Ecological Characteristics of the Lentil Strain of Pea Seedborne Mosaic Virus

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ABSTRACT

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The seed and aphid transmission characteristics of the lentil strain of pea seedborne mosaic virus (PSbMV-L) were examined and commercial lentil fields surveyed for the presence of this virus. PSbMV-L was seed-transmitted at rates of 5 and 0.5%, respectively, in *Lens culinaris* 'Tekoa' and *Pisum sativum* 'Tempter'. PSbMV-L was present as a seedborne contaminant in seeds of the USDA lentil plant introduction (germ plasm) accessions and in seed-increase plots at levels as high as 16.7%. Single pea aphids (*Acyrthosiphon pisum*) transmitted PSbMV-L at frequencies of 50–90% after both short and long acquisition access periods. Aphid transmisson rates decreased with increasing temperature. PSbMV-L was not detected in surveys of commercial lentil fields but the presence of PSbMV-L as a seedborne contaminant of lentil germ plasm accessions constitutes a potential threat to U.S. lentil production.

The lentil (*Lens culinaris* L.) strain of PSbMV (PSbMV-L) was first discovered as a seedborne contaminant of the U.S. lentil germ plasm collection (4) and is distinguished from the pea isolates by nonpathogenicity to most pea (*Pisum sativum* L.) cultivars, quantitative serology, intrinsic particle instability, pathogenicity in *L. culinaris* independent of the homozygous recessive *sbv* gene (3), which confers resistance to the pea isolates of PSbMV, and by its apparent confinement to a natural inoculum reservoir in *Lens* (vs. *Pisum*) seed. Lentil germ plasm accessions, when infected from seed, are usually symptomless; however, in greenhouse tests, PSbMV-L induces severe symptoms and yield loss in the commonly grown commercial lentil cultivars (Fig. 1).

The objective of this study was to examine some of the ecological and epidemiological characteristics of the PSbMV-L, particularly its seed- and aphid-transmission characteristics. Also presented are survey data describing the degree of PSbMV-L distribution in *Lens* germ plasm accessions, repository seed-increase plots, and commercial lentil fields.

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MATERIALS AND METHODS

Seed transmission. Fifty 2-wk-old *L. culinaris* 'Tekoa' plants were inoculated with PSbMV-L and allowed to grow to maturity under greenhouse conditions. Resultant seeds were harvested and planted and the seedlings tested for PSbMV-L by enzyme-linked immunosorbent assay (ELISA) (1) 2 wk after emergence. Seedling infection was also determined by local-lesion assay on *Chenopodium amaranticolor* Coste & Reyn.

Seed transmisson incidence of PSbMV-L in 250 seeds from infected *P. sativum* 'Tempter' plants was estimated. Seeds harvested from plants 17 wk after PSbMV-L-inoculation were planted and resultant seedlings were assayed for PSbMV-L by ELISA 2 wk after emergence.

Seeds of lentil plant introduction (PI) accessions that had shown viruslike symptoms in 1980 seed-increase plots were obtained from the Western Regional Plant Introduction Station, Pullman, WA. Thirty seeds per line (sample size normally distributed) were planted in the greenhouse. Two-week-old seedlings were individually tested by ELISA and positive reactions for PSbMV-L were also assayed on *C. amaranticolor*. Seeds of these lentil germ plasm accessions were also grown out and tested by ELISA to compare the incidence of infected seed before and after seed increase in USDA plots near Pullman, WA.

Aphid transmission. Two-week-old Tekoa lentil plants inoculated with PSbMV-L by means of third-instar pea aphids (*Acyrthosiphon pisum* (Harris)) were used as virus-source plants. Aphids cultured on Tekoa plants were not starved before being given access to PSbMV-L-source plants. Acquisition access periods (AAP), timed from the first observed aphid feeding probe, are

