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I am submitting herewith a thesis written by Brenda S. Kennedy entitled "Occurrence and Distribution of Soybean Viruses in Tennessee." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Bradford B. Reddick
Bradford B. Reddick, Major Professor

We have read this thesis
and recommend its acceptance:

James W. Kelly
Fred J. Allen

Accepted for the Council:

C. W. Mink
Vice Provost
and Dean of The Graduate School

OCCURRENCE AND DISTRIBUTION
OF SOYBEAN VIRUSES
IN TENNESSEE

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ABSTRACT

Four commercial soybean fields were surveyed for virus occurrence in each of nineteen counties in the major production areas of Tennessee in 1987 and 1988. Six leaf samples, five symptomatic and one asymptomatic, were collected from each soybean field per county. Virus isolates were identified using the Protein A sandwich ELISA (PAS-ELISA) technique. Samples were tested with antisera specific to alfalfa mosaic virus (AMV), bean pod mottle virus, (BPMV), bean yellow mosaic virus (BYMV), cowpea chlorotic mottle virus (CCMV), cucumber mosaic virus (CMV), peanut mottle virus (PMV), peanut stunt virus (PSV), southern bean mosaic virus (SBMV), soybean mosaic virus (SMV), tobacco ringspot virus (TRSV), tomato spotted wilt virus (TSWV) and white clover mosaic virus (WCMV). In 1987, 49% of the leaf samples collected tested positive for virus. AMV, BPMV, BYMV, CCMV, CMV, PSV, SBMV, SMV, TRSV and TSWV were detected. BPMV was the most frequently detected, occurring in 75% of the leaf samples that tested positive for virus. Other viruses occurred in less than 5% of the total positive samples. In 1988, 32% of the leaf samples collected tested positive for virus. AMV, BPMV, BYMV, CCMV, CMV, PSV, SBMV and TRSV were detected. BYMV was the most frequently detected virus, occurring in 33% of the positive leaf samples followed by BPMV (30%) and TRSV (11%). This is

the first report of BPMV, CCMV, SBMV and SMV in any crop in Tennessee and the first report of AMV, BYMV, CMV, PSV, TRSV and TSWV occurring in soybeans in this state.

Soybean cultivars in trials at the Agricultural Experiment Stations in Milan, Knoxville and Greenville, Tennessee were evaluated for virus occurrence and disease incidence. Thirty-one percent of the leaf samples collected tested positive for virus infection. AMV, BPMV, BYMV, CCMV, CMV, PSV, SBMV, SMV, TRSV and TSWV were detected from the leaf samples collected at Milan. BPMV, BYMV and SMV were detected at Knoxville. Alfalfa mosaic virus was the only virus found at Greenville. Virus incidence ranged from 0 to 9% with SMV being the most frequently detected virus occurring in 49% and 79% of the leaf samples that tested positive for virus at Milan and Knoxville, respectively.

In 1988, soybean cultivars 'Essex', 'Forrest', 'TN 5-85' and 'York' were inoculated with BPMV, or SMV, or BPMV and SMV at Knoxville and Milan, Tennessee to evaluate the effect of the viruses on yield. All cultivars were equally susceptible to BPMV infection. BPMV incidence ranged from 2-20% in cultivars inoculated with BPMV alone or in cultivars inoculated with BPMV and SMV. No BPMV was detected in plants not inoculated with BPMV. Little or no SMV occurred in cultivar 'York' with any treatment; however SMV incidence ranged from 4-69% in 'Essex', 2-42% in 'Forrest' and 7-75% in 'TN 5-85'. Based on the analysis of

variance, there were significant differences ($P \leq 0.05$) among blocks and cultivars but no significant differences among virus treatments or cultivar x treatment at Knoxville. However, significant differences ($P \leq 0.01$) were observed among all variables at Milan. According to Tukey's mean separation test ($P \leq 0.05$) there was a significant decrease in yield among cultivars inoculated with BPMV/SMV at Milan when compared to all other treatments. No significant differences were found among treatments at Knoxville. Significant differences were found among the cultivars at both locations. At Milan, yields of the cultivar Essex were significantly higher than Forrest, TN 5-85 and York. Yields of TN 5-85 were also significantly higher than Forrest and York. At Knoxville, cultivar TN 5-85 yielded significantly higher than Essex but not significantly higher than cultivars Forrest and York. According to Tukey's mean separation test ($P \leq 0.05$), there were differences in yield due to cultivar x treatment interactions at both locations; however no correlation between virus incidence and yield differences were apparent.

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I. INTRODUCTION

Soybean is Tennessee's leading field crop in terms of acreage and dollar value. In 1987, 1.3 million acres were grown in Tennessee, averaging 23 bushels per acre with a total value of \$158.1 million (18). Tennessee presently ranks fifteenth in the United States in soybean production; therefore diseases which affect yield are of considerable importance.

Maximum yield losses attributed to virus diseases of soybeans have been reported from 10-100% (102). Alfalfa mosaic virus (AMV), bean pod mottle virus (BPMV), bean yellow mosaic virus (BYMV), cowpea chlorotic mottle virus (CCMV), cucumber mosaic virus (CMV), peanut mottle virus (PMV), peanut stunt virus (PSV), southern bean mosaic virus (SBMV), soybean mosaic virus (SMV), tobacco ringspot virus (TRSV), tomato spotted wilt virus (TSWV) and white clover mosaic virus (WCMV) infect soybeans in the United States (102). AMV, BYMV, CMV, PSV, TRSV and TSWV have been reported to infect other crops in Tennessee (66, 86); however no previous study of viruses infecting soybeans has been conducted. The objectives of this study were to evaluate: 1) the occurrence of any or all twelve viruses named above in soybeans in Tennessee, 2) the recommended soybean cultivars for virus occurrence and disease

incidence, and 3) the effects of BPMV, or SMV, or BPMV and SMV on yield of four soybean cultivars.

II. LITERATURE REVIEW

The soybean, Glycine max (L.) Merr., was first introduced into the United States as a forage crop in 1785 and has since developed into one of the major field crops produced in the country (52). Soybeans are a primary source of vegetable oil and protein (102). As soybean acreage has expanded throughout the world, diseases which affect its growth are important.

Virus diseases of soybeans can cause from 10-100% yield reduction depending on the virus, time of infection, cultivar planted and the percentage of plants infected (102). AMV, BPMV, BYMV, CCMV, CMV, PMV, PSV, SBMV, SMV, TRSV, TSWV and WCMV are known to infect soybeans in the United States (102).

Alfalfa mosaic virus

Alfalfa mosaic virus represents its own virus group, having bacilliiform particles of different lengths, the largest about 60 nm in length, in which four species of single-stranded RNA are packaged separately (56). AMV is worldwide in distribution and infects over 430 species of dicotyledonous plants including many herbaceous and woody hosts. This virus is responsible for diseases of economical importance in alfalfa, potato and tobacco crops. Numerous strains have been reported on the basis of host range and

physical/chemical properties. AMV is sap transmissible and is transmitted in the non-persistent manner by 14 aphid species. Seed transmission has been reported up to 10% in commercial alfalfa seed (56).

In 1960, Allington et al. (3) reported the first natural occurrence of AMV infecting soybeans in Nebraska. The disease occurs sporadically in soybeans planted near alfalfa fields. Allington et al. (3) reported that AMV is transmitted by the pea aphid, Macrosiphum pisi (Harris) from alfalfa to adjoining soybeans. Because soybeans are not a preferred host, this insect feeds only briefly on soybeans which results in sporadic distribution of the disease in the field. Its effect on yield of soybeans has not been determined.

Symptoms of systemic infection in soybeans appear as a bright yellow mottle on leaves which is similar to symptoms of iron or manganese deficiency (3, 102).

Bean pod mottle virus

Bean pod mottle virus has 30 nm isometric particles and is a member of the comovirus group (99). The genome consists of two pieces of single-stranded RNA encapsidated in two nucleoprotein particles, both of which are required for infection, as well as a RNA-free particle consisting only of the protein subunits. BPMV causes economic loss in

beans (Phaseolus spp.) and soybeans (99). BPMV isolates have been reported but have not been compared to the type strain that was originally isolated from common bean in South Carolina. BPMV is sap transmissible and is transmitted by several beetle vectors (99). Seed transmission of BPMV has been reported in low levels (0.10%) for the Nebraska isolate in soybean cultivar 'Williams' (64).

In 1955, Skotland (103) reported the first natural occurrence of BPMV in soybeans in eastern North Carolina. The disease has been reported previously affecting varieties of bean (Phaseolus vulgaris L.) in South Carolina (120). It has been found to infect soybeans in Arkansas (110), Illinois (69), Virginia (106), Kentucky (39), Mississippi (80), Kansas (97), Nebraska (64), and North Carolina (93).

BPMV symptoms on soybeans consist of a green to yellow mottle on newly expanding leaves (112). Symptoms are most obvious during periods of rapid growth and cool temperatures. In older plants and during high temperatures, symptoms often become masked and the disease is not easily recognized (112). In 1980, Schwenk and Nickell (97) reported necrosis of the terminal bud and a green stem symptom. BPMV was detected in the pith of soybean stems which remained green with petioles still attached after pod set. Stems which are green at maturity make harvest difficult.

In 1963, Ross (87) demonstrated in greenhouse experiments that the bean leaf beetle Cerotoma trifurcata (Forster), could transmit this virus for at least 2 days after acquisition feeding. In field experiments, Walters (111) reported transmission of BPMV to 'Black Valentine' bean and 'Dortchsoy 67' soybean by the bean leaf beetle. In 1970, Horn et al. (50) reported four chrysomelid beetles as vectors of BPMV. In 1971, Patel and Pitre (78) demonstrated that the stripped blister beetle Epicauta vittata (Fabricius) was a potential vector of BPMV. Percent transmission by these five beetle species is relatively low, ranging from 1 to 15%, as compared to that of the bean leaf beetle which is 63% (50, 78, 111).

The perennial legume Desmodium paniculatum (L.) D.C. is a natural host for BPMV (72). Walters and Lee (113) determined that 53% of the bean leaf beetles tested transmitted BPMV to soybeans from infected Desmodium plants. These tests indicate that Desmodium spp. may be an important reservoir of BPMV.

Disease incidence of BPMV in commercial soybean fields has been reported as high as 75 to 100% (50, 103, 111, 113). In field experiments soybean yield losses as high as 53% have been reported (49).

Ross (90) reported that plants in the V1 or V2 stage (33) were more susceptible and yielded less due to BPMV

infection than plants inoculated 4 and 5 weeks later. In 1970, Walters (112) found a decrease in yield loss from 26 to 15% by delaying inoculations with BPMV from the V2 stage to the pre-R1 stage. Windham and Ross (114) studied the phenotypic response of six soybean cultivars to BPMV infection and reported that yield reductions were correlated with leaf area and leaf rugosity. Plants inoculated with BPMV at the V2 stage exhibited more severe foliar symptoms, stunting and yield reduction than plants inoculated at the V9 growth stage. Windham and Ross (115) noted that symptoms on plants growing in the field were more noticeable on tall soybean lines (100-120 cm) than adjacent shorter lines (78-85 cm). Differences in plant height may indirectly affect symptom severity, since the taller plants were infected with BPMV earlier than the shorter plants. Ross (94) noted significant yield losses in both early-and late-planted soybeans when large populations of the bean leaf beetle were present. The growth stage of the plants when infected with BPMV was important. When vector activity and virus infection coincides with the seedling stage, maximum yield loss occurs.

