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HOST RANGE AND SOME CHARACTERISTICS OF A SOYBEAN MOSAIC VIRUS ISOLATED IN KANSAS

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TABLE OF CONTENTS

HOST RANGE AND SOME CHARACTERISTICS OF A SOYBEAN MOSAIC VIRUS ISOLATED IN KANSAS

INTRODUCTION

The economic importance of soybean virus diseases has been receiving significant recognition in recent years. Significant losses in yield due to soybean mosaic virus (SMV), bean pod mottle virus (BPMV), and tobacco ringspot virus (TRSV) have been reported from many soybean growing areas of the world, more particularly from the U.S.A.

One effect of these viruses on soybeans is the production of seeds with discolored seed coats. The color of the patches or streaks which originate from the hilum is characteristic of the variety, and may be either brown or black. Such seed is considered qualitatively poor. Of concern here is the role played by certain plant virus infections in seed coat mottling. Thus a more mottling has been observed in soybean plants infected by SMV, BPMV, and TRSV (4, 13, 22). While both SMV and TRSV are seed-borne, BPMV has not been shown to be seed transmitted (21, 22).

These investigations were conducted to identify and partially characterize a seed-borne, mechanically transmissible virus isolated by Dr. A.Q. Paulsen from soybean seedlings. The virus was identified by host range, physical properties in crude sap, mode of transmission and serology. Based on the results of these studies, it was concluded that the virus was soybean mosaic virus.

REVIEW OF LITERATURE

Symptoms Induced by SMV on Soybeans

Clinton in 1916 (3) was the first to describe the symptoms of soybean mosaic in the soybean cultivar Hollyhock in Connecticut. He reported the presence of chlorosis or crinkling of the leaves. Gardner and Kendrick (10) subsequently made additional observations on the symptoms induced by SMV. They reported that pods on infected plants were stunted, flattened, less pubescent and curved more than healthy ones. Later Conover (4) described in more detail the symptoms induced by SMV in several soybean cultivars. He observed that with the cultivar Bansei the first visible symptom was a yellowish vein-clearing in minor veins of developing trifoliate leaves. This was transitory and appeared 6-14 days after inoculation. Leaves that subsequently developed were dark green in color with puffy areas present on the major veins. Leaf margins frequently curved downward and the tips upwards. Leaves became coarse, leathery and brittle at maturity. Diseased plants were stunted and set fewer pods than normal plants. Many of the pods produced by diseased plants were empty. Leaves of oily-type cultivars were somewhat wrinkled. Similar symptoms have been reported by Heinze and Kohler (12), Quantz (20), Galvez (9), and Quiniones and Dunleavy (21). On the cultivar Merr the symptoms induced were chlorotic and necrotic spots, vein-clearing, green mosaic, crinkling of the leaves, and browning of the tips. On Dortchsoy 67, Hill and Hood soybean, chlorotic mottling with some rolling under and distortion of leaflets was induced, while on Lee a mild green systemic mottling was observed.

Symptoms on Other Hosts

While Heinze and Kohler (12) and Conover (4) failed to find other hosts showing symptoms, Heinze and Kohler (12) recovered the virus from Phaseolus vulgaris and Vicia sativa. The P. vulgaris cultivars 'Burpee's Stringless', 'Stringless Green-Pod', and 'Stringless Green Refugee' were also found to be hosts by Conover (4).

In 1961, 13 legumes were reported to be invaded systemically (20). Later workers have reported additional hosts (9, 21, 31). Except for local infection of two Chenopodium species (9, 21), SMV has been mainly limited to the Leguminosae.

SMV induced local to systemic infection in P. vulgaris cultivars. After mechanical inoculation dark brown local lesions with necrosis of the inoculated leaves were observed on cultivars Furose, Kentucky Wonder, Mom bacher Speck, Processor, Ranger, Rival, Topcrop (19), Idaho Refugee (20, 31), Black Valentine, and Great Northern U.I-123 (31). On Idaho Refugee small dark brown lesions followed by veinal necrosis and death of the inoculated leaves were observed. On Black Valentine and Great Northern U.I-123, chlorosis and death of both inoculated and opposite non-inoculated primary leaves were induced. SMV was not recovered on subsequently formed leaves, indicating that it did not infect systemically. Virus was localized without symptoms on Genfer Market, Metis, Saxa, Sultan, Wachs Rheinland, Great Northern U.I-15, U.I-31, Pinto U.I-72, Pinto U.I-III, Red Mexican U.I-34 (20), Burpee's Stringless Green-pod, Bountiful, Tender Green A-468, Kinghorn Wax and Tender Green. On Doppelte Hollandische 'Prinze B' (20), both local and systemic symptoms were induced. Symptoms observed on this variety included local brown ringspots and yellowing of

inoculated leaves. Systemic vein-clearing, mottling, yellowing and wilting of tips as well as a deformation of side shoots followed. SMV systemically infected without symptoms on the cultivars Great Northern, Red Kidney, and Thord Green (9).

Systemic hosts other than P. vulgaris cultivars have also been reported by Quantz (20), Galvez (9), Walters (31), Quiniones and Dunleavy (21).

On P. lathyroides there was necrosis and yellowing of inoculated leaves (9, 20, 31). Systemic chlorotic spotting, a light green mosaic, wavy leaflets and occasional browning of the leaf tips followed. Dolichos falcatum is a systemic host with the symptoms consisting of brown necrotic lesions on the inoculated leaves and necrosis of the tip (20). On Cyamopsis tetragonoloba, local lesions, stunting, and systemic mottling were induced (21, 31). On Lupinus albus systemic vein-clearing, a green type of mosaic, rolling of leaflets and deformation were observed (20, 31). On Trigonella coerulea SMV induced a systemic mosaic while on T. foenum-graecum vein-clearing was observed (20). Mosaic with curling of the leaves was observed on Cassia occidentalis. Chlorotic spots similar to local lesions with narrower leaflets were observed on Sesbania exaltata (9). On Canavalia ensiformis there was epinasty of the inoculated leaf. This was followed by a systemic mottle with the leaflets rolling under and some necrotic mottling. On Lespedeza stipulacea 'Korean' and 'Climax' a systemic mottle and top necrosis were induced while on L. Striata 'Kobe' a systemic green mottle developed (31). Chlorotic mottling, stunting, distortion and necrosis of young leaflets was observed on Stizolobium deeringianum 'Early Speckled' (31). Intense mottling with extreme rolling under and distortion of leaflets was observed on G. ussuriensis (31).

Local lesion hosts for SMV other than P. vulgaris cultivars have been reported in the Leguminosae and Chenopodiaceae. For instance, in the Leguminosae, Phaseolus lunatus was locally infected and gave chlorotic local lesions (31). On Vigna sinensis 'Ironkley' (20) and 'Monarch' (31) chlorosis of the inoculated leaves was observed. Dolichos biflorus (21) and D. lablab (9, 21) were found to be local lesion hosts. Two species of Chenopodiaceae, Chenopodium album (9) and C. quinoa (20, 21) were also reported as local lesion hosts.

The legumes Hippocrepis multisiliquosa and Lotus tetragonolobus were systemic but latent hosts (20).

Incubation temperature has been observed to influence the severity of symptoms on soybeans. Walters (31) observed that at 20 C the symptoms were more severe on soybeans than at 27 C. Severe symptoms developed at 18.5 C while masking occurred at 29.5 C (4).

SMV Isolates

The occurrence of SMV strains has been reported by Ross (24) and Quiniones and Dunleavy (21). Ross was able to separate two strains, SMV-1 and SMV-2, on the basis of symptoms induced on Lee and Hill soybeans. On these hosts SMV-1 induced more severe symptoms than SMV-2, but both strains produced a synergistic reaction with BPMV. Quiniones and Dunleavy (21) observed that an isolate designated SMV-M caused milder symptoms compared to SMV-NC or SMV-O strains. SMV-O also incited severe symptoms and reduced seed size more than either SMV-M or SMV-NC in double infections with BPMV.

Recently Ross (25) reported on variation among seven isolates of SMV in pathogenicity to soybeans, Lespedeza stipulaceae 'Rowan' and Kentucky

Wonder Wax Pole (KWP) bean. Symptoms induced by these isolates varied on 24 varieties of soybeans tested and included systemic mottling, necrotic lesions on inoculated leaves, systemic necrotic lesions and plant necrosis. Some isolates also infected certain soybean cultivars without symptoms. On Rowan lespedeza symptoms ranged from no observable effects to dwarfing, straplike leaves, shoot necrosis or a very mild mottling. Ogden soybean and the introductions P.I. 96,983 and P.I. 170,893 were found resistant to all seven isolates. All seven caused local lesions on KWP bean. The number of lesions induced on KWP bean depended on the cultivar of soybean used as production host and with the virus isolate. This indicated that some SMV isolates were able to replicate more in some varieties than in others. KWP bean was susceptible to all isolates. On the basis of symptoms induced on Rowan lespedeza and soybeans, Ross grouped isolates 1, 5 and 7 together; 2, 4, and 6 belonged to a second group, and isolate 3 formed a third group.

Methods of Transmission

Mechanical inoculation by rubbing the inoculum on the upper leaf surface previously dusted with an abrasive appears to be the most common method of experimental transmission of SMV (4, 9, 10, 31). Earlier workers have also transmitted SMV by (a) pricking with a needle at the nodes and rubbing the inoculum into that area; (b) inoculating into slits formed with a scalpel at growing tops or on petioles; and (c) by taking out one leaf at the node and inoculating at this region. Rubbing the inoculum onto the lower surface of the leaf has also been done (10).

Aphid Transmission

SMV is aphid-transmissible. Heinze and Kohler (12) were the first to

show that various species of aphids including Doralis frangulae Koch, D. rhamni Boyer, D. fabae Scop, Macrosiphon solanifolii Ashm, Ancecorthum pseudo-solani Theob, Myzus ornatus Leing, M. persicae Sulz and Neomyzus circumflexus Buckt could also transmit SMV. They also reported that A. pseudo-solani and M. persicae were the most efficient vectors. Using these species they observed that less than 30 min feeding time was enough for both virus acquisition and transmission, indicating SMV is stylet-borne.

Seed Transmission

Many workers have demonstrated SMV transmission in soybean seeds (4, 9, 10, 12). It has been reported that the percentage transmission in seed varied with the cultivar (4, 12, 21). For instance, 13% transmission (10) was reported in Hollyhock soybean, while Heinze and Kohler (12) observed 40% seed transmission in another variety of soybean. In Lincoln soybean, 2-75% seed transmission was observed by Conover (4), and 19% by Galvez (9). On the other hand, transmission of SMV was reduced to a level below that from plants infected with SMV alone when BPMV was also present (26).