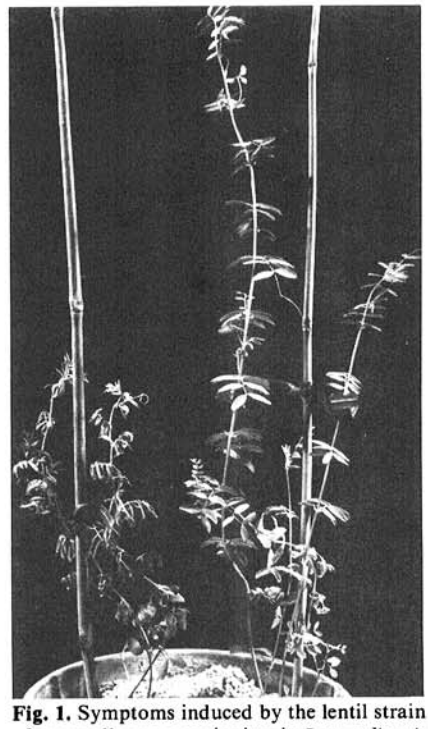

Fig. 1. Symptoms induced by the lentil strain of pea seedborne mosaic virus in *Lens culinaris* 'Tekoa.' (Left) Infected plant and (right) healthy plant.

noted in Results. Fifteen plants per AAP were inoculated with single aphids previously given timed access to PSbMV-L-infected Tekoa plants. Transmission access periods (TAP) of 45 min were provided. Aphid-inoculated plants were examined for PSbMV-L symptoms and assayed by ELISA 3 wk after inoculation to determine the frequency of virus transmission. These aphid-transmission tests were repeated in four sequential experiments.

One hundred Tekoa plants were grown in each of three growth chambers maintained at 18, 27, or 35 C (12-hr photoperiod). Plants were aphid-inoculated with PSbMV-L when 2 wk old to determine the effect of temperature on aphid transmission. Low-spectrum (cool-white) fluorescent and standard incandescent lights (14-hr photoperiod) provided illumination of 130–155 lux. PSbMV-L-infected Tekoa lentil source plants were grown under the same conditions. Pea aphid colonies reared on Tekoa plants were placed in each growth chamber 3 days before transmission experiments. As before, plants were inoculated by means of one aphid (3- to 5-min AAP and 45-min TAP per temperature) per plant. Tekoa test plants were individually assayed by ELISA 3 wk later to ascertain frequency of PSbMV-L transmission at each temperature.

Survey of commercial lentil fields. Commercial lentil fields in the Palouse area of eastern Washington were surveyed for PSbMV-L in July 1980 and June 1982. Thirty-five individual plant samples were taken from 11 commercial fields in 1980 and 63 samples were taken from 10 fields in 1982. Sites sampled are shown in Figure 2. Plants were ELISA tested for PSbMV-L. Fourteen PI lines grown near Pullman, WA, for seed increase and horticultural evaluation were also selected in 1982 for ELISA because they showed viruslike symptoms. Samples concluded from ELISA to contain PSbMV-L were also tested by immunosorbent electron microscopy (ISEM) (2).

RESULTS AND DISCUSSION

Seed transmission. Seeds from plants of lentil cultivar Tekoa that had been inoculated with PSbMV-L in the greenhouse transmitted PSbMV-L at a rate of 5% (2/42). Thirteen lentil PI accessions, when examined in seed increase plots near Pullman, WA, in 1980, showed symptoms approximating those induced by PSbMV-L in Tekoa plants. Seeds still available from seven of the stocks used to plant these seed-increase plots were tested for transmission of PSbMV-L, and five of seven contained detectable amounts of seedborne PSbMV-L (Table 1). Seedborne PSbMV-L was also detected in seeds harvested from four of these 13 seed-increase plots. No PSbMV-L was detectable in PI 432126 after seed increase, indicating that virus-free plants of this accession may have outyielded seed-infected plants, in effect diluting the number of infected seeds under the growing conditions of the plots. Conversely, the rate at which PSbMV-L was seed-transmitted in PI 432112 seems to have increased, indicating that aphid vectors may have spread the virus within the plot.

ELISA of 191 Tempter pea plants from seed harvested from PSbMV-L-infected mother plants detected one PSbMV-L-infected plant, indicating a seed-transmission rate of 0.5%. These results were confirmed by assay on *C. amaranticolor*. Reported non-seed-transmissibility of PSbMV-L in *P. sativum* 'Tempter' (4) was apparently based on a sample size too small to detect this rate of transmission. Thus, although PSbMV-L is infectious to Tempter, it is seed-transmitted in this cultivar at very low rates. Conversely, the pea strain of PSbMV is seed-transmissible at rates as high as 90% in some pea selections (5). Even low levels of PSbMV-L seed transmission could be epidemiologically important, however, because of the capacity of the pea aphid to transmit the virus, facilitating secondary spread. Of 120 pea cultivars tested for susceptibility to PSbMV-L, only six (Avon, Coronet, Nugget, Quincy, Sounder, and Tempter) were susceptible.

