Dynamics of Symptom Development of the Seed-Borne Pea Fizzletop Virus

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ABSTRACT

Pea fizzletop virus (PFV) isolates produced uniform symptom profiles on selected hosts, and exhibited uniform properties. Most seedlings from PFV-infected seed exhibited discernible degrees of downward leaf roll, vein protrusion, and/or veinbanding when 1 to 3 weeks old, and were visually detectable with high accuracy. Plants with such symptoms were usually stunted, and robust plants were free of pea fizzletop symptoms. Germination vigor was not affected by the presence of PFV within the seed. PF symptoms became transient in 6- to 8-week-old plants, and under field conditions most infected plants appeared normal at full bloom stage. Five to 10% of the symptomless, vigorous plants from infected "seedlots" (an experimental sample of seed supplied by a commercial source) were found to contain PFV. PFV in these symptomless plants was not detectable by assay until plants were 5 to 8 weeks old.

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Pea fizzletop virus (PFV) is seed-borne in peas (11), and induces perceptible but regressive symptoms in seedlings arising from infected seed (6). Not all infected seedlings exhibit readily identifiable symptoms (11). However, because at least a portion of the PFV-infected seedlings consistently express downward rolling of leaves and stunting (6), all contaminated seedlots have been visually detectable in standard greenhouse tests at Corvallis. Seedlings exhibiting very mild symptoms and subject to being overlooked were no factor in conclusions of seedlot contamination. A limited base of information was therefore sufficient for locating PFV-contaminated seed stocks, and affected seed companies effectively implemented methods developed here for locating these stocks. Subsequent eradicative measures instituted by these companies were so effective that no commercial fields of peas were found to contain PFV-infected plants during 1970 or 1971.

This paper characterizes the development of PF symptoms in seedlings infected from seed, evaluates the diversity of crude physical properties and of symptom types and intensities induced by PFV isolates, examines the degree to which seedling infection remains masked and thus escapes visual detection, and evaluates the possibility of obtaining virus-free plants from contaminated seed stocks.

MATERIALS AND METHODS.—Six pea "seedlots" (experimental samples of seed supplied by a commercial source) known to contain PFV-infected seed, were used. These seedlots, designated C1 through C6, were industry selections based on WR Perfection cultivar. In preliminary experiments (three to five trials of 105 seeds each), the seedlots produced the following average percentages of seedlings with PF symptoms: C1, 29%; C2, 24%; C3, 22%; C4, 32%; C5, 21%; C6, 4%. Selections represented by these seedlots were similar phenotypically, and indistinguishable in terms of PF symptoms exhibited by each.

All data refer to infection by seed-transmission. All aspects of symptom development thus pertain to naturally infected plants. For brevity, the term "infected seedlot" is used. The samples were assumed to be representative of the source both in germ plasm and virus content. In most cases, the seedlot source represented a limited quantity of seed from breeder plots.

Seedlots were evaluated in the greenhouse during all seasons, and three also were tested under field conditions. In the greenhouse, standard test units consisted of 105 seeds/seedlot, and 15 seeds/1-gal container of sand-soil-peat mix mulched with coarse sand. Plants were maintained in insect-free rooms at ca. 22 C during 5-week test periods. Supplemental full-spectrum fluorescent lighting was used in winter when daily sunlight energy dropped below ca. 200 langleys.

Additional tests were conducted in controlled environment chambers (day/night temperature 27/18 C, 12-hr photoperiod, 2,500 ft-c light, fluorescent and incandescent illumination). Ninety seeds from infected seedlots were planted singly into containers for these tests. Following seedling emergence, the plants were arranged into groups according to growth rate, and those exhibiting PF symptoms were labeled by earliest date of symptom appearance. Correlations between infection and growth rate were determined. Two weeks after emergence and again 3 weeks after emergence, plants with symptoms were arrayed according to symptom intensities (SI). Ten SI ratings from 10 to 100 were used, and the data were expressed as the percentage of maximum symptom severity, and frequency distributions of SI were determined.

