

A VARIANT OF BEAN POD MOTTLE VIRUS

and

ALTERED ROOT MORPHOLOGY OF BEAN POD MOTTLE VIRUS
INFECTED SOYBEANS

by

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A VARIANT OF BEAN POD MOTTLE VIRUS

INTRODUCTION

Bean pod mottle virus (BPMV) was described by Zaumeyer and Thomas (16) in 1948. It has been reported by several authors (8,9,11,14) to cause a disease of bean and soybeans in southeastern United States. Hosts reported include Phaseolus vulgaris L. (Pinto, snap, wax), Glycine max (L.) Merr. (soybeans, all cultivars), and other Leguminosae (8,11,16). Cowpea, Vigna unguiculata (L.) Walp., is mentioned both as a host (7, 12) and as a non-host (9).

Purified BPMV contains three centrifugal components. Two components, designated middle (M) and bottom (B) are ribonucleoprotein and the third component designated top (T) is an RNA-free protein shell (3,15). Both M and B are required for infection (11). Semancik and Bancroft (12) reported differential stability of M and B to purification. Chloroform at high pH destroyed bottom component, increasing the M:B ratio. The remaining particles were not immune to further degradation. All three centrifugal components exist as two electrophoretic forms, fast (F) and slow (S); the proportion of S increases with time after infection (10). Specific infectivity of F is greater than that of S (5). BPMV is serologically related to cowpea mosaic (13), radish mosaic virus (4) and red clover mottle virus (7).

In 1973 and 1974 virus isolates collected from infected soybeans in 11 Kansas counties were identified serologically as

BPMV. Only one obtained from Chautauqua county in 1973, and designated BPMV-KS, caused systemic symptoms on cowpea. Since this isolate caused symptoms of bud blight on infected soybean and because of its pathogenicity to cowpea, it was compared to a known strain of BPMV and other related viruses.

MATERIALS AND METHODS

The BPMV-KS isolate was identified as BPMV by its strong reaction to BPMV antiserum (ATCC PVAS-9). To minimize contamination by other strains or viruses, five successive single local lesion transfers were performed on Pinto bean. A BPMV sample received from C. L. Niblett (10) and originally obtained from J. B. Bancroft was designated BPMV-T and used as a reference strain.

All host plants were grown under natural light in a greenhouse. They were inoculated by rubbing carborundum-dusted leaves with a homogenate (1:3, w/v) of BPMV-infected tissue in 0.1 M potassium phosphate buffer pH 7.1 (either 0.01M buffer or tap water was used for assays on Pinto bean). Plants were then rinsed with tap water and covered with wet paper towels. When large numbers of plants were inoculated, inoculum was mixed with carborundum, 0.02 g/50 ml, and applied with a spray gun at 4.92 Kg/cm². Propagation hosts for both BPMV isolates were Dwarf Horticultural bean and Columbus soybean.

Leaves and stems of plants were harvested seven days or more after inoculation and stored at -20 C. Leaves were later homogenized in two volumes of 0.1 M potassium phosphate buffer and then stirred with an equal volume of a 1:1 (v/v) mixture of chloroform and n-butanol (1). The homogenate was strained through cheesecloth and centrifuged at 15,000 g for 30 minutes in a Beckman J-21 centrifuge using 250-ml bottles. The aqueous layer was centrifuged 70 minutes at 360,000 g in a Beckman L2-65B and the pellet resuspended overnight. Two more cycles of differential centrifugation, 12,000 g for 10 minutes and 360,000

g for 70 minutes, completed the purification procedure.

In an alternate purification procedure that allowed study of the virus from small samples, five to 40 g of fresh tissue were homogenized with 60 ml buffer and the mixture strained through cheesecloth. It was then centrifuged 25 minutes at 45,000 g in an International B20-A centrifuge. Polyethylene glycol (PEG) and NaCl were added to the supernatant to a final concentration of 8% and 0.2M respectively, and the mixture stirred at 4 C for 2 hours. After centrifugation at 12,00 g for 10 minutes, each pellet was resuspended for a minimum of 1.5 hours in 6 ml 0.01 M phosphate buffer. These preparations were used for electrophoretic comparisons.

