westchester Station	May 4	May 4 and 18	May 20

Table 2 Incidence of *M. vaccinii-corymbosi* infected twigs and blossoms.

Year	Province	No. of fields	Final percentage infected shoots					Percent of fields with 5% or more infection
			0	0.1 - 4.9	5.0 - 9.9	10.0 - 24.9	>25.0	
1980	N.S.	36	5	16	7	4	4	42
1981	N.S.	31	5	17	1	3	5	29
1982	N.S.	61	15	20	7	12	7	43
1980	P.E.I.	7	–	3	4	–	–	57
1982	P.E.I.	5	–	3	–	2	0	40
1982	N.B.	3	–	2	1	–	–	33
	Total	143	25	61	20	21	16	–
Percent of Total		100	18	43	14	15	11	–

Table 3. Weekly precipitation records (mm).

Year	Location	May				June		
		1 - 7	8 - 14	15 - 21	22 - 28	29 - 4	5 - 11	12 - 18
1980	Kentville	1.4	18.2	5.8	1.6	16.2	24.2	37.2
	Nappan	10.9	25.1	19.5	5.9	10.7	14.5	35.6
	Parrsboro	6.4	32.2	11.4	7.8	6.2	15.2	41.8
	Truro	2.8	40.0	35.6	3.2	2.0	16.6	35.6
	Yarmouth	6.8	41.4	4.4	0.5	37.8	17.6	35.2
	Ave.	5.7	31.4	15.3	3.8	14.6	17.6	37.1
1981	Kentville	19.3	7.4	19.2	69.2	15.9	33.0	15.8
	Nappan	13.9	5.7	19.2	46.3	26.2	40.4	3.8
	Parrsboro	17.0	14.2	17.6	36.8	11.8	44.8	12.6
	Truro	24.4	12.2	22.8	42.4	5.2	32.2	11.0
	Yarmouth	19.0	12.4	6.0	40.5	28.4	56.0	9.0
	Ave.	18.7	10.4	17.0	48.0	17.5	41.3	10.4
1982	Kentville	0.4	25.0	6.8	12.4	3.4	0.0	26.4
	Nappan	0.0	38.0	13.5	9.9	6.0	0.0	26.3
	Parrsboro	1.4	19.0	12.8	12.0	7.8	0.0	23.2
	Truro	3.4	23.4	2.4	5.2	2.8	0.0	22.0
	Yarmouth	0.0	7.6	4.4	10.8	0.0	15.8	16.2
	Ave.	1.0	22.6	8.0	10.1	4.0	3.2	22.8

Twig blight infections first appeared on May 20 in 1982, May 21 in 1981 but were not recorded until May 28 in 1980 (Table 1). Based on this information, the wet periods shown in Fig. 2 and the fact that ascospores are mature the first week of May it would appear that between 10 and 17 days are required for twig blight symptoms to show after infection occurs. Repeated attempts to infect lowbush blueberry plants with ascospores or conidia in the greenhouse were unsuccessful but twig blight appeared on lowbush blueberries located in the field on the Research Station at Kentville 11 days following inoculation with ascospores obtained from apothecia collected in a commercial blueberry field (Lockhart, unpublished results). The chance of this being a natural infection was minimal because no other twigs in this location showed blight symptoms. In 1982 evidence indicates that twig blight infections occurred between 10 and 17 days

before symptoms appeared on twigs. Leaf wetness was recorded on the micrologger on May 6 (first day of operation) with an extended wet period from May 10 to 17 (P. Dzikowski, personal communication) in the Westchester station field where the first twig blight symptoms were observed on May 20. Rainfall likely occurred between May 3 and 6 prior to the micrologger installation. Low rainfall amounts were recorded on May 3 and 4 in the surrounding weather stations at Kentville, Parrsboro and Truro but considerable rainfall was recorded for the second week of May (Table 3). Apparently wet periods (Fig. 2) in the first two weeks of May are important to disease development as only 29% of the fields (i.e. 9 of 31 fields) had blight infections over 5% in 1981 when there were less wet days in the second week of May with a period of five days without rain in all locations except Truro. For 1980 and 1982 there was apparently ample precipation in this period (Table 3) for twig blight infections to take place with 42 and 43% of fields (i.e. 15 of 36 fields and 26 of 61 fields) having infection greater than 5% (Table 2).

Twig and blossom blight can be a serious threat to approximately 40% of the lowbush blueberry (6.4 million kg) crop each year. Another 43% of the fields are infected with low levels of twig and blossom blight. Complete crop losses have occurred in areas subject to wet soils and extensive precipitation or foggy weather during early May. Severe infections cause complete defoliation with loss of blossoms (Fig. 1). Growers with a history of Monilinia twig and blossom blight in their blueberry fields are advised to apply recommended fungicides to control the disease blight symptoms at bud break followed by applications at 7-10 day intervals up to midbloom.

The fungicide, triforine has been shown to be effective against the primary stages of the disease (5,7). In two years of fungicide evaluation in Nova Scotia this has been confirmed (3,4).

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Fungi associated with the roots of clover in Alberta. I. *Olpidium brassicae* and *Ligniera* sp.

J.P. Tewari and P. Bains[1]

Olpidium brassicae and *Ligniera* sp. are described on the roots of *Trifolium hybridum* from Alberta. Both are new disease records for Canada.

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Olpidium brassicae et *Ligniera* sp. sont décrits sur des racines de *Trifolium hybridum* en provenance d'Alberta. Tous deux sont mentionnés pour la première fois sur cet hôte au Canada.

Introduction

This communication is the first of a series on fungi associated with the roots of clover and reports the occurrence of two zoosporic fungi.

Materials and methods

Roots of *Trifolium hybridum* (alsike clover) were collected from the fields and roadside from Alsike, Alberta during August and early September, 1982. The roots were cleared and stained according to the method of Phillips and Hayman (9), mounted in lactophenol and examined by light microscopy.

Results and discussion

Olpidium brassicae

In the material examined both the root hairs and the superficial root cells (Fig. 1) had zoosporangia and resting sporangia. The zoosporangia were spherical to elongate and had single or multiple discharge tubes (Figs.2,3). In some cases only perforations were evident in place of the discharge tubes. The resting sporangia were stellate in shape and were often surrounded by a vesicle (Figs. 3,4). In general the fungus conformed to the descriptions given by Barr(2) and Karling(7).

In Canada, on different clovers, *O. brassicae* is known to occur on *T. pratense* and *T. procumbens* (2; Dr. D.J.S. Barr, personal communication) and its occurence on *T. hybridum* in Alberta is a new disease record at least for Canada. In future, it is proposed to study the distribution of this fungus in Alberta as it is known to be a vector of certain plant viruses (2,5).

Ligniera *sp.*

Many apparently normal looking roots were found to be infected with a plasmodiophorid and had cells with cystosori. The cystosori were globose to highly irregular in shape and had a few to numerous cysts which did not appear to be arranged in a single layer (Figs. 5-7). The cysts often had verrucose walls (Fig. 6). Several stages showing the development of cystosori from plasmodia were seen. Many root nodules also had cystosori (Fig. 8). Despite extensive search the zoosporangia were not found. According to Karling (6) and Barr (1,3) both *Ligniera* and the closely related genus *Polymyxa* have cystosori that are indistinguishable from each other and that these genera can only be distinguished on the basis of their zoosporangial states. However, the cystosori of *Polymyxa* are reported to consist usually of a single layer of cysts (1,3). In the fungus under study the cystosori are usually a few cysts thick. "*Ligniera* appears to be scarcely more than a convenient dumping ground for species which cause little or no hypertrophy and develop cystosori of indefinite size, shape and structure" (6).

Dr. J.S. Karling to whom photographs of the fungus under study were sent for comments, confirmed (personal communication) that it should be classified "as a species of *Ligniera* until the time the zoosporangia and zoospores are discovered. The occasional occurrence of verrucose resting spores suggests that it might be *L. verrucosa*, but this characteristic may occur rarely in other species."

Ligniera or *Polymyxa* are, so far, not reported on clovers from Canada (1,3,4; Dr. D.J.S. Barr, personal communication). It should be mentioned that in contrast to *Polymyxa*, *Ligniera* is not known to be a vector of plant viruses (1,8).

