STUDIES ON A SEED PERPETUATED CONDITION IN COMMERCIAL SEED LOTS OF PEAS WITH SYMPTOMS SIMILAR TO THOSE DESCRIBED FOR PSbMV

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An abnormal condition observed in pea seed produced in the Mount Vernon area of northwestern Washington manifests seed symptoms similar to those associated with Pea Seed-borne Mosaic Virus (PSbMV) (3). The most common seed symptoms observed are small seed, mottled colored seed, seed with split seed coats, smooth seed in wrinkled seeded varieties and seed with a dark colored band at the suture (5). This condition has been observed in western Washington from 1983 through 1988 and is more prevalent in plantings made in late May and June than in plantings made in April or early May. The condition is seed perpetuated and the severity of the disorder *can* be reduced by selecting seed from plants free of the condition. In 1985, single plants producing only normal seed were selected from an experimental lot expressing severe seed symptoms. Seed from plants with only normal seed was bulked (lot H602B) and compared with non-selected seed (lot H600). Lot H602B assayed by the Prosser, Washington, ELISA laboratory as 0% PSbMV and lot H600 assayed 28 and 32% positive for PSbMV. Abnormal seed, mottled, split seed coats, dark bands at the suture, were observed in both seed lots when grown in Mount Vernon in 1986. A trace was observed in H602B and a high percentage in lot H600. The history of the seed production of the two lots was the same with the exception of the single plant selections made that were free of the observed seed condition. In 1986, a similar seed condition was observed in several commercial cultivars of peas planted in the later part of the season in Mount Vernon, WA. and grown to dry seed maturity (2).

METHODS AND RESULTS

Fal1 of 1986. Residual seed from several seed lots grown in the field in 1986 and which exhibited seed symptoms were planted in the greenhouse. Seedlings from these lots were visually rated for foliar symptoms -in the 6 to 8 node stage of development (cotyledon node equals 0). Seedlings were rated as (a) PSBM+; plants with obvious and typical symptoms of PSbMV infection, (b) suspect; symptoms resembling PSbMV, but not severe and (c) healthy; no foliar symptoms. A total of 580 samples were assayed through the Prosser, Washington, ELISA laboratory. The results of these assays and the relationship of foliar symptoms and ELISA data are presented in Table 1.

Table 1. Relationship of foliar symptoms and ELISA tests.

Foliar symptoms	No. samples	ELISA positive
PSBM+	117	115
Suspect	241	134
Healthy	222	14

The results of this initial study indicated that the observed symptoms in the greenhouse were induced by PSbMV. Due to the potential occurrence of PSbMV in commercial seed lots additional tests were conducted in 1987.

1987. Two studies were conducted in 1987: (1) ELISA assay of field grown single plants comparing ELISA results with seed symptoms and (2) greenhouse evaluation of six commercial seed lots. The same six seed lots were tested by Hampton and Mink (4).

Comparison of ELISA tests (tissue collected at full bloom to flat pod stage of development) from single plant selections (data from ELISA laboratory at Prosser, Washington) with seed symptoms did not establish a strong linkage between seed symptoms and ELISA data. A total of 729 plants were sampled and 207 were ELISA positive, 143 questionable and 380 negative. Ninty-seven percent of the ELISA positive plants produced seed(s) with virus-like symptoms and 60% and 44%, respectively, of the ELISA questionable and ELISA negative plants produced seed(s) with virus-like symptoms.

The six commercial seed lots were evaluated in the greenhouse. Sixty single plants of each of the 6 varieties were grown in 10 cm plastic pots. Fifteen samples were collected from each of the lots, at full pod development, and tested for PSbMV (ELISA laboratory at Prosser, Washington). All samples tested negative for PSbMV. Seed was harvested from each of the single plants and observed for virus-like symptoms. Symptoms were observed on a small percentage of seed from some of the plants, however, the symptoms were not typical of those observed in the field. Symptoms consisted of slight mottling in seed color, seed flatter than normal and split seed coats.

1988. Because of the apparent presence of PSbMV in commercial seed lots tested in 1986 and the relationship between positive ELISA results and seed symptoms in 1987, an additional test was conducted in 1988. Residua] seed of four of the six lots assayed in 1987 were tested using a modified protocol for growing seedlings and a more sensitive ELISA assay. The greenhouse protocol for this test included planting a total of 172 seeds in 5 x 5 x 7.5 cm seedling trays, growing seedlings under supplemental light providing 100 mkmols m^{-2} s^{-1} for 12 h in each 24 h cycle, and transplanting individual seedlings at the 5-6 node stage of development into 10 cm plastic pots. Seedlings were examined at time of transplanting for PSbMV-like symptoms and rated as "PSBM+", "Suspect" or "Healthy". No seedlings were rated as PSBM+ and all "Suspect" seedlings were transplanted into 10 cm pots. In addition to the "Suspect" seedlings, 20 "Healthy" seedlings from each lot were also transplanted into 10 cm pots. At the time of transplanting the top node was aseptically removed to prolong the vegetative growth cycle of seedlings and enhance development of previously observed virus-like symptoms.

Tissue samples were collected from apical growth of pea plants at full pod development and ELISA tested by J. Hagen. Each plant sampled was rated with respect to virus-like symptoms when tissue was collected for ELISA assay. Tissue from healthy and PSbMV infected pea plants of the same age and growing in the same greenhouse were included as controls.

Antiserum prepared by the Pillsbury Company was used with the direct method of ELISA similar to that described by Clark and Adams (1). An IgG

coating concentration of 2 mkg/ml, a sample dilution of 1:4 (sample:buffer; v:v), and an IgG/alkaline phosphatase at 0,5 mg/ml were used for all tests. Incubation times were at least 16-24 h at 4°C for each step.

In addition to 200 ELISA tests of tissue from the four seed lots (Table 2), 15 samples were assayed by R.O. Hampton (USDA, ARS, Corvallis, OR). Results from these assays were in agreement with those of Hagen in Table 2.

Table 2. Relationship of foliar symptoms and ELISA tests.

Foliar symptoms[1]	No. of plants[2]	Number assayed[3]	+PSbMV ELISA
PSBM+	0	0	0
Suspect	213	150	0
Healthy	483	46	0

1- Foliar symptoms at 5-6 node stage of development
2- Number of plants observed in each foliar class
3- Number of samples assayed for each foliar class

Summary. The results of assay of the 4 seed lots in 1988 substantiated data obtained in 1987 in Mount Vernon as well as those obtained by Hampton and Mink (4). These data establish that the four commercial seed lots were free of detectable PSbMV and indicate that the virus-like seed symptoms observed in 1986 in the commercial seed lots were not induced by PSbMV. However, the abnormal seed condition observed in seed and associated with late planting in Mount Vernon, WA., has been observed in the seed harvested from these seed lots. This virus-like seed symptom has been reduced in several commercial varieties by selecting seed from plants free of the condition. Results obtained in Mount Vernon for the past 5 years strongly suggest that this seed problem is accentuated by environmental conditions and is seed perpetuated. Additional research is currently being conducted to determine the cause and to develop a protocol for growing seedlings of peas to accentuate seedling symptoms associated with this virus-like seed disorder.

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