Chapter One
Literature Review

History and Economic Importance of Soybean to Virginia

The records from Virginia Historical Society (VHS) show that soybean, *Glycine max* (L.) Merrill (Family Fabaceae, Tribe Phaseolea*e*), was first grown in great quantities in the state during the early twentieth century, in the 1910s and 1920's. The crop was initially introduced as a forage crop for livestock, but later gained prominence as a food crop. This followed an introduction of soybean into the United States of America (USA) by Samuel Bowen, an East India Company employee, who brought the seeds back to England from China and was interested in contracting with farmers to grow the bean in Savannah, Georgia. In 1765, Henry Yonge planted the first soybean in the United States, on his farm in Thunderbolt (Savannah), Georgia (Hymowitz and Harlan, 1983; Hartman et al., 1999; Hymowitz, 2004). Although Virginia's agriculture was founded on tobacco, which has always been the main export crop, soybean has eventually gained a strong foothold as a major crop. Currently, soybean is "the largest hectarage crop in Virginia", but still ranks second as a revenue earner for the state. Its position as a major currency generator is only surpassed by tobacco (http://www.vaes.vt.edu/tidewater/soybean/ retrieved 4. 5. 2004). Soybean provides an employment base to approximately 50,000 families who are directly involved in research and agricultural production, while another 10,000 families are engaged in processing different products from the crop. Indirectly, another 20,000 families are dependent on soybean, as support groups that provide financial support for production, and facilitate the marketing of the crop. Thus the soybean industry employs over 1.09% of the state's total population (USDA, National Agricultural Statistics Services, 2004). Producers in the commonwealth earned an average of US$75,974,600 per year between 1999 and 2003, from the sales of soybean (Table 1.1).

Soybean can be processed into oils for domestic, institutional and industrial uses, as well as providing soybean meal and soybean cake that are utilized in formulating rations to feed livestock (Lusas, 2004). In Virginia, 90% of the seeds are sold to

processors for oil extraction and 10% are consumed locally, while the fodder is used for feeding livestock.

Virginia ranks approximately twentieth overall among other states in terms of soybean production, nationwide. The USA has in recent years produced more soybean than any other country in the world, approximately 47% of the world's total production, but this may be changing because Brazil, Argentina and Mexico have all increased their production and may soon lead the USA in soybean production (Sonka et al., 1999). In 2003, soybean production declined in the USA by 5% from 2002 (http://www.Soyatech.com, retrieved 4. 17. 2004). Although different states in the USA have often produce different amounts of soybean, the trends over the years have shown increased hectarage planted compared to earlier years. States in which soybean is grown, either as a major or a minor crop, are shown in Figure 1.1.

Glycine max is grown in Virginia as a full season (FS) crop or a double crop (DC). In FS cropping, the emphasis is placed on crop rotation, because soybean is planted in rotation with crops like corn, peanut, cotton or vegetable crops. In the DC, growers aim to diversify and maximize production by growing soybean after a main crop, usually small grains (wheat or barley), in the same growing season.

Table 1.1. The total hectares (hect) planted and harvested, yield (m^3/hect), price in US dollars per cubic meter (m^3) and total dollars earned between 1999-2003 in Virginia, from soybean sales. (Adapted from http://www.nass.usda.gov:81/ipedb/ (retrieved 3. 20. 2004)).

	Planted	Harvested	Yield	Production	Price	Value
Year	Hectares	Hectares	m^3/hect	m^3	US$/$m^3$	US$(000)
1999	190,350	178,200	2.3	415,800	128.57	53,460
2000	198,450	194,400	3.3	646,800	124.29	80,391
2001	202,500	194,400	3.1	596,400	122.86	73,274
2002	194,400	178,200	2.0	354,200	158.29	56,066
2003	202,500	194,400	2.9	571,200	204.86	117,016

Diseases of Soybean

Although soybean has maintained a good showing among crops in the state, its potential to generate more revenue is being challenged by a number of diseases caused by viruses, bacteria, phytoplasma, fungi, and nematodes. These phytopathogens (pathogens) affect the crop before, during or after production in the field. The ability to realize higher yields will greatly depend on adequate management of these disease problems. Hartman et al. (1999) reported that more than 100 pathogens affect soybean. About 35 of these pathogens are economically important because they cause quality and yield reductions.