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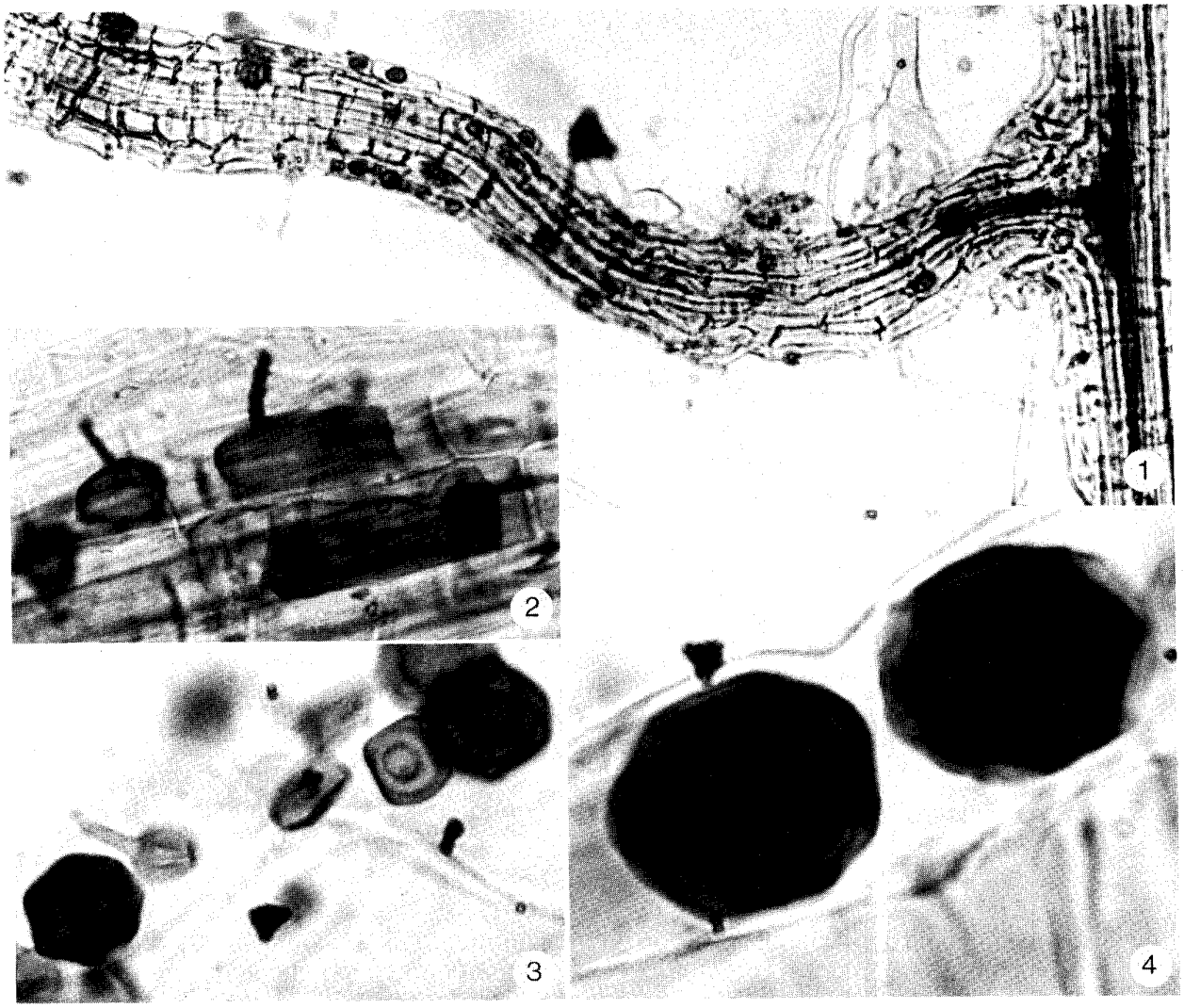
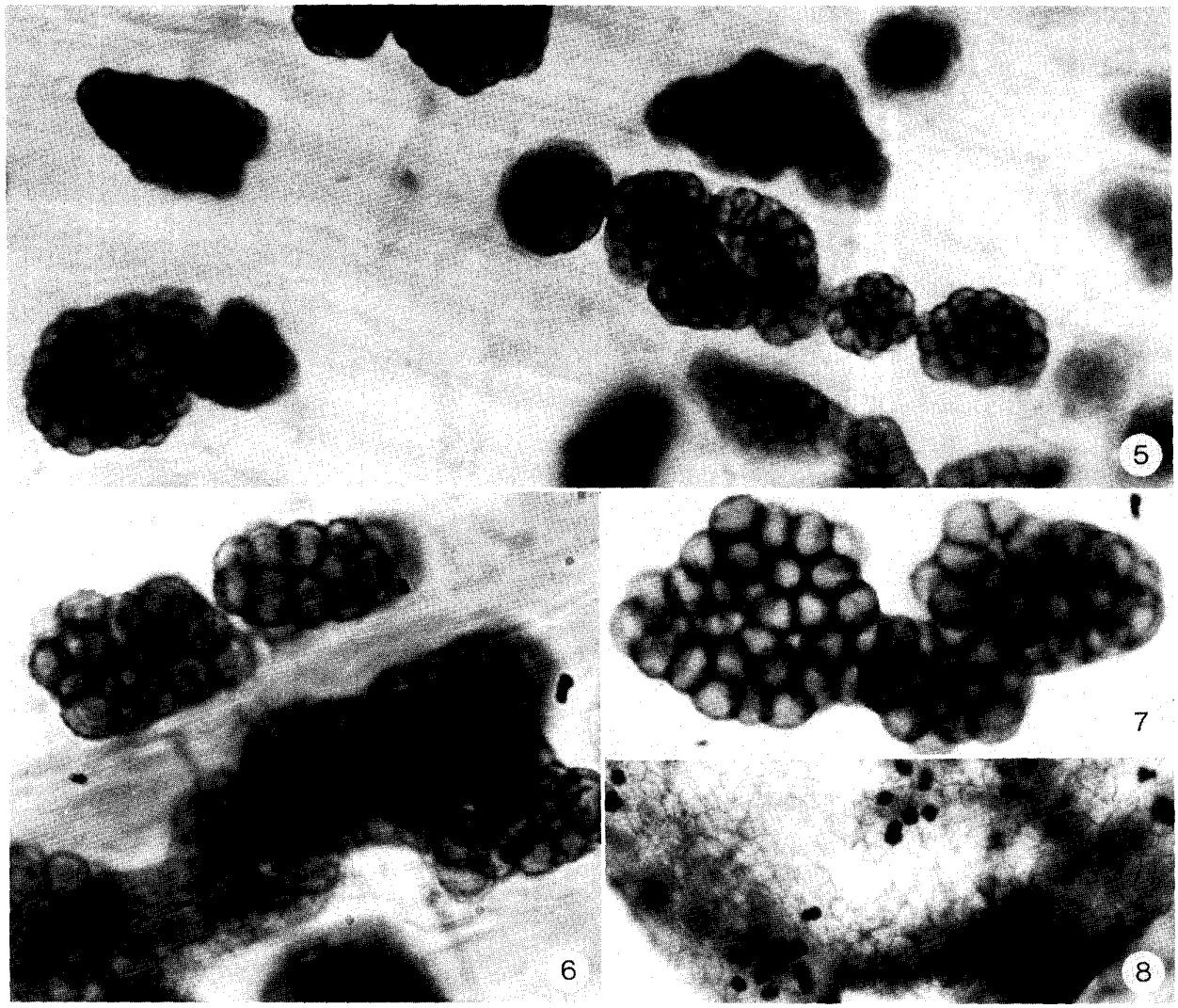


Figs. 1-4. *Olpidium brassicae*

1. An infected rootlet. × 250.
2. Zoosporangia. × 700.
3. Zoosporangia and resting sporangia. × 1700.
4. Resting sporangia. × 2000.



Figs. 5-8. *Ligniera* sp.

5-7. Cystosori in the root cells.5 × 850;6,7 × 1400. 8. Cystosori in the root nodule. × 150.

Acknowledgement

Thanks are greatfully acknowledged to Dr. J.S. Karling for his comments on the identity of the two fungi and to Dr. D.J.S. Barr for information on the records of *O. brassicae*, *Ligniera* sp. and *Polymyxa* sp. from Canada.