BPMV is often found in soybean plants also infected with SMV (102). Ross (88) reported yield reductions of 80% when soybean plants were inoculated with both BPMV and SMV; whereas separate inoculations with SMV or BPMV caused losses of 41% and 13%, respectively. Similarly, Quiniones et al.

(84) noted that soybean yield was reduced 18% by infection with SMV, 10% by BPMV, and 66% by infection with both. The presence of BPMV can increase seed mottling and reduce seed size in SMV-infected plants; the extent of the effect of the two viruses may vary depending on the strain of SMV involved (84, 88). Ross (90) reported that inoculations in the V2 stage with BPMV and SMV, altered seed characters more than later inoculations. BPMV also reduced the amount of SMV seed transmission when BPMV inoculations preceded SMV inoculations. He concluded that the earlier the SMV infection occurred then, the greater was the percentage of seed transmission. Plants infected with BPMV prior to flowering may hinder SMV establishment in the embryo and cause reductions in SMV seed transmission (90).

Calvert and Ghabrial (20) reported a greater concentration of BPMV in soybean plants doubly infected with BPMV and SMV than plants infected by one virus regardless of the timing, sequence, or means of inoculation of the two viruses. Virus concentration of BPMV varied with leaf position on plants infected by a single virus and appeared to be related to the severity of the symptoms developed on individual leaves. Leaves with severe symptoms due to single virus infections had a higher virus titer than leaves with mild symptoms. Virus concentration in leaves of similar symptoms in doubly infected plants were higher than

any leaf from a single virus infection. Tu et al. (108) reported that a mixed infection of SMV and BPMV reduced nodules more than single infections.

Scott et al. (98) tested 169 commercial varieties and 123 G. max plant introductions and found no resistance to BPMV; however four other species within the Glycine genus had members that were immune to BPMV infection. They concluded that if resistance to BPMV is to be introduced into commercial soybean varieties, the possibility of interspecific crossing must be considered.

Bean yellow mosaic virus

Bean yellow mosaic virus has flexuous rod-shaped particles, 750 nm in length which contain single stranded RNA and is a member of the potyvirus group (14). It causes diseases worldwide in many species of Leguminosae and infects a number of non-legumes, especially Liliiflorae. BYMV is sap transmissible and is transmitted in the non-persistent manner by more than 20 species of aphids (14). A low percentage of seed transmission has been reported in pea, white sweet clover and lupine. Transmission in this manner has not been reported in soybeans (14, 79). BYMV can be distinguished from other closely related members (bean common mosaic virus, soybean mosaic virus) of the potyvirus group serologically and by its symptoms, host range, and type of inclusion bodies (14).

In 1934, Pierce (79) isolated BYMV from 'Red Valentine' common bean in a field in Wisconsin. In host range experiments conducted in the greenhouse, soybeans and other legumes were found to be susceptible to the virus. This was the first report of BYMV in soybeans. BYMV has since been reported naturally infecting soybeans in Illinois (24), Kentucky (39), Montana (1) and New York State (83).

The initial symptoms of BYMV on soybeans are similar to those produced by the soybean mosaic virus. A yellowish vein-clearing develops first in the minor veins of the trifoliate leaves. Newer leaves exhibit a characteristic yellow mottle along the major veins. As these leaves mature, rusty necrotic spots can develop in the yellowed areas (24).

In 1975, Provvidenti (83) reported incidence of BYMV in commercial soybean fields in New York State ranged from 5 to 25%. In field tests, BYMV-infected plants became moderately stunted with a reduction in seed size and seed number per pod. He reported that the soybean cultivars 'Corsoy', 'Cutler 71', 'Swift', and 'Williams' were resistant to BYMV. The effects of BYMV on the agronomic performance of soybeans has not been determined.

Cowpea chlorotic mottle virus

Cowpea chlorotic mottle virus is a member of the bromovirus group. The virus particles are isometric in shape, 25nm in diameter and contain single-stranded RNA which composes 24% of the particle weight. Three particles are present, making it's genome tripartite (5). CCMV mainly infects species within the Leguminosae, however there have been reports of infection in some members of Cucurbitaceae, Solanaceae, and Chenopodiaceae (5). Five naturally occurring strains have been reported (5). The type strain (CCMV-T) isolated from cowpea (Vigna unguiculata (L.) Walp.), the soybean strain (CCMV-S) and a strain isolated from Desmodium are serologically identical (5). The bean yellow stipple strain (BYSV) of CCMV, and the strain isolated from cowpeas in Arkansas, designated CCMV-A are serologically distinct (36). CCMV strains are transmitted at low levels by the bean leaf beetle, C. trifurcata, and the spotted cucumber beetle, Diabrotica undecimpunctata howardi Barber (48). This virus naturally infects cowpeas and soybeans in the Southeastern United States and has recently been reported in several legumes in Central America (5, 10).

A soybean strain of CCMV was first isolated by Kuhn in 1968 (61) from mottled soybeans at Experiment, GA. The soybean isolate, designated CCMV-S is serologically

identical to the strain previously isolated from cowpeas, but differs in virus production and symptom expression (61). Early infection of CCMV-S on soybean causes a mild mottle on the second and third trifoliolate leaves. Newer growth becomes severely mottled with distinct light and dark areas (61).

In 1968 and 1969, Harris and Kuhn (46) determined that with early infection of CCMV-S (100%), yield of 'Davis' soybeans was reduced 20-31% and plant maturity was delayed slightly. In chemical composition tests, protein content was increased and total oil content was decreased in CCMV-S infected seed. However, these changes were so small that there would be little effect on the commercial utilization of the infected seed (45). As in cowpea, there was no evidence of seed transmission of CCMV-S in 'Davis' soybeans in field and greenhouse tests (46).

The reservoir of CCMV infection for cowpea and soybeans has not been determined. In 1979, Demski and Chalkey (27) concluded from the results of a 5 year field study, that the percentage of natural CCMV-infections cowpea ranged from 1 to 18%, and natural infections in soybean did not exceed 1% in any test plot. Spotted cucumber beetles and bean leaf beetles were collected weekly to monitor vector populations and to study host feeding preferences. The average number of spotted cucumber beetles collected was the same for both cowpea and soybean; bean leaf beetles were present in both

crops but in low numbers. Spotted cucumber beetles preferred soybeans to cowpeas in 8 of 8 feeding tests. The beetle numbers did not correlate with the incidence of disease in the field. It appeared that virus movement from cowpea or soybean to other cowpea or soybean did not take place (27).

In 1971, Harris and Kuhn (46) found 18 of 26 soybean cultivars that exhibited a hypersensitive type of resistance to CCMV-S. Boerma et al. (13) established that this hypersensitivity was controlled by a single dominant gene, designated Rcv. Bijaisoradat and Kuhn (10) screened 533 soybean lines and reported new types of resistance based on virus concentration and symptom severity. Unlike the hypersensitive response to CCMV-S, these types of resistance are more complex and not well defined. Paguio et al. (77) tested six representative soybean genotypes against six known strains of CCMV to determine which genes were related to resistance. CCMV-S and the other five strains tested reacted similarly to each other with respect to virus accumulation in systemically infected leaves to the six different soybean genotypes, indicating that the resistance is stable to known strains. However, two new strains CCMV-D and CCMV-N overcame the hypersensitive type of resistance. Therefore, incorporation of more than one type of resistance in soybean cultivars is advisable (77).

Cucumber mosaic virus

Cucumber mosaic virus has icosahedral particles about 28 nm in diameter and is the type member of the cucumovirus group (34). Single-stranded RNA which consists of four molecular species, makes up 18% of the particle weight. CMV is found in temperate regions worldwide and has a very wide host range. It causes diseases of economic importance to many cucurbits and to many dicotyledonous and monocotyledonous crops and weed species. A number of strains of CMV have been reported which makes the virus often difficult to identify from symptoms alone (34). CMV is sap transmissible and is transmitted in the non-persistent manner by more than 60 aphid species. Seed transmission has been reported in 19 species, many of which are weed species. The persistence of CMV in weed seeds may play an important part in the dissemination of the virus (34).

In 1948, CMV was isolated from diseased pea plants in Wisconsin (42). A number of leguminous plants including soybeans were susceptible to this isolate in greenhouse tests. In 1958, the soybean stunt virus (SSV), a strain of cucumber mosaic virus, was isolated from soybean plants in Japan (44). The natural occurrence of this virus in soybeans has not been reported in the United States. CMV

symptoms on soybean consist of a mild leaf mottle and chlorotic rings on systemically infected leaves (102).

Peanut mottle virus

Peanut mottle virus is a member of the potyvirus group which is characterized by flexuous filamentous particles about 750 nm in length (11). The virus infects mostly species within the Leguminosae. PMV is sap transmissible, and is transmitted in the non-persistent manner by several aphid species. Seed transmission has been reported only in peanuts (11). Five serologically indistinguishable strains have been reported in commercial peanuts in the U.S. (74). The most prevalent strain of PMV causes a mild mottle and is designated M2. This isolate is reported in commercial soybeans (62). PMV naturally infects and causes economic loss to both peanuts and soybeans and is reported in the United States in all areas where commercial peanuts are grown (25, 60, 62). It occurs in peanuts in Venezuela, Japan, West Malaysia and India (29, 101). The disease in soybeans becomes predominant when peanuts are grown in close proximity (25).

PMV was originally isolated in 1965 from peanuts in the U.S. during an investigation of a ringspot disease (60). In host range experiments, 16 species of Leguminosae including G. max were found to be susceptible to the virus (60). The

first natural occurrence of PMV in soybeans was reported in Georgia in 1971 (62) and is the most prevalent soybean virus in that state (25). It has been reported infecting soybeans in Virginia and South Carolina (25), Australia and East Africa (7, 29).

The first symptoms of PMV on soybeans are small chlorotic areas on the first or second trifoliolate leaf. Chlorotic areas enlarge, forming dark green islands on the young leaves. Chlorotic patches and line patterns have been reported on the third and fourth trifoliolate leaves. Older leaves have a general mosaic, similar to symptoms caused by other viruses (29, 62).

In field tests, yields of PMV-infected soybeans were reduced by 5-28%. This suggests that PMV may cause significant yield losses when plants become infected early in the growing season. In greenhouse tests, PMV caused a significant reduction in plant height, root and shoot weight, and pod number. Protein content was increased, and total oil content decreased in seed from PMV-infected soybeans (29).

In field surveys from 1971 through 1973, PMV was recovered from soybeans in the southern area of Georgia where 95% of the peanuts are grown (29). No PMV infections were found in soybean plants sampled outside the peanut belt. In 1974, PMV was found in each of 117 peanut fields surveyed in Georgia. The source of primary inoculum

appeared to be infected seed (75, 76). These surveys indicate that the source of PMV for soybeans is probably infected peanut (25).

