Chapter Five

Enhanced Synergistic Interactions of New Isolates of BPMV and SMV on Hutcheson Soybean

ABSTRACT

Double inoculation of soybean with *Bean pod mottle virus* (BPMV), a member of the Genus *Comovirus*, Family *Comoviridae*, isolates V-S98-1 and V-S98-15, together with *Soybean mosaic virus* (SMV) a member of the Genus *Potyvirus*, Family *Potyviridae*) isolates 2K-38, S98-51 and S98-52, was compared to single inoculation with each of the isolates, on Hutcheson, Hutcheson Roundup Ready® (RR BC3) and Hutcheson Roundup Ready® (RR BC5). The double inoculations caused severe foliar symptoms across all three cultivars with or without herbicide treatment, compared to the single inoculation of either BPMV or SMV isolates, respectively. The double inoculations also caused significant reduction in plant height, with Hutcheson and Hutcheson Roundup Ready® (RR BC5) being substantially affected by B1+S52 (BPMV V-S98-1+SMV S98-52) and B15+S52 (BPMV V-S98-15+SMV S98-52), respectively. Seed weight was also severely reduced by the same virus isolate combinations on the same cultivars. BPMV (B1)+SMV (S52) caused 100% reduction in seed weight on Hutcheson, as did B15+S52 on Hutcheson Roundup Ready® (RR BC5). Severe seed weight reduction was also caused by B1+S38 and B15+S38 across all cultivars. SMV 2K-38, S98-51 and S98-52 are SMV resistance breaking strains in Virginia soybean, while BPMV V-S98-1 and V-S98-15 are virulent isolates which have been isolated in Virginia. These combinations on Hutcheson and Hutcheson Roundup Ready® therefore pose a severe threat to soybean productivity, emphasizing the need for resistance to both viruses.

KEYWORDS: Isolates, BPMV, SMV, double inoculation, Roundup Ready®, herbicide resistance

INTRODUCTION

The realization that a single soybean plant may be infected with both *Bean pod mottle virus* (BPMV, Genus *Comovirus*, Family *Comoviridae*) and *Soybean mosaic virus* (SMV, Genus *Potyvirus*, Family *Potyviridae*) in the field, remains the biggest problem soybean growers face in trying to increase quality and reduce yield losses. In double inoculations of susceptible soybean cultivars with BPMV and SMV, yield losses of up to 86% occur compared to 8-25% in single SMV and 10% in the case of BPMV (Hartman et al., 1999). This synergistic behavior is known to enhance the titer of BPMV (Calvert and Ghabrial, 1983; Anjos et al., 1992). Both BPMV and SMV cause seed coat mottling (Hobbs et al., 2003). Mottling lowers sellability and the market value of food grade soybean, especially in the Japanese market (Griffis and Wiedermann, 1990; Sullivan, 2003). It is also known that both BPMV and SMV enhance infection by *Phomopsis* species (Abney and Ploper, 1994; Hartman et al., 1999; Koning, et al., 2001; 2003), which cause further discoloration and deterioration of soybean seed.

Although seed transmission of BPMV is only estimated at 0.1%, there are indications that seed may be one of the main sources of initial inoculum for this disease in soybean (Krell et al., 2003). Seed transmission of SMV ranges between 5% and 75% depending on the cultivar (Hill, 1999). Both viruses are vectored by insects in the field with BPMV being transmitted by leaf beetles, *Cerotoma trifucata* Foster (Insecta: Coleoptera: Chromellidae) in a semi-persistent manner. SMV, however, is transmitted by at least 32 species of aphids (Insecta: Homoptera: Aphididae) in stylet-borne or non-persistent manner (Irwin et al., 2000). Species of aphids include *Myzus persicae* (Berger and Pirone, 1986; Gibson and Rice, 1986) and *Rhopalosiphum maidis* (Bottenberg and Irwin, 1992b), among others. Even though the low level of seed transmission, especially that of BPMV, may give a false impression that there is no serious problem to growers, vector presence and activity in the presence of a low inoculum level may produce a very different result. The main problem of BPMV is associated with beetle vectors that spread the virus in soybean (Giesler 2002). As stated by Calvert and Ghabrial (1983), while confirming the work by Ross (1968), in the presence of a small amount of BPMV inoculum and a large number of beetle vectors, significant yield loss can occur. While most of the work on the effects of dual inoculation of BPMV and SMV on soybean had

important implications in the late 1960's up to the early 1980's, the study took a secondary role until the late 1990's and early 2000's, when it was realized that the incidence of BPMV was on the rise.

