VIRUSES OF CLOVERS AND ALFALFA IN ESSEX COUNN, ONTARIO, 1970-73

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Abstract

surveys of legume viruses in cultivated and wild clovers and alfalfa in Essex County were made in **1970-73.** The incidence of alfalfa mosaic virus averaged **11%** in alfalfa (Medicago sativa) crops in the first year, and **44%** in the second and later years of cut. Bean yellow mosaic, alfalfa mosaic, red clover vein mosaic, and pea streak viruses occurred at low levels in red clover (Trifolium pratense) crops, though they were frequently encountered in wild clovers. White clover mosaic virus and tobacco ringspot type viruses were also isolated from wild clovers. Bean common mosaic, pea common mosaic, clover yellow mossic, and clover yellow vein viruses were not encountered in this survey.

Resume

De **1970 à 1973,** on a effectué dans le comté d'Essex, des relevés de virus de légumineuses dans les trèfles cultivés et sauvages et dans la luzerne. La fréquence du virus de la mosafque de la luzerne a atteint en moyenne **11%** dans les cultures de luzerne (Medicago sativa) durant la premiere année, et **44%** pendant la seconde et les autres années de coupe. La mosaïque jaune du haricot, la mosaïque de la luzerne, la mosafque des nervures du trêfle rouge et la bigarrure du pois ne se sont manifestées qu'à un faible degré dans les cultures de trefle rouge (Trifolium pratense), bien qu'on ai pu fréquemment les trouver chez les trèfles sauvages. On a également isolé sur ces derniers le virus de la mosafque du trèfle blanc et ceux de la tache annulaire du tabac. Dans cette enquête, on n'a pas trouvé de virus de la mosafque commune du haricot et du pois, de la mosaïque jaune et de la mosafque jaune des nervures du trèfle.

In Essex County, Ontario, red clover (Trifolium pratense L.) and alfalfa (Medicago sativa L.) occupy about **4%** of the arable land as forage crops and, being perennials, provide a likely source of viruses for soybeans, beans, peas, tomatoes, and peppers. Legume viruses recorded in southwestern Ontario include those inciting alfalfa mosaic **(AMV),** soybean mosaic, bean mosaic, tobacco ring spot **(TRSV),** red clover vein mosaic (RCVMV), pea enation mosaic, pea common mosaic **(PCMV),** pea streak (PSV), bean yellow mosaic (BYMV), and white clover mosaic (WCMV) **(1,** 6, **22).**

In many areas of Ontario, eastern Canada and the **USA,** red clover is a frequent source of BYMV, **PCMV,** RCVMV, **PSV,** and less frequently of AMV, WCMV, and clover yellow mosaic virus (CYMV) **(10, 12, 19, 20, 22, 24).** white clover (Trifolium repens *L.)* is often infected with WCMV, AMV, and less frequently with RCVMV and PSV **(1, 19, 20, 22).** AMV, often at high levels of incidence, is usually found in alfalfa **(2, 5,** 10, **18, 26),** which may also contain WCMV, CYMV, BYMV, tobacco streak virus, and TRSV **(7, 10, 19, 20).** CYMV has been found only in and west of Wisconsin, Kentucky, and Oklahoma **(1, 7, 20),** and clover yellow vein virus (CWV) only in Quebec, Pastern Canada **(22),** and North Carolina **(15).**

Spread of BYMV from red clover into Leans has been detected for 200 m downwind and 60 m upwind **(10).** Although AMV has been reported to spread from clover and alfalfa into beans and soybeans, several workers note that AMV was not observed to spread, or that it spread very much less than BYMV **(2, 5,** 10, **26).**

viruses reduce the productivity of legume crops and resistance to winter temperatures of clovers, though not always of alfalfa **(7, 21,** 23, refs. in **12).** White clover may lose up to half of its yield from strains of AMV and BYMV, especially in combination, and also from CYMV **(14, 17, 21).** AMV is often mild or symptomless in alfalfa **(2, 5, 26),** but high levels of infection caused yield losses of **22-30%** in early cuts, and smaller losses in later cuts, in the season after inoculation **(7,** 9**).**

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Several forage legume viruses, including WCMV, CYMV and a virus related to tomato ringspot in red clover, and AMV in alfalfa, have been found to be seed transmitted (8, 11).