Seed Mottling and Effect on Yield

While SMV, BPMV and TRSV are known to cause mottling of soybean seeds, mottling has also been reported to be associated with certain other factors. Thus Woodworth and Cole (34) and Owen (17) reported some mottling associated with certain cultural conditions. More mottling was observed in plants grown in rich loam soil and at more sparce plant populations while the reverse occurred in sandy soil and denser populations. Mottling was much

greater in abnormal plants. Woodworth and Cole (36) also reported that sugar accumulation is an important factor in mottling. Ross (26) reported that some unknown environmental factors also affected mottling.

Cooper (5) maintained that mottling is controlled by genetic factors. It has been widely accepted that the causes of mottling are largely due to virus infection (14). The distribution of mottled seeds borne on a single plant varied greatly but the seeds in a pod show some similarity in degree of mottling. This has been attributed to physiological factors (36).

Available data also indicate that seeds with discolored seed coats can be obtained from healthy soybeans (22). The age of plants when inoculated is also a factor in the degree of mottling. Sequential SMV and BPMV inoculations caused more mottling on Lee and Hill soybeans than simultaneous inoculations of BPMV and SMV or in the reverse order of BPMV and SMV.

Reduction in yield due to SMV and BPMV has been reported (10, 21, 22). From SMV alone a reduction of 26-43% has been reported depending on the variety.

On Lee soybean a reduction in yield of 18% was attributed to SMV alone, 26% to BPMV alone, while a 73% reduction occurred when BPMV and SMV acted together (22). On Hill soybeans SMV reduced yield by 43%, BPMV by 14%, and together BPMV and SMV reduced yield by 81% (22).

Cross-protection and Synergistic Reactions

Cross-protection between SMV strains has been reported. The SMV isolates SMV-M and SMV-NC provided protection against each other (21).

Synergistic reactions of SMV and BPMV, and SMV and TRSV have been

reported by Ross (22), Quiniones and Dunleavy (21), and Gordon and Schmitthenner (11). The effects on yield, seed transmission and mottling have been mentioned earlier.

Severe bud blight due to TRSV and SMV together has been observed by Gordon and Schmitthenner (11). They reported that these viruses together caused a more severe bud blight than when TRSV was alone.

Physical Properties of SMV

Literature available shows a great variation in the observed properties of SMV. The reported dilution end point has varied from 1:1,000 (20) to 1:10,000 (9, 12, 21). Similarly the thermal inactivation point has been reported to be as low as 58 C (19) and as high as 70 C (21). Others have reported it to be at 60 C (12) or 62 C (9, 20). Retention of infectivity in crude extracts has been from as short as one day (12) to as long as seven days (20). Heinze and Kohler (12), Pierce (19) and Conover (4) observed that crude extracts of their isolates were infectious up to 3 days. Galvez (9) reported that extracts were not infectious after 4 days at room temperature, after 14 days at 4 C and after 120 days in the freezer (0 C).

Purification of SMV

Galvez (9) partially clarified SMV by centrifuging for 20 min at 8,000 rpm. No buffer was used for extraction. Further purification was obtained by rate-zonal density gradient centrifugation in sucrose gradients dissolved in neutral 0.02 M tris-HCl (tris (hydroxymethyl) aminomethane) buffer. Virus aggregation was a problem and adding dispersing agents to the sucrose gradients did not prevent this.

Although virus aggregation was not completely prevented, Ross (23)

claimed this was minimized by grinding leaves in 0.5 M sodium citrate containing 1% mercaptoethanol. The extract was clarified by adding n-butanol to give a final concentration of 7%. The mixture was allowed to stand overnight at 4 C, then centrifuged. The aqueous phase was collected and given two cycles of differential centrifugation. Final purification was obtained by density gradient centrifugation. He claimed that resuspension of pelleted virus was facilitated by using 0.01 M borate pH 8.3. By this method, he obtained yields of 3.6-4.4 mg of protein/100 g tissue based on spectrophotometric analysis. Such preparations were free of antigenic host constituents.

Quiniones and Dunleavy (21) purified an Iowa isolate in the following manner. Infected leaves were frozen for three days then ground in 0.01 M phosphate buffer pH 7.2. The extract was clarified with chloroform-butanol and low-speed centrifugation. Further purification and concentration was done by three cycles of differential centrifugation, with the final pellet dispersed in distilled water. It was reported that 90% of the virus was lost in the process; however, the final preparation was 30 times more infectious than crude sap. This preparation was found suitable for electron microscopy, antiserum production and serology.

Characteristics of Purified SMV

Galvez (9), using low-speed clarified extracts, observed a faint zone located 2.8 cm below the meniscus after centrifuging purified SMV through sucrose density gradient columns for 2 hr at 23,000 rpm. Infectivity was concentrated mainly at 2.0-3.2 cm below the meniscus, but small amounts of infectivity were scattered throughout the column and much more was present in the pellet. Aggregation of virus particles

during purification was encountered and no technique tried prevented this.

Some aggregation was prevented and better resuspension of pelleted virus was claimed by Ross (23) with the use of 0.01 M borate buffer pH 8.3. The SMV preparation when scanned in the ultraviolet range was found to have a broad maximum between 260 and 265 nm, a sharp minimum at 245 nm, and a slight, but significant, tryptophan shoulder, at 290 nm. He estimated the nucleic acid content of virus to be 6-7%. After rate-zonal density gradient centrifugation at 25,000 rpm for 2 hr, maximum light scattering was observed 2.7 cm below the meniscus. This zone was found highly infectious. Using schlieren optics, analytical centrifugation of preparations obtained from rate-zonal density gradient columns were found to be relatively homogeneous. Preparations containing 8 mg of protein/ml had a dilution end-point of 10^{-5}-10^{-6}.

SMV Structure

The presence of cytoplasmic inclusions in epidermal cells of SMV-infected soybeans was reported by Quiniones and Dunleavy (21). These inclusions, generally one inclusion per cell, were amorphous, nonvacuolated and ranged from 10 x 4.8 to 13.8 x 3.01 nm.

Quantz (20) gave the SMV dimensions as 748 x 12-13 nm, while Brandes and Wetter (2) listed it as 750 nm long. The range of 650-725 x 15-18 nm was reported by Galvez (9) using a Nebraska isolate. Ross (23) found the average length to be 740 nm for a North Carolina isolate. An Iowa isolate, SMV-M, was reported to be 660-720 nm long (21). Most of the work on ultrastructure was done with either leaf dip or sprayed preparations shadowed with metals.

Serology

The importance of serology in identifying plant viruses has been emphasized by van Slogteren and van Slogteren (30) and Wetter (33). Serology is a useful method of virus identification as the reaction between antiserum and virus is specific (33).

Ross (23) used the virus obtained from density gradient columns for antiserum production and for investigating the serological reactions of SMV. SMV reacted with specific antibodies to form an open flocculent type of precipitate which is considered characteristic of rod-shaped viruses. He obtained an antiserum titer of 1:2048 for purified virus and 1:4096 for clarified sap.

Quiniones and Dunleavy (21) reported that the antisera to SMV-M and SMV-NC isolates gave zero titers after absorption with their homologous antigens. After absorption with heterologous antigens, the antisera still reacted at a dilution of 1:256 with their homologous antigens.

MATERIALS AND METHODS

Culture of Plants

Seeds of soybean cultivars and other species used for host range studies were obtained from Dr. A.Q. Paulsen and from Dr. C.D. Nickell of the Agronomy Department, Kansas State University. Large-seeded legumes and other species were sown in autoclaved soil at 6-10 seeds per 4-in pot. These were later thinned to four plants per pot. Small-seeded species were sown in autoclaved soil and seedlings were transplanted when large enough to handle. The plants were grown in a greenhouse maintained at 22-26 C in the winter and at higher temperatures in other seasons. For host-range studies most legumes and cucurbits were used 10-12 days after sowing with the inoculum rubbed on the primary leaves or cotyledons. Other species were used when 4-5 leaves were available for inoculation. The plants were routinely fertilized with small amounts of 10-10-5 fertilizer.

Virus Source and Inoculation Techniques

The original inoculum was obtained from Dr. A.Q. Paulsen. This was previously isolated from seedlings grown out of mottled seeds of selection No. 118 obtained from a cross between Harosoy x C1069 (Plate 1). The mottled seeds were obtained from C.D. Nickell. The stock culture was maintained by periodic transfer to Kent or Clark 63 soybeans.

For routine transfers and host range determinations the inoculum was prepared from plants infected 13-15 days. Young leaves showing distinct mosaic symptoms were used. The leaves were ground at approximately 1:10 (wt/vol) dilution in phosphate buffer pH 8.0, 0.03 M. The resulting

PLATE I

Soybean, sample No. 118, (Harosoy x C1069). Top, mottled; bottom, unmottled, from the same seed lot.

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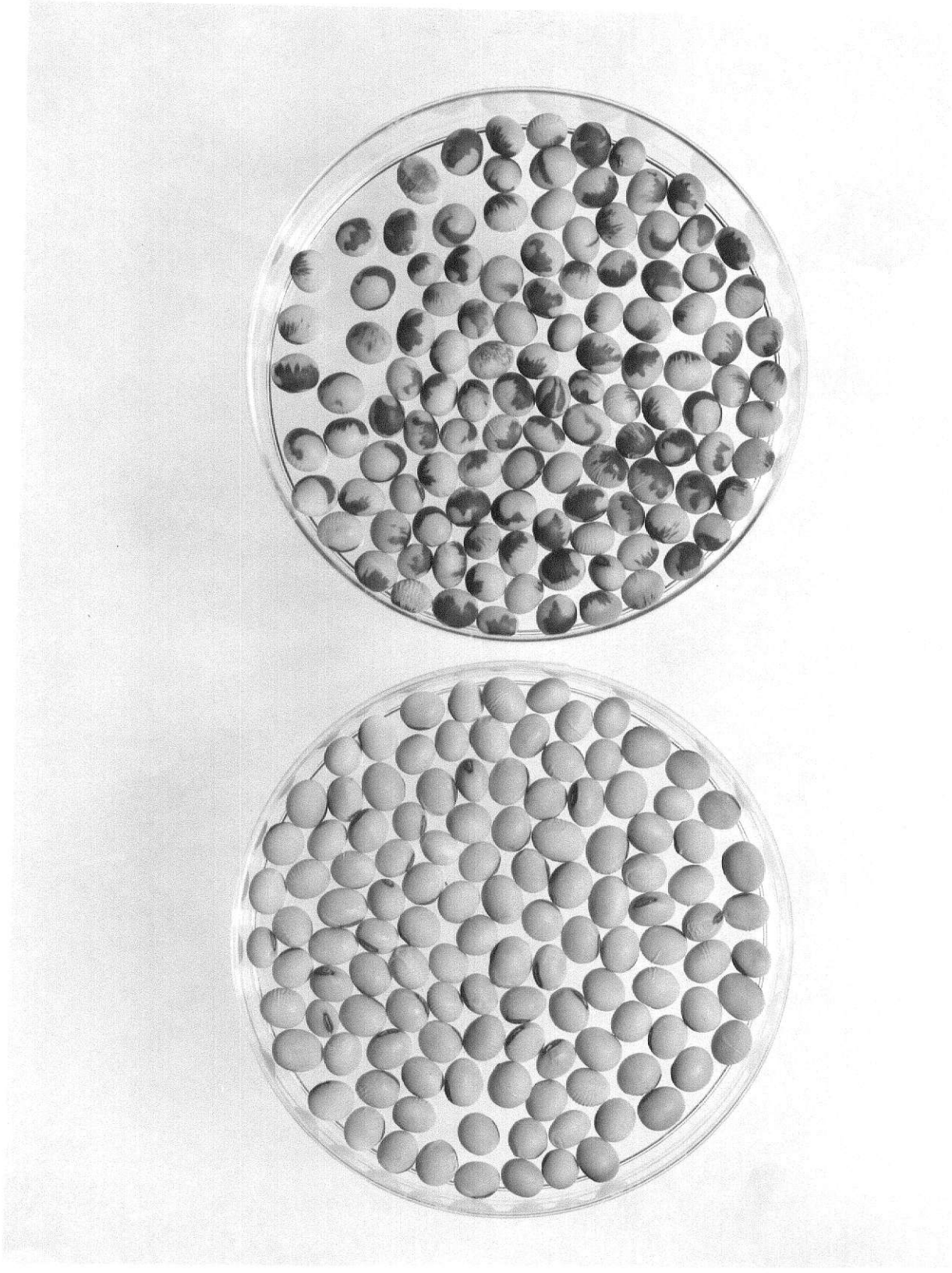