The objective of this experiment was to determine the effects of single and double inoculations of Virginia isolates of BPMV and SMV on Hutcheson, Hutcheson Roundup Ready® (RR BC3) and Hutcheson Roundup Ready® (RR BC5). While Hutcheson Roundup Ready® (RR BC3) and Hutcheson Roundup Ready® (RR BC5) are yet to be released to growers, Hutcheson is a widely grown cultivar that is resistant to most strains of SMV. However, resistance breaking strains recently have been isolated and characterized (Fayad, 2003). BPMV isolates used in this study had been shown (Chapter 2) to be severe on Hutcheson.

MATERIALS AND METHODS

Experimental design: The seeds used in this experiment were kindly supplied by G. R. Buss and planted with the help of his team from Crop and Soil Environmental Sciences, Virginia Tech, on June 27, 2003, after a delay caused by frequent rain. The experiment was done at the Glade Road Nursery, Virginia Polytechnic Institute and State University in Blacksburg. Essex, Hutcheson, Hutcheson Roundup Ready® 1 (Hutcheson RR BC5, HR1) and Hutcheson Roundup Ready® 2 (Hutcheson RR BC3, HR2) were planted in three separate blocks. The seeds were planted at 12 seeds per row in 0.46 m parallel rows, with one block each for SMV (single), BPMV (single) and BPMV+SMV (double inoculation), respectively, using split-plot design with three replications within each block. The main plot was the herbicide treatment and subplots were virus strains.

Virus isolates and inoculations: Single inoculations of two BPMV isolates V-S98-1 and V-S98-15, as described in Chapter 2, and single inoculations of two SMV isolates 2K-38 and S98-52 (Fayad, 2003), were performed on July 16, 2003 at V1-V2 stage of development. At the same time, in a third block, double inoculations were made with BPMV+SMV isolates in combinations as follows: B1+S38, B15+S38, B1+S52 and B15+S52, where B1 = BPMV V-S98-1, B15 = BPMV V-S98-15, S38 = SMV 2K-38 and S52 = SMV S98-52.

The inocula were prepared as described in Chapter 2, and held in the field in a

sterilized chilled mortar on ice. The inoculations were performed using 2.5 cm sponge brushes. After incorporating 0.5 % carborundum, the sponge was soaked in inoculum and used to rub inoculate the youngest set of trifoliolate leaflets of each plant receiving treatment(s). Non-inoculated controls were also included. For the double inoculations, SMV was inoculated first, followed approximately 30 min later by BPMV, using new inoculum and sponge for each block of SMV-inoculated plants. At 4 weeks post inoculation, the herbicide glyphosate, (RoundupPro®, 41% active ingredient, Monsanto, St Louis, MO) was applied on Hutcheson RoundupReady® (RR) BC5 and BC3 at the recommended field rate of 48 oz/acre ($0.368 g/m^{-2}$), a dilution of 14.68 ml/l. Hutcheson was not herbicide sprayed because it is susceptible to Roundup. This experiment was done in conjunction with Amer Fayad, who inoculated SMV isolates and analyzed SMV titers in the single and double inoculations, using indirect ELISA (Fayad, 2003).

Monitoring of plants: In order to estimate the effect of virus on plants, foliar symptom severity and any deformities on the vegetative parts of the plants were rated on a scale of 1-5, as described in Chapter 4. Ratings were taken at 3, 4 and 5 weeks after inoculation. At maturity, which was not based on physiological timeline in this experiment due to early frost, plant height (cm) was determined from the mean of three plants per row from all the rows in the three blocks.

For BPMV, double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) described by Clark and Adams (1976) was used. Leaves stored overnight at 4ºC were used to test for BPMV accumulation. Three leaves collected from young trifoliolates at 11 weeks after inoculation were used to prepare the antigen (virus). Six discs were made using 9 mm, # 5 cork borer, two from each of the three leaves. Fresh grinding/extraction buffer was prepared for each extraction batch as follows; phosphate buffered saline [monobasic potassium phosphate (KH_2PO_4) (1 g/l), dibasic sodium phosphate (Na_2HPO_4) (10.75 g/l), sodium chloride (NaCl) (40 g/l), and potassium chloride (KCl) (1 g/l)], pH 7.4, with 0.05% Tween (v/v) (PBS-T), plus 2% polyvinyl pyrrolidone MW = 40,000 (PVP). The egg albumin and Tween 20 at 0.2 g, and 2 g, respectively, recommended by Agdia (Elkhart, IN), were not added to 1X PBS-T. The discs were ground in 6 ml of the grinding buffer using a Tekmar® Tissumizer (Cincinnati, OH), giving a concentration of 1:50 (w:v).

