Pea Streak Virus Transmission from Alfalfa to Peas: Virus-Aphid and Virus-Host Relationships

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ABSTRACT

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Isolates of pea streak virus (PSV) derived from pea aphids (*Acyrthosiphon pisum*) occurring naturally on field-grown alfalfa (*Medicago sativa*) plants were indistinguishable serologically and by particle morphology from the Wisconsin type isolate PSV-W, differing only in pathogenicity to a few hosts. Pea aphids given acquisition access on PSV-infected alfalfa plants transmitted PSV to pea test plants (three aphids per plant) at frequencies of 25–35%, which was about half the rate of aphids fed on PSV-infected pea (*Pisum sativum*) or bell bean (*Vicia faba* var. *minor*) plants. After acquisition access periods of 1.5 ± 0.5 min on PSV-infected pea plants, apterous pea aphids retained PSV inoculativity for at least 2 hr of postacquisition fasting at 22 C. These results supported field observations of PSV spread to peas by alate pea aphids presumed to have migrated from alfalfa fields over a distance of 12.2 km. The incubation period of PSV in alfalfa plants after mechanical inoculation was 2 mo, whereas pea aphids fed on inoculated pea or bell bean plants 10–15 days after plant inoculation transmitted PSV. Aphids were unable to acquire PSV from infected alfalfa plants growing in the greenhouse or field when daytime maximum temperatures of 35–40 C prevailed. Although PSV was readily obtained from natural aphid populations on field-grown alfalfa plants, aphids removed from other proximal legume species did not transmit PSV.

Involvement of alfalfa in the natural occurrence and spread of pea streak virus (PSV) to peas was not generally recognized before 1978 (6,9). Although circumstantial evidence (3,15) indicated that an insect vector might be involved in PSV field spread, the pea aphid, *Acyrthosiphon pisum* Harris (11,12), was determined to have a limited ability to vector the Wisconsin strain of PSV. The pea aphid, however, was subsequently shown to be an efficient vector of PSV (5), and PSV transmission by *A. pisum* was found to be independent of helper agents (14). More recently, PSV was found to occur extensively in alfalfa fields of diverse agroecosystems in the Pacific Northwest (7). The purpose of this research was to assess the dissemination potential of PSV from alfalfa to peas by *A. pisum* and to compare host relationships of northwestern U.S. isolates of PSV with the Wisconsin type isolate PSV-W.

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MATERIALS AND METHODS

Isolates of PSV were obtained by inoculating young DS Perfection or Perfected Wales pea plants with pea aphids removed directly from naturally infested alfalfa stands in southeastern Washington (Gardena Bench) or southern Idaho (Magic Valley) (7). Once established in the aphid-inoculated plants, PSV isolates were morphotyped by electron microscopy of gluteraldehyde-fixed leaf-dip preparations (4), serotyped by SDS gel double-diffusion serology (10) against standardized PSV antiserum from our laboratory, propagated in pea plants, and purified by polyethylene glycol-aided differential and density-gradient centrifugation (13). The Wisconsin isolate of PSV, PSV-W provided by D. J. Hagedorn, University of Wisconsin, was used as a pathological, morphological, and serological standard. Purified virus was reestablished in pea plants to assess trueness to PSV symptom type, and in DuPuits alfalfa plants, to serve as greenhouse inoculum sources that maintained the natural virus-host relationship.

Preliminary evaluations of pea aphid colonies for ability to transmit PSV included colonies from E. S. Sylvester, University of California, Berkeley, and from J. E. Bath, Michigan State University. Data presented in this study, however, were produced with colonies collected from peas in western Oregon (Willamette Valley) and maintained virus-free on bell bean (*Vicia faba* var. *minor* (Peterm) Beck) plants. Each test of PSV transmission by pea aphids was conducted with 6- to 8-day-old viviparous apterae propagated from 10 to 15 selected reproducing females.

Aphids were fasted for 4 hr in sealed vials, placed onto PSV-source plants for acquisition periods of 1.5 ± 0.5 min, and then placed in groups of three onto DS Perfection pea test plants for 0.5–4 hr. DS Perfection plants showing initial phases of stem and leaf necrosis (10–14 days after PSV inoculation) were used as PSV sources for acquition studies because aphid transmission from alfalfa was lower and had greater seasonal variability.

RESULTS

Pea aphid acquisition of PSV. Preliminary tests of four PSV isolates, Idaho-1-1, -3-1, -3-2, and -3-3, established that aphid transmission frequencies to pea test plants were maximal after acquisition access periods of 1–3 min and were very similar among isolates. Six- to 8-day-old apterae that had been allowed 1.5 ± 0.5 min of acquisition access on PSV-infected plants of DS Perfection and transferred by sets of three to DS Perfection test plants transmitted PSV at frequencies of 40–75%.

