# Incidence of Viruses on Alfalfa in Western North America

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**ABSTRACT**

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Enzyme-linked immunosorbent assay was used to detect viruses in alfalfa leaf tissue samples taken from 216 fields at 44 widely separated locations in California, Idaho, Oregon, and Washington and southern British Columbia. Samples were collected between April 1987 and May 1988. Alfalfa mosaic virus (AMV) was found at all locations and in all but one field. Pea streak virus and pea (bean) leafroll virus were detected at 38 locations. Tobacco streak virus was found only in Idaho and Washington at seven locations, and red clover vein mosaic virus at 11 locations in the four states, but not in British Columbia. Pea seedborne mosaic virus and other members of the potyvirus group were not detected at any location. Percent of samples with AMV, pea streak virus, pea leafroll virus, tobacco streak virus, and red clover vein mosaic virus were 86, 21, 28, 4, and 3, respectively. The data indicate a higher incidence of AMV than has previously been reported over a wide geographical area. Furthermore, up to four viruses were in individual samples from several fields. Incidence of AMV in fields was not highly correlated with age of stand ($r = 0.44$), but generally fields 4–6 yr old contained 80% or more infected samples. Assays for pea enation mosaic virus indicated that this virus was in alfalfa samples, but these data were not included, because of variability in the reactions obtained with the infected control samples. Differences in percent infection were associated more with geographic location than with cultivar.

Additional keywords: alfalfa viruses

Numerous viruses in alfalfa have been reported in the United States and Canada as well as elsewhere in the world (2,3, 5,7,8,10,15,18,24). Relative incidence of viruses harbored by alfalfa in Ontario and Quebec in 1980–1981 was reported by Paliwal (20). Another study (15), conducted in 11 southeastern states, involved seven forage legume species, including alfalfa, but was restricted to small experimental plots and did not include grower fields.

Previous studies have shown that alfalfa mosaic virus (AMV) causes reductions in forage and seed yields, regeneration potential, winter survival, crude protein content, nodulation, and nitrogenase activity (1,11,12,17,19,23). Germination of seed produced on AMV-infected alfalfa plants was 31–35% less than that of seed produced on healthy plants, and seed yield per plant was 45–69% less on infected plants of two cultivars (11). A study in the United Kingdom revealed that AMV caused reductions of 15–23% in fresh weight and 15–18% in dry weight of forage of five alfalfa cultivars tested (1). AMV has a very wide host range and is known to cause damage to other crops as well. Many viruses found in alfalfa, such as pea streak virus (PSV), pea enation mosaic virus (PEMV), pea (bean) leafroll virus (PeLRV), and tobacco streak virus (TSV), are causal agents of severe diseases on other economically important crops (2,5,6,8–10,13,22). Burke (3) demonstrated that almost all 3-yr-old alfalfa plants tested from 12 cultivars contained unidentified viruses that were infectious to and produced symptoms on Stringless Green Refugee beans. Hampton (8) reported the occurrence of PSV in all fields of 5-yr-old alfalfa plantings surveyed in the Pacific Northwest and Montana. Kaiser found that chickpeas suffered significant losses when infected with isolates of AMV in experimental plots in Washington State (13). In California, AMV has been reported to cause stunting, bronzing, necrosis, and death of tomato plants in the field (14).

Because of the limited number of samples or limited areas in the cited literature, a survey was designed and set up to obtain and test samples from many fields over a large geographical area of the western United States and in British Columbia. On the basis of suggestions in the cited literature and on consultation with G. I. Mink, Prosser, Washington, the viruses chosen were AMV, PSV, PEMV, PeLRV, red clover vein mosaic virus (RCVMV), pea seedborne mosaic virus (PSbMV), and other nonspecific members of the potyvirus group.

## MATERIALS AND METHODS

Arrangements were made with county extension agents and researchers in 44 widely separated locations to assist in selecting and, in many instances, sampling 216 fields in Washington, Oregon, Idaho, California, and British Columbia. A total of 10 samples consisting of terminal portions of young alfalfa shoots, from individual plants, were collected in a preestablished pattern in each field. The basic pattern consisted of eight samples along a line that was one fourth the width or diameter of the field from the perimeter and two samples from a line through the center of the field. All distances were visually estimated. Generally, five fields (a minimum of four and a maximum of seven) were sampled in each geographic location. Most sampling was completed between April and November 1987. Two locations in California (El Centro and Blythe) were sampled in April 1988. The age of stands and cultivars grown were recorded where information was available. Samples were transported in plastic bags over ice in insulated chests to the laboratory at Prosser, Washington, where each sample was cut into small sections (~5 mm) with a new razor blade, dried over anhydrous calcium sulfate, and stored in Ziploc plastic bags at 4 C.