Coating buffer made of 0.05M sodium carbonate (1.59 g Na_2CO_3 + 2.93 g $NaHCO_3$ per liter), at pH 9.6 was used to constitute the primary coating antibody aBPMV-Hancock (kindly provided by S. A. Ghabrial, University of Kentucky), at 1:1,000. The coating antibody was prepared by adding 10 µl whole antiserum (20 µl of 1:2 in glycerin) to 10 ml of the buffer in a glass tube covered with parafilm and gently swirled. Using a multi-channel pipettor, 100 µl of the coating buffer was added to each well on a 96-well plate, which was then covered incubated at 30ºC for 2 hr. At the end of the first incubation, the primary coating antibody was shaken out and the plate blotted on paper towels. The wells were then washed out by adding 1X PBS-T to a tilted plate starting from the bottom wells and moving to the top wells. The plate was allowed to stand for 3 minutes between the washings and vigorous blotting of the plates on paper towels. After washing the plate three times using the same procedure, PBS-T was left in the wells until the antigen was ready to be added. Using a manual pipette, 200 µl of the antigen was added to each well. Each sample was added to duplicate wells. The plate was then covered with plastic film and incubated at 30ºC for 2 hr. After incubation, the antigen was shaken out and the plate washed three times using PBS-T and the procedure followed at the end of first incubation, making sure that no green residues remain on the well bottom. At the end of the last washing, PBS-T was left in the wells until ready to add the conjugate.

The BPMV enzyme conjugate was made by diluting 50 µl of Agdia anti-BPMV enzyme conjugate in 10 ml of enzyme conjugate buffer (ECI), a dilution of 1:200. The buffer was made using 100 ml of cooled (4ºC) 1X PBS-T in which 0.2 g of bovine serum albumin (BSA) and 2 g of PVP were dissolved. After shaking out the PBS-T from previous washing, the conjugate was added at 100 µl per well using the multi-channel pipettor followed by covering with a plastic film and finally incubating at 30ºC for 2 hr. The last incubation was followed by shaking out the secondary antibody then three washings with PBS-T, as described after the first incubation. At the end of the third wash, PBS-T was left in the wells until the substrate was added. The buffer was prepared by dissolving a 20 mg in 20 ml of distilled water, at least 15 minutes before the end of the last incubation, to make 0.2 M tris buffer (Sigma-FAST). A paranitrophenyl phosphate (pNPP) substrate tablet (10 mg per 20 ml) was added just before using. As soon as all the

substrate was dissolved, the PBS-T was shaken out of the plate with vigorous blotting, and 200 µl of the substrate was added to each well using the multi-channel pipettor.

The plate was incubated at room temperature for 3-15 minutes, and then read in the Spectramax plate reader (Molecular Devices, Sunnyvale, CA) by recording the absorbance at 405 nm, using the plate reader functions. The readings were taken at 15 min intervals until maximum values were attained. The readings were finally transferred to an excel program where means from duplicate wells were calculated and finally transferred to SAS for statistical analysis. Relative virus accumulation was measured using indirect ELISA for SMV, as described in Chapter 3.

Effects on seeds and yield: Although the frost of October 8, 2003 caused premature death of plants, seeds were hand harvested, machine threshed and the weight (g per row) determined.

Statistical analysis: Split-plot design was used to compare the effects of virus isolates on cultivars with the virus being the main plot and the cultivars being the subplots. The data obtained were analyzed using split-plot analysis of variance (ANOVA). Finally, a test of normality on all class variables was done using the UNIVARIATE and GLM procedures (SAS Institute, 2003; release 8.2 software 2000-2003, Cary, NC 27513). Any significant differences present at $P \leq 0.05$ were located by Tukey's Studentized (HSD) test.

