Chapter Three

Effects of Resistance-Breaking (RB) Field Isolates of Soybean mosaic virus on Resistant and Susceptible Soybeans

ABSTRACT

Soybean mosaic virus (SMV; Genus *Potyvirus*; Family *Potyviridae*), one of the most widespread viruses in soybean (*Glycine max* [L.] Merr), can be effectively managed by deployment of resistance. Hutcheson, one SMV-resistant cultivar, is widely used in the Mid-South region and carries an *Rsv*1 allele for resistance to SMV pathotypes G1-G3, the common strains in US soybean fields. Recently, resistance-breaking (RB) isolates of SMV have been detected in soybean cultivar Hutcheson in natural infections. Seven RB isolates of SMV from Virginia were inoculated on susceptible and resistant soybeans in the field in 2002-2003. The RB isolates induced severe foliar symptoms on Hutcheson and induced seed coat mottling. Hutcheson and herbicide-tolerant Hutcheson RR cultivars reacted similarly and herbicide spray had no effect on the plant-virus interactions. RB isolates did not break the resistance of the Essex-*Rsv1* or Essex-*Rsv4* and did not affect their field performance. Hutcheson and Hutcheson RR dually inoculated with RB isolates of SMV and *Bean pod mottle virus* (BPMV) showed an increase in severity of symptoms, including plant stunting. SMV accumulation increased in the dually inoculated plants. BPMV titer increased in the dual inoculations with one of the RB isolates of SMV. The results would be catastrophic if Hutcheson is infected with both viruses in the field, a definite threat with the emergence of the RB isolates of SMV and the increased spread of BPMV in soybean-growing areas.

KEYWORDS: *Glycine max*, SMV, BPMV, Mixed Infection

INTRODUCTION

Soybean mosaic virus (SMV; Genus *Potyvirus)* is a member of the Family *Potyviridae*, the largest family of plant viruses (Barnett, 1991; Shukla et al., 1994). SMV is one of the most important viruses in soybean (*Glycine max* [L.] Merr.) and occurs worldwide (Hill, 1999). Yield losses caused by SMV can reach up to 40% when plants are infected at or before floral development, and may be as high as 94%. A higher incidence of SMV infected plants in the field correlates with higher seed coat mottling. Seed transmission of SMV ranges between 0 to 68% but on average it is about 10%. Extent of virus transmission through seeds depends on the viral strains and the plant genotype and time of infection (Koning et al., 2002). Plants infected at an early stage will have reduction of pod set, seed size and weight, increase in seed coat mottling and decrease in seed quality. Late infections with SMV, on the other hand do not have significant effects on yield or seed quality (Hill, 1999; Hill et al., 1987).

Kiihl and Hartwig (1979) first reported resistance to SMV in soybeans. This resistance is regulated by single dominant genes. Roane et al. (1986) suggested a gene for gene model for the SMV-soybean interactions. SMV strains have been grouped into seven pathotypes depending on the differential reactions induced in different soybean cultivars (Cho and Goodman, 1979, 1982; Chen et al., 1991) all of which contain the *Rsv1* gene for resistance (Kiihl and Hartwig 1979; Chen et al., 1991). Cho and Goodman (1979) reported that SMV-G1, SMV-G2 and SMV-G3 were the most frequent SMV strains in natural infections in the USA. SMV-G5 and G6 constituted only 25% of all field isolates. SMV-G7 was found in only two of 98 samples and SMV-G4 was not present in any field sample.

Breeding programs for SMV resistance in Virginia have been successful. T.J. Smith released the cv. York cultivar in 1968. G.R Buss released the cv. Hutcheson in 1988 (PVP 8800138; Buss et al., 1988). Hutcheson is derived from a cross between Essex and V68-1034 (V68-1034 is in turn derived from York x PI71.506) and contains an *Rsv1* allele from York and is resistant to the low numbered strains of SMV (Chen et al., 1991). Hutcheson is important because of its high yielding and excellent combining ability. Hutcheson is widely used in the Mid-South region where cultivars of the maturity group V are grown (Ustun et al., 2001). Resistance-Breaking (RB) isolates of SMV were first detected in 1998 in Hutcheson border

rows, 10 years after its release. Prior to that, SMV had been isolated occasionally from York (Tolin, unpublished data).

