

BEAN LEAF BEETLE: IMPACT OF LEAF FEEDING INJURY ON
SNAP BEANS, HOST PLANT CHOICE AND ROLE AS A VECTOR OF
BEAN POD MOTTLE VIRUS IN VIRGINIA

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ABSTRACT

The bean leaf beetle (BLB), *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae), is a pest of commercially produced legumes in eastern Virginia and much of the soybean production areas of the United States. To better understand the economic injury level of this pest for snap bean production, field cage and manual-defoliation studies were conducted in Virginia. In the manual-defoliation study, snap bean plants had significant yield loss when $\geq 25\%$ of leaf area was removed over a two week period. In the field cage experiments, I was unable to establish beetle densities per plant that were significant enough to impact yield despite releasing up to 1,000 beetles in a cage with 60 bean plants. BLB densities averaged 2.7 BLB per plant in those high density cages.

To better understand host plant selection by BLB, laboratory and field choice experiments were conducted in snap bean, lima bean, and soybeans. Laboratory studies paired snap bean, lima bean and soybean leaf discs from plants of the same age. The field study paired the three legume species with different age groups as well as species for a total of 15 paired combinations. In laboratory studies, BLB preferred snap bean and lima bean over soybean. Field studies did not show a significant effect of bean species or age. Beetles preferred large mature (V4) foliage over small immature foliage (V1). Beetles also showed a preference for snap bean and lima bean plants over soybeans for both large and small plants.

Bean leaf beetles impact yield of soybeans directly by feeding on leaves, stems and pods, and indirectly by transmitting *Bean pod mottle virus* (Secoviridae: Comovirus) (BPMV). A survey was conducted on the Eastern Shore of Virginia to see if the epicenter of the virus was the Eastern Shore Agricultural Research and Extension Center (ESAREC). Fields were selected based on distances from the ESAREC and soybean leaves and bean leaf beetles were collected and assessed for BPMV by immunoassay using enzyme-linked immunosorbant assay (ELISA) or tissue blot immunoassay (TBIA). BPMV-positive beetle ELISA extracts were mechanically inoculated to soybeans and was able to infect BPMV to soybeans. These

soybeans then were tested by TBIA and gave BPMV-positive blots. Other areas of eastern Virginia were also surveyed for virus presence. Beetles at the ESAREC were BPMV-positive upon emergence from overwintering sites, but the virus load was low when tested by ELISA. BPMV load within the beetles was monitored and increased over time before cultivated legumes were available to feed upon. This suggests that they were acquiring virus from a source other than infected cultivated legumes. To find the potential inoculum sources of BPMV in eastern Virginia, leguminous weeds as well as perennial and winter weeds were collected and tested for BPMV using TBIA. Four weed species were found to give BPMV-positive tissue blots including: yellow wood sorrel (*Oxalis stricta*), red sorrel (*Rumex acetosella*), red clover (*Trifolium pretense*), and white clover (*Trifolium repens*). All were BPMV-positive by ELISA except white clover, which further strengthens the argument that these are a source of BPMV. However, virus could not be recovered mechanically from the weeds.

Insecticidal seed treatment of thiamethoxam on soybean seeds was evaluated to test the efficacy of the chemical as well as to evaluate the length of time the treatment would be effective in preventing beetle feeding. Treated seeds were planted in the greenhouse and the leaflets from growth stages VC – V3 were placed in Petri dishes with a single BLB for 24 hours. Beetle mortality was monitored for 72 hours and the leaf area eaten was measured. The thiamethoxam seed treatment protected soybean seedlings from beetle feeding through the V2 stage (2 trifoliates) of growth, which is approximately 21-25 days after seedling emergence and caused a mortality rate of > 70%. At the V3 growth stage (3 trifoliates), there was no effect of treatment.

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Introduction

Legume production in Virginia brings millions of dollars of revenue to the state, particularly the fresh market snap bean (*Phaseolus vulgaris L.*). Soybeans (*Glycine max L.*) are also an important crop especially on the eastern part of the state with an acreage planted of 570,000. The Bean leaf beetle (BLB) *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae) was first reported in 1875 and is native to the U.S. As this beetle has three generations in Virginia, it is a season-long pest of these legumes. The beetle has no effective biological control, thus growers have had to rely largely on insecticides to control this pest. No economic injury level has been established for this pest in snap bean production. An established threshold would benefit farmers because beetles attack both the foliage as well as the pods of the plant. Feeding preference of the beetle may play a role in pest density in the field. Since several different types of legumes are grown in Virginia establishing a preference scale may help farmers plan a pest management strategy.

This beetle is also an effective vector of *Bean pod mottle virus* (BPMV) (*Secoviridae*), a pathogen found primarily in soybeans. While foliar and pod damage from beetles is a concern in soybean production, they additionally transmit BPMV to the plants further decreasing crop yield and quality. This virus causes stunted plants and wrinkled and mottled leaves. Seeds may also have a mottled seed coat, which decreases the value. The BPMV is considered a reemerging disease in soybeans and has increased in prevalence as the BLB population and distribution has increased throughout the U.S. The goal of the research presented in this dissertation is to establish a reliable economic threshold, understand beetle feeding preferences, evaluate the prevalence of BPMV in selected counties Virginia, and design possible management strategies. The five main objectives of this research are:

1. To assess the impact of early-season BLB defoliation on snap beans.
2. Evaluate effect of known levels of defoliation on snap bean yields.
3. Determine relative preference of BLB of three bean species
4. Determine the source of BPMV as well as the percent of BPMV positive bean leaf beetles and soybean plants from various locations in eastern VA.
5. Determine if Cruiser™ insecticide soybean seed treatment can reduce BPMV incidence in soybean.

Chapter One

Bean leaf beetle literature review

Bean Leaf Beetle: Natural History

Distribution. The bean leaf beetle (BLB), *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae), is native to the U.S. and was originally described by Forster in 1771 (McConnell 1915). The first reported incidence of the beetle damaging legumes was in Kansas in 1875 (Popenoe 1876). The beetle was then observed feeding on dwarf beans and string beans near Caldwell, N.J. in 1885 by Crane (Eddy and Nettles 1930). The beetle was first reported as a pest of soybeans in Louisiana during the month of April and in Indiana in June (Webster 1887). Beetle injury on lima and wax beans was observed in Delaware in 1890 (Eddy and Nettles 1930). The beetle continued to be documented across the eastern U.S. including New York to the north and west to Minnesota, South to Mississippi, and the Gulf Coast states (Chittenden 1897).

Host plants. The BLB feeds upon a variety of legumes. When cultivated legumes such as soybeans and snap beans are unavailable, beetles feed and reproduce on native legume genera such as *Amphicarpa*, *Desmodium*, *Lespedeza* and *Stropostyles* (Chittenden 1897, Isley 1930). The preferred vegetable host plants of the BLB are in the family Fabaceae, but other crops such as pumpkin in the family Cucurbitaceae have also been documented as alternative host plants (Koch et al. 2004). This insect pest causes both direct and indirect injury to bean crops. Direct injury is caused by adults feeding directly on the pods, which may cause pod abortion in some species (Shortt et al. 1982). Pod feeding is especially damaging in snap beans where blemished fruit will get culled or the crop value will be lowered (Flood et al. 1995). Additionally, beetle feeding can introduce

pathogens into the legumes including *Bean pod mottle virus* (Secoviridae: Comovirus) (BPMV) and the fungus *Alternaria tenuissima* (Ross 1968, Sinclair and Shurtleff 1975). Additionally, indirect injury can occur through foliar feeding, which may decrease plant productivity and possible stand loss due to high infestations of the beetle (Hutchison et al. 2002).

Life Cycle and Biology

Beetles overwinter as adults, usually in woodlands surrounding the field, and become active in mid-April in Virginia (Boiteau et al. 1979), usually coinciding with spring bean planting. Adult beetles feed and mate on several legume species. A complete generation requires about 30 days. There are three generations of BLB in Virginia (Aguyoh and Masiunas 2004). The overwintering parental generation appears in May and the first generation of adults emerge in June with following generations each month until the fall. The third generation in September will overwinter and when fall arrives beetles migrate to forest edges and bury into the leaf litter. In more northern states overwintering beetles may not emerge until June or July, and only one or two generations occur.

Egg. The female lays 250-350 eggs per season, usually in clumps of 12 in the soil near host plants (Capinera 2001). The optimum depth for egg oviposition is from the soil surface to 7.6 cm below the soil surface (Waldebauer and Kogan 1973). Eggs are oval and are reddish orange in color (Eddy and Nettles 1930). The dimensions of the eggs are 0.8 mm long and 0.35 mm wide. Egg development requires 5 to 7 days (at 25°C constant temperature).

Larvae. Bean leaf beetle larvae are milky white in color and have a well-developed head capsule and thoracic legs with a soft and delicate body (Figs 1.1 and 1.3; Horn 1893). The subcylindrical body has 13 segments with the head and

legs being darker in color than the fleshy body (Chittenden 1897). The larval stage consists of three instars with the mean duration of this life stage lasting 17 days at 25°C (Chittenden 1897). Larval stage duration may be longer or shorter depending on soil temperature and moisture (Marrone and Stinner 1983a). The larvae grow from 1 mm in length at hatching to 7-10 mm at maturity (Capinera 2001). Anderson and Waldbauer (1977) found most larvae between the surface and 7.6 cm deep in the soil with no larvae seen below 15.2 cm. Larvae feed on roots and nodules of host plants.

Pupa. Once the larva reaches maturity, it builds an earthen cell in the soil in which to pupate. Pupae are whitish in color and resemble the adult without fully developed wings. Pupae range in size from 3.0-4.5 mm in length. The pupal stage duration ranges from 5-10 days depending on temperature. Soil moisture, texture, and temperature play a role in pupal mortality (Marrone and Stinner 1984). In dry soils, earthen cells can collapse killing the pupa. Larvae in these soils typically move deeper below ground to avoid desiccation (McConnell 1915). Highest survival was seen in moist organic soil and lowest in dry loamy sand soil (Marrone and Stinner 1984).

Adult. Adults emerge from the soil within a week of pupation and start to feed on legume leaves and pods (Holcomb and Fulton 1978). Beetles have a length of 3.5-5.0 mm. There is no set color or spot pattern (Fig 1.2). Pitre (1989) surveyed polymorphic forms of the BLB in Mississippi and found the greatest percentage of beetles were beige with spots (62%) followed by beige without spots (27%). Beetles that were red with spots made up 8% of the population, while red with no spots comprised 3% of the population (Pitre 1989). However, the defining characteristic for this species is a black triangular spot at the posterior portion of the pronotum (Chittenden 1897). Beetles are easily sexed by the coloration of the

area between the eyes. This area is pale yellow in males and dark in females (Eddy and Nettles 1930, Ruppel 1971). In addition, females are consistently larger than males with an average size advantage of 14 mm³ (Sims et al. 1984). The second generation of beetles emerging in late summer are the most numerous in North Carolina (Sims et al. 1984). The average longevity for female beetles is 41 days (Herzog et al. 1974). Beetles feed and mate on host plants and females oviposit at the base of the plant (Eddy and Nettles 1930).

Bean leaf beetles are effective fliers, which is important for finding food in early spring and later when dispersing within and between fields (Boiteau et al. 1979, Waldbauer and Kogan 1976). Krell et al. (2003a) showed that 90% of beetles they tested made at least one flight with most flight times lasting 30 min. or less with an average distance traveled of 166 m. Beetles may make longer flights in the spring as well as the fall when looking for emerging soybeans or to find overwintering habitats (Jeffords et al. 1983).

Damage

Bean leaf beetle adults feed primarily on the leaves and pods of the legume plant (Isley 1930). The pest status of the adult BLB depends on the crop. Important legume crops attacked in Virginia include soybean, snap bean and lima bean (USDA: NASS 2009). Foliar consumption by an adult beetle can reach 2 cm² a day (Capinera 2001), which can be devastating to young seedlings (Koch et al. 2005). Feeding injury on leaves is often described as having a “shotgun” pattern (Eddy and Nettles 1930). Early beetle injury on seedlings may result in stand loss (Koch et al. 2005). In snap beans, beetle feeding on the pods greatly diminishes the value of the crop. The BLB is an occasional pest of agronomic soybeans, but the pest status can be elevated in areas where *Bean pod mottle virus* (BPMV) (Secoviridae: Comovirus) is prevalent (Giesler 2002). This virus infects soybeans

and can cause a decrease in yield as well as a decline in seed quality (Horn et al. 1973). Transmission of the virus by beetles may also cause green stem syndrome, a plant response where stems stay green past maturity (Schwenk and Nickell 1980). In addition, beetle feeding on pods may introduce fungal pathogens, such as *Phomopsis spp.*, which discolor seeds and decrease quality (Shortt et al. 1982). Larvae feed on the roots and nodules of the bean plant (Isley 1930). The larvae prune and consume roots sections and may even consume the bark of larger roots (Waldbauer and Kogan 1975). Feeding on nodules may result in a decrease in nitrogen fixing abilities of the plant (Leonard and Turner 1918). The impact of larval feeding has not been well studied.

Pest Management

Chemical control. Foliar applications of insecticides are generally effective in controlling adult BLB. However, because of the recurrent generations, multiple sprays are often needed for season-long control. Pyrethroids are the most commonly used insecticides for BLB control. In addition, neonicotinoid insecticide seed treatments, such as thiamethoxam are effective in minimizing first generation adults and protect against larval feeding on roots (Bradshaw et al. 2008). Imidacloprid, another neonicotinoid, is systemic in stem and leaf tissue and will protect the seedlings for weeks after emergence (Koch et al. 2005). Seed treatments and foliar sprays can also be effective against the spread of BPMV (Bradshaw et al. 2008). These authors reported that the greatest yield protection was achieved when the overwintering BLB adults and the F₁ generation were both targeted. Reducing vector populations in the field was achieved by using a seed treatment of thiamethoxam followed by a foliar spray of lambda cyhalothrin.

