Host Range and Properties of Peanut Stunt Virus from Morocco

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ABSTRACT

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A virus that caused a severe disease of bean (*Phaseolus vulgaris*) in Morocco was identified as peanut stunt virus (PSV). Identification was based on host range, physical properties, particle morphology, aphid transmissibility, and serological relationships. This virus has previously been reported only from the USA and Japan. The Moroccan virus was serologically related but not identical to the eastern (PSV-E) and western (PSV-W) American strains of PSV. On the differential hosts Tennessee Greenpod bean and Perfected Wales pea the Moroccan PSV isolate also produced symptoms distinctly different from those produced by the two American virus strains. All 26 cultivars of bean tested were susceptible to infection by the Moroccan PSV isolate. The virus also was isolated from peanut (*Arachis hypogaea*) and alfalfa (*Medicago sativa*) in the vicinity of infected bean fields.

Additional key words: virus disease of bean, cucumovirus group.

Peanut stunt is a comparatively recently described virus disease, its first description dating back to only 1966 (7, 10, 12). Since that time it has been reported from several locations in the United States, and two groups of strains, PSV-E and PSV-W, have been reported there (8). To date, PSV has been reported elsewhere only from Japan (4, 13), where a different strain, designated PSV-J, has been described. Various plants have been found naturally infected by PSV (11), including peanut, tobacco, hoary-pea (*Tephrosia* sp.), soybean, crownvetch (*Coronilla varia* L.), and white clover, but most concern has been attached to the disease produced in beans (*Phaseolus vulgaris* L.) (2, 13). A virus disease of this crop, characterized by pronounced stunting, leaf mottling, rugosity, and complete loss of fruit (Fig. 1-A) was observed in 1975, 1976, and 1977 in the region of Boulaouane in Morocco. In 1977 the virus also was isolated from naturally infected peanut (*Arachis hypogaea* L.) and alfalfa (*Medicago sativa* L.) in the same region. The causal agent was identified as another variant of PSV whose properties are described here.

MATERIALS AND METHODS

Host-range experiments.—All test plants were cultivated in autoclaved soil and kept in an insect-proof, air-conditioned greenhouse. Inoculum for mechanical transmission tests was prepared by grinding infected leaf tissue in 0.05 M phosphate buffer (pH 7.1) containing 0.1% (v/v) 2-mercaptoethanol. As a routine test for systemic invasion of inoculated test plants, back inoculations were made from young leaves to cowpea [*Vigna unguiculata* (L.) Walp 'Early Ramshorn']. In addition, where evidence of local infection was lacking, back inoculations also were made from inoculated leaves.

Physical properties.—Crude sap for the determination of the physical properties was extracted from the inoculated primary leaves of Bountiful bean after local lesion appearance (8 days postinoculation). Early Ramshorn cowpea served as assay host in all tests. In the dilution test, distilled water was used as diluent. Each experiment was performed at least twice.

Insect transmission.—Nonviruliferous apterous adults of *Myzus persicae* Sulz. from a stock culture on pepper plants were used in experiments of nonpersistent aphid transmission. In separate experiments, first Bountiful bean, and then Early Ramshorn cowpea, were used as both source and assay plants. Following a 1-hr pre-acquisition fast, the aphids were allowed a single acquisition probe on source plants before being transferred in groups of five to each healthy assay plant. After 24 hr, the insects were killed by aphicide application.

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Virus purification.—Frozen infected leaf tissue of Early Ramshorn cowpea, harvested 10 days after inoculation, was homogenized 1:1 (w/v) in phosphate buffer (0.1 M, pH 7.2, containing 0.1% 2-mercaptoethanol). To the extracted crude sap, *n*-butanol was added dropwise to a final concentration of 10%. After the sap was stored overnight at 5 C, it was centrifuged at low speed. From the resulting supernatant fluid, the virus was precipitated by the addition of 10% polyethylene glycol and 2% sodium chloride. The resuspended precipitate was given two cycles of differential centrifugation. Phosphate buffer (0.03 M, pH 7.0) was used as resuspension buffer. Further purification was achieved by sucrose density-gradient centrifugation. Final preparations were suspended in distilled water.