In the field, seeds from infected and virus-free seedlots were planted in 8 replicated, 50-seed plots arranged by a completely randomized design (3). Pea plants were sprayed weekly with mevinphos applied at a rate of 0.5846 liter active material in 467 liters of water/hectare for aphid control. Growth stage at which PFV symptoms were initially expressed, symptom duration, and variability of symptom intensity were recorded once or twice weekly for a 5-week test period in the greenhouse. The same data were taken during a 102-day period for field-grown

TABLE 1. Correlation between apparent pea fizzletop symptoms for abnormal appearance of pea seedlings (seedlot C1) and assay results from *Chenopodium amaranticolor*

Description of symptom or seedling abnormality	Result of assay; no. of plants with virus/total no. assayed
Marked stunting, downward roll of leaf margins, veinbanding	37/37
Slightly less than average height, with very mild leaf roll, veinbanding and/or vein protrusion	45/45
Leaves defective in shape or color, but without rolling or vein symptoms	1/16
Healthy appearance, except traces of vein swelling; plant slightly rigid	10/44
Healthy appearance, average vigor	2/41
Healthy appearance, excellent vigor	1/121

plants until mature seed were produced. The presence or absence of PFV in plants with questionable symptoms, and in selected plants from each SI category, was tested by assay on two to three plants (10 leaves) of *Chenopodium amaranticolor* Coste & Reyn. (11).

RESULTS.–Identical symptom profiles on four hosts were produced by each of 31 PFV isolates, when taken from individual infected seedlings from seedlot C1 and tested for host reaction on two to three plants of *C. amaranticolor*, eight to ten plants of *Pisum sativum L.* 'Perfection' and 'Perfected Wales', and eight to ten plants of Bell bean (*Vicia faba* var. *minor* [Peterm.] Beck). Isolate homogeneity was also suggested by the qualitative uniformity of PF symptoms exhibited by hundreds of infected seedlings from all six seedlots. These results suggest that a single virus, PFV, was being seed-transmitted in this study. Crude physical properties for 11 of these isolates agreed with those of the pea seed-borne virus reported by Mink et al. (11). Extracts from pea plants infected with each of these 11 isolates were rendered noninfectious when diluted 10^{-4} or 10^{-5} with 0.01 M PO_4, pH 7.0 buffer; when aged at 24 C for 5 or 6 days; or when heated 10 min at 50 C.

All seedlings (Table 1) with mild to severe leaf roll and/or vein protrusion (Fig. 1) were found by assay on *C. amaranticolor* to contain PFV. Other seedling symptoms usually associated with the occurrence of PFV included stunting (Fig. 1-A), a fragile brittleness of upper stems, and a slightly cartilagelike resilience of leaves. Plants with only hints of PF-like symptoms (Fig. 1-F), 10/44 of which were infected, usually exhibited one or more of the following characteristics: slightly shorter internodes than average; slightly abnormal curl of one or more leaves; slightly more yellowish-green color than normal; or slightly more erect and rigid than normal. Miscellaneous seedling defects were probably capable of masking PFV infection, but occurred at low frequency in all but one seedlot (C6), and posed no special problem. The occurrence of PFV in seedlings with normal appearance suggested the need for further assessing masked infections.

Most infected seedlings from all six seedlots, including those from C6 with only 4% incidence of PF symptoms, exhibited clear-cut visual evidence of infection. This was true for seedlings grown in controlled environment chambers, in the greenhouse at any time of the year, or under field conditions. Plants with obvious symptoms were assigned SI ratings of 100 to 30 (Fig. 1). Plants with only traces of PF-like symptoms were assigned ratings of 20 or 10, and were considered questionable. Those with no symptoms were assigned zero ratings. The modal SI for standard tests of all seedlots was 50 to 60.

Incidence of leaf roll and vein protrusion and/or banding in 3-week-old plants was closely associated with plant stunting. Seedling populations from seedlot C4 were uniformly characterized by bimodal distributions of plant height in each of four trials. The data in Fig. 2 are from the first trial. Plants of the larger mode rarely exhibited these symptoms. No leaf symptoms of PF occurred in plants in the three taller plant-height categories, but these plants were not assumed to be virus-free (7, 9). Seedling populations from virus-free seedlots produced symmetrical, unimodal distributions of plant height.

Seedlings (Seedlots C4 and C6) selected according to earliness of emergence rather than plant growth rate included more than half those ultimately exhibiting PF symptoms. Thus, while there was an absence of PF symptoms in seedlings with excellent postemergence vigor, PF symptoms frequently occurred in plants exhibiting excellent pre-emergence vigor.