Cultural control. Mean body size of the BLB is usually omitted in basic life history reports, which is unfortunate since this information would help set reproductive parameters and help with pest management (Sims et al. 1984). Smaller female beetles may produce less offspring or have a shorter life span. Management techniques could be adjusted based upon beetle reproductive potential. Pest management strategies mainly target adults (Capinera 2001). Overwintering adults usually emerge to coincide with spring bean seedlings, which are highly attractive to the pest. Adjusting the planting date of beans may not decrease the abundance of the beetle, but may disrupt the synchrony between insect and preferred crop plant (Metcalf and Metcalf 1993). Adult BLB feeding on alternative food sources, such as grasses, cause a decrease in the beetle's fecundity (Zeiss and Pedigo 1996). Female beetles that spent 7 days or more without soybeans to feed upon had decreased oviposition rates when compared to a soybean control. This decrease in reproductive fecundity significantly reduces population numbers early in the growing season, but once legume crops were available the population recovered (Zeiss and Pedigo 1996).

Beetles will feed upon wild legume species of *Amphicarpa*, *Desmodium*, *Lespedeza* and *Strophostyles* and a few, non-legumes host plants such as *Urtica*, *Laportea*, and *Euonymus* (Helm et al. 1983; Kogan et al. 1980). Reducing alternative food sources for beetles upon emergence may also decrease beetle numbers due to limited food availability.

In addition, it has been shown that reducing the amount of redroot pigweed (*Amaranthus retroflexus* L.) and large crabgrass (*Digitaria sanguinalis* L.) in snap bean fields reduces the number of BLB found in the plots (Aguyoh and Masiusas 2004). Snap bean plants in weed-free areas also sustained less foliar and pod damage as compared to the weed infested areas (Aguyoh and Masiusas 2004).

Natural control. Bean leaf beetles have only a few known natural enemies. Adult parasitism by *Celatoria diabroticae* (Shimer) and *Hyalomyodes triangulifer* (Leow) (Diptera: Tachinidae), can reduce reproductive potential, but they are not effective in reducing population levels (Herzog 1977). Abiotic factors such as climate as well as soil type and moisture can affect eggs and larval mortality. The egg and larvae of the BLB have specific soil moisture requirements. Minimum soil moisture must reach 20% in organic soil, 11% in sandy loam soil and 2% in loamy sand for egg hatch (Marrone and Stinner 1983b). Egg and larval mortality increased linearly when placed in oven dried soil (1000 kPa), where water loss thorough the cuticle was highest (Marrone and Stinner 1984). Larval movement is also affected by moisture, with drier soils inhibiting the insects' ability to find food (Marrone and Stinner 1983a). Soil type also affects the ability of the larvae to form a pupal cell. In dry sandy clay loam, emerging adults may be unable to break through the hard crust this soil forms (Marrone and Stinner 1984).

Host plant resistance. Plants may express resistance to the insect feeding or the viruses they transmit. Snap and lima beans have no physical defense against BLB feeding, but the plants exhibit feeding tolerance. Soybeans have small hairs (trichomes) on the foliar and pod surfaces that retard phytophagous insect feeding (Levin 1973). Highly pubescent soybean cultivars such as 'Clark' with 10.33 trichomes per mm² showed significant reduction in feeding by the BLB, which may reduce BPMV spread (Lam and Pedigo 2001). Although there are no soybean cultivars that are reported to be resistant to BPMV (Ziems et al. 2007), soybean lines have developed tolerance and develop mild mottling symptoms when mechanically inoculated with BPMV (Zheng et al. 2005). There are some transgenic soybean lines expressing the capsid protein coat of the BPMV that have shown some resistance, but they are not commercially available (Reddy et al.

2001). Gunasinghe et al. (1988) and Ren et al. (2000) were also able to show that an increase in leaf and pod pubescence reduced the aphid-transmitted *Soybean mosaic virus* (SMV) in the field. Both studies showed a decrease in SMV due to a greater leaf trichome density, which inhibits probing activity of aphid vector species, reducing virus spread. Field tolerance to BPMV has also been identified in some cultivars of soybean where seed coat mottling and virus titer were low (Hill et al. 2007).

However, Redinbaugh et al. (2010) showed that reducing inoculation or acquisition by eliminating vector populations does not control the virus spread through soybean fields. They also showed that virus incidence was not decreased in fields even though cultivars associated with insect feeding resistance were used. These studies were conducted in the central United States and may not have the same impact on vector population on the east coast where the ecology is vastly different. This further suggests that germplasm development needs to focus on virus resistance instead of inhibiting virus vector herbivory (Kang et al. 2005). Because no one management tactic has shown to be the solution in limiting BPMV in the field, several management techniques may be needed to control this virus and vector.

Bean pod mottle virus

History and importance in the U.S.

Bean pod mottle virus (BPMV) was first discovered in 1947 on *Phaseolus vulgaris* L. var. 'Tendergreen' by Zaumeyer and Thomas (1948) in South Carolina. The virus was then documented in Arkansas soybeans in 1956 (Walters 1958), and was found the same year in North Carolina and Virginia (Skotland 1958). The virus was then detected in the South and North Central U.S. in the late 1960's and

early 1970's, including the states of Louisiana in 1970 (Horn et al. 1970), Kansas (Ghanekar and Schwenk 1974), Illinois (Milbrath et al. 1975), Kentucky (Ghabrial et al. 1977), Mississippi in 1978 (Pitre et al. 1979) and Ohio (Dorrance et al. 2001). The virus continued to spread across the U.S. to Nebraska (Lin and Hill 1983), South Dakota (Langham et al. 1999), Ohio (Dorrance et al. 2001), Wisconsin (Lee et al. 2001), and Indiana (Giesler et al. 2002). The virus was then documented in Ontario, Canada in 2002 (Michelutti et al. 2002). This virus is considered a major threat to soybean production in all soybean growing areas of the U.S. (Giesler et al. 2002; Fig. 1.4). Most yield reduction is due to decreased pod production, reduced pod size, and reduced seed weight (Mueller and Haddox 1980). Yield in areas of the U.S. where the virus has become endemic can experience a loss of up to 40% due to reduced pod production (Horn et al. 1973, Hopkins and Mueller 1984). Poor seed quality due to BPMV infection manifests itself as an irregular black pattern or mottling of the hilum (Tolin and Lacy 2004). The highest incidence of yield reduction was seen in plants that were infected early in the vegetative stages of development (Ross 1969).

Economic losses can also be experienced when plants also become infected with fungal pathogens such as *Phomopsis longicolla* and *Alternaria tenuissima* that cause hilum streaking and poor seed quality resulting in decreased germination rates (Schmitthenner and Kmetz 1980, Shortt et al. 1982). These fungal pathogens may sometimes be misdiagnosed as BPMV due to similar seed streaking. BPMV infects soybeans causing a disease that affects both pod production and seed quality (Horn et al. 1973). BPMV also cause hilum streaking on the soybean seeds, which further decrease the soybean seed value (Hobbs et al. 2003). Hobbs et al. (2003) found that all 82 'William' soybean plants inoculated with BPMV had significantly more hilum streaking than uninfected 'William' soybean plants. Since there are no soybean lines resistant to BPMV, they suggested planting

soybean lines that produce seeds with less streaking such as Natto L95-1805 and Natto L98-7220 (Hobbs et al. 2003).

Symptomology of BPMV

Plant response to the virus may be mild or severe depending on the growth stage infected (Ross 1963, Pitre 1970). Ross (1986) showed that soybean seedlings infected in the V1-V6 (vegetative stages) had a more severe response to the virus compared to older reproductive foliage that had been inoculated with BPMV. However, the plants had lower yields regardless of planting date (Ross 1986). Mild symptoms include slight chlorotic mottling of the foliage to severe mosaic with wrinkled leaves and patches of yellow (Giesler et al. 2002). In severe infections, BPMV may cause terminal necrosis and even death of the infected plant. This virus may also cause delayed maturity of the plant leading to “green stem” disorder (Schwenk and Nickell 1980). The disorder manifests as a soybean plant that has a green, moist stem, but the pods and seeds are ripe and dry (Hill et al. 2006). Green stem decreases harvest efficiency due to the soft plant material interfering with the operating mechanisms of the combine. However, “green stem” disorder was also seen in plants that were serologically negative for BPMV (Hobbs et al. 2006). The plant sensitivity to “green stem” disorder varies among soybean cultivars (Hill et al. 2006). The cause of “green stem” is still uncertain, but by selecting cultivars that express low sensitivity to the disorder, crops could be grown in areas where this disorder is prevalent. BPMV is also synergistic with soybean mosaic virus (SMV), and a dual infection of both viruses can cause >80% yield loss (Ross 1968, Calvert and Ghabrial 1983, Anjos et al. 1992).

Management tactics such as altered planting date, host plant resistance, or chemical control have little effect on the incidence of the virus in the field (Bradshaw et al. 2008). Even though insecticidal seed treatments and sprays that

target the F₁ generation were effective in decreasing the BLB population, the incidence of the virus was not reduced. In contrast, in another study, carefully timed insecticidal sprays that targeted emerging adults (P₁) and the first generation (F₁) were able to decrease the incidence of BPMV in the field as well as increase yield and grain quality (Krell et al. 2004).

Virus Characteristics

Taxonomy

Bean pod mottle virus has been classified in the genus *Comovirus* in the family *Secoviridae* (Sanfaçon et al. 2009) formally known as Comoviridae (van Regenmortel et al. 2000). The genera *Comovirus*, *Fabavirus*, *Nepovirus*, make up the subfamily *Comovirinae*. Bean pod mottle virus is one of 15 species in this subfamily within the family *Secoviridae*, which is part of the *Picornavirales*.

Morphology and Genomic Properties

Bean pod mottle virus has a bipartite positive-sense, single-stranded RNA genome consisting of RNA-1 (≈6.0 kb) and RNA-2 (≈3.6 kb), each of which is contained within an icosahedral particle with a diameter of 28 nm (Goldbach and Wellink 1996). Each genome segment is encapsulated separately into identical capsids. Capsids of all particles are composed of small (S) and large (L) proteins of 22 and 41 kDa, respectively. Sixty copies of each assemble symmetrically to form a capsid through non-covalent binding. These nucleocapsids separate during density gradient centrifugation into top (T), middle (M) and bottom (B) particles (Lomonosoff and Ghabrial 2001). A single RNA-1 segment fits into each B particle, and a RNA-2 segment is contained in each M particle. The 5' end of the genome has a genome-linked protein (VPg), and the 3' end has a poly (A) tract.

Both RNA segments are required for infectivity (Goldbach and Wellink, 1996). The T particles contain no nucleic acids.

Strain Diversity

Bean pod mottle virus is classified into two subgroups (I and II) that have been isolated from Virginia, Kentucky, Iowa and Arkansas (Gu et al. 2001). The subgroups were first based on nucleic acid hybridization of cloned cDNA probes designed by Gu et al. (2001). RNA-1 probes were designed using sequences of the Kentucky-Graves (G-7) and Kentucky-Hancock (K-Ha1) strains of BPMV. The G-7 probe was used to determine subgroup I strains while K-Ha1 was used to identify subgroup II strains of BPMV (Gu et al. 2001). Full and partial sequences for RNA-1 and RNA-2 can be found by searching NCBI GenBank. Codes for BPMV were submitted by MacFarlane et al. (1991) and Di et al. (1999) and are 003496.1 and 003495.1. It was observed by Mozzoni et al. (2009) that the isolates of BPMV had variable symptoms based upon which host variety and species of legume the virus infected. Virus isolates from subgroup I and II were rated based upon severity of symptoms ranging from hypersensitivity, severe mottling, intermediate mottling, and mild mosaic. The most severe symptoms were seen in *Phaseolus* spp. regardless of subgroup of BPMV (Mozzoni et al. 2007). However, symptomology varied among *Glycine max* varieties, suggesting that subgroups could be identified based upon symptomology in specific soybean species, which is similar to findings of Zheng et al. (2005). Cultivars of soybeans had variable symptomology when compared to four BPMV isolates. Zheng et al. (2005) showed that the most severe symptoms were induced by the isolate K-Hol, which is a reassortant isolate of both subgroups; this is also consistent with Gu et al. findings (2001). By using isolates of BPMV and comparing symptomology of *Glycine max*, it may be possible to develop a resistant or tolerant soybean cultivar to the most severe BPMV isolates (Mozzoni et al. 2007).

Detection methods

Detection of BPMV is possible by sampling either plant sap or BLB extracts through the use of enzyme-linked immunosorbant assay (ELISA), or by tissue blot immunosorbant assay (TBIA). Clarke and Adams (1977) described a microplate method for the detection of plant viruses using plant extracts and an enzyme-labeled specific antibody that would react with an enzyme substrate in the presence of virus. This ELISA technique was then modified for the detection of BPMV in bean leaf beetles by Ghabrial and Schultz (1982). It has been used extensively to detect BPMV in leaf sap as well as beetle extracts across the U.S. and has become an invaluable tool for tracking the spread of BPMV.

A more recent detection method of BPMV in beetles and plant sap has been the use of TBIA. This was developed by Lin et al. (1990) as a faster, less tedious immunoassay that could be done in any laboratory. The test involved blotting of leaf sap onto nitrocellulose paper and then performing a few antigen-antibody specific reactions followed by rinsing and substrate application. A positive test sample resulted in a purple precipitate. The test did not require any elaborate plate reading equipment and could be done by technicians with very little training (Lin et al. 1990). This test decreased the time, training and cost of identifying BPMV-positive plant samples. The ELISA serological detection technique was utilized by Ghabrial and Schultz (1982) for the detection of BPMV from beetle extracts. Ghabrial and Schultz used the beetles as an indicator of whether a field would have BPMV-positive foliage. Both of these techniques have been used to test leaf sap as well as beetle extracts for the presence of BPMV across the US and have been invaluable in the study of BPMV.