Serology.—All serological tests were carried out in Ouchterlony double-diffusion plates of 0.5% agarose in 0.05 M phosphate, pH 6.5. Sodium azide (0.1%) was added as a preservative. Antisera against CMV and PSV were obtained from the American Type Culture Collection (ATCC). These were, respectively, ATCC PVAS 60, and ATCC PVAS 88 (against CMV), and ATCC PVAS 39 and ATCC PVAS 62 (against PSV). Samples of antisera against four isolates of tomato aspermy virus (TAV) were provided by R. H. Lawson, U.S. Department of Agriculture, Beltsville. Antiserum against the Moroccan PSV isolate was obtained from a single rabbit following two intramuscular and two intravenous injections with gradient-purified virus. Injections were given at 10-day intervals, and the animal was bled 14 days after the final injection. This antiserum had a tested homologous titer of 1/16 in double-diffusion

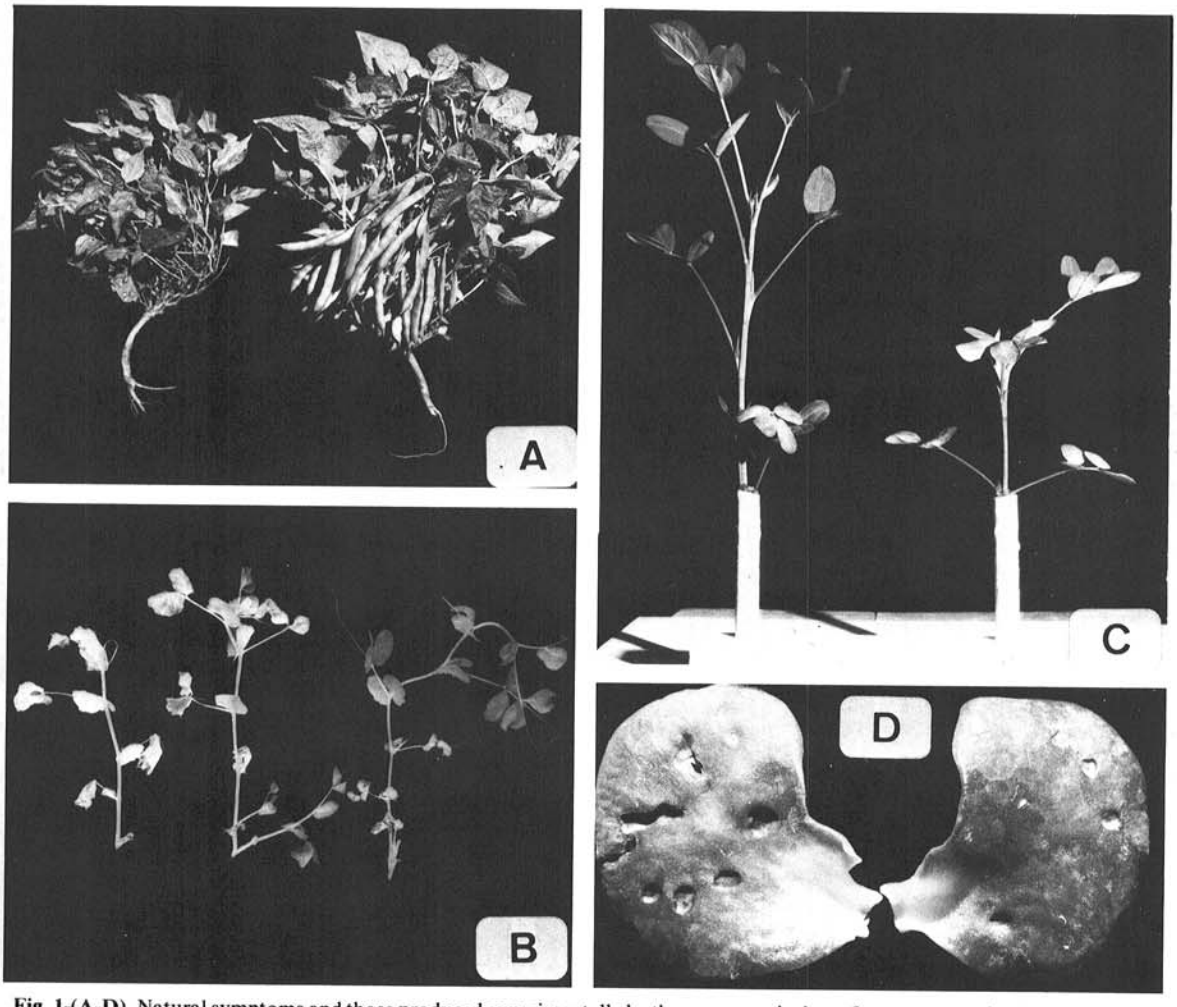

Fig. 1-(A-D). Natural symptoms and those produced experimentally by the moroccan isolate of peanut stunt virus (PSV). **A)** Effect on plant development and yield in plants of naturally infected bean cultivar Prédome. Noninfected control on the right. **B)** Strain differentiation on plants of Perfected Wales pea 20 days after mechanical inoculation. Left: plant infected by the Moroccan isolate of PSV; center: plant infected by PSV-W; right: plant infected by PSV-E. Note progressive severity of growth inhibition, chlorosis, wilt, and necrosis from right to left. **C)** Stunting effect on a plant of peanut cultivar Argentine after mechanical inoculation with the Moroccan isolate of PSV. Noninfected control of the same age on the left. **D)** Sunken necrotic local lesions on primary leaves of white lupin after mechanical inoculation with the Moroccan isolate of PSV.

plates. In addition to the three PSV isolates, two CMV isolates were used in comparative serological tests: ATCC PV 29 (Type strain), and a Moroccan CMV isolate from squash. All virus preparations used in these serological tests were purified by sucrose density-gradient centrifugation.

RESULTS

