Incidence and Effect of Alfalfa Mosaic Virus on Alfalfa

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ABSTRACT

Sixty-six percent of 349 alfalfa plants collected in Wisconsin during 1966-1968 were infected with mechanically-transmissible viruses. Alfalfa mosaic virus (AMV) was found in 59%. The AMV isolates were placed in 35 groups on the basis of differential host reaction. When 587 symptomless alfalfa plants from three widespread areas in Wisconsin were studied, 63% of the plants from 2-year-old stands and 78% from older stands were shown to contain mechanically-transmissible virus. Yields of alfalfa hay were significantly reduced by AMV. There was no significant evidence indicating that alfalfa plants exhibiting AMV symptoms were more readily winterkilled than symptomless plants. Phytopathology 60:1432-1435.

Many viruses are known to infect alfalfa, and several of these have been reported from the United States (11, 14, 15), including Wisconsin (7, 8). The present study was undertaken to evaluate the prevalence of mechanically transmissible viruses in alfalfa in Wisconsin, and to determine their effect upon forage yield and winterhardiness under natural conditions.

MATERIALS AND METHODS.—*Incidence of mechanically transmissible viruses in alfalfa fields.*—Alfalfa plants showing apparent symptoms of virus infection were collected from alfalfa fields over a 3-year period. The 1965 collections were restricted to farmer's fields and experiment station plots in southern Wisconsin. The 1966 and 1967 collections were made throughout the state. Usually only one plant was collected from each field; this was the first alfalfa plant with virus symptoms observed by the collector in that field. In 1965, only leaves were collected, and these were used as inoculum within 4 hr after the time of collection. These isolates were maintained in sweet clover (*Melilotus alba* Desv.). In 1966 and 1967, entire alfalfa plants were transported to Madison, cut back, potted, sprayed with an insecticide, and placed in the greenhouse.

The viruses were separated into groups primarily on the reactions of four differential hosts: *Phaseolus vulgaris* L. 'Bountiful'; *Pisum sativum* L. 'Perfected Wales'; *Vigna sinensis* (Endl.) 'California No. 5 blackeye'; and *Gomphrena globosa* L. Other plants which were inoculated to aid in identification included *Glycine max* (L.) Piper 'Hawkeye' and 'Harasoy 64'; *Nicotiana glutinosa* L.; *Nicotiana tabacum* L. 'Havana 38'; *Zinnia elegans* Jacq.; *Cucumis sativus* L. 'SMR 18'; and *Vicia faba* L. Assay and stock plants were grown in temp-controlled greenhouses which were regularly sprayed and fumigated to keep them free of insects. Assay plants were started from seed, and inoculated while they were young.

Inoculum was prepared by triturating diseased tissue in distilled water in a mortar with a pestle, and was applied with the forefinger to leaves of assay plants previously dusted with 400-mesh Carborundum. Each virus isolate was inoculated onto the differential hosts at least twice; some were inoculated 5 times.

After the isolates were separated into groups on the basis of host symptoms, representative members of each group were tested for serological relationship to AMV using the Ouchterlony agar double-diffusion technique.

RESULTS.—Mechanically-transmissible viruses were found in 66% of 349 alfalfa plants assayed, and 59% were shown to contain alfalfa mosaic virus (AMV) (Table 1). The viruses were classified into 46 groups on the basis of symptomatology and serological tests. Eleven of the groups, which comprised 22 of the isolates, were not shown to be AMV. The remaining 35 groups comprising 207 isolates were shown to contain AMV by serology. Present serological techniques are not adequate for differentiation of strains within AMV (1, 6, 12). The range in symptom production by different isolates of AMV was considerable, especially on beans where local lesions of various sizes and intensities of brown were noted and systemic reactions ranging from faint chlorotic foliage spots to apical necrosis and premature death were common.