Infected seedlings with SI ratings of 40 or more were visually detected by personnel in this study with 100% accuracy (Fig. 3). Plants with PF symptoms approximating SI 30 were detected almost as accurately (90%). However, accuracy decreased sharply as plants with trace symptoms, SI 20 or 10 (Fig. 1-F), were chosen. Only one of three or four plants with SI 20 and one of four to ten plants with SI 10 were found by assay to contain detectable PFV.

The collective maximum SI for seedling populations (Fig. 4) was reached when symptom development for each individual pea seedling was complete. This maximum was expressed within one week of the first definitive PF symptom, and was maintained for 2 to 3 weeks. Under greenhouse conditions, the ultimate percentage of seedlings from seedlot C1 to express symptoms (29%) had been attained 2 weeks after seedling emergence. SI had begun to decline at 4 weeks, but did not limit accurate determinations, as the tests were complete at 5 weeks. At this stage (Fig. 1-G), infected plants still exhibited mild PF symptoms, but had partially recovered from initial stunting and leaf rolling. Conversely, under field conditions only 19% of the plants from this seedlot had expressed definitive PF symptoms 6 weeks after seedling emergence; and after that time, rapidly declining SI limited visual determination of infected plants. The final percentage

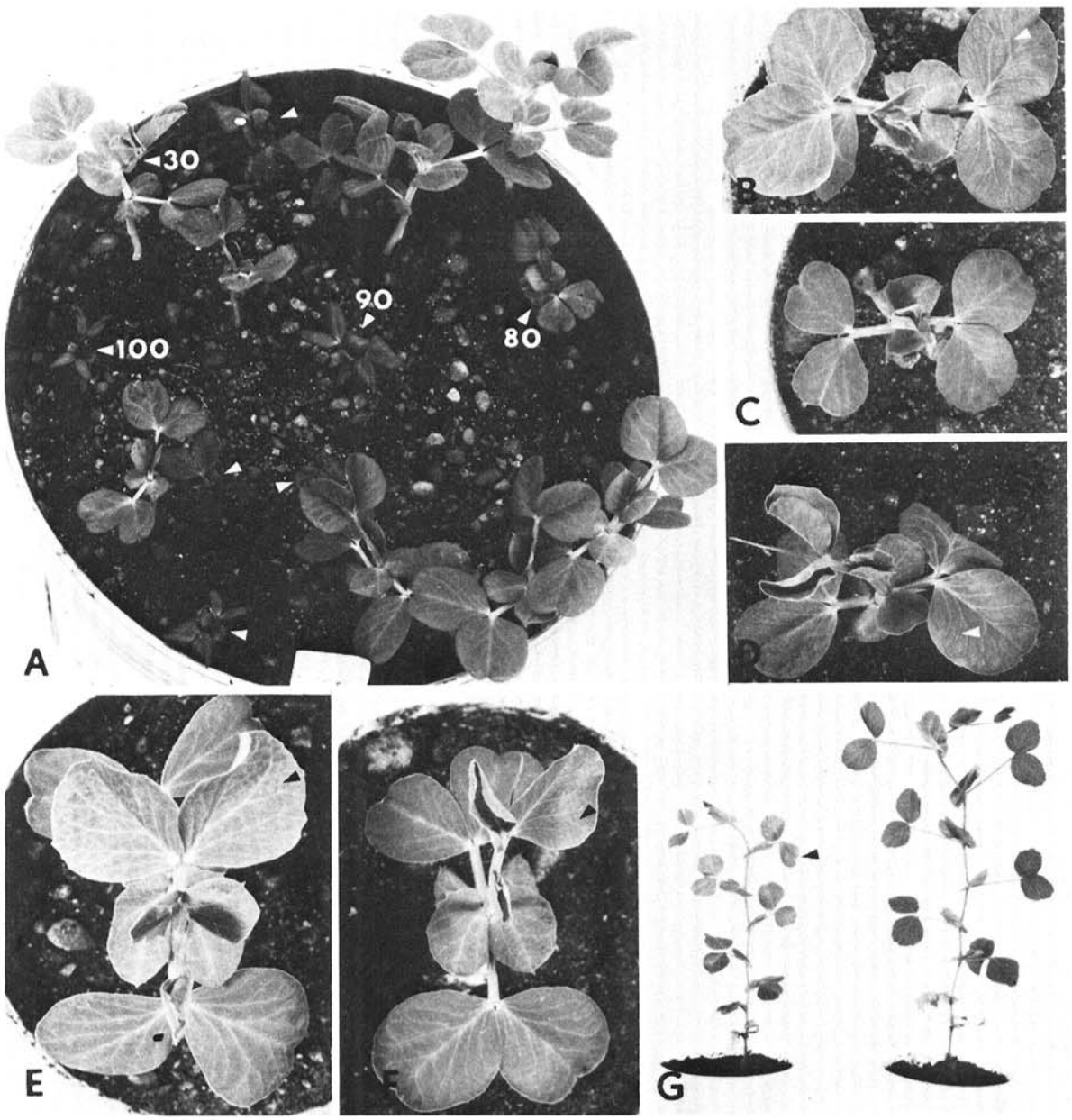
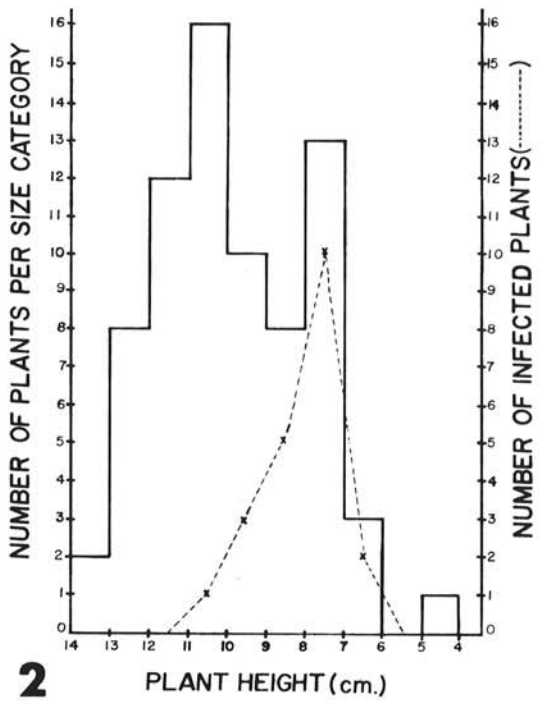
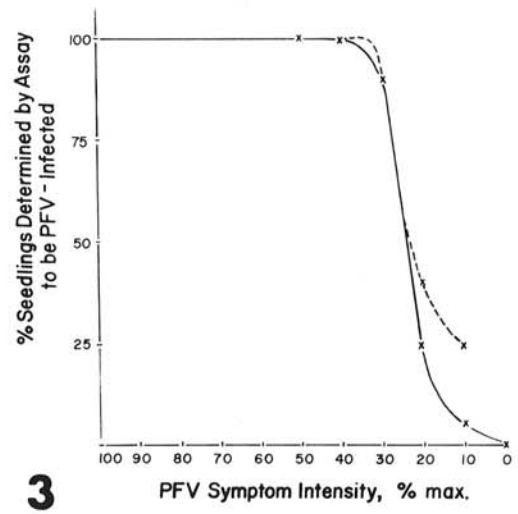
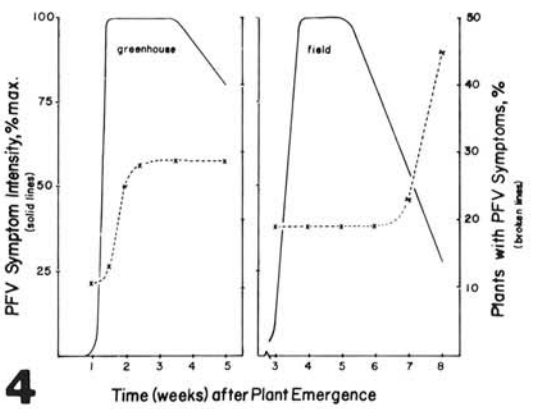


