Isolation and Characterization of Peanut Stunt Virus from Alfalfa in Spain

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ABSTRACT

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A cucumovirus was isolated from alfalfa (*Medicago sativa*) growing in the Barajas area near Madrid, Spain. After purification, the virus was identified as a strain of peanut stunt virus (PSV). In its host range, the virus (designated PSV-B) resembled previously characterized PSV strains (T, V, and W) from the United States with only minor symptom differences in a few plant species. Serologically, PSV-B was indistinguishable from PSV-W and was closely related, but not identical, to PSV-V. There was a more distant serologic relationship between PSV-B and some strains of cucumber mosaic virus (CMV) and tomato aspermy virus. Although the sedimentation rate of PSV-B was identical to previously characterized cucumoviruses, in polyacrylamide gel electrophoresis PSV-B migrated with a different mobility than either PSV-V or PSV-W, although the difference from PSV-W was very small. It coelectrophoresed with CMV strain D. The virus is different from most other cucumoviruses in that it is very unstable in solution, where it degraded into nucleoprotein particles with bacilliform structures and lower sedimentation rates. These nucleoproteins contained the separate viral RNA components. Base ratios of the RNAs of PSV-B, PSV-W, and PSV-V revealed great similarity but were significantly different from that of CMV.

Additional key words: serology, stability.

RESUMEN

Caracterización del virus del achaparramiento del cacahuete aislado de plantas de alfalfa en España.

Un cucumovirus fué aislado en España de plantas de alfalfa, en el área de Barajas cerca de Madrid, e identificado como una raza del virus del achaparramiento del cacahuete (PSV). El virus, llamado PSV-B, produce en plantas de ensayo síntomas similares a los de otras razas (T, V, y W) aisladas en USA. En ensayos serológicos, PSV-B no se diferencia de PSV-W y, aunque distinto, está estrechamente relacionado con PSV-V; PSV-B presenta una relación serológica más distante con algunas razas del virus mosaico del pepino (CMV) y el virus de la esterilidad del tomate. Mientras que PSV-B posee un coeficiente de sedimentación idéntico al de otros cucumovirus, su movilidad electroforética en gel de poliacrilamida es diferente de la de PSV-V o PSV-W, e idéntica a la de CMV-D. A diferencia con otros cucumovirus, PSV-B es muy inestable en solución, donde se degrada dando lugar a partículas nucleoproteicas de estructura baciliforme y valores de sedimentación menores. Estas partículas contienen, separadamente, los diferentes ARN virales. La proporción de bases del ARN de PSV-B, PSV-W y PSV-V es muy similar y difiere significativamente de la de CMV.

Peanut stunt virus (PSV) is classified as a member of the cucumovirus group (8). Its relationship to cucumber mosaic virus (CMV) was suspected soon after its initial isolation and association with epidemics of the stunting disease of peanut (*Arachis hypogaea* L.) in North Carolina (2) and Virginia (17) 10 yr ago. However, because the serologic relatedness of PSV to CMV was not known (18,25) and was only recently clearly defined (3), PSV has retained its separate name. The situation is further complicated by the fact that the serologic relationships of the two best characterized strains of PSV, strain V from Virginia (1,14) and PSV-W from Washington (20,21) were not known until recently (4).

Moreover, PSV is one of the most damaging viruses in the United States because it can infect numerous field and forage legumes as well as other crops. Field isolates of PSV have been obtained from soybean (*Glycine max* (L.) Merr.), bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.), clover (*Trifolium repens* L.), tobacco (*Nicotiana tabacum* L.), and other species (S. Tolin, *personal communication*). The virus causes considerable damage in these crops, and its wide host range and aphid transmissibility are important ecological factors in its geographic spread. Like other cucumoviruses, the epidemiology of PSV is characterized by sudden disease outbreaks of unexplained origin. It also has a tripartite genome, the components of which resemble those of CMV in size (14). The separated components of PSV's genome were interchangeable with those of CMV in pseudorecombination experiments in vitro (16). Because such events probably also take place in vivo, it is usually difficult to make a sharp distinction between PSV and CMV in natural cucumovirus isolates.

This article describes the purification and characterization of a cucumovirus isolated from alfalfa (*Medicago sativa* L.) in Spain. In host range and serologic characteristics, the virus is similar to PSV-W and PSV-V, but it differs considerably in stability. To our knowledge, only one other PSV isolation has been reported in Europe (5).

MATERIALS AND METHODS

Virus sources. The PSV strain described here (designated B) was isolated from alfalfa plants growing in a field in the Barajas area near Madrid, Spain, where the virus occurred in mixed infection with alfalfa mosaic virus (AMV). The two viruses were readily separated by mechanical transmission to cowpea plants (*Vigna unguiculata* L. Walp), on which PSV-B produced chlorotic local lesions and systemic chlorosis, but AMV incited only necrotic local lesions. Subsequently, PSV-B was cloned on cowpea by means of five serial single lesion transfers. The virus was increased for purification in tobacco (*N. tabacum* L. *Xanthi-nc*).