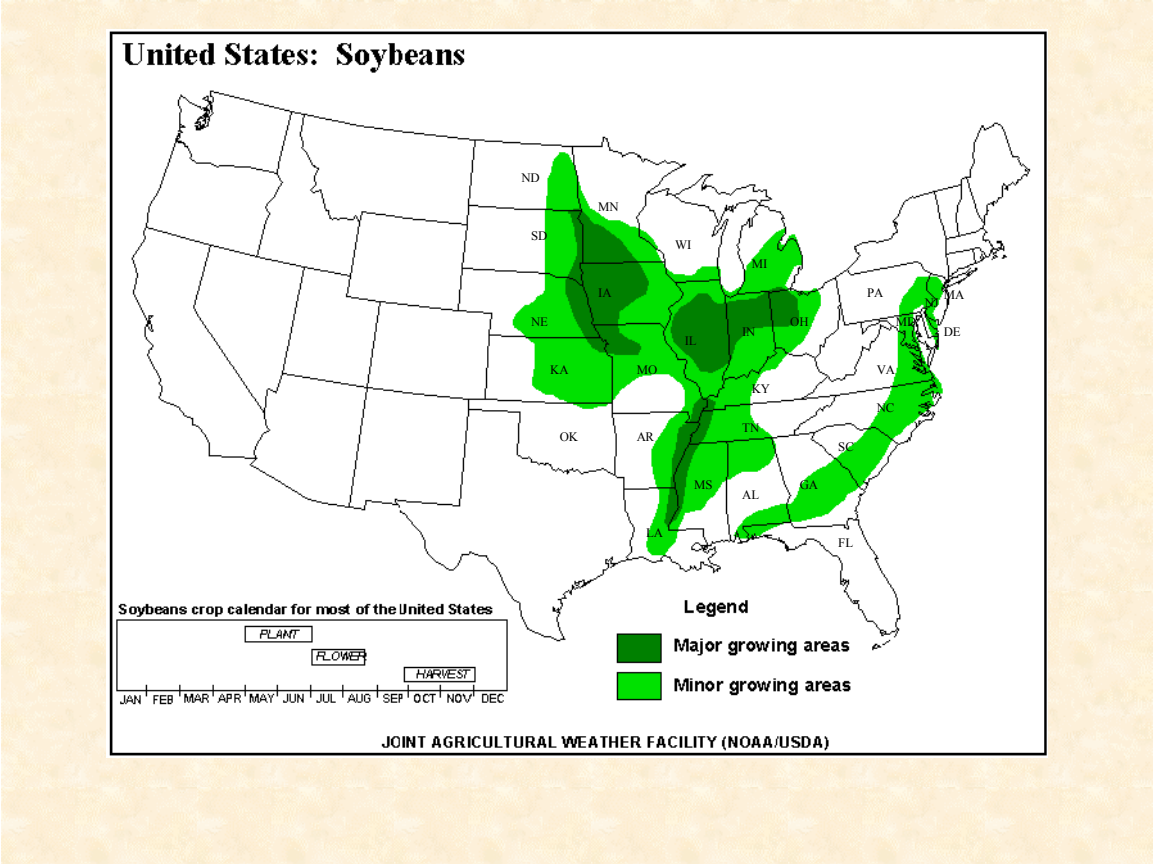

Figure 1.1. The map of United States of America, showing areas of the states that are major and minor growers of soybean. It also depicts a caption indicating the time when the crop is planted and harvested in most areas in the USA (Adapted from Joint Agricultural Weather Facility of NOAA/USDA, retrieved 3. 20. 2004).

Hartman et al. (1999) listed infectious diseases as being caused by viruses, prokaryotes such as bacteria and mollicutes, fungi and nematodes. The non-infectious disorders were listed as those stress conditions associated with abiotic factors (air, temperature, moisture and humidity), insect damage, and human activities that may introduce chemicals or pollutants into the environment. All parts of the plant including roots, stems, and foliage are susceptible to diseases (Hartman et al., 1999). Insects attack both aerial and subterranean parts of soybean (Turnipseed and Kogan, 1976; Turnipseed and Marcos, 1987). Leaf injury by insects reduces the leaf area index (LAI), which affects yields in soybean (Malone et al., 2002). Insects also transmit plant pathogens, especially viruses and phytoplasma. At least 8 major insect vectors capable of transmitting 10 virus diseases of soybean were recognized in the USA by Ford and Goodman (1976). According to Tolin and Lacy (2004), at least 46 viruses transmitted by either insects or fungi, or nematodes, including viruses whose virus/vector associations have not been confirmed; and eight bacteria as well as five phytoplasmas, cause diseases of soybean. Hartman et al. (1999) also listed at least 40 fungal pathogens of soybean and enumerated approximately 100 species of nematodes that have been associated with soybean. But in the case of nematodes, only a few species cause any major economic losses. The major nematodes species that transmit viruses belong to the order Dorylaimida, in the phylum Nematoda (Niblack, et al., 2004; Tolin and Lacy, 2004) (see also under disease ecology and epidemiology, below).