PMV is transmitted in the non-persistent manner by Aphis craccivora Koch, A. gossypii Glover, Hyperomyzus lactucae (L.), Myzus persicae (Sulzer) and Rhopalosiphum padi (L.) (11). Demski and Kuhn (28) reported 3-17% transmission of PMV from peanuts to soybeans by A. craccivora and M. persicae as compared to 20-54% transmission of PMV from peanuts to peanuts. They believe that there is a vector preference for peanuts, since PMV is spread faster and farther in peanut than soybean, with the same amount of inoculum present (29). The location and time of appearance of PMV-infected source plants indicates that aphids could be the vector in the field. However, low aphid populations have been reported during periods of virus spread, which suggests that the aphids transmitting the virus are either very efficient or another vector may be involved (25, 29).

Mild strains of PMV have also been isolated from the arrowleaf clover (Trifolium vesiculosm L.), subterranean clover (T. subterraneum L.), white lupine (Lupinus albus L.) and blue lupine (L. angustifolius L.), and the weed host, Desmodium canum (Gmel.) Schinz and Thellung) (26). These plants may be virus reservoirs during most periods of the

year. Vegetative stages of the forage legumes and and seeding dates of peanuts and soybeans often overlap, therefore a vegetative source of PMV may always be present. Currently, it is not known how important forage legume reservoirs are in the spread of the virus (26).

In 1975, Demski and Kuhn (28) determined 14 of 70 soybean cultivar and breeding lines resistant to PMV in field, greenhouse, and aphid transmission tests. One dominant gene for resistance has been identified in 'Arksoy', 'PI89784' and 'PI219789' (12, 100). A second dominant gene for resistance has been identified for soybean cultivar 'CNS' and a recessive gene in cultivar 'Peking' (19, 101).

Peanut stunt virus

Peanut stunt virus is a RNA-containing virus with isometric particles about 30 nm in diameter which makes up 16% of the particle weight (71). This virus is a member of the cucumovirus group. PSV causes diseases of economic importance in peanut, bean and tobacco and has been reported from the United States, Japan, France, Spain, the USSR, Hungary, Poland and Morocco (71, 121). PSV infects many species within the Chenopodiaceae, Compositae, Cucurbitaceae, Leguminosae and Solanaceae (71). Thirteen PSV isolates have been reported (121). PSV is sap transmissible and is transmitted in the non-persistent

manner by A. craccivora, A. spiraecola Patch and M. persicae (71). Seed transmission has been reported in peanuts at a rate of 0.1% or less, and in soybeans at a rate of 3-4%, but not through seeds of bean or cowpea (68, 71). PSV can be distinguished from cucumber mosaic virus, a closely related member of this group, serologically and by the systemic reactions it produces in cowpea, French bean and peanut (71).

In 1967, Zaumeyer and Goth (118) first reported the occurrence of PSV and found many legume species including soybeans susceptible in greenhouse studies. The first natural occurrence of PSV infecting soybeans in field plants in the United States was reported in Illinois by Milbrath and Tolin in 1973 (68). PSV has since been reported in soybeans from Kentucky (39) and Virginia (68) in the United States, and in some areas of Japan (68). Soybeans infected with PSV are stunted and less vigorous than healthy plants. Depending on the cultivar, symptoms of PSV consist of necrotic local lesions, vein clearing, and/or a general mosaic or mottle in systemically infected leaves (68, 102). The effect of PSV on yield of soybeans has not been determined; however Milbrath and Tolin (68) reported six soybean cultivars that appear to be resistant to the virus. Perennial forage legume crops are believed to be natural reservoirs for the virus (68).

Southern bean mosaic virus

Southern bean mosaic virus is a member of the sobemovirus group with isometric particles about 30 nm in diameter which sediment as a single component. The viral genome consists of one single stranded RNA molecule which makes up 21% of the particle weight. SBMV causes diseases of economic importance worldwide in bean, cowpea and urd bean (V. mungo L.) (107). Only species of Leguminosae with the exception of Gomphrena globosa L., are susceptible. Six strains of SBMV have been reported: bean (type) strain, cowpea strain, Ghana strain, severe bean mosaic strain or Mexican strain and a resistance breaking strain (107). All are serologically related but can be distinguished by immunodiffusion gels and limited host range tests. SBMV is sap transmissible and is transmitted in a circulative manner by leaf beetles, C. trifurcata and Epilachna varivestis Mulsant (107). Seed transmission of this virus has been reported in bean (1-5%), cowpea (5-40%) and soybean (2%) (54, 107).

SBMV was first isolated from bean in 1940 by Zaumyer and Harter (119). In host range experiments, SBMV infection was restricted to P. vulgaris with the exception of a Virginia variety of soybean. This was the first report of SBMV in soybeans (119). The natural occurrence of SBMV in soybeans in the United States has not been reported. This

virus has been reported affecting soybeans in the People's Republic of China and Japan (122).

Soybean mosaic virus

Soybean mosaic virus is a member of the potyvirus group, having flexuous particles 750 nm in length, which contain 6-7% single-stranded RNA by weight. This virus has a limited host range, infecting about 30 plant species within the Leguminosae and Chenopodium quinoa Willd. and C. album L. (15, 37). SMV is sap transmissible and is transmitted in the non-persistent manner by over 30 aphid species (55). A wide range of SMV isolates has been reported, many of which exhibit distinctively different properties in pathogenicity and virulence (21, 51, 89, 92).

In 1915, Clinton (23) described symptoms of SMV from soybeans at the Experiment Station in Mount Carmel, Connecticut. Five years later, Gardner and Kendrick (38) reported the disease in soybeans in Indiana and established the viral nature of the disease. Kendrick and Gardner (58) also reported 10-25% seed transmission of SMV and a reduction of seed yield ranging from 30 to 75%, which indicated that SMV has the potential to cause economic loss in soybeans. It is believed that SMV was introduced into the United States with the first soybeans brought from the Orient (102). SMV has since been reported in most major

soybean producing areas of the world. It is speculated that SMV was introduced into most areas via infected seed (102).

Symptoms of naturally infected soybeans, as described by Clinton (23), consist of a yellowish mottling of the leaves, accompanied by irregular wrinkling or puckering of the leaf tissue. Conover (24) observed similar symptoms in plants inoculated with SMV in greenhouse experiments and noted that infected leaves often become distorted, curving downwards at the sides and upwards at the tips. He also found that the symptomology of plants infected with SMV were most severe at 18.5 C and largely masked at 29.5 C (24). Diseased plants are often stunted with shortened petioles and internodes and pods produced on infected plants commonly do not bear seed (24, 58).

Seed transmission of SMV was first reported by Gardner and Kendrick (38) and is the primary source of inoculum in the field and persistence through seasons. The incidence of seed transmission of SMV in soybean ranges from 0 to over 60% depending on the cultivar (15, 16, 38, 41, 54, 81). Bowers and Goodman (16) reported that early infection with SMV prior to flowering resulted in more seed transmission than later infections. Goodman (41) investigated seed transmission among several tropical soybean lines infected with SMV. Entries previously identified as nontransmitters of SMV through seed were found to transmit the virus at very low levels (0.2%) (41). Since incidence of seed

transmission of SMV in improved cultivars is typically 5 to 20%, it seems practical to incorporate germplasm with lower rates of seed transmission into these cultivars and reduce the initial primary inoculum levels in the field (55).

Iizuka (54) demonstrated SMV to be pollen transmitted; however little has been reported on the effect of infection on timing of pollen production and its role in etiology.

Soybean seeds from SMV infected plants are often mottled (58). This condition, as described by Woodworth and Cole (116), is the formation of irregular patterns or streaking of black and brown pigments on yellow or green seeds. Ross (88) reported that seed transmission incidence was twice as high for mottled seeds as for nonmottled seeds. Others have reported soybean lines that have seed transmission but no mottling, or that show mottling but no or very low incidence of seed transmission (55, 81). Ross (91) reported that seeds from SMV-infected plants grown at 21 C during flowering and early pod set were heavily mottled; whereas those grown at a higher temperature of 32-45 C were only slightly mottled. In seed transmission tests, nonmottled and mottled seed from SMV infected plants equally transmitted the virus (91). Seed coat mottling, although often associated with virus infection, does not

always correlate with virus infection or seed transmission and can be influenced by environmental and genetic factors (55).

SMV is transmitted by many aphid species. Since aphids seldom colonize soybeans, researchers believe that transient aphids are responsible for the secondary spread of SMV (55). Schultz et al. (96) found soybeans to be efficient sources of the virus for aphid vectors since virus could be acquired from seedlings as little as five days after inoculation. Primary inoculum levels are important factors to be considered since virus movement in a field is largely governed by the number of infected plants present. Because different aphid species transmit SMV more efficiently than others and alight at different times during the growing season, timing, numbers and species composition are considered to be important factors attributing to spread of SMV (55).

In 1969, Ross (89) investigated the pathogenic variation of seven isolates of SMV and found that each isolated varied significantly in their symptom expression and their ability to infect various soybean cultivars. Cho and Goodman (21) screened 98 isolated of SMV from seeds in the USDA germplasm collection and classified seven virulence strains, based on the ability of these isolated to infect and cause symptoms in six SMV-resistant cultivars. They

also reported differences among SMV strains and in the susceptibility and symptom reactions of soybean cultivars to these strains. In order to incorporate SMV-resistance in soybeans, they concluded that breeders should evaluate segregating populations from crosses against a wide range of SMV isolates differing in virulence.

Sources of SMV resistance have been reported in soybean (21, 22). In 1979, Kiihl and Hartwig (59) reported resistance to SMV in 'PI96983' and 'Ogden' to be conditioned by a single dominant gene. Kwon and Oh (63) reported resistance conditioned by a single recessive allele in the Korean cultivar 'Kwanggyo'. Bowers and Goodman (17) reported twelve germplasm lines from maturity groups II and III that were previously ignored because of their high seed coat mottling as a sources of resistance to seed transmission of a severe isolated of SMV. Resistance to seed transmission could have a major impact on virus spread if it could be incorporated into soybean lines grown for seed production.

Tobacco ringspot virus

Tobacco ringspot virus is a member of the nepovirus group having isometric particles 28nm in diameter, with angular outlines (104). The genome consists of two single-stranded RNA molecules, both of which are encapsidated in separate particles. TRSV is sap transmissible, is world

wide in distribution and has an extremely wide host range. It causes severe damage to several agronomic crops such as soybean, tobacco, blueberry, and cucumber (104).

TRSV was first reported by R. W. Samson as reported by Allington (2), who found the disease in experimental plantings of soybeans in Indiana. In 1934, Pierce (79) observed the destructive nature of TRSV on soybeans in greenhouse experiments, but did not observe its occurrence in nature. Allington (2), in 1946 named the disease bud blight due to the characteristic curving of the terminal bud. Bud blight has been reported in Canada, Egypt, India, Turkey, and in the eastern region of the USSR (43). In North America the disease was common in some soybean producing areas of the U.S. with sporadic occurrences in the 1960's and 1970's (43). The disease is present in countries where soybeans have recently been introduced, probably via infected seed (43).

Of the many diseases caused by TRSV, bud blight of soybean is the most severe and causes the greatest economic loss (102). Allington (2) reported that plants infected at an early stage were more severely affected. In early infections young expanding leaves have a bronzed appearance, the growing point becomes necrotic and brittle, and the pith becomes reddish-brown in color. Plants infected at this stage seldom produce seed, but a high percentage infection

of this type is uncommon. Infection at or near blossom set causes the greatest economic losses as a result of reduced seed production (2). Young pods become darkly blotched, and often wither and drop within 10 days after infection (2). TRSV is also responsible for delaying nodulation, which negatively effects the efficiency of the nitrogen fixation process which can result in further yield reductions (73).