**Serology.** Enzyme-linked immunosorbent assay (ELISA) was used to detect the presence of AMV, PSV, PeLRV, TSV, PEMV, RCVMV, PSbMV, and members of the potyvirus group in the alfalfa samples. Procedures and chemical preparations were similar to those described by Clark and Adams (4). Double-antibody sandwich (DAS)-ELISA was used for AMV, PSV, RCVMV, and PeLRV, and indirect ELISA was used for detection of PEMV, PSbMV, TSV, and the potyviruses. Antiserum against AMV was provided by O. W. Barnett, Clemson University, Clemson, South Carolina; against TSV, by W. J. Kaiser, USDA-ARS, Washington State University, Pullman; against PEMV and PSbMV, by G. I. Mink, Washington

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State University; potyvirus monoclonal antibody, by AGDIA, Inc.; and antisera against PSV, RCVMV, and PeLRV, by R. O. Hampton, USDA-ARS, at Oregon State University, Corvallis. Sodium diethyldithiocarbamate trihydrate was added (0.45%) to the grinding buffer for each virus except PeLRV.

Enzyme substrate reactions were measured at 405 nm in the 96-well Falcon 3912 microtest flexible assay flat-bottomed plates (Becton Dickinson and Co., Lincoln Park, NJ), using the Bio-Tek EL 307 EIA reader (Bio-Tek Instruments, Inc., Winooski, VT), or in 40-well Gilford plates in an EIA processor/reader (Gilford Instrument Laboratories, Oberlin, OH). Controls consisted of buffer, healthy, and virus-infected plant tissue. Virus detection thresholds were established as light absorbance values at least three times those of healthy controls at 405 nm, with the infected control at an absorbance of 0.3 or higher. Samples in plates not meeting this criteria were retested. Two wells were used for each sample.

**Greenhouse bioassay.** Samples from Goldendale, Washington, and Hermiston, Oregon, were ground in 0.01 M phosphate buffer (pH 8.0), using mortars and pestles, and then inoculated on indicator host plants with cotton swabs that were predusted with 600-mesh Carborundum. Indicator plants were *Phaseolus vulgaris* L. 'Bountiful,' *Vigna unguiculata* (L.) Walp. 'California Blackeye,' *Chenopodium quinoa* Willd., and *Cucurbita pepo* L. Plants were grown in the greenhouse at 24 C under 16 hr of fluorescent light. Symptoms including veinclearing, veinbanding, systemic chlorotic lesions, leaf curl, vein necrosis, leaf drop, mosaics, stunting, localized sunken or necrotic lesions, and ring spots were recorded. This data, however, will not be presented in this paper.

## RESULTS AND DISCUSSION

Age of stands ranged from 1 to 18 yr, with about 75% of those fields 3–5 yr old. AMV was detected in 84.9% of the samples, PeLRV in 26.8%, PSV in 21.3%, RCVMV in 2.9%, and TSV in 2.2%. Neither PSbMV nor other potyviruses were detected in samples from any of the locations. AMV was detected from samples collected in all but one field, a 5-yr-old field located near Ellensburg, Washington. The number of locations and fields in which the other four viruses were found were as follows: PSV at 38 locations (126 fields), PeLRV at 38 (145 fields), TSV at seven (16 fields), and RCVMV at 11 (29 fields). Assays for PEMV indicated this virus was present, but the data will not be presented, because of variability in the reactions obtained with the infected control samples.

The month during which each location was sampled and percentages of alfalfa samples infected with the viruses included in the survey are listed in Table 1. The figures are averages of all samples from all fields at a given location. The incidence of PSV at different locations varied from 0–74%; TSV, 0–87%; RCVMV, 0–23%; and PeLRV, 0–79%. Two locations in western Washington showed a much higher incidence of TSV (Puyallup and Coupeville, at 87 and 74%, respectively) than did five others at which it was detected (between 2 and 8%). The difference is probably related to a greater prevalence of small fruits and other leguminous plants used in pastures. AMV as a single virus was found in 21 fields. Alfalfa with mixed infections include AMV and one additional virus in 92 fields, AMV and two other viruses in 84 fields, and AMV plus three other viruses in 17 fields. There were no fields in which plants were infected with five viruses. Coincidence of AMV with PeLRV occurred in 24.3% of the samples; of AMV with PSV, in 18.5% of the samples; of AMV with TSV, in 2.5% of the samples; and of AMV with RCVMV, in 2.0% of the samples. Four viruses (AMV, PSV, RCVMV, and PeLRV) were detected in four samples. Three viruses, consisting of all possible groups of three among AMV, PSV, TSV, RCVMV, and PeLRV, were detected in 7% of the samples. AMV alone was found in 41% of