The dilution end point (DEP) of both isolates of BPMV was determined by grinding fresh leaf tissue in one-half volume of 0.01 M buffer pH 7.1. This was then diluted with buffer in successive 10-fold dilutions and used to inoculate Pinto beans. The thermal inactivation point was determined by heating sap (diluted 1:2) for 10 minutes in a Thermoline Temp-Block heater at 45, 50, 55, 60, 65, 70, 75 C. Infectivity was determined on Pinto beans.

Purified virus samples were tested against antiserum of cowpea mosaic virus strain Sb (CPMV), squash mosaic virus (SqMV), tobacco ringspot virus (TRSV), tobacco streak virus (TSV), and southern bean mosaic virus (SBMV).

Sedimentation coefficients were determined on log-linear sucrose gradients (2). An SW 27 rotor was used at 25,000 rpm (82,000 g) for 3 hours at 15 C and gradients were fractionated on an ISCO fractionator scanning at 254 nm. Tobacco mosaic virus (TMV), CPMV and brome mosaic virus (BMV) were used as standards.

Several species were tested as possible host by mechanical inoculation. Both inoculated and uninoculated leaves were tested two weeks after inoculation using agar double diffusion serology. Corn (Zea mays L. 'Sweet Golden Cross Bantam', cucumber (Cucumis sativus L. 'National Pickling'), lima bean (Phaseolus lunatus L. 'Burpee Improved Large Bush'), muskmelon (Cucumis melo L. 'Hood's Heart of Gold'), pea (Pisum sativum L. 'Early Alaska', 'Dwarf Gray Sugar' and 'Laxtons Progress'), squash (Cucurbita pepo L. 'Zucchini Black'), watermelon (Citrullus vulgaris L. 'Charleston Gray'), wheat (Triticum aestivum L. 'Parker') and Pinto bean were tested. Inoculated Cherokee Wax, Dwarf Horticultural, Kentucky Wonder Wax beans, soybeans and cowpeas were tested both serologically and on Pinto bean.

Cowpea cultivars Early Ramshorn and Burgundy were tested along with eight introduction lines (P.I.) received from the USDA Plant Introduction Station at Experiment, Ga.: P.I. 164641 (India), 209971 (Japan), 255774, 255789, 255791 (Nigeria), 291385 (China), 293525, 293449 (U.S.A.). Primary leaves were inoculated and trifoliolates were assayed on Pinto beans and against BPMV-ATCC antisera to determine if infection had occurred. Four plants of each introduction were tested with each virus isolate. Six days after planting, Burgundy cowpeas were divided by leaf size into four groups and inoculated with BPMV-KS. Plants ranged in size from those that had not yet completely shed the seed coat to those with leaves 3 x 4 cm. Fifteen days later, upper leaves of all plants were assayed on Pinto bean to determine systemic infection.

Disc electrophoresis was done with 2.8% polyacrylamide gels polymerized with 10% ammonium persulfate and used without a

stacking gel. Gels were run 2.5 hours at 200 volts (2.5 amp/tube) and then scanned immediately at 260 nm with an ACTA III gel scanner to determine virus location and condition. The ratio of electrophoretic forms was determined by planimetry of the areas under the peaks on the spectrophotometer chart paper.

Specific infectivity of both isolates was determined on 8 half leaves of Pinto bean using PEG purified virus from soybeans. Using plants of identical age and condition, virus was inoculated and propagated on Dwarf Horticultural bean, transferred to a second set of Dwarf Horticultural bean, transferred to soybeans and harvested in three replications for PEG purification. The six samples were further purified and concentrated by high speed centrifugation before being diluted to $A_{260}=0.2$ and used for inoculation.