In Essex County, wheat and oat fields are usually undersown with red clover, which is cut the following season, and then ploughed under. Alfalfa is similarly undersown, but usually is cut for several years. These crops, and roadside legumes, were examined for virus infection during 1970-73.

Methods

The reactions of indicator hosts and virus particle size were the major means of identification. Indicator plants were tobacco (Nicotiana tabacum L. 'Samsun NN' and 'Haronova'), pepper (Capsicum frutescens L., 'California Wonder'), French bean (Phaseolus vulgaris L. 'Topcrop' and 'Bountiful'), pea (Pisum sativum L. 'Thomas Laxton' and 'Little Marvel'), cowpea (Vigna sinesis (Torner) Savi 'Early Ramshorn'), Chenopodium amaranticolor Coste and Reyn., broad bean (Vicia faba L. 'Long Pod'), Gomphrena globosa L., snapdragon (Antirrhinum majus L.), and other hosts useful for particular viruses. Plants were sap-inoculated by the leaf-rub method with carborundum as an abrasive. They were kept in a greenhouse maintained at 22-28 C.

AMV, BYMV, and TRSV were recognized by their typical effects on these plants. WCMV rapidly produced light green local lesions on cowpea. It infected French bean, but not C. amaranticolor, G. globosa, or the solanaceous hosts. PSV and RCVMV gave local lesions on G. globosa and C. amaranticolor. They were distinguished by particle size and by the fact that RCVMV was slower than PSV in producing lesions on G. globosa. snapdragons were checked for CYMV, and Haronova tobacco for CYVV.

Particles of the rod-shaped viruses were examined in leaf-dip preparations stained with phosphotungstic acid (pH 7.0) or shadowed with platinum - palladium (80:20) (Figs. 1-4). The mean lengths and the size range of particles, based on the examination of 5-8 isolates of each virus (n = total number of particles measured), were; RCVMV 630 nm (615-653, n = 253); WCMV 457 nm (436-473, n = 237); PSV 591 nm (562-615, n = 120); BYMV 179 nm (757-798, n = 77). Particle widths, based on 14-20 particles from 2-3 isolates of each virus, were; RCVMV 12.1 nm, WCMV 13.5 nm, PSV 12.6 nm, and BYMV 14.2 nm. These measurements agree with the published measurements for these viruses.

Local isolates of WCMV reacted positively with antiserum to this virus from the American Type Culture Collection.

Virus incidence

Estimations of viruses in clovers depended on counts of shoots or plants with virus symptoms in 6-10 quadrats of 1 M2 per field usually once during the season, with collection of 2-3 typical shoots per field for virus identification. Some collections of symptomless clovers were made early in the season in 1972 and 1973. Only 2 out of 30 collections of symptomless plants contained viruses so that reliance on symptoms appears justified. Alfalfa crops were carefully examined for virus-like symptoms in 1970 and 1971. In 1972 and 1973, 10 shoots per field, regardless of symptoms, were collected at random early in the spring and, later, from the regrowth that occurred shortly after cutting the crop, while the shoots were still only a few cm tall. These shoots were tested individually for viruses and, although their numbers were insufficient for conclusions about any one field, the results gave an estimate of virus incidence over the 30-40 fields tested each year.

