# Gamete-Seed Transmission of Alfalfa Mosaic Virus and Its Effect on Seed Germination and Yield in Alfalfa Plants

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ABSTRACT

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The percentage of alfalfa mosaic virus (AMV)-infected seeds was determined in the commercial production of seven different cultivars of alfalfa. Seed transmission of AMV ranged from 0.6 to 10.3%. The transmission of AMV through pollen and the ovules of the infected alfalfa plants was shown. Ovule transmission ranged from 0.5 to 6.0% and pollen transmission varied from 1.0 to 14.0%. Under greenhouse conditions, there was a reduction of 30.8-34.6% in germination and a reduction of 45.1-68.6% in yield of seed by AMV-infected alfalfa. No systemic infection from the infected pollen in the normal or male-sterile female parents was detected. The susceptibility of two different cultivars of alfalfa, Sonora 70 and Mesa Sirsa 034, to AMV is compared.

Alfalfa mosaic virus is a widespread and truly cosmopolitan virus that attacks alfalfa (*Medicago sativa* L.) and several other crops (1, 2, 3, 13, 17, 18, 19). Its existence has been known since 1931 (21).

In 1962, Belli (1) reported for the first time that AMV was transmitted through the seeds of alfalfa. Zschau (22) studied two strains of AMV and showed both to be seed-transmissible. Frosheiser found 1-4% seed transmission of AMV in 13 of 15 seed lots of alfalfa tested (6). He recently reported 0.5 to 26.5% transmission of AMV through pollen and 0.0 to 9.5% transmission through the ovules of infected alfalfa clones (8).

Several reports relate to the economic importance of AMV and its effect on dry- or fresh-matter yields of alfalfa plants. The conclusions of various investigators relative to the economic importance of AMV are quite contradictory (5, 7, 9, 10, 12). Zschau (22) reported that latent AMV infection generally damaged alfalfa less in the vegetative stage than in the reproductive stage, thereby affecting seed yield. However, he did not report any actual loss data.

In this paper, the results of AMV transmission through the seeds and gametes of different cultivars of alfalfa are compared. Also, the effect of AMV infection on seed germination and seed yield is discussed.

## MATERIALS AND METHODS

Tests for seed transmission of AMV were conducted with seven different cultivars of alfalfa in a greenhouse at 24-26 C and 70% relative humidity (RH). Alfalfa seeds from new crops were obtained from the Cooperative Extension, Agronomy, University of California, Davis. Three-hundred seeds of each cultivar were planted in steam-sterilized metal pots. Four-wk-old seedlings of alfalfa were used for virus assay. The seedling infection was detected by assaying the seedlings on cowpea (*Vigna sinensis* L.) (2) and on Red Kidney bean (*Phaseolus vulgaris* L.) as the test plants. This was done because of masking of AMV symptoms in some cultivars of alfalfa (2, 4, 8). Inoculum for mechanical inoculation was prepared from each alfalfa seedling by grinding it in a sterile mortar and pestle with a few milliliters of 0.05 M potassium phosphate buffer, pH 7.2. The prepared inoculum was used to inoculate six four-leaved cowpea and Red Kidney bean plants. Prior to inoculation, the leaves of these plants were dusted with corundum powder. Between 140 and 159 seedlings of each alfalfa cultivar were randomly assayed and the percentage of seed transmission was noted (Table 1).

Ovule and pollen transmission studies were done with two cultivars of alfalfa, Sonora 70 and Mesa Sirsa 034. Virus-free alfalfa plants were obtained by the assaying technique described above. Infected plants were obtained by inoculating alfalfa seedlings with a purified AMV isolate at a concentration of 0.17 mg/ml. One wk after inoculation, the alfalfa seedlings were assayed and infected plants were chosen for cross experiments.

The AMV isolate was originally obtained from naturally infected alfalfa plants and was maintained in tobacco (*Nicotiana tabacum* L. 'Havana') in an isolated greenhouse. The virus was purified by a method similar to that described by Gillaspie and Bancroft (11). This

method has been used routinely by other investigators (3, 14). The isolate was identified on the basis of its serological reaction with AMV-antiserum, particle morphology (20), and symptomatology. The isolate produced local lesions on bean and cowpea. An extinction coefficient of 5.2 $cm^2/mg$ was used to determine quantity of AMV in the preparation (15).

