Chapter Four

Variation in Seed Coat Mottling of Soybean from Plants Inoculated with *Bean pod mottle virus* (BPMV) at Three Developmental Stage

ABSTRACT

The growth stage of soybean (*Glycine max* (L.) Merrill) at the time of inoculation was determined to be a factor in the severity of seed coat mottling caused by *Bean pod mottle virus* (BPMV, Genus *Comovirus*, Family *Comoviridae*). The virus caused seed coat mottling, and reduced seed size and seed weight. The effects of different isolates of BPMV on seed coat mottling in soybean were found to vary depending on cultivar and stage of development of the plants at the time of inoculation. In 2002 and 2003, the effects of three and four BPMV isolates V-S98-1, V-S98-15 and V-S01-10, and V-W2, V-S98-1, V-S98-15 and V-S01-10, respectively, were investigated by inoculating four soybean cultivars, Bolivar, L8-379, Manokin and Williams. In both 2002 and 2003, plants were inoculated at three developmental stages; vegetative stages V1-V3 and V4-V6, and reproductive stages R1-R3, in three replicates. In 2003, non-inoculated plants were included as controls. From the results of 2002, more obvious mottling was observed in early inoculated plants (V1-V3) than in plants inoculated at V4-V6 or R1-R3 stages of development. Generally, plants inoculated at R1-R3 produced less mottled seeds than those inoculated at earlier stages. Although the trends in seed coat mottling for early inoculated plants in 2003 were similar to those in 2002, some plants inoculated at R1-R3 in 2003, had higher incidence of mottling than those inoculated at the V1-V3 stage. Seed sizes also varied from V1-V3 to R1-R3 inoculation stages, with V1-V3 showing smaller seeds followed by V4-V6 and then R1-R3. The results show that the time of planting is a crucial decision if plants are to be protected from early inoculation by over-wintering leaf beetles. The time of planting can therefore be used as an important integrated pest or disease management component. A combination of judicious use of chemicals, correct planting dates and cultural practices like weed control, may substantially reduce early infection and improve quality as well as reduce virus-induced yield losses in soybean.

KEYWORDS: Seed coat mottling, BPMV, stage of development

INTRODUCTION

Seed coat mottling in soybean is exhibited as brown or dark brown streaks that radiate from the hilum (Hobbs et al., 2003; Koning et al., 2003). Seed coat mottling occurs due to accumulations of anthocyanins and leucoanthocyanins in the seed coat. According to Todd and Vodkin (1993), the accumulation of anthocyanins, which are anthocyanidin glycosides or that of proanthocyanidins takes place under the influence of recessive alleles *i, r* and *t*. The dominant alleles *I, R* and *T* produce yellow colored soybean seed. The presence of these recessive genes will therefore lead to seed coat mottling. Low chalcone synthase (CHS) mRNA activity, in soybean varieties that have the dominant *I* allele, causes pigmentation of seeds during seed development (Wang et al., 1994). Both Senda et al (2004) and Tuteja et al (2004) have proposed that posttranscriptional silencing of the chalcone synthase gene cluster by viral suppressor sequences may be responsible for pigmentation in the seed coat of black colored soybean seeds. This is similar to the effect of a spontaneous mutation in the dominant allele *I*, which also produces black colored soybean seeds (Senda et al., 2004; Tuteja et al., 2004; Wang et al., 1994).

Viruses cause seed coat mottling in soybean (Hill, 1987; Pacumbaba, 1995; Hobbs et al., 2003; Koning et al., 2003). *Bean pod mottle virus* (BPMV, Genus *Comovirus*, Family *Comoviridae*) has been shown to cause severe seed coat mottling depending on the varieties and the season (Hobbs et al., 2003). *Soybean mosaic virus* (SMV, Genus *Potyvirus*, Family: *Potyviridae*) also causes seed coat mottling when inoculated alone or in combination with BPMV (Hill, 1987; Bottenberg and Irwin, 1992; Pacumbaba, 1995; Hobbs et al., 2003; Koning et al., 2003). Yield losses of between 3-52% occur in single inoculation with BPMV (Gergerich, 1999). In double inoculations of BPMV and SMV on a susceptible soybean plant, yield losses of up to 86% have been recorded (Hartman et al., 1999).

BPMV is transmitted between plants in the field by leaf beetles in the family Chrysomellidae, Order Coleoptera. Other families include Coccinellidae and Meloidae (Hartman et al., 1999). Beetles listed as vectors of BPMV include *Ceratoma trifurcata*

Foster, *Diabrotica balteata* LeConte, *D. undecimpunctata howardii* Barber, *D. virgifera virgifera* LeConte, *Colaspis flavida* Say, *C. lata* Schaeffer, *Epicauta vittata* Fabricius, *Epilachna varivestis* Mulsant. *Ceratoma trifurcata* is the most efficient vector for the virus in soybean (Hartman et al., 1999; Mabry et al., 2003). Beetles acquire the virus as adults during feeding and the virus passes into the hemolymph, from whence the virus is passed out when the overwintering beetles feed on young soybeans, early the following season (Krell et al., 2003). Planting dates and the time when the viruliferous beetles first come into contact with the plant can affect the incidence of BPMV and severity of mottling (Ziems et al., 2001).