For comparative purposes in this study, PSV-W (received from G.I. Mink, Washington State University, Prosser), PSV-H (a French isolate from bean [5]), PSV-V, and several CMV strains were used and propagated as described (14).

The PSV-T from *Tephrosia vogellii* (26) was used for the comparative host range study.

Host range comparison. The host range of PSV-B was determined by mechanically inoculating several plants of each test species two or more times during 10 mo. At the same time, control plants were rubbed with either healthy cowpea leaf extracts or extracts containing PSV-T and then observed for symptom development for 2–6 wk under ambient greenhouse conditions. The reactions of species in 37 genera were studied. They were: *Arachis, Antirrhinum, Atropa, Beta, Brassica, Catharanthus, Capsicum, Celosia, Cucurbita, Cucumis, Cyamopsis, Cynoglossum, Chenopodium, Datura, Dolichos, Dianthus, Gomphrena, Glycine, Helianthus, Lycopersicon, Momordica, Medicago, Nicandra, Nicotiana, Petunia, Pisum, Phaseolus, Raphanus, Saponaria, Sesbania, Salpiglossis, Tagetes, Tephrosia, Tithonia, Vicia, Vigna, and Zinnia.* Peanut plants were inoculated when 3–5 cm tall because they were more difficult to infect when larger. Plants of species that did not develop symptoms were indexed back on *Chenopodium quinoa* Willd.

Purification. The PSV-V, W, and H, CMV-S, R, and D were purified by the method of Lot et al (15).

The PSV-B was purified from fresh, infected tobacco leaves 7–8 days after inoculation. It was very unstable when purified by the method mentioned above. This method was therefore modified for PSV-B by lowering the salt concentration of the citrate extraction buffer to 0.1 M 0.1% thioglycolic acid, pH 6.5, and by including ethylenediaminotetraacetic acid (EDTA) in the solutions throughout all the steps (0.02 M in the grinding buffer, and 0.001 M in the resuspension buffers). This purification procedure produced a stable and homogeneously sedimenting component in rate zonal centrifugations in sucrose density gradients and in analytical ultracentrifugation. Virus yields ranged from 300 to 500 mg/kg of infected leaves.

Serology. An antiserum designated ps 631 was prepared by immunization of a rabbit with a preparation of PSV-B stabilized with 0.3% formaldehyde. Three doses of 1 mg of antigen each, emulsified with Freund's complete adjuvant, were injected intramuscularly at time zero and 1 and 2 wk later. This was followed by monthly booster injections. The homologous titer of ps 631 was 1/512. Other cucumovirus antisera used in this work have been described (3,4).

The antigenic properties of PSV-B and its relationships with several cucumoviruses (other PSV strains, CMV, tomato aspermy virus [TAV]) were investigated by Ouchterlony's double immunodiffusion method. The medium was 0.8% agarose (Indubiose A 37) in a veronal buffer, pH 8.6, containing 0.025 M EDTA. In these comparisons, a circular configuration of wells was used with undiluted serum in the center well and the antigens (1 mg/ml) in the peripheral wells.

Standard methods. Rate zonal centrifugation was performed with a Beckman 41 Ti rotor for 120 min at 40,000 rpm and 4 C. Gradients of 0.2–0.8 M sucrose were prepared in different buffers depending on the purpose of the experiment. They were analyzed and fractionated with an ISCO density gradient fractionator and optical unit at 254 nm.

Analytical ultracentrifugation was performed in a Spinco Model E ultracentrifuge equipped with the high intensity ultraviolet light source, monochromator, and photoelectric scanning system in an An-D rotor at 20,000 rpm and 20 C.

Polyacrylamide gel electrophoresis of intact virions and viral RNAs was performed as described (14). The RNAs were extracted by the phenol-sodium dodecyl sulfate (SDS) method (12) or by direct release of the RNA with SDS before electrophoresis (14).

Virus and RNA concentrations were estimated using extinction coefficients of $E^{1\%}_{260} = 50$ and 250, respectively (11).

Samples for electron microscopy were fixed with 0.3% formaldehyde in 0.01 M sodium phosphate buffer, pH 7, stained with 2% uranyl acetate or 2% potassium phosphotungstate, pH 6.9, and viewed and photographed with a JEOL JEM 100-B transmission electron microscope.

Base ratios were determined by polyacrylamide gel electrophoresis (22).

RESULTS

Host range. Host range and symptoms of PSV-B were essentially the same as those of PSV-T (26) in simultaneous tests and those that have been reported for other U.S. isolates of PSV (6,13,19). It differed from U.S. PSV strains from bean (6), peanut (13), and *Tephrosia* (26) only in symptoms incited in *Dolichos lablab* L. and tomato. Unlike PSV from peanuts (13) or beans (6), PSV-B incited severe systemic necrosis in *D. lablab* and usually killed the plant within 3 wk after inoculation. On the other hand, it caused only an occasional systemic chlorotic leaflet constriction on tomato plants (cv. Rutgers) instead of the reported mosaic (6) and fernleaf (19) symptoms. It incited symptoms in alfalfa like that of our strain from *Tephrosia* (26), which consisted of mild chlorotic areas on about 20% of the leaves. Plants were not noticeably stunted or otherwise affected. Four peanut cultivars (Tennessee Red, NC-2, Virginia Bunch 67, and Starr) reacted with typical stunting and mosaic to infection by the B strain and the control *Tephrosia* strain.