RESULTS

The BPMV-KS isolate was identical to BPMV-T in its dilution end point of 10^{-4} to 10^{-5} and thermal inactivation point of 65 - 70 C. It reacted strongly with BPMV-T antiserum, but not with antisera made against CPMV, SqMV, TRSV, TSV or SBMV. No indications of heterologous precipitin bands were observed in any test. Sedimentation coefficients of 54(T), 91(M), and 112(B) were determined for both isolates of BPMV. A M/B ratio of one resulted for both isolates after PEG purification from soybeans; other ratios, usually enriched B, occasionally resulted when variations of the purification methods were used. M and B each contained F and S electrophoretic forms and their ratio was dependent on the number of days post-inoculation (p.i.). Both virus isolates remained infective when stored purified at 4 C for 240 days, and 18 months in frozen tissue. Longer time periods were not tested.

BPMV-KS did not infect corn, cucumber, lima bean, muskmelon, pea, squash, watermelon or wheat. It did cause local lesions on Pinto bean and Kentucky Wonder Wax beans and systemic infection in Cherokee Wax, Dwarf Horticultural beans, soybeans and cowpea. This host range, except for cowpea, does not differ from that published for BPMV-T.

In every host, disease symptoms were less severe in plants infected with BPMV-KS than with BPMV-T. In one experiment using three replications, virus yields per 100 g of soybean tissue at 7 days p.i. averaged 1.53 mg for BPMV-KS and 2.95 mg for BPMV-T. Purified BPMV-KS had a specific infectivity (local lesions/ A_{260}) of 108, whereas the specific infectivity of purified BPMV-

T was 128.

The F/S ratio of BPMV-KS was higher than that of BPMV-T in 3 tests in Columbus soybeans harvested before 10 days p.i. Only small differences were found in plants harvested after 10 days p.i. In one replicated test, the mean F/S ratios changed from 3.0 to 0.26 for BPMV-KS and from 2.4 to 0.24 for BPMV-T (coef. of variation 12.3%) from 9 to 17 days p.i. (Fig. 1). Similar changes in F/S ratio were found for separated M and B components of both virus isolates from plants harvested 8 and 13 days p.i. Virus collected from young and old leaves of maturing plants 55 days after inoculation with BPMV-KS (Fig. 2) was purified by the PEG method and electrophoresed. No detectable F form was found in virus purified from either young or old leaves from these plants. The F/S ratio was dependent on the infection period and also on the method of purification. PEG purified virus contains an enzyme capable of F to S conversion (6). Therefore, F/S ratios must be determined immediately after purification. In four samples stored at 4 C and tested 21 days after purification, F form could not be detected.

BPMV did not infect cowpea plants inoculated when leaves were fully expanded, usually at more than 8 days after planting, and only a small number of plants became infected if plants were inoculated immediately after emergence. Cowpea lines that were susceptible to BPMV, were susceptible to both isolates in these tests only if they were inoculated when leaves were 2 to 5 cm in length. Using plants of this size, P.I. 164641, 209971, 291385, 293549 and cultivars Burgundy and Early Ramshorn were found to be systemic hosts. Cowpeas, P.I. 255774, 255759, 255791, and 293525 did not become infected after inoculation

with BPMV.

DISCUSSION

The Kansas isolate of BPMV, identified serologically and on the basis of physical and biological properties, differed from the reference strain in severity of symptoms, specific infectivity and the ratio of electrophoretic forms. The isolate was originally selected for study because its host range included cowpea, which conflicted with a published report (11). However, during the course of the investigation, other references were found which listed cowpeas as a host. This apparent discrepancy can be resolved if one considers the effect of host variability with age and host genetic composition on the virus-host relationship.

We can assume that F & S both exist in a living plant, and that F undergoes conversion to S in vivo; however, by the time the ratio is measured, it must necessarily be a function of both in vivo and in vitro conversions. The relative importance of in vitro conversion is dependent of the method of purification used. Host enzymes were probably responsible for a major part of the in vitro conversion of PEG purified preparations. Host enzymes causing conversion in CPMV were reported by Geelen et al. (6) in PEG purified preparations but were not active in chloroform-butanol purified preparations. In vitro ratios of F/S of BPMV-KS, purified with PEG, were storage time dependent, indicating in vitro conversion.

