## Chapter Three

## Reaction to and Impact of *Bean pod mottle virus* (BPMV) and *Soybean mosaic virus* (SMV) on Soybean Breeding Lines and Cultivars


### ABSTRACT

Soybean (*Glycine max* (L.) Merrill) breeding lines and cultivars in the process of being released to growers were inoculated with BPMV isolate V-S98-1 or SMV G1, in 2001, 2002 and 2003, in order to monitor their reactions to these viruses in terms of foliar symptom severity and seed coat mottling. There were no relationships between the foliar symptom severity scores, mottling severity and the seasons of plant production, demonstrating that seasonal variations have no effect on virus activity and do not affect foliar symptom severity or seed coat mottling in soybeans. In 2003, one block each inoculated with inoculated with BPMV and SMV, and a block of non-inoculated control were monitored to determine the effects of the isolates on foliar symptom severity, seed coat mottling and seed weight. The seed size, (g/100 seeds) in 2003, across all cultivars, did not vary among the treatments. Leaf blot immunoassay demonstrated that 72.6% and 85.5% of the rows were successfully inoculated with BPMV V-S98-1 and SMV G1, respectively. The overall results indicated that SMV G1-inoculated plants had higher foliar symptom severity score of 2.0, which increased to 2.6 during the second reading, while BPMV V-S98-1-inoculated plants increased from 1.1 to 2.8, and the non-inoculated ones increased from 1.1 to 1.5. However, the relative accumulation of SMV shown by enzyme-linked immunosorbent assay (ELISA) did not always correspond with foliar symptom severity scores. There were entries that recorded high foliar symptoms but showed lower ELISA values and vice versa. For example, DP5644RR had a foliar symptom severity score of 2.0 and high virus accumulation with ELISA of 1.673, and a mottling score of 4.0, a case of consistently high scores. V46NRS scored 1.0 and 2.0 at foliar symptom severity1 and 2, respectively, seed coat mottling of 4.0 and ELISA value of 1.213 and seed weight of 12.1 g, an indication of low foliar score and a high ELISA value.



**KEY WORDS:** Cultivars, Soybean breeding lines, maturity group

## INTRODUCTION

The performance of different soybean cultivars when subjected to the most virulent isolates of *Bean pod mottle virus* (BPMV, Genus *Comovirus*; Family *Comoviridae*), V-S98-1 and *Soybean mosaic virus* (SMV, Genus: *Potyvirus*; Family, *Potyviridae* strain G1, commonly found in Virginia, is an important step in determining the effectiveness of a cultivar as an ideal candidate seed source, for soybean production. Soybean cultivars released by breeders to growers for soybean production, are subject to diseases caused by viruses (Phipps and Tolin, 2001). Two viruses of importance in Virginia soybean are BPMV and SMV (Tolin and Roane, 1975; Roane and Tolin, 1977; Phipps and Tolin 2001). Reports and studies from soybean growing states show that BPMV is increasing in most areas and that the virus may become a major problem in all soybean growing regions of the USA (Giesler et al., 2002). SMV is present wherever soybean is grown worldwide (Hartman et al., 1999). These two viruses cause losses in soybean when inoculated singly or in double inoculations. Yield losses of 3-52%, 8-35%, and 86% for BPMV, SMV and BPMV/SMV, respectively, have been recorded (Hartman et al., 1999). The presence of SMV strain G1 (Cho and Goodman, 1979; Hunst and Tolin, 1982) and BPMV isolate V-S98-1 (Gu et al., 2001), have been confirmed in Virginia, as described in Chapter 2.

Although a number of soybean cultivars have resistance to SMV based on the presence of *Rsv1* gene (Roane et al., 1983; Buss et al., 1988; Chen et al., 1991), resistance to BPMV in commercial cultivars is currently unavailable. Since the identification of *Rsv1*, a number of resistance genes to SMV in different soybean cultivars have been identified (Chen et al., 1991). A potentially valuable SMV resistance gene is the *Rsv4* gene in PI 486355 and PI 88788, which are cultivars resistant to all characterized strains of SMV (Ma et al., 1995; Hayes et al., 2000; Gunduz et al., 2004). However, reports from the field show that resistance to SMV in notable cultivars like Hutcheson with *Rsv1y* is being challenged by new strains of SMV. Studies have demonstrated the existence of resistance breaking (RB) strains of SMV (Fayad, 2003).

In the USA, soybean growers select maturity groups (MG) that are adapted to particular regions. Daylength and other environmental factors influence suitability of a soybean cultivar to an area (Dong et al., 2003). The relationship between the length of

day and flowering behavior of soybean was recognized in 1920 by Gamer and Allard (Piper and Morse, 1923). This understanding of photoperiodism has enabled breeders to produce soybean cultivars suited to different regions, worldwide. In North America, soybeans are classified into 13 maturity groups (MG) based upon the effects of daylength and the period to appearance of the first flowers. These groups are then designated by Roman numerals from the earliest (000) to the latest (X) maturing. Maturity group zone, though non-specific at times, defines specific regions where particular cultivars are most suited for production. Three MG zones are (1) MG 000, 00, 0 and I, (2) MG II, III, IV, V, VI and VII and (3) MG VIII, IX and X (Heatherly and Elmore, 2004). Soybean varieties have also been categorized based on seed size as small-seeded, conventional varieties and large-seeded varieties. These categories are usually given by weight as $\leq$80 mg/seed (small), conventional weight falls anywhere from >80 mg/seed to 200 mg/seed and 220 mg/seed are grouped as large (Heatherly and Elmore, 2004).