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Detection of potato virus Y in primarily infected mature plants by ELISA, indicator host, and visual indexing

R.P. Singh[1] and J. Santos-Rojas[2]

Potato virus Y was detected in greenhouse- and field-grown seven-week-old potato plants by local lesion host *Solanum demissum* P.I. 230579 and by ELISA tests. The virus was detected in 3-4 weeks by mechanical inoculation of local lesion hosts in greenhouse-grown plants and in 4-5 weeks in field-grown plants. However, PVY was detected in these same plants by ELISA one week later. Virus concentration varied in plants inoculated at different leaf positions, reached a peak, then slowly declined. Smaller tubers (less than 30 g, and 30-60 g) from primarily infected field-grown plants gave rise to 'symptomless' plants, which were not diagnosed by visual observation, while they were diagnosed to be PVY infected by ELISA tests. Also, visual observation detected fewer plants as PVY infected from stem end than from bud end pieces of the tubers.

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Le virus Y de la pomme de terre (PVY) a été détecté chez des plants de pomme de terre semés en serre et au champ et agés de sept semaines, à l'aide de la plante indicatrice *Solanum demissum* P.1. 230579 et du test ELISA. L'inoculation mécanique de la plante indicatrice a permis la détection du virus en 3-4 semaines chez les plants de serre et en 4-5 semaines chez les plants de champ toutefois, le test ELISA n'a permis la détection du virus qu'une semaine plus tard chez les même plants. La concentration de virus varie chez les plants inoculés sur des feuilles différentes, atteint un sommet, puis diminue lentement. Les plus petits tubercules (moins de 30 g et 30 à 60 g.), provenant de plants en champ à infection primaire, ont donné naissance à des plants "sans symptômes" diagnostiqués comme non-infectés par observation visuelle alors que les tests ELISA les ont identifiés comme étant porteurs de virus. Lorsque le talon des tubercules est utilisé comme semence la technique d'observation visuelle détecte moins de plants infectés avec PVY que lorsque la couronne des tubercules est utilisée.

Introduction

For large-scale testing of potatoes for virus infection, 'Florida-test' is often used in North America. In this test whole tubers are planted after treatment with dormancy-breaking chemicals. The Florida-test has been useful in detecting mosaics caused by potato virus Y (PVY) in tubers which are even partially infected, because viruses transport within tubers when tubers have been planted and the eyes have developed into stems (2). However, the enzyme-linked immunosorbent assay (ELISA) (3) is a laboratory test and has been considered for large-scale testing of potato viruses (4, 5, 7, 8, 10, 12), in which mostly dormant tubers will be tested, which may not detect partially infected tubers.

The studies dealing with potatoes have used a mixture of primarily and secondary infected (5) or only secondary infected (8) plants. Thus, there is no information available regarding the detection of PVY from mature plants primarily infected, and also whether such late infections result in tubers with partial infection. The aim of the present study was to establish conditions for detecting PVY infection, late in the growing season, compare with indicator host and ELISA, and to determine the extent of partial infection in tubers. For this purpose, the most widely grown North American cultivar, Russet Burbank, was used and inoculations were made to different leaf positions and in late July to mimic the early flights of green peach aphid (*Myzus persicae* (Sulzer)) in New Brunswick.

Materials and methods

Potato (*Solanum tuberosum* L.) cv. 'Russet Burbank', which is widely grown in the Maritimes was obtained as a virus-indexed tuber from the Fredericton Research Station program of Dr. A.R. McKenzie, and propagated under field and greenhouse conditions. Greenhouse and field experiments were similar in most details. In the greenhouse, plants were grown in 18 cm pots and were maintained at 18-22°C with 14-hour day length. In the field experiments potato plants were selected at random from several rows.

A common strain of potato virus Y (PVY) in Red Pontiac potatoes was used for inoculation. Five plants were mechanically inoculated (1/20 dilution of potato sap) on the bottom leaf, 5 on the middle leaf (half-way to the plant) and 5 on the top leaf. Similarly, 3 plants were inoculated with buffer alone (one at each leaf position) in the same field and used as healthy controls. In addition, 3 plants infected with PVY were maintained under field conditions to serve as infected controls. The inoculation was made on July 22, 7 weeks post planting and leaf samples were collected every week for 8 or 9 weeks, up to the topkilling dates in the field. Ten leaflets were taken at random from each plant and two sets of discs

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were punched through the leaflets using a 0.5 cm cork borer. One set of discs was ground in glycine-phosphate buffer (w/v 1:20) (0.05 M glycine + 0.03 M K_2HPO_4, pH 9.2) and used to inoculate *S. demissum* plants (13), while the other set of discs was tested by the ELISA procedure.

From field-grown plants, the tubers from each plant of each leaf position group, were put in separate bags and divided into different groups according to their weights. The weight groups were, weights of less than 30 g, 30-60 g, 60-90 g, 90-120 g, and 120 g and over. From each tuber both bud and stem end were planted separately in order to determine which of these parts were infected. This was done by visual observation of symptoms in the greenhouse, and by testing of the leaves by ELISA.

S. demissum test plants were used when they had 6-8 leaves and inoculations were made on each half-leaf with separate samples. All samples were inoculated on 5 right and on 5 left half-leaves. The local lesions were averaged from 10 leaf-halves for each sample for each date.

The ELISA procedure was similar to that used in an earlier study (10), except that the enzyme γ globulin conjugate was incubated at 5°C rather than 25°C. The antisera used in this study were a gift from the International Potato Center, Lima, Peru by Dr. C. Fribourg and locally produced against several PVY isolates. The A405 nm values were measured by Titertek Multiskan (Flow Laboratory, Mississauga, Ontario, Canada).

Results

The isolates of PVY, mainly differing in host reactions and virus yields (unpublished data) were initially tested by ELISA. A Saco PVY was characterized by necrotic local lesions in Saco potato and milder leaf-drop symptoms in *Physalis* species; while a common PVY was characterized by necrotic local lesions in Saco and severe leaf-drop in *Physalis* species. All these isolates caused similar necrotic local lesions in *S. demissum*. The PVY isolates were tested against an antiserum prepared in Peru, and their homologous antisera. There was no indication of strain specificity or interference in ELISA tests. A typical reaction of Red Pontiac isolate of PVY is shown in Fig. 1A. The virus concentration based on A405 nm values was higher in tobacco plants than in potato plants, although both were significantly different than the healthy or buffer controls (Fig. 1A). Similarly, with local lesion host *S. demissum* P.I. 230579, tobacco had higher virus concentration than potato (1B) and this bioassay was able to detect PVY up to a sap dilution of 320 fold (1B). In addition, 75 mosaic samples collected from different parts of New Brunswick were tested against antisera from Peru; these all reacted positively. Thus it appears that the main strain of PVY in New Brunswick is a common strain and one antiserum can detect most of the infections from the field.

Detection of PVY from greenhouse- and field-grown plants

The results (Table 1) from greenhouse-grown plants show that PVY was detected in 4 out of 15 plants within 3 weeks of inoculation by *S. demissum* but not by ELISA. However, by the fourth week virus was detected in all the plants by *S. demissum* test as compared to 13 of the 15 by ELISA. The plants with the bottom leaves inoculated had a low detection rate throughout the experiment, while those inoculated at top leaves had high virus detection rate.

The detection of PVY by ELISA procedure in the ninth week after inoculation was unreliable because only 5 of 15 plants were found to be positive, although by the *S. demissum* test

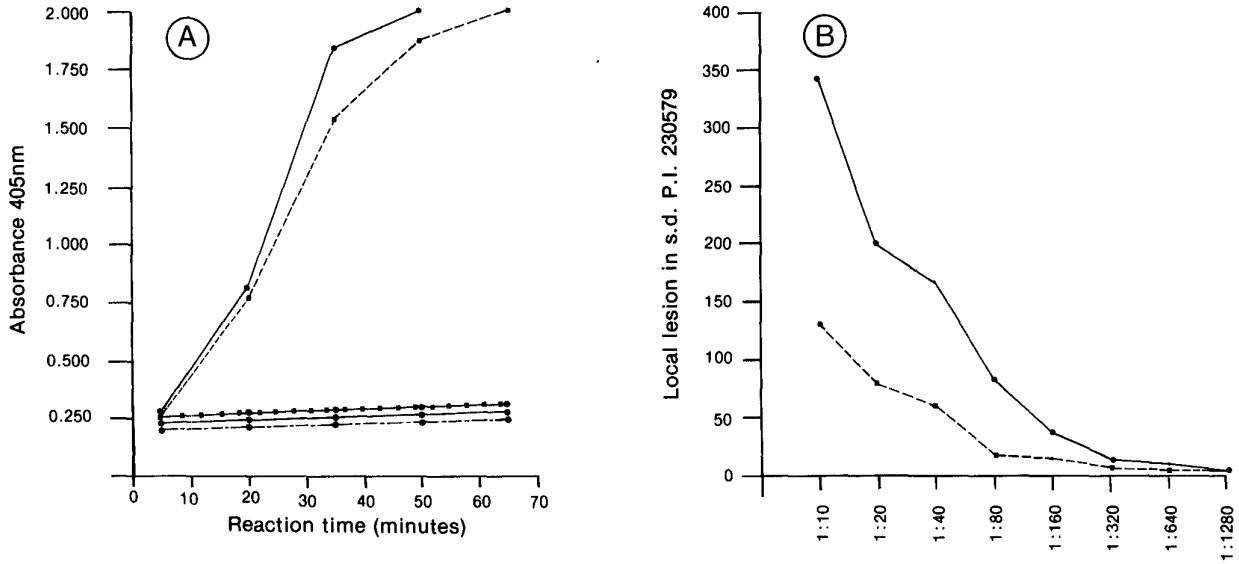

Figure 1A Absorbance values (A405 nm) for potato virus Y from tobacco (●–●–●), and from potato (-■- -■-) using γ globulins from Peru; Lower part, healthy tobacco (-■–■–), healthy potato (-●–●-) and buffer (-●– – –●-)