Results and discussion

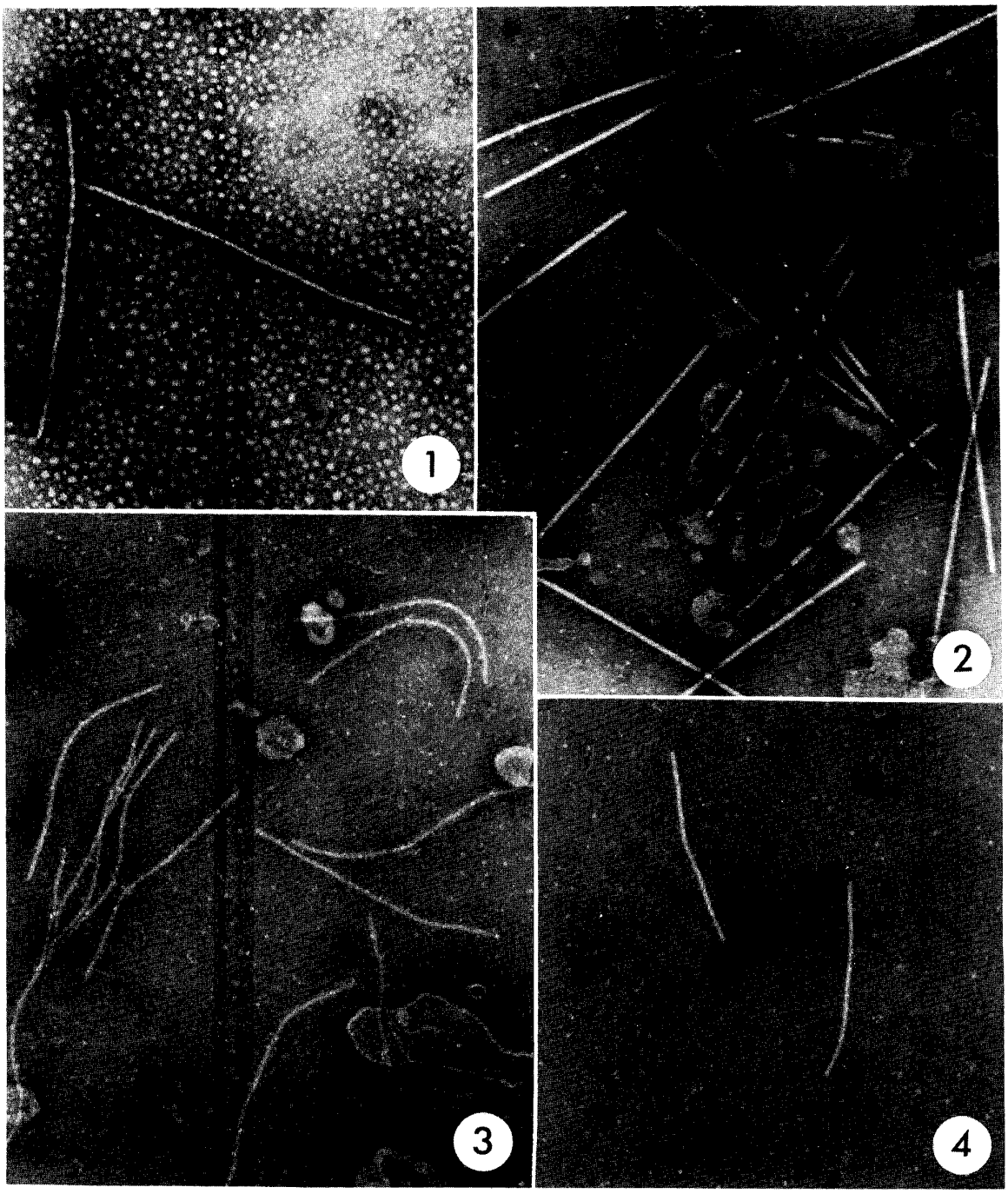
alfalfa mosaic virus

AMV occurred with unexpected frequency in alfalfa crops, infecting 11% of the shoots in first year crops, and 44% in crops in their second and later years of cut (Table 1). These levels of infection would cause estimated yield losses of 2% and 7% respectively (7, 9). The incidence of AMV in red clover field crops was low, but roadside clovers were often infected.