Properties of the virus in sap. Thermal inactivation, dilution end point, and aging in vitro of PSV-B were determined as described (26) except that systemically infected cowpea leaves served as the source of crude juice and, after treatment, bioassay was done on C. *quinoa*. These properties were similar to those reported by Kuhn (13) for the peanut strain.

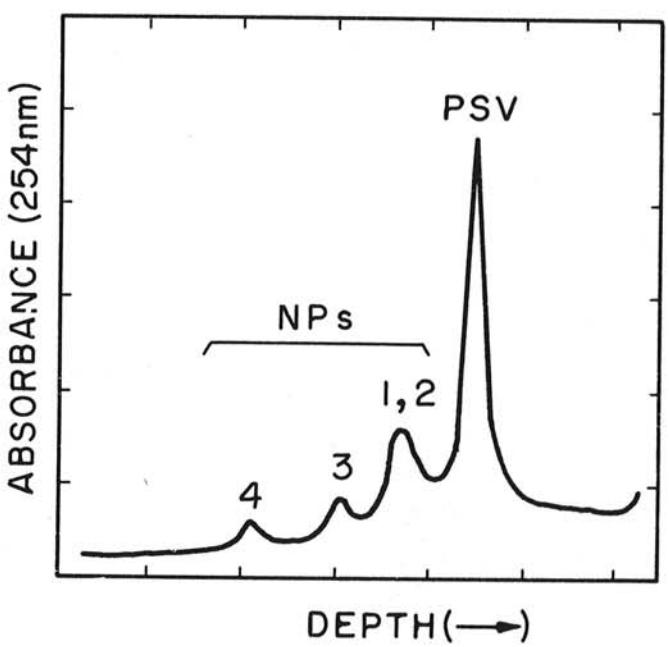

Fig.1. Sucrose density gradient centrifugation profiles of strain B of peanut stunt virus purified in the absence of EDTA showing the intact virus and degraded nucleoprotein peaks. Gradients, 0.02–0.8 M sucrose, in 0.01 M Na phosphate buffer, pH 7, were centrifuged at 4 C in a Beckman SW 41 rotor at 40,000 rpm for 120 min.

Degradation of the virus. When PSV-B was purified in the absence of EDTA, it was unstable at low ionic strength in neutral or weakly alkaline phosphate or citrate buffers or in distilled water. In addition to the virus peak, under such conditions, a number of slower sedimenting and well defined nucleoprotein peaks (NP) were found in rate zonal centrifugation experiments (Fig. 1). The same sedimentation pattern was again reproduced when the virus peak was fractionated and maintained in the above conditions (Fig. 4A, left). Except for the leading virus peak, the sedimentation pattern resembled that obtained by the degradation and/or reassociation of CMV-S at pH 10.3 (9,11). The NPs, with sedimentation coefficients of 75S, 59S, and 37S, were fractionated from the gradients and reconcentrated by ultracentrifugation for further analysis.

Each NP exhibited an ultraviolet spectrum characteristic for nucleoproteins. Their polyacrylamide electrophoretic mobilities were identical but slightly faster than those of the virion. Polyacrylamide gel electrophoresis of their RNA showed that, in order of decreasing sedimentation rates, the fractionated NPs contained the RNA components 1 + 2, 3, and 4 of PSV-B, respectively (Fig. 2). Bioassays of the virus and NP fractions showed that infectivity was primarily associated with the virus fraction. In electron micrographs of the NPs, short bacilliform structures of different lengths were observed (Fig. 3).

Stability tests. The effect of EDTA and Mg^{2+} on the stability of the virus and the NPs was investigated. Degraded virus, purified in the absence of EDTA (Fig. 1), was separated into two fractions consisting of the combined NPs and intact virus. Aliquots of these two fractions were dialyzed for 16 hr at 4 C in neutral 0.01 M sodium phosphate buffer alone and with either 0.01 M EDTA or