Host range.—The Moroccan PSV isolate infected 59 of the 65 test plants used. The host range was similar but not identical to those reported for other PSV isolates (4, 8, 12, 13). The virus infected all 26 bean cultivars that were tested. Symptoms on these plants included chlorotic and necrotic local lesions, and systemic mosaic, necrosis, leaf deformation, and stunting. Similar systemic symptoms were produced on soybean [*Glycine max* (L.) Merr. 'Lee'] and peanut (*Arachis hypogaea* L. 'Argentine', 'Florigiant', 'Virginia Bunch'). A lethal systemic reaction was produced in Early Ramshorn cowpea, chickpea (*Cicer arietinum* L.), and in all cultivars of *Pisum sativum* L. tested. The virus produced chlorotic and necrotic local lesions, without subsequent systemic invasion, in *Citrullus vulgaris* Schrad., *Cucumis anguria* L., *C. melo* L., *C. sativus* L., *Cucurbita maxima* Dcne., *C. moschata* Dcne., *C. pepo* L., *Lagenaria siceraria* (Mol.) Standl., and *Momordica charantia* L. Although most of the solanaceous test plants were susceptible to infection, systemic symptoms developed regularly only in *Datura stramonium* L., *Nicotiana clevelandii* Gray, and *Petunia hybrida* Vilm. Tomato (*Lycopersicon esculentum* Mill. 'Bonny Best') did not become infected, and the cultivars Condine Red, Géante de Saint Pierre, and Tiny Tim were infected only locally.

Apium graveolens L., *Brassica rapa* L., *Helianthus annuus* L., *Vinca rosea* L., and *Ocimum basilicum* L. were not infected by the virus.

Physical properties.—Thermal inactivation of the Moroccan PSV isolate occurred at 50-52 C. In 10-fold dilution steps, virus infectivity was lost between 10^{-4} and 10^{-5}. The longevity in vitro at room temperature (24 C) was 24-48 hr.

Insect transmission.—In a single experiment on nonpersistent aphid transmission using *Myzus persicae,* four of eight assay plants of Bountiful bean and two of five Early Ramshorn cowpeas developed typical symptoms of systemic infection.

Purification and electron microscopy.—In rate-zonal density gradient centrifugation in 10-40% sucrose gradients purified virus sedimented as a single UV-absorbing band. Material from this band was dialyzed against distilled water, fixed in cold neutralized 4% formaldehyde for 10-15 min, and stained with 2% neutralized phosphotungstic acid. When examined in the electron microscope, spherical 30-nm diameter particles typical of the cucumovirus group were observed.