Virginia is subdivided into three soybean growing zones and uses MGs, IV, V and VI. Cropping systems greatly influence choices of maturity, disease and herbicide resistance in a particular cultivar or groups of cultivars (Buss and Holshouser, 2001). At least 100 public and private cultivars and breeding lines are offered every year to growers, hence these cultivars must be subjected to tests in Uniform Variety Trials (UVT) and Virginia Soybean Variety Evaluation Tests (VSVT) or Official Variety Trials (OVT) in order to evaluate performance (Buss and Holshouser, 2001). Uniform variety trials are tests on available cultivars or lines, while official variety trials are tests by breeders on new cultivars and lines. The objectives of this study are to evaluate the reaction of breeding lines and cultivars to SMV G1 and BPMV V-S98-1, observe any quality losses, as judged by seed coat mottling, determine any relationship between seed coat mottling and maturity group or seed size, and finally determine if any seasonal variations have effects on cultivars when they are subjected to the same isolates over several growing seasons. In addition, the effects of maturity groups (MG) were considered.

## MATERIALS AND METHODS

**Experimental design and planting of seeds:** Soybean seeds used in this study (Appendix B) were those contributed by breeders for field tests and eventual recommendation for use by soybean growers, nationwide. The seeds were supplied by G. R. Buss (Crop and Soil Environmental Sciences, Virginia Tech), and also planted with the help of his team at Glade Road Soybean Nursery, Virginia Tech, Blacksburg.

In 2001, 2002 and 2003, seeds were planted using complete block design, in single rows of 1 m long with one block for BPMV and a similar block for SMV. For 2001 and 2002, planting dates were June 12 and 19, respectively. In 2003, planting was done on June 27, using the same design, but a block of non-inoculated control was included. In total, 256, 290, and 184 entries were planted in 2001, 2002 and 2003, respectively. Five cultivars Lee 68, Essex, York, V73-178 and Manokin were planted as checks, where Lee 68, Essex and Manokin are susceptible to SMV, and York and V73-178 are resistant to SMV G1, G2 and G3.

**Virus isolates and inoculation:** The effects of one BPMV isolate, V-S98-1 and one SMV strain, G1 were evaluated on soybean cultivars entered in the OVT and UVT, in 2001, 2002 and 2003. The BPMV isolate V-S98-1 was selected from the isolates collected and described in Chapter 2. The BPMV isolate also produced more severe symptoms on susceptible soybean cultivars in hill plot experiments (Chapter 2) compared to other BPMV isolates. The selection of SMV G1 was also based on the fact that it is common SMV in Virginia soybean, and had been previously used for genetic studies. SMV G1 was the same as described by Hunst and Tolin (1982). The isolates had also been shown in other studies to cause severe symptoms on most cultivars, except those resistant to SMV.

The source of inoculum for BPMV was the cv. Marshall propagated in the greenhouse and inoculated with BPMV V-S98-1 at least 3 weeks before the time of field inoculation. Inoculum was prepared by grinding 25 g of young refrigerated leaflets harvested from young trifoliolate leaves, after removing the stems. The tissues were ground in a Waring Blendor (Model 5011, Waring Corp., New Hartford, CT) containing 225 ml of cold 0.01 M sodium phosphate buffer at pH 7.0. The mixture was then strained through four layers of cheesecloth into a 600 ml beaker placed in a container of crushed

ice. Another 25 ml of the buffer was used to rinse the cup and bring the volume to 250 ml. For each 250 ml of inoculum, 1.25 g of 600 mesh carborundum (Buehler, Lake Bluff, IL) was added and agitated, thus bringing the final concentration to 1 g of tissue per 10 ml of buffer, and 0.5% carborundum. To avoid any deterioration of the virus, the inoculum was stored in a container placed in crushed ice throughout the time of inoculation.

The SMV inoculum was prepared by grinding 25 g of fresh leaves harvested from cv. Essex or Lee 68 propagated in the greenhouse and inoculated with SMV G1, and inoculated for at least 2.5-3 weeks, as above. The tissues were ground in 250 ml of 0.5 M sodium citrate buffer, 0.5% carborundum was added. Inoculum was stored in ice throughout the time of use.

The inoculum was applied by force with an artist's air brush, spraying approximately 0.2 ml per plant, on the lower surface of one of the young trifoliolate leaves, at a pressure of approximately 4.57 kilograms per square centimeter ($kcm^{-2}$) (Roane et al., 1983; Chen et al., 1991). An "oily", water-soaked spot on the lower part of an inoculated leaf, confirmed the penetration of the leaf cuticle by the inoculum. The air brushes were properly rinsed with buffer to ensure no cross contamination occurred and were filled with one virus until a complete inoculation of all the plants was done, before changing to the second virus.

**Assessment of responses of plants to virus treatment:** Two methods of foliar assessment were used to score symptom severity of BPMV and SMV inoculated plants. The scores were either numerical on a scale of 1-5, as described in Chapter 2, and for SMV the scores were marked in 2001, as susceptible or resistant (S or R). The S or R scale was not used with BPMV because there are no commercial cultivars with resistance to BPMV. Because not all breeding lines and cultivars were replicated across all the three years, only twenty four that were replicated in each year are considered in detail.

**Immunosorbent assays:** In 2003, plant response to virus inoculation was also assessed using leaf blot immunoassay for SMV and BPMV, as described in Chapter 2. Samples from the non-inoculated control were also tested to assess natural spread of virus in the plot. In 2003, relative virus accumulation of SMV in selected cultivars from the UVT

was quantified in order to ascertain the association between virus titer, symptom severity and the severity of seed coat mottling.