**Table 1.** Percentage of virus-infected alfalfa samples from survey in western North America as detected by enzyme-linked immunosorbent assay

| State or province Sampling site | Month[a] sampled | Virus[b] AMV | PSV | TSV | RCVMV | PeLRV |
|---|---|---|---|---|---|---|
| British Columbia | | | | | | |
| Creston | June | 88[c] | 18 | 0 | 0 | 36 |
| Penticton | June | 96 | 52 | 0 | 0 | 32 |
| California | | | | | | |
| Blythe | April | 76 | 20 | 0 | 0 | 14 |
| Davis | Nov. | 67 | 20 | 0 | 6 | 20 |
| El Centro | April | 73 | 52 | 0 | 0 | 37 |
| Fresno | Nov. | 85 | 31 | 0 | 0 | 31 |
| Modesto/Turlock | Nov. | 93 | 21 | 0 | 21 | 67 |
| Orland | Sept. | 100 | 0 | 0 | 14 | 0 |
| Salinas | Nov. | 77 | 12 | 0 | 6 | 38 |
| San Luis Obispo | Nov. | 85 | 0 | 0 | 0 | 58 |
| Vacaville | Sept. | 97 | 0 | 0 | 0 | 15 |
| Idaho | | | | | | |
| Caldwell | May | 100 | 3 | 0 | 0 | 67 |
| Fairfield | July | 48 | 2 | 2 | 0 | 12 |
| Pocatello | July | 80 | 10 | 0 | 0 | 0 |
| Rexburg | Sept. | 49 | 37 | 0 | 0 | 0 |
| Twin Falls | July | 82 | 23 | 0 | 0 | 0 |
| Weiser | Sept. | 96 | 14 | 2 | 6 | 18 |
| Oregon | | | | | | |
| Corvallis | Aug. | 88 | 12 | 0 | 0 | 36 |
| Hermiston | June | 98 | 29 | 0 | 0 | 52 |
| Klamath Falls | Sept. | 92 | 26 | 0 | 0 | 40 |
| Milton-Freewater | Aug. | 76 | 0 | 0 | 18 | 60 |
| Ontario | May | 88 | 14 | 0 | 2 | 8 |
| Redmond | Aug. | 82 | 6 | 0 | 0 | 10 |
| Roseburg | Sept. | 100 | 0 | 0 | 0 | 57 |
| Salem | Oct. | 81 | 34 | 0 | 20 | 18 |
| Woodburn | Sept. | 96 | 16 | 0 | 12 | 62 |
| Washington | | | | | | |
| Bellingham | May | 100 | 5 | 5 | 0 | 22 |
| Colville | Aug. | 98 | 26 | 0 | 0 | 18 |
| Coupeville | May | 87 | 0 | 74 | 0 | 39 |
| Ellensburg | June | 58 | 20 | 0 | 0 | 12 |
| Ephrata | April | 98 | 22 | 0 | 0 | 0 |
| Goldendale | June | 72 | 24 | 4 | 0 | 5 |
| Mt. Vernon | May | 88 | 8 | 8 | 0 | 13 |
| Okanogan | Aug. | 100 | 46 | 0 | 0 | 36 |
| Pasco | April | 100 | 35 | 0 | 0 | 79 |
| Pend Oreille Co. | Aug. | 80 | 33 | 0 | 0 | 53 |
| Prosser | June | 100 | 40 | 0 | 0 | 4 |
| Pullman | July | 76 | 28 | 0 | 0 | 26 |
| Puyallup | May | 87 | 13 | 87 | 0 | 0 |
| Sequim | May | 84 | 4 | 0 | 0 | 6 |
| Spokane | Aug. | 63 | 9 | 0 | 0 | 34 |
| Vancouver | Sept. | 100 | 18 | 0 | 0 | 10 |
| Walla Walla | May | 100 | 74 | 0 | 0 | 16 |
| Yakima | April | 97 | 60 | 0 | 5 | 38 |

[a] Month in which samples were collected from fields in the location.
[b] AMV = alfalfa mosaic virus, PSV = pea streak virus, TSV = tobacco streak virus, RCVMV = red clover vein mosaic virus, and PeLRV = pea leafroll virus.
[c] Percentages are the means of seven to 10 samples in four to seven fields at each location.