Soybeans dually inoculated with SMV and *Bean pod mottle virus* (BPMV; Genus *Comovirus*, Family *Comoviridae*) display more severe symptoms and yield losses than those infected each of the two viruses independently (Calvert and Ghabrial, 1983). BPMV is present in major soybean growing areas of the US, mainly the South and the Midwest (Giesler et al., 2002). BPMV is probably present in all US soybean-producing states but this is not confirmed since no surveys have been done to assess its natural occurrence (Giesler et al., 2002). Yield losses due to BPMV can reach up to 52% (Gergerich, 1999). No soybean cultivars have been identified with resistance to BPMV (Giesler et al., 2002). BPMV and SMV show a synergism in terms of BPMV titers in the doubly infected plants versus plants singly infected with BPMV (Anjos et al., 1992, Calvert and Ghabrial, 1983). There was no difference whether BPMV is inoculated before or after SMV did not make a difference. BPMV titer was always higher in the doubly inoculated plants. SMV titer did not change with the change in order of virus application (Calvert and Ghabrial, 1983). These observations are consistent with the conclusion of Shukla et al. (1994) that if potyviruses occur in a complex with other unrelated viruses, the effects on yield quantity and quality are more severe.

Since Hutcheson is resistant to the common SMV strains in the US and since the occurrence of SMV isolates that break its resistance appeared quickly, we feel this poses a threat to the durability of the resistance and success of this cultivar. In addition, our preliminary tests in 1997 and 1998 showed that Hutcheson was not only susceptible to SMV, but was also severely affected by two Virginia isolates of BPMV. Our aim in this study was to determine the potential effects of naturally-occurring resistance-breaking (RB) isolates of SMV on Hutcheson, and on Hutcheson with the glyphosate herbicide tolerance gene (Hutcheson RR) since the latter is being widely used for herbicide management, under field conditions. We looked at the effects of seven RB isolates of SMV on Hutcheson and Hutcheson RR and two RB isolates in dual inoculations with two isolates of BPMV. Effects examined included symptom severity, plant height, seed size, average yield, percent germination, and virus seed transmission. We also investigated the effect of the herbicide Roundup on virus-inoculated plants.

MATERIALS AND METHODS

Virus Isolates and Maintenance

Seven RB isolates (Table 3.1) were used in field inoculations. These were 2K-13i, 2K-22, 2K-24, 2K-38, 2K-40, S98-51 and S98-52, all isolated from Virginia (described in the previous chapter). Greenhouse-grown plants of Hutcheson were inoculated using a 1:10 (leaf tissue:0.01M neutral sodium phosphate extraction buffer, pH 7.0) inoculum of SMV from each of the RB isolates of SMV. Inoculum was extracted from leaves of SMV-infected plants using a mortar and pestle, rubbed onto carborundum-dusted unifoliolate leaves using a moistened pestle, and rinsed off with tap water. Cultures were passed to new Hutcheson host plants at the V1-V2 growth stage each 2-3 weeks, and were also preserved by freezing at -80^{o}C.

Field Inoculations of Soybean with Seven RB Isolates of SMV in2002:

All plantings were done the first week of June 2002 and inoculations carried out on the 22 June 2002. Field-grown plants of cvs. Essex, Hutcheson, Essex-*Rsv1*, and Esse*x*-*Rsv4* were inoculated at the V2 to V3 growth stage with the RB inoculum containing 1% carborundum. Two leaves of each plant were rub-inoculated using a sponge, and were not rinsed with water. Each hill plot (described by Koning et al., 2002) consisted of 12 plants (thinned 3 weeks later to 10 plants) of one of the four soybean varieties (Essex, Essex-*Rsv1*, Essex-*Rsv4*, Hutcheson). Seeds were kindly provided by G.R. Buss (Dept. Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia). Eight treatments were applied: seven strains of SMV virus plus non-inoculated control in three replications (blocks). Each block had 32 hill plots (four cultivars x eight treatments). Every group of four hill plots, one of each cutlivar, received the same treatments. The experimental design was considered a factorial RCBD. Data collected consisted of symptom severity (see below) 3 weeks after inoculation, plant height (cm; average height of 4 plants at maturity), total yield, average yield per plant (since not all seeds germinated, plant number varied between hill plots), weight of 100 seeds, percentage germination (100 seeds of each cultivar/treatment from each block). Symptom scoring was based on a scale of 1-5: 1 = no symptoms, 2 = slight mosaic, 3 = slight mosaic and slight leaf deformation, 4 = severe mosaic and leaf deformation, 5 = severe mosaic, leaf deformation and stunting. Germination tests were conducted in plastic beds placed randomly on