Fifteen healthy and 15 infected alfalfa plants, all of equal age and planted in separate 15-cm diameter clay pots, were used for each cross experiment. All possible crosses except crosses No. 3 and 7 (Table 2) were made simultaneously. The cross pollinations for each cultivar were made in a separate greenhouse, but under rather similar conditions. The seed was produced at 24-26 C, under supplemental fluorescent light on a 16-hr photophase. Each of the cross pollinations were made under 2 × 1 × 0.75-m cages in an attempt to prevent pollen contamination from other plants. Alfalfa leaf cutter bees (*Megachile rotundata* Fab.) were used for pollination during the flowering period of the plants. Fifty bees were placed in each cage. For ovule transmission studies, the flowers were emasculated by clipping the standards before pollination could occur. This was done using a method similar to that described by Frosheiser (8).

To determine accurately the transmission frequency of AMV through gametes, another clone of alfalfa (AA 1207-white flower) was used. This clone was used as the malesterile female parent and the difficulties associated with emasculation were eliminated. The possible crosses were made during the flowering periods of the alfalfa plants using the two cultivars, Sonora 70 and Mesa Sirsa 034 (Table 3). Alfalfa leaf cutter bees and seedling assays were used as in the previous experiments. In the above experiments, the healthy mother plants were assayed three times at 1-mo intervals to determine if there was any systemic infection from the infected pollen.

For seed germination studies, 1,000 seeds from each of the cross pollinations were chosen randomly and were planted in 10 replications in steam-sterilized metal pots. Germination tests for both cultivars were done at the same time and under rather similar conditions.

An analysis was conducted to determine if statistical differences existed between yield or germination of each cultivar related to the effects of virus infection (Table 2). The unpaired "Student's" *t*-tests (between two comparable distributions) were used. Except where otherwise stated, differences discussed in relation to the tests were statistically significant at either $P = 0.05$ or $P = 0.01$. Although the cross-pollination tests for both alfalfa cultivars, Sonora 70 and Mesa Sirsa 034, were done

TABLE 1. Transmission of alfalfa mosaic virus (AMV) in commercially grown alfalfa seed

| Cultivar | Transmission (%) |
|---|---|
| Sonora 70 | 0.6 |
| Mesa Sirsa 034 | 6.3 |
| Lahonton 799 | 1.3 |
| Moopa 69 (112) | 6.7 |
| El Unica (1148) | 5.4 |
| Niogaro N-71 | 10.3 |
| Caliverde | 8.2 |

TABLE 2. Alfalfa mosaic virus (AMV) transmission through pollen and ovules in cross pollinations between infected and noninfected alfalfa plants and concomitant effects of virus infection on seed germination and seed yield

| Cross No. | Cross Description[a] | Transmission[c] | Germination[d] (%) | Seed per plant[e] (g) |
|---|---|---|---|---|
| 1 | H[b] × H(M.S.) | 0.0 | 94f | 1.37h |
| 2 | H × I(M.S.) | 10.1 | 90f | 1.21h |
| 3 | EI × H(M.S.) | 4.0 | 86f | ...[f] |
| 4 | I × I(M.S.) | 12.0 | 65f | 0.43h |
| 5 | H × H(S) | 0.0 | 98g | 2.99i |
| 6 | H × I(S) | 1.0 | 95g | 2.81i |
| 7 | EI × H(S) | 0.5 | 89g | ...[f] |
| 8 | I × I(S) | 2.0 | 64g | 1.64i |

[a]Abbreviations: H = healthy; I = infected; E = emasculated; M.S. = cultivar Mesa Sirsa; and S = cultivar Sonora.

[b]Used as mother plants for seed preparation.

[c]Number of seedlings assayed: 145-150 per cross for Mesa Sirsa and 200 per cross for Sonora.

[d]Mean percent germination. Means numbers followed by the same letter are statistically different from each other according to unpaired "Student's" *t*-tests that were made between each of the two comparable distributions ($P = 0.05$, $P = 0.01$).

[e]Mean weight (g) of seeds per plant. Means numbers followed by the same letter are statistically different from each other according to unpaired "Student's" *t*-tests that were made between each of the two comparable distributions ($P = 0.05$, $P = 0.01$).

[f]No data available.

TABLE 3. Alfalfa mosaic virus transmission through pollen and ovules in crosses between infected and noninfected alfalfa plants

| Cross[a] | Seedlings assayed (no.) | Transmission through ovules (%) | Transmission through pollen (%) |
|---|---|---|---|
| HA[b] × H (M.S.) | 100 | 0.0 | 0.0 |
| IA × H (M.S.) | 100 | 6.0 | N/A |
| HA × I (M.S.) | 100 | N/A[c] | 14.0 |
| HA × I (S) | 100 | N/A | 2.0 |

[a]Abbreviations: H = healthy; I = infected; A = AA 1207, a male-sterile female parent; M.S. = cultivar Mesa Sirsa 034; and S = cultivar Sonora 70.