Serology.—In double-diffusion tests the Moroccan virus gave either no reaction, or else a very faint precipitin line, when tested against antisera to either of the two strains of CMV, ATCC PVAS 60, or ATCC PVAS 88. Both the type strain of CMV (ATCC PV 29) and the Moroccan CMV isolate from squash reacted positively against these two CMV antisera (Fig. 2-B). The Moroccan PSV isolate also reacted positively when tested against antisera to three TAV isolates. The Moroccan PSV isolate, and both the type and Moroccan squash isolates of CMV gave strong positive reactions when tested against antiserum to PSV-E (ATCC PVAS 62, Fig. 2-A). The precipitin lines of the two CMV isolates fused with each other, but spurred with those of the Moroccan PSV isolate. Against antiserum to PSV-W (ATCC PVAS 39) the Moroccan PSV isolate reacted only weakly, in contrast to PSV-W and PSV-E (Fig. 2-C). When all three PSV isolates were tested against an antiserum to an isolate of the Moroccan virus (Fig. 2-D), all reacted positively, with mutual spur formation or intersection of precipitin lines. The intersection of the precipitin lines suggests that a serological difference exists between the PSV-M used for antiserum production and the purified preparation of the same virus used in the antigen wells. Approximately 1 yr elapsed between preparation of the antiserum and purification of virus for the test reported in Fig. 2-D. It is possible that during the course of repeated transfers some component or components present in the original virus isolate was eliminated. If this did occur, then the virus referred to above as PSV-M may consist of a mixture of types that differ serologically and possibly in other properties.

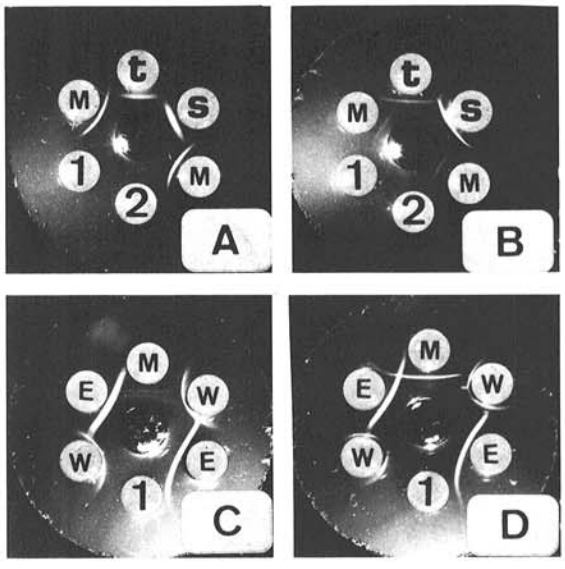

Fig. 2-(A-D). Serological differentiation of the Moroccan peanut stunt virus (PSV) isolate from cucumber mosaic virus (CMV) and PSV strains E and W. **A)** Reaction of two different CMV strains and the Moroccan isolate of PSV against PSV-E antiserum (ATCC PVAS 62). **B)** Reaction of two different CMV strains and the Moroccan PSV isolate against CMV-antiserum (ATCC PVAS 88). The inner lines around the antiserum well are considered to be nonspecific. **C)** Reaction of the Moroccan PSV isolate and PSV strains E and W against PSV-W antiserum (ATCC PVAS 39). **D)** Reaction of the Moroccan PSV isolate, PSV-E, and PSV-W against antiserum to the Moroccan PSV isolate. Abbreviations: M = Moroccan PSV isolate; E = PSV strain E; W = PSV strain W; t = CMV type strain (ATCC PV 29); s = Moroccan CMV isolate from squash; 1 = control preparation from *Vigna unguiculata*; and 2 = control preparation from tobacco.