a greenhouse bench. All seedlings from the germination tests were tested for SMV by tissue blot immuno assay to determine the percentage of virus transmission. Selected plants were used for inoculation of healthy Hutcheson in the greenhouse to verify the presence of virus.

Field Inoculation of Hutcheson and Hutcheson RR with seven RB Isolates of SMV in 2002

A split plot design was used for this experiment. Hutcheson and herbicide tolerant Hutcheson (Hutcheson RR BC3, provided by G.R. Buss) were grown in parallel rows. Eight treatments were applied (same as above) and completely randomized over three blocks in each Hutcheson and Hutcheson RR BC3. The Hutcheson RR row was sprayed with Roundup® at the recommended rate of 1.5 lb a.i. per acre (0.168g/sq meter) three weeks after inoculation. Data collection was similar to the above experiment except that no data on seed germination and virus transmission through seeds were collected.

Single SMV and Dual SMV/BPMV Experiments 2003

A single virus inoculation experiment was done in 2003 using three RB isolates of SMV and SMV-G1 (Hunst and Tolin, 1982), the common strain. Planting date, delayed by heavy rains, was 27 June 2003 and inoculation date was 16 July 2003. The experimental design consisted of a split plot with the main factor either herbicide treatment or no herbicide treatment replicated over three blocks. The four soybean cultivars used were Essex, Hutcheson, 99VPI-120 (Hutcheson RR BC5), and 99VPI-67 (Hutcheson RR BC3). Five treatments were applied on rows with 12 plants in all varieties in both the sprayed and un-sprayed plants. Rows were thinned to ten plants 2 weeks later. The treatments were completely randomized over the blocks and included three RB isolates 2K-38, 2K-40, and S98-52 in addition to SMV-G1 and non-inoculated controls. The herbicide RoundupPro® (41% active ingredient) treatment was applied on 99VPI-120 and 99VPI-67 4 weeks after inoculation at the recommended rate of 48 oz/acre (0.368g/sq meter) with final working dilution of 14.68ml/l. Data collected included symptom severity (3, 4 and 5 weeks after inoculation) using the same scale as above, plant height (cm, average of four plants from each row at maturity), and relative virus titer (11 weeks after inoculation) (see below). A frost on 8 October 2003 killed plants prematurely, so yields could not be determined.

The dual infection experiment was done using a three block (replication) split-plot design with herbicide as the main factor (same as above). Essex was not included in this test. The five treatments included the non-inoculated controls, and four combinations of SMV and BPMV as follows: 2K-38/BPMV-1, 2K-38/BPMV-15, S98-51/BPMV-1 and S98-52/BPMV-15. The strain identity of the BPMV-1 was S98-1 and that of BPMV-15 was S98-15 (Gu et al., 2002). To prevent confusion between the designation of S98 for both SMV and BPMV, we designated the strains of BPMV as BMPV-1 and BPMV-15 in this paper. Field inoculations and scoring of BPMV were performed by L. Mackasmiel. Treatments were completely randomized over each block. Data collection was as described above.