Indirect ELISA was done on SMV inoculated plants as described by Koenig (1981), using fresh trifoliolate leaves from symptomatic plants taken 11 week post-inoculation. One leaf was selected from each of three symptomatic plants per row. Extraction of fresh tissue was done using 0.05 M sodium carbonate at pH 9.6, 1% polyvinyl pyrrolidone MW = 40,000 (PVP) for SMV. Six leaf disks (9 mm) made using a # 5 cork borer were ground in 6 ml of the grinding buffer using Tekmar® Tissumizer (Cincinnati, OH), giving an end dilution of 1:50 (w/v). The extracts were then incubated overnight in Dynatech Immuno™ 96-well plates at 4°C. Duplicate wells containing 200 µl of antigen extract were used for each sample. After incubation the plates were washed 3 times using 1X phosphate buffered saline + 0.05% Tween-20, PBS-Tween (PBS-T) (Agdia) at room temperature. The plates were then incubated at 37°C for 2 hr with polyclonal antibody (1:10,000) raised against SMV particles (Hunst and Tolin, 1982). This was followed by three washings with PBS-Tween, then the plates were incubated at 37°C with goat anti-rabbit IgG (whole molecule) conjugated with alkaline phosphate (1:15,000) (Sigma-Immuno Chemicals St Louis, MO). The plates were washed three times as above with PBS-Tween. After the final wash, paranitrophenyl phosphate (pNPP) substrate (Sigma®) was added and absorbance recorded at 405 nm every 15 min using Spectramax plate reader (Molecular Devices, Sunnyvale, CA) up to a maximum of 60 min.

**Assessment of effects on seed:** In 2001, 2002 and 2003, the seeds were hand harvested and machine threshed from three plants per row, except in 2001 when all the threshing was done by hand. Severity of seed coat mottling was determined on a scale of 1-4, where 1 was 0% mottling, 2 = 1-25% mottling, 3 = 26-75% mottling, 4 = 75-100% mottling.

**Statistical analysis:** Complete block design was used to compare the effects of virus isolates on cultivars. The data obtained were analyzed using PROC REG and 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

When the results of foliar symptom severity scores and seed coat mottling scores across all the 24 breeding lines and cultivars were subjected to regression analysis, no seasonal effects were established. The analysis shown in Table 3.1 therefore indicate that there were no relationships among the foliar symptom severity scores, mottling severity and the seasons of plant production.

The results of foliar symptom severity and seed coat mottling scores on five selected individual cultivars during the three years (2001, 2002, and 2003) show that there were variations in response(s) among the different cultivars and lines, Figures 3.1 and 3.2 for BPMV and SMV, respectively. Manokin treated with BPMV V-S98-1, for example, showed high foliar symptom during 2001 season but had reduced symptom severity in 2002 as well as in 2003. However, the mottling score remained the same throughout the three seasons. DP5644RR exhibited progressively higher severe foliar symptom during the three seasons, while the mottling scores fluctuated from 4.0 in 2001, to 1.8 (2002) and then increased to 4.5 in the third year. The same trend was observed in SMV-treated plants (Figure 3.2). The results of indirect ELISA showed consistent absorbance values that ranged from 0.078 at foliar symptom severity scores of 3.0 and 4.0 for 7522nRR to 1.673 at foliar symptom severity of 3.0 and 4.0 for DP5644RR. However, the relative SMV accumulation, did not always correspond with foliar scores, since there were occasions when high foliar symptoms showed lower ELISA values and vice versa. The ELISA value was high for Lee 68 but the cultivar had low foliar symptom severity score. Conversely, S46-W8 had both high foliar symptom severity score of 2.0 and 3.0, low virus accumulation with ELISA reading of 0.105, and a seed coat mottling score of 2.8.

The results of leaf blot immunoassay of all 62 rows sampled in 2003 are shown in Table 3.2. The results demonstrate that 72.6% and 85.5% of the rows were successfully infected with BPMV V-S98-1 or SMV G1, respectively. The non-inoculated plants showed infection in 4.8% of the rows. These represented 15, 17 and one infected plant(s)

out of 20 plants in each 1 m row, for BPMV, SMV and non-inoculated controls, respectively.

The overall effects of SMV G1, BPMV S98-1 and no virus treatments in 2003, are shown in Tables 3.3. The effect of BPMV on symptom severity and seed size were different from the control and SMV, but the effect of SMV though analyzed together, consisted of both susceptible and resistant cultivars and lines. Seed sizes (g per 100 seeds) were therefore not different among treatments. Table 3.4 shows the responses to BPMV S98-1 inoculation and the non-inoculated control also distributed by MG relative to foliar symptom severity, seed coat mottling and seed weight. The non-inoculated control plants scored low at 1.0 during the first and second foliar symptom severity readings and mottling scores were consistently lower. Seed weights were high with only eight cultivars recording less than 10 g per 1 m row. Four out of six MG 5E registered < 10 g per row indicating small seeded cvs. BPMV-inoculated plants showed lower first foliar severity scores except the two of the five checks. At the second foliar symptom severity reading a value as high as 4.0 was recorded for RT-3799N. The seed mottling scores were also consistently higher than those recorded for corresponding non-inoculated entries. BPMV had little effect on seed size, as in twelve of 19 lines, seeds from inoculated plants were equal to or greater than non-inoculated plants. The seed weights recorded for MG 5E were all <10 g.