Host range

To date, BPMV appears to have a narrow host range consisting of four plant species, *Desmodium canadense* (L.), *D. paniculatum* (L.), *Glycine max* (L., Merrill), and *Phaseolus vulgaris* (L.). However, other plant species may be infected by mechanical means (Krell et al. 2003b). Studies have been conducted, to determine a definitive source of primary inoculum for BPMV. *D. paniculatum* was identified as a perennial host of BPMV (Moore et al. 1969). Walters and Lee (1969) confirmed BLB transmission of BPMV from *Desmodium paniculatum* to soybeans.

Vectors of BPMV

Phytophagous insects are the most common means by which viruses are transmitted horizontally in the field. While 70% of known plant viruses are transmitted by aphids (Hemiptera: Aphidae), some beetle families are also capable of transmitting viruses through leaf feeding. To transmit viruses, insects must first acquire the virus particles from an infected host plant and be able to retain the viable pathogen until deposition into plant material occurs. The BLB is the most common vector of BPMV to soybeans. It has several generations a year and is likely responsible for localized spread of BPMV as well as long distance spread due to their seasonal flight capacity (Boiteau et al. 1979, Krell et al. 2003a). Both sexes and all polymorphic forms of BLB are fully capable of transmitting the virus in the field (Pitre 1989). Although the Mexican bean beetle, *Epilachna varivestis* (Coleoptera: Coccinelidae) (Cockbain and Bowen 1975), striped blister beetle, *Epicauta vittata* (Coleoptera: Meloidae) (Patel and Pitre 1971), spotted cucumber beetle *Diabrotica undecimpunctata howardii* (Coleoptera: Chrysomelidae) (Hobbs and Fulton 1979), Japanese beetle *Popillia japonica* (Coleoptera: Scarabaeidae) (Wickizer and Gergerich 2007) and western corn rootworm *Diabrotica virgifera*

virgifera (Coleoptera: Chrysomelidae) (Mabry et al. 2003) can also transmit BPMV, the BLB is considered the most efficient vector of BPMV.

The virus is present in a non-persistent or semi-persistent manner on the mouthparts as well as in the hemolymph of the beetle (Ross 1963; Hartman et al 1999). Movement of the virus into the hemocoel of the vector is unnecessary for virus retention of transmission (Wang et al. 1992). The virus is transmitted easily by mechanical means, is relatively stable and antigenic. Inoculation of a plant virus into foliar tissue was thought to be a simple process of virally contaminated beetle mouth parts macerating foliage (Smith 1965). However, most viruses that are mechanically transmitted cannot be vectored by leaf-feeding beetles, suggesting that virus transmission by chrysomelids is complex and involves various biological factors concerning beetles, viruses and plant interactions (Fulton et al. 1987). Beetles transmit virus particles to plant cells by depositing BPMV contaminated regurgitant onto the wounded surface of the leaf tissue. Since leaf-feeding beetles lack salivary glands the regurgitant acts as a lubricant for the movement of mouthparts during feeding (Snodgrass 1935). Viruses spread by beetles are able to be infective in the presence of regurgitant whereas non-beetle transmitted viruses are non-infective when mixed with regurgitant (Gergerich et al. 1983). These non-beetle transmissible viruses are not inactivated, but prevented from binding to the plant cell by some morphological phenomenon that beetle-transmitted viruses possess (Gergerich and Scott 1988).

Ecology

Although BPMV has been established in the U.S. for several years, little is known about the ecology and epidemiology of this pathogen. There are three hypothesized sources for this virus: infected seed, overwintered viruliferous beetles, and perennial host plants (Giesler et al. 2002). Several studies have

shown low incidence (< 1%) of seed-borne virus with only 1 positive out of 1,000 seeds evaluated (Lin and Hill 1983, Ross 1968). While others have shown no transmission at all to seeds (Schwenk and Nickell 1980, Krell et al. 2003b). Overwintered beetle transmission of the virus was found to be very low as well (Mueller and Haddox 1980). Over-wintering BLB are capable of transmitting BPMV, but at low rates. In Iowa, Krell et al. (2003b) demonstrated that 1 out of 194 overwintered beetles sampled were serologically positive for BPMV. The regional location of soybean fields will determine which beetle species vector will be most prevalent. Growers in the southeastern U.S. have a greater possibility of the BLB being the primary vector of BPMV even though the Mexican bean beetle may be found in fields. For soybean fields located in Illinois, the western corn rootworm is probably the most prevalent virus vector (Mabry et al. 2003).

Walters et al. (1972) showed that serologically positive beetles, through an immunological technique, declined as the winter progressed. This further strengthens the proposition of Horn et al. (1973) and Krell et al. (2003b) that the primary source of BPMV inoculum is a perennial host plant. The first perennial host plant of BPMV was identified as *Desmodium* spp. by Moore et al. (1969). Several researchers have searched for a natural reservoir for this virus and have often found *Desmodium* spp. as an important source for this virus (Moore et al. 1969, Walters and Lee 1969, Lee and Walters 1970, Krell et al. 2003b, Bradshaw 2007). No other naturally occurring infected plant species has been identified, which suggests that the host range of the BLB is far greater than the host plant range of the virus it transmits.

While no single management tactic has proven completely effective in eliminating BPMV from the landscape, vector suppression may be effective when used in combination with other management techniques (Pedersen et al. 2007). Developing a soybean cultivar with field tolerance or host plant resistance may be

the most effective long term approach to dealing with BPMV (Bradshaw et al. 2008, Krell et al. 2004).

BPMV in Virginia

Virginia harvested 230,671 hectares of soybeans with a crop value of \$160 million in 2008. Accomack and Northampton Counties, VA on the Delmarva Peninsula produced 44,000 metric tons of soybeans in 2008 with a value of \$17 million (USDA NASS 2008). In 2007, a BPMV-positive soybean sample was obtained from the Virginia Tech Eastern Shore AREC in Accomack County. A survey to determine where the virus has become endemic in Virginia needs to be performed. In addition, management techniques for virus / vector control need to be evaluated and Virginia soybean growers should be advised on the possible yield loss caused by this virus.

References

- Aguyoh, J.N., and J.B. Masiunas. 2004. Interaction of insects and weeds in a snap bean agroecosystem. *HortScience* 39: 287-290.
- Anderson, T.E., and G.P. Waldbauer. 1977. Development and field testing of a quantitative technique for extracting bean leaf beetle larvae and pupae from soil. *Environ. Entomol.* 6: 633-636.
- Anjos, J.L., Jarlfors, U., and S.A. Ghabrial. 1992. Soybean mosaic potyvirus enhances the titer of two comoviruses in dually infected soybean plants. *Phytopathology* 82: 1022-1027.
- Boiteau, G., J.R. Bradley, Jr., and J.W. Van Duyn. 1979. Bean leaf beetle: Flight and dispersal behavior. *Ann. Entomol. Soc. Am.* 72: 298-302.
- Boiteau, G., J.R. Bradley, Jr., and J.W. Van Duyn. 1979. Bean leaf beetle: Emergence patterns of adults from overwintering sites. *Environ. Entomol.* 8: 427-431.
- Bradshaw, J.D., M.E. Rice, and J.H. Hill. 2007. No-choice preference of *Cerotoma trifurcata* (Coleoptera: Chrysomelidae) to potential host plants of bean pod mottle virus (Comoviridae) in Iowa. *J. Econ. Entomol.* 100: 808-814.
- Bradshaw, J.D., L.P. Pedigo, J.H. Hill, and M.E. Rice. 2008. Evaluation of management strategies for bean leaf beetles (Coleoptera: Chrysomelidae) and bean pod mottle virus (Comoviridae) in soybeans. *J. Econ. Entomol.* 101: 1211-1227.
- Calvert, L.A., and S.A. Ghabrial. 1983. Enhancement by soybean mosaic virus of bean pod mottle virus titer in doubly infected soybean. *Phytopathology.* 73: 992-997.
- Capinera, J.L. 2001. *Handbook of Vegetable Pests*. Academic Press, New York. 93 pp.
- Chittenden, F.H. 1897. The bean leaf beetle. *USDA Div. Entomol. Bull.* 9: 64-71.

- Clarke, M.F., and A.N. Adams. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for detection of plant viruses. 34: 475-483
- Cockbain, A.J., and R. Bowen. 1975. Diseases of field beans (*Vicia faba* L.) Rothamsted Experiment Station Report for 1975. Part . 261-263 pp.
- Di, R., C.C. Hu, and S.A. Ghabrial. 1999. Complete nucleotide sequence of bean Pod mottle virus RNA1: Sequence comparisons and evolutionary relationships to other comoviruses. *Virus Genetics*. 18: 129-137.
- Dorrance, A.E., D.T. Gordon, A.F. Schmitthenner and C.R. Grau. 2001. First report of *Bean pod mottle virus* in soybean in Ohio. *Plant Dis*. 87: 308-312.
- Eddy, C.O., and W.C. Nettles. 1930. The bean leaf beetle. S.C. Agric. Exp. Stn. Bull. 265: 1-25.
- Flood, B., G. Hein, and R. Weinzierl. 1995. Beans. In: Foster, R., Flood, B. (Eds.), *Vegetable Insect Management*. Meister Publishing Co., Willoughby, OH.
- Fulton, J.P., R.C. Gergerich, and H.A. Scott. 1987. Beetle transmission of plant viruses. *Annu. Rev. Phytopathol*. 25: 111-123.
- Gergerich, R.C., and H.A. Scott. 1988. Evidence that virus translocation and virus infection of non-wounded cells are associated with transmissibility by leaf-feeding beetles. *J. Gen. Virol*. 69: 2935-2938.
- Gergerich, R.C., H.A. Scott, and J.P. Fulton. 1983. Regurgitant as a determinant of specificity in the transmission of plant viruses by beetles. *Phytopathology* 73: 936-938.
- Ghabrial, S.A., C.M. Pickard, and R.E. Studkey. 1977. Identification and distribution of virus diseases of soybean in Kentucky. *Plant Dis. Rep*. 61: 690-694.
- Ghabrial, S.A., and F.J. Schultz. 1983. Serological detection of bean pod mottle virus in bean leaf beetles. *Phytopathology* 73: 480-483.

- Ghanekar, A.M., and F.W. Schwenk. 1974. Seed transmission and distribution of tobacco streak virus in six cultivars of soybeans. *Phytopathology* 64: 112-114.
- Giesler, L.J., S.A. Ghabrial, T.E. Hunt, and J.H. Hill. 2002. Bean pod mottle virus: a threat to U.S. soybean production. *Plant. Dis.* 86: 1280-1289.
- Goldbach, R.W., and J. Wellink. 1996. Comoviruses, molecular biology and replication. pp. 35-76. In: Harrison, B.D. and A.F. Murrant (eds.). *The Plant Viruses*. New York: Plenum Press.
- Gu, H., A.J. Clark, P.B. De Sa, T.W. Pfeiffer, S.A. Tolin, and S.A. Ghabrial. 2001. Diversity among isolates of Bean pod mottle virus. *Phytopathology* 92: 446-452.
- Gunasinghe, U.B., M.E. Irwin, and G.E. Kampmeier. 1988. Soybean leaf pubescence affects aphid vector transmission and field spread of soybean mosaic virus. *Ann. App. Biol.* 112: 259-272.
- Hartman, G.L., J.B. Sinclair, and J.C. Rupe (ed.) 1999. *Compendium of Soybean Diseases* 4th ed. APS Press, St. Paul, MN.
- Helm, C.G., M.R. Jeffords, S.L Post, and M. Kogan. 1983. Spring feeding activity of overwintered bean leaf beetles (Coleoptera: Chrysomelidae) on nonleguminous hosts. *Environ. Entomol.* 12: 321-322.
- Herzog, D.C., C.E. Eastman, and L.D. Newsom. 1974. Laboratory rearing of the bean leaf beetle. *J. Econ. Entomol.* 67: 794-795.
- Herzog, D.C. 1977. Bean leaf beetle: Parasitism by *Celatoria diabroticae* (Shimer) and *Hyalomyodes triangulifer* (Leow). *J. Ga. Entomol. Soc.* 12: 64-68.
- Hill, C.B., G.L. Hartman, R. Esgar, and H.A. Hobbs. 2006. Field evaluation of green stem disorder in soybean cultivars. *Crop Sci.* 46: 879-885.
- Hill, J.H., N.C. Koval, J.M. Gaska, and C.R. Grau. 2007. Identification of field

- tolerance to bean pod mottle and soybean mosaic viruses in soybean. *Crop Sci.* 47: 212-218.
- Hobbs, H.A., and J.P. Fulton. 1979. Beetle transmission of cowpea chlorotic mottle virus. *Phytopathology.* 69: 255-256.
- Hobbs, H.A., G.L. Hartman, Y. Wang, C.B. Hill, R.L. Bernard, W.L. Pedersen, and L.L. Domier. 2003. Occurrence of seed coat mottling in soybean plants inoculated with bean pod mottle virus and soybean mosaic virus. *Plant Dis.* 87: 1333-1336.
- Hobbs, H.A., C.B. Hill, C.R. Grau, N.C. Koval, Y. Wang, W.L. Pedersen, L.L. Domier, and G.L. Hartman. 2006. Green stem disorder of soybean. *Plant Dis.* 90: 513-518.
- Holcomb, C.E., and J.P. Fulton. 1978. Rearing the bean leaf beetle, *Cerotoma trifurcata*, for plant virus vector studies. *Plant Dis. Rep.* 62: 12-14.
- Hopkins, J.D., and A.J. Mueller. 1984. Effect of bean pod mottle virus on soybean yield. *J. Econ. Entomol.* 13: 1135-1137.
- Horn, N.L., L.D. Newsom, and R.L. Jensen. 1970. Effects of virus diseases on soybean in Louisiana. *La. Agric.* 13: 12.
- Horn, N.L., L.D. Newsom, and R.L. Jensen. 1973. Economic injury thresholds of bean pod mottle virus and tobacco ringspot virus infection of soybeans. *Plant. Dis. Rep.* 57: 811-813.
- Hutchison, W.D., E.C. Burkness, and T. Rabaey. 2002. New seed treatments for snap beans: first-year results. *Proceedings of the Midwest Food Processors Association, Annual Meeting, February 26-27, La Crosse WI, USA*, pp. 27-37.
- Isley, D. 1930. The biology of the bean leaf beetle. *Ark. Agric. Exp. Stn. Bull.* 248.
- Jeffords, M.R., C.G. Helm, and M. Kogan. 1983. Overwintering behavior and

