Chapter Five

Mechanisms of the Soybean *Rsv3* Gene for Resistance to *Soybean mosaic virus*

ABSTRACT

Resistance to *Soybean mosaic virus* (SMV; Genus *Potyvirus*; Family *Potyviridae*) in soybean (*Glycine max* [L.] Merr.) is controlled by single dominant genes at three distinct loci, *Rsv1*, *Rsv3* and *Rsv4*. The mechanisms of resistance at the *Rsv3* locus were investigated in Harosoy and the Williams isoline L29 by tracking virus replication and movement. Two other cultivars with two genes for resistance to SMV were also tested: Columbia with *Rsv3* and *Rsv4*, and Tousan 140 with *Rsv1* and *Rsv3*. Greenhouse-grown plants of each line were inoculated with SMV strains G1, G6 and G7 to monitor events during the infection process. Leaves were sampled over time and tested for the presence of SMV by immuno-printing. Number and size of infection sites and proportion of leaf area infected were determined. Results confirmed that the SMV resistance conditioned by *Rsv3* in Harosoy is strain specific and its mechanisms involve restriction of virus accumulation and movement, both cell-to-cell and long distance with G6 and G7. Interactions of L29 and SMV confirmed that *Rsv3* in L29 conditions an extreme resistance (ER) with no detectable virus to both G6 and G7. The *Rvs3* alleles in Harosoy and L29 are different, therefore we designated them at $Rsv3^{hs}$ and $Rsv3^{hd}$. Cultivars carrying both *Rsv1* and *Rsv3* or *Rsv3* and *Rsv4* genes were resistant to all strains tested. These results support the use of gene pyramiding to insure a wider and more durable resistance to SMV.

Keywords: *Glycine max*, SMV, Virus Resistance

INTRODUCTION

Three genes for resistance to *Soybean mosaic virus* (SMV; Genus *Potyvirus*; Family *Potyviridae*) *Rsv1*, *Rsv3* and *Rsv4* have been identified in soybeans (*Glycine max* [L.] Merr.) that map to three different loci and react differently to SMV strains. SMV infects soybeans worldwide (Brunt et al., 2003) and reduces yields significantly (Hill 1999, Hill et al., 1987). SMV is classified into seven strain groups, G1-G7, based on the differential responses (resistance, necrosis and susceptibility) of a set of soybean cultivars carrying alleles at the *Rsv1* locus (Chen et al., 1991; Cho and Goodman 1979, 1982). Eight alleles of *Rsv1* have been identified (Buss et al., 1989, Chen et al., 1991, Chen et al., 2001, Ma et al., 2002) in soybeans that react differently to different strains of SMV. Various alleles of the *Rsv1* locus conditions either necrosis and resistance reactions to SMV-G1 through G4 but susceptibility to SMV-G5 through G7 (Chen et al., 1994). A second resistance gene *Rsv2* was described in OX670 (derived from Raiden) (Buzzell and Tu, 1984). However, Gunduz et al. (2001) showed that OX670 has two genes for resistance at two distinct loci. One is allelic to *Rsv1* and is derived from Raiden; the other is allelic to *Rsv3* derived from Harosoy. The *Rsv2* gene designation was therefore dropped. Roane et al. (1986) suggested a gene-for-gene model for the SMV-soybean interactions.

The responses of *Rsv3*-containing cultivars are summarized in Table 5.1. Harosoy is susceptible to SMV-G1 through G4, resistant to SMV-G5, G6 and G7. However, the *Rsv3* gene in Harosoy is not completely dominant and heterozygous plants (e.g., F1 of Harosoy x Lee 68) for this gene showed lethal necrosis to SMV-G7 (Gunduz et al., 2001). *Rsv3* mediated resistance was first identified from cv. Columbia (Buzzell and Tu, 1989). This cultivar is resistant to SMV strains G1-G7. The *Rsv3* derived from Columbia conditions systemic necrosis to SMV-G1 in the heterozygous state and the *Rsv4* derived from this cultivar conditions resistance to all strains of SMV (Ma et al., 1995). Another allele at the *Rsv3* locus was reported from Hardee (Buss et al., 1999), which is susceptible to strains G1-G4 and resistant to strains G5-G7. Isoline L29 (Bernard et al., 1991), derived from a cross between Williams and Hardee, contains an *Rsv3* gene from Hardee (Buss et al., 1999). L29 is susceptible to SMV-G1 through G4 and resistant to SMV-G5 through G7 (Gunduz et al., 2001). The *Rsv3* locus maps to soybean linkage group MLG B2, which contains a cluster of disease resistance genes (Jeong et al., 2002). Tousan 140, another

cultivar resistant to SMV, is resistant to SMV-G1 through G7 conditioned by the two distinct loci *Rsv1* and *Rsv3*. Chen et al. (1993) and Ma et al. (1995) reported the presence of two independent genes for resistance to SMV from PI 486355. One is allelic to *Rsv1*; the other is not allelic to either *Rsv1* or *Rsv3,* and so was designated as *Rsv4*. Hayes et al. (2000) mapped the *Rsv4* locus to linkage group Dlb.