In Table 3.5, the effect of SMV G1 on cultivars, are shown based on the same parameters considered for BPMV-inoculated plants. Small ($\leq$80 mg/seed) seeded lines MFS-553, MFS-591 and RT-3975, a large seeded cultivar, belonging to MG 5E, 5L and 3, respectively, recorded similar foliar symptom severity scores between 2.5-5.0, and ELISA values of 1.038, 1.235 and 1.108, respectively. However, the seed coat mottling scores were 1.3, 2.4 and 4.0 for MFS 553, MFS 591 and RT-3975, respectively. Seed weight of RT-3975 was reduced by SMV inoculation by 65%. The same variations among the medium conventional seeded cultivars were also observed. All the MG 5E plants recorded high ELISA values of 0.8525 to 1.6725 with seed weight <10 g, only two cultivars showed seed weights < 10 g on MG 4E and 4L in SMV-inoculated plants.

The relative distribution of foliar symptom severity scores (Foliar1 and Foliar2) of 62 soybean cultivars and lines planted in 2003 and inoculated with BPMV, SMV and

non-inoculated control are shown in Figure3.3. Figure 3.4 shows the relative distribution of seed weight (g) of the same 62 cultivars and lines. The relative distribution shows that foliar symptom severity scores taken during the first reading (foliar1) were distributed 1.0-3.0 for BPMV, 1.0-4.0 for SMV and 1.0-2.0 for non-inoculated plants. However, foliar2 were 1.0-3.0 for non-inoculated control and 1.0-5.0 each for BPMV and SMV, respectively. The relative distributions of cultivars and lines based on seed size and foliar symptom severity, showed a very narrow, narrow, and wide distributions for non-inoculated, BPMV and SMV, respectively. Medium (conventional) seed and large seed weights were similarly distributed.

## DISCUSSION

All breeding lines and cultivars to BPMV V-S98-1 and SMV G1, used in this study, exhibited varied responses that are cultivar dependent. This is because the level of foliar symptom severity recorded for each cultivar did follow any uniform pattern but was randomly distributed among different cultivars, and over the different years (Figures 3.1 and 3.2). The overall analysis to show the relationship between foliar symptom severity, seed coat mottling and season variations, did not detect any significant interactions. This therefore shows that foliar symptom severity scores may not be reliable as predictors of seed coat mottling. Although some cultivars scored higher on foliar symptom severity, seed coat mottling scores were relatively low.

The results of the comparison of the overall mean of foliar symptom severity and seed size (g/100 seeds) across all 62 breeding lines and cultivars for each block treated with BPMV or SMV, and non-inoculated control, in 2003 show that SMV G1 had high foliar symptom severity scores at the first reading, which increased in the second reading. BPMV V-S98-1 increased from 1.1 to 2.8 and the non-inoculated also had increased from 1.1 to 1.5. SMV G1 remained unchanged. The overall seed size, (g/100 seeds), across all cultivars did not vary among the treatments. An indication that some field spread may have contributed to more infection, or that severity increased over time. At the first reading there were more entries showing more severe symptoms with SMV than with BPMV. In both Figures 3.3 and 3.4 some natural spread in the field or from seed

transmission may have contributed to the number of cultivars exhibiting symptoms, especially in the control.

Breeders and growers use foliar symptom severity to select for resistance to virus. When high foliar symptoms are observed in the field, this is anticipated to reflect high seed coat mottling when the seeds are harvested from the diseased plants. The relationship between SMV accumulation and seed coat mottling was shown to be inconsistent across cultivars and years (Koning et al., 2003). Our ELISA results also showed mixed relationship among entries and breeding lines in terms expression of foliar symptom severity and seed coat mottling for SMV. For detection of virus in the seed, ELISA remains a reliable method, but the absence of seed coat mottling does not mean the absence of virus in the seed. Methods of detecting viruses in growing plants that growers and breeders can use therefore remains a critical factor in predicting the effects of viruses on quality and yield. To assist growers and breeders, more research into ways monitoring virus disease during a growing season and its effect on seed quality and yield loss must be done in order to create reliable methods comparable to ELISA that can be used in the field. Even though ELISA may have shown a number of inconsistent values to foliar symptom severity scores and seed coat mottling in the cultivars and lines, it is still a more reliable method of quantitating virus accumulation.

The study has shown that it is unreliable to predict seed coat mottling from foliar symptom severity, even though foliar symptoms have always been used to assess resistance. Our study suggests that better methods such as ELISA and molecular techniques need to be exploited in order to come up with ways to relate seed coat mottling and foliar severity symptoms in the field. At present, selection for non-mottled seeds remains the only way of reducing the impact of BPMV on seed quality.

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**Table 3.1.** The results of regression analysis to establish the relationship between foliar symptom severity and seed coat mottling, of 24 breeding lines and cultivars, among the seasons 2001, 2002 and 2003. The plants were treated with *Bean pod mottle virus* (BPMV), or *Soybean mosaic virus* (SMV) and non-inoculated control, in 2003. The readings were taken at 30 days post inoculation (dpi) and 66 dpi.

| **Source** | **DF** | **Squares** | **Square** | **F Value** | **Pr>F** |
|---|---|---|---|---|---|
| Model | 2 | 0.07163 | 0.03581 | 0.03 | 0.9679 |
| Error | 69 | 75.66712 | 1.09662 | | |
| Corrected Total | 71 | 75.73875 | | | |

| **Variable** | **DF** | **Parameter Estimate** | **Standard Error** | **t Value** | **Pr>\|t\|** |
|---|---|---|---|---|---|
| Intercept | 1 | 19.52645 | 302.60261 | 0.06 | 0.9487 |
| Year | 2 | -0.0833 | 0.15115 | -0.06 | 0.9562 |
| Cultivar | 23 | -0.00445 | 0.01783 | -0.25 | 0.8037 |