Three isolates were considered to be clover yellow mosaic virus (CYMV) on the basis of host range, symptomatology, particle length (576 mμ), and a positive serological reaction. The particles were observed to be of a flexuous rod-type in electron microscopic studies using Brandes' (2, 3) dip technique. This is the first report of the occurrence of this virus in Wisconsin.

MATERIALS AND METHODS.—*Presence of viruses in symptomless plants.*—Five hundred and eighty-seven symptomless alfalfa plants were assayed in 1967 from three different locations in Wisconsin, with two ages of stands being sampled from each location. Samples were collected from symptomless plants in a random manner on 15 August 1967 and 25 October 1967 by sampling each field ca. 10-20 yards in from the perimeter and then on the diagonals. The top 3-4 inches of foliage of each plant were collected, using suitable precautions against cross-contamination. Within 24 hr, the collected samples were triturated in a mortar with a pestle and distilled water. The brie was rubbed onto

TABLE 1. Incidence of mechanically transmissible alfalfa viruses in Wisconsin alfalfa plants collected over a 3-year period

Year collected	No. fields studied	No. plants assayed	% Plants with:	
			Virus	AMV[a]
1965	40	45	57.8	53.3
1966	158	165	70.9	65.5
1967	115	139	61.9	54.0
Sum or mean	313	349	63.5	57.6

[a] Alfalfa mosaic virus.

primary leaves of 14-day-old Bountiful beans which had been dusted with 400-mesh Carborundum. Plants were maintained in the greenhouse at 21-26 C until symptoms developed or for 2 weeks, at which time they were discarded. Most symptoms became apparent on the 3rd day after inoculation, and became more intense during the next 2-3 days.

RESULTS.—The percentages of virus-infected symptomless plants from 2-yr-old alfalfa stands were 35, 64, and 89, compared with the older stands which yielded 64, 85, and 86 (Table 2). The percentage was lower in the central than in the southern and western Wisconsin fields. There were differences in symptoms produced by different samples from each field (Table 2). This indicated that there was more than one virus strain in each field. The identity of the viruses was not determined.

MATERIALS AND METHODS.—*Effect of AMV on alfalfa yields.*—Rooted stem cuttings of Glacier alfalfa clone G4 were inoculated on 16 April 1967 with AMV isolate 536 (an apparently typical and genetically stable isolate) using Carborundum and the forefinger. The noninoculated control cuttings were virus-free as determined by inoculation to Bountiful bean. The alfalfa cuttings were transplanted into the field 1 week after inoculation. Noninoculated cuttings were transplanted before the inoculated cuttings to avoid contamination. The plants were sprayed weekly with alternate applications of Cygon and Malathion to control insects.

The experimental design used was a randomized complete block with six replications and two treatments (inoculated and noninoculated). Each treatment in each replication consisted of five rows, 1 foot apart, with 12 plants/row spaced 9 inches apart. Each treatment was bordered with three rows of Vernal alfalfa seeded heavily at time of transplanting and thinned to a 9-inch spacing. A few of the transplants did not survive and were replaced on 21 June to obtain 100% stands.

Hay was harvested on 10 July, 11 August, and 25 September by cutting with virus-free pruning shears approximately 5 cm above ground level. Noninoculated plots were harvested before inoculated plots to avoid contamination. Wet wt were recorded immediately after cutting, and dry wt were obtained after 4-5 days in forced air ovens at 52-55 C. Since significant differences between treatments were obtained in the first cutting, three randomly selected plants in each plot were measured for the following yield components in the second and third cutting: stems per plant, mean stem length, leaves per plant, leaves per stem, and leaves per cm of stem. Many of the plants died from winter injury.

The experiment was repeated in 1968 using stem cuttings of Vernal alfalfa clones V4, V6, and V8. Similar procedures were used except that the plot was not sprayed for insect control, because this practice was not effective in controlling spread of virus in 1967.