extract was applied onto Carborundum-dusted leaves with a cheesecloth pad. The plants were covered with wet paper toweling after inoculation to prevent rapid desiccation, then incubated in the greenhouse at 22-26 C in winter and at prevailing temperatures in water-cooled greenhouses in summer (26-40 C).

Methods of Assay

Phaseolus vulgaris 'Kentucky Wonder Wax Pole' (KWP) bean was the assay host chosen. Primary leaves of 10-12 day-old plants were generally used at which time the leaves were most susceptible. The half-leaf method of assay was employed for all experiments. For best lesion formation, an incubation temperature of 30 C is required (J.P. Ross, personal communication to Dr. A.Q. Paulsen). To obtain this, the method used by Ross was often employed. Leaves of KWP bean were detached soon after inoculation, floated in 2% sucrose solution in petri dishes, and incubated at 30 C in a bacteriological incubator. Lesion counts were made 3-5 days after inoculation.

RESULTS

Experimental Host Range

SMV was mechanically inoculated to 42 species belonging to 9 families (Table 1). For most leguminous species, 20-25 seedlings were inoculated. For soybean cultivars 60-80 seedlings were tested. With non-leguminous species, five to six plants were inoculated. To determine if local or systemic infection occurred and to find symptomless hosts, back inoculations were made onto primary leaves of KWP bean using a 1:10 dilution of sap in 0.03 M phosphate buffer pH 8.3. The assays were made 15-20 days after inoculation. Most of these experiments were performed during summer. Out of 42 species in 9 families, only 6 species in the Leguminosae were found susceptible (Table 1).

The 15 cultivars of soybeans tried were susceptible to SMV (Table 1) but some variation in the severity of symptoms was observed. A transitory vein-clearing in leaves developing above inoculated leaves was the first symptom observed. This appeared 4-5 days after inoculation. Dark-green puffy spots, also transitory, appeared 5-6 days after inoculation on the first trifoliate leaf of some cultivars, notably Bansei, Clark 63, Kent, Harosoy, Hill, Lincoln, and Wayne. Later, systemic symptoms such as distorted, misshappen leaves having wavy margins were observed on Bansei, Clark 63, Kent, Harosoy, Hill, Lincoln and Wayne (Plates 2 and 3). Milder mosaic symptoms were observed on Amsoy, Beeson, Calland, Cutler and Shelby. Most of the varieties tested developed necrotic local lesions. These appeared later than initial systemic symptoms, usually 15-16 days after inoculation. Almost all the cultivars tested produced mosaics that were dark green in color, and leaves that were leathery and brittle.

PLATE II

Mosaic symptoms on cultivars (A) Hill, (B) Harosoy, (C,D) Kent, and (E) Lincoln.

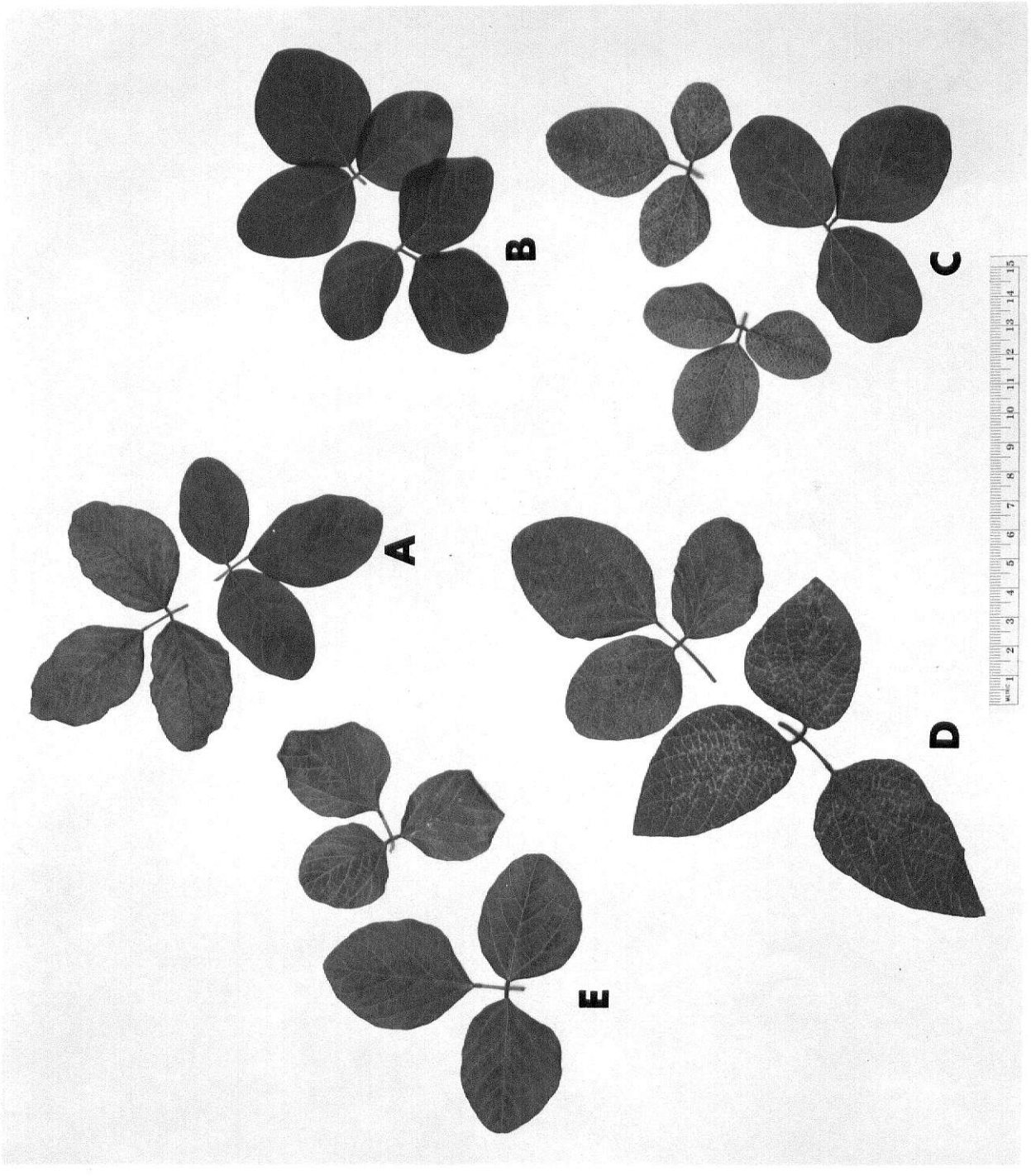
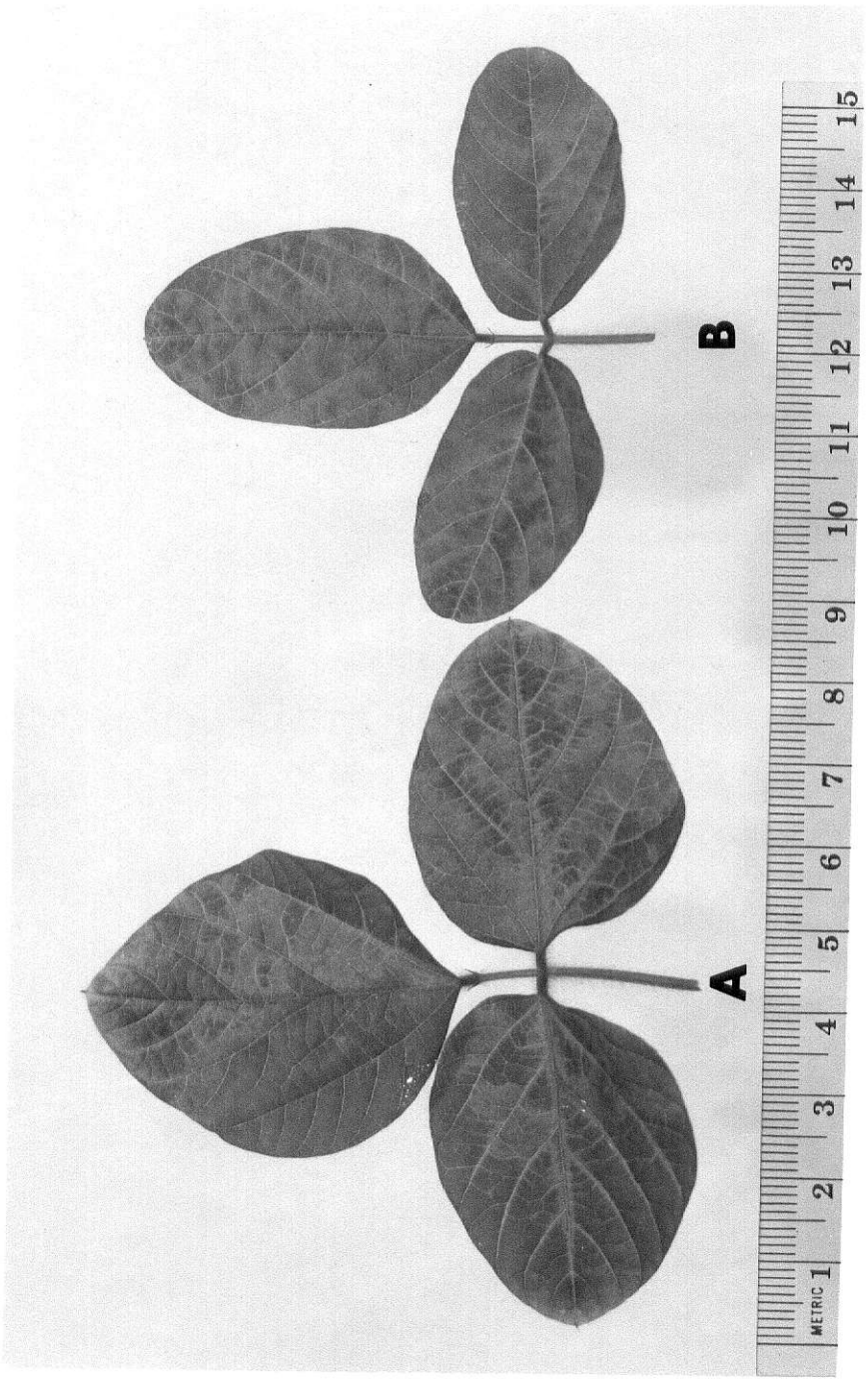

A
B
C
D
E

PLATE III

Mosaic symptoms on soybean varieties (A) Kent and (B) Hill.