Aphid transmission. High rates of PSbMV-L transmission by the pea aphid occurred with only one aphid per test plant. When aphids were allowed a half-log progression of AAP on infected lentil plants and TAP of 45 min, PSbMV-L was transmitted at rates higher than 50% between 3 and 33 min, at lowered rates (15–40%) between 100 and 333 min, and at markedly greater rates (40–95%) after AAP of 1,000 and 3,333 min (Fig. 3). This general pattern of bimodality was previously reported for the potato aphid and the standard pea strain of PSbMV (6) but at much shorter AAP. This distinction prompted reevaluation of transmission patterns within the 1- to 120-min interval.

For this purpose, pea aphids were provided access to either infected Tekoa lentil or infected Tempter pea plants for AAP of 1 through 118 min (Fig. 4). Transmission peaks (70–80%) occurred after AAP of 3–5 min and declined rapidly (10–12%) with AAP of 5–10 min, with transmission maxima at about 60

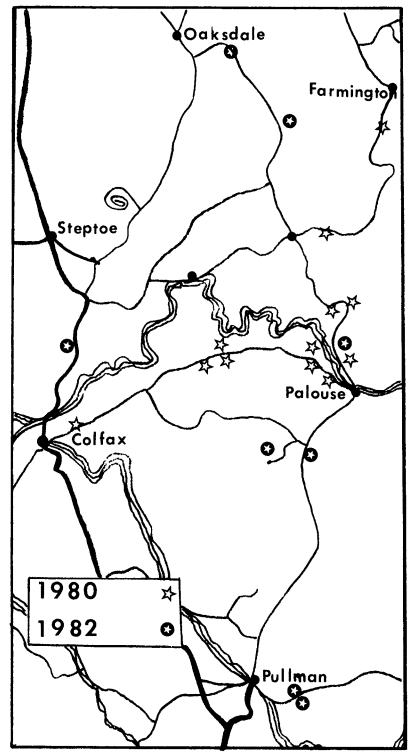

Fig. 2. Commercial lentil field sampling sites (1980 and 1982) in the Palouse area of eastern Washington.

Table 1. Seed transmission rates of the lentil strain of pea seedborne mosaic virus in *Lens culinaris* germ plasm accessions before and after seed increase

USDA Plant Introduction	Percentage of infected seed[a]	
	Seed planted in plots for increase	Seed from increase plots
185602	NA[b]	12.0%
299603	NA	0
297772	NA	0
299325	NA	0
299185	NA	0
299265	NA	0
299233	10%	12.5%
432077	0	0
432112	3.5%	13.8%
431655	11–18%	16.7%
432126	3.4%	0
432121	0	0
432218	3.5%	0

[a] Percentages based on tests of 17–30 seeds per line.
[b] NA = not available for testing.

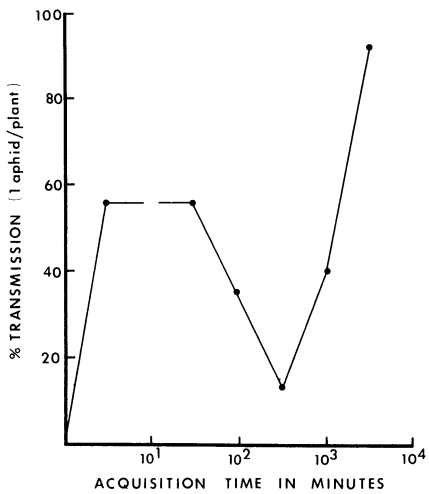

Fig. 3. Long-term bimodal transmission of PSbMV-L by *Acyrthosiphon pisum* provided acquisition access periods of 3–3,333 min. Infected *Lens culinaris* 'Tekoa' plants were the PSbMV-L source and healthy Tekoa seedlings were used as test plants.