Enzyme Linked Immuno Sorbent Assay- ELISA

Tissue samples were collected from the uppermost trifoliolate leaves from the field 11 weeks after inoculation treatments were applied. Extractions were done from fresh tissue in extraction buffer (0.05 M sodium carbonate pH 9.6; 1% polyvinyl pyrrolidone MW=40,000 (PVP) for SMV or PBS-T plus 2% PVP for BPMV) using the Tekmar® Tissumiser (Cinncinnati, OH). Six leaf discs (5 mm) were ground in 6 ml, giving an end dilution of 1:50 (w:v). For SMV, Extracts were incubated in Dynatech Immulon™ uncoated 96-well plates overnight at 4°C. Wells were washed 4-5 times with PBS-Tween at room temperature, incubated at 37°C with polyclonal antibodies (1:10,000) raised against SMV particles (Hunst & Tolin, 1982) followed by 4-5 washes with PBS-Tween at room temperature. Plates were incubated at 37°C with goat anti-rabbit IgG (whole molecule) conjugated with alkaline phosphatase (1:15,000) (Sigma-Immuno Chemicals, St Louis, MO). For BPMV tests conducted by L. Mackasmiel, plates were coated with BPMV antisera (1:10,000) for 90 min at 30°C , rinsed, incubated with BPMV extracts overnight at 4°C, rinsed and treated with antibodies against BPMV conjugated with alkaline phosphatase at 1:200 (Agdia®) After rinsing a final time with PBS-Tween, we added paranitrophenyl phosphate (pNPP) substrate (Sigma®) and recorded absorbance at 405 nm over time using the Spectramax plate reader (Molecular Devices, Sunnyvale, CA).

RESULTS

The effects of field isolates of SMV that break the resistance of Hutcheson were investigated on susceptible and resistant soybean cultivars. Essex, Essex-*Rsv1*, Essex-*Rsv4*, Hutcheson and two herbicide tolerant Hutcheson were inoculated with seven RB field isolates of SMV. Yield and other agronomic performance parameters were measured. Results from the Hutcheson and Hutcheson RR experiment in 2002 showed no significant difference between the two cultivars. Statistical analyses ($P \leq 0.05$) for all yield parameters including average yield per plant, weight of 100 seeds and plant height (data not shown) showed no significant difference. Symptom severity was affected by virus treatment. Isolates 2K-38, 2K-40, S98-51 and S98-52 induced the most severe symptoms (Table 3.2).

Average yields per plant for Hutcheson and Essex (Table 3.3) were significantly affected by different RB isolates at $P \leq 0.1$ and $P \leq 0.05$, respectively. SMV isolate 2K-38 reduced the yield of Hutcheson by 43% whereas all the other isolates did not significantly reduce yield compared to the non-inoculated controls. S98-52 reduced the yield of Essex by 38%. Overall, Essex-*Rsv1* and Essex-*Rsv4* Essex produced higher yield per plant (data not shown). Average yield per plant was similar for Essex-*Rsv1* and Essex-*Rsv4*, both of which are resistant to SMV.

Symptom severity was scored on a scale of (1-5), was affected by the virus treatment applied. RB isolates 2K-38, 2K-40 and S98-52 induced severe symptoms on Hutcheson (Table 3.4). All the other isolates induced less severe symptoms on this cultivar. Essex-*Rsv1* and Essex-*Rsv4* remained free of symptoms except for 2K-38 which induced mild mosaics that were confirmed as a serologically positive SMV by tissue blot immunoassay. All controls remained free of symptoms.

Plant height, seed germination, and seed coat mottling were measured from field plants inoculated with the RB isolates of SMV. Plant height was not affected by any of the treatments thus virus symptoms did not include stunting. Effect of RB isolates on seed germination was tested. Percentage germination was not significantly affected by any of the treatments; however greatest reductions were seen with 2K-38, 2K-40, and S98-52 (Table 3.5). About 10% of the seedlings (total n= 2100) from the germination tests were abnormal and were used for inoculation on healthy Hutcheson plants in the greenhouse. Less than 4% of those became

diseased and tested positive SMV in tissue blot immuno-assay. These were soybeans infected with 2K-22 and 2K-38. Seed coat mottling was scored as high mottling, low mottling or non-mottling (Figure 3.1). Seeds from Hutcheson plants inoculated with the RB isolates 2K-22, 2K-38 and S98-52 showed high mottling whereas seeds from plants inoculated with the RB isolates 2K-40, S98-51 and 2K-13i had low mottling. Seeds from non-inoculated plants showed no mottling.

The most severe of the RB field isolates of SMV, were used in field inoculations in a repeat of the experiment conducted in 2002. No yield data were collected since the planting was delayed by about 5 weeks from the previous years due to unfavorable weather conditions. In addition, frost in the second week of October 2003 killed all green tissue and prevented maturation of the pods. Pods did not fill normally, rendering yield and seed analysis meaningless.