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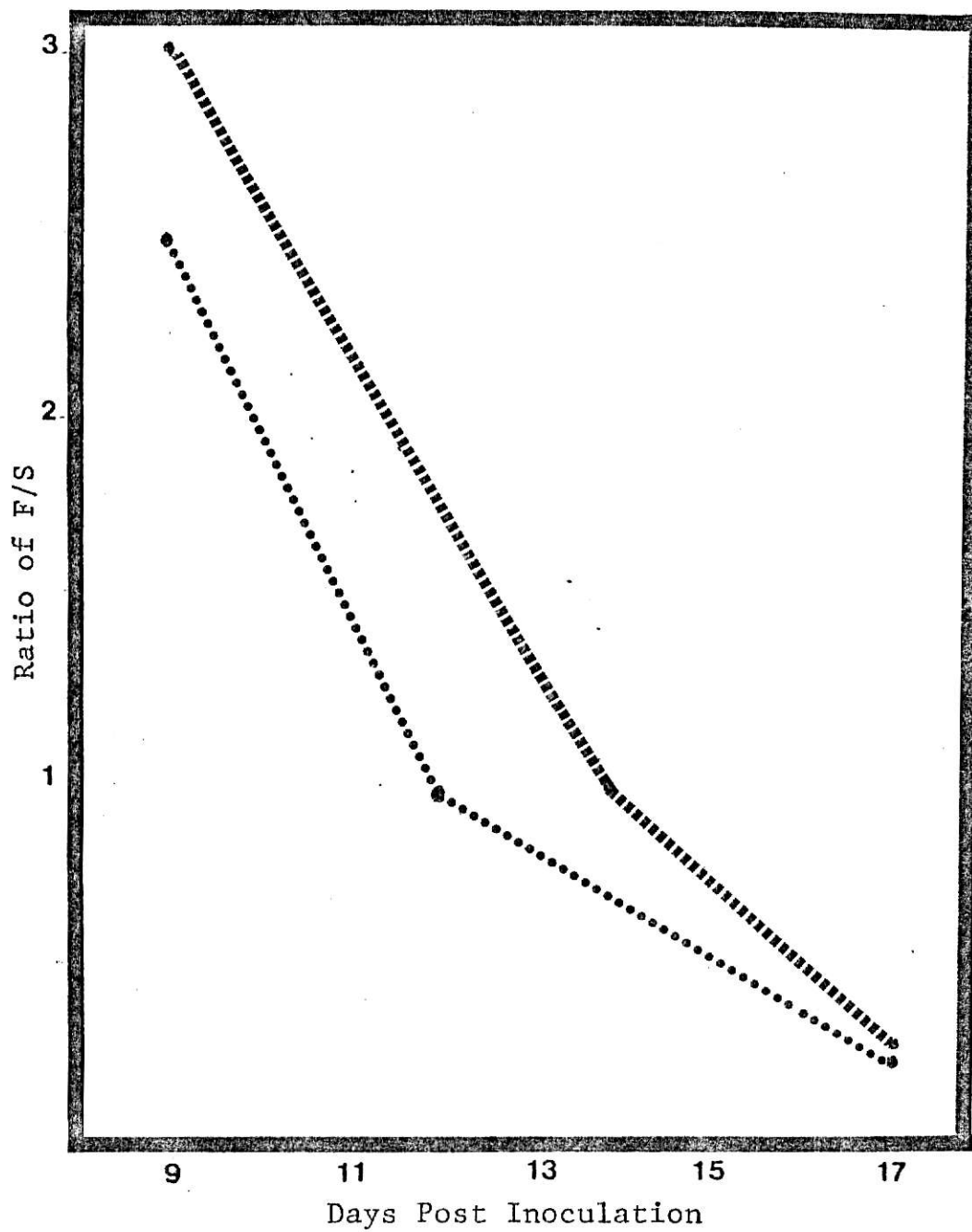


Fig. 1. A comparison of the ratio of electrophoretic forms (F and S) of BPMV-KS (-----) and BPMV-T (.....) purified from soybeans on increasing days post-inoculation.