RESULTS

Symptom severity: The symptom severity for Hutcheson, Hutcheson RoundupReady® (RR) BC5 and BC3, inoculated with BPMV, SMV or double BPMV+SMV inoculations, respectively, are shown in Table 5.1. In general, both BPMV and SMV isolates showed different severities of foliar symptoms across the three entries. The effect of herbicide treatment was non-significant across all treatments and the control. Single BPMV inoculation with V-S98-1 (B1) resulted in a significantly higher foliar symptom severity, than did V-S98-15 (B15), but both were also different from the control. The two SMV isolates, 2K-38 (S38) and S98-52 (S52) also had significantly ($P \leq 0.05$) higher foliar scores than SMV G1 and the control. SMV G1 and the control treatments were consistently similar in value across all SMV single inoculations, as expected because

Hutcheson is resistant to this strain of SMV. Lack of symptoms in the control indicates that the natural spread of SMV was low or late.

The double inoculations, BPMV+SMV all resulted in highly significant ($P \leq 0.0001$) increase in foliar scores compared to the single inoculations and the control. This indicates substantial synergy between the viruses. A combination B1+S52 caused highly significant foliar damage with scores of ≥ 3.0, which were similar to B15+S52, although B15+S52 had one score of 2.7 (Table 5.1). These were higher than the foliar scores exhibited by B1+S38, and B15+S38 in Hutcheson but not always in the RR lines.

Virus accumulation: Synergistic interaction was also demonstrated by measuring the comparative virus titer by ELISA. The extent of enhancement by double inoculation, calculated as a ratio of ELISA value for double versus single inoculations (BPMV and SMV), is shown in Tables 5.2 and 5.3. In Table 5.2, virus accumulations in BPMV and SMV single inoculations did not deviate from the controls even though natural spread was detected in the controls. ELISA values in the control still remained lower than the inoculated plants. Double inoculated plants were significantly higher ($P \leq 0.05$) and all the ELISA values were different from all the controls. In Table 5.3, the ratios of double inoculations (BPMV+SMV) were compared to the single BPMV or single SMV (SMV+BPMV:SMV). The two ratios were then compared to each other. The values obtained provided the level of enhancement of each virus. Sixteen (84.2%) out of 19 values showed higher titer for BPMV and only three (15.8%) out of 19 were relatively higher for SMV.

Hutcheson Roundup Ready® (RR) BC3 and BC5, with herbicide treatment accumulated high titer of both viruses, as shown in Table 5.3, for all single virus treatment and cultivar interactions. Because of natural spread in the controls, some virus treatments showed no significant difference even though ELISA values still exhibited high virus titer. All double-inoculated treatments had significantly higher accumulation of both SMV and BPMV, but there were relatively more BPMV enhancement compared to SMV.

Effect on plant growth and yield: The mean plant height (cm) based on three plants from each row, is summarized in Table 5.4. BPMV and SMV single inoculations had similar effects on plant height compared to the BPMV+SMV double inoculations, which

had significant ($P \leq 0.05$) reduction in height, especially the B1+S52 and B15+S52 on Hutcheson Roundup Ready® (RR) BC3 and BC5 lines with or without herbicide treatment, even though natural infection also affected the heights of Hutcheson RR BC5 control plants.

Effects on seeds: The mean seed weights (g per row) of the three cultivars were determined by harvesting seeds after the plants dried out. The mean seed weights are shown in Table 5.5. The double inoculations resulted in very highly significant ($P \leq 0.0001$) yield reduction. BPMV isolates V-S98-1 and V-S98-15 in combination with SMV S98-52, caused 100% seed weight reduction on Hutcheson and Hutcheson Roundup Ready® (RR) BC5, without herbicide treatment. High seed weight reductions were also recorded in B1+S38 and B15+S38 treatments across all cultivars. BPMV single inoculation reduced seed weights in two, five and four Hutcheson, Hutcheson Roundup Ready® (RR) BC5 and BC3, respectively. While SMV single inoculation reduced only one each of Hutcheson Roundup Ready® (RR) BC5 and BC3, respectively, but it must be realized that the control plants were naturally infected late and thus affected the comparison (Table 5.5).

DISCUSSION

It has been shown in this study that doubly-inoculated plants produced lower yields than those singly-inoculated, and that up to 100% yield loss may be realized in Hutcheson and Hutcheson Roundup Ready® (RR) BC5. Yield reductions of 3-52% due to BPMV pathogenesis, and up to 85% in mixed inoculations and pathogenesis with SMV, have been reported by Ross (1968).