Incidence of Viruses in Virginia and USA

A number of viral diseases of plants have had mixed effects on soybean production in Virginia by reducing quality and yield, while other viral diseases have not had notable effects in this regard (Phipps and Tolin, 2001). Based on published reports, mass media, communications with other scientists, electronic media such as web pages and unpublished data, there is an increase in the incidence of some plant viruses in Virginia. An example of a thrips-vectored virus that recently impacted crop production is *Tomato spotted wilt virus* (TSWV, Genus *Tospovirus,* Family *Bunyaviridae*) (van Regenmortel et al., 2000), which adversely affected production of tomato in the state and the USA, generally in 2001 (http://edis.ifas.ufl.edu/BODY_PG101; retrieved 3. 20. 2004). *Bean pod mottle virus* (BPMV, Genus *Comovirus*, Family: *Comoviridae*) is

becoming a real problem in states where most of the soybean is grown (Giesler et al., 2002). *Soybean mosaic virus* (SMV, Genus *Potyvirus*, Family: *Potyviridae*) (Barnett, 1991; Shukla et al., 1994) has been shown to exist in all areas where soybean is grown, worldwide. An increase in incidence is normally associated with a combination of factors, as will be discussed under disease ecology and epidemiology, but it suffices to state at this point that in all these cases, the density and movement of the vector highly influences the incidence of viruses.

Other Viruses in Virginia Soybean

BPMV and SMV are not the only viruses affecting soybean production. According to Phipps and Tolin (2001), and Tolin (personal communication, unpublished), viruses which are known to cause diseases in soybean, and have also been found in soybeans grown in Virginia, include: *Alfalfa mosaic virus* (AMV, Genus *Alfamovirus*, Family *Bromoviridae*); *Peanut mottle virus* (PMV, Genus *Potyvirus*, Family *Potyviridae*); *Peanut stunt virus* (PSV, Genus *Cucumovirus*, Family *Bromoviridae*); *Tobacco ringspot virus* (TRSV, Genus *Nepovirus*, Family *Comoviridae*), *Tobacco streak virus* (TSV, Genus *Ilarvirus*, Family *Bromoviridae*) and *Tomato ringspot virus* (TmRSV, Genus *Nepovirus*, Family *Comoviridae*). Also present and potentially infecting soybean are *Clover yellow vein virus* (CYVV, Genus *Potyvirus*, Family *Potyviridae*); *Bean yellow mosaic virus* (BYMV, Genus *Potyvirus*, Family *Potyviridae*); *Soybean dwarf virus* (SDV, Unassigned, Family *Luteoviridae*) and *Tomato spotted wilt virus* (TSWV, Genus *Tospovirus*, Family *Bunyaviridae*).

Effects of Viruses on Soybean Production

Approximately 46 viruses are known to infect soybeans in the field (Tolin and Lacy, 2004). Symptoms exhibited by diseased plants will vary from yellowed to crinkled leaves, mosaic leaves, stunted plants, persistent green stems, deformed pods and mottled seeds. Some symptoms may lead to deterministic field diagnosis of disease. However, most symptoms overlap and often require laboratory techniques, such as serology or polymerase chain reaction (PCR) methods, for diagnosis.

The effects of pathogenic viruses on soybean, and the yield losses caused by them, can be devastating to growers. Diseases caused by viruses are causing quality and yield losses in *G. max* grown in Virginia (Phipps and Tolin, 2001). Reports from

Southern United States of America's Soybean Diseases loss estimates (SUSASD), show that viruses contributed 0.76% yield loss between 1974 and 1994 (Wrather et al., 1995). The estimated loss caused by viruses accounted for 0.1-0.12% of the total 3.3-6.8% yield losses in Virginia, from all enumerated diseases in soybeans. These estimates included soybean grown in the state between 1991 and 1999 (SUSASD). It is difficult to verify the accuracy of these reports, since the methods of data collection and data analysis were not given.

Yield and quality losses in soybean caused by viral diseases can be high. BPMV causes yield loss ranging between 3 and 52% in single inoculations (Gergerich, 1999). But BPMV dually inoculated with SMV on the same soybean plants, displays more severe symptoms and yield losses (Calvert and Ghabrial, 1983). BPMV and SMV exhibit synergism in terms of increased symptom severity in the doubly infected plants compared to plants singly infected with BPMV (Calvert and Ghabrial, 1983; Anjos et al., 1992). The BPMV titer from greenhouse and field plants was always higher in the doubly inoculated plants than in plants singly inoculated with BPMV (Calvert and Ghabrial, 1983). The ratio of BPMV titer in doubly to singly inoculated plants varied from 1.34 to 6.14 in different leaves, but the ratio of SMV ranged from 0.87 to 1.29 (the details of this interaction will be discussed in Chapter 5). In field experiments, no difference in symptom severity was observed when BPMV was inoculated before or after SMV, nor did SMV titer change (Calvert and Ghabrial, 1983). While soybean cultivars like Hutcheson that show resistance to SMV are known (Buss et al., 1988), no soybean cultivars have been identified to date with resistance to BPMV (Giesler et al., 2002). Losses from SMV can be 8-35%, but losses of 94% have been reported (Hartman, et al., 1999).