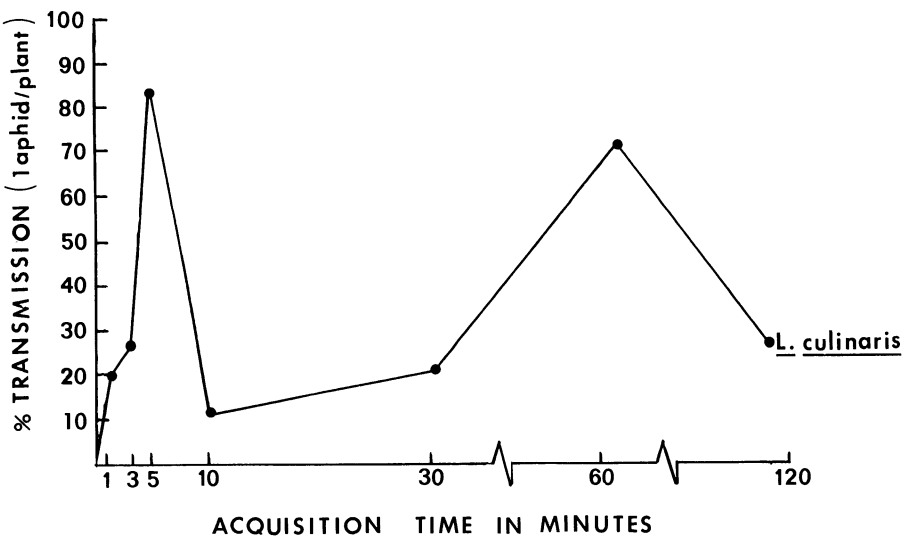

Fig. 4. Short-term bimodal transmission of PSbMV-L by *Acyrthosiphon pisum* provided acquisition access periods of 1–120 min. Infected *Lens culinaris* 'Tekoa' or *Pisum sativum* 'Tempter' were the source plants. Healthy Tekoa seedlings were used as test plants.

min. These time-transmission results match those previously reported for the pea strain (6). Thus, although attempts to integrate short- and long-term trials into a single experiment were not successful, it is obvious that PSbMV-L can be transmissible by the pea aphid in a trimodal manner. More extensive tests could reveal that polymodal transmission patterns are acquisition characteristics of relatively undisturbed aphid populations on plants infected with styletborne viruses.

Bimodal transmission is not yet a commonly reported phenomenon among aphid-transmissible viruses. The mechanisms effecting the process are unknown. Because bimodal or polymodal transmission may be functions of specific virus-vector interactions, care was taken to use an *A. pisum* biotype present in the lentil-growing areas. Such transmission after both short- and long-period aphid probes would appear to favor virus survival and spread, ie, impose fewer limitations on the styletborne mode of virus transmisson.

Aphid-transmission rates were temperature-dependent. Frequencies of aphid transmission decreased from 81% at 18 C to about 25% at 27 C and to 15% at 35 C. The titer of PSbMV-L in infected plants as measured by ELISA decreased as a linear function of temperature. PSbMV-L was barely detectable in individual plants grown at 35 C. These results could reflect temperature effects on the virus, host, or vector, and/or interactions thereof.

Survey of commercial lentil fields. PSbMV-L was not detected in 35 samples taken in 1980 from 11 commercial lentil fields or in 63 samples taken in 1982 from 10 commercial lentil fields. However, PSbMV-L was detected in 1980 in seed-increase plots of lentil PI accessions previously reported to be virus-infected (4). Of 14 PI accessions observed in 1982 seed-increase plots with slight viruslike symptoms, PI 185602 (separately found to be infected in seed-transmission studies) (Table 1) and PI 299222 contained PSbMV-L. The other 12 accessions contained no ELISA-detectable PSbMV-L (PIs 212611, 244046, 251029, 251784, 298224, 299176, 299188, 299249, 299253, 299260, 299316, and 345632).

PSbMV-L as a potential pathogen of commercial lentils. The ease with which PSbMV-L is seed- and aphid-transmissible should facilitate its rapid spread from infected *Lens* germ plasm accessions to proximal lentil plantings during germ plasm seed increases. The absence of PSbMV-L in Palouse commercial lentil fields could be due to frequent daytime high temperatures of 30–35 C during the lentil growing season, although constant temperatures in that range (in growth chamber tests) would have exceeded normal temperature stress in the Palouse. Greater potential field spread might be expected in unusually moderate summers.

The presence of PSbMV-L as a seedborne contaminant of the lentil germ plasm collection and its aphid-transmissibility constitute a potential threat to the approximately 50,000 ha of commercial lentils grown in eastern Washington. There are inherent dangers in planting infected germ plasm near commercial lentil fields because the commonly grown cultivars are susceptible to PSbMV-L. After establishment in commercial seedstocks, the virus could be expected to persist through seed transmission and to be spread by aphids. Likewise, breeding or genetic use of infected germ plasm exposes other proximal lentil materials to potential PSbMV-L-infection by aphid transmission.

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