A
B
METRIC
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12
13
14
15

Infected plants matured later than non-infected ones. The pods were much flattened and many were either empty or contained small seeds. Two exceptions were Harosoy and Beeson in which seeds were not badly flattened and size reduction was only slight.

A variety of symptoms was expressed on legumes susceptible to SMV. Five to six days after inoculation tiny necrotic local lesions, black in color, were observed on P. vulgaris 'Kentucky Wonder Wax Pole' (Plate 4), 'Royalty Purple Pod', 'Scotia' and 'Wade'. The lesions enlarged slightly and some veinal necrosis occurred when the lesions formed close to the veins. Adjacent lesions tended to coalesce to form large necrotic areas. On the cultivar Romano chlorotic local lesions were observed (Plate 5). Red Kidney and Pencil Pod Wax beans were systemically infected without symptoms, while Bountiful and Burpee's Stringless Green-Pod were infected locally without symptoms.

A systemic chlorotic mottling with downward rolling of leaflets developed on P. lathyroides (Plate 6). A systemic chlorotic mottling with downward rolling of leaflets was followed by the appearance of tiny necrotic local lesions on Canavalia ensiformis. Necrotic local lesions followed by a systemic chlorotic mottling was observed on Cyamopsis tetragonoloba. A chlorotic systemic mottle often accompanied by vein-banding and slightly ruffled leaves was observed in Cassia florida.

PLATE IV

Necrotic local lesions on *Phaseolus vulgaris* 'Kentucky Wonder Wax Pole' (KWP) bean. Photograph taken seven days after inoculation.

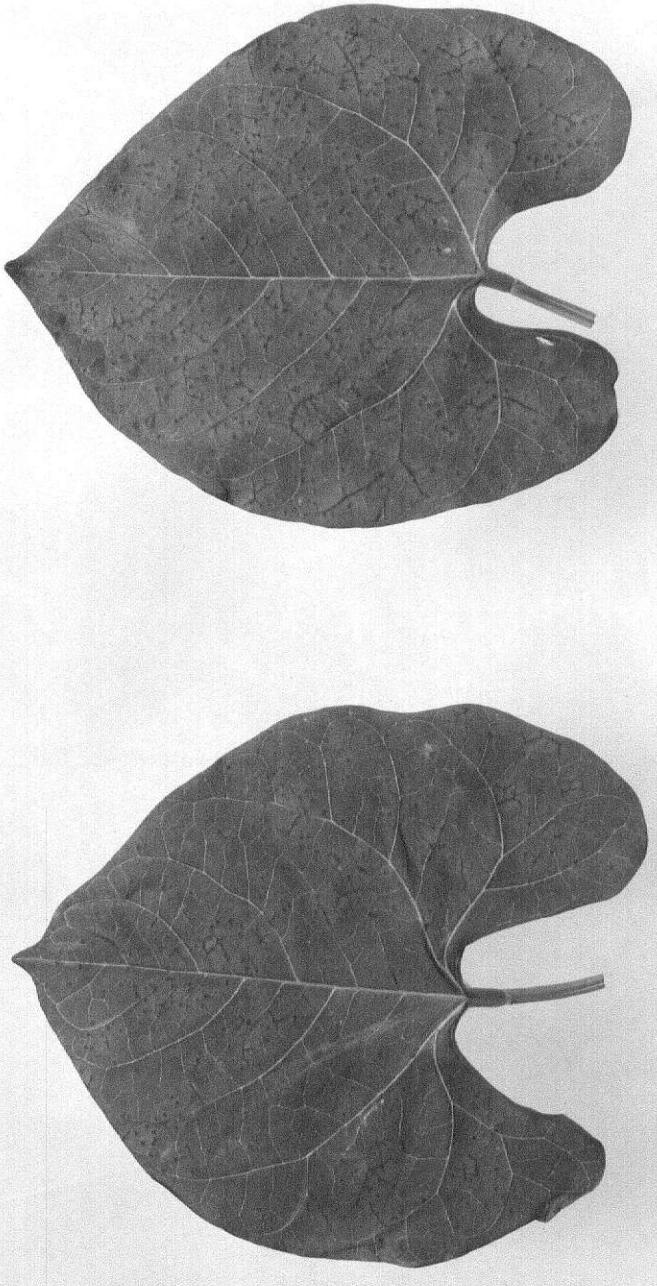


PLATE V

Chlorotic local rings on Romano bean as they appear 15 days after inoculation.

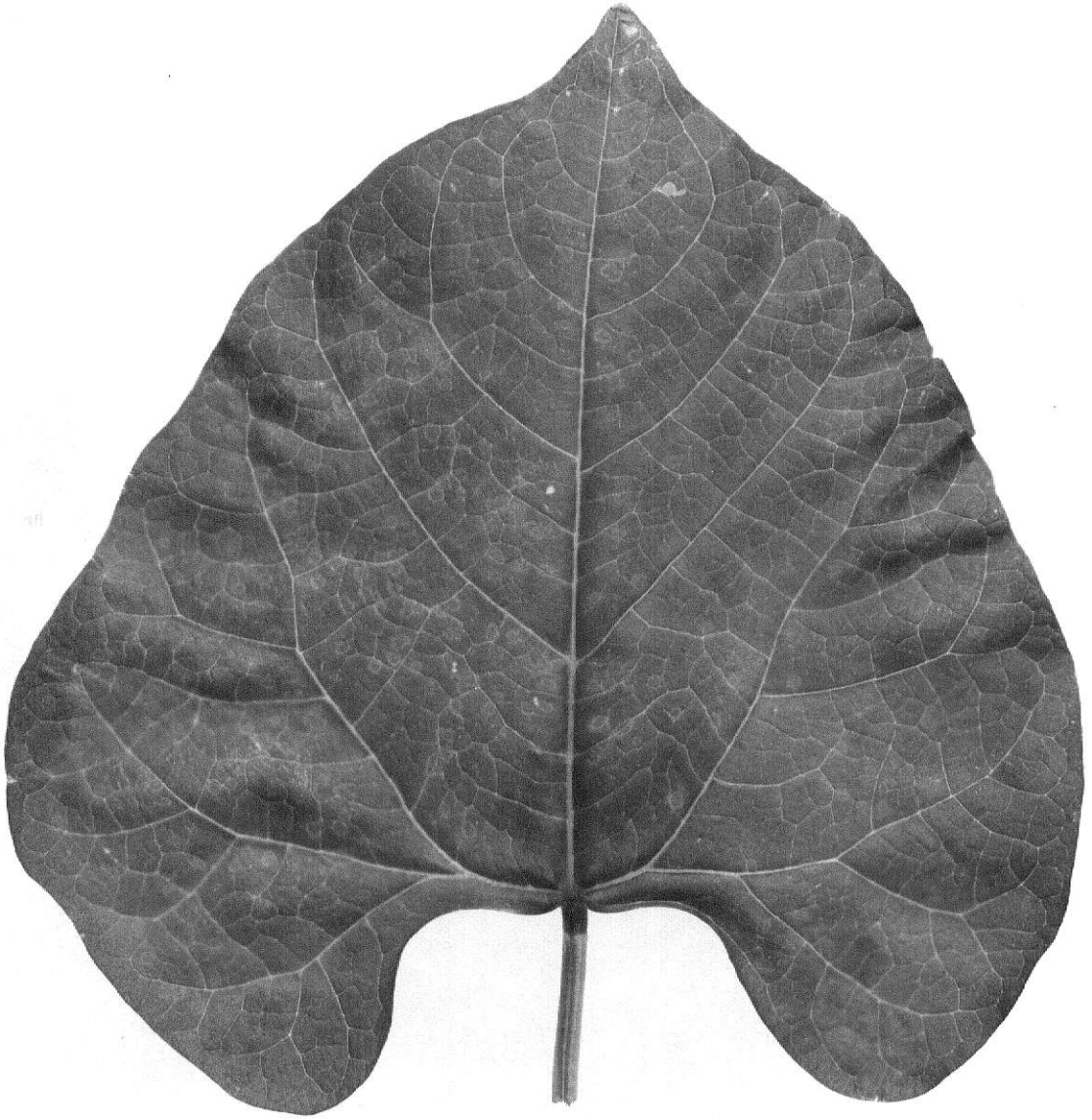
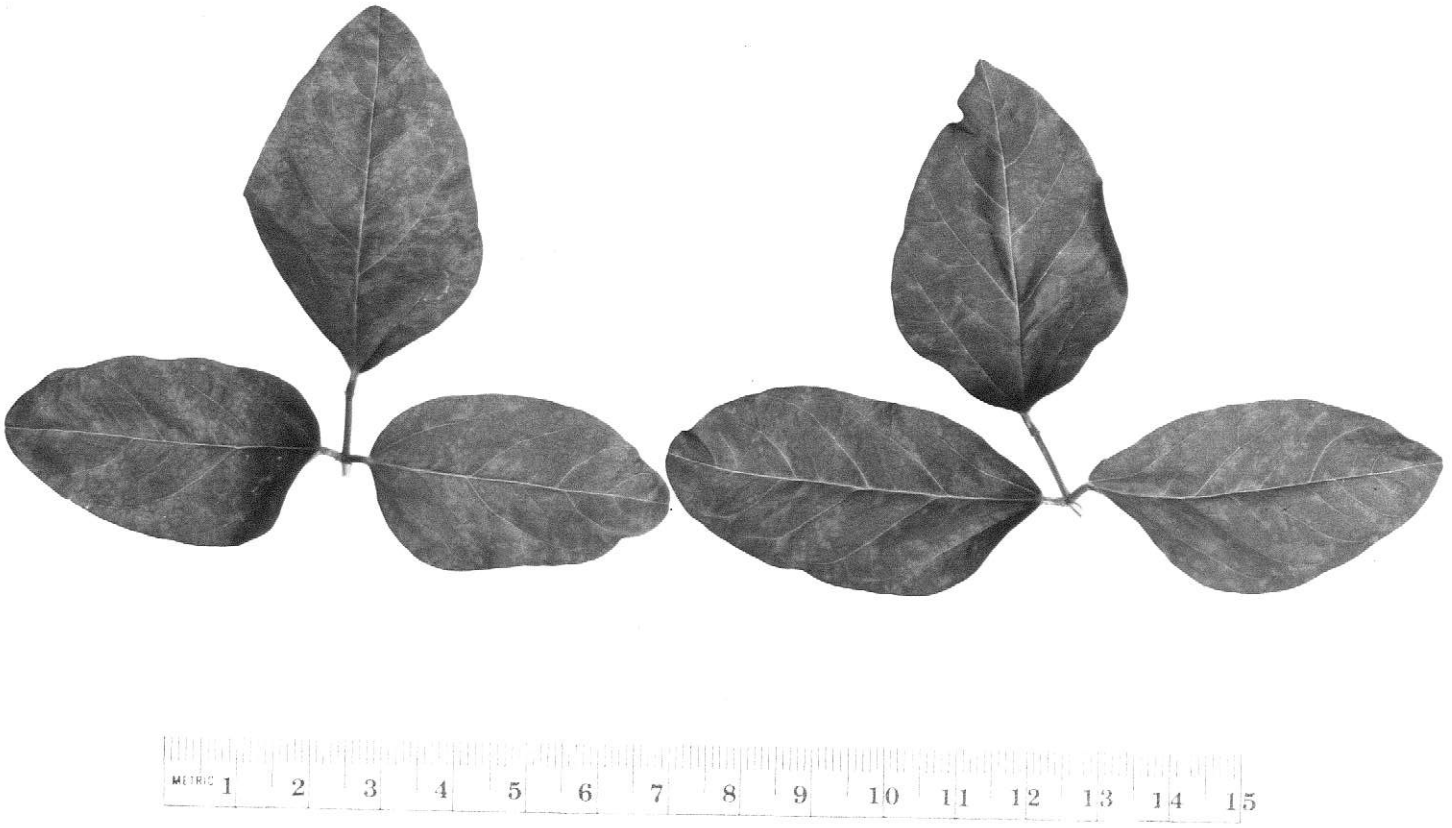