Isolates of AMV could be divided into two main groups based on their effects on French beans: those which produced small local lesions on the primary leaves, usually without becoming systemic, and those which multiplied in local areas with diffuse edges, causing chlorosis and vein necrosis on the inoculated leaves, and later becoming systemic and causing necrosis in uninoculated leaves. These two types of isolate were obtained about equally from alfalfa, and both were isolated from red and white clovers and from yellow sweet clover (Melilotus officinalis L.).

About half the AMV isolates from alfalfa in Essex County became systemic in French beans, as observed in Indiana (3), whereas in England only 1.7% of isolates from alfalfa were of this type (9).

Isolates from Essex County that became systemic in French beans usually produced very mild symptoms in tobacco, moderate symptoms in pepper, and did not infect cucumber. Systemic strains described by Zaumeyer and Patino (26, 27) also showed these tendencies, but those isolated by Houston and Oswald (13) gave typical symptoms on solanaceous hosts. Four of 71 Essex County isolates tested resembled the "alfalfa yellow mosaic" virus in producing an intense systemic yellow mosaic in cowpea (25). A few isolates produced small green rings on the inoculated primary leaves of 'Bountiful' French beans as described by Frosheiser (8).



Figures 1-4. Virus particles from leaf-dip preparations, stained with phosphotungstic acid, pH 7.0, X 66.875. 1) Bean yellow mosaic virus, 2) Red clover vein mosaic virus, 3) Pea streak virus, 4) White clover mosaic virus.

Table 1. Legume viruses in Essex county, Ontario, 1970–73

Source plants	virus*	Number of times isolated from source plant collections[a]	No. of fields or locations	Number of fields or locations with infection level (%) indicated[a]				Average infection (%) in all fields of each crop and year of cut
				Trace	Up to 10	11–50	51–100	
Field crops								
Red clover, 1st year of cut	none		13					
	AMV	9	4	1	3			0.2
	BYMV	29	14	5	8	1		1.1
	PSV	4	4	4				
	RCVMV	1	1	1				
Red clover, 2nd year of Cut	BYMV	4	1			1		20.0
Alfalfa, 1st year of cut	none	324	16					
	AMV		19	4	5	8	2	11.1
Alfalfa, 2nd year or older	none		9					
	AMV		51	1	5	29	22	44.4
Yellow sweet clover	none		2					
	AMV	1	1		1			
	BYMV	2	2	1		1		
	RCVMV	1	1	1				
Alsike clover	none		2					
	AMV	1						
	BYMV	9	1		1			
Roadsides and waste ground								
Red clover	none		18					
	BYMV	9	9	7	2			
	PSV	1	6	4	2			
	RCVMV	3	3	2	1			
	TRSV[b]	9	9	8	1			
	WCMV	4	4	2			2	
Alsike clover	none		6					
	AMV	1	1	1				
	BYMV	1	1			1		
	PSV	1	1	1				
	TRSV[b]	2	1		1			
	WCMV	1	1			1		
white clover	none		Y					
	AMV	3	3				3	
	RCVMV	3	2		2			
	TRSV[b]		2		2			
	WCMV	8	7	1	2	3	1	
Yellow sweet clover	none		3					
	AMV	6	4	2		1	1	
	BYMV	8	6	2	2	1	1	
	PSV	1	1	1				
	RCVMV	3	2	2				
	TRSV[b]	3	3	2	1			
Alfalfa	none		4					
	AMV	17	3	2		1		

[a] Collections and estimates of infection were of plants with symptoms, except from alfalfa fields, where in 1972 and 1973 random samples of plants were collected. viruses in plants with mixed infections are recorded separately.

[b] Includes tobacco and tomato ringspots and possibly other ringspot types.