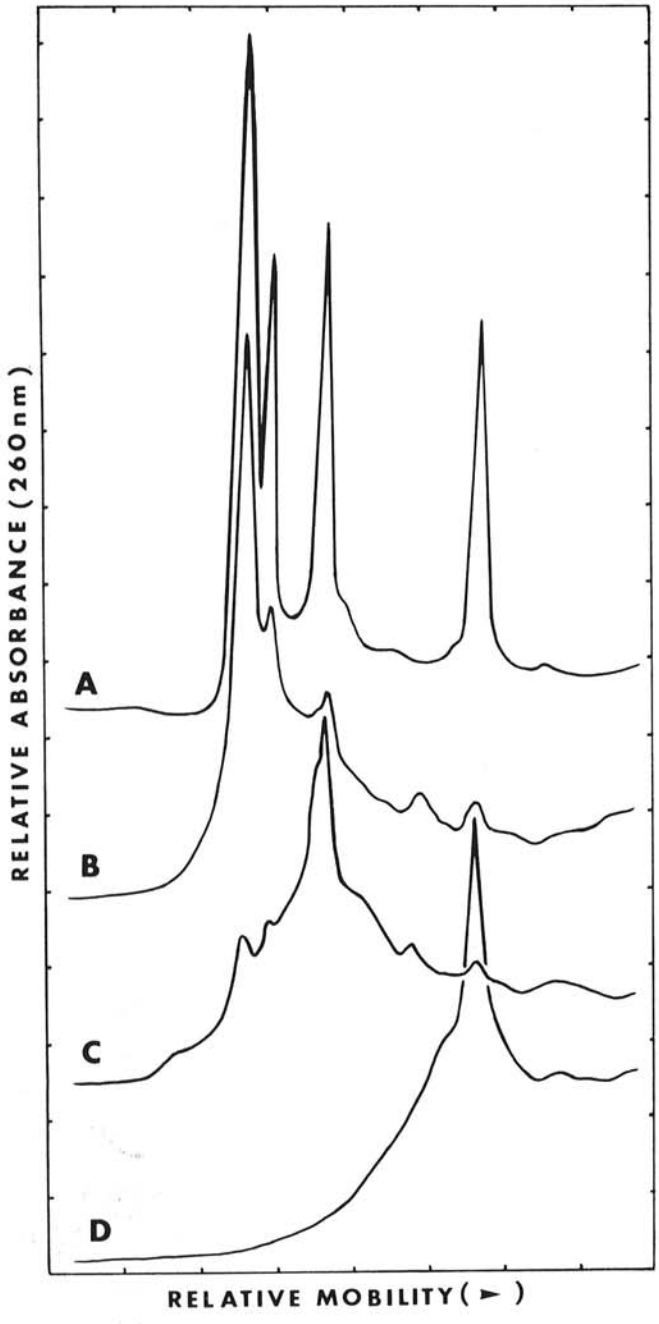

Fig.2. Polyacrylamide gel (2.4%) electrophoretic patterns showing the RNA component composition of **A)** peanut stunt virus strain B, **B)** degradation products nucleoprotein (NP) 1 + 2, **C)** NP 3, and **D)** NP 4.

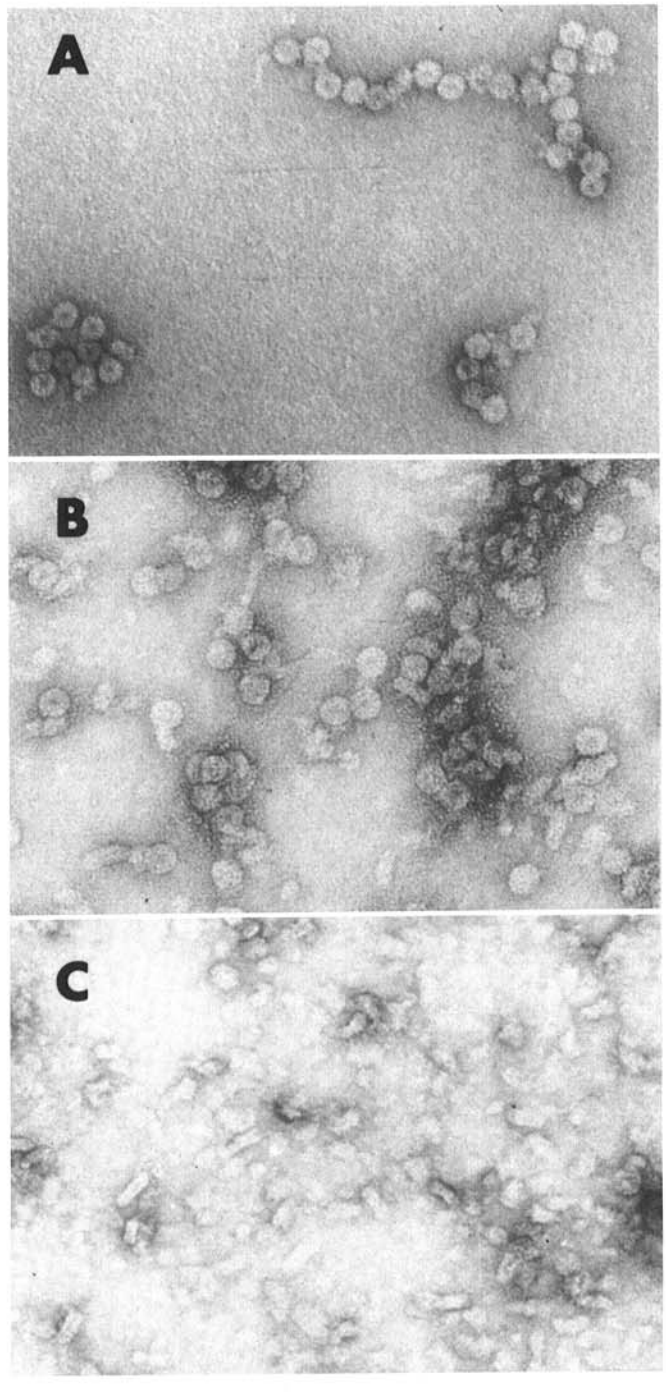

Fig. 3. Electron micrographs of: **A)** peanut stunt virus (PSV) strain B (× 90,000) purified with EDTA (cf. Fig. 4-D, left); **B)** PSV-B (× 125,000) purified without EDTA (cf. Fig. 1); **C)** nucleoprotein structures (× 125,000) (cf. Fig. 4-D, right).