Retention of PSV by pea aphid during postacquisition fasting. After 1.5 ± 0.5 min of acquisition access to PSV (Idaho-3-2)-infected pea plants, aphids were fasted from 1 to 120 min in sealed vials at 22–24 C and then placed by sets of three on each of 18–25 pea test plants (Fig. 1). Transmission percentages per post-acquisition fasting period were widely scattered ($r^2 = 0.22$; $r = 0.47$, significant at the 0.01 level), manifesting expected variability in PSV acquisition, retention, and transmission by aphid triplets starved for identical or similar periods. Analysis of aphid-group-transmission percentages relative to fasting periods, however, demonstrated a gradual

decrease in PSV transmissibility during the 120-min test interval. An average of 18% of the aphid triplets retained and transmitted PSV when acquisition access was followed by 120-min fasting periods.

PSV characteristics. PSV isolates derived from aphids occurring naturally on alfalfa and separated where necessary from mixtures with alfalfa mosaic virus (AMV) by density gradient centrifugation consisted of 630-nm particles typical of carlaviruses; they reacted identically with PSV-W against our PSV antiserum in SDS immunodiffusion tests and were tested for trueness to type by pathogenicity-symptomatology on selected hosts. Isolate Idaho-3-2, representative of numerous isolates from alfalfa, differed from PSV-W principally in its noninduction of lesions on inoculated leaves of *Chenopodium amaranticolor* (Table 1). Isolate Idaho-3-2 only occasionally induced mild mosaic symptoms in inoculated plants of DuPuits alfalfa or local lesions on inoculated leaves of *Gomphrena globosa*.

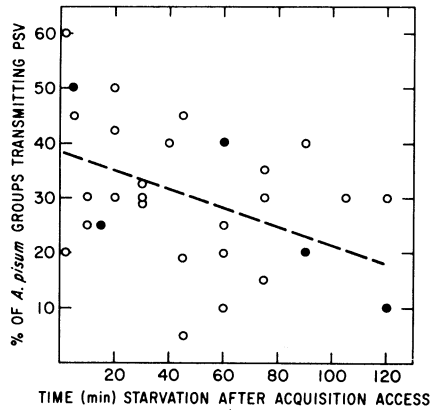

Fig. 1. Retention of pea streak virus (PSV) by pea aphids (*Acyrthosiphon pisum*) provided 1.5 ± 0.5 min of acquisition feeding on PSV-infected DS Perfection pea plants, then fasted for 1–120 min. After prescribed fasting periods, aphids were placed in groups of three on 18–25 DS Perfection test plants. Thirty-four datum points represent percentages of infected plants per test. • = Identical results in two tests. Regression equation: $y = 38.52 - 0.17x$. Coefficient of determination (r^2) = 0.22.

Inoculum potential of PSV hosts. Aphid acquisition-transmission of PSV from infected pea, bell bean, and alfalfa plants was compared at selected post-inoculation intervals to assess these hosts as virus-acquisition sources. PSV transmission rates of ⩾60% were frequently obtained when aphids were fed on PSV-infected pea or bell bean plants approaching maximum symptom development yet remaining succulent. Aphids probed reluctantly and were unable to acquire PSV from collapsed or partially wilted tissues. After the collapse of upper portions of PSV-infected bell bean plants, adventitious shoots frequently arose near the bases of such plants and persisted for several weeks with only mild streak symptoms. Aphids that were fed on these shoots readily transmitted PSV but at average rates lower than aphids fed on diseased terminals before tissue collapse.

In contrast to 10- to 15-day incubation periods of PSV in inoculated pea and bell bean plants, the incubation period of PSV in DuPuits alfalfa plants was at least 2 mo. Pea aphids that were fed on PSV-inoculated plants before that post-inoculation interval were unable to transmit PSV. Maximum rates of PSV transmission by aphids fed on infected alfalfa plants ranged from 25 to 35% or about half that of aphids fed on infected pea or bell bean plants. Type isolate PSV-W was infectious to DuPuits alfalfa, with an incubation period and other virus-host relationships that were indistinguishable from the northwestern U.S. isolates of PSV.

Aphids reared at 22 C were repeatedly (in four trials) unable to transmit PSV from infected alfalfa plants maintained in the greenhouse during summer months (maximum temperatures 35–40 C). Likewise, pea aphids removed from field-grown alfalfa plants in southern Idaho in July during sustained daytime maximum temperatures of 35–40 C transmitted PSV at rates ranging from zero to three plants per 120 test plants on each of which six to eight native pea aphids had fed. Similar tests in May (maximum temperatures 20–25 C) typically resulted in 10–20% PSV transmission rates.

In southern Idaho, where PSV-infection of pea crops occurs annually, PSV was not readily transmissible from leguminous plants other than alfalfa by means of naturally occurring aphids. Neither *A. pisum* nor *Nearctaphis bakerii* (Cowen) found on red clover (*Trifolium pratense*) plants in that area transmitted PSV to experimental pea test plants. Likewise, *A. pisum* from indigenous *Lathrus odoratus* L., *Melilotus officianalis* L. (all known PSV hosts), and an unknown aphid species from *Robinia pseudoacacia* L. also failed to transmit PSV. Alfalfa thus appears to be a principal, if not the exclusive, inoculum reservoir from which PSV is transmitted to peas in southern Idaho.