Results of the 2003 experiment using single SMV inoculations with SMV-G1 and the RB isolates 2K-38, 2K-40 and S98-52 showed no significant effect of the herbicide. Analysis of variance (ANOVA; $P \leq 0.05$) was performed on the non-sprayed plots to assess the effects on the RB. Our results showed no significant difference for all traits (i.e., plant height, symptom severity, relative virus titer) between the two Hutcheson RR (99VPI-120 and 99VPI-67). Essex, however, was significantly different from the other three cultivars. SMV-G1, as expected, did not induce symptoms on any cultivar except Essex. All the other RB isolates induced symptoms on all the cultivars, with significantly higher severity on Hutcheson and the two Hutcheson RR than on Essex (Table 3.6). Plant height was not significantly affected by any of the treatments (data not shown).

Relative virus titer was measured by ELISA and reading absorbance values at 405 nm (peak absorbance for the substrate). Our results showed that relative virus titer was significantly affected by the treatments (Table 3.7). Essex inoculated with SMV-G1 showed higher ELISA values than the three other cultivars, quantitatively confirming Essex allowed virus replication and accumulation. In contrast, no virus accumulation was detected in the SMV-G1 inoculated Hutcheson, a cultivar resistant to this strain of SMV. RB isolates accumulated to levels equal to that of SMV-G1 in Essex. ELISA values of the non-inoculated controls reflected some natural spread of RB isolates. Levels of natural spread of SMV to non-inoculated controls increased over

the duration of the experiment and reached up to 90% in several rows (9/10 plants). SMV symptoms in these rows began appearing as early as 3 weeks after treatments were applied. Three weeks later, more plants became infected and showed more severe symptoms. All non-inoculated plants were tested serologically by tissue blot immuno assay to confirm natural infection with SMV (data not shown).

Soybean plants were inoculated with both SMV and BPMV to assess the effect of dual inoculations in field-grown Hutcheson and herbicide tolerant Hutcheson. Results from the double SMV/BPMV inoculation experiment showed no statistically significant difference between the spray/ non-spray plots and among the three cultivars Hutcheson, 99VPI-120 and 99VPI-67 ($P \leq 0.05$). Since the experiment was unbalanced for the 2K-38 combination with the other two BPMV strains (i.e. unequal replication), comparison between single and double inoculations was done on all the single virus treatments and the S98-52/BPMV-1 and S98-52/BPMV-15. All the parameters tested were significantly different ($P \leq 0.05$) between the single and the double virus inoculation plots (Tables 3.8-9).

SMV and BPMV interacted synergistically on soybean plants doubly-inoculated with the two viruses. Symptom severity across Hutcheson and the two Hutcheson RR cultivars is presented in Tables 3.6. Plant height was reduced by more than 50% in the doubly-inoculated plants versus S98-52 alone or any other single virus treatment (Table 3.8). Symptoms were more severe in the doubly inoculated soybeans versus the single SMV or the single BPMV (Table 3.8). Higher SMV accumulation was detected in the dual versus single inoculations (Table 3.9). The ELISA value for SMV was lower in S98-52-inoculated plants than in the dually inoculated plants indicating that SMV titer increased more than 2 fold in the presence of . In contrast, BPMV titer was higher in Hutcheson doubly inoculated with S98-52/BPMV-1 than in S98-52/BPMV-15 (Table 3.9).

DISCUSSION

The effects of several new isolates of SMV are described that break the resistance of the soybean cv. Hutcheson. Hutcheson is a maturity group V (MG V) used for its excellent performance in the Mid-South region (Ustun et al., 2001). Hutcheson is derived from York and is resistant to SMV G1-G3 (Chen et al., 1991), the common strains of SMV in the US (Cho and Goodman, 1979). These two characteristics make it a cultivar of choice for growers in southern soybean-growing states. Seven RB isolates from Virginia induce severe symptoms on the susceptible soybean cultivars Hutcheson and Essex under field conditions. The extent of symptom severity is dependent on the RB isolate. Essex-*Rsv1* and Essex-*Rsv4*, however, were resistant to all the RB isolates. Essex-*Rsv1* is an Essex isoline derived from PI 96983 (G. Buss, unpublished data). Essex-*Rsv4* is an isoline of Essex (G. Buss unpublished data) with the *Rsv4* resistance gene derived from V94-5152 (Buss et al., 1997) that is resistant to all strains of SMV (Ma et al., 1995). These two isolines remained resistant in the field, except for occasional plants that showed mild symptoms, and gave higher yields than did Essex and Hutcheson. These results demonstrate that the genes for resistance in Essex isolines are still effective.