Figure 1B Bioassay of PVY using *Solanum demissum* P.I. 230579 from tobacco (-●–●-) and from potato (-■- -■-) at various sap dilutions.

Table 1. Detection of PVY in Russet Burbank potatoes grown in the greenhouse and inoculated seven weeks post planting.

Treatment	Plant No.	Time in weeks: Local lesion assay 3	4	5	6	7	8	9	ELISA* 3	4	5	6	7	8	9
PVY top leaf	1	+	+	+	+	+	+	+	–	+	+	+	+	+	–
	2	–	+	+	+	+	+	+	–	+	+	+	+	+	+
	3	–	+	+	+	+	+	+	–	+	+	+	+	+	–
	4	–	+	+	+	+	+	+	–	+	+	+	+	+	+
	5	–	+	+	+	+	+	+	–	+	+	+	+	+	+
PVY middle leaf	1	+	+	+	+	+	+	+	–	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	–	+	+	+	+	+	–
	3	–	+	+	+	+	+	+	–	+	+	+	+	+	+
	4	–	+	+	+	+	+	+	–	+	+	+	+	+	–
	5	–	+	+	+	+	+	+	–	+	+	+	+	–	–
PVY bottom leaf	1	–	+	+	+	+	+	–	–	+	–	+	+	–	–
	2	–	+	+	–	+	+	+	–	–	+	+	+	+	–
	3	–	+	+	+	+	+	+	–	+	+	+	+	+	–
	4	–	+	+	–	+	+	+	–	–	+	+	+	+	–
	5	+	+	+	+	+	+	+	–	+	+	+	+	–	–
Healthy control	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PVY tobacco		+	+	+	+	+	+	+	+	+	+	+	+	+	+
Healthy tobacco		–	–	–	–	–	–	–	–	–	–	–	–	–	–

* The A405 nm value twice or more than the healthy control was used to classify + PVY; each sample was replicated 4 times in the plate.

14 of the 15 were found to be positive (Table 1). In the field-grown plants (Table 2), the virus detection was delayed by 2 weeks. The earliest PVY detected by the *S. demissum* test was in the fifth week, while by the ELISA procedure was in the sixth week. Contrary to greenhouse-grown plants, in field-grown plants the virus infection from leaves inoculated at various positions did not differ significantly. There was more variation in virus detection from the field-grown plants than those from the greenhouse. All of the plants were found to be positive by ELISA in the eighth week whereas only 12 of 15 were detected by the *S. demissum* test (Table 2).

In the greenhouse- and field-grown plants the inoculated leaves from those plants which were inoculated at bottom and mid-points became chlorotic then necrotic and dropped off, while in those plants inoculated on the top leaves, no leaf-drop occurred. The symptoms, mottle and various degrees of mosaic in the new growth, were obvious in greenhouse-grown plants, but were seldom observed under field conditions.

The concentration of virus based on A405 nm value increased sharply in both greenhouse- and field-grown plants and then declined (Fig. 2A,B). The highest values were obtained in 4 weeks and 6-7 weeks in greenhouse- and field-grown plants, respectively. The local lesion assay followed closely the same pattern (Fig. 2C) in the greenhouse-grown plants, but the number of lesions fluctuated with inoculum obtained from the field-grown plants (2D).

Detection of PVY infection by visual-indexing and ELISA

The infections of tubers in the groups classified according to the weights of the tubers showed that smaller tubers (less than 30 g, and 30-60 g) either showed partial infection or

Table 2. Detection of PVY in Russet Burbank pototoes grown in the field and inoculated seven weeks post planting.

Treatment	Plant No.	Time in weeks: Local lesion assay 3	4	5	6	7	8	ELISA* 3	4	5	6	7	8
PVY top leaf	1	–	–	–	+	+	+	–	–	–	+	+	+
	2	–	–	–	+	+	+	–	–	–	–	+	+
	3	–	–	–	+	+	+	–	–	–	+	+	+
	4	–	–	+	+	+	+	–	–	–	–	+	+
	5	–	–	+	+	+	+	–	–	–	–	+	+
PVY middle leaf	1			+	+	+	+	–	–	+	+	+	+
	2	–	–	–	+	+	+	–	–	–	+	+	+
	3	–	–	+	+	+	+	–	–	–	+	+	+
	4	–	–	–	+	+	+	–	–	–	+	+	+
	5	–	–	+	+	+	–	–	+	–	+	–	+
PVY bottom leaf	1	–	–	+	+	–	–	–	–	–	–	–	+
	2	–	–	+	+	+	+	–	–	–	+	+	+
	3	–	–	+	+	+	+	–	–	–	+	+	+
	4	–	–	+	+	+	+	–	+	–	+	+	+
	5	–	–	+	+	+	+	–	–	–	+	–	+
PVY potato control	1	+	+	+	+	+	+	+	+	+	+	–	+
	2	+	+	+	+	+	+	+	+	+	+	–	+
	3	+	+	+	+	+	+	+	+	+	+	+	+
Healthy potato control	1	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–	–
PVY tobacco		+	+	+	+	+	+	+	+	+	+	+	+
Healthy tobacco		–	–	–	–	–	–	–	–	–	–	–	–

* The A405 nm value twice or more than the healthy control was used to classify + PVY; each sample was replicated 4 times in the plate.

failed to show clear mosaic symptoms in the greenhouse (Table 3). Of the 99 tubers tested, only 78 and 85 were diagnosed as mosaic showing by visual indexing from stem and bud ends, respectively. However, when tested by the ELISA method, all plants, irrespective of weights or tuber ends, were found positive for PVY. The potato plants which failed to show clear mosaic symptoms were rechecked by 'ELISA KIT' (Boehringer-Mannheim) and by local lesion assay. All samples caused local lesion in *S. demissum* but they were very low (average from 10 leaves, 5 to 25), but reacted positively in 'ELISA KIT' (A405 values 0.299 to 1.987). There was no clearcut correlation with lesion number and ELISA values.

Discussion

From this study it is clear that diagnosis of infected plant either in the field, from late infection or by planting smaller tubers for visual indexing in the greenhouse, can be misleading. In earlier studies in Europe (1) small tubers were found to have lower percentage of infection; however, in the present study, they were infected, but failed to show clear symptoms. This may be due to different cultivar used and also longer period of time between inoculation and harvest of the tubers. Since both stem and bud ends of each tuber, irrespective of weight or position of leaf inoculated on the plant, yielded tubers which were positive for PVY by ELISA, this procedure can be relied upon to detect most of the infection in routine laboratory testing.