The majority of commercially-utilized soybean germplasm that is resistant to SMV contains the *Rsv1* gene for resistance. This germplasm has been highly effective in controlling the common SMV strains G1-G3. However, recent emergence of *Rsv1* resistance-breaking isolates of SMV (see chapters 2 and 3) underscores the need to incorporate additional resistance genes and to understand the basis of resistance for non-*Rsv1* based loci. In this study, we determined (i) the different reactions of four different soybean cultivars containing *Rsv3* (Columbia, Harosoy, L29, and Tousan 140) to SMV, (ii) the virus accumulation in the different cultivars, (iii) the extent of virus cell-to-cell movement, (iv) the extent of long distance movement, and (iv) the potential effect of gene pyramiding on SMV resistance. The mechanisms of resistance of *Rsv3* are compared to that of *Rsv4* (described in previous chapter), which is non-necrotic, non-strain specific and involve restriction to cell-to-cell and long-distance virus movement.

MATERIALS AND METHODS

Genetic Material and Growth Conditions

The soybean cultivars used in this study were Columbia, Harosoy, L29, and Tousan 140 containing the *Rsv3* gene for resistance to SMV, and Essex (*rsv*), which is susceptible to all SMV strains. Test plants (6/pot) were grown from seed in 11x10 cm pots filled with MetroMix360® (Scotts-Sierra Horticultural Products Co., Marysville, OH). Osmocote® 18 %N, 6 %P, 12 %K, (Scotts-Sierra Horticultural Products Co., Marysville, OH) was used as a supplemental fertilizer. G. R Buss (Dept. Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA) graciously supplied seeds of all cultivars. Tests were conducted under greenhouse conditions in May-June of 2001. Two other biological replicates were done on January 2001 and October 2001.

Virus Inoculations:

SMV strains G1 (Hunst and Tolin, 1982), G2, G5, G6 and G7 (Cho and Goodman 1979) were used as virus inocula (G1, G5, G6 and G7 are ATCC accessions PV571, PV573, PV612 and PV613, respectively, submitted by S.A Tolin). Virus cultures were maintained in the greenhouse (see Chapter 4). Virus inoculations were carried as described in Chapter 4.

Leaf-Immunoprint Analysis:

Virus movement in plants (both cell-to-cell and long distance) was monitored by immunological detection of virus in imprints of individual leaves as described in chapter 4. Sampling dates were 2, 4, 9, 14, 19 and 26 days post inoculation (dpi). Total number of infection sites, average size of an infection site, and total leaf area infected were determined from digitized images of immunoprints of each of three leaves per treatment using the Alpha Innotech Imager (Mod 4912-2010/0000) and the Alpha Innotech Analysis software (Alpha Innotech Corporation, San Leandro, CA). Analysis of variance was performed using SAS statistical package (North Carolina State University, Raleigh, NC).

Enzyme Linked Immuno Sorbent Assay (Indirect-ELISA)

Accumulation of virus in inoculated leaves was measured by quantitative indirect-ELISA. Three inoculated leaves were sampled from three plants of the same cultivar inoculated with each strain. Extractions (10 ml buffer/1 g tissue) were done from fresh tissue by adding extraction buffer (0.05 M sodium carbonate pH 9.6; 1% polyvinyl pyrolidone MW (40,000) PVP) using extraction bags and roller ball bearing grinders (Agdia®). Extracts were then diluted with the same buffer to bring the final dilution to 1:50. Samples were tested for relative virus titer by indirect ELISA as described in Chapter 4. Absorbance at 405 nm was recorded using the Spectramax plate reader (Molecular Devices, Sunnyvale, CA).