Seed coat mottling by BPMV is on the increase because of the increased incidence of BPMV in all soybean growing areas in the USA (Giesler et al., 2002). It is important to examine the critical stage at which plants are most vulnerable to BPMV. Studies by Ross (1969), Krell et al. (2003), and Ziems et al. (2003) have shown that severity of BPMV is associated with the growth stage of the plant when the virus is inoculated in plants. The objectives of this study are to determine the interactions among different isolates of BPMV on soybean cultivars and estimate the severity of seed coat mottling, and to study the effects of seed coat mottling on seedling germination and development.

MATERIALS AND METHODS

Soybean cultivars and virus isolates: Soybean used for this study included the cultivars Williams, Manokin, Bolivar, and the breeding line L8-379. These cultivars represented maturity groups (MG) III (Williams, L8-379), IV (Manokin), and V (Bolivar). Resistance to SMV was present in L8-379, an isoline of Williams with the *Rsv1* gene, and Bolivar, a newly released cultivar with the *Rsv1y* allele (Tyler and Young, 2004). The seeds were kindly provided by G. R. Buss (Crop and Soil Environmental Sciences, Virginia Tech). These experiments were conducted in the field at the Glade Road Nursery at Virginia Polytechnic Institute and State University, Blacksburg, Virginia in 2002 and 2003.

Four Virginia isolates of BPMV V-W2, V-S98-1, V-S98-15, and V-S01-10, collected and maintained in the greenhouse as explained in Chapter 2, were used. These

isolates have been classified in the study by Gu et al. (2002) into strain subgroups I (V-S98-1) or II (see Chapters 1 and 2).

Experimental design and virus inoculations: In 2002, seeds were hand planted on June 11 in 2.0 m rows, 24 seeds per row, with row spacing of 30 cm, using a completely randomized plot in split-plot design. The time of inoculation was the main plot, with virus and cultivars being the sub-plot and split-plot, respectively. In 2003, an expanded experiment used four soybean cultivars planted on July 3, 2003, in 0.5 m rows, at 12 seeds per row, with row spacing of 30 cm, using a completely randomized plot in split-plot design. The time of inoculation was the main plot, with virus and cultivars being the sub-plot and split-plot, respectively. The treatments were replicated three times for each virus isolate, inoculation time, and cultivar. Three replications of each cultivar were not inoculated, as controls.

The inoculum for each virus strain used in 2002 was prepared by taking 7 g of fresh leaf tissue from soybean cv. Marshall inoculated 1-2 weeks before inoculum preparation. The leaf tissue was ground in a Waring Blendor (Model 5011, Waring Corp., New Hartford, CT) in 50 ml of 0.01 M phosphate buffer at pH 7.0 (at 1:10, w/v), using separate containers for each isolate. The sap was strained through four layers of cheesecloth. Additional buffer was added to bring the final volume to 70 ml, which was then divided into three equal parts and placed in sealed tubes for use at different times in the field. Each aliquot of 20-25 ml of inoculum was sufficient to inoculate 60 plants. The tubes were stored at -20º C for at least 24 hr, but not longer than two months, before use. In 2003, approximately 25 g of frozen leaf tissue was ground in 250 ml of buffer for each virus strain using the same procedure, as described above.

In 2002, the planting was done during a period of drought hence seedling emergence was very uneven. The seedlings were therefore tagged as being at three different stages V1-V3, V4-V6, and R1-R3 (Johnson, 1997). During seedling selection and tagging, care was taken to replicate each virus isolate and treatment three times. Inoculations were done on July 18, 2002 (30 dap), coincident with the R1 stage of the plants that emerged early. At this date, all the late emergence plants were only at the V1-V3 stage. The inoculum was first thawed then placed in a chilled mortar and an aliquot of carborundum equal to 0.5% (w:v) was added. Plants were inoculated by rubbing the

inoculum onto the upper surface of the youngest fully-expanded trifoliolate leaves. To avoid contamination among isolates, all inoculations of each strain were made to all plants before other isolates were done. Control plants of each cultivar that were not inoculated were also included in the design, as explained above.

In 2003, plants seeded on June 27 germinated uniformly and were inoculated at three different growth stages, V1-V3, V4-V6 and R1-R3. The first inoculation at V1-V3 was done on July 12, 2003 at 15 days after planting (dap) for all the cultivars. This was followed by the second inoculation on August 2, 2003 for all the cultivars at 30 dap (V4-V6). However, last inoculation for R1-R3 was done at two different stages in order to accommodate early blooming cultivars, Williams and L8-379 (on August 9, 2003) at 37 dap, and the late bloomers, Bolivar and Manokin (on August 26, 2003), 54 dap. In 2002, all tagged plants were harvested, however, in 2003 only plants in the rows of Williams and L8-379 were harvested.

Monitoring effects of BPMV on plants: In 2002, plants were not monitored for foliar symptoms severity due to poor germination, therefore no data were collected. In 2003, the severity of infection was scored for each virus treatment using the changes in the green color of the foliage and any deformation on the leaf or stem of the plant. These scores were assigned for each row on a rating scale of 1-5, with 1 being for a leaf with no observable color change, 2 for a leaf with slight, light green/dark green mosaic coloration, 3 for a leaf with obvious and increased mosaic color, 4 for a leaf with severe mosaic and chlorotic colors and 5 for very extreme mosaic color as well as leaf or stem distortion. The mean score for the three rows of each treatment were compiled for comparisons. The first scoring was done 46 dap while the second scoring was at 67 dap.

