INVESTIGATION OF PURPORTED NON-ELISA-DETECTABLE LATENT PEA SEEDBORNE MOSAIC VIRUS INFECTION IN SELECTED U.S. COMMERCIAL PEA SEEDLOTS

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A 1986 report from Northwest Washington (3) indicated that pea seedborne mosaic virus (PSbMV) existed pervasively in commercial U.S. pea seedlots in a latent form not detectable by conventional ELISA serology. This report also suggested that in the Northwest Washington coastal environment PSbMV concentration increased in these lines and produced epidemics of the pea seedborne mosaic disease. A large number of PSbMV-infected pea lines were being grown in the Northwest Washington Research and Extension Center experimental plots, but aphid-inoculation of commercial lines from these infection sources was considered unlikely.

The possibility that U.S. commercial seedlots might contain a high incidence of seed with latent PSbMV infection demanded further investigation. Accordingly, we obtained sub-samples from six commercial seedlots (see Table 1) reported to have been infected with PSbMV (3). The possible presence of PSbMV in these seedlots was examined comprehensively by inspection of plants and by ELISA serology using anti-PSbMV rabbit immunoglubulin G produced in our respective laboratories. Results are presented below.

Methods and Materials. DAS ELISA methodology (double-antibody sandwich enzyme-linked immunosorbent assay) essentially as reported by Clark and Adams (2) was used in this investigation. Several satisfactory immunoglobulins had been produced by our laboratories for standardized ELISA detection of PSbMV. However, new high-titer antiserum against purified virus of both the P1 (1,6) and P2 (1,5) PSbMV pathotypes was produced, in case prior antisera lacked the sensitivity, either qualitatively or quantitatively, necessary to detect trace amounts of PSbMV in latent infections. Low background A405 values (i.e., near-zero reactions with healthy-plant controls) by reactants prepared from this antiserum facilitated repeated plate readings up to 72 h. Prolonged readings enabled us to detect slow-developing ELISA reactions that otherwise might have escaped detection. Positive controls included 5,000 to 10,000-fold dilutions of infected-plant homogenates and purified PSbMV diluted to 10 mg/ml, both of which were consistently detectable by this approach.

Preliminary tests of seedlots reported to contain latent PSbMV infection consisted of planting either 100 seeds (Corvallis) or 200 seeds (Prosser) of each seedlot in growth chambers and growing plants for 6 weeks (Corvallis) or to maturity (Prosser). During that time plants, of all seedlots were tested by ELISA once (Corvallis) or twice (Prosser).

Follow-up tests consisted of planting 300 seeds each of the six commercial seedlots in experimental field plots at two Oregon coastal locations, Siletz and Yachats, approximating northwest Washington coastal conditions, growing the plants to maturity, and harvesting seeds for ELISA tests of "second-generation" plants. Natural spread of two indigenous viruses, red clover vein mosaic virus and pea enation mosaic virus,

occurred in these plots, suggesting the presence of aphid vectors capable of spreading PSbMV (i.e., multiplying its incidence) had it been present in trace amounts.

Seeds harvested from these plots were visually graded and any showing testa symptoms even mildly suggestive of PSbMV were partitioned for separate planting and testing (see Table 1). Plants from these seeds were tested by two approaches. First, 180-200 seedlings representing each cultivar were sampled and tested by ELISA 20 days after emergence. A second set of plants were grown to maturity, during which all plants were routinely observed for PSbMV symptoms during growth, and selected plants were tested for PSbMV infection by ELISA at two or more times during growth and development.

Pea plants grown in field plots or greenhouses at Mt. Vernon were also tested by ELISA during 1986-88, to investigate possible PSbMV-like isolates not detectable by conventional anti-PSbMV antiserum or by anti-P1 + P2 antiserum. During this evaluation, 17 plants with PSbMV-like symptoms were sent to Corvallis from Mt. Vernon, as representative examples of diseased plants observed in experimental plots. These samples were both tested by ELISA and established in the greenhouse as reference inoculum sources. In December 1988, 19 greenhouse-grown plants with symptoms suggestive of PSbMV were submitted for ELISA testing, some from pea cvs. 'Bolero[1], 'Headliner', 'Puget', and 'Sundance' previously reported (3) to be PSbMV-infected, and some representing homozygous sbm/sbm (PSbMV-resistant) genotypes.

Results and Discussion. Three hundred plants from each of six commercial seedlots were grown in growth chambers, rigorously assayed by ELISA, and found to be free of detectable PSbMV. Twenty plants sampled from Oregon-coastal seed-increase plots planted with these seedlots were tested for possible PSbMV by ELISA, and were free of detectable PSbMV. No PSbMV-like symptoms developed on any of several hundred greenhouse-grown plants and no PSbMV was detectable by ELISA in plants from second-generation seeds of the six commercial seedlots (Table 1).

Of 17 plants with PSbMV-like symptoms from experimental plots at Mt. Vernon and ELISA tested at Corvallis, 15/17 produced typically positive PSbMV-ELISA results at Corvallis and yielded PSbMV isolates typical of the P1 pathotype.

In the course of these investigations, no atypical PSbMV isolates or serotypes were detected. None of the 19 greenhouse-grown (Mt. Vernon) plants with PSbMV-like symptoms contained ELISA-detectable PSbMV, whereas in the same tests (a) a known PSbMV-inf ected plant included to test the system was strongly ELISA positive and (b) a 10,000-fold dilution of PSbMV-infected plant homogenate was readily detectable. No mechanically transmissible virus was detected by infectivity assays of these same plant samples.

In view of the results from these several experimental approaches, we know of no factor that would have limited our ability to detect PSbMV in the six commercial seedlots tested, had the virus been present.

In our experiments, plants from first- and second-generation seeds provided no evidence of an increasing PSbMV concentration when the cultivars were grown under either coastal or greenhouse environments. The results suggest that the seedlots submitted to us, and investigated as possible PSbMV-inoculum sources, contained no detectable traces of PSbMV. It seems unlikely therefore that the ca 100% incidence of PSbMV in

Mt. Vernon experimental plots in 1986 (3) was attributable to such seedlots as had been submitted to us as exemplary PSbMV inoculum sources.

Implication of a non-PSbMV-related, seed-transmissible seed-symptom-inducing phenomenon or agent in pea (4) warrants further pathological and perhaps genetic or physiological investigation.

Table 1. Results from tests of six commercial seedlots for latent PSbMV infection by DAS ELISA (double-antibody sandwich enzyme-linked immunosorbent assay).

	Number of infected plants/Number of plants tested					
		Tests of plants from 2nd generation seed				
Commercial Seedlot	1st generation preliminary results [1]	Seedling tests	Plant test 6 weeks [2]		Plant test 12 weeks [2]	
'Bolero' VFN 2832	0/200, 0/100	0/185	0/90	0/4	0/69	0/2
'Early Frosty' RP 55114	0/200, 0/100	0/190	0/31	0/4	0/29	0/3
'Headliner' GP 96165	0/200, 0/100	0/205				
'Puget' 715-271	0/200, 0/100	0/190	0/5		0/3	
•Scout' 86 ML BLK 10	0/200, 0/100	0/180	0/46	0/17	0/29	0/1
'Sundance' 91045	0/200, 0/100	0/195	0/46	0/14	0/40	0/7

— Upper line, Prosser data; lower line, Corvallis data.

_ First column, plants from seeds with normal appearance; second column, plants from seeds with any seedcoat abnormality (i.e., in lieu of typical PSbMV-induced seed symptoms).

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6. Hampton, R.O. and G.I. Mink. 1975. Descriptions of Plant Viruses No. 146, CMI/AAB.

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