Fig. 1. Pea fizzletop virus-induced symptoms in pea seedlings arising from infected seed (seedlots C1, C4). SI = symptom intensity (10-100%). **A)** Two-week-old seedlings, 8/15 of which exhibited marked leaf rolling (pointers) ranging from SI 30 to 100. Unmarked plants differed in height, but were symptomless (SI 0). **B, C, D)** Small, symptomless plant (C) contrasted with infected seedlings (B, D) exhibiting vein protrusion and banding, perhaps the most characteristic single PF symptom in young seedlings (SI 50). **E, F)** Aberrant symptom types: plant entirely symptomless before three-node stage, with third leaf exhibiting vein and veinlet clearing SI 40 (E); and plant symptomless except third leaf exhibiting "trace" vein protrusion, SI 10 (F). **G)** Five-week-old infected seedling (left) which had partially recovered from virus-induced stunting (healthy plant, right) and exhibited only slight leaf symptoms (arrow).







of infected plants (46%) therefore could not be presumed accurate.

Transience of PF symptoms under field conditions revealed the elusive nature of this disease, and explained its survival in commercial breeding selections during careful, harsh roguing practices by seed industry personnel. Initial symptoms were mild and atypical for any known pea disease; and these disappeared by midseason.

Five to 10%, respectively, of the healthiest, most vigorous pea plants from seedlots C1 and C4 were found to contain PFV (Table 2). These were selected from seedling populations 2 to 3 weeks after emergence, isolated, and assayed on *C. amaranticolor* at 2-week intervals. Simultaneous with detection of PFV by assay, infected plants exhibited mild leaf roll and vein protrusion on young foliage. The lag period during which the virus increased in these plants to quantities detectable by assay was 5 to 8 weeks, much the same phenomenon as reported previously for another host-virus system (4). Increasing strictness in choosing the most excellent plants in successive trials of seedlot C4 caused a correspondingly reduced incidence of plants with masked infection.

DISCUSSION.—Results from the study indicated close uniformity among numerous isolates of PFV, and that the seed-transmitted virus in seedlot C1 was the same as that reported by Mink et al. (11). This virus, called "pea seed-borne virus" by Stevenson & Hagedorn (16, 17), was initially reported to have particle lengths less than 750 nm (5, 15). PFV is being investigated serologically and morphologically at Prosser, Wash. and Corvallis, Ore., to determine its relationship to pea seed-borne mosaic virus of Japan (8), to pea leafroll mosaic virus of The Netherlands, (1, 2), each having 750-nm particle-length modes, and to pea leaf rolling mosaic virus of Czechoslovakia (12) which has a particle-length mode of 700 nm.

Results also suggested that visual inspection of 200 to 500 seedlings/seedlot constituted a feasible standard test for seed-borne PFV. Most infected plants from all seedlots expressed symptoms, and even trace occurrence of leaf roll or vein protrusion was indicative of seedling infection by PFV. Plants with truly latent infections were demonstrated in two seedlots, but this phenomenon would not have limited accurate detection of PFV-infected seedlots.

Although recovery of virus-free plants from infected seedlots was not completed in this study,

Fig. 2-4. 2) Frequency distribution of 25-day-old plant heights, in relation to numbers of infected plants within each class, seedlot C4. Note bimodal distribution; absence of virus-induced symptoms in most vigorous plants. **3)** Accuracy of concluding seedling infection (plant-by-plant) based on symptoms of different intensities, seedlot C1. Divergence of dotted line depicts range of visual sensitivity or judgment among four technical and research persons. **4)** Time-related visual detectability of pea fizzletop virus (PFV) -infected pea seedlings (population) under greenhouse and field conditions, seedlot C1. Note accuracy limitation produced by declining symptom intensity (SI) in the field.