Presence of virus was confirmed by leaf blot immunoassays, as described in Chapter 2. The middle leaves in the youngest set of trifoliolates were selected from three symptomatic plants in each row for leaf blot immunoassay. In addition, three leaves were selected from three symptomatic plants from a few rows for quantitative analysis of BPMV titer using double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), refer to Chapter 5.

Assessment of effects of BPMV on seeds: The plants were harvested on December 11 and on November 5, for growing seasons 2002 and 2003, respectively. For 2003, only

seeds from Williams and L8-379 could be weighed, because Manokin and Bolivar failed to reach maturity, hence they were not harvested.

Two methods of determining the effects of BPMV isolates on seed coat mottling were used. The first method involved evaluation of the incidence of seed coat mottling by counting the number of mottled seeds from a set of 100 randomly selected seeds, from each row of Williams and L8-379. The incidence of mottling was determined as a percentage of seeds selected (0-100%). From these values the mean incidence per treatment and time of inoculation was calculated. A second method was used to determine the proportion of mottled seeds by weighing all mottled seeds per row and calculating the values as a percentage of the weight of all the seeds harvested in each row. The values obtained were used to compute the mean proportion of mottled seeds per treatment at each time of inoculation.

In addition, seed size was measured by taking the weight (g) of seeds per row from two sets of 100 randomly selected seeds, and the mean weight of seeds per row was determined, and used to calculate the mean values for each treatment and time of inoculation.

Seedling assessment: Seedlings were grown in the greenhouse using seeds harvested from the rows of Williams and L8-379, as explained above. The seeds were grown in plastic trays at a spacing of 6 cm by 6 cm from each other. Each row on the tray consisted of 10 non-mottled seeds and 10 mottled seeds, if all the seeds were available (two rows of L8-379 had less than 10 mottled seeds). For mottled seeds, a gradient of mottling severity was used to place the seeds on the tray, hence one half of each row had non-mottled seeds and the other half had mottled seed. From the end of non-mottled seeds, less mottled to severely mottled seeds were planted up to the end of each row.

The seedlings were scored for germination, seedling height taken from the soil surface to the tip of emerging leaves (later trifoliolates), and color of foliage post-emergence. Seedling vigor and foliage color or any deformities associated with development, were also noted. Leaf blot immunoassay was performed on symptomatic plants to confirm the presence of BPMV or SMV that may be seed borne or seed transmitted.

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

The levels of seed coat mottling in 2002 are shown in Figure 4.1. There was more mottling in Bolivar inoculated at V1-V3 compared to Manokin and L8-379. Moderate mottling was exhibited at V4-V6 stage inoculation in Bolivar, less in Manokin and none in L8-379. At R1-R3, Bolivar still showed more mottling compared to the other cultivars but there was a reduced level of mottling across all cultivars.

Effect of inoculation time on symptom severity and virus accumulation: The effect of BPMV isolates on the cultivars inoculated at three different stages of development in 2003 was assessed using foliar scores, leaf blot immunoassay and double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). The effectiveness of virus inoculation on all plants, measured by leaf blot immunoassay was done on September 24, 2003 at 75 days after the first inoculation (dafi). The results are shown on Table 4.1. The V1-V3 inoculation showed infection rate per row ranging from 58.3 to 100%, V4-V6 inoculation was 54.5 to 100% and R1-R3 produced 0% to 75% infection. For non-inoculated control plants, 12 out of 68 plants that were leaf blotted tested positive for BPMV, one, three, four and four plants each of Bolivar, L8-379, Manokin and Williams, respectively. An overall infection rate of those plants sampled was 17.6%. This showed that most of our inoculations were successful, and that there was some natural spread of the virus in the plot.

Foliar score was used to grade the symptom severity on both inoculated and non-inoculated plants. The results are shown in Table 4.2. The first reading recorded similar foliar scores across the two cultivars, Williams and L8-379, at V4-V6, R1-R3, which was similar to the control, when all the BPMV isolates are compared. All isolates induced

symptoms 31 days after inoculation at V1-V3, except V-W2 inoculated on L8-379. The second reading of foliar scores at 67 dap were more severe, especially at V1-V3 (52 dafi), across all isolates. On L8-379, both V-S98-1 and V-S98-15 gave a score of 3.0. BPMV V-S01-10 also gave greater symptom severity for both Williams and L8-379. Bolivar and Manokin were similarly affected by BPMV isolates (Table 4.3). The earlier inoculations exhibited severe mottling at both the first and second readings, respectively. Both BPMV V-S98-1 and V-S98-15 severely affected Bolivar and Manokin, with reading of 3.0 and 2.8 being recorded at second reading for V1-V3. In general, the pattern of foliar symptoms severity was consistent, except that Williams and the isoline had higher scores at R1-R3 than did Bolivar and Manokin.

The virus accumulation determined by DAS-ELISA is shown in Table 4.4. Only selected cultivars and lines were tested. Bolivar inoculated at V1-V3 showed the highest ELISA value of 2.457, high foliar scores of 3.0 and high seed coat mottling of 100%. These resulted in the lowest seed weight of 7.5 g per 0.9 m row. Other readings were not consistent across the parameters measured. Williams inoculated at V4-V6 showed no foliar symptom yet gave values for ELISA as high as those inoculated at V1-V3 and showing symptoms. It recorded 87% seed coat mottling and high seed weight of 13.2 g. Overall, high ELISA values showed high values for seed coat mottling and seed weight.