RESULTS.—In 1967, the inoculated G4 plots yielded significantly less hay ($P = .05$) than the noninoculated ones in the first cutting (Table 3). The differences were all quite large, with a 30% reduction in dry matter. None of the differences in the second cutting were significant, though they were fairly large. The differences in the third cutting were small. There were no significant differences between noninoculated and inoculated plants with respect to any of the five yield components in the second and third cutting. As the season progressed, the noninoculated plants became infected with AMV, apparently by insect transmission. Plants sampled at random from noninoculated plots all assayed positively for AMV on Bountiful bean.

One cutting of hay was harvested from the Vernal clones on 3 November 1968, because of the late transplanting. The inoculated plots yielded somewhat less wet and dry wt than the noninoculated ones, but the reductions were not statistically significant. The differences in yield components between inoculated and

TABLE 2. Presence of mechanically transmissible viruses in symptomless alfalfa plants from three areas in Wisconsin as determined by inoculation on Bountiful bean

Area of Wisconsin	Age of stand, yr.	Field size, acres	Variety	No. alfalfa plants producing indicated symptoms on Bountiful bean					
				No symptoms	Chl/Nec[a]	Lesions[b]	Chl/Nec + lesions	Chl/Nec + epinasty	Chl/Nec + lesions + epinasty
South-central	2	7	Vernal	10	79	6			
South-central	15	12	Vernal	14	32	11	39		
Central	2	25	Vernal	64	33	1			
Central	4	30	Vernal	36	41	19	4		
Southwest	2	3	Vernal	36	45	5	5	4	5
Southwest	5	5	Kansas Common	14	58	4	12	5	

[a] Chlorosis and/or necrosis of veins.
[b] Red-brown local lesions.

TABLE 3. Effect of alfalfa mosaic virus on yield of Glacier alfalfa clone G4 in 1967[a]

Cutting no. and date	Measurement	Alfalfa plants		Inoculated, % change	Coefficient of variability
		Noninoculated	Inoculated		
First	Dry wt (g)	1,121	744	—30.9*[b]	14.8
10 July	% dry matter	28.8	25.8	—10.4*	4.7
Second	Dry wt (g)	1,019	930	— 8.81	14.7
11 Aug.	% dry matter	30.4	30.5	0	5.4
Third	Dry wt (g)	1,423	1,415	— 0.53	3.1
25 Sept.	% dry matter	21.5	21.3	— 0.93	310.3

[a] Inoculated 9 May 1967; transplanted 16 May 1967.
[b] * = Reduction significant at .05 level.

noninoculated plots were not statistically significant, except that noninoculated plants had greater numbers of leaves per plant than inoculated plants. Number of stems per plant and plant height were reduced 32 and 16%, respectively. The coefficients of variability (CV) were fairly high, and may explain why significant differences were not obtained for these components.

MATERIALS AND METHODS.—*Effect of AMV on alfalfa winterkilling.*—To study the effect of 26 AMV isolates on the winterkilling of alfalfa, 8,400 alfalfa plants were produced in the greenhouse from stock clones. The plants were inoculated when they were approximately 100-120 days old in late July and early August, with inoculum obtained from 26 AMV isolates maintained in sweet clover. The plants were transplanted into the field in September 1966. The plants were placed in rows 9 inches apart, with a 6-inch spacing between plants and 42 plants/row.

In a second winterkilling study, data were taken from the 1967 yield experiment on Glacier alfalfa clone G4 previously described. Each plot contained 60 plants and had 100% stands when observed in November 1967. Final winterkill notes were taken in April 1968, and were based on six replications.

RESULTS.—The amount of killing due to winter conditions varied from 8%, in plants inoculated with AMV isolate 5, to 37%, in plants inoculated with isolate 24 (Table 4). There was 33% winterkilling of noninoculated controls. The frequency of symptom expression varied between virus isolates, ranging from 3 to 48%, depending upon the isolate. There was no apparent association between those plants displaying symptoms and those winterkilled. Thus, on the average, winterkilling occurred as frequently among symptomless plants as among plants showing symptoms.