Seed Coat Mottling Associated with Viruses

Seed coat mottling shows as irregular patterns of brown to dark brown colorations that originate close to the hilum then spread on to the testa toward the sides on soybean seeds of yellow or greenish seed coat color. These discolorations often cover the whole seed in severely mottled seeds. The pattern is commonly referred to as "bleeding hilum". Severe mottling lowers the desirability of soybean produced for human consumption. This is mainly the case in edible soybean production and marketing, where prices as high

as US$571.43 to US$628.57 per cubic meter are paid when demand is high for high quality soybean (Sullivan, 2003). Poor seed quality resulting from discoloration and low seed weight can lead to heavy losses in revenue to the growers. BPMV is one of the most economically important viruses of *G. max* because it causes seed coat mottling, reduced seed size and possibly seed weight (Giesler et al., 2002; Hobbs et al., 2003). Both BPMV and SMV induce seed coat mottling by enhancing an accumulation of anthocyanins or leucoanthocyanins, producing dark irregular bands on yellow or greenish seeds (Todd and Vodkin 1993). Seed coat mottling caused by SMV may vary from 0% to greater than 95% (Koning et al., 2003).

Other Causes of Seed Discoloration

A number of abiotic and biotic factors cause seed discoloration that may also lower the quality of seeds during production or storage. Abiotic factors such as a low temperature of 15°C or below, occurring at the flowering stage are associated with brown discoloration of hilum in soybean seed (Takahashi and Abe, 1994; Morrison et al., 1998). Other environmental factors such as dampness and high humidity, especially during storage, can greatly affect seed coat color and lower soybean seed quality.

Fungal infection by *Diaporthe/Phomopsis* complex, commonly referred to as *Phomopsis* spp. causes soybean seed discoloration. *Cercospora kikuchii* (Matsumoto and Tomoyasu) Gardner, and *Alternaria spp*., can also occur on pods (Abney and Plopper, 1994). Infection of soybean by BPMV or SMV predisposes seeds to *Phomopsis* spp. (Abney and Plopper, 1994; Koning et al., 2001; Koning et al., 2003).

Another important factor that causes seed coat discoloration and changes in seed coat texture is the genetic make up of a cultivar. The wild relative of *G. max* known as *Glycine soja* Seib. et Zucc, a native plant of China, produces black seeds naturally (Gai et al., 1999, 2000). However, *G. max* has been domesticated and bred to produce yellow or yellowish-white seeds. Dominant genes at the loci *I R* and *T* determine seed coat color in soybean because of their influence on the accumulation of anthocyanins (anthocyanidin glycosides) or proanthocyanidins. The presence of recessive alleles (*i r* and *t*) leads to high accumulation of anthocyanins, but the dominant genes suppress anthocyanin accumulation resulting in yellow seeds (Todd and Vodkin, 1993). The genotypes *I R T, i R T, i r T, i R t* and *i r t* produce yellow, black, brown, imperfect-black and buff soybean

seeds, respectively (Lindstrom and Vodkin, 1991). During seed development, soybean varieties that have the dominant *I* allele expressed a low chalcone synthase (CHS) mRNA activity compared to the pigmented seeds (Wang et al., 1994). Post-transcriptional silencing of the chalcone synthase gene cluster by viral suppressor protein has been proposed by Senda et al. (2004) and Tuteja et al. (2004) as being 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 (Lindstrom and Vodkin, 1991; Wang et al., 1994).

Distribution and Importance of BPMV in Virginia and the USA

BPMV causes severe disease in soybean grown in the southern, eastern, and some parts of north-central states in the USA (Lin and Hill, 1983; Ghabrial and Hershman, 1990). The virus was first reported from bean, *Phaseolus vulgaris* cv. Tendergreen in Charleston, South Carolina (Zaumeyer and Thomas, 1948). BPMV was first isolated from soybean in the USA, in Arkansas (Walters, 1958), and in North Carolina and Virginia in the same year (Skotland, 1958). Since then, it has been reported in Louisiana (Horn et al., 1970), Iowa (Quiniones and Dunleavy, 1971; Krell et al., 2003), Kansas (Ghanekar and Schwenk, 1974), Illinois (Milbrath et al., 1975), Kentucky (Ghabrial et al,. 1977, 1990), Mississippi (Pitre et al., 1979), Nebraska (Lin and Hill, 1983), South Dakota (Langham et al., 1999), Indiana (Giesler et al., 2001), Ohio (Dorrance et al., 2001), and Wisconsin (Lee et al. 2001). The distribution of BPMV in North Carolina was also studied in mid-1980s (Ross and Butler, 1985). Currently, BPMV is considered a major disease problem in both major and minor soybean growing areas of the USA, mainly the South and the Midwest, but it poses a severe threat in central and northern Great Plains (Giesler et al., 2002). Outside the USA, BPMV has been reported in Ontario, Canada (Michelutti et al., 2002), Brazil and Peru (Fribourg and Pérez 1994; Anjos et al., 1999).