Effect of inoculation time on yield and seed quality: In 2003, only two cultivars Williams and the isoline, L8-379 were harvested because Bolivar and Manokin failed to reach physiological maturity. The effects of both late planting and early frost did not allow the two late maturity group to produce physiologically mature seeds.

The yield loss relative to time of inoculation was both virus and cultivar dependent. The mean total weights (g) of all the seeds harvested from each row and calculated as the mean of each treatment are shown in Table 4.5. For both cultivars, the earlier inoculations at V1-V3 caused a greater yield reduction compared to V4-V6 and R1-R3 inoculations. Only V-S98-1 had a higher yield at V1-V3 of 102.6 g with L8-379. Most of the R1-R3 were higher in total weight than the second and first inoculations, respectively.

The mean seed weight (g/100 seeds), which is a measure of seed size, was used to determine the effect of virus on soybean productivity. Two batches of 100 randomly

selected seeds were weighed and the mean weight determined per row. The mean of the three rows per treatment was then computed. The results are shown in Table 4.6. The results are fairly concentrated around the values recorded for the controls. At significance level of $P \leq 0.0001$ which was computed by transforming the data using the square root of seed coat mottling, the mean separation procedure showed some significant differences between virus treatments. Specifically, the V4-V6 inoculations of V-S01-10 to L8-379, and R1-R3 inoculation of V-S98-15 to Williams, as well as V1-V3 inoculation of V-S98-1 to Williams caused loss in seed weight. But even with the isolates that exhibited severe foliar symptoms, the differences were very minimal.

The results of the effect of BPMV isolates on Williams and L8-379, measured as seed coat mottling, are shown in Table 4.7. Mottling was determined by randomly selecting 100 seeds from the seeds harvested from each row and counting the number of mottled seeds. This number was then used to calculate the mean incidence as percentage of seeds selected. The results show that different levels of seed coat mottling were observed across the two cultivars, at different stages of growth and the time of inoculation, among different isolates. At V1-V3, the isolates inoculated on L8-379 caused higher mottling than V4-V6 and R1-R3, except V-W2, which had 18.7% compared to 34.7 and 19.7% at V4-V6 and R1-R3, respectively. On Williams, the seeds from plants inoculated with the isolates V-W2, V-S98-1 and V-S98-15 at V1-V3 were more highly mottled at V1-V3 than those inoculated at V4-V6 and R1-R3. BPMV V-S01-10 inoculated Williams produced seeds with lower mottling at R1-R3 than at V1-V3 and V4-V6, while V-W2 had lower mottling at V4-V6 or R1-R3.

The mean proportion (w/w) of mottled seed was determined by weighing (g) all the seeds harvested from each row, followed by complete sorting and weighing separately all mottled seeds from each row (Table 4.8). These results show varying responses across the two cultivars with both V-W2 and V-S98-1 giving higher proportions of total seeds harvested from R1-R3 compared to V1-V3 and V4-V6 in both cultivars. BPMV V-S98-15 and V-S01-10 were consistent across the two cultivars, recording a high proportion of mottled seed weight at V1-V3 followed by V4-V6 and then R1-R3 being the lowest.

Seedling assessment: Germination of seedlings grown from seeds harvested from the two cultivars Williams and the isoline, L8-379, following virus treatment or non-inoculated control, were assessed by determining the germination of 30 non-mottled seeds and 30 mottled seeds from each treatment, 10 from each row. The results of seedling germination are shown in Figures 4.2 and 4.3 for L8-379 and Williams, respectively. The seedlings were grown from seeds of plants inoculated at V1-V3 using BPMV isolates V-W2, V-S98-1, V-S98-15, V-S01-10 and non-inoculated control. Across the two cultivars, stage of growth at inoculation and virus treatment, non-mottled seeds had consistently higher germination than mottled seeds. These differences were also consistent with the other two stages of growth at the time of virus inoculation (Data not shown).

The results of seedling heights showed a range in height between non-mottled and mottled seeds. The overall mean height of seedlings grown from non-mottled and mottled seeds of L8-379 and Williams were each 4.4 cm and 3.6 cm, respectively. Seedlings from non-inoculated seeds of L8-379 were 4.3 cm and 3.6 cm for non-mottled and mottled seeds, respectively, while those from non-inoculated Williams seeds were 4.5 cm and 3.8 cm, respectively. Based on the time of inoculation, the overall height of seedlings grown from L8-379 seeds were 4.4, 4.4 and 4.5 cm at V1-V3, V4-V6 and R1-R3, respectively, for non-mottled seeds. The mottled seedlings recorded 3.7, 3.6 and 3.6 cm at V1-V3, V4-V6 and R1-R3, respectively. At V1-V3, V4-V6 and R1-R3, the overall mean height for Williams seedlings were 4.5, 4.4 and 4.3 cm for non-mottled, and 3.4, 3.6 and 3.9 for mottled seeds, respectively. Observations on deformities at emergence and post emergence (data not included), also confirmed that seedlings from non-mottled seeds had fewer deformities or other symptoms associated with mottling or poor germination. Five plants tested from L8-379 mottled seeds by leaf blot immunoassay were positive for BPMV.