- spring colonization of soybean by the bean leaf beetle (Coleoptera: Chrysomelidae) in Illinois. *Environ. Entomol.* 12: 1459-1463.
- Kang , B.C., I. Yeam, and M.M. Jahn. 2005. Genetics of plant virus resistance. *Annu. Rev. Phytopathology* 45: 581-621.
- Koch, R.L., E.C. Burkness, and W.D. Hutchison. 2004. Confirmation of bean leaf beetle, *Cerotoma trifurcata*, feeding on cucurbits. *J. Insect Sci.* 4:5 6.
- Koch, R.L., E.C. Burkness, W.D. Hutchison and, T.L. Rabaey. 2005. Efficacy of systemic insecticide seed treatments for protection of early-growth-stage snap beans from bean leaf beetles (Coleoptera: Chrysomelidae) foliar feeding. *Crop Prot.* 24: 734-742.
- Kogan, M., G.P. Waldbauer, G. Boiteau, and C. Eastman. 1980. Sampling bean leaf beetles on soybean, pp.201-236. *In* M. Kogan and D.C. Herzog [eds.], *Sampling methods in soybean entomology*. Springer, New York.
- Krell, R.K., L.P. Pedigo, J.H. Hill, and M.E. Rice. 2003a. Characterization of bean leaf beetle (Coleoptera: Chrysomelidae) flight capacity. *J. Kans. Entomol. Soc.* 76: 405-416.
- Krell, R.K., L.P. Pedigo, J.H. Hill, and M.E. Rice. 2003b. Potential primary inoculum sources of bean pod mottle virus in Iowa. *Plant Dis.* 87: 1416-1422.
- Krell, R.K., L.P. Pedigo, J.H. Hill, and M.E. Rice. 2004. Bean leaf beetle (Coleoptera: Chrysomelidae) management for reduction of bean pod mottle virus. *J. Econ. Entomol.* 97: 192-202.
- Lam, W.-K.F., and L.P. Pedigo. 2001. Effect of trichome density on soybean pod feeding by adult bean leaf beetles (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 94: 1459-1463.
- Langham, M.A.C., D.C. Doxtader, J.D. Smolik, and R.A. Scott. 1999. Outbreak of

- a viral disease affecting soybean in South Dakota. *Phytopathology* 90: S45 (Abstr.).
- Langham, M.A.C., R.C. Gergerich, and H.A. Scott. 1990. Conversion of *Comovirus* electrophoretic forms by leaf-feeding beetles. *Phytopathology* 80: 900-906.
- Lee, F.N., and H.J. Walters. 1970. A virus isolated from *Desmodium* related to bean pod mottle virus. *Phytopathology*. 60: 585.
- Lee, M.E., N.C. Kurtzwell, and C.R. Grau. 2001. Prevalence and agronomic effects of viruses in Wisconsin soybeans. North Central Division of the Amer. Phytopathol. Soc. P-2002-0015-NCA.
- Leonard, L.T., and C.F. Turner. 1918. Influence of *Cerotoma trifurcata* on the nitrogen gathering functions of the cowpea. *Jour. Am. Soc. Agron.* 10: 256-261.
- Levin, D.A. 1973. The role of trichomes in plant defense. *Q. Rev. Biol.* 48: 3-15.
- Lin, M.T., and J.H. Hill. 1983. Bean pod mottle virus: Occurrence in Nebraska and seed transmission in soybeans. *Plant Dis.* 67: 230-233.
- Lin, N.S., Y.H. Hsu, and H.T Hsu. 1990. Immunological detection of plant viruses and a mycoplasma-like organism by direct tissue blotting on nitrocellulose membranes. *Phytopathology* 80: 824-828.
- Lomonosoff, G.P., and S.A. Ghabrial. 2001. Comoviruses. Pages 239-242 in *Encyclopedia of Plant Pathogens*, vol. 1. O. Maloy and T. Murray, eds. John Wiley & Sons, New York.
- Mabry, T.R., H.A. Hobbs, T.A. Steinlage, B.B. Johnson, W.L. Penderson, J.L. Spencer, E. Levine, S.A. Isard, L.L. Domier, and G.L. Hartman. 2003. Distribution of leaf-feeding beetles and bean pod mottle virus (BPMV) in Illinois and transmission of BPMV in soybean. *Plant Dis.* 87: 1221-1225.
- MacFarlane, S.A., M. Shanks, J.W. Davies, A. Zlotnick, and G.P. Lomonosoff.

1991. Analysis of the nucleotide sequence of bean pod mottle virus middle component RNA. *Virology* 183: 405-409.
- Marrone, P.G., and R.E. Stinner. 1983a. Bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae): Physical factors affecting larval movement in soil. *Environ. Entomol.* 12: 1283-1285.
- Marrone, P.G., and R.E. Stinner. 1983b. Effects of soil physical factors on egg survival of the bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae). *Environ. Entomol.* 12: 673-679.
- Marrone, P.G., and R.E. Stinner. 1984. Influence of soil physical factors on survival and development of the larvae and pupae of the bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae). *Can. Entomol.* 116: 1015-1023.
- McConnell, W.R. 1915. A unique type of insect injury. *Econ. Entomol.* 8: 261-266.
- Metcalf, R.L., and R.A. Metcalf 1993. *Destructive and Useful Insects: Their Habitat and Control*, 5th ed. McGraw-Hill, New York.
- Michelutti, R., J.C.Tu, D.W.A. Hunt, D. Gagnier, T.R. Anderson, T.W. Welacky, and A.U. Tenuta. 2002. First report of *bean pod mottle virus* in soybean in Canada. *Plant Dis.* 86:330.
- Milbrath, G.M., M.R. McLaughlin, and R.M. Goodman. 1975. Identification of bean pod mottle virus from naturally infected soybeans in Illinois. *Plant Dis. Rep.* 59: 982-983.
- Moore, B.J., H.A. Scott, and H.J. Walters. 1969. *Desmodium paniculatum*, a perennial host of Bean pod mottle virus in nature. *Plant Dis. Rep.* 53: 154-155.
- Mozzoni, L., P. Chen, and R. Gergerich. 2009. Differentiation of Bean pod mottle virus isolates based upon host symptoms. *Eur. J. Plant Pathol.* 124: 133-140.
- Mueller, A.J., and A.W. Haddox. 1980. Observations on seasonal development of

- bean leaf beetle, *Cerotoma trifurcata* (Forster) and incidence of bean pod mottle virus in Arkansas' soybean. J. Ga. Entomol. Soc. 15: 398-403.
- Patel, V.C., and H.N. Pitre. 1971. Transmission of Bean pod mottle virus to soybean by the striped blister beetle, *Epicauta vittata*. Plant Dis. Rep. 55: 628-629.
- Pedersen, P., C. Gran, E. Cullen, and J.H. Hill. 2007. Potential for integrated management of soybean virus disease. Plant Dis. 91: 1255-1259.
- Pitre, H.N. 1970. Insect and experimental transmission studies in relation to bean pod mottle virus disease of soybeans. Miss. Farm Res. 33:1.
- Pitre, H.N. 1989. Polymorphic forms of the bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae), in soybeans in Mississippi: Transmission of bean pod mottle virus. J. Entomol. Sci. 24: 582-587.
- Pitre, H.N., V.C. Patel, and B.L. Keeling. 1979. Distribution of bean pod mottle disease on soybeans in Mississippi. Plant Dis. Rep. 63: 419-423.
- Popenoe, E.A. 1876. A list of Kansas Coleoptera. Trans. Kan. Acad. Sci. 5: 21-40.
- Reddy, M.S.S., S.A. Ghabrial, C.T. Redmond, R.D. Dinkins, and G.B. Collins. 2001. Resistance to *Bean pod mottle virus* in transgenic soybean lines expressing the capsid polyprotein. Phytopathology 91: 831-838
- Redinbaugh, M.G., J.E. Molineros, J. Vacha, S.A. Berry, R.B. Hammond, L.V. Madden, and A.E. Dorrance. 2010. Bean pod mottle virus spread in insect-feeding-resistant soybean. Plant Dis. 94: 265-270.
- Ren, Q., T.W. Pfeiffer, and S.A. Ghabrial. 2000. Relationship between soybean pubescence density and soybean mosaic virus field spread. Euphytica. 111: 191-198.
- Ross, J.P. 1963. Transmission of bean pod mottle virus in soybeans by beetles. Plant Dis. Rep. 47: 1049-1050.
- Ross, J.P. 1968. Effect of single and double infections of soybean mosaic and

- bean pod mottle viruses on soybean yields and see characteristics. *Plant Dis. Rep.* 52: 344-348.
- Ross, J.P. 1969. Effect of time and sequence of inoculation of soybeans with soybean mosaic and bean pod mottle viruses on yields and seed characters. *Phytopathology* 59: 1404-1408.
- Ross, J.P. 1986. Response of early-and late-planted soybeans to natural infection by bean pod mottle virus. *Plant Dis.* 70: 222-224.
- Ruppel, R.F. 1971. An asymmetrical grandmorph of *Cerotoma facialis*. (Coleoptera: Galerucidae). *Mich. Entomol.* 4: 1-2.
- Sanfacon, H., J. Wellink, O.L. Gall, A. Karasev, R. van der Vlugt, and T. Wetzell. 2009. Secoviridae: a proposed family of plant viruses within the order *Picornavirales* that combines the families *Sequiviridae* and *Comoviridae*, the unassigned genera *Cheravirus* and *Sadwavirus*, and the proposed genus *Torradovirus*. *Arch. Virol.* 154: 899-907.
- Schmitthenner, A.F., and K. Kmetz. 1980. Role of *Phomopsis* spp. in the soybean seed rot problem. *Proc. World Soybean Res. Conf.* 2nd. Westview Press, Boulder, CO. 355-366.
- Schwenk, F.W., and C.D. Nickell. 1980. Soybean green stem caused by bean pod mottle virus. *Plant Dis.* 64: 863-865.
- Shortt, B.J., J.B. Sinclair, C.G. Helm, M.R. Jeffords, and M. Kogan. 1982. Soybean seed quality losses associated with bean leaf beetles and *Alternaria tenuissima*. *Phytopathology* 72: 615-618.
- Sims, S.R., P.G. Marrone, F. Gould, R.E. Stinner, and R.L. Rabb. 1984. Ecological considerations of bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae), size variation in North Carolina. *Environ. Entomol.* 13: 300-304.
- Sinclair, J.B., and M.C. Shurtleff (eds.) 1975. *Compendium of soybean diseases*.

- Am. Phytopathol. Soc., St. Paul, MN. 69 pp.
- Skotland, C.B. 1958. Bean pod mottle virus of soybeans. *Plant Dis. Rep.* 42: 1155-1156.
- Smith, K.M. 1965. Plant virus-vector relationships. *Adv. Virus Res.* 11: 61-96.
- Snodgrass, R.E. 1935. Principles of insect morphology. pp. 154-155. McGraw-Hill, New York.
- Tolin, S.A., and G.H. Lacy. 2004. Viral, bacterial, and phytoplasmal diseases of soybean. *Soybeans: Improvement, Production, and Uses*, 3rd ed, Agronomy Monograph no. 16. 765-819.
- United States Department of Agriculture [USDA]: National Agricultural Statistics Service (NASS). 2008.
(http://www.nass.usda.gov/Data_and_Statistics/index.asp). Accessed April 10, 2009.
- United States Department of Agriculture [USDA]: National Agricultural Statistics Service (NASS). 2009.
(http://www.nass.usda.gov/Data_and_Statistics/index.asp). Accessed May 25, 2010.
- van Regenmortel, H.M.V., C. Fauquet, D. Bishop, E. Carsten, M. Estes, S. Lemon, J. Maniloff, J., M. Mayo, D. McGeoch, C. Pringle, and R. Wickner. 2000. *Virus taxonomy: Classification and nomenclature of viruses: Seventh Report of the International Committee on Taxonomy of Viruses*. Academic Press, New York. 1167 pp.
- Waldbauer, G.P., and M. Kogan. 1973. Sampling for bean leaf beetle eggs: Extraction from the soil and location in relation to soybean plants. *Environ. Entomol.* 2: 441-446.
- Waldbauer, G.P., and M. Kogan. 1975. Position of bean leaf beetle eggs in soil near soybeans determined by a refined sampling procedure. *Ibid.* 4: 375-380.

- Waldbauer, G.P., and M. Kogan. 1976. Bean leaf beetle: Phenological relationship with soybean in Illinois. *Environ. Entomol.* 5: 35-44.
- Walters, H.J. 1958. A virus disease complex in soybeans in Arkansas. (Abstr.) *Phytopathology* 48: 346.
- Walters, H.J., and F.N. Lee. 1969. Transmission of Bean pod mottle virus from *Desmodium paniculatum* to soybean by the bean leaf beetle. *Plant Dis. Rep.* 53: 411.
- Walters, H.J., F.N. Lee, and K.E. Jackson. 1972. Overwintering of bean pod mottle virus in bean leaf beetles. (Abstr.) *Phytopathology* 62: 808.
- Wang, R.Y., R.C. Gergerich, and K. Kim. 1992. Noncirculative transmission of plant viruses by leaf-feeding beetles. *Phytopathology* 82: 946-950.
- Webster, F.M. 1887. A new enemy to the bean and cowpea. Dept. Agric. Report: 152.
- Wickizer, S.L., and R.C. Gergerich. 2007. First report of Japanese beetle (*Popillia japonica*) as a vector of *Southern bean mosaic virus* and *Bean pod mottle virus*. *Plant Dis.* 91: 637.
- Zaumeyer, W.J., and H.R. Thomas. 1948. Pod mottle, a virus disease of beans. *Phytopathology* 38: 81-96.
- Zeiss, M.R., and L.P. Pedigo. 1996. Timing of food plant availability: Effect on survival and oviposition of the bean leaf beetle (Coleoptera: Chrysomelidae). *Environ. Entomol.* 25: 295-302.
- Zheng, C.M., P.Y. Chen, T. Hymowitz, S. Wickizer, and R. Gergerich. 2005. Evaluation of *Glycine* species for resistance to bean pod mottle virus. *Crop Prot.* 24: 49-56.
- Ziems, A.D., L.J. Giesler, G.L. Graef, M.G. Redinbaugh, J.L. Vacha, S.A. Berry, L.V. Madden, and A.E. Dorrance. 2007. Response of soybean cultivars to Bean pod mottle virus infection. *Plant Dis.* 91: 719-726.