Since there is a marked preference in the field for top and bottom leaves of potato by *M. persicae* (6) and our mechanical inoculation tests from the field experiments show almost similar infection rate on bottom and top inoculated

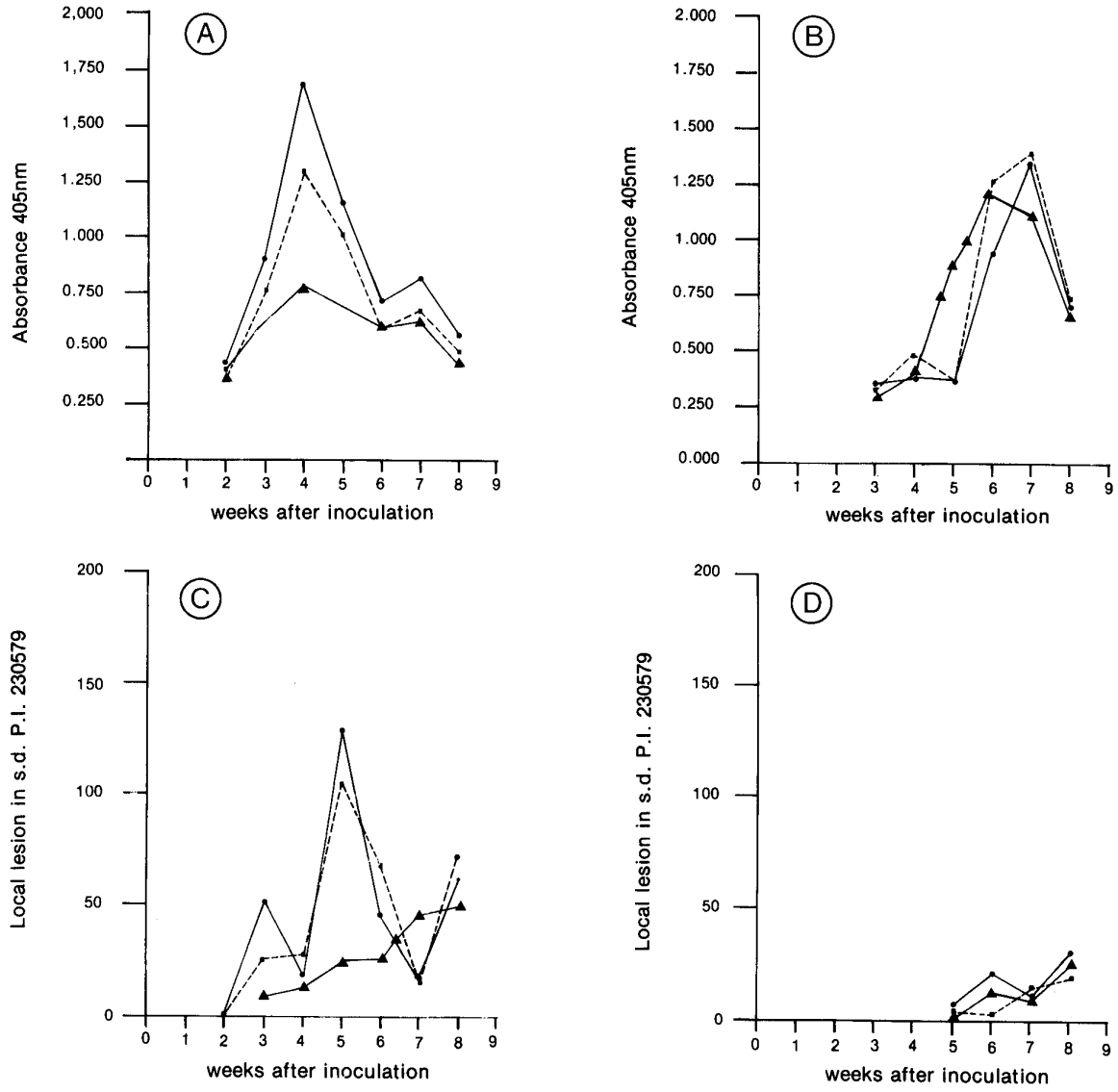

Figure 2A Absorbance values (A405) from greenhouse-grown PVY infected plants, inoculated at various leaf positions, top leaf (-●-●-), middle leaf (- ■ - - ■ -), and bottom leaf (-▲—▲-).

Figure 2B Similar to 2A, except field-grown plants.

Figure 2C Bioassay of PVY using *Solanum demissum* P.I. 230579 from greenhouse-grown PVY infected plants, inoculated at various leaf positions, to leaf (-●-●-), middle leaf (- ■ - - ■ -), and bottom leaf (-▲—▲-).

Figure 2D Similar to 2C, except field-grown plants.

plants, the finding that such plants infected at mature stage can be detected by ELISA is of significance.

The observation that PVY was detected sooner by bioassay using *S. demissum* P.I. 230579 than ELISA indicates that infectivity assay in this host is more sensitive than ELISA. This is not surprising because the ELISA test has been shown to be less sensitive than bioassay with certain other host-virus combinations (9,11). However, ELISA was reported to be superior than A6 test for PVY in another study (12).

The greater ease of PVY detection and higher concentration of the virus in greenhouse-grown plants may be the reflection of the optimum environmental conditions, favoring the virus multiplication rather than any intrinsic properties of plants or the methods used. It is similar to those of German potato variety Leo, studied in the greenhouse (8), where a peak virus concentration was reached, then it declined slowly.

Table 3. Detection of PVY infection from stem and bud end of Russet Burbank pototo tubers after primary infection under field conditions.

Treatment	Weight grams	Number of tubers tested	Visual-Symptoms positive		ELISA positives	
			Stem end	Bud end	Stem end	Bud end
PVY top leaf	less than 30	3	1	2	3	3
	30-60	5	3	3	5	5
	60-90	5	4	4	5	5
	90-120	1	1	1	1	1
	over 120	18	16	16	18	18
PVY middle leaf	less than 30	2	0	2	2	2
	30-60	2	2	2	2	2
	60-90	8	7	6	8	8
	90-120	3	3	3	3	3
	over 120	18	18	18	18	18
PVY bottom leaf	less than 30	2	0	2	2	2
	30-60	3	0	0	3	3
	60-90	2	2	2	2	2
	90-120	8	5	6	8	8
	over 120	19	16	18	19	19
Total		99	78	85	99	99

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Washing of potatoes to remove nematodes and to observe effect on storage rot diseases

J. Kimpinski and H. W. Platt[1]

Washing of potatoes in the laboratory indicated that only a few second-stage juveniles of the clover-cyst nematode, *Heterodera trifolii*, had been present in soil on the surface of potato tubers. No mature dead females, or cysts were detected. About 90% of the soil adhering to tubers at harvest was removed by a commercial washing procedure. Clover-cyst nematodes were not detected on tubers which had passed through the commercial wash. Washing did not appear to encourage storage rot diseases of potatoes under recommended storage conditions.

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Le lavage des tubercules de pommes de terre en laboratoire n'a permis de découvrir dans le sol à la surface des tubercules que quelques nématodes juvéniles du deuxième stades de développement, appartenant à l'espèce *Heterodera trifolii*. Aucune femelles adultes mortes et aucun cystes ne furent détectés. Le procédé de lavage commercial permet d'enlever environ 90% du sol adhérant aux tubercules après la récolte. On n'a pas retrouvé *Heterodera trifolii* sur les tubercules ayant subi un tel lavage. De plus, le lavage des tubercules de pommes de terre ne semble pas encourager le développement des pourritures d'entrepôt aux conditions d'entreposage recommandées.

Introduction

The clover-cyst nematode, *Heterodera trifolii* Goffart, 1932, is a common parasite of forage legumes in eastern Canada (3,6,7) but it usually does not cause detectable damage (5). Although this nematode species does not attack potatoes it may survive as a cyst in soil attached to the tubers. Cysts are mature, dead females which have developed into spherical sac-like structures with tough leather-like walls, and each may contain several hundred viable eggs that are protected from adverse conditions for extended periods.

Several countries which import seed potatoes from the Maritime provinces have expressed concern about the possible entry of clover-cyst nematodes along with the tubers. One way of alleviating the problem is to wash the tubers prior to storage or shipment. Therefore a test was conducted to assess the effectiveness of commercial washing for soil and nematode removal. In addition to the nematode observations, samples of washed and unwashed potatoes were monitored for the development of storage rot diseases.

Materials and methods

Soil and root samples were collected during August 1981 from fields of Red Pontiac potatoes near Bayview and Linkletter, Prince Edward Island. Soil samples were mixed thoroughly and placed in modified Baermann pans (4) for 7 days. Potato roots were washed free of soil and held in a mist chamber (1) for 7 days. Juveniles of the clover cyst nematode and other vermiform nematodes were recovered by these methods, and examined under the compound microscope.

A few days before harvest, 151 kg and 168 kg of tubers were dug from the Bayview and Linkletter sites, respectively. All the tubers from each site were washed in the laboratory for 4 minutes in 60 litres of water in a rotary washer. Organic matter and other particles were removed from the surface of the wash water with a fine screen (44 μm aperture) and placed on white filter paper. This material was examined with a stereomicroscope at 30 to 60 magnification for nematode cysts which have specific gravities lower than water and float on the surface. The wash water was passed through a fine screen (36 μm aperture), and the sediment at the bottom of the rotary washer was processed through a sugar-flotation technique (2), for recovery of vermiform nematodes.