**Table 2.** Percentage of virus-infected plants in alfalfa cultivars from survey in western North America as detected by enzyme-linked immunosorbent assay

| Cultivar[a] | Virus[b] | | | | |
|---|---|---|---|---|---|
| | AMV | PSV | TSV | RCVMV | PeLVR |
| Agate (9) | 97 (9) | 13 (3) | ... | 30 (2) | 63 (6) |
| Apollo II (7) | 83 (7) | 40 (5) | 30 (1) | 20 (1) | 42 (6) |
| Blazer (5) | 90 (5) | 62 (4) | ... | ... | 17 (4) |
| Ladak (3) | 73 (3) | 20 (2) | ... | ... | 43 (3) |
| Ranger (7) | 53 (7) | 43 (6) | 10 (1) | ... | 13 (3) |
| Saranac (3) | 93 (3) | 10 (2) | 10 (1) | ... | 13 (3) |
| Spectrum (3) | 73 (3) | 10 (1) | 60 (1) | ... | 10 (1) |
| Thor (4) | 100 (4) | ... | ... | ... | 33 (3) |
| Trumpetor (14) | 81 (14) | 40 (6) | 69 (7) | 10 (1) | 29 (7) |
| Vernal (13) | 91 (13) | 42 (12) | 20 (1) | ... | 32 (9) |
| WL 316 (8) | 95 (8) | 37 (7) | ... | ... | 50 (3) |

[a] Cultivars occurring three or more times in the survey are included. The number of fields is shown in parentheses.

[b] AMV = alfalfa mosaic virus, PSV = pea streak virus, TSV = tobacco streak virus, RCVMV = red clover vein mosaic virus, and PeLRV = pea leafroll virus.

the samples, and none of the viruses assayed in this survey were detected in 11.4% of the samples.

Alfalfa cultivars that occurred three or more times in the survey, and the percentage of virus infected samples for each, are listed in Table 2. Differences between cultivars are associated more with geographic location than with cultivar. For example, the cultivar Ranger, which had the lowest incidence of AMV, was grown in three locations in Idaho where the AMV infection rate was lower and in one location (Okanogan) in Washington, where the cultivar was at an indicated 100% infection.

Incidences of viruses in this survey differ in some respects from those in other surveys. AMV, with an overall infection rate average of 85%, was somewhat higher than generally reported in the literature. TSV was found only in Idaho and Washington and at low frequencies, except for at two locations in western Washington, where it was detected in over 70% of the samples. The virus is known to occur in western Washington in small fruits (21). By comparison, a Canadian survey showed a range of incidence of AMV from 2 to 78% and TSV from 0 to 16%, with PSV at 10%, in 24 fields in Ontario and Quebec (20). Hampton reported approximately 40% incidence of AMV and PSV in 5-yr-old or older alfalfa fields in southeastern Washington (8). In a similar study in the southeastern United States, the incidence of AMV and RCVMV in small experimental plots was found to be 3.9 and 3.1%, respectively (15). McLaughlin and Ensign (16) reported that two of three alfalfa samples from western Idaho were positive for AMV, but that all three were negative for RCVMV. During an epidemic (in 1980) of PeLRV in peas in southern Idaho, PeLRV was found in 30 of 31 alfalfa samples from nearby fields (10). Incidence of AMV in fields in this survey was not highly correlated with age of stand ($r = 0.44$), but generally fields 4–6 yr old contained 80% or more infected samples. Fields 1 yr old ranged from 50 to 70% infection, 2-yr-old fields ranged from 40 to 90% infection, and 3-yr-old fields ranged from 10 to 100% infection. One 7-yr-old and one 8-yr-old field contained 20% infection. It is possible that death of virus-infected plants in older fields may give an apparent reduction in infection level, in the event that infected plants die more rapidly than uninfected and resistant plants, or if death occurs more rapidly than the onset of new infections. This could provide opportunity for selection for resistance in alfalfa to geographical strains of one or more of the viruses.

Greenhouse bioassays on indicator host plants revealed many viruslike symptoms that differed from those associated with the viruses discussed in this paper. The causal agents were not identified, but it appears likely that alfalfa harbors viruses other than those identified in this study.


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