Features of BPMV and Comoviruses

Taxonomy

At the taxonomic level, BPMV ranks as a species in the genus *Comovirus* in the family *Comoviridae* (van Regenmortel et al., 2000). There are 64 species in this family, which consists of 15 species in the genus *Comovirus*, four in the genus *Fabavirus* and 34

in the genus *Nepovirus*. In addition, there are ten species that are temporarily assigned to the genus *Nepovirus* and one virus that has not been assigned to any genus (van Regenmortel et al., 2000).

The National Center for Biotechnology Information's (NCBI) GenBank has BPMV's accession numbers as NC_003496.1/NC_003495.1, for RNA1/RNA2. The International Committee on Taxonomy of Viruses' (ICTV) decimal code for BPMV is given as 18.0.1.0.003. The decimal code means that BPMV is the third (alphabetically) virus species in the first (*Comovirus*) genus of the 18th family (*Comoviridae*).

Morphology and Genomic Properties

BPMV is an icosahedral comovirus (van Regenmortel et al., 2000). Each non-enveloped particle has a diameter of 28 nm and has a positive sense, single stranded, bipartite RNA genome. It is a T=3 virus like the picornaviruses. Members of the comoviruses and picornaviruses have evolved from a common ancestor and the proteins which form the virions have a similar ultrastructure. In a T=3 virus such as poliovirus there are 3 different subunits per 60 triangles. BPMV is different because it is composed of 60 triangular units. A single protein is composed of 3 antiparallel beta-barrel proteins subunits. Each of the 60 units consists of two types of protein subunits, small (22kDa) and large (42kDa), encoded by RNA2, that are non-covalently linked with interfaces stabilized by hydrophobic interactions (Goldbach and Wellink, 1996).

BPMV has a segmented genome consisting of two single-stranded, linear RNA molecules, named RNA1 and RNA2. Both genome segments are encapsidated separately into different types of particles that separate during the density gradient centrifugation procedure into top (T), middle (M) and bottom (B) particles. A single RNA1 segment fits into each B particle, while an RNA2 segment is contained in each M particle. The T particles contain no nucleic acids. RNA1 segment is 5995 nucleotides (nt) (Di et al., 1999) and RNA2 segment is 3688 nt. MacFarlane et al. (1991) estimated 3662 nt for RNA2. The 5' end of the genome has a genome-linked protein (VPg), and the 3' end has a poly (A) tract. Both RNA1 and RNA2 are required for infectivity (Goldbach and Wellink, 1996).

RNA1 (5995 nt) produces a polyprotein of 202 kDa which is processed into 5 mature proteins 32kDa, 58kDa, VPg (4 kDa), 24 kDa and 87 kDa. These are processed

into regulator (protease co-factor), nucleotide binding protein (helicase), genome linked protein, protease produced from a 110 kDa intermediate and polymerase produced from a 110 kDa intermediate, respectively. The RNA2 (3688 nt) produces a polyprotein of 105 or 95 kDa processed into 4 functional proteins, movement protein (cell-to-cell-movement), cell-to-cell-movement protein, derived from a 58 kDa product, large coat protein, and small coat protein (Goldbach and Wellink, 1996; Gu et al., 2001).

Strain Diversity

BPMV field isolates from Virginia, Kentucky, Arkansas and Iowa were classified into two subgroups, I and II, based on nucleic acid hybridization of cloned cDNA probes by Gu et al. (2001). They also found that there are reassortant strains between the two subgroups (I/II). The probes were designed from RNA1 of BPMV strains, Kentucky-Graves (K-G7) and Kentucky-Hancock (K-Ha1). The percentage nucleotide sequence identity between strains K-G7 a prototype of subgroup I, and K-Ha1 a prototype of subgroup II were determined to be 85.6% and 86.9% for RNA1 and RNA2, respectively (Gu et al., 2001). In the NCBI GenBank, full sequences for RNA1 and RNA2 are available (MacFarlane et al., 1991; Di et al., 1999), and also partial sequences.

BPMV has always been confined to the USA. However, there are reports that Canada, Brazil and Peru have also reported BPMV in soybean (Fribourg and Pérez 1994; Anjos et al., 1999; Michelutti et al., 2002). This spread of BPMV to other countries, which may have taken place through seed, may bring about new strain diversity of the virus in the future, as soybean production increases in most of these countries.

Features of SMV and Potyviruses

Taxonomy

SMV is a species in the genus *Potyvirus*, in the family *Potyviridae*. The ICTV decimal code for SMV is 00.057.0.01.061 and the virus accession number is 57010061 (Shukla, 1994; van Regenmortel et al., 2000). Other genera in the family *Potyviridae* are *Bymovirus, Ipomovirus, Macluravirus, Rymovirus* and *Tritimovirus*.

Morphology and Genomic Properties

The non-enveloped virions contain one molecule of linear single stranded RNA. *Potyvirus* particles are flexuous (non-rigid) rods of 750 nm long and 11-15 nm in diameter. Both the axial canals and the helices are obscure. The total genome length is

about 9588 nt. The complete genome sequences of SMV genomic RNA are present in the NCBI GenBank (Jayraham et al., 1992).