RESULTS

Symptom Appearance

The phenotype of *Rsv3* conditioned responses to SMV was compared to those of the susceptible cv. Essex. Inoculated leaves of Essex showed chlorotic lesions with rust-colored margins at the point of infection, and veinal chlorosis 9 days after inoculation (dpi) with SMV-G1, G6 and G7. Non-inoculated first and second trifoliolate leaves showed typical mosaic and chlorotic veins by the same time (see Chapter 4. Figure 4.1A). SMV invaded the leaf vasculature by 9 dpi and invaded the whole leaf by 19 dpi as shown by leaf immunoprints (see Chapter 4, Figure 4.3A-D). Two cultivars, L29 and Harosoy, which were homozygous for the *Rsv3* but did not contain other genes for SMV resistance were susceptible to SMV-G1 showing chlorosis in the inoculated leaves and systemic mosaics in the upper leaves. Figure 5.1 shows symptomatic L29 plants inoculated with SMV-G1 (A) and SMV-G2 (B), at 14 dpi. These cultivars were resistant to SMV strains G6 and G7 showing no symptoms (Figure 5.1C-D).

We also assessed symptom development in cultivars having multiple SMV resistance genes using Tousan 140 (*Rsv1/Rsv3*) and Columbia (*Rsv3/Rsv4*). As expected, these cultivars showed resistance to all SMV strains since *Rsv1* conditions resistance to SMV-G1-G4, *Rsv3* appears to protect against strains G5-G7, and *Rsv4* confers strain-independent resistance (i.e., G1-G7; see Chapter 4). These cultivars remained free of symptoms for 26 dpi, the duration of the experiment, with all SMV strains tested (data not shown). However, a few inoculated unifoliolates turned gray and detached from the stem after 19 dpi. No necrosis was observed for any cultivar-strain combination.

Virus Movement in Inoculated Leaves

Leaf immunoprints were used to assess virus accumulation in the SMV-inoculated leaves of the *Rsv3* containing cultivars (Figure 5.2). Since the rate of viral spread varied with different strains:cultivar combinations, the number of initial infection sites were assessed using tissue immunoprints that represented the maximum number of foci prior to coalescence (Figure 5.3). The mean number of infection sites from three leaves of each cultivar inoculated with SMV-G1, G6 and G7 showed similar responses in Essex, and the two cultivars homozygous for the *Rsv3*

gene, L29 and Harosoy to SMV-G1 (Figure 5.3). In the susceptible interactions of these *Rsv3*-carrying cultivars with SMV-G1, virus was first detected as small infection foci (0.5-0.7 mm diameter, respectively) on the inoculated leaves 4 dpi (Figure 5.4). SMV-G1 inoculated leaves of Essex, L29 and Harosoy showed similar numbers of infection foci by 4 dpi (Figure 5.3); the rate of viral spread in Harosoy and L29 was analogous to that in Essex (see Chapter 4). By 9 dpi, infection foci coalesced; SMV-G1 invaded the leaf vasculature, and invaded the whole leaf by 19 dpi. Total leaf invasion was detected in L29 and Harosoy leaves inoculated with SMV-G1 at 26 dpi (Figure 5.2 A and C), and is not different from the Essex response to all the strains tested. In contrast, no virus was detected in SMV-G6 or G7 inoculated leaves of L29 (Figure 5.2B), whereas SMV-G6 and G7 showed a few infection foci on inoculated leaves of Harosoy (Figure 5.2 D and Figure 5.3). The size of infection sites was not different between the various cultivars/strains tested and virus invasion was less than 1% of the leaf area. These results suggest that the *Rsv3* in Harosoy and L29 are different alleles and therefore we designated them as $Rsv3^{hs}$ and $Rsv3^{hd}$, respectively.

The effect of *Rsv3* on resistance to SMV was also analyzed in plants containing *Rsv3* in combination with *Rsv1* (Tousan 140), which confers resistance to SMV-G1 through G4 or *Rsv4* (Columbia), which provides strain-independent resistance (see chapter 4). Leaf immunoprints from Columbia showed no infection foci in SMV-G6 or G7 inoculated leaves (Figure 5.3). SMV-G1 inoculated unifoliolates from Columbia showed a small number of infection foci, significantly less than that from that of the susceptible Essex, L29 or Harosoy (Figure 5.3). In contrast, no infection foci were detected in SMV-G1 inoculated unifoliolates from Tousan 140 (Figure 5.3). A significantly lower number of infection foci were detected in SMV-G6 and G7 inoculated Tousan 140 at 19 dpi (Figure 5.3) with similar diameters to those in the susceptible Essex at 4 dpi (Figure 5.4). However, by 26 dpi, the virus did not spread away from the infection foci and did not invade the leaf vasculature.