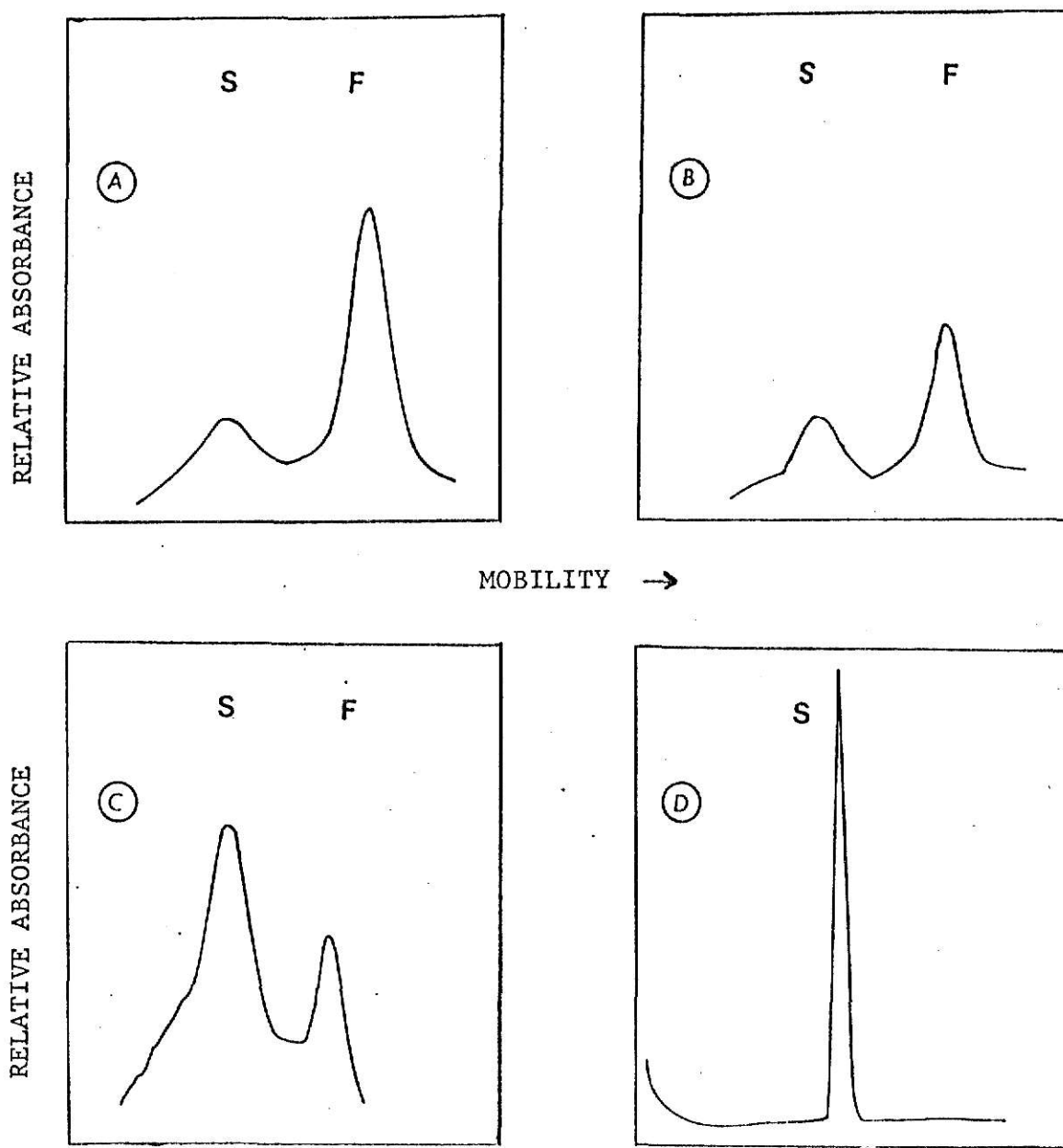


Fig. 2. Photometric scanning patterns of the Kansas isolates of BPMV on 2.8% polyacrylamide gels at (A) 6 days, (B) 8 days, (C) 13 days and (D) 55 days after inoculation. These patterns indicate a gradual shift from F to S.