TABLE 4. Effect of a representative 10 of 26 alfalfa mosaic virus isolates on the winterkilling of alfalfa

Alfalfa mosaic virus isolate	No. inoculated plants	% Alfalfa plants with virus symptoms	% Alfalfa plants winterkilled	% Alfalfa plants with virus symptoms and winterkilled
2	357	11	29	3.6
5	332	7	8	0.9
10	324	10	23	1.5
15	277	7	17	1.6
24	211	19	37	7.1
29	315	20	18	4.1
32	256	48	9	1.6
36	355	41	14	4.5
42	359	12	29	3.3
45	338	3	22	0.3
Control	385	0	33	0
Total and means for 26 isolates	8,046	Mean 18	Mean 18	Mean 3.2

In the 1967 study on Glacier alfalfa, the per cent of winterkilling was quite high, probably because of the lack of snow cover and extended periods of below-seasonal temp. In this experiment, 50% of the plants in the inoculated plots were winterkilled, while 40% of the plants in the noninoculated plots were winterkilled. This difference was not significant.

DISCUSSION.—The large amount of AMV found in Wisconsin alfalfa was expected, but the relatively small percentage of apparently virus-diseased alfalfa plants containing other viruses was not. Alfalfa is inferred to be a reservoir host of the pea enation mosaic virus (9, 13), and this virus, if present, should have been easily identified in the present study. The fact that it was not isolated, however, may help to explain why this disease is not important on processing peas in Wisconsin. The studies which indicated that AMV consisted of many strains agreed with those of Frosheiser (4).

Our experiments were particularly striking in showing that symptomless plants can contain a high percentage of virus-infected plants. Only Mueller (10) has reported virus incidence of this magnitude in alfalfa, but he did not find such high percentages in young Rhode Island alfalfa stands. Gibbs (5) surveyed 68 farms in Great Britain and found that 11% of the plants sampled contained AMV, but suggested that incidence was actually greater than this. He pointed out that the method of sampling is important, since there are probably more infected plants on the perimeter than in the middle of the field, but he did not attempt to outline any sampling procedure. Our procedure included all areas of the fields, and demonstrated that alfalfa stands can become severely infected by the end of the 2nd year. Stands older than 2 years contained considerably more virus than younger stands in two of the three areas studied, while incidence was about the same in the third area.

A possible and likely explanation for the significant yield reductions only in first cuttings of AMV-infected alfalfa is that, as the season progressed, the noninoculated plants progressively became infected with AMV

due to insect spread. There were very few or no AMV-free plants in the noninoculated plots at the end of the season. Also, environmental conditions may have been such that differences did not have a chance to develop. Growing conditions for hay production were quite good during the last half of the 1967 season, which might have obscured possible differences developing primarily during times of stress.

The yield differences obtained in the first 1967 cutting were unexpected because no visible virus symptoms were observed during the season. In contrast, none of the differences for any of the yield components studied were significant. This was expected, since yields were not significantly different in the second and third cuttings.

These experiments indicate that AMV may have a deleterious effect on the yield of alfalfa hay. The virus spread very rapidly in small plots in spite of insect-control efforts. Spread in large fields might be slower than in experimental plots, but may be rapid in fields up to 7 acres in size as indicated by the 90% infection of a 2-year-old commercial field.

Our results indicated no positive relationship between virus infection, as measured by symptoms, and winterkilling of alfalfa. It is significant that 33% of the noninoculated plants were winterkilled. This percentage is quite high when compared to the 18% winterkill-mean for inoculated plants. The data are slightly skewed, in that the control plot contained most of the clones involved (385 out of 400), while some of the virus isolates were inoculated onto sets of clones with fewer plants. These sets were sometimes the less winter-hardy clones which winterkilled nearly 100% in the control. Even so, all data were consistent in indicating no positive relationship between winterkilling of alfalfa and presence of virus symptoms, and agree with those of Frosheiser (4), who found no increase in winterkilling of alfalfa in Minnesota due to AMV infection.

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