Systemic Movement of SMV

Systemic movement of SMV was tracked by immunoprints. In the susceptible reactions, virus moved away from the inoculated plants to the upper leaves and resulted in total invasion of the plants. By 9 dpi, SMV-G1 invaded the vasculature of the inoculated leaf of L29 (Figure 5.5A), and was detected in the petioles of both the inoculated unifoliolate and non-inoculated first trifoliolate leaves and the veins and adjacent cells of the latter (B). No virus accumulation was detected in the non-symptomatic L29 SMV-G7 inoculated unifoliolates (C) and non-inoculated trifoliolate leaves 19 dpi (D). Similarly, in Harosoy, SMV-G1 invaded the vasculature of the inoculated unifoliolate leaves by 9 dpi (E) and entered the non-inoculated upper leaves (F) and moved out of the veins to invade the leaf tissue by 9 dpi (G). Figure 5.5 confirms the correlation between visual symptoms of SMV on non-inoculated first trifoliolates and virus accumulation in leaf tissue.

Relative Virus Accumulation

Indirect ELISA was used to measure virus accumulation quantitatively. Results in Table 5.2 show the relative virus titer in L29 and the susceptible Essex inoculated with SMV-G1, G2, G5, G6 and G7. Data show mean absorbance reading at 405 nm from 3 different inoculated leaves sampled at 14 dpi, the date at which virus showed maximum invasion in the susceptible reactions. All three leaves from SMV-G1 or G2 inoculated L29 leaves were positive and were essentially the same. All samples from SMV-G5, G6 or G7 inoculated L29 leaves tested negative for SMV. The relative virus titer in the SMV-G1 or G2 inoculated L29 was similar. All values for Essex were positive for SMV showing no difference between the strains. These results confirmed those from the immunoprints for SMV-G1, G6 and G7 from this study and G2 and G5 (S.A Tolin, unpublished data).

DISCUSSION

The *Rsv3*-containing cultivars showed three major responses to the three strains of SMV tested. These were either: (i) susceptibility with chlorosis and local and systemic mosaic symptoms, (ii) limited virus replication and movement with no visible lesions, or (iii) extreme resistance (ER) with no symptoms and no necrotic lesions developing even after 4 weeks. These responses were strain specific for the *Rsv3* containing cultivars tested. The L29 isoline of Williams with the *Rsv3* derived from Hardee was susceptible to SMV-G1 and G2. This gene provided no limitation to virus accumulation and spread from sites of initial entry as shown by ELISA data on relative virus titers 14 dpi. *Rsv3* in L29 had also no effect on long distance movement of G1 and G2. SMV moved from the inoculated leaves to the first trifoliolates by 9 dpi and invaded the entire leaf area 10 days later. In contrast, L29 was resistant to SMV strains G6 and G7 with no detectable virus in the leaf immunoprints (both inoculated unifoliolates and non-inoculated trifoliolates) and no detectable virus accumulation in the inoculated leaves (Table 5.2). The *Rsv3* locus in L29 therefore appears to function as an extreme resistance response to the higher numbered strains. These data support the observations reported by Buss et al. (1999) on *Rsv3* response to SMV.

Cultivar Harosoy was susceptible to SMV-G1 with 100% invasion of the inoculated leaf and the development of typical symptoms of SMV in upper non-inoculated trifoliolates. Harosoy was resistant to strains G6 and G7 but did not completely inhibit virus entry and replication as did L29. Few infection foci were detected but the cell-to-cell movement of the virus was reduced relative to that of SMV-G1 on Harosoy. The long distance movement, on the other hand, was inhibited since no virus accumulation was detected in the upper non-inoculated trifoliolate leaflets. This does show, however, that virus replication is extremely reduced but not inhibited as in the case of L29 and extreme resistance to the same G6 and G7 strains. This is the first evidence that the responses of Harosoy and L29 to strains of SMV are different and that alleles at the *Rsv3* locus in these cultivars are different. We designate them as $Rsv3^{hs}$ and $Rsv3^{hd}$, respectively.

The response of Tousan 140, the cultivar with two genes *Rsv1* and *Rsv3,* was not as we expected. Although resistant to all strains, the response was an ER type only to SMV-G1. This however does agree with the study by Gunduz et al (2002) where Tousan 140 showed a necrotic

type of response to SMV-G1 and SMV-G2, therefore inhibiting the virus from invading the whole leaf area. A few infection foci were detected in SMV-G6 and G7 inoculated unifoliolates of Tousan 140 (Figure 5.3) that did not increase in size over time and did not invade the vasculature and the rest of the leaf, suggesting a restriction of virus movement and accumulation. The results of Gunduz et al (2002) also indicated that this cultivar was resistant to these two strains, but did not define the type of resistance. Our results, therefore, have characterized the resistance in Tousan 140 as both ER to SMV-G1 and restriction to virus accumulation and movement in response to the other strains tested. The resistance to SMV-G1 is derived from the *Rsv1* and the resistance to SMV-G6 and G7 is derived from *Rsv3*. Data from number of initial infection sites and corresponding diameter, and percentage leaf area infected, suggest that the *Rsv3* allele in Tousan 140 is similar to that of Harosoy but not L29. The two genes for resistance in this cultivar show a potential for gene pyramiding in breeding for disease resistance to confer resistance to a range of strains of the same virus. Incorporating the *Rsv1* and *Rsv3* genes in soybeans for resistance to SMV will protect soybeans from SMV G5 and G6-like isolates that have emerged to break the resistance of *Rsv1*.