The epidemiology of this disease is not well understood. In greenhouse experiments, the grasshopper Melanoplus differentialis (Thomas) and five species of thrips have been reported to transmit TRSV inefficiently (9, 31, 67). No efficient insect vector of TRSV has been identified. However many still believe that an aerial vector is responsible for disease spread in the field because the most devastating effects of the disease occur around the margins of the fields first and then inward as the season progresses (2, 9, 47, 67). Weed hosts adjacent to soybean fields may be virus reservoirs for possible aerial vectors (4, 109). Tuite (109) tested for TRSV in plants from an adjoining soybean field where bud blight was epidemic and found Ambrosia artemisifolia L. (ragweed), Daucus carota L. (wild carrot), Erigeron strigosus Muhl. (fleabane), Rumex acetosella L. (red sorrel), Taraxacum officinale Weber. (common dandelion), Trifolium repens L. (white clover) to be

symptomless hosts. Those species with symptoms were Melilotus spp. (sweet clover), and Trifolium pratense L. (red clover) (109).

The nematode, Xiphinema americanum Cobb, is a vector of TRSV, but its efficiency in transmission to soybeans is low (9, 35, 65). Its importance as a vector has been questioned many times, since virus transmitted in this manner remains primarily in soybean roots and rarely moves to the foliage (9).

In 1954, Desjardens et al. (30) reported 78% seed transmission of TRSV in 'Lincoln' soybeans when inoculated artificially in the greenhouse. Athow and Bancroft (4) reported 100% seed transmission of TRSV from naturally infected soybean plants in field tests. They concluded that the efficiency of seed transmission appeared to be dependent upon the time of infection of the plants. Yang and Hamilton (117) found seed transmission was dependent on infection of the megagametophyte. Plants infected with TRSV produced 0-2,000 pollen grains per flower and healthy plants produced 4,000-6,000. There was a decrease in pollination of infected plants, because pollen grains produced shorter germ tubes than those from healthy plants. They concluded that poor germination and slow germ tube elongation suggest that pollen does not play a significant roll in seed transmission

but it may be an important factor in yield reduction. Seed transmission is believed to be the primary source of natural dissemination of the virus.

Tomato spotted wilt virus

Tomato spotted wilt virus has membrane-bound RNA particles 70-90 nm in diameter which consists of 20% lipid, 7% carbohydrate and 5% RNA (53). TSWV causes a range of chlorotic, necrotic, stunting and enation symptoms. It can infect at least 166 plant species in 34 families, including 7 monocotyledonous families, and is common in temperate and subtropical regions of the world (53). TSWV is physically and chemically one of the most unstable plant viruses, but is readily sap transmissible when neutral buffers containing reducing agents are used. This virus is acquired by the larval stage of thrips Thrips tabaci Lindeman, Frankliniella schultzei (Trybom), F. occidentalis (Pergande) and F. fusca (Hinds) but is transmitted only by the adult stage. Seed transmission has been reported in Cineraria and tomato (53).

The natural occurrence of TSWV in soybeans has not been reported. In host range studies, a local reaction of TSWV in soybeans appears as necrotic flecks with halos and the leaves turn orange in color (102).

White clover mosaic virus

White clover mosaic virus is a single component RNA-containing virus, with 6% RNA by weight and elongated particles 480 nm x 13 nm (8). It is a member of the potato x virus group, and infects mainly members of the Leguminosae. WCMV is worldwide in distribution and causes mosaic, vein-clearing and mottle diseases of various clover and pea species. Three strains of WCMV have been reported and can be distinguished on the basis of symptom expression in various hosts. WCMV is sap transmissible, but normally not by arthropod vectors. Seed transmission has been reported in T. pratense (8).

WCMV was first reported in soybeans during a host range study of a virus isolated from white clover (T. repens) in Indiana (6). Symptoms on soybeans consist of small local necrotic lesions and systemically infected leaves exhibit vein clearing and general chlorosis (102). The natural occurrence of this virus in soybeans has not been determined.

III. MATERIALS AND METHODS

Virus propagation

In order to have positive controls for use in Protein A sandwich enzyme linked immunosorbent assay (PAS-ELISA) (32), known virus isolates of AMV, BPMV, BYMV, CCMV, CMV, PMV, PSV, SBMV, SMV, TRSV, TSWV and WCMV respective host plants were inoculated and maintained in a greenhouse in 4 inch clay pots in a sterile soil mixture of 1 part Promix (Premier Brands Inc., New Rochelle, N.Y.) to 1 part sand. Inoculum was prepared by grinding 1.0-1.5 g of infected tissue in 1.0 ml 0.03M sodium phosphate buffer, pH 7.2, containing 0.02M 2-mercaptoethanol with a mortar and pestle. The plants were dusted with 600 mesh carborundum and the virus suspension was rubbed onto healthy leaves with a gauze pad.

Antisera

The source of antisera to the viruses used in this study are listed in Table 1. Initially, no satisfactory antisera were available to either BPMV or SMV and attempts were made to produce antisera to these viruses.

Table 1. Virus isolates, source, antisera and propagative hosts used in this study.

Virus isolates	Virus source	Antisera source	Propagative host
AMV	Barnett	Barnett	<u>Glycine max</u> (L.) Merr. cv. 'Peking'
BPMV	Scott	Sherwood	<u>Glycine max</u> (L.) Merr. cv. 'Ransom'
BYMV	Scott	Reddick	<u>Glycine max</u> (L.) Merr. cv. 'Lee'
CCMV	Scott	Barnett	<u>Glycine max</u> (L.) Merr. cv. 'Davis'
CMV-S	Barnett	Barnett	<u>Nicotiana tobacum</u> L. cv. 'B21'
PMV	Sherwood	Sherwood	<u>Glycine max</u> (L.) Merr. cv. 'Ransom'
PSV-E	Barnett	Barnett	<u>Phaseolus vulgaris</u> L. cv. 'Bountiful'
SBMV	Scott	Scott	<u>Phaseolus vulgaris</u> L. cv. 'Cherokee Wax'
SMV	Hill	Hill	<u>Glycine max</u> (L.) Merr. cv. 'Essex'
SMV-G1	Tolin	Kennedy	<u>Glycine max</u> (L.) Merr. cv. 'Essex'
TRSV	Reddick	Reddick	<u>Glycine max</u> (L.) Merr. cv. 'Young'
TSWV	Reddick	Sherwood	<u>Nicotiana tobacum</u> L. cv. 'B21'
WCMV	Barnett	Barnett	<u>Phaseolus vulgaris</u> L. cv. 'Tendergreen'

Note: AMV = alfalfa mosaic virus; BPMV = bean pod mottle virus; BYMV = bean yellow mosaic virus; CCMV = cowpea chlorotic mottle virus; CMV = cucumber mosaic virus; PMV = peanut mottle virus; PSV = peanut stunt virus; SBMV = southern bean mosaic virus; SMV = soybean mosaic virus; TRSV = tobacco ringspot virus; TSWV = tomato spotted wilt virus; WCMV = white clover mosaic virus.

Virus purifications

Bean pod mottle virus

BPMV was maintained in G. max 'Ransom.' Infected leaf tissue was ground in a 0.03M sodium phosphate buffer, pH 7 containing 0.02M 2-mercaptoethanol (1-1.5:1, w/v) and the first true leaves of 7 to 10 day-old soybean seedlings were inoculated. Systemically infected leaves were harvested 17 to 20 days after inoculation. BPMV was purified using a method developed by Steere (105) with some modifications.

One-hundred to 200 grams of infected leaf tissue were homogenized in a Waring blender with 0.03M potassium phosphate, pH 7.0, containing 1% 2-mercaptoethanol; buffer volume was 1.5x tissue weight. One volume of cold (4 C) chloroform and n-butanol (1:1, v/v) was then added for every 2 volumes of tissue homogenate and blended for 30 sec. This mixture was separated into three layers by low speed centrifugation for 20 min at 8,000xg. The upper straw-colored aqueous layer containing the virions was saved, filtered through glass wool and placed in a separatory funnel and maintained overnight at 25 C. The aqueous phase was clarified further by low speed centrifugation for 20 min at 10,500xg. The supernatant was collected and centrifuged for 4 1/2 h at 86,300xg to produce the virion pellets. The supernatants were then discarded and the virion pellets were

resuspended in 0.5-1.0 ml 0.03M potassium phosphate, pH 7.0, and placed on a mechanical shaker overnight at 5 C. The resuspended pellets were then layered on 10-40% continuous sucrose gradients (0.5 ml of virion suspension per gradient) and centrifuged for 3 h at 25,000xg. To evaluate and collect virion zones, the gradients were placed on an ISCO Density Gradient Fractionator. The virion peak was collected and concentrated by centrifugation for 3 h at 265,800xg. The pellet was resuspended in 1.0 ml 0.03M potassium phosphate, pH 7.0 and virion concentration estimated spectrophotometrically using 8.7 as the extinction coefficient.

Soybean mosaic virus

The SMV-G1 isolate was maintained in G. max 'Essex.' SMV infected leaf tissue was prepared following the method described earlier, and the first true leaves of 7 to 10 day-old soybean seedlings were inoculated. Systemically infected leaves were harvested 17 to 20 days after inoculation. SMV was purified following a method developed by Jones (57) with some minor modifications (85).

Two-hundred to 300 grams of infected leaf tissue were homogenized in a Waring blender with 0.5M potassium phosphate, pH 7.0, + 1.0M urea + 0.5% thioglycolic acid + 0.01M sodium diethyldithiocarbamate in a 1:2.5, w/v. Cold chloroform (4 C) was added to the homogenate (1.0 ml per

gram tissue) and emulsified by blending at high speed for 1 min. The emulsion was separated by low speed centrifugation for 15 min at 8,000xg. The straw-colored, aqueous phase was saved by filtering through glass wool. To precipitate the virions, 4% polyethylene glycol (PEG 8,000) (w/v) and 0.25M sodium chloride were added to the aqueous phase. The mixture was stirred for 1 h at 4 C and the precipitate was collected by low speed centrifugation for 20 min at 10,500xg. The supernatant was discarded and the pellets were resuspended in 0.5M potassium phosphate, pH 7.0, + 1.0M urea (0.1 volume of the initial aqueous phase volume) for 1-3 h on a mechanical shaker at 4 C. The partially purified virions were treated with 1% triton X-100 (v/v) and stirred for 1 h at 5 C. Following a low speed centrifugation for 10 min at 4100xg, the supernatant was collected and subjected to a second PEG/NaCl precipitation as above. Pellets were resuspended in 4.0 ml 0.5M potassium phosphate, pH 7.0, + 1.0M urea and 0.8 grams cesium sulfate was added and then layered onto a cushion of 0.8 ml of 53% cesium sulfate (0.79 grams of cesium sulfate + 0.705 ml 0.5M potassium phosphate + 1.0M urea). An equilibrium cesium sulfate gradient was formed by centrifugation for 16-18 h at 86,300xg. The virion band was removed and diluted with 2 volumes of 0.5M potassium phosphate + 1.0M urea, and was centrifuged for 90 min at 265,800xg. The resulting pellet was resuspended in

1.0 ml 0.03M tris buffer. Virion concentration was estimated spectrophotometrically using 2.4 as the extinction coefficient.