PLATE VI

Systemic mosaic symptoms on *P. lathyroides*.


METRIC
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14
15

TABLE 1. Reactions of plant species mechanically inoculated with SMV in the greenhouse.

Family and Species	Cultivar	Symptom Expressed[a]
Aizoaceae		
Tetragonia expansa (Murr.) Thunb.		0
Amaranthaceae		
Gomphrena globosa L.		0
Apocynaceae		
Vinca rosea L.		0
Chenopodiaceae		
C. album L.		0
C. amaranticolor Coste and Reyn.		0
C. ambrosioides L.		0
C. capitatum (L.) Asch.		0
C. foetidum Lam.		0
C. murale L.		0
C. rubrum L.		0
C. quinoa Willd.		0
Cruciferae		
Rhaphanus sativus L.		0
Cucurbitaceae		
Cucumis sativus L.	Chicago Pickling	0
Leguminosae		
Arachis hypogaea L.		0
Canavalia ensiformis DC.		N, SM
Cassia florida		C
Cicer arietinum L.		0

Crotalaria anagyroides H.B.K.		0
C. incana L.		0
C. spectabilis Roth.		0
Cyamopsis tetragonoloba (L.) Taub.		SM, NLL
Dolichos biflorus L.		0
Glycine max (L.) Merr.	Amsoy	SM, NLL
	Beeson	SM, NLL
	Bansei	SM
	Blackhawk	SM
	Clark	SM, NLL
	Clark 63	SM, CLL, NLL
	Calland	SM, NLL
	Cutler	SM, NLL
	Harosoy	SM, CLL
	Hill	SM, NLL
	Kent	SM, CLL, NLL
	Lincoln	SM, CLL
	Patterson	SM, NLL
	Shelby	SM, NLL
	Wayne	SM, NLL
Lathyrus latifolius L.		0
Medicago sativa L.	Cody	0
	Moapa	0
Phaseolus angularis Wight.		
P. coccineus L.		0
P. lathyroides L.		SM
P. lunatus L.	Fordhook 242	0

	Henderson Bush Lima	0
P. vulgaris L.	Black Valentine	0
	Bountiful	AL
	Cherokee Wax	0
	Great Northern	0
	Gratiot	0
	Kentucky Wonder Wax Pole	NLL
	Michigan Navy Beans	0
	Pencil Pod Wax	AS
	Pinto	0
	Pinto U.I.-III	0
	Red Kidney	AS
	Romano	CLR
	Royalty Purple Pod	NLL
	Saginaw	0
	Sanilac	0
	Scotia	NLL
	Burpee's Stringless Green-pod	AL
	Landreths Stringless Green-pod	0
	Surecrop Wax	0
	Tenderpod	0
	Tendercrop	0
	Topcrop (Bush bean)	0
	Top Notch Golden Wax	0
	Wade	NLL
	Wren's Egg	0

Pisum sativum L.	Alaska	0
	Wando	0
Vicia faba L.		0
Vigna cylindrica Skeels		0
Vigna unguiculata (Ter.) Savi	Black-eye	0
	Brown Sugar Crowder	0
	Early Ramshorn	0
	White Lady	0
	White Sugar Crowder	0
Scrophulariaceae		
Torenia fournieri Lind.		0
Solanaceae		
Datura stramonium L.		0
Lycopersicon esculentum Mill.		0
Nicandra physalodes (L.) Gaertn.		0
Nicotiana glutinosa L.		0
N. rustica L.		0
N. tabacum L.	Havana 38	0
	Xanthi-nc	0
Physalis floridana Rydb.		0

---

a

AL - localized without symptoms

AS - systemic without symptoms

C - chlorosis, chlorotic

CLR- chlorotic local rings

LL - local lesions

N - necrotic, necrosis

O - no symptoms, no reaction on KWP

S - systemic

SM- systemic mottling

Seed Transmission

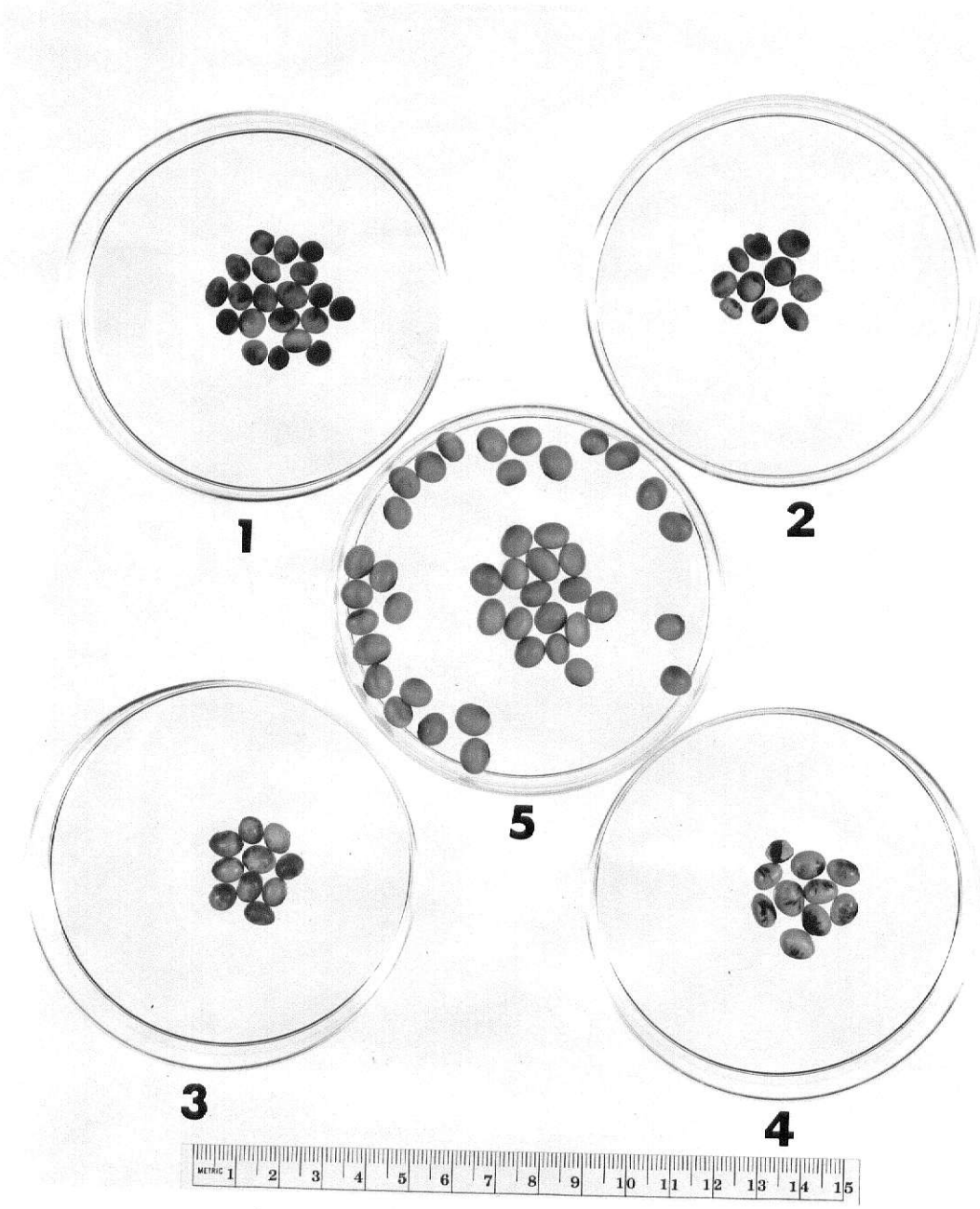
To determine virus transmission through the seed 14 soybean cultivars (Table 2) were selected. Seedlings growing in the greenhouse were inoculated at the primary leaf stage, then allowed to set seed. These were incubated at 22-24 C. The percentage of mottled seeds was determined from the harvested seeds. Forty to fifty mottled seeds were planted of Beeson, Clark 63, and Calland and for the other cultivars as many as were available. The seedlings that came up were observed for foliar symptoms. Because of the numbers involved, plants showing possible infection as malformed leaves and mild mosaic were individually assayed for virus and only limited assays were made from normal-looking plants.