Strain Diversity

A number of SMV strains have now been recognized and used in different studies to establish the genetics of SMV resistance. Cho and Goodman (1979) recognized seven strain groups of SMV known as G1-G7 that were differentiated on the basis of reaction of differential host genotypes. Two other strains C14 and G7A were later added (Buzzell and Tu, 1984; Lim, 1985). At least 14 strains have been reported from Canada, China, Japan and Korea (Cho et al., 1977; Takahashi et al., 1980; Tu and Bruzzell, 1987; Gai et al., 1989). The pathogenic relationship among the strains from different places is being studied. New Korean strains of SMV have been identified (Chen et al., 2004).

A number of soybean cultivars have resistance to one or more strains, but resistance is often allelic. Chen et al. (1991) studied the genetic relationships among different strains of SMV and soybean cultivars, and found that there are at least two genes that condition resistance to SMV. They found that single resistance genes in 'Ogden', 'York', 'Marshall', and 'Kwangyo' were also the alleles at the *Rsv1* locus and were assigned the gene symbols, *Rsv1-t*, *Rsv1-y*, *Rsv1-m*, and *Rsv1-k*, respectively. *Rsv4,* a gene isolated from LR2 (a progeny of PI486355 x Essex), is a completely dominant locus that confers resistance to SMV in both homozygous and heterozygous conditions (Ma et al, 1995). PI486355 contained two genes for SMV resistance, one of which is at the *Rsv1* locus and was designated *Rsv1-s*. These six *Rsv1* alleles confer differential reaction to SMV strains G1 through G7 (Chen et al., 1993; Ma et al., 1995). Genotypes resistant to one strain are often susceptible to other strains and commonly grown cultivars are susceptible to multiple strains. Cultivar reactions to SMV vary from resistant (symptomless) to susceptible (mosaic). Bowers and Goodman (1991) established an interaction between strains SMV G1 to G7 and different soybean cultivars relative to seed transmission.

Disease Ecology and Epidemiology

The occurrence of any plant disease requires the dynamic interactions between virulent plant pathogens, susceptible plant hosts and an optimum environment (Agrios, 1997; Irwin et al., 2000). Although viruses do not actively penetrate plant tissues, several

routes of entry are exploited to move from one host plant to another (Hull, 2002). These movements can be vertical, thus involving movement from one plant generation to the next, or they can also involve horizontal movement between plants in the same field, during a growing season. New cropping areas are associated with new viruses (Tolin and Lacy, 2004). Sites that help vectors over-winter or act as reservoirs for viruses will favor the spread of diseases in soybean. Increased use of no-tillage or minimum tillage increases weeds and plant materials necessary for over-wintering beetles or vegetation where aphids can breed in early spring. These weeds may also be acting as reservoir hosts for viruses (Krell et al., 2003). But changes in the weather that will enhance rapid and sustained vector increase, especially warm winters, may also play an important part in increasing survivability of vectors or help them move to areas usually restricted by cold winters (Giesler et al., 2001).

Seed as a Source of BPMV and SMV

Seeds are the basic input for production of most crops in the field as well as the final product in any agricultural production (Agarwal and Sinclair, 1997). Soybean, a major world food crop, is produced from seed. Both biotic and abiotic factors contribute greatly to the quality of seed during growth and development of the crop. The value of soybean to a producer depends on the quality of seeds both at planting and during harvesting. One biotic factor that contributes to loss of seed quality is disease. Diseases in soybean are caused by pathogens such as viruses, bacteria and fungi among others.

Seeds provide a suitable and a major route of vertical transmission of pathogens from one plant generation to the next (Hull, 2002). Only eight viruses are known to be transmitted through soybean seed (Tolin and Lacy, 2004). Seed transmission of BPMV in soybean seed remains a center of controversy, as it is documented at only 0.1% (Hartman et al., 1999). An even lower seed transmission of BPMV of 0.012 to 0.037% has been documented (Krell et al., 2003). While some researchers have confirmed its seed transmission (Lin and Hill, 1983), others have not shown evidence of seed transmission of the virus in soybean (Skotland, 1958; Ross 1963; Schwenk and Nickell, 1980). This conflicting situation therefore raises the question whether BPMV is seed-borne or seed transmitted, or both. A seed-borne virus is on the seed coat or between the seed coat and the cotyledon, while a seed transmitted virus is incorporated in the embryo

during the development of the seed. Enzyme-linked immunosorbent assay (ELISA) has been used to detect viruses in seeds and in plants (Lister, 1978).