Hutcheson and two Hutcheson RR lines had similar interactions with the RB isolates of SMV in the field. A preliminary experiment in 2002 showed that the RB isolates induced similar symptoms between the two cultivars. However, no significant effect on yield or plant height could be detected ($P \leq 0.05$). This might have been due to the fact that the blocks were very different. Two factors contributed to this discrepancy among the three blocks: the third block was at the end of the field where the soil drained and rabbits moved into this end of the field and fed on the plants. Therefore, this experiment was repeated the following year using these and other RB isolates. Phenotypic data showed no difference between Hutcheson and those lines with the RR gene. This was the first direct comparison of RR and non-RR cultivars in terms of extent of response to SMV resistance to RB isolates. Herbicide treatment had no effect on the interaction between Hutcheson plants and the RB isolates. Virus titer, symptom severity, and plant height were similar between sprayed and non-sprayed plants.

The effects of RB isolates on seed coat mottling, seed germination and transmission of virus through seeds were investigated. Seed germination was not significantly reduced by any of

the inoculations ($P \leq 0.05$). However this study was based on only 300 seeds for each cultivar and inoculations. More tests need to be done to assess the potential reduction of seed germination, especially since two of the isolates, 2K-40 and S98-52 reduced Hutcheson germination by over 20% from the non-inoculated control. All RB isolates caused mottling of the seed coat in Hutcheson. Seed coat mottling is an important factor affecting the grade of seed quality and has been found to be an increasing problem in the Midwestern US (Hobbs et al., 2003).

Single inoculation with SMV showed some difference in symptom severity on Hutcheson but not on Essex. However all the RB isolates induced less severe symptoms on Essex compared to the SMV G1 (Table 3.6). This is surprising since Essex is fully susceptible to all strains of SMV. These results support the hypothesis that the fitness of RB strains is reduced in natural populations (Garcia-Arenal and McDonald, 2003). There was no major difference in the response of the Hutcheson and Hutcheson RR cultivars to the different RB isolates.

Virus accumulation did not differ in Essex and Hutcheson cultivars inoculated with any of the RB isolates (Table 3.7). Virus titer as measured by indirect ELISA was higher for all the RB compared to SMV-G1 inoculated Hutcheson, 99VPI-120 or 99VPI-67. These are extremely resistant to SMV-G1 and are expected to permit no replication of this strain. No significant difference in relative virus titer was detected for the different RB isolates tested ($P \leq 0.05$).

Double infection with both SMV and BPMV significantly increased the extent of symptom severity, reduced plant height and increased the titer of SMV (Table 3.7-3.9). The effect on the first two parameters was expected and is similar to those reported previously (Anjos et al., 1992; Calvert and Ghabrial, 1983). However increased titers of SMV in the dual infection were not expected and do not agree with previous findings on the synergistic relationship between these two viruses. Anjos et al. (1992) and Calvert and Ghabrial (1983) reported an increase of BMPV levels, but not of SMV levels, in soybean plants dually inoculated with SMV and BPMV. Our results showed that BPMV titer increased with one of the two RB isolates of SMV tested. Additional greenhouse experiments are needed to confirm the effect on virus titer with dual inoculations.

SMV isolates that break the resistance of *Rsv1*^{y} in the cultivar Hutcheson have the potential to affect the normally excellent performance of Hutcheson in the field. These RB isolates have the potential to become established in soybean growing areas wherever Hutcheson

or the herbicide tolerant Hutcheson cultivars are grown. Since Hutcheson is not resistant to BPMV, an increasing problem reported in the major soybean growing areas, there is a probability that both viruses will come in contac. The results of our work suggest that double infections would create severe yield losses. It is therefore very critical to eliminate seed-borne infection from Hutcheson.