TABLE 2. Latent pea fizzletop virus (PFV) infection determined by successive assays of symptomless plants from infected seedlots on *Chenopodium amaranticolor*

Seedlot	Trial no.	No. selected symptomless plants	Age of plants (weeks) when assayed	No. plants with latent infection
C1	I, G105[a]	6	2, 4, 6	0
		8, inf[b]	2, 4, 6	8 (2, 4, 6)[c]
	II, G210	15	2, 4, 6, 8	1 (8)
C4	I, C73	10	3, 5, 9, 11	2 (5, 9, 11)
	II, C73	10	5, 7, 11, 13	1 (7, 11, 13)
	III, G400	30	10, 12	2 (10, 12)

[a] G = greenhouse; C = controlled environment chamber; population from which plants were selected.
[b] Infected plants with obvious PFV symptoms, as controls.
[c] No. in parentheses indicate plant age when determined to contain PFV.

results presented suggest this could be accomplished by (i) selecting the healthiest plants from populations of 200 to 500 seedlings when 3 to 5 weeks old; (ii) isolating and maintaining them until 10 weeks old and assaying them at that time on *C. amaranticolor*, (iii) harvesting seed from virus-free plants and repeating this cycle; and (iv) increasing seed produced in second cycle for preliminary performance testing.

Eventually, direct Chenopodium assays of grouped samples of pregerminated pea seed may be feasible, as suggested for seed-borne lettuce mosaic virus (LMV) by Pelet (13) and developed by Marrou & Messiaen (10). This procedure appears to yield information more efficiently, more precisely, and with greater certainty than that obtained by visual examination of lettuce seedlings, as reported by Rohloff (14). However, this procedure does not determine the percentage of seed transmission (10). Also, failure to detect latent infection in 3-week-old seedlings in the present study suggests that detection of PFV in the seeds from which they arose would not have been possible by direct assay.

LITERATURE CITED

1. BOS, L. 1969. Enige ontwikkelingen bij het onderzoek over virus-ziekten van peulvruchten. Jubileumuitgave 30 Jaren P.S.C. Inst. voon Plantenziekundig Onderz. p. 139-149.
2. BOS, L. 1970. The identification of three new viruses isolated from Wisteria and Pisum in the Netherlands, and the problem of variation within the potato virus Y group. Netherlands J. Plant Pathol. 76:8-46.
3. FEDERER, W. T. 1955. Experimental design, p. 86. The MacMillan Company, New York.
4. HAMPTON, R. O. 1963. An attempt to detect virus-free buds in latent virus-infected sweet cherry. Phytopathology 53:718-719.
5. HAMPTON, R. O. 1969. Characteristics of virus particles associated with the seed-borne pea fizzletop disease. Phytopathology 59:1029 (Abstr.).
6. HAMPTON, R. O., & J. R. BAGGETT. 1970. Host effects and diagnostic symptoms of pea fizzletop disease. Plant Dis. Reptr. 54:355-358.
7. HAMPTON, R. O., & E. W. HANSON. 1968. Seed transmission of viruses in red clover: evidence and methodology of detection. Phytopathology 58:914-920.
8. INOUYE, T. 1967. A seed-borne mosaic virus of pea. Ann. Phytopathol. Soc. Japan 33:38-42.
9. LISTER, R. M., & A. F. MURANT. 1967. Seed transmission of nematode-borne viruses. Ann. Appl. Biol. 59:49-62.
10. MARROU, J., & C. M. MESSIAEN. 1967. The Chenopodium quinoa test: a critical method for detecting seed transmission of lettuce mosaic virus. Int. Seed Testing Ass. Proc. 32:49-58.
11. MINK, G. I., J. KRAFT, J. KNESEK, & A. JAFRI. 1969. A seed-borne virus of peas. Phytopathology 59:1342-1343.
12. MUSIL, M. 1970. Pea leaf rolling mosaic virus and its properties. Biologia (Bratislava) 25:379-392.
13. PELET, F. 1965. Dosage du virus de la Mosaique de la laitue par indexage de le graine sur Chenopodium quinoa Willd. Rev. Hort. Suisse 38:7-10.
14. ROHLOFF, I. 1967. The controlled environment room test of lettuce seed for identification of lettuce mosaic virus. Int. Seed Testing Ass. Proc. 32:59-64.
15. STEVENSON, W. R., & D. J. HAGEDORN. 1969. A new seed-borne virus of peas. Phytopathology 59:1051-1052 (Abstr.).
16. STEVENSON, W. R., & D. J. HAGEDORN. 1970. Effect of seed size and condition on transmission of the pea seed-borne mosaic virus. Phytopathology 60:1148-1149.
17. STEVENSON, R. W., & D. J. HAGEDORN. 1971. Reaction of Pisum sativum to the pea seedborne mosaic virus. Plant Dis. Reptr. 55:408-410.