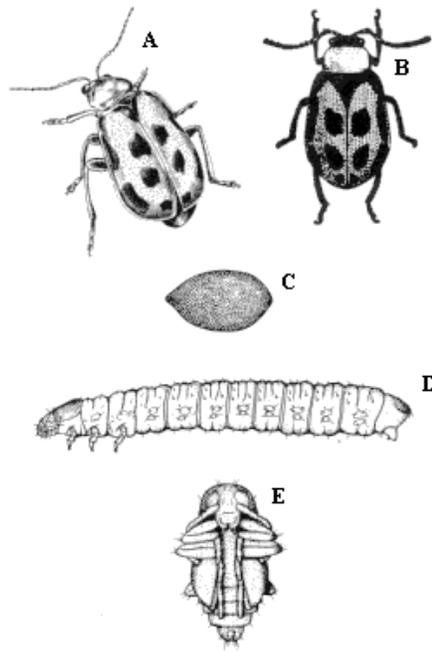


<http://www.ent.iastate.edu/soybeaninsects/node/138>
Picture courtesy of Iowa State University Cooperative Extension. 2010.

Figure 1.1. Bean leaf beetle larvae on legume cotyledon.



Figure 1.2. Adult bean leaf beetle color and spot pattern variations in Illinois.
http://ipm.illinois.edu/fieldcrops/insects/bean_leaf_beetle/index.html
Picture courtesy of University of Illinois Cooperative Extension. 2010.



Bean leaf beetle. A-B, Adults. C, Egg. D, Larva. E, Pupa.

[http://ipm.ncsu.edu/ag295/html/bean leaf beetle.htm](http://ipm.ncsu.edu/ag295/html/bean_leaf_beetle.htm)
Drawing by P. Kooaroon (A) and Mei-Jung Lin (B).
Picture courtesy of North Carolina State University. 2010.

Figure 1.3. Life stages of the bean leaf beetle.

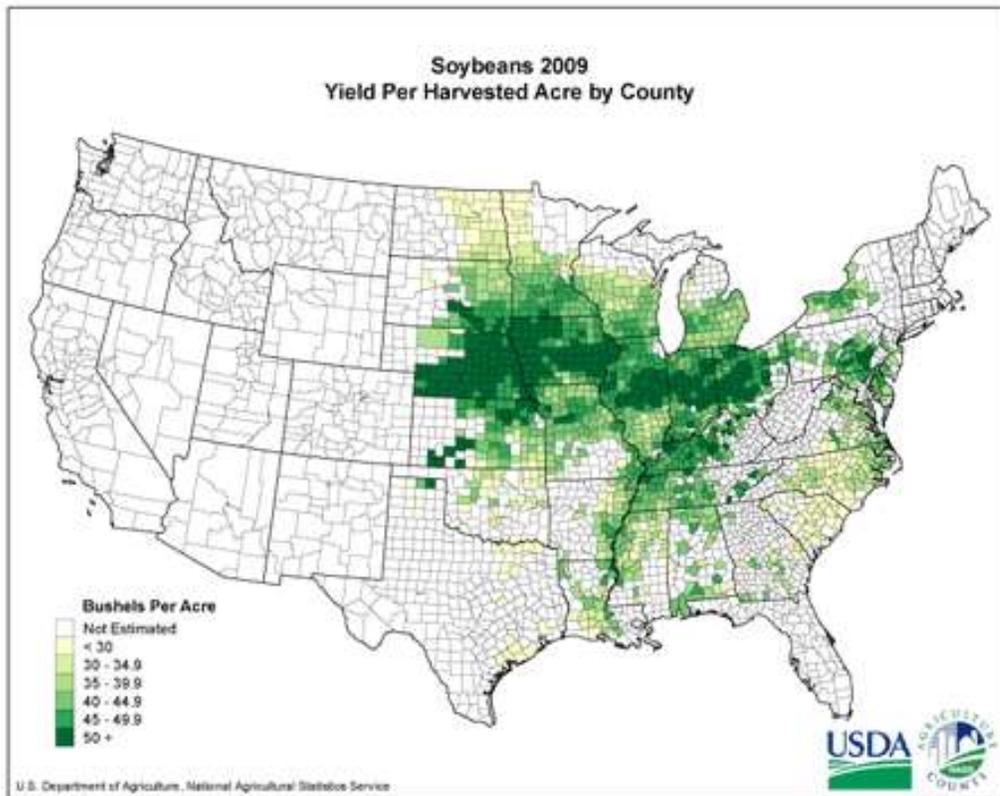


Figure 1.4. Major soybean growing areas in the U.S.A. as represented by dark green coloring based on bushels per acre. Map courtesy of National Agricultural Statistics Service. http://www.nass.usda.gov/Charts_and_Maps/Crops_County/sb-pl.asp. 2010.

Chapter 2

Effect of bean leaf beetle leaf feeding on snap bean yield

Abstract

Bean leaf beetle (BLB), *Cerotoma trifurcata* (Forster), is a common pest of bean crops in the eastern and central U.S. Adults feed on leaves and pods of soybeans, (*Glycine max* L.), snap beans, (*Phaseolus vulgaris* L.), and other legumes. In 2007 and 2008 at two locations in Virginia, we conducted field studies to determine the impact of early-season BLB defoliation on ‘Bronco’ snap bean yield. In one study, we simulated BLB feeding using a leaf-hole punch that removed 0, 25, 50 or 75% of the total leaf area. Two groups were evaluated, with the first group receiving two hole-punch sessions one week apart simulating early-season defoliation pressure, and the second group receiving four weekly defoliation sessions representing season-long feeding pressure. For both two-week and four-week defoliations, there was a strong negative linear relationship between defoliation and pod yield in both irrigated and non-irrigated snap beans. Significant yield loss occurred at 25% defoliation in three of the eight experiments, at 50% defoliation in seven of the eight experiments, and 75% defoliation in all eight experiments.

In 2006 and 2007 walk-in exclusion cages were used to house a range of BLB densities on ‘Bronco’ snap beans in the field. In 2006, cumulative mean BLB densities ranged from 0 to 1.4 per plant or 0 to 92 beetle-days among the cages. The cages with the highest beetle densities resulted in >50% defoliation at 17 days after beetle release. There was a significant correlation between beetle density and defoliation in the cages, and also between beetle density and whole plant mass and

bean yield. In 2007, cumulative mean densities in the cages ranged from 0 to 2.7 BLB per plant or 130 beetle-days. Although these densities were higher than those obtained in 2006, the levels of defoliation only reached a maximum of 18%. Consequently, although there was a significant correlation between beetle density and defoliation in 2007, there was no effect on snap bean yield. The differences in results obtained in these two studies indicate that other variables can impact the beetle density/defoliation/yield loss relationship in snap beans.

Introduction

The bean leaf beetle (BLB), *Cerotoma trifurcata* (Forster), (Coleoptera: Chrysomelidae) is native to North America where it is a common pest of soybeans (*Glycine max*), lima beans (*Phaseolus lunatu*), and snap beans (*Phaseolus vulgaris*) in some regions. In Virginia, *C. trifurcata* is multivoltine and overwinters as an adult in leaf litter (Capinera 2001). Adults injure plants by chewing holes in leaves and pods. Most economic loss in soybeans occurs from adults feeding on the pod and the base of the peduncle, which can damage the seed as well as allow secondary infection by plant pathogens (Shortt et al. 1982, Smelser et al. 1992b). However, significant yield loss can also occur from sustained leaf feeding (Hunt et al. 1994), particularly when plants are seedlings in the V1 to V4 stage of growth (Hunt et al. 1995). When BLB adults feed on young unfurling trifoliates, the leaf area removed is exaggerated because the leaves have not yet fully expanded. Moreover, Hunt et al. (1994) demonstrated that soybean plants that experienced sequential simulated-BLB defoliation, as compared to cotyledon removal or a one-time defoliation session, showed a significant yield loss of 70%. Smelser et al. (1992b) calculated the relationship of BLB days to yield loss in soybeans to be 0.015 g of seed per beetle per day, and from this estimated an economic injury level of 214 beetles per m². In comparison to

soybeans, little research has been done to quantify the effect of BLB defoliation on snap beans. Koch et al. (2005) showed that adult feeding on cotyledon (V1-stage) snap beans can cause significant stand loss, stunted plants, and reduced pod yields.

The goal of this study was to quantify the relationship of BLB defoliation and subsequent yield loss in snap beans through the use of both mechanical defoliation techniques and cage studies with varying beetle densities. This information will help to determine an economic injury level (EIL) for BLB on early-stage snap beans.

Materials and Methods

Manual Defoliation Study. The experiment was conducted in 2007 and 2008 at two locations, the Virginia Tech Eastern Shore Agricultural Research and Extension Center (ESAREC) located in Painter and the Hampton Roads Agricultural Research and Extension Center (HRAREC) located in Virginia Beach. At each location and in each year, ‘Bronco’ snap beans were planted on rows 91 cm apart with 6 – 10 plants per 0.3 m row, according to commercial recommendations (Kuhar et al. 2007), including pre-plant applications of ammonium nitrate fertilizer at 22.7 kg [AI]/ha and 0.37 kg [AI]/ha of S-metolachlor (Dual Magnum, Syngenta Crop Protection, Inc., Greensboro, NC), a pre-emergence herbicide. The specific dates of planting and other experimental procedures are given. The experiment used a randomized complete block design (RCBD). At 25 spatially-isolated locations (plots) in each field, plants were thinned to four seedlings per meter representing a single plot/replication of four defoliation levels. Bean plants at the Virginia Beach location were irrigated daily for both study years, and not irrigated at Painter. A drench of thiamethoxam (Platinum 2SC, Syngenta Crop Protection, Inc., Greensboro, NC) at 0.14 kg

[AI]/ha was applied at cotyledon stage to prevent natural BLB defoliation (Koch et al. 2005). The first defoliation session took place when the 1st true leaves appeared (see Table 2.1 for dates). Before each defoliation session, leaf area was measured using a leaf area meter (LI-COR model no. LI-3100). Leaf area from two randomly-selected plants in each of the defoliation categories was calculated. Data obtained from these plants were used to determine the cumulative area of leaf (cm²) needed to obtain 0, 25, 50 and 75% defoliation. Then the number of holes to be punched was based on the area removed by a standard paper hole-punch (Staples model 10573-CC), which removed 0.3 cm² per punch. In addition, a larger hole-punch (machined and assembled by hand) was used that removed 5 cm² per punch when over 100 punches were needed. Two groups were used to assess the impact of defoliation on yield. Treatment one represented early-season BLB defoliation only and had two weekly sessions of hole punching, while treatment two represented season-long defoliation and had four weekly sessions. At harvest (60 days after planting), all pods were hand-picked per plant and measured in grams using a digital scale.

The effects of defoliation level (0, 25, 50, and 75%) and duration (2 or 4 weeks) on yield were analyzed using a two-way ANOVA. Because the interaction of defoliation and duration on snap bean yield was significant at the Painter location, the effect of defoliation level on yield was analyzed separately for both 2-week and 4-week sessions, as well as for location and year. In addition, regression analysis was used to describe the relationship between percent defoliation and yield.

Field Cage Study. The experiment was conducted in 2006 and 2007 at the ESAREC. On 18 May 2006 and 28 May 2007, ‘Bronco’ snap beans were planted according to commercial recommendations for Virginia (Kuhar et al. 2007),

including pre-plant applications of ammonium nitrate fertilizer at 22.7 kg [AI]/ha and 0.37 kg [AI]/ha of S-metolachlor herbicide. Rows were 91 cm apart with 6 – 10 plants per 0.3 m. At six locations in the field, plants in an area of ~0.001 ha were caged. Each steel cage frame was 3.65 x 3.65 x 1.82 m and was covered with a fine-mesh thrips screen (32 x 32 mesh Lumite[®] screen). Access to the inside of the cage was through a metal zipper on the side of the cage. The cage was held in place in the field by metal tent stakes pushed into the ground through preformed metal grommets in the cages. At cotyledon stage within each cage, plants were thinned to a density of 100 plants per cage in 2006. In 2007 plants were thinned to 60 plants in order to increase beetle density per plant. Each cage represented a different beetle density. Adult BLB were collected from nearby snap beans and soybeans and were placed in cages on 1 June 2006 and 13 June 2007, when the bean seedlings had their first true leaves. Beetle densities released in 2006 were 0, 100, 200, 300, 400 and 500 beetles in each of the treatment cages, respectively. In 2007 beetles were placed in the cages at the following densities: 0, 120, 240, 360, 690, and 1000. At 3-day intervals, the number of BLB per twenty random plants per cage was recorded. From these data, beetle-days were determined by plotting the mean beetle density per plant over days in the cage. The area under the curve equaled the number of “beetle-days”. Percent defoliation by the beetles was calculated by removing all leaves from five random plants per cage and measuring leaf area using a LI-COR model no. LI-3100 leaf area meter (LI-COR Inc., Lincoln, NE). The leaf area consumed by beetles was compared to a control leaf with no beetle feeding in or to photocopy cutouts of the leaves with the feeding holes intact.

At 60 days after planting, whole plant weights as well as pod weights of 50 plants per cage were recorded. The quantitative relationship between beetle-days

and defoliation, as well as beetle-days and yield were determined using polynomial regression.