Table 3.1. Resistance-breaking (RB) field isolates of SMV (described in chapter 2).

Isolate	**Year**	**Location of source**	**Cultivar**
S98-51	1998	Virus nursery, Blacksburg VA (yield loss trials)	Hutcheson
S98-52	1998	Virus nursery, Blacksburg VA (yield loss trials)	Hutcheson
2K-13i	2000	Blacksburg breeding nursery, VA	PI556950
2K-22	2000	AREC[a], Warsaw, VA (crossing and demonstration blocks)	PI507389
2K-24	2000	AREC, Warsaw, VA (yield trials)	RR-52 Hutcheson
2K-38	2000	Virus nursery, Blacksburg VA (border row)	Hutcheson
2K-40	2000	Virus nursery, Blacksburg VA (border row)	Hutcheson

[a] Agricultural Research and Education Center.

Table 3.2. Symptom severity of Hutcheson and Hutcheson RR inoculated with each of seven resistance-breaking (RB) isolates of SMV in 2002. Symptoms were scored 3 weeks after inoculation. Data represent the mean of scores from three replications.

	Symptom severity based on a scale 1-5[a]	
RB isolate	**Hutcheson**	**Hutcheson RR BC4**
2K-13i	2	2.8
2K-22	2.7	2.7
2K-24	2.3	2.7
2K-38	4	3.7
2K-40	3.8	3.3
S98-51	3.2	3
S98-52	3.8	4
Control	1	1.6

[a]1 = no symptoms, 2 = slight mosaic, 3 = slight mosaic and slight leaf deformation, 4 = severe mosaic and leaf deformation, 5 = severe mosaic, leaf deformation and stunting

Table 3.3. Average yield per plant from Hutcheson and Essex inoculated with seven resistance-breaking (RB) isolates of SMV in 2002**.**

	Average yield (g) per plant from three replications in 2002			
SMV Isolate	**Hutcheson**		**Essex**	
2K-13i	17.053	**A**	12.193	**ABC**
2K-22	16.353	**A**	10.347	**BC**
2K-24	17.000	**A**	17.760	**A**
2K-38	8.780	**B**	10.667	**BC**
2K-40	12.177	**AB**	11.423	**BC**
S98-51	17.260	**A**	15.387	**AB**
S98-52	12.220	**AB**	8.427	**C**
Control	15.360	**A**	13.627	**ABC**

ANOVA and LSD mean separation at $P \leq 0.1$ for Hutcheson (LSD=6.81) and $P \leq 0.05$ for Essex (LSD=5.68). Means not followed by the same letter within a column are significantly different**.**

Table 3.4. Symptom severity of Essex, Hutcheson, Essex-*Rsv1* and Essex-*Rsv4* scored 4 weeks after inoculation with SMV in 2002.

RB isolate	Symptom severity based on a 1-5 scale[a]			
	Essex	**Hutcheson**	**Essex-*Rsv1***	**Essex-*Rsv4***
2K-13i	2.3	2	1	1
2K-22	3.17	2.17	1	1
2K-24	2.5	1.83	1	1
2K-38	3	4.17	2	1.7
2K-40	3	3.17	1	1
S98-51	2.83	2.7	1	1
S98-52	3	3.17	1	1
Control	1	1	1	1

[a] 1 = no symptoms, 2 = slight mosaic, 3 = slight mosaic and slight leaf deformation, 4 = severe mosaic and leaf deformation, 5 = severe mosaic, leaf deformation and stunting.

Table 3.5. Percentage of germination of seeds collected from Hutcheson after inoculation with seven resistance-breaking (RB) isolates of SMV in 2002.

Strain	Percentage germination
2K-13i	75.7
2K-22	78.3
2K-24	70.3
2K-38	64.0
2K-40	59.3
S98-51	77.7
S98-52	60.0
Control	77.3

ANOVA analysis at $P<0.05$ showed no significant reduction to percentage germination.

Table 3.6. Symptom severity in soybean cultivars inoculated with SMV in 2003.

