Pea Streak and Alfalfa Mosaic Viruses in Alfalfa: Reservoir of Viruses Infectious to Pisum Peas

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ABSTRACT

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Alfalfa (*Medicago sativa*) plant samples from four diverse northwestern U.S. production areas were tested by enzyme-linked immunosorbent assay for pea streak virus (PSV) and alfalfa mosaic virus (AMV). Plants from all fields 5 yr or older in all areas contained PSV and AMV; neither virus was detected in two plantings that were less than 2 yr old. At least 40% of the sampled plants were PSV-infected in five of seven geographic locations; the other two locations contained 13 and 24% infected plants. Incidence of PSV-infected plants was independent of proximal plantings of peas or other known PSV hosts. Coincidence of PSV and AMV in sampled alfalfa plants exceeded 60% in five of seven locations. No seedborne PSV was demonstrated in 670 alfalfa seedlings grown from seed obtained from PSV-infected fields, whereas at least 2% frequency of seedborne AMV was detected in the same seedlings.

Additional key words: enzyme-linked immunosorbent assay, Idaho, Montana, Oregon, *Pisum sativum*, red clover vein mosaic virus, Washington

Fourteen viruses that induce streaking symptoms in pea (*Pisum sativum* L.) consisting of brownish purple streaks in or on the stem, plant stunting, and localized or whole-plant necrosis were enumerated by Bos (2,3). At least eight other viruses capable of inducing such symptoms can now be added to this number. Incidence of two pea-streak-inducing viruses, pea streak virus (PSV) (3), and alfalfa mosaic virus (AMV) (12) in alfalfa stands is reported in this paper. Although PSV was once suggested to be transmitted by aphids from alfalfa to peas (17), it has since been reported and widely assumed to be nonpathogenic to alfalfa (7,13,18). Involvement of alfalfa in the natural occurrence and dissemination of PSV therefore has not been generally recognized (3,6). Nevertheless, Beczner (1) documented PSV as an alfalfa pathogen in Hungary, and alfalfa was recently concluded to be the principal PSV inoculum reservoir in the Pacific Northwestern United States (10,11). Nelson et al (14) also reported isolating PSV from alfalfa stands in Arizona. Hampton (8) has proposed that alfalfa latent virus, included by Graham et al (6) as an alfalfa pathogen, should be considered a strain of PSV.

This paper reports the relative incidence of PSV and AMV in alfalfa stands of distinctive agricultural areas of the northwestern United States.

MATERIALS AND METHODS

Because long-term studies had indicated that alfalfa was a principal reservoir of PSV, incidence of this virus in alfalfa grown in four northwestern U.S. agricultural areas was estimated. These areas were the Willamette Valley of western Oregon, a nonirrigated minor hay-producing area not well suited to alfalfa; the Gardena Bench of southeastern Washington, an alfalfa seed-production area that historically has been associated with destructive PSV and AMV spread to pea fields; the Magic Valley of southern Idaho, the area of the northwestern United States in which natural spread of these viruses to peas is currently most consistent; and the Jefferson Valley, Shields Basin, and Townsend area of southwestern Montana, which is isolated from commercial plantings of peas and contains large alfalfa acreages from which hay is produced for winter livestock feed. The Willamette Valley was selected because peas in that area are rarely infected by PSV; red clover vein mosaic virus is usually associated with streaking symptoms of peas in that area (16). Southwestern Montana locations were chosen because their isolation from peas precluded involvement of this PSV host in PSV epidemiology in alfalfa stands. The incidence of PSV in alfalfa stands in the latter locations relative to that of AMV was anticipated as a measure of the inherence of PS in alfalfa. An isolated field of DuPuits alfalfa near Mesa, WA, was sampled because it comprised the first planting of this cultivar in the United States, with seed imported from France. Fields near Townsend, MT, were sampled because of the lower elevation and milder climate that contrasted with that of Shields Basin.

Incidence of PSV in fields of alfalfa plants and in alfalfa seedlings was assessed by enzyme-linked immunosorbent assay (ELISA). Plants were sampled and tissues were desiccated under vacuum between layers of silica gel and stored at −34 C until assayed. For testing, desiccated tissues were homogenized and diluted 20-fold with standard ELISA virus buffer (4). Because PSV and AMV were known to occur in mixed infections (9), plant samples were assayed simultaneously for both viruses. Extracts from infected tissue, healthy tissue, and buffer controls were included in test plates, and detection thresholds were computed as final-reaction absorbance of 405 nm wavelength light (A_{405}) values that exceeded those of healthy controls by at least four standard deviations. Most samples containing either PSV or AMV exceeded threshold values by 10-fold or more. Absorbance values (A_{405}) representing optimized ELISA reactions were recorded on a Gilford PR-50 EIA recording photometer. Immunogammaglobulin for ELISA was prepared by one-step column chromatography using DEAE Affi-Gel Blue (Bio-Rad Laboratories, Richmond, CA 94804). Use of coating antibody, buffers, antigen preparations, enzyme conjugate, and substrate was similar to that described by Clark and Adams (4).

Initial tests for seedborne viruses consisted of abrading Carborundum-dusted DS Perfection pea plants with triturated composite alfalfa seedling

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samples. The identity of transmitted virus was determined by SDS immunodiffusion serology.

RESULTS

Inoculum reservoir of PSV. PSV was detected in alfalfa plantings of all four production areas sampled (Table 1). Incidence of PSV in alfalfa fields in southwestern Montana, notwithstanding the absence of commercial pea fields in the area, was comparable with that of southern Idaho and southeastern Washington. Incidence of PSV was substantially lower in alfalfa fields of western Oregon and Shields Basin, MT, than other locations. Incidence of AMV, which is seed-transmitted in alfalfa (5) and aphid-transmissible (9,15), was greater than that of PSV at all locations except Shields Basin. The coincidence of PSV and AMV in alfalfa tissue samples exceeded 60% in all locations except Mesa, WA, and Shields Basin.