AMV - alfalfa mosaic virus; BYMV - bean yellow mosaic virus; PSV - pea streak virus; RCVMV - red clover vein mosaic virus; TRSV - *see* (b) below; WCMV - white clover mosaic virus.

The frequency of AMV in clovers and alfalfa in Essex County may be related to the presence of many solanaceous crops, especially tomatoes, peppers, eggplants (Solanum melonsena L.), and tobacco, as well as to large acreages of soybeans and green beans. Pratt *(22)* did not isolate AMV from clovers in other areas of Ontario, though it was found in Quebec. AMV was the most frequently isolated virus in red clovers in Washington (10), where large acreages of field beans and alfalfa are grown, and it was frequent in Rhode Island (19). Frosheiser (8) suggested that most initial AMV infections in alfalfa stands may arise from infected seeds.

Bean yellow mosaic virus

BYMV (Fig. 1) occurred widely in red and yellow sweet clovers on roadsides and waste ground. It was the most commonly found virus in red clover field crops, but its incidence in these crops, which normally are kept for only 1 year of cutting, was low (Table 1). Most isolates produced bright mosaics without necrosis in French bean, but a few produced necrosis, as described in the literature (4, 16). Necrotic and mild isolates were recovered from white bean plots at Harrow.

BYMV was estimated to affect 10–50% of the plants in red clover fields in Wisconsin,

Minnesota, Idaho, and Kentucky, compared with 2–10% for AMV (12, 24).

Red clover vein mosaic virus and pea streak virus

These viruses (Figs. 2, 3) occur frequently in red clover crops in Wisconsin, Minnesota, Idaho, and Kentucky (12, 24), but in Essex County red clover crops they were found only infrequently. This may be because pea crops are less frequent, except possibly near Windsor, than in some U.S.A. areas. They were, however, often encountered in roadside clovers in Essex County (Table 1), and RCVMV has been recorded in peas in the county (6). Pratt (22) encountered these viruses in red and white clovers in other parts of Ontario and eastern Canada.

White clover mosaic virus

This virus (Fig. 4), recorded once (1) in clovers in Essex County, was often isolated from white and red clovers on roadsides and in orchards but not from field crops of clovers. White clover patches were often extensively infected with the virus.

Tobacco ringspot and related viruses

Viruses of the tobacco ringspot type, recorded once from red clover field crops in Essex County (6) were frequently encountered in clovers on roadsides and waste ground. In cross-protection tests on local isolates of this type, using known isolates of TRSV and tomato ringspot virus, one local isolate behaved like TRSV, one like tomato ringspot virus, and one behaved like TRSV but did cause some lesions on plants previously inoculated with TRSV. The local tomato ringspot virus isolate, compared with TRSV, caused fewer necrotic patterns on the upper leaves of tobacco, caused more rapid necrosis of C. amaranticolor and did not infect spinach (Spinacia oleracea L.). However, it behaved like TRSV in infecting snapdragon and lima bean (Phaseolus limensis MacF.).

Other viruses

Pea common mosaic and bean common mosaic have been recorded in Essex County (6), but were not encountered in this survey. Clover yellow mosaic virus and clover yellow vein virus have not been found in Essex County.

Acknowledgments

We wish to thank Dr. R. Stace-Smith, Research Station, Canada Agriculture, Vancouver, for supplying the tobacco and tomato ringspot virus isolates used in this study; S. Itz and E. Ford, Electron Microscope Centre, Chemistry and Biology Research Institute, Canada Agriculture, Ottawa, and E. Murray, Canada Agriculture, Harrow, for their competent technical assistance: and Drs. G. H. Haggis, R. C. Sinha, J. W. Aylesworth, and W. G. Bonn for reading and commenting on the manuscript. One of us (J.F.B.) is indebted to Drs. G. H. Haggis and Y. C. Paliwal for advice in the relevant areas of electron microscopy and virology at the initiation of this study.

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