Potatoes were washed at harvest on location with a commercial washer in which the tubers passed under high-pressure spray nozzles. About 150 kg of washed tubers from the Linkletter site were rewashed in the laboratory to assess the effectiveness of the commercial washer for removing soil and nematodes.

Samples of washed and unwashed potatoes were monitored during the winter for development of storage rot diseases. Stem ends and wounds were examined and samples were cultured on selective media for determination of fungal and bacterial disease organisms.

Results

Worm-shaped, second-stage juveniles of *H. trifolii*, the motile infective stage which invades roots of suitable hosts, were recovered from the August soil samples in relatively low numbers at both locations (Table 1). No clover-cyst nematode juveniles were recovered from the potato roots (data omitted).

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Table 1. Number of clover-cysts nematodes recovered from the surface of potato tubers.

Location	Weight of tubers (kg)	Number of second-stage juveniles			Number of cysts
		Initial count[a]	In sediment[b]	In water[c]	
Bayview[d]	151	100	0	0	0
Linkletter[d]	168	450	15	17	0
Linkletter[e]	150	450	0	0	0

[a]Per kg of field soil from samples obtained during August 1981.
[b]Approximately 100 cc for the field samples and 10 cc in prewashed potatoes.
[c]Approximately 60 litres.
[d]Field sample.
[e]After commercial wash.

No cysts were observed in the particulate matter removed from the surface of the wash water. A few dead second-stage juveniles were recovered from the wash water and sediment of the Linkletter sample.

Virtually all of the soil was removed from the surface of the tubers in each field sample during the washing process in the laboratory. About 90% of the soil adhering to tubers was removed by the commercial washing. Detailed examination of the wash water and sediment from previously washed tubers revealed that the incidence of clover-cyst nematodes had been reduced to a level which was not detectable (Table 1).

The microbial cultures on selective media disclosed the presence of *Fusarium* spp. and *Erwinia* spp. on both washed and unwashed tubers. However, there were no obvious differences in the levels of storage rots between washed and unwashed potatoes under recommended storage conditions.

Discussion

The results indicated that only a few second-stage juveniles were present in soil adhering to tubers. This was not a matter of great concern since this stage of the clover-cyst nematode is very susceptible to desiccation and would survive only a few days under normal storage conditions or during transit. However, if nematode juveniles or cysts are of concern, for example in the export of seed, then an adequate commercial washing procedure should remove more than 90% of the soil from the tubers and reduce the occurrence of clover-cyst nematodes to levels which approach zero. In addition, the technique does not appear to enhance storage rot diseases of potatoes under recommended storage conditions. As an added control measure, the soil can be treated at planting with a nematicide to further reduce nematode levels, and a fungicide could be applied to the washed tubers to control fungal storage rots.

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

John G. McDonald and Michio Suzuki

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 détecté à un niveau assez bas dans les légumineuses fourragères de l'Ile-du-Prince-Edouard. En se basant sur 90 échantillons par champ, testés 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 première 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 réservoir viral ne semble pas constituer une menace pour la culture de la pomme de terre étant donné le peu d'importance du calicot de la pomme de terre, causé par AMV.

Introduction

Alfalfa mosaic virus (AMV) is worldwide in distribution and has a wide natural host range (3,6). It is particularly prevalent 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 susceptibility to 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 importance of this virus in crop production.

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 sativa* 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.

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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 (A280=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 μul 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 (unpublished data 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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Survey of eastern Ontario alfalfa fields to determine common fungal diseases and predominant soil-borne species of *Pythium* and *Fusarium*[1]

P. K. Basu[2]

One hundred alfalfa (*Medicago sativa*) fields in 12 counties of eastern Ontario were surveyed in 1981. Based on symptoms and isolations from host tissues and soil samples, the following pathogens were detected. *Phoma medicaginis* (spring black stem), *Colletotrichum trifolii* (anthracnose), *Phytophthora megasperma* f. sp. *medicaginis* (root rot), and *Sclerotinia trifoliorum* (sclerotinia crown and stem rot) were found in 19, 10, 9, and 1 fields, respectively. *Pseudopeziza medicaginis* (common leaf spot) and *Stemphylium botryosum* (Stemphylium leaf spot) were present in most fields while *Peronospora trifoliorum* (downy mildew) occurred sporadically. *Verticillium albo-atrum* (wilt) was not found. *Pythium irregulare*, *Fusarium oxysporum* and *F. equiseti* were widely distributed in soil, but their population densities seemed to bear no significant relationship to fields visually rated as being in good, fair or poor condition; however, in laboratory tests, certain isolates of each of the latter three fungi were pathogenic to alfalfa seedlings.

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Cent champs de luzerne (*Medicago sativa*) dans 12 comtés de l'est de l'Ontario furent inventoriés en 1981. En se basant sur les symptômes et les isolations faites à partir des tissues des plantes hôtes et des échantillons de sol, les pathogènes suivants ont été détectés. *Phoma medicaginis* (tige noire printanière), *Colletotrichum trifolii* (anthracnose), *Phytophthora megasperma* f. sp. *medicaginis* (pourridié phytophthoréen) et *Sclerotinia trifoliorum* (flétrissure sclérotique) ont été identifiés respectivement dans 19, 10, 9 et 1 champs. *Pseudopeziza medicaginis* (tache commune) et *Stemphylium botryosum* (tache stemphylienne) étaient présents dans la plupart des champs tandis que *Peronospora trifoliorum* (mildiou) ne l'était que sporadiquement. *Verticillium albo-atrum* (flétrissure verticillienne) n'a pas été isolé. *Pythium irregulare*, *Fusarium oxysporum* et *F. equiseti* sont largement distribués dans le sol mais il ne semble pas exister une relation significative entre leurs densités de population et les champs visuellement classés bons, passables ou pauvres.

Introduction

In conjunction with recent surveys for Verticillium wilt (*Verticillium albo-atrum* Rienke & Berth.) of alfalfa (*Medicago sativa* L.) across Canada (2), an effort was made to obtain information on the occurrence of other common fungal diseases (9) and soil-borne pathogens (7, 8) in eastern Ontario. Although winterkill is believed to be one of the major factors for alfalfa decline (4), the importance of root and crown rot pathogens, such as, species of *Phytophthora*, *Pythium*, *Fusarium*, *Aphanomyces*, and other fungi is well documented (6, 7, 10, 12, 14, 15, 17). The incidence of foliage diseases (3, 5) also cannot be ignored.

Materials and methods

Based on the production acreage of alfalfa and its mixtures (1), 100 alfalfa fields were chosen in a stratified-random manner (18) from the 12 counties of eastern Ontario (Fig. 1). Plants, within a 2 m wide W-shape path (3) in each field, were examined for disease symptoms (9) and pathogens were isolated from host tissues to confirm diagnosis when required. Soil samples (500 g) near plant roots were taken from each of 10 approximately equally spaced sites along the path; these were bulked, mixed, sieved, and tested for the presence of *Phytophthora*, *Pythium*, and *Fusarium* species using selective media (13, 17, 19). *Phytophothora megasperma* Drechs. f. sp. *medicaginis* Kuan & Erwin (11) was detected by the alfalfa seedling baiting method (13); species of *Pythium* and *Fusarium* were isolated from dilution plates as described by Singh and Mitchell (17), and Wensley and McKeen (19), respectively. The pathogenicity of representative isolates was tested by the inoculum layer technique (16), using 40 alfalfa seedlings per treatment in duplicate pots. The names of alfalfa cultivars and field size were noted, and the condition of each field was rated visually as good, fair or poor, depending upon the crop growth and stand. Fields were visited a few days before the first, second or third cut. Paired *t* tests (18) were performed to determine if there were significant differences in the population densities of the fungi in good, fair, and poor fields.