Incidence of PSV among four alfalfa cultivars of southeastern Washington exceeded 40% in fields that were 5 yr or older (Table 2). Incidence of AMV in these fields was equal to or slightly exceeded that of PSV and, again, mixed infections were common. Neither PSV nor AMV was detected in alfalfa plantings that were 9 or 21 mo old.

To examine a possible means by which PSV becomes established in alfalfa fields, we tested seeds from the 5 and 16-yr-old Ranger and 18-yr-old Vernal alfalfa plantings for transmission of PSV (Table 2). Sixty-six sets of three or four 6-wk-old seedlings were tested by assay on Cascade pea plants. Of five sets that induced infection on test plants, all were found by standard and SDS immunodiffusion serological tests to contain AMV but not PSV. Subsequently, 51 sets of eight 3-wk-old seedlings were tested for PSV and AMV by ELISA. Twelve sets contained AMV, whereas none contained detectable quantities of PSV. On the assumption that only one infected seedling occurred in each set found to contain AMV, the rates of AMV seed transmission would have been 5/264 (2%) and 12/408 (3%), which are in general agreement with previous results (5).

Viruses in naturally infected peas. Streak-affected pea plants in disease outbreaks in Washington and Idaho have been found by us to contain PSV with or without AMV. Red clover vein mosaic virus, monitored in some of the samples of this study, was detected only rarely in alfalfa plants. In contrast, this virus is widespread in red clover in western Oregon and western Washington (16), from which it is transmitted annually to peas by the pea aphid and comprises the principal pea-streak-inducing virus in that geographic area.

Table 1. Incidence of pea streak virus (PSV) and alfalfa mosaic virus (AMV) in alfalfa fields of four northwestern production areas as determined by enzyme-linked immunosorbent assay

Production area	No. of fields tested	PSV[a]	AMV[a]	Coincidence of AMV with PSV[b]
Oregon (Rickreall)	4	3/24	18/24	3/3
Washington I (Gardena)	5	24/57	28/57	18/24
II (Mesa)	1	7/16	10/16	2/7
Idaho (Twin Falls)	5	17/30	19/30	12/17
Montana I (Jefferson Valley)	20	28/60	48/60	24/28
II (Shields Basin)	6	9/37	6/37	2/9
III (Townsend)	4	19/35	25/35	15/19

[a]Number of plants infected per number of plants tested. Samples tested simultaneously for PSV and AMV.
[b]Number of plants containing PSV (denominator) that also contained AMV (numerator).

Table 2. Incidence of pea streak virus (PSV) and alfalfa mosaic virus (AMV) in southeastern Washington alfalfa fields relative to cultivar and age of stand as determined by enzyme-linked immunosorbent assay

Alfalfa cultivar	Age of planting (yr)	PSV[a]	AMV[a]	Coincidence of AMV with PSV[b]
Ranger	5	3/7	3/7	3/3
	16	13/17	13/17	9/13
Vernal	0.75	0/8	0/8	...
	18	8/17	12/17	6/8
DuPuits	20	7/16	10/16	2/7
Agate	1.75	0/8	0/8	...

[a]Number of plants infected per number of plants tested. Samples tested simultaneously for PSV and AMV.
[b]Number of plants containing PSV (denominator) that also contained AMV (numerator).

DISCUSSION

Two bodies of evidence suggest that PSV is somehow inherent to alfalfa. First, PSV epidemics in peas are consistently associated with massive migratory flights of the pea aphid from alfalfa at the first cutting for hay (11). In the absence of such aphid flights, we have not observed a single outbreak of pea streak in peas. Second, as presented in this paper, the consistent incidence of PSV in alfalfa stands over a large geographic area, including diverse climates and flora, logically opposes requisite involvement of alternative PSV hosts. Yet, the means by which PSV becomes established in an isolated alfalfa field was not determined in this study. Once the virus is established in a given alfalfa planting, aphid transmission of PSV to other alfalfa fields in the vicinity is plausible.

The ~2-yr time lag observed between alfalfa planting and eventual pervasion of PSV in resulting stands, corresponding to companion greenhouse results (11), contrasts with the rapid (10–15 days) increase in infected-plant frequencies in natural PSV epidemics. This unique time lag seems likely to complicate discernment of the origin of PSV in alfalfa. Although our tests of about 670 alfalfa seedlings failed to detect seedborne PSV, we consider seed transmission of the virus the most plausible explanation of its origin in isolated alfalfa stands, perhaps at trace frequencies and/or with protracted low virus concentrations after transmission. Investigations into this fascinating possibility and phenomenon face the challenging prospect of detecting one infected seedling among perhaps thousands tested or of testing seedlings over long periods while maintaining them free of contamination, each with attending requirements for undisputed proof. Yet, ELISA technology certainly opens the opportunity to this formidable and significant task.

Alfalfa fields, particularly in eastern Washington, remain productive for 15–20 yr. During this period, interestingly, the incidence of neither PSV nor AMV increases to 100%. This may suggest that PSV-resistant genotypes exist within the type-mixtures common to alfalfa cultivars (synthetics). The shorter average longevity (4–6 yr) of alfalfa stands in the Shields Basin and nonirrigated western Oregon locations, however, almost certainly contributes to the lower incidence of the viruses in alfalfa plants there.

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