The cultivar Columbia was resistant to all SMV strains used in this study. No SMV was detected in either inoculated unifoliolates and non-inoculated trifoliolates for 26 dpi with G6 or G7 inoculated plants. The *Rsv3* in Columbia conditioned an extreme resistance to these two strains but not to strain G1. Columbia has been reported to give a hypersensitive reaction (HR) in response to SMV-G1 (Buzzell and Tu, 1989). We did not observe any necrotic lesions on SMV-G1 inoculated plants in our study, but we detected a few infection foci on the inoculated leaves (Figure 5.3). The virus movement was, however, restricted (Figure 5.4) and less that 1 % of the whole leaf area was invaded after 4 weeks following infection. However, necrosis in Columbia was induced by SMV strains in another experiment suggesting environmental conditions play a role in the necrotic response. Ma et al. (2002) also reported that cultivar Columbia was not necrotic to strains G1, G2, G3, G5, G6 and G7 of SMV and that no systemic symptoms were present in the upper non-inoculated leaves. Ma et al. (2002) considered Columbia to be resistant to all these SMV strains. Our present results distinguish the type of resistance in Columbia to the strains of SMV tested. However, since *Rsv4* in Columbia conditions resistance to all SMV strains, the resistance conferred by this cultivar to strains G5-G7 could not be compared to that conferred by other *Rsv3* alleles until genetic crosses to separate the two genes have been done.

In summary, the response of the *Rsv3* gene is strain specific conferring resistance to SMV G1, G6 and G7. Restriction to virus movement appears to be involved in resistance but is not the major mechanism for this gene, in contrast to the *Rsv4* (Chapter 4; Gunduz et al., 2004), which has been reported as non-strain specific and non-necrotic and its main mechanism is restriction to virus movement (Chapter 4; Gunduz et al., 2004; Ma et al., 1995). In addition, the *Rsv3* gene conditions both a reduction in virus accumulation (with restriction to movement) and an ER similar to that of *Rsv1* (PI 96983) to SMV-G1 (data not shown), in response to the various SMV strains. This work is the first to distinguish between the alleles of *Rsv3* locus based on the interaction with strains of SMV. Our study shows potential for better resistance to SMV using gene pyramiding, especially to the *Rsv1* resistance-breaking isolates of SMV.

Table 5.1. Reported responses of *Rsv3*-containing cultivars to strains of *Soybean mosaic virus*.

		Susceptibility	Resistance	Reference
Essex	*rsv*	G1-G7		Chen et al., 19991
Hardee	*Rsv3*	G1-G4	G5-G7	Buss et al., 1999
L29	*Rsv3*	G1-G4	G5-G7	Buss et al., 1999
Harosoy	*Rsv3*	G1-G4	G5-G7	Gunduz et al., 2001
Tousan 140	*Rsv1/Rsv3*		G1-G7	Gunduz et al., 2002
Columbia	*Rsv3/Rsv4*		G1-G7	Ma et al., 1995

Table 5.2. Relative virus-titer from Essex and L29 inoculated with *Soybean mosaic virus*. Values represent ELISA absorbance at 405 nm from 3 different inoculated leaves of L29 and Essex with SMV strains G1, G2, G5, G6, G7 and buffer inoculated as healthy controls at 14 dpi.

Cultivar	**Absorbance at 405 nm of ELISA of leaves from plants inoculated with SMV strains**											
	G1		G2		G5		G6		G7		Buffer Control	
L29 (*Rsv3*)	2.58	+	2.52	+	0.07	-	0.08	-	0.06	-	0.08	-
Essex (*rsv*)	2.66	+	NT	NT	2.54	+	2.72	+	2.83	+	0.10	-

(+): Values greater than 0.0845 or 0.1235 (mean of healthy plus 3 x standard deviation of healthy) are considered positive for L29 and Essex, respectively.

(-): Values less than 0.0845 or 0.1235 (mean of healthy plus 3 x standard deviation of healthy) are considered negative for L29 and Essex, respectively.

NT: not tested.

Figure 5.1. Response of the soybean cultivar L29 inoculated with SMV strains G1 (A), SMV-G2 (B), SMV-G6 (C), or SMV-G7 (D), 14 days post inoculation.