The percentage of mottled seeds was higher in Amsoy (26%), Beeson (29%), Calland (25%), Cutler (30%), and Patterson (27%) than in others. The color of the mottled area varied with the variety but a dark brown to black color was the most common. A brown color was present on Amsoy, Bansei, Beeson, Harosoy and Patterson, while the black color occurred on Clark, Clark 63, Calland, Cutler, Kent, Lincoln and Wayne. The discolored area was larger in Amsoy, Calland, Clark 63, and Cutler, whereas this occurred as short streaks or small patches on Beeson and Harosoy (Plate 7).

Seed transmission was obtained in Amsoy (33%), Beeson (8%), Calland (5%), Clark (8%), Clark 63 (11%), Cutler (2%), Lincoln (28%), Patterson (16%), and Shelby (43%), but not in Haroscy, Hill, Kent and Wayne (table 2). Very few seeds were obtained in most cultivars so that if seed transmission was actually low, then it could have been missed. Where seed transmission was obtained, the seedlings had primary leaves with fairly wavy margins that cupped downward. A mild mosaic was also present.

PLATE VII

Mottled seeds of soybean varieties (1) Clark 63, (2) Calland, (3) Amsoy, (4) Cutler grown in the greenhouse, and Healthy Kent (5) seeds included for comparison.


1
2
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METRIC 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

TABLE 2. Percentage seed coat mottling and seed transmission of SMV in soybean cultivars.

Soybean Cultivar	No. Seeds Mottled/ No. Seeds Harvested	% Mottled	No. Seedlings/ No. Seeds Sown	No. Plants with Symptoms	% Seed Transmission
Greenhouse Grown-Plants					
Amsoy	30/118	26	12/30	4	33
Beeson	51/176	29	13/51	1	8
Calland	49/196	25	19/49	1	5
Clark	19/130	15	12/19	1	8
Clark 63	89/560	16	27/40	3	11
Cutler	28/92	30	28/28	6	2
Harosoy	3/18	17	3/3	0	0
Hill	7/50	14	0/7	0	0
Kent	5/105	44	5/5	0	0
Lincoln	11/92	11	7/11	2	28
Patterson	7/26	27	7/7	1	16
Shelby	7/135	5	7/7	3	43
Wayne	12/60	20	11/12	0	0
Field-Grown Plants					
Bansei	95/268	35	189/268	43	22
Kent	233/441	53	245/441	12	5
Lincoln	65/476	14	339/476	11	3

Very few seeds were harvested from greenhouse-grown plants, which limits the usefulness of the data. Ten to 20 seedlings of Bansei, Kent and Lincoln were inoculated at the primary leaf stage in the greenhouse. Two weeks after inoculation these were transplanted to an area outside the Mosaic Greenhouses to simulate field conditions. The plants were kept watered and fertilized. While all plants showed symptoms of infection, the symptoms were mild on Kent. Only a few of the pods formed were flattened and most pods had 2-3 seeds each. Bansei plants on the other hand were dwarfed, the leaves were leathery and brittle, and they showed a rugose green mosaic with leaves cupped downwards. Pods bore one to two seeds each and some remained small. On Lincoln the symptoms were considerably less severe than on Bansei but not as mild as in Kent.

Of the three cultivars grown in the field, Bansei gave the highest percentage seed transmission (22%) but did not give the most mottling (35%). On the other hand, 53% of the Kent seeds were mottled, but only 5% seed transmission was observed. Lincoln had 14% mottled seeds, with 3% seed transmission.

Transmission by Aphids

Nonviruliferous Myzus persicae Sulz. were maintained on Datura stramonium and Medicago sativa. The experiments were conducted with aphids that were starved for 10 min before acquisition feeding. Ten to fifteen adults were allowed to feed for 5 min on either Clark 63 soybeans or Phaseolus lathyroides that were showing distinct symptoms. The aphids were then transferred to a healthy soybean seedling and allowed to feed for 5 min before they were manually removed. To insure that all aphids were killed, plants were also sprayed with Kelthane. Seedlings infected

in this manner showed mosaic symptoms after 8-10 days incubation at 24 C, indicating that the aphids transmitted SMV in a stylet-borne manner. It was observed that symptoms appeared earlier on test plants when virus was acquired from P. lathyroides than from soybeans. Back assays to KWP bean from the resulting infected plants gave typical lesions due to SMV.

Choice of Production and Assay Hosts

Of the local lesion hosts tested, the greatest numbers of lesions were induced on KWP bean. The lesions appeared within 4-5 days after inoculation under proper incubation temperatures. The seedlings could be used in 10-12 days after sowing at which time primary leaves suitable for half-leaf assays were available. The leaves remained fairly susceptible for at least three days. However, incubation temperatures of 30 C were necessary for best lesion production.

Soybeans were used as the propagative hosts. Kent and Clark 63 soybeans were selected among several tested as they consistently gave the highest virus concentration.

Effect of Age of Infection on Virus Concentration

To determine whether the age of infection would affect virus concentration, Kent soybeans were inoculated with SMV. The plants were assayed 10-26 days after infection at 2-day intervals using a 1:10 dilution in 0.03 M pH 8.0 phosphate buffer. Leaves showing systemic infection were used as the source of inoculum. In a single trial it was found that virus concentration increased up to a maximum at 12-14 days after inoculation and gradually decreased thereafter. No virus was detected in plants infected 26 days.

Hill, Clark 63 and Kent soybeans were also compared as propagative hosts. For this, a 1:10 dilution was prepared from plants 14 days after inoculation. Clark 63 and Kent soybeans induced more lesions on KWP bean than Hill and therefore were used subsequently as propagative hosts.

Experiments to Increase the Susceptibility of the Assay Host

Several methods of increasing the susceptibility of the assay host were tried. For these tests, a 1:10 dilution of fresh inoculum was used.

Effect of Pre- and Post-inoculation Darkening

KWP bean seedlings were covered 24 hr before or after inoculation. A slight increase in lesion numbers was observed with pre-inoculation darkening, but the results did not warrant routine use of this method. Lesion numbers were slightly lower in plants darkened after inoculation than in control plants given ordinary lighting conditions.

Effect of Incubation Temperature

After inoculation plants were moved to separate greenhouses or growth chambers kept at 22-24 C, 28-30 C, or 28-32 C. Inoculated leaves were also detached, floated in 2% sucrose in petri dishes and incubated at 22-24 C, 28-30 C, or 28-32 C. It was found that better symptoms and more lesions were observed at 28-30 C.

Effect of pH and Concentration of Buffer

For these tests phosphate buffer was used. For the effect of pH, buffer of 0.10 M concentration was used at pH 6.5, 7.0, 7.5, and 8.0.

It was observed that extracts in pH 7.0 buffer were the most infectious. Further experiments therefore were made using pH 7.0 buffer at 0.01, 0.03, 0.05, and 0.1 M. Extracts in distilled water served as controls. It was found that 0.03 M gave the most lesions. Inoculum prepared in distilled water was found to produce more local lesions on Royalty Purple Pod bean than on KWP bean. On the other hand, more lesions were induced on KWP bean in the presence of phosphate.

Properties of SMV in Crude Extracts

Dilution End-Point

Inoculum was prepared from 14-day infected Clark 63 soybeans using leaves with distinct systemic symptoms. A weighed quantity of leaves was ground in 0.03 M phosphate buffer, pH 8.0 to make a 1:10 dilution. Further dilutions of 1:50, 1:100, 1:500, 1:1,000, 1:5,000, 1:10,000 were made from this. The preparations were then assayed for infectivity on KWP bean by the half-leaf method. No lesions were obtained at a 1:5,000 dilution, but a few showed at 1:1,000.

Thermal Inactivation Point

A 1:1 (wt/vol) dilution in 0.03 M pH 8.0 phosphate buffer was prepared from plants infected for 14 days. The extract was drawn into thin capillary glass tubes and one end sealed. In a preliminary trial the extract was heated for 10 min in a water bath at temperatures of 55-90 C using 5° intervals. It was observed that some infectivity was retained after heating at 55 but not at 60 C. Subsequent trials involved heating extracts at 2° intervals in temperatures of 55 C to 65 C. Extracts were

infectious at 57 C, but not at 59 C.

Longevity in Vitro

A 1:1 (wt/vol) dilution of extract was prepared in 0.03 M phosphate buffer pH 8.0. This was stored in a sealed vial at 22-24 C. Inoculations were made daily for 4 days. Virus infectivity was retained for only one day.

Tolerance to Desiccation

Systemically-infected soybean leaves were diced into small pieces with a razor blade and desiccated over $CaCl_2$ at 4 C. The desiccated material was infectious up to 5 months, the longest time tried.

Effect of Freezing and Thawing

One-gram samples of systemically-infected soybean leaves were frozen or were stored in a refrigerator at 4 C or in greenhouse at 22-24 C. After 1-2 hr the tissues were ground separately in 0.03 M phosphate buffer pH 8.0, to give a 1:10 dilution, and each extract divided into two parts. One-half of each extract was either frozen, refrigerated or stored at greenhouse temperature, respectively, while the other half was assayed for infectivity immediately. It was found that extracts from tissues held at refrigeration and greenhouse temperatures were infectious but that freezing inactivated the virus completely.

Cross-protection Experiments

Cross-protection experiments were performed using Kent soybeans as this host gave necrotic local lesions when inoculated with SMV. Two

other known isolates, SMV-M from Iowa (provided by S.S. Quiniones) and SMV-DTG from Ohio (provided by D.T. Gordon) also induced necrotic local lesions on this host. These isolates were inoculated separately to seedlings in the primary leaf stage. The challenge virus was then inoculated onto trifoliate leaves showing distinct mosaic symptoms, 21-25 days after inoculating the first or protecting isolate. No lesions were induced by the challenge virus on the leaves to which it was introduced, indicating that cross-protection among these isolates could have occurred.

SMV, SMV-M and SMV-DTG were inoculated to young Wayne soybeans. Symptoms were observed on the inoculated plants. Fifteen days after the SMV inoculation, TRSV was inoculated to leaves showing SMV symptoms. Necrotic local lesions due to TRSV were observed six days later. This indicated the absence of cross-protection between TRSV and SMV.

Purification

Eight to twelve Kent soybean seeds were sown per 4-in. pot. Ten days after sowing, the seedlings were inoculated with leaf extracts of SMV, SMV-M or SMV-DTG isolates freshly prepared in 0.03 M phosphate buffer pH 7.0. Leaves were harvested 14 days after inoculation and stored in the refrigerator at 4 C if not used the same day.

