Alfalfa Mosaic Virus Transmission to Seed Through Alfalfa Gametes and Longevity in Alfalfa Seed

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ABSTRACT

Direct assay of alfalfa seed on *Phaseolus vulgaris* 'Bountiful' plants was as effective as seedling assay on bean to detect alfalfa mosaic virus (AMV) in alfalfa *(Medicago sativa)* seeds.

AMV was transmitted to alfalfa seeds at a much higher frequency through male gametes (pollen) than through females gametes (ovules). The transmission frequency through pollen in all tests ranged from 0.5 to 26.5% and transmission through the ovules ranged from 0 to 9.5%. Transmission through both pollen and ovules was much less at a constant temp of 29 ± 1 C than at 18 ± 2 C or 24 ± 2 C. Transmission at alternating temp of 29 C and 22 C was greater than at 29 C but less than at 18 C and 24 C constant.

AMV seed transmission differed considerably among AMV strains and in different tests, and somewhat among alfalfa clones.

The percentage of virus-infected seeds was not reduced significantly in a seed lot containing about 20% infected seeds after storage for 5 yr at −18 C, 4 C, or 21-27 C. The percentage of infected seeds in two commercial seed lot samples, stored under refregeration for 4 yr, also was not reduced appreciably.

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Alfalfa mosaic virus (AMV) in alfalfa (*Medicago sativa* L.) is widely distributed and is present in most alfalfa fields. As the age of the stands increases, the incidence of infected plants increases; up to 80% infected plants have been found in 4-yr-old fields (3, 7, 11). Limited studies have shown that AMV may reduce forage yield (5, 10).

AMV transmission through alfalfa seed was reported in Italy (1) and Germany (12) in 1962 and in the U.S.A. in 1964 (2). Belli (1) found 2.1% infected seed in a 'Vernal' seed lot and 0.8% in a 'California Common' seed lot produced in the U.S.A. and 2.2-5% infected seeds in varieties grown in Italy. I assayed 56 alfalfa seed lots from the seed-producing areas of western U.S.A. (6) and found no infected seeds in eight seed lot samples. The other 48 seed lot samples contained 0.2-6% infected seeds. Hampton (8, 9) found that several viruses were readily transmitted through seed of red clover (*Trifolium pratense* L.).

After crossing an alfalfa plant infected with a local lesion AMV strain with a plant infected with a strain causing systemic symptoms in bean, I detected the presence of both strains in the seed from the cross by assaying the alfalfa seedlings on 'Bountiful' bean *(Phaseolus vulgaris* L.) plants. The results indicated that AMV could be transmitted through both the male (pollen) and female (ovules) gametes.

The results of studies made to compare transmission through pollen and ovules, the effect of temp on transmission frequency and the longevity of the virus in alfalfa seeds are reported in this paper.

MATERIALS AND METHODS.—Alfalfa plants were crossed in the glasshouse or growth room to provide the seed used for AMV transmission studies. Individual plants were grown in a loam-sand mixture in 13-cm clay pots. Except where stated otherwise, the crosses were made and seed produced at about 21 C, with supplemental fluorescent light to provide a 16-h light period.

The AMV strains were obtained from naturally infected alfalfa plants and were maintained in alfalfa plants in isolation in a glasshouse.

For pollen transmission studies, pollen from AMV-infected plants was transferred to virus-free plants, and for ovule transmission studies, the procedure was reversed. The flowers of normal female plants were emasculated to prevent contamination due to selfing, or male-sterile plants were used. The flowers were emasculated by clipping the standards before tripping the flowers. After tripping, the pollen and anthers were removed by suction; the flowers then were dipped in 57% ethyl alcohol for 5 s and rinsed in distilled water. Pollen from another plant was transferred to the stigma 15-30 min after rinsing.

Because AMV is usually masked in alfalfa (5), seedling infection was detected by assaying the seedlings on Bountiful bean leaves. The seedlings were grown in an insect-free growth room at 21 C and assayed on bean when the seedlings were in the 2-3 trifoliolate leaf stage. Alfalfa shoots 3-6 cm long were folded between forceps and crushed on glass slides. The expressed juice was then gently rubbed on unifoliolate bean leaves, usually one leaf per seedling, previously dusted with 600-grit silicon carbide.