Plant heights were also adversely affected especially by B1+S52 (BPMV V-S98-1+SMV S98-52), and B15+S52 (BPMV V-S98-15+SMV S98-52) combinations compared to the other isolates, demonstrating that there are differences among the isolates. It has also shown that reduction in plant heights occur and yield reduction of up to 100% depending on the soybean cultivar and BPMV and SMV isolates, may take place. Because only one year of data has been presented, there is a need to document the seasonal effects on the isolates and isolate combinations in order to ascertain the effects of existing isolates and the effects of seasons on soybean production. On foliar scores,

those combinations that had BPMV isolates V-S98-1 and V-S98-15 had higher scores. Although they did not differ from the single inoculations of SMV, they differed from BPMV. Again, B1+S52 and B15+S52 still had the highest scores.

It was shown in this study that sixteen (84.2%) out of the total 19 treatments showed higher titer for BPMV and only three (15.8%) out of 19 was relatively higher for SMV. The enhancement for BPMV to SMV was between 1.01 and 1.61, and the overall enhancement of BPMV was 2.04 whereas that for SMV was 1.75. The results of the study therefore disagree with (Fayad, 2003) that SMV titer increases in the double inoculated plants, when compared to single BPMV inoculation. The finding agrees with (Ross, 1968; Calvert and Ghabrial, 1983). In 1968, Ross found that SMV, a potyvirus enhances the titer of BPMV, a comovirus when both are inoculated in the same susceptible soybean plant. Later, Calvert and Ghabrial (1983) found the same results when BPMV and SMV were inoculated on the same plant. BPMV ranged from 1.67 to 2.66 times higher than in single inoculation and SMV ranged from 1.16 to 3.20 times than that in a single inoculation. The study by Anjos et al. (1992) on joint inoculations of BPMV and SMV on soybean also demonstrated that there is elevated accumulation of BPMV. They proposed that the comovirus (BPMV) might possibly be utilizing the replication machinery of SMV, since both utilize similar non-structural gene products, hence the elevated titers of BPMV during such synergistic interactions. The basis for this synergistic behavior is finally being elucidated. Some studies have now shown that the post-transcriptional gene silencing is responsible for the enhancement of BPMV titer (Giesler et al., 2002).

This study has demonstrated that double inoculation of BPMV and SMV causes high virus titer of BPMV, but other studies have also shown increased BPMV and SMV accumulations (Ross, 1968; Calvert and Ghabrial, 1983; Anjos et al., 1992) and (Fayad, 2003), respectively.

These findings demonstrate that higher losses than previously thought may be taking place in Hutcheson and the isolines. Hutcheson is a popular cultivar with growers in Virginia. But Hutcheson is susceptible to SMV breaking strains (Fayad, 2003). Hutcheson is also susceptible to the BPMV isolates used in this study. The existence of these BPMV and SMV isolates and strain combinations in Virginia is bound to

predispose Hutcheson and its isolines to combined inoculations in the field and that will lead to adverse reduction in quality and yield losses in soybean from growers. There is a need to incorporate more resistance or high tolerance to BPMV in Hutcheson and its isolines.

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Table 5.1. Comparison of mean symptom severity (1-5) of Hutcheson, Hutcheson RoundupReady® (RR) BC5 and BC3, treated in single inoculation with *Soybean mosaic virus* (SMV), *Bean pod mottle virus* (BPMV) isolates, and in double inoculations with both SMV and BPMV. Hutcheson Roundup Ready soybeans were either herbicide treated or untreated.

Virus	Treatment	Herbicide	Hutcheson	Hutcheson RR BC3	Hutcheson RR BC5
BPMV	**Control**	-	1.0a	1.0a	1.0a
	V-S98-1 (B1)	-	1.7b	1.7b	2.3c
	V-S98-15 (B15)	-	1.3a	2.3c	1.3a
	Control	+	-	1.0a	1.0a
	V-S98-1 (B1)	+	-	2.0c	2.3c
	V-S98-15 (B15)	+	-	1.3a	1.7b
BPMV+SMV	**Control**	-	1.0a	1.0a	1.0a
	B1+S38	-	2.5d	2.3d	2.5d
	B15+S38	-	2.5d	3.0e	3.0e
	B1+S52	-	3.3e	3.0e	3.0e
	B15+52	-	3.3e	-	3.3e
	Control	+	-	1.0a	1.0a
	B1+S38	+	-	2.4d	2.9e
	B15+38	+	-	3.0e	2.8d
	B1+S52	+	-	3.3e	3.3e
	B15+S52	+	-	2.7d	3.0e
SMV	**Control**	-	1.0a	1.0a	1.0a
	2K-38 (S38)	-	2.3c	2.3c	2.3c
	S98-52 (S52)	-	2.3c	2.7d	2.5d
	G1	-	1.0a	1.0a	1.0a
	Control	+	-	1.0a	1.0a
	2K-38 (S38)	+	-	1.7bc	2.0c
	S98-52 (S52)	+	-	2.3cd	2.0c
	G1	+	-	1.0a	1.0a

Numbers within a column followed by the same letter as their corresponding control are not significantly different ($P \leq 0.05$; Tukey's Studentized Range (HSD) test).