Ross' (23) method was followed. Leaves, buffer and the Waring blendor jar were pre-cooled to about 4 C. Four hundred to five hundred g tissue was used for SMV isolate and 200-300 g each for SMV-M and SMV-DTG. The leaves were homogenized in 0.5 M sodium citrate pH 8.0 containing 1% 2-mercaptoethanol to give a 1:3 dilution. The homogenate was squeezed through two layers of cheesecloth. To every 100 ml of extract, 7 ml of

n-butanol was added with stirring. The mixture was then refrigerated overnight. This was centrifuged at 12,100 g for 10 min and the aqueous phase collected and filtered through glass wool. Virus was pelleted by centrifuging at 147,100 g for 2 hr in an International B-60 ultracentrifuge. Half of the resulting pellets were resuspended in 0.01 M borate buffer pH 8.3 and the other half in 0.01 M pH 73. Buffer was added at the rate of 2 ml per tube and the pellets allowed to resuspend overnight in the refrigerator. Suspensions in the same buffer pH were pooled together and then centrifuged at 12,100 g for 10 min. The supernatant fractions were collected and given a second high speed centrifugation. Borate buffer for resuspending the pellets was added to give an equivalent of 1.3 ml per 100 g tissue. Pellets were again allowed to resuspend by standing overnight in the refrigerator. A final low speed centrifugation gave a partially purified virus preparation that was light green in color with very little opalescence attributable to virus. These were stored in the refrigerator until assayed for infectivity, relative concentration, and serological relationships. Absorbance at 260 and 280 nm were also read.

Photometric Scanning

A Hitachi Perkin Elmer Model 124 spectrophotometer was used to determine the absorbance (A) of a 1:20 dilution of the virus preparation. This gave an average A 260/280 ratio of 1.26 for SMV, 1.33 for SMV-M, and 1.32 for SMV-DTG. Based on the A 260 readings the relative concentrations (Extinction coefficients, $E^{0.1\%}_{260}$) in mg/ml of the purified preparations are: 0.008 for SMV; 0.01 for SMV-M; and 0.011 for SMV-DTG, using an $E^{0.1\%}_{260} = 3$ (Iscotables, Instrumentation Specialties Co., Inc.,

Lincoln, Ne., 1967).

Infectivity Assays

Dilutions of 1:10, 1:20, 1:40, 1:80, 1:160, and 1:320 of the above preparations were made in 0.01 M, pH 8.3 borate buffer and were then inoculated to 12-day old KWP beans. Lesions were observed and counted after five days. The SMV preparation was infectious up to 1:160 but not at 1:320. Virus was still infectious seven days after purification with an average lesion count of 39 per half leaf at a 1:160 dilution.

Serology

The microprecipitin test under oil and ring interface (15, 29) tests were used to determine the serological relationships of SMV. The antisera used in these tests were provided by Dr. S. Quiniones for two Iowa isolates, SMV-M and SMV-O, and by Dr. W. Langenberg for a Nebraska isolate (SMV-N).

For the microprecipitin tests, plastic petri dishes free of scratches were selected and 7-8 small squares per row were drawn with a red wax pencil. A series of two-fold dilutions from 1:10-1:80 of the purified SMV, SMV-DTG and SMV-M were made in 0.01 M borate buffer pH 8.3. The antisera were diluted in 0.85% saline to give a 1:10 and 1:20 dilution. A drop of the reactants were placed into the grid squares with micro-pippettes equipped with a No. 27 gauge hypodermic needle, starting with the lowest concentration. The reactants were covered with heavy mineral oil, incubated at room temperature and read after 4, 8 and 24 hr.

Similar dilutions of the antigens were prepared for the ring interface test. The antisera were diluted to 1:20 and 1:40 with 20% glycerine.

The reactants were layered into small serological tubes (6 x 50 mm) with disposable micropipettes, with the antiserum below. Readings were taken after 30 min, 1 and 2 hr incubation at room temperature.

In both tests, the controls were saline alone, buffer alone, saline plus serum, and saline plus virus. No precipitates were observed in any of these controls. No precipitates were obtained with SMV-M and SMV-O antisera tested against any of the antigens used in microprecipitin tests. With SMV-N antiserum diluted to 1:20, a small amount of precipitate was present at a dilution of 1:160 for all three antigens (Table 3). At 1:40 antiserum dilution a precipitate was obtained at a dilution of 1:160 for SMV, and SMV-DTG antigens, while SMV-M reacted only at 1:20.

Some mixing resulted while layering the reactants in the ring interface tests. However, positive reactions (Table 4) were obtained at a 1:160 dilution of the three antigens against a 1:40 dilution of SMV-N antiserum. SMV also reacted positively when tested against SMV-M antiserum. With SMV-O antiserum diluted to 1:40, a precipitate was observed with SMV, SMV-M and SMV-DTG when these were used at a 1:80 dilution.

Density Gradient Centrifugation

Gradient columns for rate-zonal centrifugation were prepared according to Brakke (1). The gradients, prepared at least 18 hr before use, were made by layering 4, 7, 7, 7, and 6 ml, respectively, of 10, 20, 30, 40 and 50% sucrose dissolved in 0.01 M pH 8.3 borate buffer. One ml of the SMV preparation (0.008 mg/ml) was layered on top of one column. One ml of SMV-DTG (.012 mg/ml) was layered on a second column. Purified cowpea mosaic virus (CPMV, 50 mg/ml) and tobacco mosaic virus (TMV, 50 mg/ml) were layered separately over 2 other columns in which 0.1 M

TABLE 3. Serological reactions of SMV, SMV-M, and SMV-DTG tested by the microprecipitin tests under oil.

Reciprocal of Antiserum Dilution	Reciprocal of Antigen Dilution: SMV 20	SMV 40	SMV 80	SMV 160	SMV-DTG 20	SMV-DTG 40	SMV-DTG 80	SMV-DTG 160	SMV-M 20	SMV-M 40	SMV-M 80	SMV-M 160
SMV-N												
20	+[a]	+	+	+	++	++	+	+	+	+	+	+
40	+	+	++	+	++	++	+	+	+	-	-	-
Saline + buffer	-[b]	-	-	-	-	-	-	-	-	-	-	-
Saline	-	-	-	-	-	-	-	-	-	-	-	-
Buffer	-	-	-	-	-	-	-	-	-	-	-	-
Saline + Antiserum	-	-	-	-	-	-	-	-	-	-	-	-

[a] Positive reaction, density of precipitate varying from slight (+) to a little more (++).

[b] No precipitate observed.

TABLE 4. Serological reactions of SMV, SMV-M, SMV-DTG tested by the ring interface test.

Reciprocal of Antiserum Dilution	Reciprocal of Antigen Dilution											
	SMV				SMV-DTG				SMV-M			
	20	40	80	160	20	40	80	160	20	40	80	160
SMV-N												
20	+	+	+	+	M	+	+	+	M	M	+	+
40	+	M	+	+	+	M	+	+	+	-	+	+
SMV-M												
20	0	0	++	+	0	0	0	0	0	0	0	0
40	0	0	-	++	0	0	0	0	0	0	0	0
SMV-O												
40	++	+	+	0	M	+	++	0	++	M	+	0
Saline	-	-	-	-	-	-	-	-	-	-	-	-
Buffer	-	-	-	-	-	-	-	-	-	-	-	-
Saline + serum	-	-	-	-	-	-	-	-	-	-	-	-
Saline + virus	-	-	-	-	-	-	-	-	-	-	-	-

[a] Key to table symbols: +, precipitate observed; -, no precipitate; 0, this dilution was not tried; M, reactants mixed.

phosphate buffer, pH 7 was used. These served as calibration standards. These were then centrifuged for 2 hr at 109,844 g in the SB-110 rotor of the International ultracentrifuge at 4 C. The tubes were observed for the presence of visible zones. None were observed in either of the SMV isolates. When the gradients were fractionated with an ISCO model D Density-gradient Fractionator (Instrumentation Specialties Co., Inc., Lincoln, Nebraska) and scanned at 254 nm with an ISCO UA-2 UV analyzer, a peak was obtained at 11.5 ml for the SMV preparations. Two peaks were observed for CPMV, one at 8.25 ml, and the other at 10.2 ml. TMV banded at 15.3 ml. The SMV bands were collected and tested for infectivity on KWP bean without further treatment. These were not infectious. On the other hand, the pellet observed at the bottom of the tube in which the SMV isolate was run was slightly infectious, giving 15 lesions per half-leaf.

A second experiment was tried in which clarified sap extracts of SMV were used. For this, SMV was produced in Bansei soybeans. The plants were inoculated with extracts obtained from Bansei seedlings infected through the seed. Galvez' (9) method was used with some modifications. A 1:1 (wt/vol) dilution of extract was prepared in (A) 0.02 M, pH 7.3 tris-HCl, and in (B) 0.01 M pH 8.3 borate. One percent 2-mercaptoethanol was added to the buffers used for grinding the tissues. To half of extract A 7% butanol was added and this was designated C. Half of extract B was also clarified with butanol and designated D. The extracts were allowed to stand overnight at 4 C and centrifuged at 7,710 g for 20 min the next day. Two ml each of the supernatant fractions were then layered separately on sucrose gradients prepared in the appropriate buffers. These were centrifuged at 90,433 g for 2 hr.

When examined visually, faint bands were found in the tubes containing SMV. A single band each was observed in A, C and D. These were located at approximately 1.5 cm below the meniscus. Three bands were observed in preparation B, located at 1.5, 2.3 and 2.9 cm below the meniscus. Preparation C was fractionated through the gradient fractionator; however, no peaks were observed, probably because of the presence of interfering host constituents. Two ml samples collected and assayed for infectivity were not infectious. For the other three samples, therefore, the bands were individually collected with a hypodermic syringe equipped with No. 23 gauge needle. These were tested for infectivity without further treatments. The single bands in preparations A and D were not infectious. Similarly, no infection was obtained with the top two bands of B, but the third band was slightly infectious, giving 11 lesions per half-leaf on KWP bean.

DISCUSSION

At least three viruses known to infect soybeans can induce seed coat mottling on this host. These are SMV, TRSV and BPMV. SMV and TRSV are seed-transmissible (4, 6, 9, 11, 21, 22), while BPMV is not (21, 22). The three viruses can be identified on the basis of host range, vectors and properties.

The suggested indicator hosts for TRSV are tobacco, Chenopodium amaranticolor, Vigna unguiculata and Datura stramonium (16). For BPMV, Phaseolus vulgaris 'Pinto', 'Pencil Pod Wax', 'Tendergreen' (27) are the indicator hosts. For SMV Cyamopsis tetragonoloba, P. lathyroides, Canavalia ensiformis and KWP bean (9, 20, 21, 31) are useful hosts. SMV is transmitted in a stylet-borne manner by aphids (4, 9, 20). TRSV has several vectors including grasshoppers (7), nematodes (8) and mites (28).