AMV was also detected in alfalfa seeds by direct seed assay. The scarified seeds were placed on wet filter paper in petri dishes in a refrigerator, usually overnight, until used. Individual swollen seeds were crushed in a droplet of 0.5% $Na_2HPO_4 \cdot 7H_2O$ on a glass slide with a glass spatula and then rubbed on silicon carbide-dusted Bountiful bean leaves, one seed per leaf. After each seed was applied, the spatula was rinsed in water, then in alcohol, and flamed.

The inoculated bean plants were incubated in the glasshouse at about 24 C. Local lesions appeared in 2-4 days and systemic infection was detected in 6-10 days.

RESULTS AND DISCUSSION.—*Comparison of seed and seedling assays.*—Two seed lots, each containing

TABLE 1. Alfalfa mosaic virus (AMV) detection in seed by direct seed and seedling assay on 'Bountiful' bean

AMV strain	Method	Transmission (%)	Seeds or seedlings tested (no.)
F1	Seed assay	20.8	144
F1	Seedling assay	21.6	134
U21	Seed assay	22.5	142
U21	Seedling assay	20.4	142

TABLE 2. Alfalfa mosaic virus (AMV) transmission through pollen and ovules in crosses involving infected and noninfected alfalfa plants

Cross[a]	Seedlings infected with AMV		
	Transmission through pollen (%)	Transmission through ovules (%)	Seedlings tested (no.)
U6/1249(E) × 5		1.6	185
U6/1249(E) × 247		2.1	334
5 × U6/1249	6.1		346
247 × U6/1249	9.5		296
D8/20 × 5		0.3	338
D8/20 × 247		0.6	307

[a] Number alone identifies the clone. Numerator prefixed with a letter denotes the AMV strain with which the clone (denominator) is infected. 20 is the male-sterile clone 20 DRC. Other clones are normal. (E) = Emasculated.

about 20% infected seeds were assayed on bean leaves; both the direct seed assay and the seedling assay were used. One seed lot contained seeds infected with AMV strain F1 that causes local lesions on bean leaves. The second lot contained seeds infected with AMV strain U21, which is systemic in bean plants. The percentage of infected seeds detected was essentially the same in both assay methods (Table 1). The small differences were most likely due to the distribution of the infected seeds in the seed lots.

Pollen and ovule transmission.—Earlier studies established that AMV could be transmitted through both pollen and ovules. To determine the transmission frequency through ovules, I emasculated the flowers of clone 1249 infected with AMV strain U6 (U6/1249) and pollinated them with pollen from virus-free plants of clones 5 and 247. In addition, I also pollinated plants of the male sterile clone 20 infected with AMV strain D8 (D8/20) with pollen from healthy plants of clones 5 and 247. To determine the transmission frequency through pollen, I pollinated virus-free plants of clones 5 and 247 from U6/1249. The seedling assay was used to detect infected seeds. The transmission frequencies through pollen and ovules are given in Table 2. AMV transmission was about four times greater through pollen than through ovules. Transmission through the ovules of D8/20 was very low.

Further experiments were performed with other AMV strains and different clones. The male-sterile clone (20) was used to eliminate the need for emasculation. Two normal clones (5 and 1348) were used as the pollen parents and four AMV strains (U21, F1, D8, and R2) were used in a number of combinations (Table 3). Strains F1 and D8 cause local lesions in inoculated Bountiful bean leaves and strains U21 and R2 are systemic in plants of this variety.

Percentage of transmission varied among virus strains. F1 and U21 had a much higher transmission frequency than D8 and R2. Transmission of R2 was very low and transmission of D8 was intermediate between the high and low strains. In the first experiment, transmission through ovules of D8/20 was greater than through the pollen from D8/5. In the second experiment transmission was five times greater through the pollen in this same strain and clone combination. Evidently the condition of the parent plants had a great effect on transmission frequency. With a few exceptions, pollen transmission was much greater than ovule transmission in these two experiments. Pollen transmission ranged from 0.5 to 26.5% and ovule transmission ranged from 0 to 7.7%.