**Table 3.2.** The percentage of rows sampled that were infected, across 62 breeding lines and cultivars for each block treated with *Bean pod mottle virus* (BPMV), or *Soybean mosaic virus* (SMV) and non-inoculated control, in 2003, as determined by leaf blot immunoassay.

| **Virus** | **Positive Blots** | **Total Rows Sampled** | **% of Rows Infected** |
|---|---|---|---|
| BPMV | 45 | 62 | 72.6 |
| SMV | 53 | 62 | 85.5 |
| Non-inoculated (Control) | 3 | 62 | 4.8 |

**Table 3.3.** Comparison of the overall mean of foliar severity symptoms (1-5) and seed size (g/100 seeds) across 62 breeding lines and cultivars for each block treated with *Bean pod mottle virus* (BPMV), or *Soybean mosaic virus* (SMV) and non-inoculated control, in 2003. The readings were taken at 30 days post inoculation (dpi) and 66 dpi.

| **Virus Treatment** | **Foliar Score 1 (1-5)** | **Foliar Score 2 (1-5)** | **Seed Size (g per 100 seeds)** |
|---|---|---|---|
| Non-inoculated (Control) | 1.1a | 1.5a | 10.6a |
| BPMV | 1.5b | 2.8b | 10.4a |
| SMV | 2.0c | 2.6b | 10.2a |
| HSD | 0.3 | 0.4 | 1.0 |

Numbers in the column followed by the same letter are not significantly different ($P \leq 0.05$; Tukey's Studentized Range (HSD) test)

**Table 3.4.** The foliar symptom severity scores (1-5), seed coat mottling scores (1-4) of twenty four selected breeding lines and cultivars, treated with *Bean pod mottle virus* (BPMV) and non-inoculated plants in 2003, distributed by maturity groups (MG). The foliar readings were taken at 30, 67 days post inoculation (dpi). Mottling scores and seed weight were taken after seed harvest.

| | | Non-inoculated Plants | | | | BPMV-inoculated Plants | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Cultivar** | **MG** | **Foliar1[a]** | **Foliar2** | **Seed Coat Mottling** | **Seed Wt (g)** | **Foliar1** | **Foliar2** | **Seed Coat Mottling** | **Seed Wt (g)** |
| MANOKIN | 4E | 1.0 | 1.0 | 1.0 | 9.7 | 2.0 | 3.0 | 2.0 | 8.9 |
| Essex | 5 | 1.0 | 1.0 | 1.8 | 12.4 | 2.0 | 3.0 | 1.6 | 8.7 |
| V73-178 | 5 | 1.0 | 1.0 | 1.0 | 9.0 | 2.0 | 3.0 | 1.4 | 8.4 |
| York | 5 | 1.0 | 1.0 | 1.0 | 10.7 | 2.0 | 3.0 | 3.0 | 9.5 |
| Lee 68 | 6 | 1.0 | 1.0 | 2.0 | 8.2 | 2.0 | 3.0 | 3.0 | 7.5 |
| RT-3799N | 3 | 1.0 | 1.0 | 1.0 | 13.4 | 1.0 | 4.0 | 2.5 | 14.6 |
| RT-3975 | 3 | 1.5 | 1.0 | 1.5 | 13.8 | 2.0 | 3.0 | 1.8 | 13.8 |
| DKB44-51 | 4E | 1.0 | 1.0 | 1.5 | 10.4 | 1.0 | 3.0 | 3.0 | 10.9 |
| DP4344RR | 4E | 1.0 | 1.0 | 1.5 | 11.9 | 1.0 | 2.5 | 2.8 | 12.7 |
| DP4690RR | 4E | 1.0 | 2.5 | 2.5 | 10.8 | 1.0 | 3.0 | 3.0 | 10.2 |
| RT-4098 | 4E | 1.0 | 2.0 | 1.0 | 12.8 | 1.0 | 3.0 | 2.5 | 12.6 |
| S46-W8 | 4E | 1.0 | 1.0 | 2.0 | 11.7 | 1.0 | 3.0 | 2.7 | 12.2 |
| V442NRR | 4E | 1.0 | 1.0 | 1.5 | 10.7 | 1.0 | 3.0 | 3.5 | 11.4 |
| V462NRS | 4E | 1.0 | 1.0 | 1.5 | 13.6 | 1.0 | 3.0 | 3.0 | 12.3 |
| 7522nRR | 4L | 1.5 | 1.0 | 2.0 | 7.8 | 2.0 | 3.0 | 1.8 | 8.8 |
| DK4868 (RR) | 4L | 1.0 | 1.0 | 1.5 | 11.7 | 1.0 | 3.0 | 4.0 | 12.0 |
| DP4748S | 4L | 1.0 | 1.0 | 2.0 | 12.0 | 1.5 | 3.0 | 4.0 | 12.5 |
| RT-4980 | 4L | 1.0 | 1.0 | 1.5 | 13.2 | 1.0 | 3.0 | 1.8 | 12.6 |
| DP5414RR | 5E | 1.0 | 1.0 | 2.5 | 10.1 | 1.5 | 3.0 | 5.0 | 9.7 |
| DP5644RR | 5E | 2.0 | 3.0 | 2.0 | 7.7 | 1.5 | 3.0 | 4.5 | 8.8 |
| MFS-553 | 5E | 1.0 | 1.0 | 1.0 | 5.3 | 1.0 | 3.0 | 2.1 | 5.7 |
| RT-5001N | 5E | 1.0 | 1.0 | 2.0 | 8.2 | 1.5 | 3.0 | 2.0 | 9.9 |
| RT-557N | 5E | 1.0 | 1.0 | 2.0 | 10.2 | 1.5 | 3.0 | 2.6 | 9.1 |
| MFS-591 | 5L | 2.0 | 1.0 | 1.0 | 5.1 | 1.0 | 3.0 | 1.3 | 4.7 |

[a]Two foliar symptom severity scores (1-5), foliar 1 and foliar 2 taken at 30, 67 days post inoculation.