Seed transmission of SMV has been confirmed wherever soybean is grown, worldwide (Hill et al., 1987; Pacumbaba, 1995). A vertical transmission from one plant generation to the next occurs in the seed and provides the initial source of inoculum in the field (Irwin et al., 2000). The virus is transmitted in the seed at greater than 30%, depending on the severity of infection, cultivar and the strain of the virus. However, there is no significant difference between mottled and normally colored non-mottled seeds, in terms of seed transmission of SMV (Hill et al., 1980; Pacumbaba, 1995). The same is true for mottled seeds from BPMV infected plants, indicating that seed transmission of BPMV or SMV cannot be predicted based on seed coat mottling.

The correlation between seed coat mottling and seed transmission or vice versa, appears to be insignificant. This has made it difficult to predict the level of BPMV or SMV infection when the seeds are grown in the field, however, there is a good indication that seeds from SMV infected plants show 92% mottling compared to 96% mottling in seeds from plants doubly infected with BPMV and SMV (Quiniones et al, 1971). The finding indicated that there is a correlation between seed infection by BPMV and SMV, and seed coat mottling in soybean (Quiniones et al, 1971). SMV transmission in the seed varies from 5 to 75% depending on the cultivar (Hartman et al., 1999).

Vectors as a Source of BPMV and SMV

Arthropod vectors are the most common means of transmitting viruses horizontally between hosts in the field. Insects especially aphids (Homoptera: Aphididae), transmit in excess of 70% of plant viruses under natural conditions. Damage to plant tissues caused by other animals including humans, abiotic agents such as strong wind, high temperature and moisture stress, may greatly lower plants' defense responses, which may create points of entry, and a suitable environment for viruses resulting in disease development (Agrios, 1997).

In order to transmit viruses, vectors have to acquire the virus from an infective source (host) and retain the pathogen for a length of time. After this period, which can be very short or long depending on the virus, the pathogen can be passed on to another host. BPMV is efficiently transmitted by the bean leaf beetle, *Cerotoma trifucata* Forster, in a

non-persistent or semi-persistent manner (Ross, 1963; Walters, 1964; Wang et al., 1992, 1994; Hartman et al., 1999). This means that the virus can be acquired and immediately passed to the uninfected host or the virus gets into the beetle's gut and is passed into the hemolymph. *Epilachna varivestis* Mulsant and *Diabrotica undecimpunctata howardii* Barber, are also known to transmit BPMV, but less efficiently compared to *C. trifucata* (Hartman et al., 1999). Over-wintering beetles can transmit the virus during the early part of spring when they feed on a suitable host, by passing the virus through their regurgitant into the plant tissue. A low transmission rate of 1 in 194 samples of beetle transmission of BPMV by over-wintering beetles was estimated (Krell et al., 2003). Serological techniques have been used to detect the presence of BPMV in bean leaf beetles (Ghabrial and Schultz, 1983). They used the method to accurately predict incidences of BPMV in most locations in Kentucky.

In the case of aphids, the vectors that transmit SMV, the virus is acquired and transmitted in a non-persistent, or stylet-borne manner. The virus is acquired and released after a very short time, usually 30 seconds, when aphids probe plant hosts for suitable plants to feed. Approximately 32 species of aphids from 15 different genera can spread SMV between plants in the field. They called this a "zig-zag" transmission (Irwin et al., 2000). This horizontal transmission also forms an important epidemiological parameter associated with the spread of the disease in non-persistent transmission of aphid-borne viruses (Irwin et al., 2000).

The presence of soybean aphid (*Aphis glycines* Matsamura) in the USA is a matter of concern to growers because this is a new vector that was recently introduced into the nation from China. The presence of this vector was confirmed during 2001 field surveys in Virginia. Any conditions that favor rapid development and high population build up of this aphid, and large wind masses that can transport them over long distances, may greatly influence epidemiology of virus diseases in soybean in Virginia. Long distance travel in wind masses is an important epidemiological parameter associated with the spread of the aphid-borne diseases (Ruesink and Irwin, 1986). *A. glycines* overwinters on *Rhamnus* spp., and has been proven to transmit every SMV isolate (Clark and Perry, 2002). From the time of its first detection in Wisconsin in July 2000, it had

spread to 21 US states, including Virginia, and three Canadian provinces by the end of 2003 (Venette and Ragsdale, 2004).

Other Plants as Reservoirs of BPMV and SMV

The primary inoculum source for BPMV remains generally speculative. Clover and other legumes growing in the field may be acting as reservoir hosts for BPMV or SMV. From the 1930s, *Desmodium* spp. were reported from Arkansas as a food plant for bean leaf beetle (Isely, 1930). *Desmodium paniculatum* has also been known as a natural host for BPMV (Moore et al., 1969). In a recent survey, BPMV was only detected in one alternate host, *Desmodium canadense*, among naturally occurring plant species collected from the field in Iowa (Krell et al., 2003). No alternate hosts have been found for SMV (Hill et al., 1980; Tolin and Lacy, 2004).