	Symptom severity (1-5) on four soybean cultivars[a]			
Strain	Essex	Hutcheson	99VPI-120	99VPI-67
SMV-G1	3 C[b]	1 E	1 E	1 E
S98-52	2 D	3.8 AB	3.7 AB	3.8 AB
2K-38	1.7 D	3.3 BC	3.5 BC	3 C
2K-40	2 D	3.8 AB	3.8 AB	4.2 A
Control	1 E	1 E	1 E	1 E

[a] 1 = no symptoms, 2 = slight mosaic, 3 = slight mosaic and slight leaf deformation, 4 = severe mosaic and leaf deformation, 5 = severe mosaic, leaf deformation and stunting.

[b] ANOVA and LSD mean separation at $P \leq 0.05$. Numbers not followed by the same letter are significantly different. LSD (0.284) mean separation at $P \leq 0.05$.

Table 3.7. Relative virus titer from indirect ELISA absorbance at 405 nm from single SMV inoculations in 2003.

	Absorbance values at 405 nm from four soybean cultivars in 2003			
Strain	Essex	Hutcheson	99VPI-120	99VPI-67
SMV-G1	2.22 **A**	0.77 **EF**	0.11 **F**	0.13 **F**
S98-52	1.78 **AB**	1.68 **ABC**	0.83 **DEF**	1.54 **ABCDE**
2K-38	1.87 **AB**	2.18 **A**	1.81 **AB**	1.96 **A**
2K-40	1.69 **ABCD**	2.08 **A**	2.11 **A**	2.03 **A**
Control	0.84 **CDEF**	0.88 **CDEF**	0.69 **EF**	1.08 **BCDE**

Relative virus accumulation in leaves of four soybean cultivars inoculated singly with four SMV isolates. Values are mean absorbance at 405 nm of duplicate wells in ELISA at 1:50 dilution of field-collected leaves, 11 weeks after inoculation.

Numbers not followed by the same letter are significantly different. LSD (0.86) mean separation at $P \leq 0.05$.

Table 3.8. Relative symptom severity and plant height between single and double inoculated Hutcheson, 99VPI-120 and 99VPI-67.

Virus Inoculum	Symptom severity (1-5)	Plant Height (cm)
SMV-G1	1 **F**	64.3 **A**
S98-52	3.8 **CD**	55.8 **AB**
2K-38	3.3 **D**	55.9 **AB**
2K-40	3.9 **BC**	50.5 **B**
BPMV-1	2 **E**	54.7 **AB**
BPMV-15	2.3 **E**	52.8 **AB**
S98-52/BPMV-1	4.6 **A**	29.7 **C**
S98-52/BPMV-15	4.5 **AB**	31.2 **C**
Control	1 **F**	61.1 **A**
LSD	0.60	9.851

Numbers not followed by the same letter are significantly different. LSD mean separation at $P \leq 0.05$. Mean separation is individual for each of the three observations.

Table 3.9. Comparison of relative virus titer in single SMV and BPMV versus double SMV/BPMV inoculations from Hutcheson.

	ELISA Value		Comparative Titer of Double :Single	
Treatment (SMV/BPMV)	SMV[a]	BPMV[b]	SMV	BPMV
SMV-G1	0.34 D	-		
2K-38	1.98 B	-		
2K-40	2.06 B	-		
S98-52	1.35 C	-		
BPMV-1	-	1.48		
BPMV-15	-	2.52		
S98-52/BPMV-1	2.36 AB	2.11	1.75	1.43
S98-52/BPMV-15	2.96 A	1.35	2.20	0.87
Control	0.88 D	0.13		

[a]Relative accumulation of four SMV isolates inoculated singly to four soybean cultivars. Values are mean absorbance at 405 nm of duplicate wells in ELISA at 1:50 dilution of field-collected leaves 11 weeks after inoculation.

Numbers not followed by the same letter are significantly different. LSD mean separation at $P \leq 0.05$. LSD for SMV is 0.38.

[b] Relative accumulation of BPMV in singly or dually inoculated soybean. ELISA values were collected by L. Mackasmiel and have not yet been statistically analyzed.

Figure 3.1. Effect of the RB isolates of SMV on seed coat mottling of Hutcheson.

A= no seed coat mottling; this includes the non-inoculated controls

B= high seed coat mottling; this includes 2K-22, 2K-38 and S98-52

C= Low seed coat mottling; this includes 2K-24, 2K-40, S98-51 and 2K-13.

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