0.01 M $MgCl_2$. Other aliquots of these two fractions also were first treated with 0.3% formaldehyde and then dialyzed against the solutions mentioned. All dialyzed samples were then analyzed by rate zonal centrifugation on sucrose gradients (Fig. 4). Dialysis against sodium phosphate buffer alone caused renewed partial degradation of the virus fraction and an equilibrium condition was reached with the NPS fraction (Fig. 4-A). However, EDTA had a stabilizing effect on the virus (Fig. 4-B), but Mg^{2+} ions failed to stabilize and induced aggregation of the degradation products (Fig. 4-C). Virus or NPs that had first been fixed with formaldehyde remained stable (Fig. 4-D).

Like several strains of CMV and PSV-V (18), PSV-B was dissociated in the presence of 1 M LiCl in 0.01 M Tris HCl, pH 7.2. Like cowpea ringspot virus, a newly identified cucumovirus (23), PSV-B precipitated in 0.02 M sodium acetate buffer, pH 5.

Virion polyacrylamide electrophoresis. When PSV-B was electrophoresed for 16 hr at 3 mA/gel at ambient temperature alone and combined with PSV-V, PSV-W, and each of the CMV strains S, R, and D, it had the same electrophoretic mobility as CMV-D (14) but was slower than PSV-W (Fig. 5).

Sedimentation coefficient. When isolated in the presence of EDTA, PSV-B sedimented monodispersely in 0.02 M Na phosphate, pH 7.2, buffer containing 0.002 M EDTA. At a concentration of 0.2 mg/ml, its $S_{20,w}$ was 89.7S.

RNA component composition and electrophoretic mobilities. Polyacrylamide electrophoresis of PSV-B in 2.4% gels in the presence of SDS, like PSV-V (14), revealed four principal RNA components (Fig. 2). Sometimes RNA 3 was resolved as two components. The electrophoretic mobilities of the four PSV-B RNA components were compared with those of PSV-V and CMV-D RNA in 3% gels, by coelectrophoresing the RNA preparations in all combinations of two. In this way, PSV-B RNA 2 was found to have a mobility intermediate between CMV-D RNA 2 and PSV-V RNA 2 (14). The other RNA components of PSV-B coelectrophoresed with those of CMV-D and PSV-V RNA.

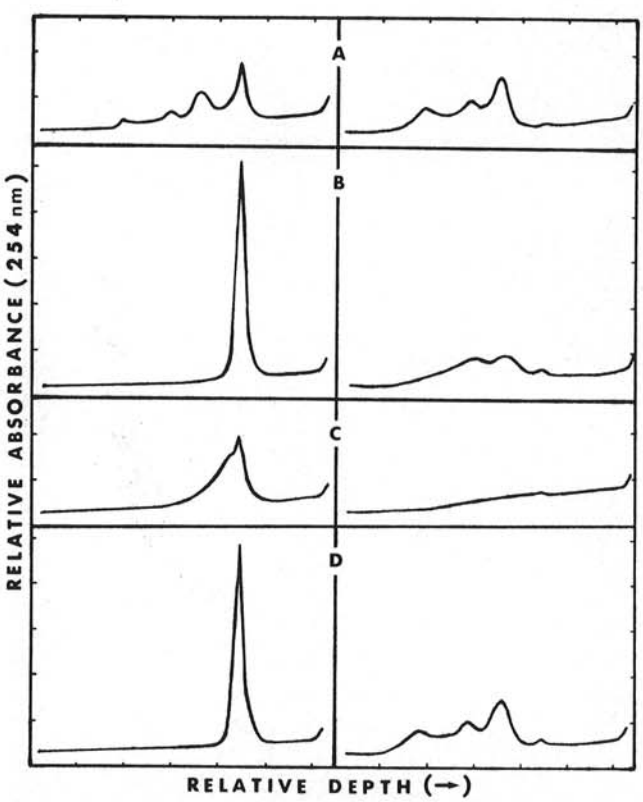

Fig. 4. Sedimentation profiles of peanut stunt virus strain B (left) and nucleoproteins fraction (right) in sucrose density gradients. Samples were dialyzed overnight at 4 C against 0.01 M phosphate buffer: **A**) alone, **B**) plus 0.01 M EDTA, **C**) plus 0.01 M $MgCl_2$. **D**) Control samples containing 0.3% formaldehyde and dialyzed as described.

Base ratios. The base ratios of the RNAs of PSV-B, PSV-V, and PSV-W were determined concurrently with those of CMV-S and turnip yellow mosaic virus (TYMV) as standards. Table 1 shows the similarity of the base ratios of the PSV strains but shows the significant difference with the base ratio of CMV. The base ratio of the PSV strains also was quite different from the previously determined set of values for PSV-W (21).