Figure 5.1-Continued

C

D

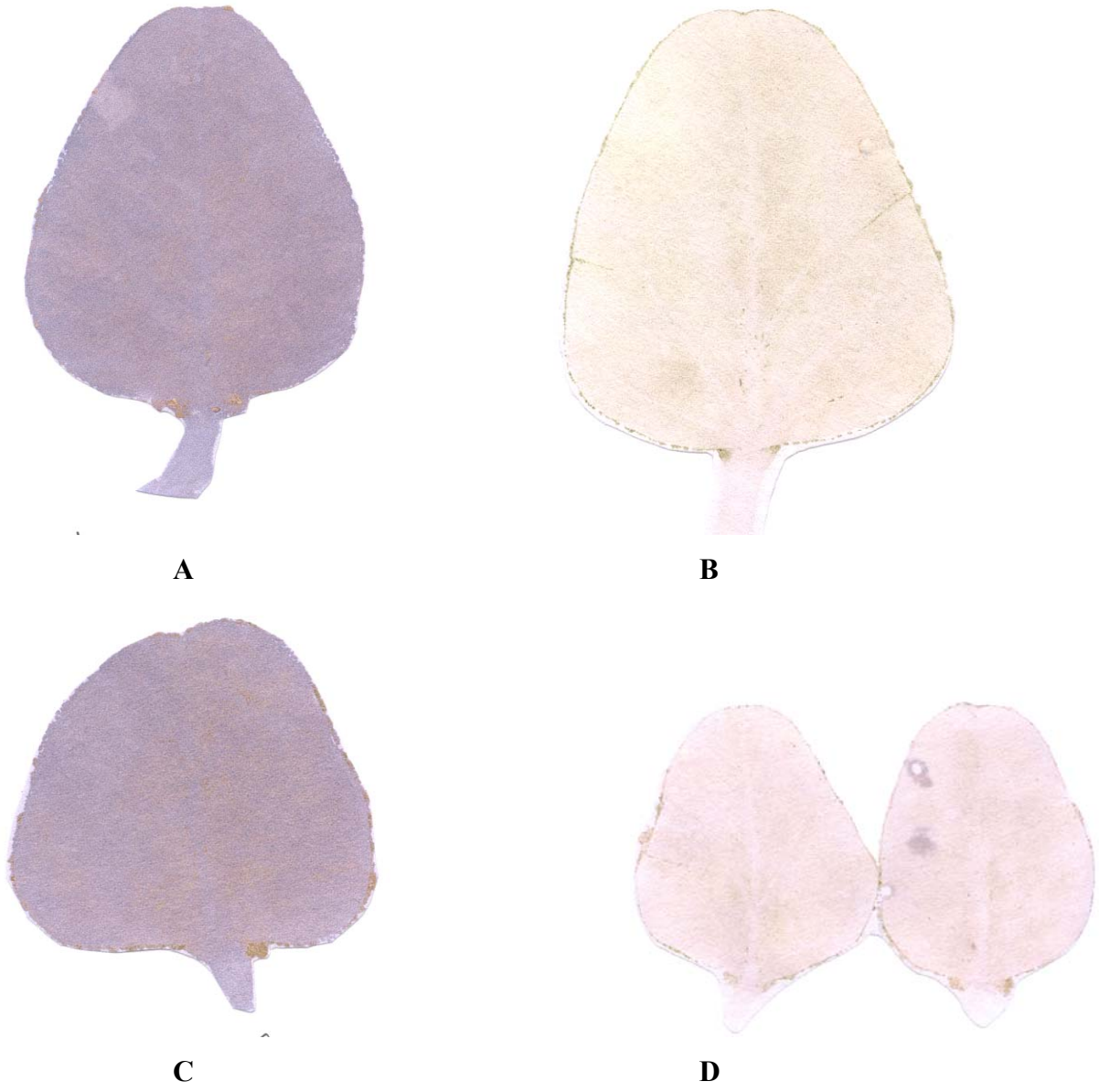


Figure 5.2. Leaf immunoprints of SMV G1 and G7 inoculated unifoliolate leaves of L29 and Harosoy at 26 dpi. (A) and (B): L29 inoculated with SMV-G1 and G7 respectively. (C) and (D): Harosoy inoculated with SMV-G1 and G7, respectively.