DISCUSSION

Seed coat mottling by BPMV is on the increase because of the increased incidence of BPMV in all soybean growing areas in the USA (Giesler et al., 2002). The time at which the virus first comes into contact with the host plant has been suggested as

a critical factor in combating the disease. The results of foliar scores demonstrate that those plants inoculated at V1-V3 showed more severe symptoms at the second reading (67 dap) than those inoculated at later stages, V4-V6 and R1-R3. Although our leaf blot immunoassay only detected 61.7% infectivity, it must be realized that a negative leaf blot test does not mean that a plant is not infected. It may only be possible that the virus titer is too low to be detected using this technique.

The mottling results show that seeds from those plants that were inoculated early (V1-V3) generally had higher mottling scores than those inoculated at V4-V6 and R1-R3 for most of the stages of growth and virus interactions. Hobbs et al. (2003) confirmed the effect of BPMV on seed coat mottling by inoculating soybean plants in insect proof cages. Even though BPMV exhibited less severe symptoms than SMV in their experiment, it caused seed coat mottling in both Williams and L8-379. Our study shows that both Williams and L8-379 exhibited higher seed coat mottling at V1-V3 than at V4-V6 and R1-R3, but mixed responses were also observed at V1-V3.

The results of mean seed weight and seed size show that foliar symptoms may not be a suitable predictor of the impact of BPMV on soybean plants. Although V-S98-1 and V-S98-15 exhibited severe foliar symptoms, the virus has not significantly reduced the mean seed yield or size. The effect of the SMV resistance gene in L8-379 did not reduce the effects of BPMV. This was also shown by Hobbs et al. (2003), where the non-seed coat mottling gene in Williams and the SMV resistance gene in the isoline did not reduce BPMV effects. The effect of BPMV and SMV in doubly inoculated plants may therefore remain a major problem in cultivars that are susceptible to SMV and can be severely affected by BPMV.

The results of seedling germination, seedling height and seedling deformities show that mottled seeds are more likely to produce seedlings with poor growth characteristics, especially at the early stages of development. Even though survivability was not tested, those seedlings from mottled seeds showed poor germination, slower growth as determined by height taken each day after germination, and showed more distorted and stunted seedlings than the non-mottled seeds. Because no field tests were performed, it was impossible to gauge how vigorous the plants could have performed in the field. But it fair to say from the greenhouse results that they could have produced less

vigorous plants. Plants from mottled seeds could have also more likely grown at a slower rate than those from non-mottled seeds.

The results of this study confirm that BPMV causes higher seed coat mottling in early inoculated plants than in later inoculated plants. These results agree with those found by Hobbs at el. (2003) and Krell et al. (2003). Although earlier studies by Ross (1986) and Calvert and Ghabrial (1983) demonstrated the effect of time of infection by BPMV and the effect the virus on doubly inoculated soybean plants, the importance of determining the exact time of inoculation and its effect on quality and yield is being revisited as more and more areas begin to realize problems of BPMV infection. The importance of knowing when the initial inoculum source may become a source of infection cannot be over emphasized. Although over-wintering beetles may not be responsible for a substantial amount of inoculum, they still contribute a source that may combine with the low seed transmission of 0.1%, to cause rapid spread of BPMV in field grown soybean, especially when there is high vector density and activity. Trap cropping early in the year combined with chemical control of the over-wintered beetles, followed by proper timing of planting to avoid the first generation of beetles that may follow, can greatly reduce the chances of early infection in the full season plants. Seeding of double crop soybean to avoid the second generation leaf beetles may greatly reduce early infection, reduce use of chemical control and enhance quality while reducing yield losses. Avoiding early infection is a sure method of avoiding high losses due to BPMV and may also be effective against SMV as well.

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Table 4.1. The relative percentage of plants infected after inoculation at three growth stages with four *Bean pod mottle virus* (BPMV) isolates, as determined by leaf blot immunoassay. Leaf blots were taken 75 days post first inoculation.

Time	Positive Plants	Total Plants Sampled	% Infected
V1-V3	324	410	79.0
V4-V6	164	264	62.1
R1-R3	134	279	48.0
Control	12	68	17.6

Table 4.2. The mean symptom severity of Williams and L8-379 inoculated at three different stages of development using four *Bean pod mottle virus* isolates and non-inoculated control. Stages of development for soybean are designated as vegetative V1-V3, V4-V6 and reproductive R1-R3. The readings were taken at 46 days after planting (dap) and 67 dap.