Results

Manual Defoliation Study. Based on two-way ANOVA, the interaction between defoliation level and number of defoliation sessions was significant at the Painter location in both years of the experiment (Table 2.3). Although this interaction was not significant at the Virginia Beach location (Table 2.3), the effect of defoliation level on bean yield was analyzed separately by the number of defoliation sessions, location, and year. In all eight experiments (2 years x 2 locations x 2 defoliation sessions), there was a significant treatment effect of defoliation level on bean yield ($P < 0.05$; Figs. 2.1 and 2.2). Significant yield loss occurred at 25% defoliation in three of the eight experiments, at 50% defoliation in seven of the eight experiments, and 75% defoliation in all eight experiments. There was a strong correlation in both years (2007 and 2008) and at both locations between defoliation level and snap bean pod yield (Table 2.2). This correlation was similar for both the two-defoliation sessions as well as the four-defoliation sessions. In Painter, where beans were not irrigated, the two-defoliation sessions had a linear relationship of $b = 1.104x$; $r^2 = 0.6428$ in 2007 and $b = 0.7912x$; $r^2 = 0.9919$ in 2008. The four-defoliation sessions for this same location had similar results with a linear relationship of $b = 1.1257x$; $r^2 = 0.9614$ in 2007 and $b = 1.144x$; $r^2 = 0.9966$ in 2008.

In Virginia Beach, under irrigated conditions, the two-defoliation session treatment had a linear regression of $b = 0.7334x$, $r^2 = 0.5308$ and the four-defoliation session had a regression of $b = 0.7732x$, $r^2 = 0.8399$ for 2007. Similarly in 2008 at this same location, the two-defoliation sessions treatment had

a linear regression of $b = 0.7359x$, $r^2 = 0.7134$ and the four-defoliation sessions treatment had a linear regression of $b = 0.9646x$, $r^2 = 0.8945$.

Field Cage Study. Table 2.4 represents the total number of BLB released per cage and the mean number of BLB observed per plant during a two-week period for both years. In 2006, cumulative mean BLB densities ranged from 0 to 1.4 per plant or 0 to 92 beetle-days among the cages. The cages with the highest beetle densities resulted in a high amount defoliation (>50%), and concomitantly, there was a significant correlation between beetle density and defoliation at 17 days after beetle release in the cages, and also between beetle density and whole plant mass and bean yield (Table 2.5). The relationship between beetle-days and percent defoliation fit a two-degree polynomial curve ($b = -0.0127x^2 \pm 1.5742x$; $r^2 = 0.9155$; Fig 2.3). The relationship between beetle density and yield only explained 38% of the variability in the data and is therefore, not shown.

In 2007, cumulative mean densities of BLB in the cages ranged from 0 to 2.7 BLB per plant or 130 beetle-days. Although these densities were higher than those obtained in 2006, the levels of defoliation only reached a maximum of 18% (Fig. 2.4). Nonetheless, a significant correlation between beetle density and defoliation occurred in 2007 with an r-value of 0.91; $P < 0.001$ (Table 2.5). However, neither whole plant mass, nor bean pod yield correlated with beetle density (Table 2.5).

Discussion

Based on my manual defoliation experiments conducted across a range of conditions, including irrigated versus non-irrigated snap beans, and two defoliation sessions versus four weekly defoliations, the results were similar in that 50% and 75% defoliation resulted in significant yield loss in virtually all of the experiments.

In three of the eight experiments defoliation levels of 25% also resulted in significant yield loss. These results are similar to Greene and Minnick (1967) who reported that early-season defoliation of ‘Harvester’ snap beans can be overcome if the total leaf area removed is less than 25% and does not occur around bloom. Further, in late vegetative stages, they showed that removing 25% of the foliage resulted in a 37% yield loss. My results in snap beans are also comparable to research in soybeans by Todd and Morgan (1972), who reported that an early-season infestation of phytophagous insects would not cause yield losses if defoliation levels were kept below 33%. Similar results were also seen from the manual defoliation of soybeans conducted by Hammond (1982). In that study, a single manual hole-punch defoliation of 36.9% during the V1 stage did not cause a reduction in yield for soybeans.

In my experiments, the overall yield (control and treatments) for the experiments conducted in Painter, VA was reduced by 50% due to lack of rainfall in 2007 (Fig. 2.2). The rainfall during the reproductive stage of the spring snap beans (May – July) was 8.38 cm, which is 3.15 cm less than the 67-year average rainfall (11.53 cm). Similar yield reduction was also seen by Caviness and Thomas (1980) in irrigated and non-irrigated soybeans where they had a reduction of over 20% due to drought. Nonetheless, despite the yield differences between irrigated and non-irrigated snap beans, the relative effect of defoliation on yield was relatively consistent across locations.

In soybeans, Smelser and Pedigo (1992a) showed a significant linear relationship between BLB cumulative density or beetle-days and BLB leaf injury. My results from the field cage experiments with snap beans were variable and inconclusive. In 2006, a range of densities from 0 to 1.4 BLB per plant or 0 to 92 beetle-days resulted in >50% defoliation at the highest beetle densities, and a

significant correlation between beetle density and defoliation, and also between beetle density and whole plant mass and bean yield. However, in 2007, a greater range of beetle densities from 0 to 2.7 BLB per plant or 130 beetle-days resulted in a maximum defoliation level of only 18%, and no correlation between beetle density and snap bean yield. The differences in results obtained in these two experiments indicate that other variables can impact the beetle density/defoliation/yield loss relationship in snap beans. Such variables include the health and age of beetles, the health and developmental stage of the snap bean plants, and weather conditions, which can impact both the plant and beetle mortality. Moreover, the relationship between leaf area consumption by beetles and % defoliation level on plants is a dynamic process because the holes caused by beetle feeding in leaves continue to expand as the leaf unfurls making the hole larger than it was originally (Hammond 1989, Hunt et al. 1995). Nonetheless, although the quantitative relationship needed in order to calculate an economic injury level for BLB on early-stage snap beans may still be unknown, my experiments reiterate the need for BLB and other insect control in order to prevent foliar feeding injury exceeding 25%.

References

- Capinera, J.L. 2001. Handbook of Vegetable Pests. Academic Press, New York. 93 pp.
- Caviness, C.E., and J.D. Thomas. 1980. Yield reduction from defoliation of irrigated and non-irrigated soybeans. *Agron. J.* 72: 977-980.
- Hammond, R.B., and L.P. Pedigo. 1982. Determination of yield-loss relationships for two soybean defoliators by using simulated insect-defoliation techniques. *J. Econ. Entomol.* 75: 102-107.
- Hammond, R.B. 1989. Effects of leaf removal at soybean growth stage V1 on yield and other growth patterns. *J. Kansas Entomol.* 62: 96-102.
- Hunt, T.E., L.G. Higley, and J.F. Witkowski. 1994. Soybean growth and yield after simulated bean leaf beetle injury to seedlings. *Agron. J.* 86: 140-146.
- Hunt, T.E., L.G. Higley, and J.F. Witkowski. 1995. Bean leaf beetle injury to Seedling soybean: consumption, effects of leaf expansion, and economic injury levels. *Agron. J.* 87:183-188.
- Isley, D. 1930. The biology of the bean leaf beetle. *Ark. Agric. Exp. Stn. Bull.* 248.
- Greene, G.L., and D.R. Minnick. 1968. Snap bean yields following simulated insect defoliation. *Proc. Fla. State Hort. Soc.* 80: 132-134.
- Koch, R.L., E.C. Burkness, W.D. Hutchison, and T.L. Rabaey. 2005. Efficacy of systemic insecticide seed treatments for protection of early-growth-stage-snap beans from bean leaf beetle (Coleoptera: Chrysomelidae) foliar feeding. *Crop Prot.* 24: 734-742.
- Kuhar, T.P., S.B. Phillips, R.A. Straw, C. Waldenmaier, and H.P. Wilson. 2007. Commercial vegetable production recommendations. Pub. No. 456-420.
- Lam, W., and L.P. Pedigo. 2000. A predictive model for the survival of

- overwintering bean leaf beetles (Coleoptera: Chrysomelidae). *Environ. Entomol.* 29: 800-806.
- Shortt, B.J., J.B. Sinclair, C.G. Helm, M.R. Jeffords, and M. Kogan. 1982. Soybean seed quality losses associated with bean leaf beetles and *Alternaria tenuissima*. *Phytopathology* 72: 615-618
- Smelser, R.B., and L. Pedigo. 1992a. Bean leaf beetle (Coleoptera:Chrysomelidae) herbivory on leaf, stem, and pod components of soybean. *J. Econ. Entomol.* 85: 2408-2412.
- Smelser, R.B., and L. Pedigo. 1992b. Soybean seed yield and quality reduction by bean leaf beetle (Coleoptera: Chrysomelidae) pod injury. *J. Econ. Entomol.* 85: 2399- 2403.
- Todd, J.W., and L.W. Morgan. 1972. Effects of hand defoliation on yield and seed weight of soybeans. *J. Econ. Entomol.* 65: 567-570.

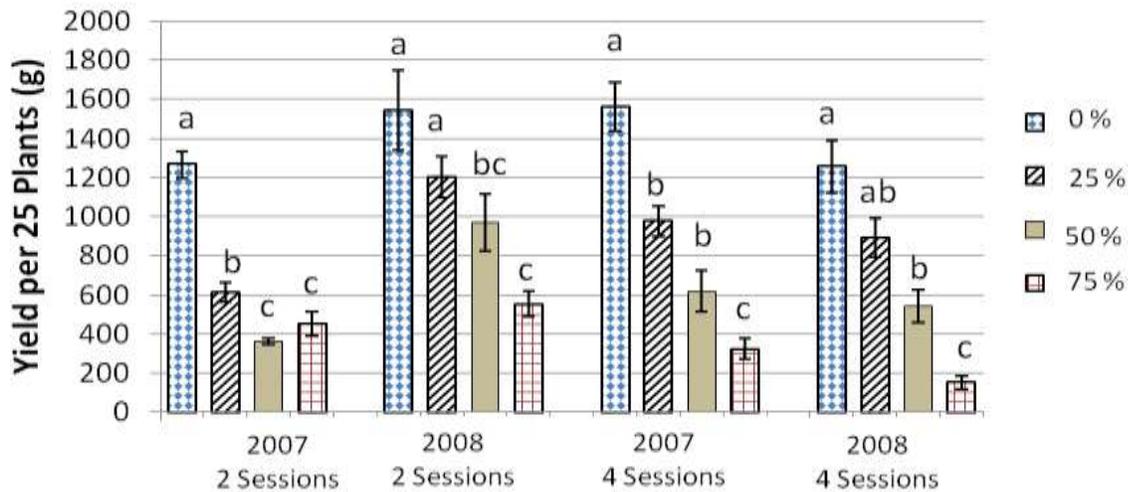


Figure 2.1. Pod yield (mean \pm SE per 25 plants) of manually-defoliated ‘Bronco’ snap beans grown in Virginia Beach, VA with daily irrigation in spring 2007 and 2008. Sessions refers to the number of weekly defoliations at designated levels (0, 25, 50, or 75%). Bars surmounted by the same letters within the same year and treatment are not significantly different as determined by ANOVA ($P < 0.05$) and Tukey’s HSD. .

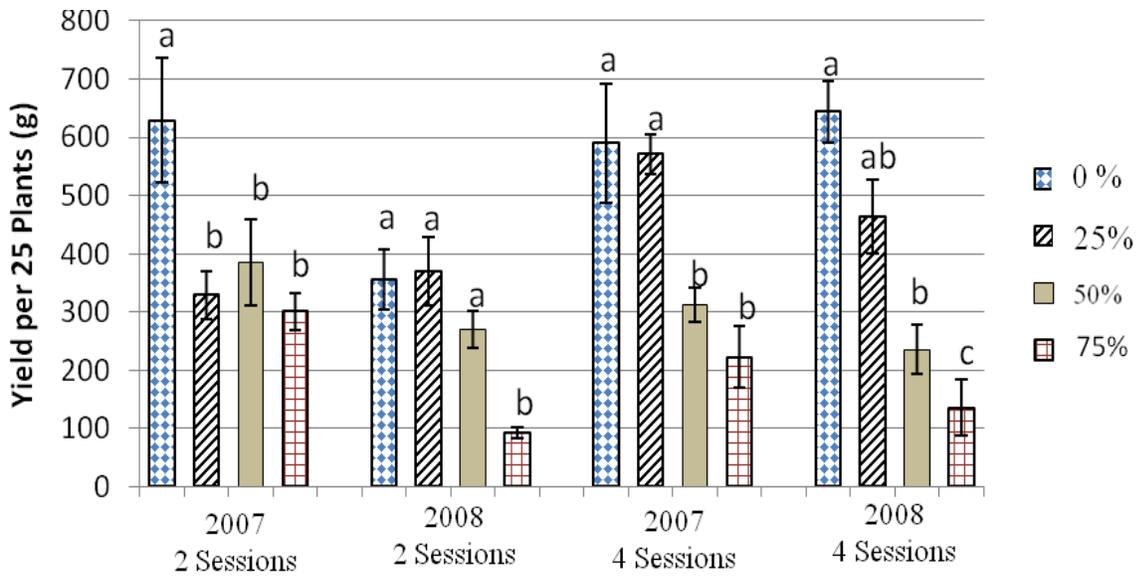


Figure 2.2. Pod yield (mean \pm SE per 25 plants) of manually-defoliated ‘Bronco’ snap beans grown in Painter, VA with little to no irrigation in spring 2007 and 2008. Sessions refers to the number of weekly defoliations at designated levels (0, 25, 50, or 75%). Bars surmounted by the same letters within the same year and treatment are not significantly different as determined by ANOVA ($P < 0.05$) and Tukey’s HSD.

Table 2.1. Dates for planting, manual defoliation sessions, and harvest of four simulated defoliation experiments conducted on snap beans in Virginia from 2007 to 2008.