The SMV isolate investigated was identified on the basis of its host range, properties in crude sap, seed and aphid transmission, cross-protection and serological reactions. The virus was easily transferred mechanically to known SMV indicator hosts, KWP bean, Cyamopsis tetragonoloba, Canavalia ensiformis, and P. lathyroides. Typical symptoms were observed as reported earlier by Walters (31), Galvez (9), Quiniones and Dunleavy (21), Quantz (20) and Ross (23).

Host range data confirm the observations of other workers that SMV has a narrow host range. The isolate investigated seemed to be more limited in hosts in that no species outside of Leguminosae was infected. It would therefore appear more limited in its hosts than the isolates studied by Galvez (9) and Quiniones and Dunleavy (21) who reported infection of some Chenopodium species.

Some new hosts were found for SMV. They are Cassia florida which was infected systemically, Romano bean on which chlorotic local ringspots were induced, and Royalty Purple Pod bean on which tiny necrotic local lesions developed.

The temperature at which inoculated plants were incubated appeared important in symptom expression. Thus in soybean cultivars, more severe symptoms appeared when plants were grown in cooler temperatures of about 18-22 C. More seedlings showed seed infection also when grown at these temperatures. However, the incubation period was longer than when plants were kept at 24-30 C. At the higher temperatures, symptoms were generally milder, and often in some varieties symptoms were transitory. Seedlings grown from mottled seeds looked normal, and virus concentrations were generally less in such plants. The effect of temperature on symptom expression may be important, particularly when assessing the amount of seed transmission of SMV based on symptoms alone and without other methods of assay.

On the other hand, warmer temperatures of about 24-28 C favored symptom development on other legumes. Of particular interest is the effect of temperature on lesion production in KWP bean, the assay host used. The data on SMV confirmed Ross results on the requirement for high temperatures (30 C) for maximum susceptibility of this host. While detaching the inoculated leaves, floating in 2% sucrose then incubating these at 30 C was somewhat inconvenient, the best results were obtained in this manner.

The observed temperature effects on symptom expression in suscepts confirm the observations of Conover (4) and Walters (31), who reported that best symptoms on soybeans appeared at 18.5-20 C and that masking

occurred when temperatures were above 27 C.

The 15 varieties of soybeans tested were all susceptible. Symptoms varied from mild to severe. In most cultivars necrotic local lesions were observed. The percentage of seed mottling and seed transmission also varied with the cultivars tested. For instance, only 5% mottling was observed on Kent, whereas this went up to 30% on Cutler. Seed transmission also varied from 5% in Calland to 43% in Shelby. The seeds generally were small and usually flattened. In the field grown cultivars Kent gave the highest percentage of mottled seeds, whereas Bansei gave the highest percentage of seed transmission.

Myzus persicae transmitted SMV in a stylet-borne manner. The data agrees well with reported aphid transmission of this virus (4, 9, 20). It is interesting that test plants showed symptoms earlier when the aphids acquired the virus from P. lathyroides than from soybeans. This may be related to the feeding habits of M. persicae. Observations indicated that P. lathyroides was a preferred host with the aphids feeding more actively here than in soybeans. It is also possible that the aphids acquired more virus in this host in the process of feeding. Also, at higher temperatures as during summer when these experiments were performed, it could be that virus concentration in P. lathyroides was higher than in soybeans. No studies were done to determine the concentration of SMV in P. lathyroides, however.

SMV reached a maximum concentration in plants 12-14 days after inoculation and then gradually decreased, so that after 26 days no virus could be detected. This may indicate that virus inactivation occurred in vivo. Quiniones and Dunleavy (21) obtained maximum virus concentration with the SMV isolates they used 25 days after inoculation. They used

Bansei soybean as the production host and whether there may be differences in peaks of virus titers with the cultivar or the virus isolates used was not tested. Ross (25) reported that some isolates of SMV produced higher concentrations in some soybean cultivars than in others.

Phosphate is known to increase the number of lesions on beans mainly by increasing host susceptibility (35). It was therefore the buffer chosen in experiments on the effect of buffer concentration and pH on SMV. On KWP bean, it was found that the greatest lesion numbers developed with neutral phosphate when used at 0.03 M. It is interesting that when Royalty Purple Pod bean was used as the local lesion host, more lesions developed when extracts were prepared in distilled water. The reason for this is not known, and no further experiments were done to determine why this should be so.

Extracts of SMV were infectious at 1:1000 dilution but not at 1:5000, and infectivity was retained for only a day. In both these properties, the results agree with those of Quantz (20). Some infectivity was observed after heating at 57 but not at 58 C. This observation agrees with that of Pierce (19). The low virus yield, thermal death point, and short activity in extracts may be due to the properties of the virus isolate itself or to the low virus concentration in the production host.

Freezing of both leaves and buffered extracts completely inactivated SMV. On the other hand, Galvez (9) reported that infectivity was retained in frozen extracts up to four months. Again this may be a characteristic of the isolate itself.

Two attempts to purify SMV using Ross' (23) technique did not yield a preparation of sufficient virus titer useful for studying the virus in pure form. Several factors and reasons may explain the low yields of

purified virus obtained. Kent soybean was the production host, and infected leaves were harvested 14 days after inoculation. At this time virus was at maximum concentration, based on infectivity assays to determine the effect of age of infection on concentration.

Aggregation and denaturation were serious problems in attempts to purify SMV. Evidence that these occurred was shown in density gradient centrifugation. Thus bands that were located above the infectious band were not infectious whereas the pellets were. Density gradient centrifugation of crude and purified preparations also provided evidence that SMV did occur at low concentrations in soybeans and that most of what was present was lost in the preparative techniques. It could be that choice of a better production host and some manipulation of and changes in the buffers and ionic concentrations used might reduce this. These were not investigated in these studies.

Serologically, the SMV investigated reacted positively with antiserum prepared for three known SMV isolates. The extent of this serological relatedness is not known as both antigen and antisera were of limited quantities to permit extensive study of this area.

Cross-protection experiments also provided evidence that the isolate studied is related to other known SMV isolates. The absence of the second seed-borne virus, TRSV, was shown conclusively by the lack of protection upon challenge-inoculation with TRSV in soybeans. Serological assays made by Dr. A.Q. Paulsen to determine the presence of BPMV, CPMV, and TRSV in crude and purified preparations also provide evidence that only SMV was associated with the seed-borne virus originally isolated from Harosoy x C1069 seeds.

It is concluded that Cyamopsis tetragonoloba, Canavalia ensiformis,

Cassia florida, and KWP and Romano beans may be useful as indicator hosts of the SMV isolate investigated. Identification would be more specific by the use of other methods, however, including seed and aphid transmission, structure, serology and other properties of the virus.

The SMV isolate studied failed to infect systemically certain legumes which have been reported as systemic hosts. Also, in repeated trials, *Chenopodium album* and *C. quinoa* were insusceptible. In comparison to the two other isolates (SMV-M and SMV-DTG) used here, the Kansas SMV gave milder symptoms on soybeans. Also, based on the limited data obtained, particularly with field-grown soybeans, the Kansas isolate induced less mottling and less seed transmission than published reports. These differences may indicate inherent characteristics of the Kansas isolate and would reflect variations in the isolates themselves.

SUMMARY

A virus originally isolated from mottled Harosoy x C1069 seeds grown in Kansas was identified as soybean mosaic virus (SMV) on the basis of host range, methods of transmission, properties in crude sap, serology and cross-protection.

Clark 63 and Kent soybeans were found to be good propagative hosts, while Kentucky Wonder Wax Pole (KWP) bean was considered a good local lesion host. It was necessary to incubate the inoculated leaves of KWP bean at 30 C for maximum susceptibility.

SMV infected 6 legume species out of 21 tested. Cassia florida and Phaseolus vulgaris 'Romano' and 'Royalty Purple Pod' beans were found as new hosts. On C. florida a systemic mosaic was induced while only local necrotic lesions developed on the beans. On other hosts, symptoms varied from localized chlorotic and necrotic spots to a systemic mosaic. Twenty-one nonleguminous species belonging to 8 families were not infected.

The virus was seed-borne in soybean. In limited tests, mottling percentage as well as seed transmission varied with the cultivar, but was generally low. A reduction in pod set and seed size was observed.

The aphid Myzus persicae Sulz. transmitted the virus in short acquisition and infection feeding periods. This indicated it is stylet-borne.

In Kent soybeans virus concentration increased up to 14 days after inoculation and decreased gradually thereafter. Greatest lesion counts were obtained with 0.03 M phosphate buffer pH 7.0. In crude extracts, some infectivity was detected at 1:1000 but not at 1:5000 dilution. Virus tolerated heating at 57 C. Crude extracts kept at 22-26 C were

infectious only for a day. Diced tissue was still infectious five months after desiccation. Freezing leaves and extracts inactivated the virus.

SMV protected Kent soybeans against challenge-infection by two known soybean mosaic virus isolates, SMV-M and SMV-DPG.

By the microprecipitin and ring interface tests, SMV reacted serologically to SMV-M, and SMV-O, antisera but at a lower titer than the homologous antigen. SMV also reacted positively with antiserum of a Nebraska isolate. Cyamopsis tetragonoloba, Canavalia ensiformis, P. lathyroides, Cassia florida and Phaseolus vulgaris 'Kentucky Wonder Wax Pole' may be used as indicator hosts.

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HOST RANGE AND SOME CHARACTERISTICS OF A SOYBEAN MOSAIC VIRUS ISOLATED IN KANSAS

by

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A seed-borne virus obtained from mottled Harosoy x C1069 soybeans was identified as soybean mosaic virus (SMV) by host range, methods of transmission, properties in crude sap, and serology. Of 42 species in 9 families only 6 leguminous species were susceptible. Cassia florida and Phaseolus vulgaris 'Romano' and 'Royalty Purple Pod' were found as new hosts. Virus was both seed- and aphid-transmitted. Clark 63 and Kent soybean varieties were used as the production hosts and P. vulgaris 'Kentucky Wonder Wax Pole' (KWP) bean as the assay host. Crude extracts were infectious at a dilution of 1:1000 but not 1:5000 and after heating for 10 min at 57 C. Crude extract was infectious for only one day. Desiccated tissues stored at 4 C were still infectious after 5 mo. Freezing inactivated SMV. SMV gave a positive serological reaction to three known SMV isolates. Protection was obtained in soybeans against Ohio and Iowa isolates. Soybean cultivars showed differences in percent of mottling and seed transmission.