Stage of Growth at Inoculation		**Cultivar**			
Time	**Virus**	**L8-379**	**Williams**	**L8-379**	**Williams**
	Treatment	Reading 1	Reading 1	Reading 2	Reading 2
		(46 dap)		(67 dap)	
	Control	1.0a[a]	1.0a	1.7a	1.7a
V1-V3	V-W2	1.0a	1.3a	1.7a	1.7a
(15 dap)	V-S98-1	2.0ab	2.0ab	3.0b	2.7b
	V-S98-15	2.0ab	2.0ab	3.0b	2.5b
	V-S01-10	1.3a	1.5a	2.2ab	2.0ab
V4-V6	V-W2	1.0a	1.0a	2.0ab	1.7a
(30 dap)	V-S98-1	1.2a	1.2a	1.7a	1.7a
	V-S98-15	1.0a	1.2a	1.7a	1.7a
	V-S01-10	1.0a	1.0a	1.0a	1.3a
R1-R2	V-W2	1.0a	1.0a	1.7a	1.7a
(37 dap)	V-S98-1	1.0a	1.0a	2.0ab	2.0ab
	V-S98-15	1.0a	1.0a	1.3a	1.3a
	V-S01-10	1.0a	1.0a	1.7a	1.7a

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

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

Table 4.3. The mean symptom severity of Bolivar and Manokin inoculated at three different stages of development using four *Bean pod mottle virus* isolates and non-inoculated control. Stages of development for soybean are designated as vegetative V1-V3[a], V4-V6 and reproductive R1-R3. The readings were taken at 46 days after planting (dap) and 67 dap.

Stage of Growth at Inoculation		**Cultivar**			
Time	**Virus**	**Bolivar**	**Manokin**	**Bolivar**	**Manokin**
	Treatment	Reading 1	Reading 1	Reading 2	Reading 2
		(46 dap)		(67 dap)	
	Control	1.0a[a]	1.0a	1.0a	1.3a
V1-V3	V-W2	1.2a	1.7ab	1.3a	2.0b
(15 dap)	V-S98-1	2.5b	2.2b	3.0c	2.8c
	V-S98-15	1.7ab	2.0b	2.3b	2.2b
	V-S01-10	1.0a	2.0b	1.3a	2.3b
V4-V6	V-W2	1.0a	1.0a	1.0a	1.2a
(30 dap)	V-S98-1	1.2a	1.2a	1.8ab	1.3a
	V-S98-15	1.0a	1.0a	1.3a	1.7ab
	V-S01-10	1.0a	1.0a	1.0a	1.3a
R1-R2	V-W2	1.0a	1.0a	1.0a	1.3a
(37 dap)	V-S98-1	1.0a	1.0a	1.2a	1.3a
	V-S98-15	1.0a	1.0a	1.0a	1.7ab
	V-S01-10	1.0a	1.0a	2.0b	1.5ab

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

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

Table 4.4. The relative[a] foliar symptom severity score (Readings 1 and 2), seed coat mottling, total seed weight and ELISA values of four selected cultivars. These cultivars were inoculated using BPMV V-S98-1 at V1-V3 and V4-V6 stages of development (15 and 30 days after planting, respectively).

Cultivar/Stage of Inoculation	Foliar Score (1-5) 1	Foliar Score (1-5) 2	Seed Coat Mottling (%)	Total Seed Weight (g)	ELISA
Bolivar (V1-V3)	3.0	3.0	100	7.5	2.457
Bolivar (V4-V6)	1.5	2.0	100	8.1	1.208
Williams (V1-V3)	2.0	2.0	41	15.9	1.224
Williams (V4-V6)	1.0	1.0	87	13.2	1.412
L8-379 (V1-V3)	2.0	3.0	94	15.4	1.536
Manokin (V1-V3)	2.0	3.0	95	9.1	1.047

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

[a]The values shown in the Table were not statistically analyzed because only a few readings were taken.

Table 4.5. The mean of total weights (g) of all the seeds harvested per row from each virus treatment. Williams and L8-379 were inoculated at three different stages of development using *Bean pod mottle virus* isolates and non-inoculated control. Stages of development for soybean are designated as vegetative V1-V3[a], V4-V6 and reproductive R1-R3.

Virus Treatment	**Total weight of seeds harvested from cultivars**					
	L78-379			**Williams**		
Treatment	**V1-V3**	**V4-V6**	**R1-R3**	**V1-V3**	**V4-V6**	**R1-R3**
Control	100.7a	100.7a	100.7a	114.5a	114.5a	114.5a
V-W2	96.5ab	89.6ab	122.8a	98.9ab	111.9a	123.9a
V-S98-1	102.6a	82.9ab	65.7b	78.9b	53.6c	106.8a
V-S98-15	79.4b	60.1b	91.4ab	87.0b	84.3b	64.2bc
V-S01-10	75.3b	107.2a	100.8a	93.5ab	95.1ab	103.8a

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

[a]Inoculation at V1-V3 was 15 days after planting (dap), V4-V6 at 30 dap, and R1-R3 at 37 dap.

Table 4.6. The mean seed weight (g/100 seeds) of mottled seeds from two randomly selected batches of 100 seeds of Williams and L8-379. The plants were inoculated at three different stages of development using *Bean pod mottle virus* isolates and non-inoculated control. Stages of development for soybean are designated as vegetative V1-V3[a], V4-V6 and reproductive R1-R3.

Stage of Development at Inoculation		**Weight (g) per 100 seeds of cultivars**	
Time	**Virus Treatment**	**L8-379**	**Williams**
V1-V3	Control	15.5a	16.0a
V1-V3	V-W2	15.4a	16.0a
	V-S98-1	15.9a	13.7bc
	V-S98-15	15.0a	15.7a
	V-S01-10	15.0a	15.5a
V4-V6	V-W2	14.5b	15.4a
	V-S98-1	15.7a	14.6b
	V-S98-15	14.9b	15.4a
	V-S01-10	12.2c	15.4a
R1-R2	V-W2	14.9b	16.0a
	V-S98-1	14.4b	15.3a
	V-S98-15	14.9b	13.3bc
	V-S01-10	15.9a	16.2a

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

[a]Inoculation at V1-V3 was 15 days after planting (dap), V4-V6 at 30 dap, and R1-R3 at 37 dap.