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ALTERED ROOT MORPHOLOGY OF BEAN POD MOTTLE VIRUS
INFECTED SOYBEANS

The effect of virus diseases on the root systems of plants is characterized by reduced growth, disturbances in uptake of water or ions and disturbances in the production of metabolites (9). Root morphological aberrations have received little attention except for reports by Gold and Faccioli (5) and Panopoulous et al. (7) on curly-top virus (CTV) infected plants grown in fog culture. While conducting experiments on the response of soybean cultivars to bean pod mottle virus (BPMV), a growth aberration was noted on the roots of infected plants. This study was undertaken to determine if the aberrations were virus induced and if there were any internal manifestations of the disease.

MATERIALS AND METHODS.--Soybeans, Glycine max L., cultivars 'Columbus' and 'Marshall' and an experimental line, K2-70-16, were planted in pots 10 x 25 cm deep containing vermiculite. When plants became 6 to 15 cm long, they were removed from the germination pot and inoculated with a Kansas isolate of BPMV by spraying the cotyledons and hypocotyls with a high pressure air gun at 5 Kg/cm². Inoculum was prepared by grinding fresh BPMV-infected tissue in 0.1M K₂HPO₄, pH 7.1, straining it through cheesecloth and adding 0.02 g carborundum/50 ml. After inoculation, the stem of each plant was wrapped in glass wool and the roots fed through a hole in the lid of a plastic barrel (80 cm deep x 60 cm diameter)(5). Four inoculated and four non-sprayed control plants were transplanted to each barrel lid in alterna-

ting sequence. In one experiment involving 16 plants, controls were sprayed with an extract from healthy soybeans. Moisture and nutrients were provided by two compressed air jets that produced a fog inside the barrel. Eight liters of Hoagland's solution (6), 0.1 strength except for KNO_3 which was full strength, were recycled continually throughout the experiment.

Experiments were terminated when roots reached the liquid at the bottom of the barrel (usually 20 days). Materials for light microscope examination were fixed 5 days in a 5% formaldehyde:5% propionic acid:90% ethanol solution. They were then dehydrated through a graded ethanol:tert-butanol series (8), embedded in paraffin, sectioned and stained $2\frac{1}{2}$ hours in safranin and 30 seconds in fast green. Serial sections were made of 11 diseased roots (four cross sectional and seven longitudinal), along with corresponding sections of six healthy roots.

Five-cm lengths of three roots were assayed for presence of virus by agar double diffusion tests with BPMV antiserum and on Pinto beans, a local lesion host. Twenty-four inoculated plants were assayed by each method. A total of 36 control and 60 inoculated plants were grown. The occurrence of disease symptoms was tested under various growing conditions; two plants were allowed to grow for 5 days after reaching nutrient solution at bottom of barrel and 12 plants were grown in soil. Soil was washed from the roots of soil-grown plants 23 days post-inoculation.

RESULTS AND DISCUSSION.--Control soybean roots were straight with no bends and only slight curves. Secondary roots hung away from the main root and grew to a length of approxi-

mately 10 cm while the primary root grew to 40 cm. Roots of virus-inoculated plants began to show bends 24 to 48 hours after leaf symptoms appeared, usually 5 days after inoculation. No root abnormalities occurred on plants serving as controls and no differences in reaction were seen between the 3 cultivars grown. Leaf sap from 20 plants was tested serologically and on Pinto bean for the presence of virus; 17 had root crooks and 14 of these gave positive reaction to both tests. Every plant with a detectable amount of virus had root deformities.