Antisera production

Purified virions were prepared for injection by mixing with Freund's incomplete adjuvant (Sigma, St. Louis, MO) (1 ml of antigen + 1 ml of adjuvant). Rabbits were injected subcutaneously and intramuscularly at weekly intervals for three weeks, followed by one booster injection two weeks later. The rabbits were bled at weekly intervals starting one week after the booster injection. The blood was collected and stored overnight at 5 C. The serum was separated from the red blood cells by two cycles of centrifugation for 10 min at 4100xg. The serum was then collected and mixed with an equal volume of glycerol + 1% sodium azide and stored at -20 C.

Commercial soybean virus survey

In 1987 and 1988, a survey was conducted in the State of Tennessee to detect the natural occurrence and distribution of twelve viruses infecting commercial soybeans. Four soybean fields in each of nineteen counties representing the major production areas of East, Middle and West Tennessee were surveyed for virus infection.

Six leaf samples were taken from each of the four soybean fields per county. Five of these samples were leaves with virus symptoms such as mosaic, mottle or leaf distortion. A sample was also taken from a symptomless plant to use as a healthy comparison. Each sample consisted of three leaflets (one trifoliolate). All samples were placed in a zip-lock bag with a moistened paper towel, transported on ice, and stored at -20 C for further evaluation.

Soybean cultivar trials survey

In 1987, soybean cultivar trials at the Milan, Knoxville and Tobacco Experiment Stations were scouted for virus infection.

Plants with virus-like symptoms were counted and percent incidence calculated on the basis of row size and number of seeds planted. Up to fifteen leaf samples with virus-like symptoms were collected per cultivar. Cultivars without symptoms were not sampled. All samples were placed in a zip-lock bag with a moistened paper towel and transported on ice, and stored at -20 C.

Yield comparison test

An experiment was conducted to determine the effects of single and double infections of BPMV and SMV on the seed yield loss of four soybean cultivars commonly grown in Tennessee. The cultivars that were used are 'Essex', 'Forrest', 'TN 5-85' and 'York'. Cultivars Essex and Forrest are susceptible to both viruses. York is resistant to SMV. Cultivar TN 5-85 and York reactions to BPMV were unknown (114).

Experiments were planted in an incomplete block design with three replications at the Milan Experiment Station in Milan, TN and the Knoxville Plant Sciences Farm Laboratory in Knoxville, TN on May 17, 1988 and May 12, 1988, respectively. At Milan, individual plots consisted of 4 rows spaced 40 inches apart, 30 feet in length and bordered by one row of okra. In Knoxville, individual plots consisted of 4 rows spaced 36 inches apart, 20 feet in length and bordered by one row of okra. Border rows of okra were planted to try to eliminate additional virus spread between treatments. The incomplete block design used was generated by a computer program developed by W. L. Sanders and J. F. Schneider of the Knoxville Experiment Station. The treatments were uninoculated plants, plants inoculated with BPMV, or SMV, or BPMV and SMV. Inocula used for field tests were obtained by macerating young, symptomatic, BPMV-

infected 'Ransom' soybean leaves or SMV-infected 'Essex' soybean leaves 1:10 (w/v) in 0.03 M phosphate buffer, pH 7.2, containing 600-mesh carborundum. The macerates were mixed 1:1 for plants inoculated with both BPMV and SMV. Inoculations were performed by inoculating every fifth plant with an artist airbrush (60 psi of CO₂) (82) at the Milan and Knoxville locations on June 8, 1988 and June 13, 1988, respectively. On July 17, 1988 at Milan and July 25, 1988 at Knoxville, fifteen leaf samples were randomly collected and evaluated for BPMV and SMV infection by the PAS-ELISA technique. The two center rows of plots were trimmed to 30 feet at Milan and 16 feet at Knoxville before harvest. Cleaned seed were weighed and moisture levels calculated for each plot. Seed weights were adjusted to 13% moisture content before yield was calculated. Data from both locations were combined and analyzed with the general linear models procedure (PROC GLM) of the Statistical Analysis System (SAS) by using an incomplete block design without the recovery from interblock information (95).

PAS-ELISA

Soybean leaf samples were evaluated for virus presence by PAS-ELISA developed by Edwards and Cooper (32). Twelve polystyrene microtitre plates (Dynatech, LTD, McLean, VA) were rinsed with distilled water. Protein A in 0.05M sodium carbonate buffer, pH 9.6 (1 mg/ml), was added to each plate

(200 μ l per well) and incubated at 30 C for 2 h. The unbound protein was then removed by rinsing four times with phosphate-buffered saline 0.02M phosphate, 0.15M NaCl, and 3mM KCl, pH 7.3 (PBS) + 0.05% tween 20 (PBS-tween) following each step. Antisera for each virus (AMV, BPMV, BYMV, CCMV, CMV, PMV, PSV, SBMV, SMV, TRSV, TSWV and WCMV) was added at a 1:1000 dilution in PBS-tween to each well of its corresponding plate and incubated for 2 h at 30 C. Sap was then expressed from the thawed soybean leaves by a roller-type leaf squeezer and diluted 1:5 with PBS-tween. Forty-six samples, replicated twice, were placed in the wells of all twelve plates and stored overnight at 5 C. Known positive (infected) and negative (uninfected) samples were also added as controls for each ELISA plate. A second layer of each respective antisera was added as above. Protein A alkaline phosphatase was diluted 1:1000 in PBS-tween and added to each of the wells and incubated for 2 h at 30 C. In the final step, substrate (p-nitrophenylphosphate, 1 mg/ml) in 10% diethylalamine was added at room temperature and light absorbance values were recorded with a Dynatech Minireader II. Wells containing PBS-tween (negative controls) instead of leaf sap were used to calibrate the Dynatech Minireader II. Positive and negative thresholds were determined for each ELISA plate by multiplying three times the mean absorbance value of the healthy controls. If

three times the mean healthy control value was less than 0.10, the positive-negative threshold was 0.10.

IV. RESULTS AND DISCUSSION

Commercial soybean virus survey

In the 1987 survey, 278 samples were collected and 136 tested positive in twelve of the nineteen counties surveyed for one or more of the following viruses: AMV, BPMV, BYMV, CCMV, CMV, PSV, SBMV, SMV, TRSV, and TSWV (Table 2). Neither PMV nor WCMV were detected in any county. BPMV was the most prevalent virus detected and was found throughout the major soybean producing counties of West Tennessee and Robertson county in Middle Tennessee. Seventy-five percent of the samples collected that tested positive for virus, tested positive for BPMV (Table 2). All other single virus infections were less than 5% of the total positive samples. Mixed infections occurred in five counties and contributed to 16% of the positive samples. The largest combination of viruses detected in a sample was AMV/CCMV/CMV/PSV. In 15% of the mixed infections, BPMV was one of the viruses. The most prevalent co-infected virus combination was BPMV/SBMV and BPMV/PSV detected in 3 and 2 counties, respectively.

In 1988, 376 samples were collected and 121 tested positive for virus infection. AMV, BPMV, CCMV, CMV, PSV, SBMV and TRSV were detected in seventeen of the nineteen counties surveyed (Table 3). PMV, SMV, TSWV, and WCMV were not detected in any county. Of the eight viruses found, BYMV was most prevalent in that it was detected in 33% of

Table 2. Frequency distribution of the viruses detected in the 1987 soybean virus survey.

County	AMV	BPMV	BYMV	CCMV	CMV	PSV	SBMV	SMV	TRSV	TSWV	AMV/CCMV/ CMV/PSV	BPMV/BYMV	BPMV/CMV	BPMV/PSV	BPMV/SBMV	BPMV/TRSV	BPMV/TSWV	no. positive samples	no. samples tested
<u>East Tennessee</u>																			
Blount																		0	8
Monroe				1				1			1							3	15
Fentress			3															3	21
<u>Middle Tennessee</u>																			
Bedford																		0	11
Giles		1																1	9
Hickman																		0	0 ^a
Humphreys																		0	0 ^a
Lincoln																		0	0 ^a
Maury					1													1	6
Robertson		9	1															10	16
Rutherford				1		2												3	16
<u>West Tennessee</u>																			
Dyer		10											1	4	1			16	25
Gibson		12							4									16	22
Haywood		4																4	10
Lake		17													2	2	1	22	24
Lauderdale		19							1									20	24
Madison		8																8	23
Obion		14							1					3	2			20	24
Tipton		8										1						9	24
Total	0	102	4	2	1	2	0	1	5	1	1	1	1	7	5	2	1	136	278
Infection % ^b	0	75	3	2	1	2	0	1	4	1	1	1	1	6	4	2	1		

Note: AMV = alfalfa mosaic virus; BPMV = bean pod mottle virus; BYMV = bean yellow mosaic virus; CCMV = cowpea chlorotic mottle virus; CMV = cucumber mosaic virus; PSV = peanut stunt virus; SBMV = southern bean mosaic virus; SMV = soybean mosaic virus; TRSV = tobacco ringspot virus; TSWV = tomato spotted wilt virus.

^a No diseased soybeans were observed or collected from four fields in this county.

^b Percent infection of soybeans with individual viruses or multiple viruses was calculated by dividing the number of the respective positive virus samples by the total number of all positive samples and then rounding to the nearest whole number.

Table 3. Frequency distribution of the viruses detected in the 1988 soybean virus survey.

County	AMV	BPMV	BYMV	CCMV	CMV	PSV	SBMV	TRSV	AMV/CMV	AMV/BPMV/	BYMV/CMV/	SBMV/TRSV	BPMV/CMV	BPMV/BYMV/	CMV	BPMV/BYMV/	CMV/SBMV	BYMV/CMV	BYMV/SBMV	BYMV/CMV/	SBMV	CMV/PSV	no. positive samples	no. samples tested
<u>East Tennessee</u>																								
Blount	5								1														6	12
Monroe			3																				3	30
Fentress			17					1															18	24
<u>Middle Tennessee</u>																								
Bedford			4		3			1		1	4	1	1			3	1						19	24
Giles	1			1		1		3															6	24
Hickman			3		1											1	2						7	12
Humphreys					1		3													1			5	24 ^a
Lincoln																							0	0 ^a
Maury																					1		1	12
Robertson		9	5																				14	17
Rutherford			3					1															4	12
<u>West Tennessee</u>																								
Dyer		6	2					2															10	23
Gibson		1	3																				4	24
Haywood		3						2															5	24
Lake		10						2															12	24
Lauderdale								1															1	23
Madison		3																					3	24
Obion																							0	19
Tipton		3																					3	24
Total	6	35	40	1	5	1	3	13	1	1	4	1	1	1	1	5	2	1					121	376
Infection % ^b	5	30	33	1	5	1	3	11	1	1	4	1	1	1	1	5	2	1						

Note: AMV = alfalfa mosaic virus; BPMV = bean pod mottle virus; BYMV = bean yellow mosaic virus; CCMV = cowpea chlorotic mottle virus; CMV = cucumber mosaic virus; PSV = peanut stunt virus; SBMV = southern bean mosaic virus; TRSV = tobacco ringspot virus.

^a No diseased soybeans were observed or collected from four fields in this county.