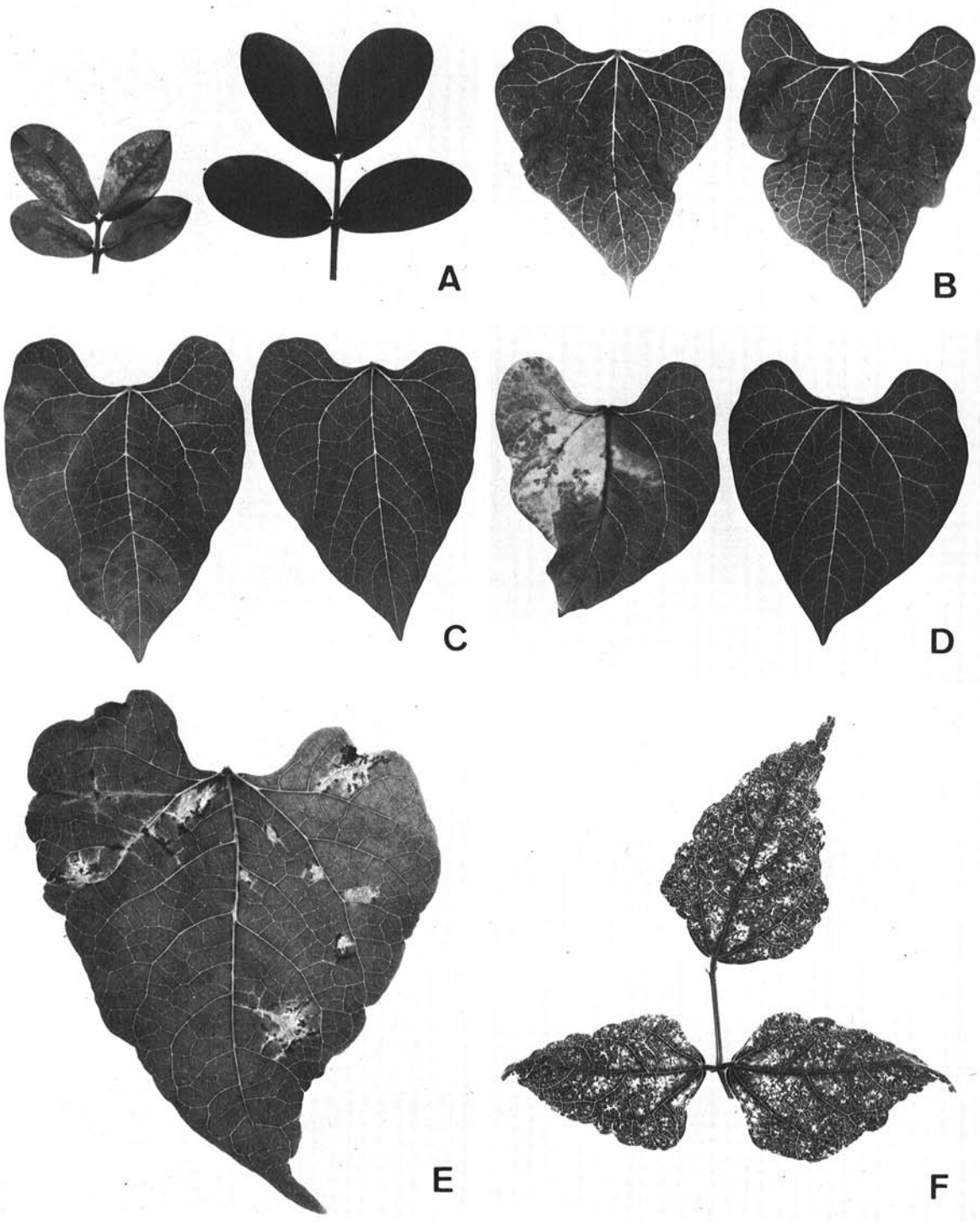

Fig. 3-(A-F). Test plant reactions to mechanical inoculation with the Moroccan isolate of peanut stunt virus (PSV-M). **A)** Systemic leaf mottle in a plant of peanut Argentine. Healthy leaf on the right. **B)** Two primary leaves of Tennessee Greenpod bean after mechanical inoculation with strain PSV-E. **C)** and **D)** Primary leaves of Tennessee Greenpod bean after mechanical inoculation with the Moroccan isolate of PSV. Healthy leaves on the right. **E)** Local, and **F)** Systemic symptoms of the Moroccan PSV isolate on lima bean.