Root symptoms appear as dramatic changes in the direction of root growth beginning at the tip and causing the root to grow horizontally. Most commonly two right angle turns occurred about 1 cm apart. These turns, not necessarily in the same plane, resulted in a lateral displacement of the growing point (Fig. 1). Curls, twists, and combinations of turns also occurred. Normal periodic initiation of secondary roots did not seem to be affected by disease symptoms; however, numerous turns and "Z" bends appeared in the secondary roots. Abnormalities persisted as the root continued to grow downward. Up to five such affected areas were counted on a single root, each separated by a section of normal appearing root. Root sections growing in nutrient solution also exhibited sharp bends. Roots of both infected and virus-free plants grown in soil developed a multitude of naturally occurring sharp bends; it was not possible to determine if these were virus-induced.

Dried BPMV-infected plants weighed 33.5% ($P=0.01$) less than healthy controls. Root length for virus-infected plants varied from 13.3% ($P=0.01$) shorter than controls after 16 days in one experiment to 19% shorter ($P=0.05$) after 11 days in an-

other. Differences occurred about 5 days post-inoculation and increased thereafter.

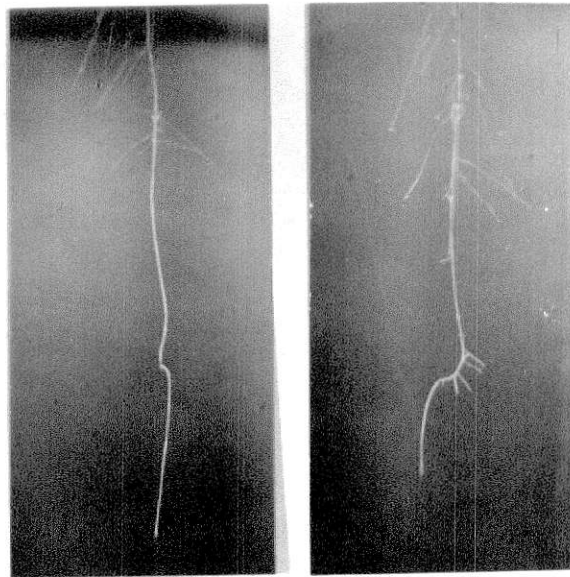
Serial sections of the roots revealed long rows of safranin-sensitive parenchyma cells opposite the phloem of the tetrarch root and extending for distances of about 1 mm above and below the point of root bending (Fig. 2a). The cells stained in a manner similar to that of the meristematic cells of the root tip; the nucleolar diameter of these cells was two to three times that of nucleoli of normal cells. These vertically aligned rows of cells occurred singly or in bundles of two or three contiguous rows. Up to three rows of bundles of rows of cortical cells were observed in a single cross-section. Multiple bundles were always by adjacent arms of the tetrarch phloem system, when viewed in cross section. The rows of cells tended to occur on the inside of a bend and would overlap when bends occurred close together or when the root would rotate. Cells adjacent to the deeply stained cells were not as elongated as were corresponding cells on the opposite side of the root (Fig. 2b). The wall of the cell in contact with the small group of deeply stained cells was shorter than the opposite wall, and gave the cell a crushed appearance; in cross section these cells were trapezoidal.

Tobacco ringspot virus replication and cell growth were monitored by Atchison (1) and by Atchison and Francki (2) who found that root tip cells did not elongate if they were infected before a critical moment in the elongation process. The root cells supported virus replication for a short time after initial infection; thereafter, virus levels could be maintained only by downward transport from the leaves. CTV symptom expression occurred as the end result of a sequence of metabolic de-