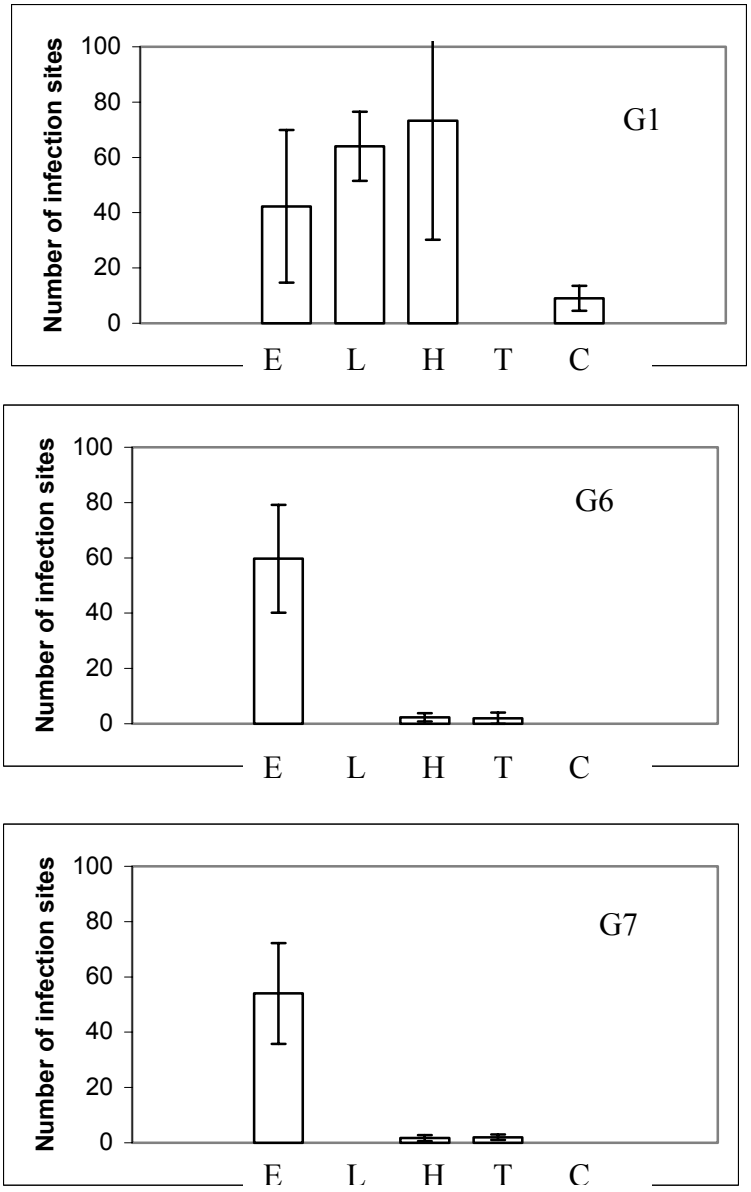


Figure 5.3. Number of initial infection sites on soybean cultivars inoculated with SMV strains G1, G6 and G7. (E= Essex; L= L29; H= Harosoy, T= Tousan140; C= Columbia).

Data represent the mean number and standard deviation (error bars)from three inoculated leaves of sampled from three different plants following inoculation with the SMV strains G1, G6 and G7. Data are derived from number of infection sites (before the virus moved into the vasculature of the leaf) from inoculated leaves of Essex and Tousan 140 sampled at 4 and 19 dpi, respectively. Data for Harosoy inoculated with G1, G6 and G7 correspond to leaves sampled at 4, 14 and 19 dpi, respectively. Data for Columbia inoculated with G1, G6 and G7 correspond to leaves samples at 9, 19 and 19 dpi respectively. Data for L29 inoculated with G1, G6 and G7 correspond to leaves samples at 4, 26 and 26 dpi.