Serologic comparison of PSV-B with other PSV strains and cucumoviruses. Antiserum prepared against PSV-B did not react with CMV or TAV but reacted with all PSV strains. In immunodiffusion tests, the precipitin lines to B and W antigens coalesced, although a spur developed between B and V or H and between W and V or H (Fig. 6-A). A similar diagram was obtained with sera against PSV-W. Reciprocally, a spur between B and V or H and between W and V or H was observed when PSV-V antisera were used (Fig. 6-B). Thus, strains B and W seem serologically indistinguishable but they can be easily distinguished from V or H.

Of the many CMV antisera, those prepared against strains belonging to the serological group DTL (3,4) did not react with PSV-B. However, there was a distinct reaction with sera against strains belonging to the S group ToRS (Fig. 6-C).

Antiserum against TAV also reacted with PSV-B (Fig. 6-D). Spurs, which occasionally appeared between the lines of different PSV strains, were sometimes less distinct and depended on the serum used. A spur was often observed between B and V or H and between W and V or H.

DISCUSSION

The data demonstrate that the cucumovirus isolated from the Barajas area in Spain is a PSV. Although a distinct host range comparison was made only with PSV-T, previous work has indicated that PSV-T is a typical PSV isolate from the southeastern United States (26). Serologically, PSV-B also fell in line with other PSV isolates. However, PSV-B resembled PSV-W much more closely than PSV-V in its reaction with antisera developed against the different PSV and CMV strains. Most notable here was the failure of PSV-B antiserum to react with CMV antigens of the DTL group and vice versa. This property is characteristic of PSV-W, since PSV-V shows a clear serologic relationship with the DTL CMVs (4). The close similarity of PSV-B and PSV-W, as opposed to PSV-V, also was apparent from the nearly identical electrophoretic mobilities of their virions.

The only property of PSV-B that differs significantly from properties of PSV-W is instability in solution. Presumably, however, this reflects only a relatively small difference between the coat proteins of the two viruses, in a region that does not affect their antigenic sites. A discrepancy was the fact that the base ratios of all PSV isolates analyzed (Table 1) were significantly different from

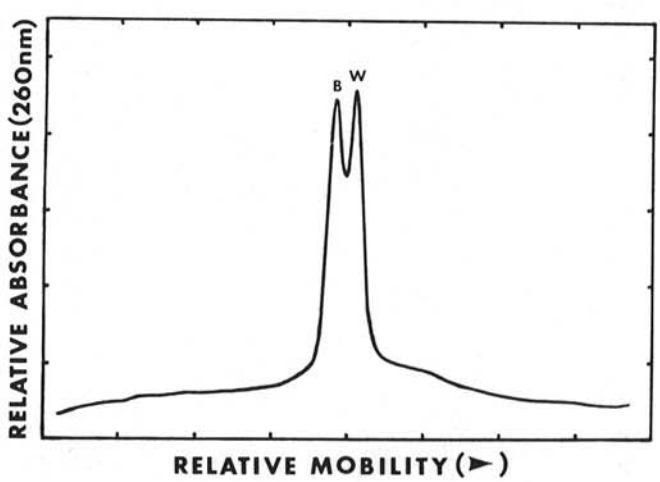

Fig. 5. Polyacrylamide gel (2.4%) electrophoresis patterns showing the difference in virion mobilities between peanut stunt virus strains B (left) and W (right).

TABLE 1. Base ratios of the RNAs of peanut stunt virus (PSV), cucumber mosaic virus (CMV), and turnip yellow mosaic virus (TYMV)

Virus	Number of determinations	Base ratio (% ± standard deviation)			
		Adenine	Guanine	Cytosine	Uracil
PSV-B	5	23.4 ± 0.3	21.8 ± 0.7	27.0 ± 0.6	27.7 ± 0.4
PSV-V	5	22.2 ± 0.3	23.0 ± 0.3	26.0 ± 0.3	28.8 ± 0.8
PSV-W	5	23.1 ± 0.4	22.6 ± 0.2	26.2 ± 0.5	28.2 ± 0.1
PSV-W	...[a]	25.7	24.5	21.0	28.8
CMV-S	5	23.3 ± 0.2	23.5 ± 0.5	23.6 ± 0.4	29.6 ± 0.3
CMV-Y	...[b]	24.3 ± 0.5	23.4 ± 0.4	23.2 ± 0.7	29.0 ± 0.7
TYMV	5	22.9 ± 0.6	17.5 ± 0.3	38.2 ± 0.4	21.3 ± 0.3
TYMV	...[c]	22.4 ± 0.1	17.2 ± 0.2	38.3 ± 0.2	22.1 ± 0.2

[a] Data from Mink et al (20).
[b] Data from Kaper et al (10).
[c] Data from Symons et al (24).