Year	Location	Planting date	Defoliation initiated	Harvest
2007	Painter	28 May	11 June	July 27
2007	Virginia Beach	22 May	1 June	July 21
2008	Painter	14 June	30 June	11 August
2008	Virginia Beach	4 June	20 June	1 August

Table 2.2. Correlation between manual defoliation level and pod yield of ‘Bronco’ snap beans in field experiments conducted in Painter, VA (under little to no irrigation) and Virginia Beach, VA (under daily irrigation) in 2007 and 2008. Data were analyzed using a correlation equation at a significance level of $P < 0.05$.

Painter 2007	r-value	p-value
Percent Defoliation and Yield (2 Sessions)	-0.80	< 0.001
Percent Defoliation and Yield (4 Sessions)	-0.95	< 0.001
<u>Painter 2008</u>	<u>r-value</u>	<u>p-value</u>
Percent Defoliation and Yield (2 Sessions)	-0.90	< 0.001
Percent Defoliation and Yield (4 Sessions)	-0.99	< 0.001
<u>Virginia Beach 2007</u>	<u>r-value</u>	<u>p-value</u>
Percent Defoliation and Yield (2 Sessions)	-0.80	< 0.001
Percent Defoliation and Yield (4 Sessions)	-0.95	< 0.001
<u>Virginia Beach 2008</u>	<u>r-value</u>	<u>p-value</u>
Percent Defoliation and Yield (2 Sessions)	-0.90	< 0.001
Percent Defoliation and Yield (4 Sessions)	-0.99	< 0.001

Table 2.3. Two-way analysis of variance results of the effects of defoliation level (0, 25, 50, and 75%) and number of manual defoliation sessions (2 wk or 4 wk) on ‘Bronco’ snap bean yield at two Virginia locations

Field Location	Year	Factor	df	F	P
Virginia Beach	2007	Percent Defoliation	3	19.75	< 0.0001
	2007	Sessions	1	1.94	0.1719
	2007	Percent Defoliation * Sessions ^a	3	0.86	0.4699
	2008	Percent Defoliation	3	45.32	< 0.0001
	2008	Sessions	1	1.94	0.1719
	2008	Percent Defoliation * Sessions ^b	3	1.58	0.2123
Painter	2007	Percent Defoliation	3	10.81	< 0.0001
	2007	Sessions	1	0.04	0.8402
	2007	Percent Defoliation * Sessions ^c	3	4.94	0.0062
	2008	Percent Defoliation	3	15.62	< 0.0001
	2008	Sessions	1	2.48	0.1238
	2008	Percent Defoliation * Sessions ^d	3	11.96	< 0.0001

Table 2.4. Densities of bean leaf beetle adults on plants over time following artificial infestation into field cages containing 100 V-1 stage ‘Bronco’ snap beans in 2006 (A) and 60 V-1 stage ‘Bronco’ snap beans in 2007 (B) in Painter, VA.

A.

Number of beetles released per cage on 1 June	2 June	5 June	8 June	11 June	14 June	16 June	Cumulative mean	Beetle days
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0
100	0.2	0.1	0.2	0.5	0.6	0.3	0.3	20
200	0.7	0.5	0.6	0.7	0.5	0.4	0.6	36
300	0.9	0.5	0.4	0.8	0.9	1.1	0.8	50
400	1.2	0.5	0.9	0.8	1.4	1.7	1.0	70
500	0.8	1.6	0.9	1.3	2.1	1.6	1.4	92

B.

Number of beetles released per cage on 13 June	15 June	18 June	21 June	24 June	27 June	Cumulative mean	Beetle days
0	0.0	0.0	0.0	0.0	0.0	0.0	0
120	0.4	0.6	1.0	0.6	0.5	0.6	29
240	0.4	0.9	0.6	0.9	0.9	0.7	54
360	0.9	2.7	2.6	2.5	2.4	2.2	70
690	0.5	2.5	3.7	3.6	3.2	2.7	99
1000	1.8	1.6	3.6	3.3	3.1	2.7	130

Table 2.5. Correlation coefficients and significance levels of bean leaf beetle density with defoliation, whole plant mass, and pod yield of ‘Bronco’ snap beans grown in exclusion cages with artificially-released beetles in 2006 and 2007 in Painter, VA.

<u>2006</u>	r-value	p-value
Percent defoliation at 17 days after artificial release of beetles	0.73372	< 0.001
Whole plant mass	-0.3843	0.006
Pod yield	-0.37287	0.007
<u>2007</u>	<u>r-value</u>	<u>p-value</u>
Percent defoliation at 17 days after artificial release of beetles	0.911603	< 0.001
Whole plant mass	-0.03759	0.79
Pod yield	0.036966	0.79

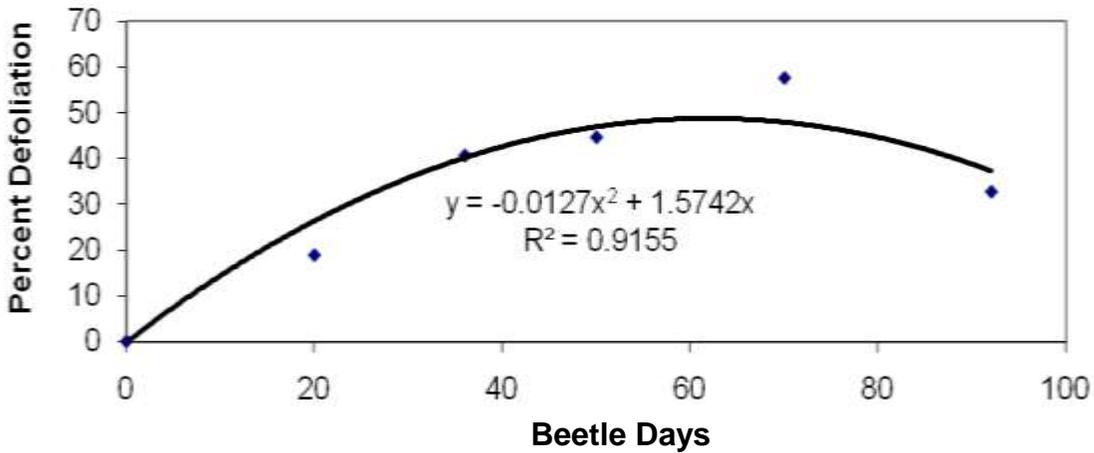


Figure 2.3. Relationship between bean leaf beetle days and percent defoliation at 17 days after artificial release into field cages in Painter, VA in June 2006.

Unbroken line represents the curve of best fit of a second degree polynomial.

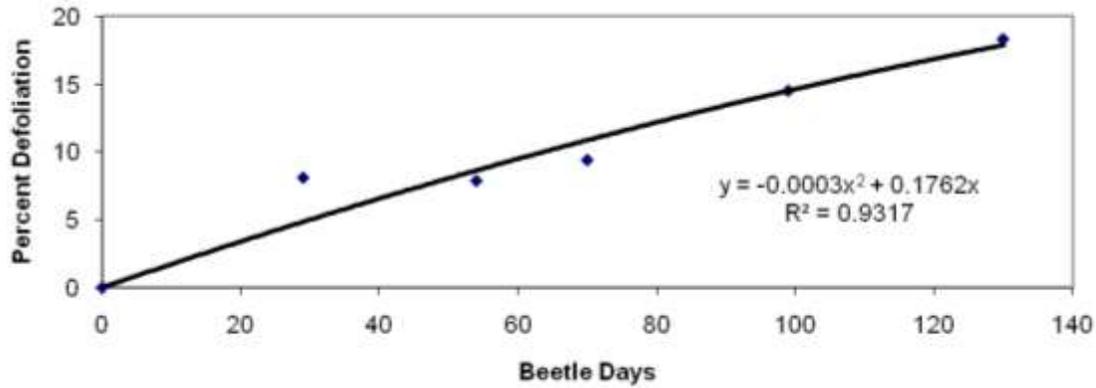


Figure 2.4. Relationship between percent defoliation by bean leaf beetles 17 days post introduction into cages in 2007 in Painter, VA. Percent defoliation was calculated by randomly selecting 5 plants from each cage and using a photocopy of the leaf to be assessed by a leaf area meter. Unbroken line represents the curve of best fit of a second degree polynomial.

Chapter 3

Bean leaf beetle feeding preference among snap beans, lima beans, and soybeans

Abstract

In the eastern and central U.S., the bean leaf beetle (BLB), *Cerotoma trifurcata* (Forster), is a pest of legume crops on which adults chew holes in leaves and scar bean pods. On the Delmarva Peninsula, snap beans, lima beans and soybeans are important crops and relatively high populations of BLB occur. From 2007 to 2009, experiments were conducted in the laboratory and field in Painter, VA to determine potential host plant preferences of BLB among the aforementioned crops. In the lab, paired choice tests with 5-cm² leaf discs of each plant revealed that beetles consumed significantly more leaf tissue of snap bean and lima bean than soybean in both years. No differences were seen between snap bean and lima bean in 2008; however, in 2009, lima beans were preferred over snap beans. In the field, numbers of beetles were recorded from two different growth stages of each of the three bean species arranged in paired combinations. Over a three week sampling period, significantly more beetles were found on larger (V4-stage) plants than smaller (V1-stage) plants, and more cumulative beetles were found on snap bean and lima bean compared with soybean. No significant differences were found between snap bean and lima bean. Thus, in the lab and field, BLB adults preferred snap bean and lima bean to soybean. This information could have implications for beetle movement and concomitant pest management when these crops are grown adjacent to one another.

Introduction

The bean leaf beetle (BLB), *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae), is oligophagous within the family Fabaceae (Kogan et al. 1980). The insect is native to North America where it is a familiar pest of snap beans, *Phaseolus vulgaris* L., and soybeans, *Glycine max* (L.) Merr., in the eastern and central U.S. (Eddy and Nettles 1930). In the absence of cultivated bean crops, BLB will also feed on leguminous weeds (Chitenden 1897, Isley 1930, Bradshaw et al. 2007), and occasionally non-leguminous hosts such as grasses, particularly in early spring if legume hosts are not available (Helm et al. 1983, Zeiss and Pedigo 1996). However, BLB longevity and reproductive success is highest on the cultivated bean crops (Zeiss and Pedigo 1996), and beetles prefer these crops to leguminous weeds such as *Desmodium* spp. (Henn 1989, Bradshaw et al. 2007). Thus, the availability of optimal host plants could play an important role in the population dynamics of this pest species (Zeiss and Pedigo 1996). On the Delmarva Peninsula, soybeans, snap beans, and lima beans, *Phaseolus lunatus* L., are important crops grown in close proximity to one another, and relatively high populations of BLB occur.

The objective of this study was to better understand the host plant preference of the BLB among soybean, snap bean, and lima bean. This information could have implications for pest management of BLB particularly in crops grown adjacent to one another.

Materials and Methods

Leaf consumption choice test. A dual-choice test was performed in the lab at the Eastern Shore Agricultural Research and Extension Center (ESAREC) in Painter, VA in 2008 and 2009, to analyze the feeding preference of adult BLB for either

‘Fordhook’ bush lima bean, ‘Bronco’ snap bean or ‘Vigero V39N4RR’ soybean foliage. Petri dishes (Fisherbrand 100 x 15 mm, Fisher Scientific, Pittsburgh, PA) were partially filled with 2% water agar to keep foliage moist. Fully expanded leaves were collected from the field the morning of the experiment and a large 5 cm² hole-punch was used to cut leaf disks. Leaf disks did not include the major leaf vein to allow for more-consumable leaf area. Leaf disks were run through a LI-COR model no. LI-3100 leaf area meter (LI-COR Inc., Lincoln, NE). to obtain the area of the disc before beetle introduction to ensure accurate measurement of area consumed by beetles. Discs were then paired and placed onto the water agar.

Adult BLB were collected from snap beans and soybeans in the field and starved for 24 hours prior to testing. In the early afternoon, a single unsexed beetle was placed in the Petri dish and allowed to feed on the leaf discs for 24 hours, after which, the leaf disks were run through the leaf area meter again to determine the area consumed by the beetle. Each beetle in a Petri dish with paired leaf disks was considered a replication. Three paired choice tests were performed, which consisted of: 1) lima bean vs. soybean, 2) snap bean vs. soybean, and 3) lima bean vs. snap bean. In 2008, 40 replications of each paired test were performed. In 2009, the paired choice test was performed again with 20 replications of each. Data were analyzed using a paired Student’s t-test with an $\alpha = 0.05$.

Host plant field preference assay. A small-plot randomized complete block experiment was conducted in spring 2007 at the ESAREC to determine if there was a beetle preference among ‘Fordhook’ bush lima beans, ‘Bronco’ snap beans, and ‘Vigero V39N4RR’ soybeans in the field. This experiment also tested if there was a preference between seedlings (V1) or larger plants (V4). All beans were seeded

in the greenhouse in 10-cm pots with Pro-mix (Premier Horticulture, Quakertown, PA). The beans to be used as the larger V4 plants were seeded in the greenhouse on 16 May 16; whereas, the V1 beans were seeded on 30 May. All beans were transplanted in the field on 6 June. Prior to transplanting, the field was treated with 56 kg/ha of nitrogen fertilizer and 0.37 kg [AI]/ha S-metolachlor pre-emergent herbicide (Dual Magnum, Syngenta Crop Protection, Inc., Greensboro, NC) at a rate of. Soybean, lima bean, and snap beans were paired in the field (5 plants each) with 2 growth stages (V1 and V4).

The experiment was arranged in a randomized complete block design with four replications. Within each replication, five sets of five plants of each of the six variety x growth stage combinations were arranged uniformly such that each treatment bordered all other treatments equally. The row spacing was 36 cm, with each row measuring 2 meters in length. Each paired row also had 36 cm between plant species. Beetles from the surrounding area naturally infested the plants for 24 hours before the first beetle count was made. Twice weekly from 7 to 24 June (during peak beetle activity) the numbers of BLB adults observed on 10 plants per block was recorded for each of the six plant species × growth stage treatments. The first beetle count was made one day after transplanting beans in the field. Beetle count data were analyzed using a two-way ANOVA with plant species, growth stage, and their interaction as treatments in the model (Ott and Longnecker 2001). When *F* values were significant by ANOVA, means were separated using Tukey-Kramer HSD at a P-value ≤ 0.05 . The results of this analysis are presented in a 2-way table of treatments means (Mead and Curnow 1983).