Effect of temp on AMV seed transmission.—Plants of clones 5 and 1348 infected with AMV F1 and AMV U21 were crossed on virus-free plants of clone 20, and virus-free plants of clones 5 and 1348 were crossed on plants of

TABLE 3. Alfalfa mosaic virus (AMV) transmission through pollen and ovules in crosses between a male-sterile and normal clones

Cross[a]	Seedlings infected with AMV (%)			
	First experiment[b]		Second experiment[c]	
	Transmission through ovules	Transmission through pollen	Transmission through ovules	Transmission through pollen
20 × U21/1348		7.6		13.5
20 × U21/5		25.7		9.7
20 × F1/1348		–[d]		11.2
20 × F1/5		26.5		7.9
20 × D8/1348		–		4.2
20 × D8/5		2.1		4.7
20 × R2/1348		–		0.5
20 × R2/5		–		0.9
U21/20 × 1348	–		2.8	
U21/20 × 5	–		6.5	
F1/20 × 1348	–		3.3	
F1/20 × 5	–		7.5	
D8/20 × 1348	6.3		0.9	
D8/20 × 5	4.2		0.9	
R2/20 × 1348	–		0.5	
R2/20 × 5	–		0.0	
D8/20 × U21/1348	7.7	5.6	–	–
D8/20 × U21/5	4.2	18.9	–	–
Avg	6.1	14.4	2.8	6.6

[a] Number alone identifies the clone. Numerator prefixed with a letter denotes AMV strain with which the clone (denominator) is infected. 20 is the male-sterile clone, other clones are normal.

[b] Number of seedlings assayed first experiment, 143-144 per cross.

[c] Number of seedlings assayed second experiment, 213-216 per cross.

[d] Dash indicates that the cross was not made.

clone 20 infected with AMV F1 and AMV U21 at temp of 18 ± 2 C, 24 ± 2 C, 29 ± 1 C, and alternating at 29 C for 16 hr and 22 C for 8 h daily. The plants were placed at the respective temp immediately after being clipped and remained at the temp until the seed was mature.

The plants held at 18 and 24 C were grown in separate glasshouses in late winter when temp could be controlled. Daylight was supplemented with fluorescent light to maintain 16-h light periods. Plants grown at 29 C and at alternating temp were grown in growth rooms under fluorescent lights with a 16-h light period. The seed was assayed directly on bean.

Except for the cross 20 × U21/1348, the transmission was similar at 18 and 24 C (Table 4). The transmission frequency was sharply reduced at 29 C. Transmission at the alternating temp increased over that at 29 C, but remained considerably lower than at 18 and 24 C. In addition to temp, the natural light to which the plants at 18 and 24 C were exposed may have affected the transmission frequency.

After the seed was harvested in all the experiments, the virus-free parents were clipped and the new growth was assayed for virus infection. All plants initially virus-free remained virus-free.

AMV survival in alfalfa seed.—An alfalfa seed lot containing about 20% AMV-infected seeds was divided into three lots and stored in coin envelopes within polyethylene bags at −18 C, 4 C, and in the laboratory (21 to 27 C). Seeds were removed from storage and assayed on bean by the seedling method after 0.5, 1, 2, 3, 4, and 5 yr of storage. The number of seedlings tested from each storage temp, each time, varied from 97 to 194, but the numbers usually ranged from 120 to 145. Although the percentage of infected seedlings varied considerably, there was no significant decrease of virus activity in the seeds during the 5 yr of storage (Table 5). No decrease in germination was apparent during the storage period.

Two commercial seed lot samples, one containing 6.0 and the other 5.8% AMV-infected seeds, were stored under refrigeration for 4 yr. At the end of the storage period, the seed lots contained 5.1 and 3.4% infected seeds, respectively.

Some of the variations in seed transmission frequency in these studies were due to effects of AMV strain, temp, and variability among alfalfa clones. The causes for many of the variations are unknown. The condition of the parent plants apparently has a great effect on the incidence of seed infection. To define these conditions, we need more precise and controlled experimentation.