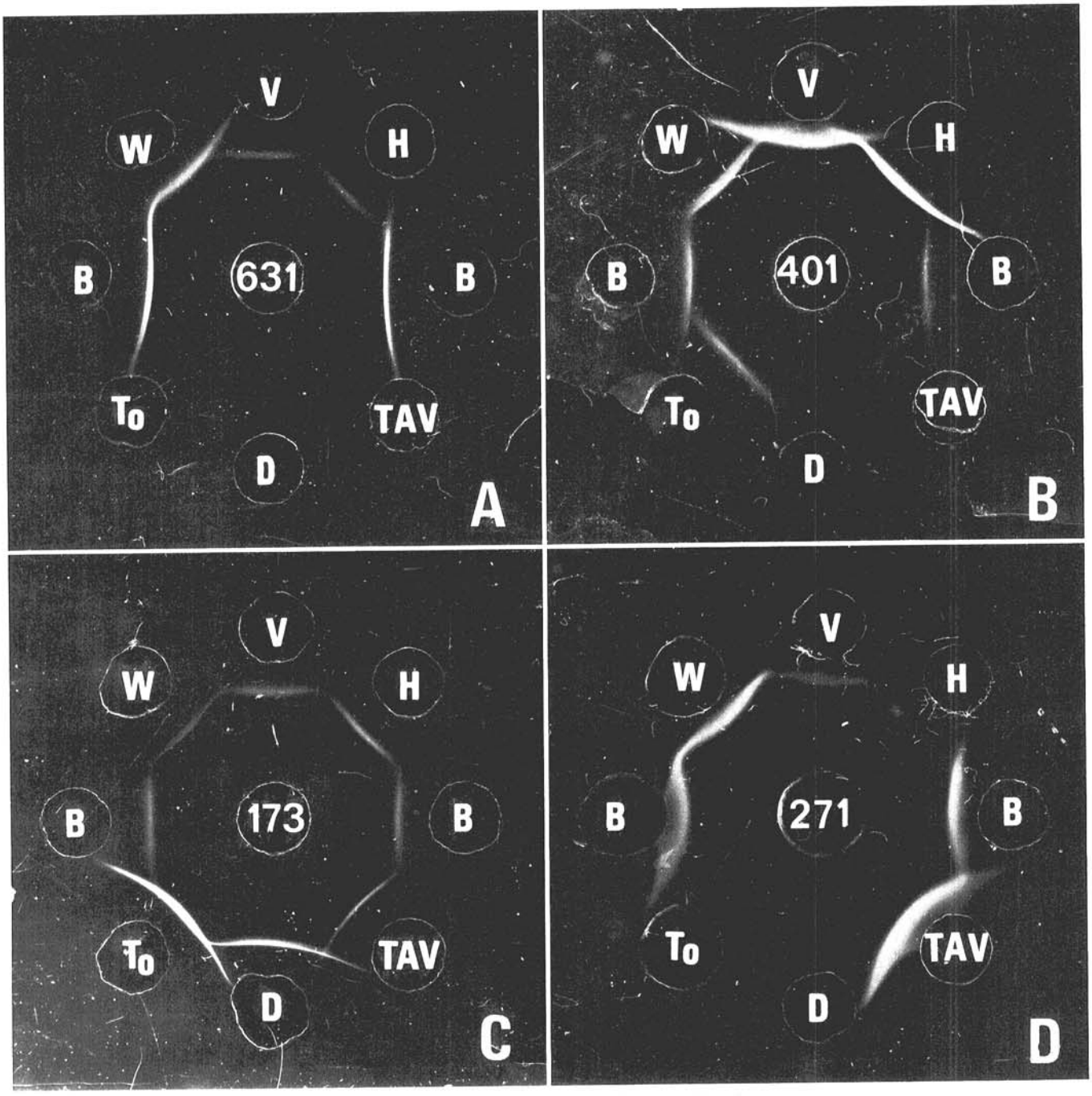

Fig. 6. Immunodiffusion serologic patterns obtained with various combinations of cucumoviruses and their antisera. Peripheral wells contain peanut stunt virus (PSV) strains B, W, V, and H; cucumber mosaic virus (CMV) strains To and D; and tomato aspermy virus (TAV) antigens. Center wells are filled with antisera to: **A**) PSV-B; **B**) PSV-V; **C**) CMV-To; **D**) TAV.

the reported base ratios for PSV-W (21). We have no explanation for the discrepancy except that different methods were used. The base ratios determined concurrently for TYMV and CMV (Table 1) agree with published values.

Confirmation of some of the taxonomic relationships established for different PSV strains is emerging from current studies of nucleotide homologies among several PSV strains (J. R. Diaz-Ruiz, S. Tolin, and J. M. Kaper, *unpublished*). In competition hybridization experiments, the RNAs of all the eastern U.S. strains of PSV competed fully with each other for complementary strands in the double-stranded RNA of PSV-V, whereas the RNAs of PSV-B and PSV-W were unable to compete. In contrast, none of the RNAs of eastern U.S. strains were able to compete with PSV-W RNA for its complementary strand, but PSV-B competed 100%. These experiments reinforce our results and suggest the existence of two rather different taxonomic groups in this group of legume viruses.

Recently, Fischer and Lockhart (7) reported the occurrence of a Moroccan strain of PSV from alfalfa. Hence, there are now two reports of natural occurrences of PSV in alfalfa. However, the serologic relationship of this Moroccan isolate to PSV-W or PSV-E was not as close as that of PSV-B. It should be interesting to extend the biochemical methods used here to a comparison of PSV-B and the Moroccan strain of PSV.