Strain differentiation.—To differentiate the Moroccan virus isolate from PSV-E and PSV-W, Tennessee Greenpod bean and Perfected Wales pea were used as differential hosts in two separate inoculation experiments. On Tennessee Greenpod bean, PSV-E produced numerous necrotic local lesions (Fig. 3-B) which later coalesced and led to complete leaf desiccation, followed by systemic stem, petiole and vein necrosis, necrotic ringspots, leaf mottle, and stunting. On the same host, PSV-W provoked no apparent local symptoms except slight rugosity of the leaf surface, but the young leaves developed a distinct mosaic and blistering. Typical of the reaction to this strain was the complete absence of necrosis. On the inoculated primary leaves of the same bean cultivar the Moroccan PSV isolate produced large chlorotic ringlike lesions and necrotic vein streaking on the lower leaf surface (Fig. 3-C, D), which caused downward rolling of the lamina. Systemic symptoms consisted of occasional stem and petiole necrosis and necrotic leaf spots. In Perfected Wales pea (Fig. 1-B), PSV-E produced a faint systemic mottle without necrosis. On this host, PSV-W caused local chlorosis, followed by leaf desiccation. Chlorotic spots and veinbanding mottle appeared on the young leaves. This developed into necrotic stem streaking, which caused the leaves to wilt and desiccate. The most severe and rapid symptom development, however, was observed with the Moroccan virus isolate. Symptoms began with necrotic, partially ringlike local lesions, which expanded and caused the leaves to desiccate. The subsequent symptoms of systemic infection consisted of mottle, leaf chlorosis, wilt, stem necrosis, vein necrosis, and finally top necrosis, which led to plant death 14-15 days after inoculation.

DISCUSSION

On the basis of particle morphology, aphid transmissibility, and physical properties, the Moroccan bean virus isolate must be classified as a member of the cucumovirus group. In biological properties, however, it differs significantly from CMV. The virus failed to produce systemic infection in any of the cucurbits tests, and either failed, or only rarely succeeded, in systemically invading the solanaceous species (*N. tabacum, N. glutinosa,* pepper, or tomato) normally infected by CMV isolates. Peanut, which has not been shown to be susceptible to CMV, was infected by the Moroccan virus. In contrast to most CMV isolates, the Moroccan bean virus systemically infected cowpea, bean, and *Chenopodium quinoa* Willd. A Moroccan CMV isolate from cowpea, which systemically infects these three species, can be distinguished readily by its ability to produce systemic infection in cucurbits, and by the diagnostic reaction evoked in Columbia Pinto and Great Northern bean (3). Isolates of CMV, which systemically infect beans, have been reported from Puerto Rico (6), France (5), Spain (1), and Germany (9). These viruses differed in host range from the Moroccan PSV isolate and were all clearly identified as isolates of CMV. The Moroccan virus reacted, but only weakly, with the CMV antisera used. The Moroccan PSV isolate also reacted with antiserum to TAV, but differs from this latter virus in its ability to infect systemically datura, bean, and *C. quinoa,* and in failure to infect systemically *N. glutinosa,* tomato, and tobacco. On the basis of biological, physical, and serological properties, the Moroccan bean virus isolate is identified as PSV, and for convenience is designated PSV-M. Serological tests, and symptoms produced on the differential hosts Tennessee Greenpod bean and Perfected Wales pea, demonstrated clear differences between PSV-M and the American strains PSV-E and PSV-W. Because of its aphid transmissibility, ability to infect such common weeds as *Stellaria media* (L.) Cyr., and the total absence of resistance in all tested bean cultivars, this virus is considered to be of potential importance to bean cultivation in Morocco.

Because infected beans, either from the field or greenhouse, failed to produce fruit, seed-transmission of PSV-M would seem to be of little importance in natural spread of this virus. In 1977, the virus was isolated from peanuts in the vicinity of fields of infected beans. Seed-transmission of PSV in peanut has been reported (8) but it was judged to be of little importance. More significant, perhaps, was the isolation of PSV-M from severely affected fields of alfalfa in the same region. This is the first report of alfalfa as a natural host of PSV. That crop may become the most important reservoir of PSV-M which is able to infect beans, peanuts, and other legumes in Morocco.

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