**Table 3.5.** The foliar[a] symptom severity scores (1-5), seed coat mottling scores (1-4), corresponding ELISA values, and seed weight (Wt)(g) of twenty four selected breeding lines and cultivars treated with *Soybean mosaic virus* (SMV) in 2003, distributed by maturity groups (MG). The readings were taken at 30 and 67 days post inoculation. Mottling scores were taken after seed harvest.

| | **Maturity group, virus treatment, foliar symptom severity, seed weight and ELISA[b]** | | | | | |
|---|---|---|---|---|---|---|
| **Cultivar** | **MG** | **Foliar1** | **Foliar2** | **Mottling** | **Seed Wt** | **ELISA** |
| MANOKIN | 4E | 1.5 | 2.0 | 3.5 | 9.5 | 0.581 |
| Essex | 5 | 1.5 | 2.0 | 1.5 | 9.1 | 0.134 |
| V73-178 | 5 | 1.0 | 2.0 | 1.8 | 11.5 | 0.106 |
| York | 5 | 1.0 | 1.0 | 2.0 | 11.0 | 0.107 |
| Lee 68 | 6 | 2.0 | 3.0 | 1.5 | 8.4 | 0.913 |
| | | | | | | |
| RT-3799N | 3 | 3.0 | 4.0 | 3.0 | 12.5 | 0.808 |
| RT-3975 | 3 | 3.5 | 5.0 | 4.0 | 4.9 | 1.108 |
| DKB44-51 | 4E | 2.5 | 5.0 | 4.0 | 9.2 | 1.232 |
| DP4344RR | 4E | 2.5 | 4.0 | 4.0 | 13.9 | 0.854 |
| DP4690RR | 4E | 2.5 | 4.0 | 3.0 | 11.8 | 1.065 |
| RT-4098 | 4E | 2.0 | 3.0 | 3.0 | 13.0 | 1.008 |
| S46-W8 | 4E | 2.0 | 3.0 | 2.8 | 12.7 | 0.105 |
| V442NRR | 4E | 1.0 | 2.0 | 4.0 | 12.4 | 0.124 |
| V462NRS | 4E | 1.0 | 2.0 | 4.0 | 12.1 | 1.213 |
| 7522nRR | 4L | 3.0 | 4.0 | 3.0 | 9.9 | 0.078 |
| DK4868 (RR) | 4L | 1.5 | 3.0 | 4.0 | 13.2 | 0.139 |
| DP4748S | 4L | 1.0 | 2.0 | 1.0 | 10.0 | 0.171 |
| RT-4980 | 4L | 2.5 | 3.0 | 4.0 | 13.7 | 0.967 |
| DP5414RR | 5E | 1.0 | 2.0 | 2.0 | 9.0 | 1.267 |
| DP5644RR | 5E | 3.0 | 4.0 | 1.8 | 8.1 | 1.673 |
| MFS-553 | 5E | 4.0 | 5.0 | 1.3 | 1.4 | 1.038 |
| RT-5001N | 5E | 2.5 | 4.0 | 2.6 | 8.7 | 1.332 |
| RT-557N | 5E | 2.5 | 3.0 | 2.0 | 7.9 | 0.853 |
| MFS-591 | 5L | 3.0 | 4.0 | 2.4 | 4.2 | 1.235 |

[a]Two foliar symptom severity scores (1-5), foliar 1 and foliar 2 taken at 30, 67 days post inoculation (dpi).

[b] ELISA values are the mean absorbance at 405 nm from a 1:50 (w/v) leaf extract by indirect ELISA for SMV.