Table 5.2: The relative virus accumulation of virus in Hutcheson, Hutcheson Roundup® Ready (RR) BC5 and BC3, singly or doubly inoculated with *Bean pod mottle virus* isolates or *Soybean mosaic virus* (SMV) isolates.

Virus	Absorbance at 405 nm of two viruses in Hutcheson and two isolines				
	Treatment	**Herbicide**	**Hutcheson**	**Hutcheson RR BC3**	**Hutcheson RR BC5**
BPMV	**Control**	-	1.337a	1.410a	0.918a
	V-S98-1	-	1.410a	1.642b	1.414b
	V-V-S98-15	-	1.478a	1.637b	1.120c
	Control	+	-	0.133a	0.639a
	V-S98-1	+	-	1.210c	1.037b
	V-V-S98-15	+	-	1.176b	1.202c
BPMV+SMV	**Control**	-	0.082a	0.996a	0.723a
	B1+S38	-	2.537b	2.431b	2.508b
	B15+S38	-	2.701b	2.741b	2.603b
	B1+S52	-	2.723b	2.419b	1.990b
	B15+52	-	2.735b	-	2.654b
	Control	+	-	0.753a	0.656a
	B1+S38	+	-	2.593b	2.767b
	B15+38	+	-	2.747b	2.725b
	B1+S52	+	-	2.877b	2.583b
	B15+S52	+	-	2.732b	2.837b
SMV	**Control**	-	0.882a	1.088a	0.695a
	2K-38	-	2.184b	1.957c	1.802d
	S98-52	-	1.685b	1.550b	0.831b
	G1	-	0.775a	0.133d	0.111c
	Control	+	-	0.248a	0.245a
	2K-38	+	-	1.288c	1.440c
	S98-52	+	-	1.647d	1.479c
	G1	+	-	0.952b	0.805b

Numbers within a column followed by the same letter as their corresponding control are not significantly different ($P \leq 0.05$; Tukey's Studentized Range (HSD) test).

Values are the mean absorbance at 405 nm from a 1:50 (w/w) leaf extract by DAS-ELISA (BPMV) or Indirect ELISA (SMV).

Table 5.3. The relative accumulation of virus calculated as the ratio of absorbance at 405 nm in ELISA for double *Bean pod mottle virus* and *Soybean mosaic virus* (BPMV+SMV) inoculation versus single BPMV inoculation or single SMV inoculation.

Treatment	Ratio of ELISA values for double:single inoculations				
	Cultivar	Herbicide	BPMV+SMV:BPMV	SMV+BPMV:SMV[a]	BPMV:SMV
B1+S38	H[b]	-	1.78	1.16	**1.53**
	HR1	+	2.67	1.92	**1.39**
	HR1	-	1.77	1.39	**1.27**
	HR2	+	2.14	2.01	**1.06**
	HR2	-	1.48	1.24	**1.19**
B1+S52	H	-	1.93	1.62	**1.19**
	HR1	+	2.49	1.75	**1.42**
	HR1	-	1.41	2.40	**0.59**
	HR2	+	2.38	1.75	**1.36**
	HR2	-	1.47	1.56	**0.94**
B15+S38	H	-	1.83	1.24	**1.48**
	HR1	+	2.27	1.89	**1.20**
	HR1	-	2.32	1.44	**1.61**
	HR2	+	2.34	2.13	**1.10**
	HR2	-	1.67	1.40	**1.19**
B15+S52	H	-	1.85	1.62	**1.14**
	HR1	+	2.36	1.92	**1.23**
	HR1	-	2.37	3.19	**0.74**
	HR2	+	2.32	1.66	**1.40**

Relative accumulation of virus calculated as ratios of BPMV+SMV:BPMV or SMV+BPMV:SMV. Values are from mean absorbance at 405 nm of duplicate wells in ELISA at 1:50 dilution of leaves sampled at 11 weeks after inoculation.