^b Percent infection of soybeans with individual viruses or multiple viruses was calculated by dividing the number of the respective positive virus samples by the total number of all positive samples and then rounding to the nearest whole number.

the samples that tested positive for virus, followed by BPMV (30%) and TRSV (11%) (Table 3). The majority of the BYMV samples were collected in Middle and East Tennessee, with the exception of Dyer and Gibson county in West Tennessee. BPMV was detected in seven of the eight counties tested in West Tennessee and Robertson county in Middle Tennessee. TRSV was found in eight of the nineteen counties surveyed, the majority which were in West Tennessee. All other single virus infections were either 5% or less of the total positive samples. Mixed infections were found in five counties and in 17% of the total positive samples. Nine different virus combinations were detected. The largest virus combination found in a leaf sample was AMV/PMV/BYMV/CMV/SBMV/TRSV from Bedford county. BYMV was detected in 14% and BPMV in 7% of the mixed virus infections. The most prevalent virus combinations were BYMV/SBMV (2 counties) and BPMV/CMV (1 county).

BPMV has been isolated from soybeans in Arkansas (110), Illinois (69), Virginia (106), Kentucky (39), Mississippi (80), Kansas (97), Nebraska (64), and North Carolina (93), however this is the first report of BPMV in Tennessee. The source of initial infection is most likely infected soybean seed (64), however wild perennial hosts such as D. paniculatum could also be contributing to initial infection via the beetle vector C. trifurcata (113).

BYMV has been isolated from soybeans in Illinois (24), Montana (1), New York State (83), and Kentucky (40). In 1983, McLaughlin (66) detected BYMV in the Middle Tennessee area from the following forage legumes: arrowleaf clover (T. vesiculosum), alsike clover (T. hybridum), red clover (T. pratense), and subterranean clover (T. subterraneum). BYMV has also been detected in half-runner beans in Middle Tennessee (Reddick personnel communication 1988). Since BYMV is not seed transmitted in soybeans (24), the presence of reservoir hosts for this virus in Tennessee indicates the potential for aphid vectors to spread this pathogen to soybeans in the field. This is the first report of BYMV infecting soybeans in Tennessee.

TRSV was the third most prevalent of the viruses detected in this survey. This virus has a wide host range, infecting such crops as tobacco and cucumber, and is seed transmitted in soybeans. Since an aerial vector for TRSV is not known at this time, the initial source for infection in soybeans is most likely infected seed. This is the first report of TRSV infecting soybeans in Tennessee.

AMV, CCMV, CMV, PSV, SBMV, SMV and TSWV were detected in low frequencies (Tables 2 and 3) throughout the two year survey. AMV, an aphid transmitted virus, was detected in Giles and Blount county in 1988. This virus has been found in association with soybeans planted adjacent to alfalfa

fields (3); however Millsap et al (70) was unable to detect AMV in forage legumes in Tennessee, but detected its presence in 'Burley' tobacco. Therefore, tobacco in close proximity to soybeans would be an excellent reservoir host for this virus and its aphid vector.

CCMV, a beetle transmitted virus, was detected in Giles, Monroe and Rutherford counties (Tables 2 and 3). This virus has been isolated from soybeans and cowpeas from the southern United States but this is the first report of CCMV in Tennessee. The primary inoculum source of CCMV for cowpea and soybean has not been determined.

CMV was detected in four counties in Middle Tennessee (Tables 2 and 3). CMV persists and is seed transmitted in many weed species, which may help in the dissemination of the virus to soybeans through aphid vectors. This is the first report of CMV infecting soybeans in Tennessee.

PSV was detected in five counties in Tennessee (Tables 2 and 3). PSV, an aphid transmitted virus, has been isolated in soybeans from Kentucky (39) and Virginia (68). PSV is one of the more prevalent viruses infecting half-runner beans in Middle Tennessee, and is also present in forage legumes across the state (Reddick personnel communication 1988; 66). Perennial forage legumes are believed to be the natural reservoirs for PSV in Virginia (68), and probably contribute to the occurrences in soybeans in Tennessee. Soybeans and half-runner beans are often

grown at the same time in Middle Tennessee which may account for the occurrence of PSV in soybeans in those areas. This is the first report of PSV infecting soybeans in Tennessee.

SBMV, a beetle transmitted virus, causes diseases of economic importance to common bean and cowpea. The importance of this disease in soybean has not been determined, but should be monitored when these crops are grown in the same vicinity of each other. This is the first report of SBMV in any crop in Tennessee.

It is surprising that SMV was only detected in one sample from Monroe county. SMV, an aphid transmitted and seed borne virus, has been reported throughout the major soybean growing areas of the southern United States (55). This is the first report of SMV in Tennessee. It is possible that an isolate of SMV could remain undetected if it was serologically different than the isolate used in the study, but not probable since SMV was detected several times in the soybean cultivar trials (see next section). Because SMV does not occur naturally in species other than G. max, the primary source of inoculum in the field spread is infected seed (55, 58). This virus, contrary to this study, often occurs simultaneously with BPMV in plants and as a result causes significant economic losses (88).

TSWV, a thrip transmitted virus, was detected in Lake and Lauderdale counties in West Tennessee in 1987 (Table 2).

This may be the first report of its natural occurrence in soybeans and is of interest since this virus has just recently been detected in tobacco, tomato and pepper in Tennessee (86). Importance of this virus in soybeans has not been determined.

PMV and WCMV were not detected in any county during the two years surveyed. Since PMV in soybeans only becomes predominant when peanuts and soybeans are grown in close proximity, it is not surprising that this disease was not detected in soybeans in Tennessee because peanuts are rarely planted in Tennessee. The natural occurrence of WCMV has not been determined in soybeans. This virus has been reported infecting forage legumes from Kentucky, North Carolina, Georgia, Alabama and Mississippi; however, McLaughlin (66) was unable to detect WCMV in forage legumes in Tennessee. It is possible that this virus is not present in the state at this time.

Soybean cultivar trials survey

At the Milan Experiment Station in West Tennessee, 43 of 157 soybean leaf samples tested positive for virus infection (Table 4). AMV, BPMV, BYMV, CCMV, CMV, PSV, SBMV, TRSV and TSWV were detected. Neither PMV nor WCMV were detected in any of the cultivars tested. SMV was the most prevalent virus. It was detected in 10 of the 28 cultivars tested, and occurred in 49% of the positive samples. BPMV

Table 4. Occurrence and percent incidence of viruses in the soybean cultivar trials at the Milan Experiment Station in 1987.

Cultivar	ELISA Virus Id	No. tested positive	No. samples tested	% virus incidence ^a
Maturity Group IV Cultivars				
Coker 393	BPMV	1	1	0.1 ^b
RA 452	AMV/BPMV/ BYMV/CMV	1	2	0.2
Stevens	BPMV	1	1	0.1
Maturity Group V Cultivars				
AgriTec AT550	AMV	1	7	0.7 ^b
AgriTec AT575	AMV	1	7	0.7
	BPMV/PSV	1		
	SMV	2		
Asgrow A5980	BPMV	1	11	1.1
	SMV	6		
Bedford	BPMV	1	6	0.6
Capehart 5646	SMV/TSWV	1	2	0.2
Coker 425	SBMV	1	4	0.4
Deltapine 105	SBMV	1	15	3.8
Deltapine 675	BYMV	1	5	0.5
Epps	BPMV	4	6	0.6
Essex	CCMV/SBMV	1	12	1.2
FFR 560	SMV	4	9	0.9
FFR 565	AMV/SMV	1	15	5.8
	SMV	1		
Forrest	SMV	1	1	0.1
Hartz 5171	TRSV	1	4	0.4
Hartz 5252	SMV	1	7	0.7
Hartz 5370	SMV	1	7	0.7
N. K. S53-34	SMV	2	5	0.5
Pioneer 9531	BPMV/SMV	1	7	0.7
	SMV	2		
Pioneer 9541	BPMV	1	5	0.5
TN-83-26	BYMV	1	15	2.3

Table 4. (continued)

Cultivar	ELISA Virus Id	No. tested positive	No. samples tested	% virus incidence ^a
Maturity Groups VI & VII Cultivars				
HSC Baldwin	BPMV	1	2	0.2 ^b
Totals		43	157	

Note: AMV = alfalfa mosaic virus; BPMV = bean pod mottle virus; BYMV = bean yellow mosaic virus; CCMV = cowpea chlorotic mottle virus; CMV = cucumber mosaic virus; PSV = peanut stunt virus; SBMV = southern bean mosaic virus; SMV = soybean mosaic virus; TRSV = tobacco ringspot virus; TSWV = tomato spotted wilt virus.

^aPercent incidence was calculated by dividing the number of plants with symptoms observed per plot by the number of seed planted per plot.

^bTwo repetitions were sampled, 480 seeds planted/rep.

was found in 10 of the 28 cultivars tested and contributed to 30% of the positive samples. Mixed infections were detected in 6 of the cultivars tested and contributed to 14% of the positive samples. Disease incidence ranged from 0.1 to 5.8%.

At the Knoxville Plant Sciences Field Laboratory 168 samples were collected and 56 tested positive for virus infection (Table 5). BPMV, BYMV and SMV were the only viruses detected and SMV was the most prevalent virus detected in that it was detected in 19 of the 24 cultivars and contributed to 79% of the positive samples. BYMV was found in 14 of the 24 cultivars (34%) and BPMV in 1 cultivar (1.8%). The only mixed virus infection detected was BYMV/SMV and occurred in 13% of the positive samples. Disease incidence ranged from 0.1 to 9% with the exception of 'Shenandoah' (53%) which only had seventeen soybean plants in the plot.

Only 2 of the 39 cultivars were collected from the Tobacco Experiment Station (Table 6). AMV was the only virus detected. Disease incidence as estimated by visual symptoms ranged from 0-9%. This agrees with the observations made during the survey of commercial fields. The ten viruses identified in the commercial survey were also detected from the survey of cultivars at Milan. AMV, BYMV, CCMV, CMV and TSWV could have been brought into the plots by their respective insect vectors from other infected

Table 5. Occurrence and percent incidence of viruses in the soybean cultivar trials at the Knoxville Plant Sciences Field Laboratory in 1987.

Cultivars	ELISA Virus Id	No. tested positive	No. samples tested	% virus incidence ^a
Maturity Group IV Cultivars				
Coker RA 451	BYMV	1	6	0.3 ^b
	SMV	4		
Dekalb Pfizer	SMV	3	4	0.2
Pennyryle	BYMV/SMV	1	2	0.2
	SMV	1		
Pioneer 9442	SMV	2	3	0.2
Stevens	BYMV/SMV	1	3	0.2
	SMV	2		
TN-4-86	SMV	2	2	0.1
Maturity Group V Cultivars				
Bay	BYMV/SMV	2	4	0.4 ^c
	SMV	2		
Bedford	BYMV	1	15	1.6
Coker 355	SMV	3	3	0.3
Coker Co82-372	BYMV	1	15	8.9
Deltapine 415	SMV	2	2	0.2
Deltapine 675	BYMV/SMV	1	3	0.3
	SMV	1		
Epps	BPMV	1	15	6.8
Essex	SMV	3	8	0.8
FFR 560	BYMV	1	9	0.9
FFR 565	SMV	2	15	4.1
Hartz 5252	BYMV/SMV	1	2	0.2
	SMV	1		
Hartz 5370	BYMV/SMV	1	6	0.6
M82-572403	SMV	2	2	0.2
N. K. S59-19	BYMV	1	8	0.8
	SMV	2		
Shenandoah	SMV	2	9	52.9
TN-5-85	BYMV/SMV	1	2	0.2

Table 5 (continued)

Cultivars	ELISA Virus Id	No. tested positive	No. samples tested	% virus incidence ^a
TN-83-26	BYMV	2	15	1.8
Y. K. 577	BYMV	4	15	3.5
	SMV	2		
Totals		56	168	

Note: BPMV: bean pod mottle virus; BYMV = bean yellow mosaic virus; SMV = soybean mosaic virus.