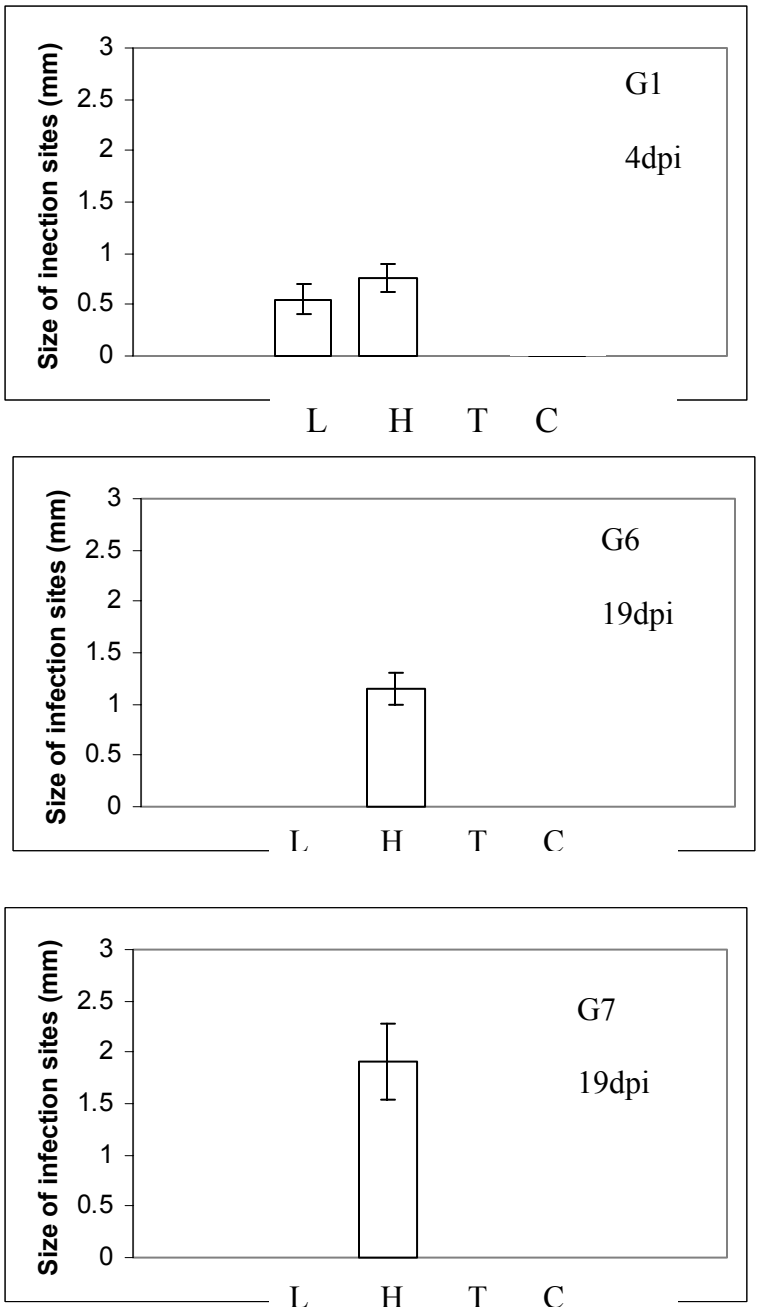
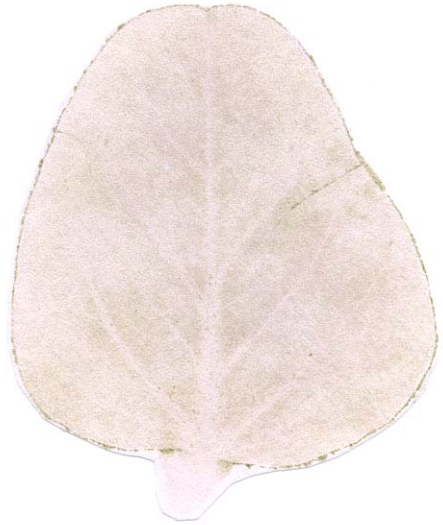


Figure 5.4. Size of initial infection sites on soybean cultivars inoculated with SMV strains G1, G6 and G7. (L= L29; H= Harosoy, T= Tousan140; C= Columbia).
Data (showing standard error bars) were derived from leaf immunoprints from cultivars Columbia, Harosoy, L29 and Tousan 140. No lesions were detected in L, T or C inoculated with G6 or G7 and T or C inoculated with G1. Size of infection sites from unifoliolate leaves inoculated with G1, G6, and G7 and sampled at 4, 19 and 19 dpi, respectively.

A **B**

Figure 5.5. Leaf immunoprints of non-inoculated first trifoliolate leaves from L29, Harosoy and Tousan 140 inoculated with SMV-G1 and G7. (A) L29 SMV-G1inoculated unifoliolate and (B) non-inoculated first trifoliolate leaflet 9 dpi. (C) L29 SMV-G7 inoculated unifoliolate and (D) non-inoculated first trifoliolate leaflet 19 dpi. (E) Harosoy SMV-G1 inoculated unifoliolate and (F-G) non-inoculated first trifoliolate leaflet at 9 and 9 dpi.

Figure 5.5. Continued



C

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E

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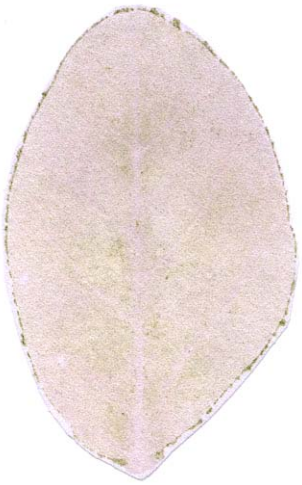

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