Disease Management

For effective disease management, deployment of integrated methods of disease control coupled with rapid, sensitive and early detection methods remain the cornerstone of avoiding severe disease outbreaks. This is particularly true in the case of viral diseases that may only show low titer, hence may be difficult to detect by symptoms. The use of serological methods and polymerase chain reaction (PCR) as well as molecular cloning techniques, have greatly improved the ability to detect diseases caused by viruses (Hampton et al., 1990; Hadidi et al., 1995). However, in the field, most crop production systems still rely solely on the use of cultural methods, disease resistance and chemical methods, as disease management techniques.

Elimination of Viruses in Propagation Materials

Clean propagation material that is free from viruses is the first line of defense growers can use to control diseases. Clean seed sources for soybean growers through seed certification programs remain the major method of excluding viruses that are transmitted in the seeds (Foster and Hadidi, 1998). However, certification for freedom from BPMV or SMV is not generally done in the USA. But as stated above, the presence of a large number of viruliferous vectors can result in high incidence and severity of BPMV infection from a small amount of inoculum. For SMV, the presence of aphids, especially *A. glycines*, can be very important in the spread of the virus in soybean. In the presence of high populations of *A. glycines* any small source of inoculum will provide a

starting point for a disease epidemic. Soybean is the aphid's principal secondary or summer host. On soybean, the aphid develops winged morphs during the summer when there are host plants in the field and this exposes thousands of acres of soybean to the risk of invasion by this vector (Ragsdale et al., 2004).

Delay of Infection Cycle

Planting time is important if the time when vectors first come into contact with the host can be determined. Delay in planting of soybean has been shown to delay occurrence of BPMV (Ross, 1986). Early planted soybean had higher beetle populations than late planted plants. Over-wintering bean leaf beetles have been shown to be less effective in transmitting the virus (Ziems et al., 2003). Planting time can therefore be planned to coincide with a low population of very active vectors, especially the second and third generations of beetles.

Introduction of Resistance in Cultivars

Resistance to BPMV is not available in any commercial soybean cultivars being used by growers, to date (Giesler et al., 2002). Experiments to incorporate resistance by using coat protein (CP), has been tried with limited success (Di et al., 1996; Reddy et al., 2001). Resistance to BPMV has been shown in the wild *Glycine* spp., hence there is every possibility that incorporation of resistance genes will be accomplished in the future, in *G. max*. Whether this method will become useful on a large scale to combat the problem of BPMV is a future prospect. Cross-protection has also been considered based on relatedness that was demonstrated from molecular analysis of BPMV isolates (Gu et al., 2001). By testing the reaction of different cultivars to different isolates of BPMV, it is possible that the level of tolerance to BPMV will be established.

Use of Chemical Pest Control

Judicious use of pesticides to reduce the population of vectors especially beetles that overwinter, may be an important component of pest management programs (Giesler et al., 2002). It has been demonstrated that the use of cultural and chemical methods in managing BPMV is a viable undertaking. Early use of chemicals to eradicate overwintering beetles and late planting of soybean might help reduce the incidence of BPMV (Ziems et al., 2003). The use of chemical control may not be very effective in reducing viruses in the case of non-persistent transmission, especially when aphids are

already present in the fields. This is because chemicals may accelerate rather than slow transmission of viruses by chemically sprayed aphids, since their probing activities may accelerate before they are killed by chemicals. Aphids are also blown over long distances by wind currents and may traverse huge land masses before landing on soybean. But in trap crops use chemicals before planting a major crop may be useful in reducing populations of existing vectors.

Summary and Objectives

There is evidence to show that an increase in the number of vectors including that of *A. glycines*, an important vector of SMV in soybean, is taking place (Ragsdale et al., 2004). The presence and the spread of *C. trifucata*, bean leaf beetle, a major vector of BPMV has been documented (Ghabrial and Schultz, 1983; Wang et al., 1992; Wang et al., 1994; Giesler et al., 2001). From these occurrences it is evident that there is a need to accumulate data and information about vectors and viruses present in soybean. It is also appropriate to document epidemiology of soybean viruses in Virginia at this time in order to provide adequate surveillance and vigilance that may better prepare the Commonwealth's agriculture to handle future virus problems in soybean production. Work on breeding of resistance in soybean for SMV has been a great success in Virginia due to the work of Buss and others, but no major activities have gone into finding a solution to the problem of BPMV. Most of the information on BPMV is based on studies by Ross in the early 1960's through the 1980's, and those from the midwest by Ghabrial and others. Since different maturity groups are used in the Midwest than in the middle Atlantic, there is a need to establish local information that will be suitable for those maturity groups used in the area.

Therefore, the objectives of this study are to:

(1)- Collect and characterize isolates of BPMV in Virginia and their effects on soybean.

(2)- Compare the reaction of new soybean breeding lines to BPMV and SMV and their effect on seed coat mottling.

(3)- Determine the interaction between time of inoculation and four different BPMV isolates on the severity of seed coat mottling in soybean.

(4)- Study the effect of double inoculation of BPMV and SMV on seeds and yields of Hutcheson soybean.

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