^aPercent incidence was calculated by dividing the number of plants with symptoms observed per plot by the number of seeds planted per plot.

^bFour repetitions were sampled, 480 seeds planted/rep.

^cTwo repetitions were sampled, 480 seeds planted/rep.

Table 6. Occurrence and percent incidence of viruses in the Group V soybean cultivar trials at the Tobacco Experiment Station in 1987.

Cultivar	ELISA Virus Id	No. tested positive	No. samples tested	% virus incidence ^a
Capeheart 5646	AMV	1	1	0.05
Pioneer 9581	AMV	1	1	0.05
Total		2	2	

Note: AMV = alfalfa mosaic virus.

^aPercent incidence was calculated by dividing the number of plants with symptoms observed per plot by the number of seeds planted per plot (4 reps were sampled, 480 seeds planted per rep).

crops or weed hosts. However, BPMV, PSV, SBMV, SMV or TRSV have been reported to be seed transmitted in soybean (30, 54, 58, 64, 68); therefore infected seed or alternate crops or weed hosts could serve as the primary inoculum source. Only BPMV, BYMV and SMV were detected in Knoxville. The presence of BPMV and SMV could be explained by seed transmission and/or by vector transmission of these viruses. BYMV has not been reported to be seed transmitted in soybeans and could have been aphid transmitted from adjacent BYMV-infected clover. The nine viruses not detected at Knoxville (AMV, CCMV, CMV, PMV, PSV, SBMV, TRSV, TSWV and WCMV) may not have been established in weed hosts or their vectors may not have been present. AMV was the only virus detected at the Greenville location. This virus has been reported to infect Burley tobacco in Tennessee (70); therefore its presence in soybean at the Greenville location is not unexpected since tobacco is the primary crop grown at this location.

SMV was most prevalent virus detected at the Milan and Knoxville Plant Sciences Field Laboratory; however only one sample was positive for SMV throughout the commercial field survey. Possible explanations are: 1) more samples were collected per acre in the soybean cultivar trials than during the commercial survey, increasing the possibility of detecting more SMV; 2) commercial soybean fields are usually planted with a single cultivar; whereas the cultivar trials

included several genotypes. Thus, if SMV seed transmission levels were high in a few cultivars and were transmitted by aphids to other cultivars, SMV levels would be higher at Milan and Knoxville than throughout the state.

Due to low disease incidence, yield differences could not be detected. The survey of viruses in the soybean cultivar trials was not conducted in 1988.

Yield Comparison Test

Disease incidence of plants inoculated with BPMV, SMV or BPMV and SMV at the Milan Experiment Station and the Knoxville Plant Sciences Field Laboratory is listed in Tables 7 and 8, respectively. BPMV incidence levels ranged from 2 to 20% in plants inoculated with BPMV alone or in plants inoculated with BPMV and SMV. No BPMV was detected in plants not inoculated with BPMV. This indicates that there was little or no transmission of BPMV by its vectors at either location. All four cultivars tested were equally susceptible to BPMV infection, indicating no resistance in these cultivars to this virus. Little or no SMV was found in the resistant cultivar York; however Essex, Forrest and TN 5-85 had 20-69% SMV incidence in SMV-inoculated plants at both locations. Low incidence levels of SMV (2-9%) were observed in the uninoculated or BPMV-inoculated plants at Milan indicating low levels of vector or seed transmission

Table 7. Percent incidence of BPMV, SMV, or BPMV and SMV occurring in four soybean cultivars at the Milan Experiment Station.

Cultivar	Treatment	Infection ^a		
		BPMV	SMV	BPMV/SMV
		-----%		
Essex	Uninoculated	0.0	4.4	0.0
	BPMV	17.8	6.7	2.2
	SMV	0.0	66.7	0.0
	BPMV/SMV	4.4	68.9	0.0
Forrest	Uninoculated	0.0	2.2	0.0
	BPMV	0.0	6.7	6.7
	SMV	0.0	33.3	0.0
	BPMV/SMV	2.2	31.1	6.7
TN 5-85	Uninoculated	0.0	6.7	0.0
	BPMV	15.5	8.9	0.0
	SMV	0.0	59.9	2.2
	BPMV/SMV	13.3	22.2	4.4
York	Uninoculated	0.0	0.0	0.0
	BPMV	19.9	0.0	0.0
	SMV	0.0	0.0	0.0
	BPMV/SMV	0.0	2.2	0.0

Note: BPMV = bean pod mottle virus; SMV = soybean mosaic virus.

^abased on PAS-ELISA

Table 8. Percent incidence of BPMV, SMV, or BPMV and SMV occurring in four soybean cultivars at the Knoxville Plant Sciences Field Laboratory.

Cultivar	Treatment	Infection ^a		
		BPMV	SMV	BPMV/SMV
		-----%		
Essex	Uninoculated	0.0	62.2	0.0
	BPMV	2.2	46.7	2.2
	SMV	0.0	68.8	0.0
	BPMV/SMV	0.0	48.9	15.6
Forrest	Uninoculated	0.0	20.0	0.0
	BPMV	8.9	28.9	4.4
	SMV	0.0	42.2	0.0
	BPMV/SMV	0.0	35.6	8.9
TN 5-85	Uninoculated	0.0	75.4	0.0
	BPMV	2.2	55.6	8.9
	SMV	0.0	66.7	0.0
	BPMV/SMV	6.7	51.1	20.0
York	Uninoculated	0.0	0.0	0.0
	BPMV	17.8	0.0	0.0
	SMV	0.0	0.0	0.0
	BPMV/SMV	20.0	4.4	0.0

Note: BPMV = bean pod mottle virus; SMV = soybean mosaic virus.

^abased on PAS-ELISA

(Table 7). However, high levels of SMV (20-75%) were found in the uninoculated or BPMV-inoculated plants at Knoxville indicating high levels of vector or seed transmission (Table 8).

Based on the analysis of variance, there were significant differences ($P \leq 0.05$) among blocks and cultivars but no significant differences among virus treatments or cultivar x treatment at Knoxville (Table 9). However, significant differences ($P \leq 0.01$) were observed among all variables at Milan (Table 9).

According to Tukey's mean separation test ($P \leq 0.05$) there was a significant decrease in yield among cultivars inoculated with BPMV/SMV at Milan when compared to all other treatments (Table 10). No significant differences were found among treatments at Knoxville (Table 10). Significant differences were found among the cultivars at both locations (Table 10). At Milan, yields of the cultivar Essex were significantly higher than Forrest, TN 5-85 and York. Yields of TN 5-85 were also significantly higher than Forrest and York. At Knoxville, cultivar TN 5-85 yielded significantly higher than Essex but not significantly higher than cultivars Forrest and York.

According to Tukey's mean separation test ($P \leq 0.05$), there were differences in yield due to cultivar x treatment interactions at both locations. Essex plants inoculated with SMV at Milan with an incidence level of 67% SMV had a

Table 9. Summary of the analyses of variance for virus treatment effects on yield at the Milan and Knoxville location.

Source	df	Mean Square	
		Milan	Knoxville
Block	11	24.43**	87.24*
Cultivar	3	122.40**	113.48*
Treatment (Trt)	3	37.62**	18.25
Cultivar*Trt	9	37.29**	43.86
Error	21	6.07	35.67

*, ** indicates significance at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 10. Least-square mean yields for virus treatments and cultivars at the Milan and Knoxville locations.

Factor	Yield	
	Milan	Knoxville
	-----bu a ⁻¹ -----	
<u>Virus Treatment</u>		
Uninoculated	60.99+0.80	43.99+2.08
BPMV	61.73+0.81	45.30+1.89
SMV	61.69+0.77	45.32+1.84
BPMV/SMV	57.31+0.83	42.35+1.99
HSD ^a (0.05) = 2.69	HSD (0.05) = 6.53	
<u>Cultivar</u>		
Essex	65.10+0.79	39.57+1.84
Forrest	58.34+0.83	44.91+1.93
TN 5-85	61.17+0.78	47.65+1.89
York	57.11+0.84	44.83+2.02
HSD (0.05) = 2.69	HSD (0.05) = 6.53	

Note: BPMV = bean pod mottle virus; SMV = soybean mosaic virus.

^aHonest significant difference based on the 5% level of significance.

significant yield increase of 13% when compared to the uninoculated plants which had an incidence level of 5% SMV (Table 7 and Figure 1). BPMV-inoculated Essex plants, with incidence levels of 18% BPMV and 7% SMV, were not significantly different in yield from the uninoculated plants but were significantly higher in yield than plants inoculated with both viruses (Table 7 and Figure 1). BPMV/SMV-inoculated Essex plants with incidence levels of 4% BPMV and 70% SMV were significantly lower in yield than the other treatments (Table 7 and Figure 1). However, this decrease in yield is probably not a synergistic interaction since the incidence level of the plants testing positive for both BPMV and SMV in PAS-ELISA was estimated to be 0% (Table 7). Essex plants inoculated with SMV at Knoxville with an incidence level of 69% SMV also had a significant yield increase of 19% when compared to the uninoculated plants which had an incidence level of 63% SMV (Table 8 and Figure 1). Yield of SMV-inoculated Essex plants were also significantly higher than the other virus treatments which had incidence levels of SMV ranging from 47-49% (Table 8). Uninoculated Essex plants or plants inoculated with BPMV or BPMV/SMV were not significantly different in yield from each other (Table 8 and Figure 1).