LITERATURE CITED

1. BOATMAN, S., J. M. KAPER, and S. A. TOLIN. 1973. A comparison of properties of peanut stunt virus and cucumber mosaic virus. (Abstr.) Phytopathology 63:801.
2. COOPER, W. E. 1966. A destructive disease of peanut. Plant Dis. Rep. 50:136.
3. DEVERGNE, J. C., and J. L. CARDIN. 1975. Relations sérologiques entre cucumovirus (CMV, TAV, PSV). Ann. Phytopathol. 7:255-276.
4. DEVERGNE, J. C., and J. L. CARDIN. 1976. Caractérisation de deux sérotypes du virus du Rabougrissement de l'Arachide (PSV). Ann. Phytopathol. 8:449-459.
5. DOUINE, L., and J. C. DEVERGNE. 1978. Isolement en France du virus du Rabourgrissement de l'Arachide (peanut stunt virus, PSV). Ann. Phytopathol. 10:79-92.
6. ECHANDI, E., and T. T. HEBERT. 1971. Stunt of beans incited by peanut stunt virus. Phytopathology 61:328-330.
7. FISCHER, H. U., and B. E. L. LOCKHART. 1978. Host range and properties of peanut stunt virus from Morocco. Phytopathology 68:289-293.
8. HARRISON, B. D., J. T. FINCH, A. J. GIBBS, M. HOLLINGS, R. J. SHEPHERD, V. VALENTA, and C. WETTER. 1971. Sixteen groups of plant viruses. Virology 45:356-363.
9. KAPER, J. M. 1976. Molecular organization and stabilizing forces of simple RNA viruses. V. The role of lysyl residues in the stabilization of cucumber mosaic virus strain S. Virology 71:185-198.
10. KAPER, J. M., T. O. DIENER, and H. A. SCOTT. 1965. Some physical and chemical properties of cucumber mosaic virus (strain Y) and of its isolated ribonucleic acid. Virology 27:54-72.
11. KAPER, J. M., and J. L. M. C. GEELEN. 1971. Studies on the stabilizing forces of simple RNA viruses. II. Stability, dissociation, and reassembly of cucumber mosaic virus. J. Mol. Biol. 56:277-294.
12. KAPER, J. M., and C. K. WEST. 1972. Polyacrylamide gel separation and molecular weight determination of the components of cucumber mosaic virus RNA. Prep. Biochem. 2:251-263.
13. KUHN, C. W. 1969. Effects of peanut stunt virus alone and in combination with peanut mottle virus on peanut. Phytopathology 59:1513-1516.
14. LOT, H., and J. M. KAPER. 1976. Physical and chemical differentiation of three strains of cucumber mosaic virus and peanut stunt virus. Virology 74:209-222.
15. LOT, H., J. MARROU, J. B. QUIOT, and C. ESVAN. 1972. Contribution a l'etude du virus de la mosaique du concombre (CMV). I. Methode de purification rapide du virus. Ann. Phytopathol. 4:25-38.
16. MARCHOUX, G. 1975. Propriétés biologiques et génétiques des ARN du virus de la mosaique du concombre. Ph.D. Thesis, University of Marseille, France. pp. 99-107.
17. MILLER, L. I., and J. L. TROUTMAN. 1966. Stunt disease of peanuts in Virginia. Plant Dis. Rep. 50:139-143.
18. MINK, G. I. 1969. Serological relationships among cucumber mosaic virus, tomato aspermy type viruses, and peanut stunt virus. Phytopathology 59:1889-1893.
19. MINK, G. I. 1972. Peanut stunt virus. Plant Virus Description No. 92. Commonw. Mycol. Inst. Assoc. Appl. Biol., Herts., Berks., England.
20. MINK, G. I., T. T. HEBERT, and M. J. SILBERNAGEL. 1967. A strain of peanut stunt virus isolated from beans in Washington. (Abstr.) Phytopathology 57:1400.
21. MINK, G. I., M. J. SILBERNAGEL, and K. S. SAKSENA. 1969. Host range, purification, and properties of the western strain of peanut stunt virus. Phytopathology 59:1625-1631.
22. MORRIS, T. J., and J. S. SEMANCIK. 1974. Nucleotide composition of RNA by polyacrylamide gel electrophoresis. Anal. Biochem. 61: 48-53.
23. PHATAK, H. C., J. R. DIAZ-RUIZ, and R. Hull. 1976. Cowpea ringspot virus: A seed-transmitted cucumovirus. Phytopathol. Z. 87: 137-142.
24. SYMONS, R. H., M. W. REES, M. N. SHORT, and R. MARKHAM. 1963. Relationships between the ribonucleic acid and protein of some plant viruses. J. Mol. Biol. 6:1-15.
25. TOLIN, S. A., and S. BOATMAN. 1972. The serological relationship between peanut stunt virus and cucumber mosaic virus. (Abstr.) Phytopathology 62:793
26. WATERWORTH, H. E., R. L. MONROE, and R. P. KAHN. 1973. Improved purification procedure for peanut stunt virus, incitant of Tephrosia yellow vein disease. Phytopathology 63:93-98.