The relatively high incidence of AMV-infected seed lots and the longevity of the virus in the seeds suggest that infected seeds are an important factor in the worldwide distribution of AMV and are the initial source of AMV-infected plants in many alfalfa stands. The incidence of infected plants increases very rapidly under heavy infestation by pea aphids [*Acyrthosiphon pisum* (Harris)] (11). As the incidence of infected plants in the seed field increases, the incidence of AMV-infected seed produced in the field also increases. This points out the necessity for using virus-free parent plants to produce the first generation seed of a variety. Alfalfa plants often become infected with AMV without any evident symptoms after several seasons in field nurseries. Asexual progeny of AMV-infected clones are always infected under usual methods of propagation. If these clones are used as parents, the likelihood of considerable AMV-infected seeds in the resultant seed is very great. Therefore, virus free plants should always be used as parental stock for first generation seed production. This, combined with adequate insect control in seed fields, should reduce AMV-infected alfalfa seeds to a minimum. A method for freeing alfalfa clones from AMV has been described (4).

TABLE 4. Percentage of alfalfa mosaic virus (AMV)-infected seed produced at four temperature regimes

Cross[a]	Temperature and % infected seed			
	18 C	24 C	29 C	29/22 C[b]
20 × F1/5	18.5	20.6	2.7	10.4
20 × F1/1348	12.9	14.8	1.4	4.8
20 × U21/5	13.7	–[c]	4.0	8.4
20 × U21/1348	22.1	4.8	0.0	2.0
F1/20 × 5	5.5	2.8	0.0	1.4
F1/20 × 1348	3.4	3.4	0.6	0.3
U21/20 × 5	9.9	8.1	2.0	–
U21/20 × 1348	7.6	5.5	0.7	–

[a] Number alone identifies the clone. Numerator prefixed with a letter denotes the AMV strain with which the clone (denominator) is infected. 20 is the male-sterile clone; other clones are normal.

[b] 29 C plus light for 16 h and 22 C, dark for 8 h.

[c] Dash indicates that the cross was not made.

TABLE 5. Percentage alfalfa mosaic virus (AMV)-infected seedlings from an alfalfa seed lot stored at three temperatures for various periods of time[a]

Storage period, (yr)	Storage temperatures and percentage of infected seedlings		
	21-27 C	4 C	−18 C
0.5	17.6	19.9	23.5
1	22.7	16.9	22.3
2	20.0	16.5	22.7
3	19.6	16.0	14.7
4	19.5	17.8	12.4
5	18.0	18.1	20.5

[a] The seed lot contained about 20% infected seeds when placed in storage.

LITERATURE CITED

1. BELLI, G. 1962. Rilievi ed esperienze sulla transmissione per seme del virus del mosaico dell'erba medica e dimostrazione della sulla esclusione in cloni vite virosati. Ann. Fac. Agric. di Milano. Vol. 10 (1961). 15 p.
2. FROSHEISER, F. I. 1964. Alfalfa mosaic virus transmitted through alfalfa seed. Phytopathology 54:893 (Abstr.).
3. FROSHEISER, F. I. 1964. Prevalence of alfalfa mosaic virus in alfalfa in Minnesota. Plant Dis. Rep. 48:506-507.
4. FROSHEISER, F. I. 1969. Freeing alfalfa clones from alfalfa mosaic virus by heat treatment. Phytopathology 59:391-392.
5. FROSHEISER, F. I. 1969. Variable influence of alfalfa

mosaic virus strains on growth and survival of alfalfa and on mechanical and aphid transmission. Phytopathology 59:857-862.
6. FROSHEISER, F. I. 1970. Virus-infected seeds in alfalfa seed lots. Plant Dis. Rep. 54:591-594.
7. GIBBS, A. J. 1962. Lucerne mosaic virus in British lucerne crops. Plant Pathol. 11:167-171.
8. HAMPTON, R. O. 1967. Seed transmission of viruses in red clover. Phytopathology 57:98 (Abstr.).
9. HAMPTON, R. O., and E. W. HANSON. 1968. Seed transmission of viruses in red clover: Evidence and methodology of detection. Phytopathology 58:914-920.
10. HENSON, L., and S. DIACHUN. 1957. Effect of a strain of alfalfa mosaic virus on the yield of clonally propagated Atlantic alfalfa. Phytopathology 47:15 (Abstr.).
11. MUELLER, W. C. 1965. Progressive incidence of alfalfa mosaic virus in alfalfa fields. Phytopathology 55:1069 (Abstr.).
12. ZSCHAU, K. VON, and C. JANKE. 1962. Samen Übertragung des Luzernemosaicvirus an Luzerne. Nachrichtenblatt fur den Deutschen Pflanzschutzdienst. 16:94-96.