Results and discussion

Since the major emphasis in this survey was to detect Verticillium wilt, plants showing wilting, yellowing or stunting were collected and cultured to isolate pathogens. None of the 64 plants with these symptoms gave any evidence of *V.*

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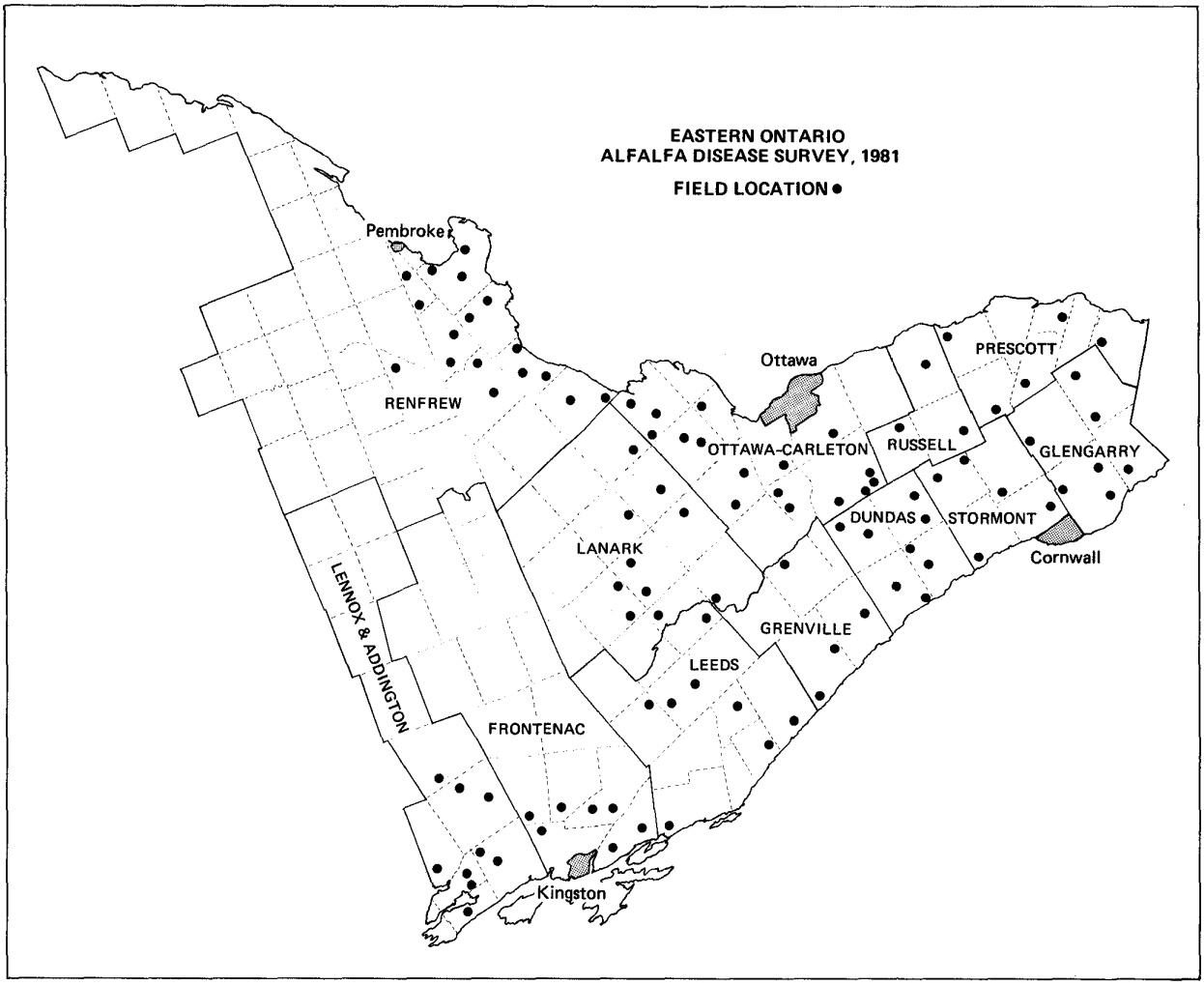

Figure 1. Showing approximate locations of alfalfa fields surveyed in eastern Ontario.

albo-atrum being present, indicating that the disease has not yet reached a detectable level in this area. Laboratory experiments at Ottawa showed that this pathogen can remain viable in alfalfa stems for at least 6 months through a temperature range from -5° to 35°C, and this is comparable to local outdoor temperatures (unpublished). Therefore, all suggested precautions (2) should be taken to prevent its introduction to this area through infected alfalfa stems.

It was noteworthy that a number of the above 64 samples yielded the spring black stem and anthacnose pathogens, *Phoma medicaginis* Malbr. & Roum var. *medicaginis* Boerema and *Colletotrichum trifolii* Bain; these two diseases were found in 19 and 10 fields, respectively. Common leaf spot (*Pseudopeziza medicaginis* (Lib.) Sacc.) and Stemphylium leaf spot (*Stemphylium botryosum* Wallr.) were found in most fields while downy mildew (*Peronospora trifoliorum* de By.) occurred sporadically as observed in previous studies (3). Sclerotinia crown and stem rot (*Sclerotinia trifoliorum* Erikss.) was encountered in one field. *P. megasperma* f. sp. *medicaginis* was detected in soil samples from 9 fields that appeared in good condition with no distinct root rot patches evident.

Pythium irregulare Buisman, *Fusarium oxysporum* Schlecht, and *F. equiseti* (Corda) Sacc. were widely distributed in eastern Ontario alfalfa fields but their population densities varied greatly from field to field within each county as indicated by large standard error values (Table 1). There were 3 poor, 17 fair and 80 good fields. The population densities of each of these fungi (*P. irregulare*, *F. oxysporum* and *F. equiseti*) in poor and fair fields compared with 20 randomly chosen good fields did not differ significantly, as determined by paired *t* tests. Thus visual rating of fields as good, fair or poor showed no relationship with the population of fungi obtained. However, certain isolates of each of the three species were pathogenic to alfalfa seedling in laboratory tests (Table 2). Isolates of *P. irregulare* from Prescott, Leeds and Frontenac counties were virulent but those from other counties were not. Similarly, isolates of *F. oxysporum* also varied in virulence.

Table 1. Mean number of propagules of *P. irregulare, F. oxysporum* and *F. equiseti* per gram of soil from alfalfa fields in twelve counties of eastern Ontario.

County	No. of fields	*P. irregulare*	*F. oxysporum*	*F. equiseti*
Dundas	8	62.5 (13.9)*	1100.0 (280.3)	850.0 (219.6)
Frontenac	8	160.0 (40.5)	750.0 (244.2)	200.0 (151.2)
Glengarry	7	205.7 (49.1)	400.0 (174.6)	171.4 (119.0)
Grenville	4	65.0 (25.0)	900.0 (640.3)	100.0 (100.0)
Lanark	10	90.0 (23.3)	760.0 (300.8)	200.0 (89.3)
Leeds	8	192.5 (41.2)	800.0 (239.0)	0.0
Lennox & Addington	9	195.5 (37.5)	1688.9 (273.1)	133.3 (94.3)
Ottawa-Carleton	16	265.0 (44.1)	900.0 (226.6)	350.0 (162.8)
Prescott	5	96.0 (38.7)	400.0 (219.1)	220.0 (80.0)
Renfrew	17	177.6 (19.5)	988.2 (191.3)	352.9 (136.7)
Russel	3	153.3 (6.7)	0.0	266.2 (133.3)
Stormont	5	304.0 (50.4)	1840.0 (449.0)	640.0 (240.0)

*Standard error in parenthesis

Table 2. Range of virulence* of representative isolates of *Pythium irregulare, Fusarium oxysporum* and *F. equiseti* from 10 countries of eastern Ontario.

County	*P. irregulare*	*F. oxysporum*	*F. equiseti*
Dundas	0.0	2.6	5.0
Frontenac	29.2	17.5	0.0
Grenville	0.0	5.6	0.0
Lanark	0.0	20.0	0.0
Leeds	50.0	17.1	0.0†
Lennox & Addington	0.0	0.0	0.0
Ottawa-Carleton	3.0	34.5	0.0
Prescott	68.2	5.7	0.0
Renfrew	0.0	0.0	7.5
Stormont	2.1	2.8	0.0

*Based on percentage of alfalfa seedlings showing brownish discoloration or lesions on roots.

† This isolate was from Glengarry to replace Leeds where *F. equiseti* was not found.

Most of the isolates of *F. equiseti* were non-pathogenic; only two from Dundas and Renfrew counties caused discoloration of alfalfa roots. It was clear, that different isolates of the same fungus varied in virulence; and this suggests the possible existence of races which cannot be verified until constancy of virulence can be demonstrated on specific alfalfa cultivars.

The various alfalfa cultivars encountered in this survey (such as, Saranac, Vernal, Iroquois, Pioneer, Anchor, Thor, Vista and others) were found to be representative of the common cultivars grown in Ontario. The incidence of diseases, however, did not appear to be restricted to specific cultivars or areas visited. The total field area surveyed was 650.8 ha representing about 0.6% of the production acreage of alfalfa and its mixtures (1).