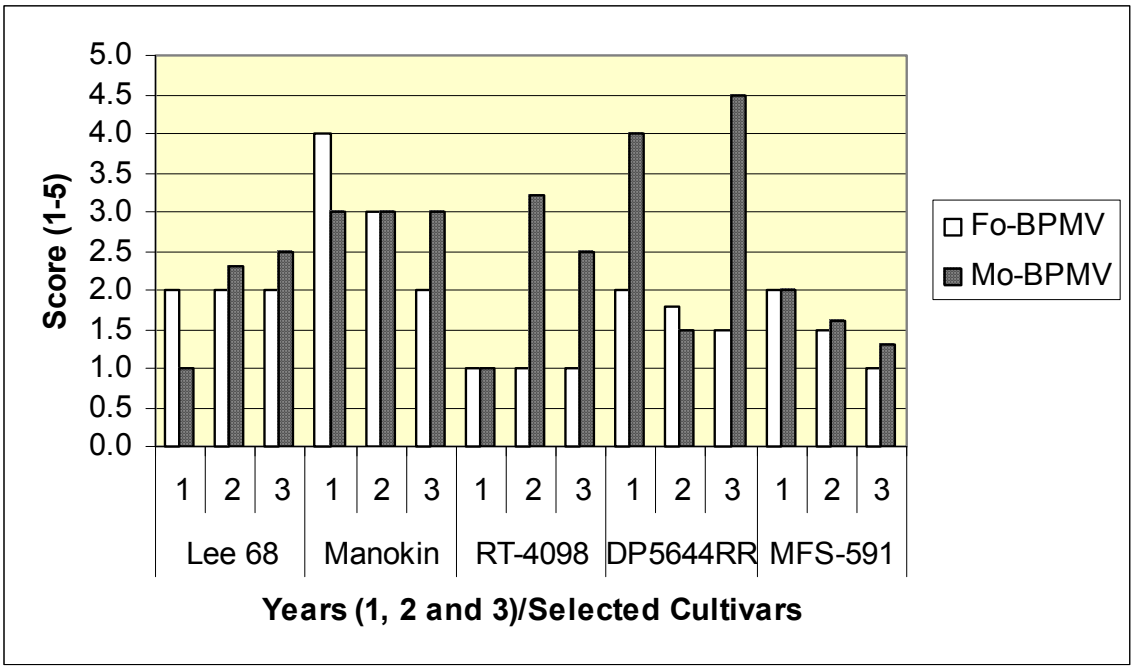



**Figure 3.1.** The foliar symptom severity scores (Fo-BPMV) and seed coat mottling (Mo-BPMV) scores of five of twenty four breeding lines and cultivars treated with *Bean pod mottle virus* (BPMV) in 2001, 2002 and 2003 (1, 2 and 3). The foliar readings were taken at 30 days post inoculation. Mottling scores were taken after seed harvest.

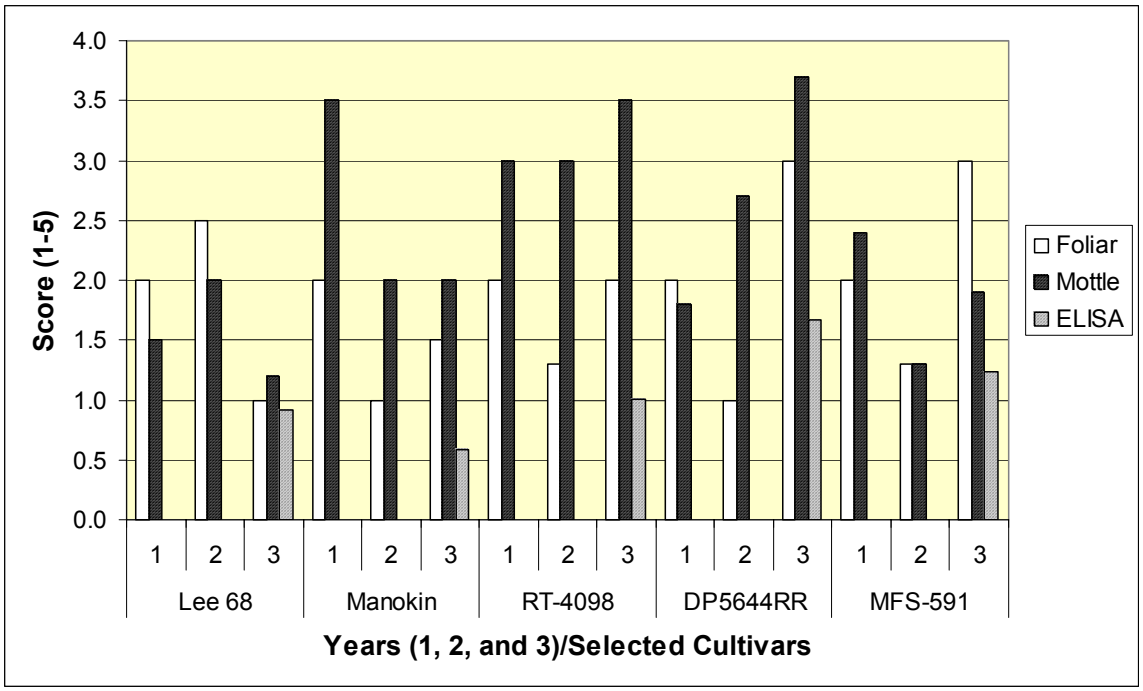
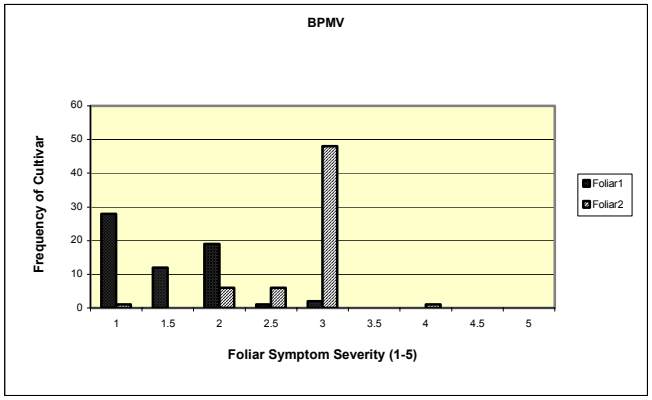
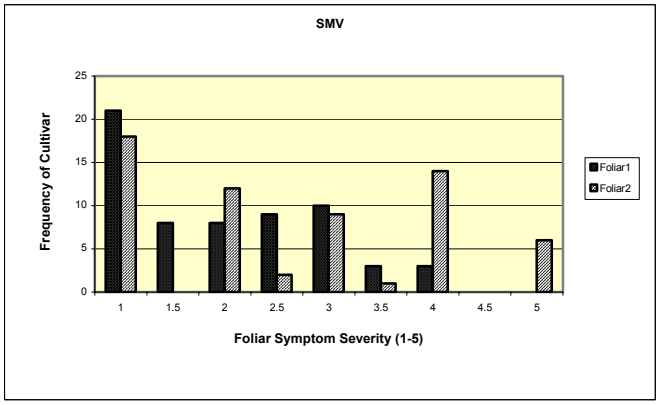