Table 4.7. The mean incidence (number) of mottled seeds per 100 randomly selected seeds from each row of Williams and L8-379, inoculated at three different stages of development using *Bean pod mottle virus* isolates and non-inoculated control. Stages of development for soybean are designated as vegetative V1-V3[a], V4-V6 and reproductive R1-R3.

Virus Treatment	**Incidence of seed coat mottling**					
	L8-379			**Williams**		
Treatment	**V1-V3**	**V4-V6**	**R1-R3**	**V1-V3**	**V4-V6**	**R1-R3**
Control	6.7a	6.7a	6.7a	13.0a	13.0a	13.0a
V-W2	18.7b	34.7bc	19.7b	30.7b	19.3ab	24.3ab
V-S98-1	48.7bc	45.0bc	36.3bc	73.0c	49.0b	49.7b
V-S98-15	54.0c	54.0c	33.0bc	71.7c	65.0c	48.9b
V-S01-10	43.7bc	29.2bc	16.3b	49.0b	50.7b	20.3ab

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

[a]Inoculation at V1-V3 was 15 days after planting (dap), V4-V6 at 30 dap, and R1-R3 at 37 dap.

Table 4.8. The mean proportion (w/w) of the total weight of all mottled seeds per row expressed as a percentage of all seeds harvested in each row. Williams and L8-379 were inoculated at three different stages of development using *Bean pod mottle virus* isolates and non-inoculated control. Stages of development for soybean are designated as vegetative V1-V3[a], V4-V6 and reproductive R1-R3.

Virus Treatment	**Proportion by weight of mottled seed**					
	L8-379			**Williams**		
Treatment	**V1-V3**	**V4-V6**	**R1-R3**	**V1-V3**	**V4-V6**	**R1-R3**
Control	2.6a	2.6a	2.6a	4.9a	4.9a	4.9a
V-W2	6.6a	7.3a	10.2a	18.4ab	14.9ab	14.4ab
V-S98-1	22.6b	7.4a	28.4b	35.2b	18.6ab	34.3b
V-S98-15	40.8c	10.4a	9.6a	40.8c	28.8b	17.9ab
V-S01-10	17.4b	5.4a	4.1a	25.5b	18.5ab	10.7a

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

[a]Inoculation at V1-V3 was 9 days after planting (dap), V4-V6 at 30 dap, and R1-R3 at 37 dap.

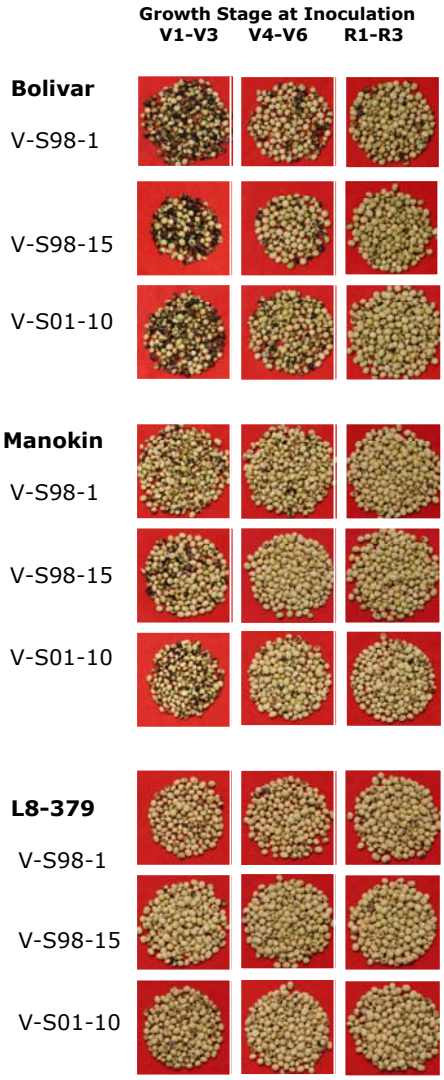

Figure 4.1. Seed coat mottling exhibited by seeds harvested from field-inoculated plants in 2002. The plants were inoculated at vegetative stages V1-V3, V4-V6 and reproductive stages R1-R3, respectively, using BPMV strains (V-S98-1, V-S98-15 and V-S01-10). Bolivar showed more mottling at V1-V4 than Manokin and L8-379. Similar patterns were observed at V4-V6 and R1-R3.