[b]Used as mother plants for seed preparation.

[c]N/A = not applicable.

TABLE 4. Observations[a] of the effect of alfalfa mosaic virus (AMV) on two cultivars of alfalfa

| Tests | Alfalfa cultivars: Mesa Sirsa 034 (%) | Sonora 70 (%) |
|---|---|---|
| Pollen transmission | 10.1-14.0 | 1.0-2.0 |
| Ovule transmission | 4.0 | 0.5 |
| Reduction in seed yield | 68.6 | 45.1 |
| Reduction in seed germination | 30.8 | 34.6 |
| Infection from AMV isolate | 100.0 | 60.0 |

[a]The data were obtained from different experiments and were not subjected to statistical analysis.

simultaneously and under similar conditions but in separate greenhouses, owing to our knowledge of relative variations (coefficient of variation) statistical comparisons were not made between the data for seed yield or germination of these two cultivars. The data shown in Table 4 are based, therefore, on observations.

## RESULTS

The inoculated test plants developed local lesions within 4 or 5 days. In some cases, systemic infections appeared in 10-15 days after inoculation. Mechanical inoculations using the purified AMV isolate gave 100% infection in Mesa Sirsa and 60% infection in Sonora under similar conditions.

Seed produced by seven common cultivars of alfalfa obtained from Fresno County, California, was infected with AMV. The percentage of seed transmission ranged between 0.6 and 10.3% (Table 1). The seeds were samples from commercial seed production fields infected with AMV. However, there were no data on the level of AMV infection in the fields from which the seeds were collected.

Alfalfa mosaic virus was transmitted through both pollen and ovules. Percentage of transmission varied between two different cultivars of alfalfa tested. Ovule transmission ranged from 0.5 to 6.0% and pollen transmission ranged from 1.0 to 14.0% (Tables 2, 3, 4). In these experiments, no systemic infection from the infected pollen was detected in the normal and male-sterile female parents. Apparently the infected pollen causes only the resulting seed to become infected and not the healthy pollinated plant.

Under greenhouse conditions, the virus infection greatly reduced seed germination and yield. A percentage reduction of 30.8 - 34.6 in seed germination and a percentage reduction of 45.1 - 68.6 in seed yield was observed when 100% of the plants were infected (Tables 2, 4). The statistical analysis revealed these significant reductions ($P = 0.01$) by comparing the data for crosses 1 and 4 of Mesa Sirsa and for crosses 5 and 8 of Sonora (Table 2). These effects were associated with poorly developed flowers and seeds.

## DISCUSSION

The detection of AMV infection in the commercial seed production of common cultivars in California and the results of other investigations (5, 8) show that infected seeds play an important role in the epidemiology of AMV and may be the primary source of inoculum in the new alfalfa-growing areas. Therefore, the production of virus-free seeds should be viewed seriously.

Transmission of AMV occurred through both male gametes (pollen) and female gametes (ovules) of the infected alfalfa plants. However, the rate of transmission through the ovules was much lower than through pollen. This is a confirmation of the results reported earlier for other alfalfa clones (8).

The economic importance of AMV in alfalfa has been considered minor. However, the results here show 45.1-68.6% reduction in seed yield and 30.8-34.6% reduction in seed germination when 100% of the plants were infected. In addition to being a seed-borne pathogen in alfalfa and several other crops (13, 19), AMV also has a wide host range (2), numerous strains or variants (2, 3, 5, 7, 16, 22), is effectively transmitted by aphids (2, 3, 7), and has overwintering hosts such as alfalfa and sugar beet (18); these facts indicate that it has the potential to become a more important pathogen in the future.

There are significant statistical differences ($P = 0.05$) in seed yield and germination between crosses 1 and 2 for Mesa Sirsa and also between crosses 5 and 6 for Sonora. It appears that the pollen from the infected plants is the reason for these differences. Since AMV affects the mother plants during the propagation stage (5, 7, 9, 10, 22) it is possible that the gametes also are affected. This adverse effect on the gametes is probably responsible for the high reduction in seed yield and germination.

Based on the data in Table 4, Sonora appears to be less susceptible to AMV than Mesa Sirsa. The low rate of AMV transmission through the seed of Sonora and lower infection from mechanical inoculation produced results to support this possibility.

In cross experiments, the utilization of alfalfa leaf cutter bees has provided for a natural pollination. As a result, the possibility of abnormalities that might have occurred if hand pollination was utilized were reduced.

Although the results of this research indicate significant reduction in seed yield and germination, more comprehensive yield studies should be conducted to determine the economic significance of AMV in alfalfa and other susceptible crop hosts.

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