DISCUSSION

Earlier studies (1,2) in eastern Oregon and Washington indicate that alfalfa is a favored fall, winter, and early spring host of the pea aphid. In southern Idaho, pea aphids typically increase to large populations in alfalfa by May and June, when the first cutting for hay occurs. The cutting operation forces migration flights of viviparous alatae that result, especially within 1.5–5 km downwind (gentle breezes [1]) in transmission of PSV and AMV to susceptible leguminous hosts. Severe streak epidemics in peas have frequently appeared to result from single major aphid flights. Aphid acquisition of PSV from infected pea plants in our studies, however, indicates that secondary PSV spread from field-infected pea plants could also occur. In some instances, pea fields in eastern Washington were invaded by massive pea aphid flights of unknown origin, followed in 2 wk by apical or whole-plant necrosis of 70–100% of the plants. The distance upwind to the nearest alfalfa field in one such instance was 12.2 km. Retention of PSV by *A. pisum* migrating alatae in this case could have been as long as 2 hr, a possibility supported by experimental retention data of this study.

Historically, pea crops of eastern Oregon and Washington were reportedly attacked by mosaic-type diseases before 1950, whereas viral diseases associated with pea aphid flights from alfalfa in subsequent years were characterized by "tip blights", "yellow tops", and "wilts". This shift was described in 1954 by

Table 1. Reactions of plant hosts to inoculation with Idaho and Wisconsin isolates of the pea streak virus

Plant host[a]	Pea streak virus isolates	
	PSV-Idaho-3-2	PSV-Wisconsin
Chenopodium amaranticolor	−/−	$LL_n/^{-b}$
Gomphrena globosa	$(LL_n)/-$	$LL_n/-$
Medicago sativa	lat/lat, (m)	lat/lat
Phaseolus vulgaris[c]	lat/−	lat/−
Pisum sativum	W/St, k, N	W/St, k, N
Spinacea oleracea	−*/−*	−/lat
Trifolium pratense	−*/lat	−/lat
Vicia faba var. *minor*	LL_n/Str, N, r	LL_n/Str, N, r
Nonhosts[d]	...	...

[a] Cultivars of all hosts, as reported in Phytopathology 68:989-997.

[b] Inoculated leaves/systemic symptoms. Symbols are: − = absence of symptoms and virus; −* = no symptoms, tissue not assayed; LL_n = localized, necrotic lesions; ( ) = variable symptoms; lat = latent infection; m = mild mosaic; W = wilting, death; St = plant stunting; k = localized tissue necrosis; N = whole-plant necrosis; r = recovery after whole-plant necrosis; Str = necrotic streaking of leaves and stems.

[c] Only cultivar Black Turtle Soup was found to be susceptible in inoculated leaves.

[d] Nonhosts included *Antirrhinum majus, Cucumis sativus, Datura stramonium, Glycine max, Lycopersicon esculentum, Nicotiana glutinosa, N. tabacum, Petunia hybrida, Phlox drummondii, Trifolium repens,* and *Vigna unguiculata. D. stramonium* and soybean cultivar Bragg were sometimes infected locally.

McWhorter (8). In attemping to account for the viruses causing pea-plant terminals to become "wilted, yellowed, spotted as by a fungus, or shrivelled and dried up," he concluded (correctly, we believe) that AMV was one of the incitants. He was not aware of PSV, known at that time to occur only in Wisconsin (3). The symptoms described our current experience with PSV in alfalfa, however, and the capacity of our purified PSV isolates to induce such symptoms in pea cultivars commonly grown in the 1940s and 1950s all indicate that PSV was probably present in the diseased plants described. Factors triggering the shift from mosaic-to necrosis-inducing viruses, though inviting inquiry, are unknown.

The unexpectedly long PSV incubation periods in inoculated alfalfa plants observed in our studies could explain conclusions by earlier investigators (3,16) that PSV was not pathogenic to alfalfa. This phenomenon may also contribute to the previously observed (7) low incidence of PSV-infected plants in alfalfa plantings less than 2 yr old.

Earlier studies (11,12), from which the pea aphid was considered an inefficient vector of PSV, may have tested nonrepresentative pea aphid biotypes or PSV isolates with limited aphid transmissibility. Several aphid clones in our study from Oregon, California, and Michigan, as well as unselected indigenous populations in southern Idaho, readily transmitted numerous PSV isolates occurring in the Pacific Northwest. Three Oregon pea aphid colonies, moreover, transmitted both Idaho and Wisconsin isolates of PSV at frequencies as high as 75%, in agreement with previous results (4). There can be no doubt that *A. pisum* has the innate ability to transmit a range of naturally occurring PSV isolates.

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