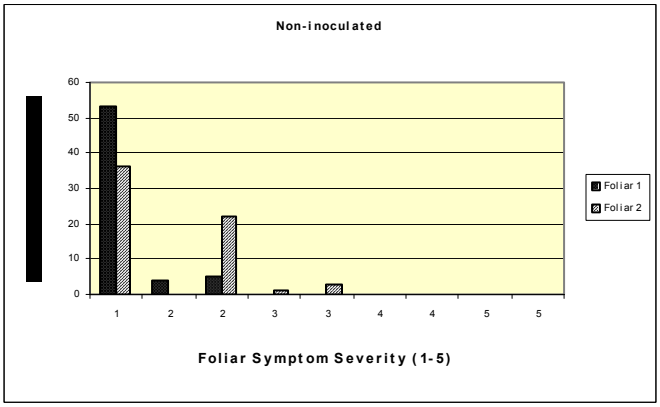

**Figure 3.2.** The foliar symptom severity scores (foliar), seed coat mottling scores (mottle), and corresponding ELISA values in 2003, of five out of twenty four selected breeding lines and cultivars treated with *Soybean mosaic virus* (SMV) in 2001, 2002 and 2003 (1, 2 and 3). The foliar readings were taken at 30 days post inoculation. Mottling scores were taken after seed harvest.




(A)

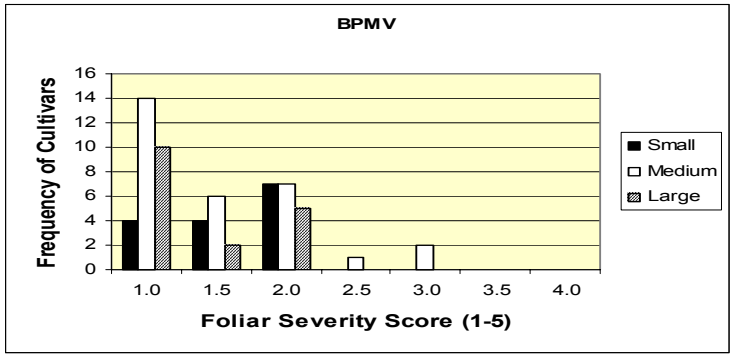



(B)

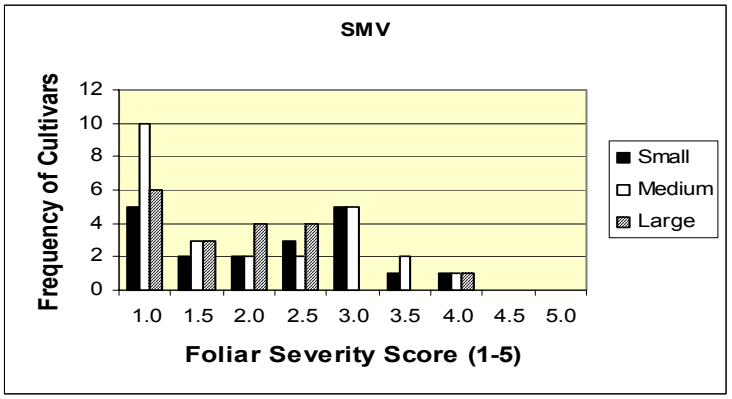



(C)

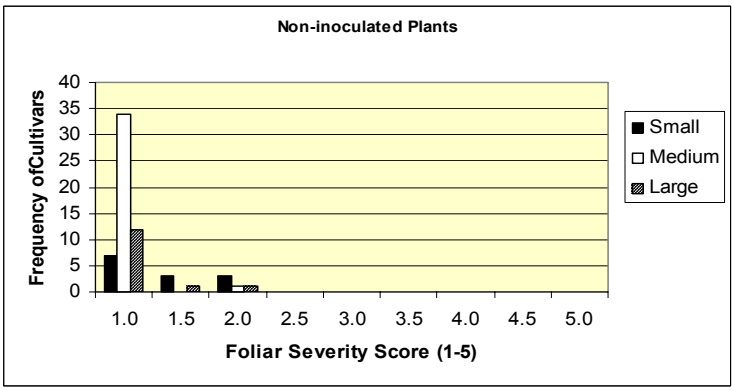
**Figure 3.3.** The relative distribution of 62 soybean lines and cultivars based on the level of foliar symptom severity score (1-5). The plants were inoculated with *Bean pod mottle virus* (BPMV) (A), *Soybean mosaic virus* (SMV) (B), or were non-inoculated (C) in 2003. The foliar readings were taken at 30 (foliar1) and 67 (foliar2) days post inoculation.




(A)




(B)




(C)

**Figure 3.4.** The relative distribution of 62 soybean lines and cultivars based on categorization by seed weight (small, medium and large) and the level of foliar symptom severity score (1-5). The plants were inoculated with *Bean pod mottle virus* (BPMV) (A), or *Soybean mosaic virus* (SMV) (B), and non-inoculated control (C) in 2003. The foliar readings were taken at 30 days post inoculation.