In the Milan tests, yields of BPMV-inoculated Forrest plants were significantly increased by 5% when compared to the uninoculated plants which had an incidence level of 2%

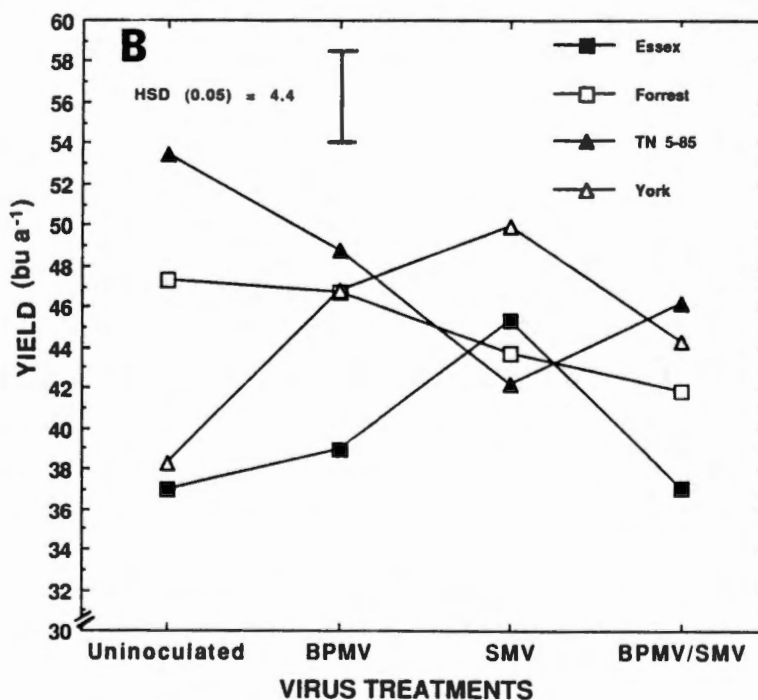
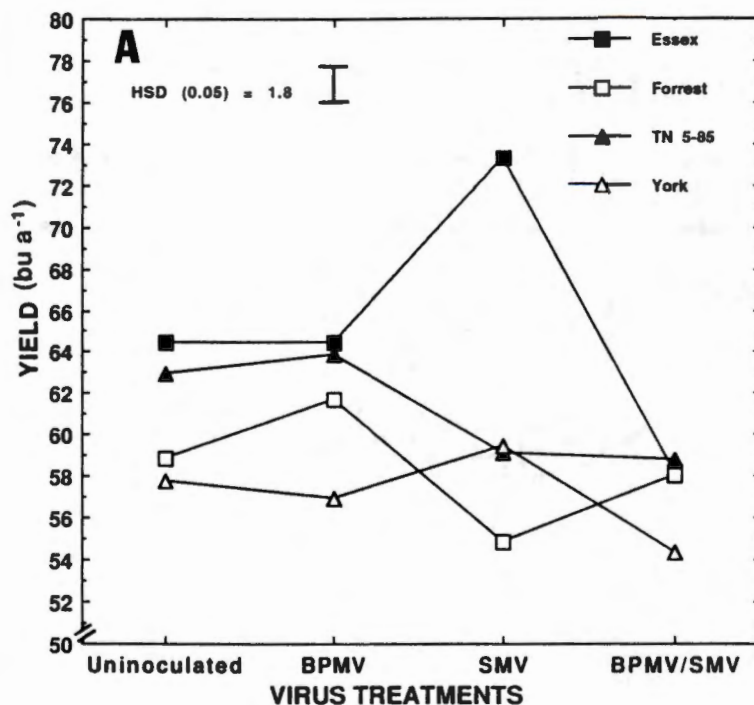


Figure 1. Comparison of mean yields of four soybean cultivars when uninoculated or inoculated with bean pod mottle virus (BPMV) or soybean mosaic virus (SMV) or BPMV and SMV at Milan (A) and Knoxville (B).

SMV (Table 7 and Figure 1). The effect of BPMV on cultivar Forrest at this location could not be evaluated since estimated incidence levels of the plants testing positive for BPMV in PAS-ELISA were 0% (Table 7). SMV-inoculated Forrest plants with an incidence level of 33% SMV yielded significantly lower than any of the other treatments (Table 7 and Figure 1). BPMV/SMV-inoculated Forrest plants with an incidence level of 7% BPMV/SMV were not significantly different in yield than the uninoculated plants (Table 7 and Figure 1). However, BPMV/SMV-inoculated Forrest with incidence levels of 31% SMV, yielded significantly higher than plants inoculated with SMV with incidence levels of 33% SMV (Table 7 and Figure 1). At the Knoxville location, BPMV-inoculated Forrest plants with an incidence level of 9% BPMV and 29% SMV were not significantly different in yield when compared to the uninoculated plants which had incidence levels of 0% BPMV and 20% SMV (Table 8 and Figure 1). Yield of SMV-inoculated Forrest plants were significantly lower (7%) than plants inoculated with BPMV (Table 8 and Figure 1). SMV-inoculated Forrest plants with an incidence level of 42% SMV were significantly lower (8%) in yield when compared to the uninoculated plants which had an incidence level of 20% SMV (Table 8 and Figure 1). The individual effects of BPMV and SMV on yield of Forrest could not be evaluated due to the spread of SMV into BPMV inoculated and uninoculated treatments (Table 8). BPMV/SMV-inoculated

Forrest plants with incidence levels of 9% BPMV/SMV and 37% SMV were significantly lower in yield than plants inoculated with BPMV or not inoculated at all (Table 8 and Figure 1).

At Milan, BPMV-inoculated TN 5-85 plants with incidence levels of 16% BPMV and 9% SMV were significantly higher in yield than the other virus treatments but were not significantly different than the uninoculated plants which had an incidence level of 7% SMV (Table 7 and Figure 1). SMV-inoculated TN 5-85 plants with an incidence level of 60% SMV were significantly lower in yield than the uninoculated or BPMV-inoculated plants, but were not significantly different from plants inoculated with both viruses (Table 7 and Figure 1). BPMV/SMV-inoculated TN 5-85 plants with incidence levels of 13% BPMV only, 22% SMV only and 4% BPMV/SMV (both) were significantly lower in yield than the uninoculated which had an incidence level of 7% SMV or BPMV-inoculated plants which had incidence levels of 9% SMV and 15% BPMV (Table 7 and Figure 1). At Knoxville, yields of TN 5-85 when inoculated with any of the virus treatments were significantly reduced when compared to the uninoculated plants (Figure 1). TN 5-85 plants inoculated with BPMV with incidence levels of 2% BPMV and 56% SMV yielded significantly lower (9%) than the uninoculated plants which had an incidence level of 75% SMV (Table 8 and Figure 1). BPMV-inoculated TN 5-85 plants also yielded significantly

higher (14) than plants inoculated with SMV which had an incidence of 67% SMV. SMV-inoculated TN 5-85 plants yielded significantly lower than any of the virus treatments (Figure 1). TN 5-85 plants inoculated with SMV yielded significantly lower (21%) than the uninoculated plants which had an incidence level of 75% SMV (Table 8 and Figure 1). TN 5-85 plants when inoculated with both BPMV and SMV had incidence levels of 7% BPMV only, 51% SMV only and 20% BPMV/SMV (both) and yielded significantly lower (14%) than the uninoculated plants which had an incidence level of 75% SMV, but not significantly different from plants inoculated with only BPMV (Table 8 and Figure 1).

At Milan, SMV-inoculated York plants yielded significantly higher than when inoculated with BPMV or BPMV/SMV, but were not significantly different from the uninoculated plants (Figure 1). This is not surprising that there was no significance between SMV-inoculated and uninoculated since cultivar York is resistant to SMV and did not become infected when inoculated only with SMV (Table 7). BPMV-inoculated York plants with incidence levels of 20% BPMV yielded significantly lower than when inoculated with SMV but were not significantly different from the uninoculated plants (Table 7 and Figure 1). York plants inoculated with both BPMV/SMV with an incidence level of 2% SMV yielded significantly lower than any of the other treatments (Table 7 and Figure 1). At Knoxville, yields of

cultivar York were significantly increased when inoculated with any of the virus treatments when compared to the uninoculated plants. SMV-inoculated York plants at Knoxville yielded significantly higher than any of the other treatments (Figure 1). BPMV-inoculated York plants with an incidence level of 18% BPMV yielded significantly higher (19%) than the uninoculated plants which did not test positive for virus infection (Table 8 and Figure 1). BPMV/SMV-inoculated York plants with incidence levels of 20% BPMV and 4% SMV also yielded significantly higher than the uninoculated plants, but yielded significantly lower than plants inoculated with SMV alone (Table 8 and Figure 1).

Single virus effects on yield did not correlate with incidence levels. Significant yield increases and decreases were observed at Knoxville from SMV-inoculated plants and BPMV-inoculated plants when compared to the uninoculated plants; however these differences were confounded due to high SMV-incidence levels in the uninoculated plants or BPMV-inoculated plants, with the exception of cultivar York (Table 8 and Figure 1). At Milan, SMV-inoculated Essex plants with incidence level of 67% SMV yielded significantly higher than all other treatments; whereas SMV-inoculated Forrest and TN 5-85 plants with SMV incidence levels of 33% and 60%, respectively, were reduced in yield when compared to uninoculated or BPMV-inoculated plants. The incidence of

BPMV was less than 20% in any treatment or cultivar. In only three instances were significant yield differences observed in BPMV-inoculated plants, two of which were at Knoxville which were confounded by high SMV incidence levels and one at Milan which had 0% BPMV only, 7% SMV only and 7% BPMV/SMV (both) incidence levels. The effects of BPMV/SMV treatments on yield also did not correlate with incidence levels because of low BPMV incidence levels.

It appears that the effects of these viruses on yield of the four cultivars tested at Milan and Knoxville were minimal. Some possible explanations are: 1) the virus isolates used were not virulent enough to cause yield losses in these four cultivars, 2) plants may not have been inoculated at an early enough growth stage to cause significant yield differences, 3) incidence levels of 20% with BPMV were not high enough to cause significant yield losses in these cultivars, 4) yield differences between the SMV-inoculated and uninoculated plants were confounded due to high SMV-incidence levels in the uninoculated plants.

V. SUMMARY

During the commercial soybean virus survey in 1987, AMV, BPMV, BYMV, CCMV, CMV, PSV, SBMV, SMV, TRSV and TSWV were detected. BPMV was the most frequently detected virus and was found throughout the major soybean producing counties of West Tennessee and Robertson county in Middle Tennessee. In 1988, AMV, BPMV, BYMV, CCMV, CMV, PSV, SBMV and TRSV were detected. BYMV was the most frequently detected virus, followed by BPMV and TRSV. The majority of the BYMV samples were collected in Middle and East Tennessee, with the exception of Dyer and Gibson county in West Tennessee. BPMV was detected in seven of the eight counties tested in West Tennessee and Robertson county in Middle Tennessee. TRSV was found in eight of the nineteen counties surveyed, the majority of which were in West Tennessee. This is the first report of BPMV, CCMV, SBMV and SMV in any crop in Tennessee and the first report of AMV, BYMV, CMV, PSV and TSWV occurring in soybeans in this state.

Although disease incidence was not calculated in individual fields, virus incidence appeared low since it was sometimes difficult to collect five diseased samples from the majority of the counties in Middle and East Tennessee.

During the soybean cultivar survey at the Milan Experiment Station, AMV, BPMV, BYMV, CCMV, CMV, PSV, SMV, TRSV and TSWV were detected. BPMV, BYMV and SMV were

detected at the Knoxville Plant Sciences Field Laboratory. Alfalfa mosaic virus was the only virus detected at the Tobacco Experiment station. SMV was the most frequently detected virus at Milan and Knoxville. Disease incidence as estimated by visual symptoms ranged from 0-9%. This agrees with the observations made during the commercial survey.

In the yield comparison test, single virus effects on yield did not correlate with incidence levels due to low incidence levels of BPMV and high incidence levels of SMV in the BPMV-inoculated and uninoculated plants. The effects of BPMV/SMV treatments on yield also did not correlate with incidence levels because of low BPMV incidence. It appears that the effects of these viruses on yield of the four cultivars tested at Milan and Knoxville were minimal.

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VITA

Brenda S. Kennedy was born in Sullivan, Illinois, on March 12, 1964. She received her Bachelor of Science Degree in Botany and Environmental Biology from Eastern Illinois University in August of 1986. In September, 1986 she accepted a research assistantship at the University of Tennessee, Knoxville and began study toward a Master's degree under the supervision of Dr. Bradford B. Reddick. In May of 1989, she received her Master of Science Degree with a major in Entomology and Plant Pathology.

Brenda S. Kennedy is a member of the National and Southern Division of the American Phytopathological Society.