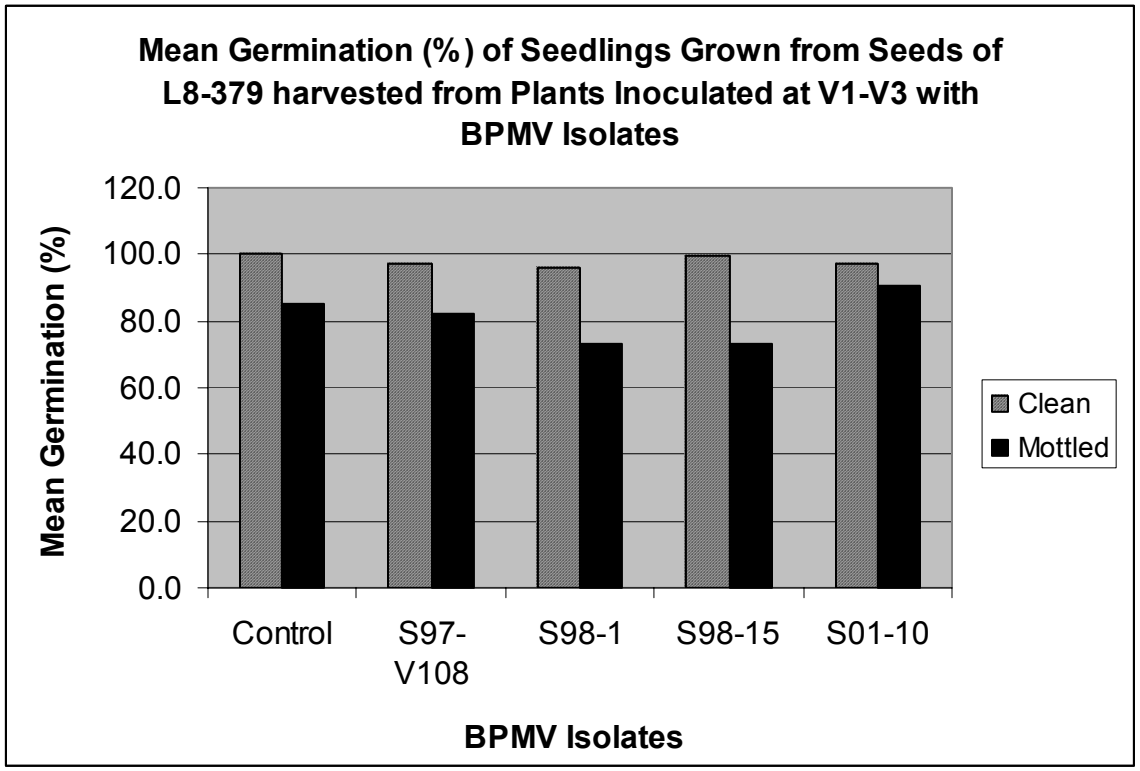

Figure 4.2. The mean germination percentage of seedlings grown from seeds of L8-379 harvested from plants inoculated at V1-V3 with BPMV isolates

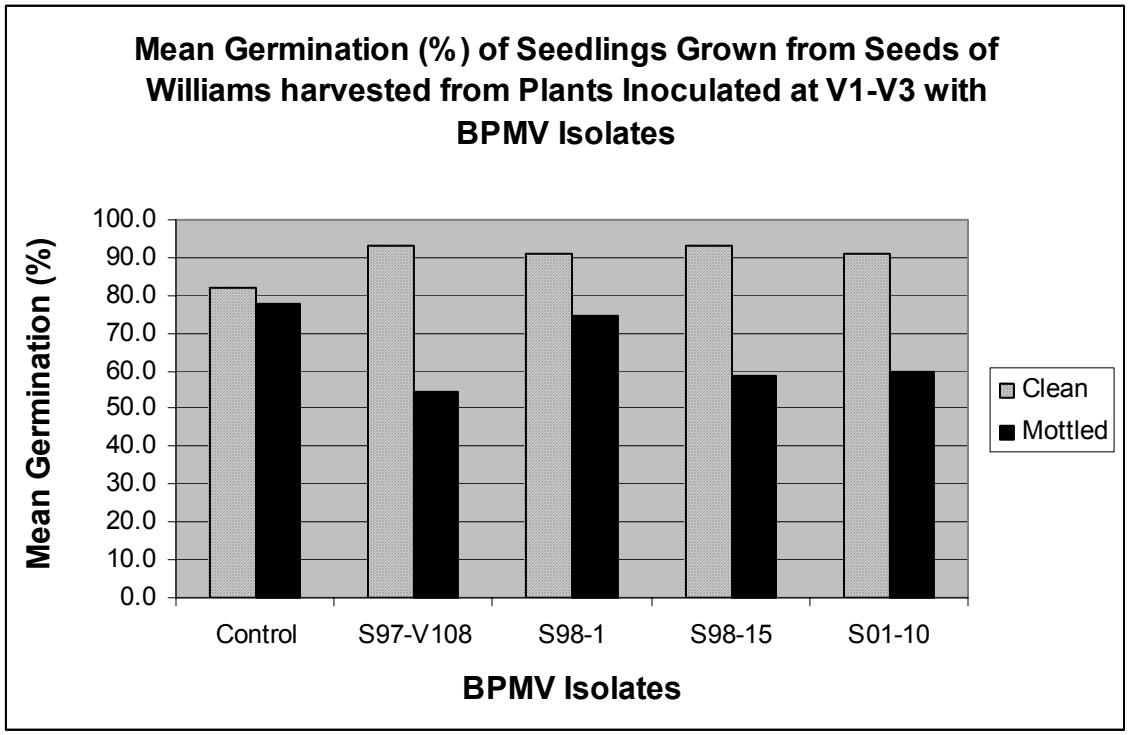

Figure 4.3. The mean germination percentage of seedlings grown from seeds of Williams harvested from plants inoculated at V1-V3 with BPMV isolates.