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A recurrence of tomato corky root in Ontario

W. R. Jarvis[1]

Corky root rot of tomato, caused by *Pyrenochaeta lycopersici*, was found in several greenhouses in southwestern Ontario in 1982 and 1983, the first records since 1944.

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La maladie des racines liégeuses de la tomate, causée par *Pyrenochaeta lycopersici*, a été identifiée dans plusieurs serres du sud-ouest de l'Ontario en 1982 et 1983 pour les premières fois depuis 1944.

Introduction

Corky root rot was found in a greenhouse crop of tomatoes, *Lycopersicon esculentum* Mill., at Chatham, in southwestern Ontario in the summer of 1982. The cv. Vendor was being grown in plastic greenhouses in steam-sterilized soil groundbeds but the crop was unthrifty. Many of the plants wilted on hot days. The disease was found in cv. MR13 in several other greenhouses in the Leamington area in 1982 and 1983. Symptoms typical of corky root rot (1-5, 7) occurred in the roots of both cultivars and some roots also had microsclerotia of *Colletotrichum coccodes* (Wallr.) Hughes. Isolations from surface-sterilized portions of affected roots yielded cultures of the so-called gray sterile fungus (GSF) (2), known since 1966 as *Pyrenochaeta lycopersici* Schneider & Gerlach (6). The roots also yielded cultures of *C. coccodes*.

The isolates of *P. lycopersici* alone first induced symptoms typical of brown root rot and, later, corky root rot, in tomato seedlings of the cv. MR13 grown in sterilized potted soil infested with the fungus cultured on sterile rye seed. Infesting the soil with chopped infected roots also induced symptoms in seedlings.

The last report of this disease in Ontario was made by Richardson and Berkeley in 1944 (5), before its etiology was defined, and the causal fungus identified as *P. lycopersici*. *Colletotrichum coccodes* is often associated with *P. lycopersici* but is not itself an incitant of corky root rot.

Acknowledgment

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A suggestion for the survey and reporting of native plant pathogens

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By reviewing distribution records of conifer pathogens, it was found that some may have originated from specific refugia and may not have colonized the entire geographic range of their hosts. Host indexes and checklists often refer to large geographic areas and do not reflect the presence or absence of pathogens in specific geographic areas. Suspected refugia should be surveyed to determine the presence or absence of pathogens. The presence of a pathogen might indicate host resistance to it; whereas the absence of such may suggest that the host should be protected from the pathogen.

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En étudiant les données sur la distribution d'un groupe de pathogènes des conifères, on a trouvé que certains pouvaient provenir de refuges précis et ne pas avoir colonisé toute l'aire géographique de leurs hôtes. Souvent, les répertoires et listes d'hôtes couvrent de grandes étendues géographiques et n'indiquent pas la présence ou l'absence des pathogènes dans des régions précises. Des relevés devraient être effectués aux endroits que l'on soupçonne d'être des refuges pour préciser ce fait. La présence d'un pathogène pourrait indiquer une résistance de l'hôte, tandis que son absence pourrait signifier qu'il faudrait protéger l'hôte contre ce pathogène.

Introduction

Of importance to plant pathologists is finding the geographic origins of pathogens (Stevens 1961). The geographic origin will often lead to finding the most variation in the pathogen and several evolved resistant reactions in the host (Leppik 1967). Conversely, susceptible hosts in a geographic area not inhabited by the pathogen could be severely damaged by the pathogen if it were introduced, as has happened to North American white pines by the introduction of the blister rust fungus.

Within North America there are certain native pathogens with limited distribution which perhaps could expand their range if introduced to new areas. This is most clearly evident from the distribution patterns of dwarf mistletoes (Hawksworth & Wiens 1972) and introduction experiments (Smith & Wass 1979).

Recent information suggests that conifers native to British Columbia survived ice ages in several different refugia (von Rudloff & Nyland 1979; von Rudloff *et al.* 1981; Warner *et al.* 1982; Wheeler & Guries 1982), and therefore a particular species or closely related species may have more than one recent geographic origin. This may also be true of the pathogens, or perhaps some pathogens could have been excluded from some of the refugia.

These conifer species or subspecies now often overlap in distribution and interbreed within their group. Pathogens limited in their spread, such as dwarf mistletoes (*Arceuthobium* spp.) and soil fungi, may still be spreading to new geographic areas; whereas wind-borne pathogens are largely limited by environmental conditions or are isolated within or excluded from certain areas by geographic barriers such as island populations of shore pine (*Pinus contorta* Dougl. var. *contorta*) and mountaintop populations of coastal alpine-fir (*Abies lasiocarpa* (Hook.) Nutt.).

The purpose of this exercise was to examine some distribution records of Pinaceae pathogens in British Columbia for anomalies which may suggest the origins of the pathogens and which may indicate any host populations which may be free of certain pathogens.

Results and Discussion

The only native soil-borne root pathogens, *Phellinus weirii* (Murr.) Gilbertson and *Armillaria mellea* (Vahl.: Fr.) Kumm. are widely distributed (Baranyay & Bauman 1972) and, without further species subdivision and geographic reporting, no inferences can be drawn.

The distribution (Baranyay & Bauman 1972) of *Arceuthobium americanium* Nutt.: Engelm. on lodgepole pine (*Pinus contorta* var. *latifolia*) in the southern one-half of the province and *A. douglasii* Engelm. on Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) in the southern interior suggest migration from southern refugia rather than northern or coastal refugia. The presence of *A. tsugense* (Rosendahl) G.N. Jones shore pine pathotype on *P. contorta* only on the south coastal region in specialized habitats (Wass 1976) suggests survival in coastal refugia. The pathotype on western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and mountain hemlock (*T. mertensiana* (Bong.) Carr.) is coastal in distribution, being absent from interior populations as well as northern and southern limits of these hosts (Hawksworth & Wiens 1972; Fowells 1965). This distribution suggests development and spread from the shore pine pathotype gene centre. The severity of damage also suggests that this is a relatively new host-parasite combination.

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Records of important pathogens which are lacking, possibly because the pathogen has not reached the host, for Queen Charlotte Islands (QCI) populations of shore pine and for coastal mountain top populations of coastal alpine fir include: *Arceuthobium tsugense* pine pathotype; *Coleosporium asterum* (Diet.) Syd.; *Cronartium coleosporioides* Arth.; *C. comandrae* Peck; *C. comptoniae* Arth.; *Lophodermella montivaga* Petr.; *Melampsora medusae* Thuem.; *Scirrhia pini* Funk & A.K. Parker; and *Delphinella abietis* (Rostr.) E. Muell.; *Dermea rhytidiformans* Funk & Kuijt; *Isthmiella abietis* (Dearn.) Darker, respectively.

The origin of coastal spruce cone rust (*Chrysomyxa monesis* Ziller) appears to be on the QCI, since it is the only cone rust on the islands and it is only occasionally found on the continent, and then only on the alternate host.

Host indexes and checklists often cover large geographic areas and do not reflect native host gene origins. Listings covering a smaller geographic area or specific host gene origins would more likely lead to the finding of the geographic origin of native pathogens, where resistance may be found, and may indicate which native populations of hosts should be kept free of pathogens by limiting introductions. The QCI appears to be one such important area. To further this end, the distribution of all pathogens within possible refugia should be examined and their presence or absence reported.

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Author Index to Volume 63

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Titles should be concise and informative providing, with the Abstract, the key words most useful for indexing and information retrieval.

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Figures should be planned to fit, after reduction, one column (maximum 84 × 241 mm) or two columns (maximum 175 × 241 mm), and should be trimmed or marked with crop marks to show only essential features. Figures grouped in a plate should be butt-mounted with no space between them. A duplicate set of unmounted photographs and line drawings is required. Figures should be identified by number, author's name, and abbreviated legend.

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Les articles et les communiqués sont publiés en anglais ou en français. Les manuscrits (l'original et une copie) et toute la correspondance qui s'y rapporte doivent être envoyés à M. H.S. Krehm, Service des programmes de recherche, Direction de la recherches, ministère de l'Agriculture du Canada, Ottawa (Ontario) K1A 0C6.

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Les titres doivent être courts et révélateurs en contenant, avec le résumé, les mots clés les plus utiles pour le classement et l'extraction de l'information.

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Les tableaux doivent être numérotés en chiffres arabes et avoir un titre concis. Ils ne devraient pas avoir de lignes verticales. Les renvois doivent être identifiés par un signe typographique particulier (* † § # ¶ ** ††) surtout lorsqu'il s'agit de nombres.

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