[a]Analysis of virus accumulation for SMV in single and double inoculations were performed by A. Fayad.

[b]H = Hutcheson, HR1 = Hutcheson Roundup Ready® 1 (Hutcheson RR BC5) and HR2 = Hutcheson Roundup Ready® 2 (Hutcheson RR BC3).

Table 5.4: The plant height (cm) of Hutcheson, Hutcheson RoundupReady® (RR) BC3 and BC5, treated in single inoculations with *Soybean mosaic virus* (SMV) isolates or single inoculations with *Bean pod mottle virus* isolates, and in double inoculations with both SMV and BPMV. Hutcheson Roundup Ready soybeans were either herbicide treated or untreated.

Virus	Treatment	Herbicide	Hutcheson	Hutcheson RR BC3	Hutcheson RR BC5
BPMV	**Control**	-	57.9a	59.2a	58.7a
	V-S98-1	-	54.8a	51.6ab	54.1a
	V-S98-15	-	52.9ab	53.7a	51.1ab
	Control	+	-	52.0a	59.3a
	V-S98-1	+	-	53.4a	52.6a
	V-S98-15	+	-	47.7ab	49.3ab
BPMV+SMV	**Control**	-	63.4a	59.4a	56.9a
	B1+S38	-	44.4b	43.4b	41.7b
	B15+S38	-	44.2b	35.4c	38.2c
	B1+S52	-	22.7c	31.6c	34.7c
	B15+52	-	27.9c	-	31.9c
	Control	+	-	62.9a	62.9a
	B1+S38	+	-	40.6b	40.0b
	B15+38	+	-	42.7b	42.7b
	B1+S52	+	-	24.3c	26.2c
	B15+S52	+	-	36.1c	25.5c
SMV	**Control**	-	62.6a	60.3a	60.5a
	2K-38	-	58.3a	58.9a	50.3b
	S98-52	-	54.1b	54.4b	58.8a
	G1	-	66.1a	61.3a	59.0a
	Control	+	-	63.3a	66.2a
	2K-38	+	-	63.5a	61.0a
	S98-52	+	-	50.1b	54.9b
	G1	+	-	59.8b	70.0a

Numbers within a column followed by the same letter as their corresponding control are not significantly different ($P \leq 0.05$; Tukey's Studentized Range (HSD) test).

Table 5.5: The mean seed weight (g per row) of Hutcheson, Hutcheson Roundup® Ready (RR) BC3 and BC5, treated in single inoculations with *Soybean mosaic virus* (SMV) isolates or single inoculations with *Bean pod mottle virus* isolates, and in double inoculations with both SMV and BPMV. Hutcheson Roundup Ready soybeans were either herbicide treated or untreated.

Virus	Treatment	Herbicide	Hutcheson	Hutcheson RR BC3	Hutcheson RR BC5
	Control	-	56.0a	21.7b	55.5a
BPMV	V-S98-1	-	39.3b	33.8a	20.1c
	V-S98-15	-	37.0b	32.9a	15.3c
	Control	+	-	39.2a	32.5a
	V-S98-1	+	-	24.4b	14.6c
	V-S98-15	+	-	22.1b	13.1c
BPMV+SMV	**Control**	-	55.8a	31.3a	14.2a
	B1+S38	-	9.5d	10.5c	3.2e
	B15+S38	-	1.3e	4.9f	0.3f
	B1+S52	-	0.0f	1.7f	2.3e
	B15+52	-	1.9e	-	0.0f
	Control	+	-	22.4a	19.6a
	B1+S38	+	-	2.6f	2.8f
	B15+38	+	-	2.7f	2.7f
	B1+S52	+	-	2.9f	2.6f
	B15+S52	+	-	2.7f	2.8f
SMV	**Control**	-	50.7a	33.0ab	27.1a
	2K-38	-	37.8b	37.0ab	16.5b
	S98-52	-	30.4b	27.7b	18.8b
	G1	-	63.2a	32.5ab	25.9a
	Control	+	-	35.0a	23.4a
	2K-38	+	-	33.6a	21.0b
	S98-52	+	-	14.4c	15.9c
	G1	+	-	36.7a	22.1a

Numbers within a column followed by the same letter as their corresponding control are not significantly different ($P \leq 0.05$; Tukey's Studentized Range (HSD) test).