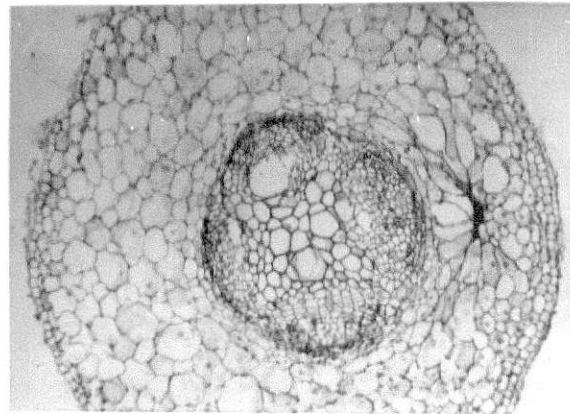
rangements that were influenced by light intensity and growing conditions (7). The growth of healthy roots reflect environmental factors and consists of interlocking and overlapping cycles that are influenced by shoot growth (3). Aberrations caused by BPMV occurred as intermittent events resulting from virus invasion. The symptoms observed could be a result of virus amplifying a normal plant cycle or from virus induced fluctuations in plant growth. Gillaspie and Bancroft (4) reported for soybeans a multi-week "flush" and "recovery" cycle that correlated with virus yield and with leaf symptoms. Root bending and "recovery" reported here did not occur with a regularity that directly corresponded to any environmental condition and did not occur with the same frequency as the previously reported virus yield cycle.

Figure 1. Abnormal root growth patterns of BPMV infected soybean roots grown in fog culture.

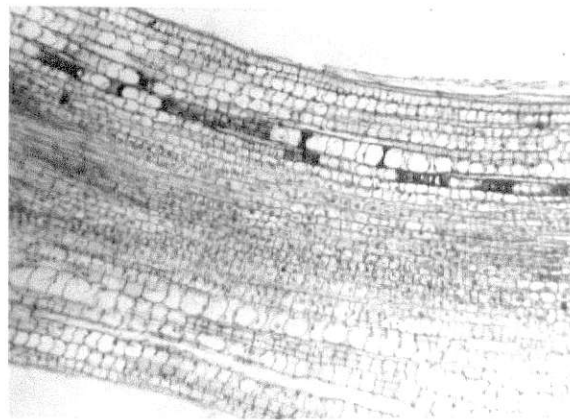
Figure 2. Sections of BPMV infected soybean roots at point of bend showing distorted and heavily staining cortical cells: (A) cross section (B) longitudinal section.



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ABSTRACT

An isolate of bean pod mottle virus was recovered from soybeans in southeast Kansas, initially propagated on cowpeas and designated BPMV-Ks. The virus was identified as BPMV by host range, dilution end point, thermal inactivation point, serological relationships, sedimentation coefficients and electrophoretic heterogeneity.

BPMV-KS differed from a reference strain of BPMV in virulence, as determined by lower yield from purification, reduced specific infectivity, milder symptoms on soybean and Dwarf Horticultural bean and in rate of conversion of electrophoretic forms from fast (F) to slow (S). Ratios of electrophoretic forms were a function of time, F predominated in early infection, while S became predominant by 11 to 13 days post-inoculation.

Pathogenicity to cowpea was found to be dependent on host variability and age of the host plant at the time of inoculation. Cowpeas, Vigna unguiculata, P.I. lines 164641, 209971, 291385, 293549 and cultivar Early Ramshorn and Burgundy were found to be systemic hosts of both strains, while cowpea P.I. lines 255774, 255789, 255791, and 293525 were non-hosts.

ALTERED ROOT MORPHOLOGY OF BEAN POD MOTTLE VIRUS-INFECTED

SOYBEANS GROWN IN A NUTRIENT FOG CULTURE

ABSTRACT

Root tips of soybeans grown in a nutrient fog culture exhibited morphological deformities beginning five to seven days post-inoculation of hypocotyl and cotyledons with bean pod mottle virus. Most commonly a right angle bend occurred at the tip of the tap root and later at tips of secondary roots. Horizontal growth of up to 1 cm was commonly followed by another sharp downward bend. Deformities occurred up to five times on a single 70 cm long tap root. Safranin-stained serial sections of paraffin-embedded roots revealed up to three contiguous rows of deeply stained parenchyma cells associated with the inner surface of the root bend. These rows, about 2 mm in length, paralleled the stele and occurred opposite the phloem. In cross section the row was made up of two to six cells which were smaller than normal. Infected plants harvested 16 days post-inoculation weighed 33% less than controls and had detectable levels of virus in the roots.