Results

Leaf consumption choice test. Leaf area consumed by each beetle was calculated by subtracting the area remaining from the starting leaf area. In 2008, BLB consumed more snap beans ($P < 0.0001$) and lima beans ($P < 0.001$) than soybean and no differences were seen between snap bean and lima bean ($P = 0.14$) (Table 3.1). In 2009, snap beans ($P < 0.001$) and lima beans ($P < 0.001$) were chosen over soybeans; however lima beans ($P < 0.05$) were preferred over snap beans (Table 3.1).

Host plant field preference assay. Bean leaf beetle densities peaked on 11 June and decreased steadily over the remaining two weeks of sampling (Fig. 3.1). Because beetle densities were relatively low on most sample dates, data were pooled across all sample dates for analysis. The 2-way ANOVA model was highly significant ($F = 6.63$; $df = 5, 162$; $P < 0.0001$). The main effects of plant species ($P < 0.0012$) and plant growth stage ($P < 0.0049$) were significant, and the interaction of those factors was not ($P > 0.098$). Significantly more beetles were found on snap bean and lima bean compared with soybean and no differences were found between snap bean and lima bean (Fig 3.2). Also, significantly more beetles were found on larger (V4) plants than smaller (V1) plants (Fig. 3.3).

Discussion

The results of my experiments showed that, when given a choice, BLB adults preferred to feed on leaves of snap bean or lima bean over soybean, and that when plants were of similar growth stage in the field, more beetles were found on snap bean and lima bean over soybean. Henn (1989) also found a strong preference in BLB for varieties of bean, *Phaseolus* spp., over soybean, but no preference among *Phaseolus* species. Although the physiological or

morphological basis for this apparent host plant preference in BLB is not known, plant pubescence could be a major factor. Trichomes are the first plant structures contacted by insects during host plant acceptance (Smith 1989). Further, high trichome densities in soybean varieties have been associated with decreased feeding injury by numerous insects (Chiang and Norris 1983, Elden and Lambert 1992, Lambert et al. 1992, Cannon and Bach 1996), including bean leaf beetle (Lam and Pedigo 2001). Although this variable was not measured in our study, it is known that snap beans and lima beans have very low trichome densities relative to soybeans.

Another potential explanation for the preference of snap beans may be due to coevolution of the beans and the BLB. *Phaseolus vulgaris* originated in North or South America (Gepts and Debouck 1991), where the BLB is endemic (Eddy and Nettles 1930). Soybeans originated in Asia, and thus, did not co-evolve with the beetle.

The preference of BLB for snap bean and lima bean over soybean could have implications on pest management, particularly if these crops are planted adjacent to one another, and if beetles will disperse from one crop to the next. Waldbauer and Kogan (1976) observed BLB leaving mature soybean plants to feed on younger late-planted soybeans. However, these same authors also reported that BLB adults were not significantly more abundant in snap bean fields that were adjacent to soybean plots. Thus, the pest management implications of the BLB host plant preference for snap bean and lima bean over soybean may be insignificant. Additional research on beetle dispersal in the field is needed to better understand this relationship.

References cited

- Boiteau, G., J.R. Bradley, JR., and J.W. Van Duyn. 1979. Bean leaf beetle: Emergence patterns of adults from overwintering sites. *Environ. Entomol.* 8: 427-431.
- Bradshaw, J.D., L.P. Pedigo, J.H. Hill, and M.E. Rice. 2007. No-choice preference of *Cerotoma trifurcata* (Coleoptera: Chrysomelidae) to potential host plants of bean pod mottle virus (Comoviridae) in Iowa. *J. Econ. Entomol.* 100: 808-814.
- Cannon, A.J, and C.E. Bach. 1996. Effects of soybean trichome density on Mexican bean beetle (Coleoptera: Coccinellidae) development and feeding preference. *Environ. Entomol.* 25:1077-1082.
- Chiang, H.S, and D.M. Norris. 1983. Morphological and physiological parameters of soybean resistance to agromyzid beanflies. *Environ. Entomol.* 12:260-265.
- Chittenden, F.H. 1897. The bean leaf beetle. USDA Div. Entomol. Bull. 9: 64-71.
- Eddy, C.O., and W.C. Nettles. 1930. The bean leaf beetle. S.C. Agric. Exp. Stn. Bull. 265: 1-25.
- Elden, T.C, and L. Lambert. 1992. Mechanisms of potato leafhopper resistance in soybean lines isogenic for pubescence type. *Crop Sci.* 32:1187-1191.
- Gepts, P., and D. Debouck. 1991. Origin, domestication, and evolution of the common bean (*Phaseolus vulgaris* L.). In Schoonhoven, A. van, and O. Voysest. Common beans: research for crop improvement. pp 7-53.
- Helm, C.G., M.R. Jeffords, S.L. Post, and M. Kogan. 1983. Spring feeding activity of overwintered bean leaf beetles (Coleoptera: Chrysomelidae) on non-leguminous hosts. *Environ. Entomol.* 12: 321-322.
- Henn, T. 1989. Analysis of bean leaf beetle feeding behavior in dual-choice preference testing of legumes. M.S. thesis, University of Illinois, Urbana.

- Isley, D. 1930. The biology of the bean leaf beetle. Ark. Agric. Exp. Stn. Bull. 248.
- Kogan, M., G.P. Waldbauer, G. Boiteau, and C. Eastman. 1980. Sampling bean leaf beetles on soybean, pp.201-236. *In* M. Kogan and D.C. Herzog [eds.], *Sampling Methods in Soybean Entomology*. Springer, New York.
- Lam, W.-K.F., and L.P. Pedigo. 2001. Effect of trichome density on soybean pod feeding by adult bean leaf beetles (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 94: 1459-1463.
- Lambert, L., R.M. Beach, T.C. Kilen, and J.W. Todd. 1992. Soybean pubescence and its influence on larval development and oviposition preference of lepidopterous insects. *Crop Sci.* 32:463-466.
- Mead, R., and R.N. Curnow. 1983. *Statistical Methods in Agriculture and Experimental Biology*. Chapman and Hall/CRC, New York, NY.
- Ott, R.L., and M. Longnecker. 2001. *An Introduction to Statistical Methods and Data Analysis*, 5th ed. Duxbury, Thompson Learning Inc., Pacific Grove, CA.
- Smith, C.M. 1989. *Plant Resistance to Insects: A Fundamental Approach*. Wiley, New York.
- Waldbauer, G.P., and M. Kogan. 1976. Bean leaf beetle: Phenological relationship with soybean in Illinois. *Environ. Entomol.* 5: 35-44.
- Zeiss, M.R., and L.P. Pedigo. 1996. Timing of food plant availability: effect on survival and oviposition of the bean leaf beetle (Coleoptera: Chrysomelidae). *Environ. Entomol.* 25: 295-302.

Table 3.1. Mean \pm SE of leaf area consumed by *C. trifurcata* in a 24 hour period of three legume species. Paired choice tests determined beetle feeding preference for 2008 – 2009 at the Eastern Shore Agriculture Research and Extension Center in Painter, VA. Data for each pair were analyzed separately using a student's *t*-test at the 0.05 level of significance.

Year	Paired Combinations	Mean \pm SE		T - value	Significance
		A	B		
2008	A. Lima bean vs. B. Soybean	0.57 \pm 0.1	0.16 \pm 0.22	5.32	P < 0.0001
	A. Snap bean vs. B. Soybean	0.54 \pm 0.11	0.11 \pm 0.07	5.14	P < 0.0001
	A. Snap bean vs. B. Lima bean	0.23 \pm 0.1	0.36 \pm 0.1	1.68	P = 0.14
2009	A. Lima bean vs. B. Soybean	0.33 \pm 0.1	0.01 \pm 0.03	6.01	P < 0.001
	A. Snap bean vs. B. Soybean	0.31 \pm 0.1	0.03 \pm 0.08	4.2	P < 0.001
	A. Snap bean vs. B. Lima bean	0.07 \pm 0.1	0.31 \pm 0.13	1.73	P < 0.05

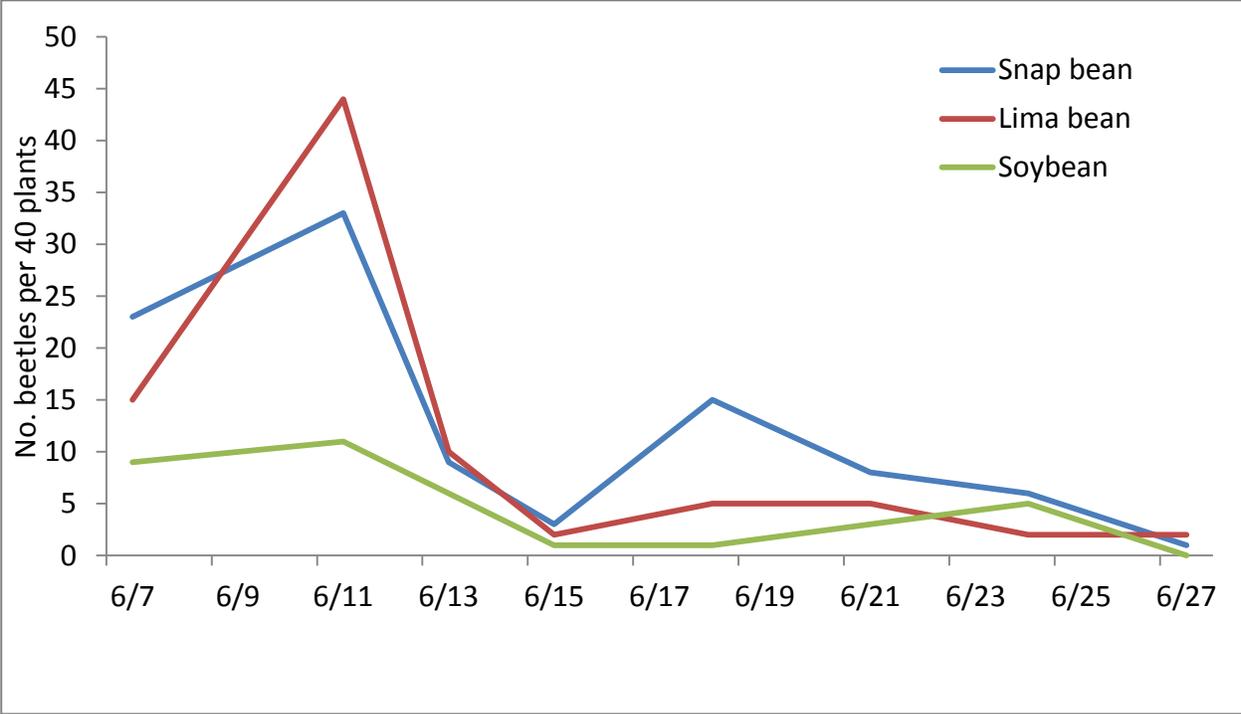


Fig. 3.1. Counts of bean leaf beetle adults on snap beans, lima beans, and soybeans (small and large plants combined for each species) during a host plant choice field experiment conducted in June 2007 in Painter, VA.

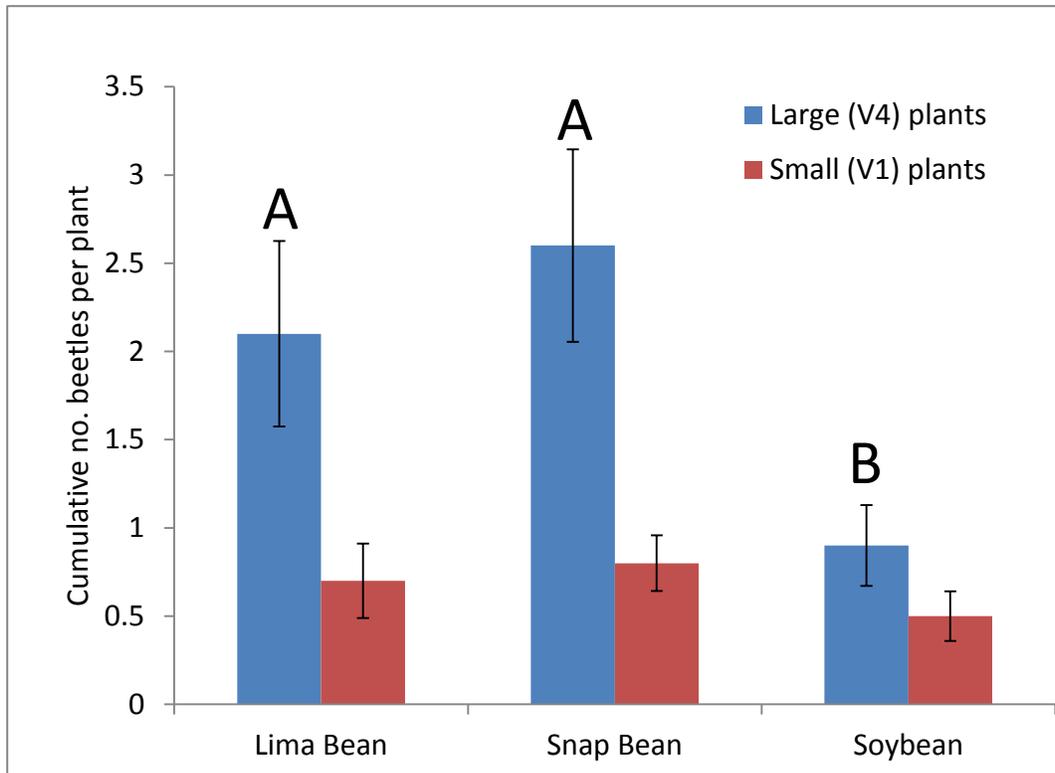


Fig. 3.2. Mean \pm SE cumulative counts of bean leaf beetle adults on snap beans, lima beans, and soybeans during a host plant choice field experiment conducted in June 2007 in Painter, VA. Columns surmounted by a letter in common are not significantly different according to Tukey HSD at $P > 0.05$.

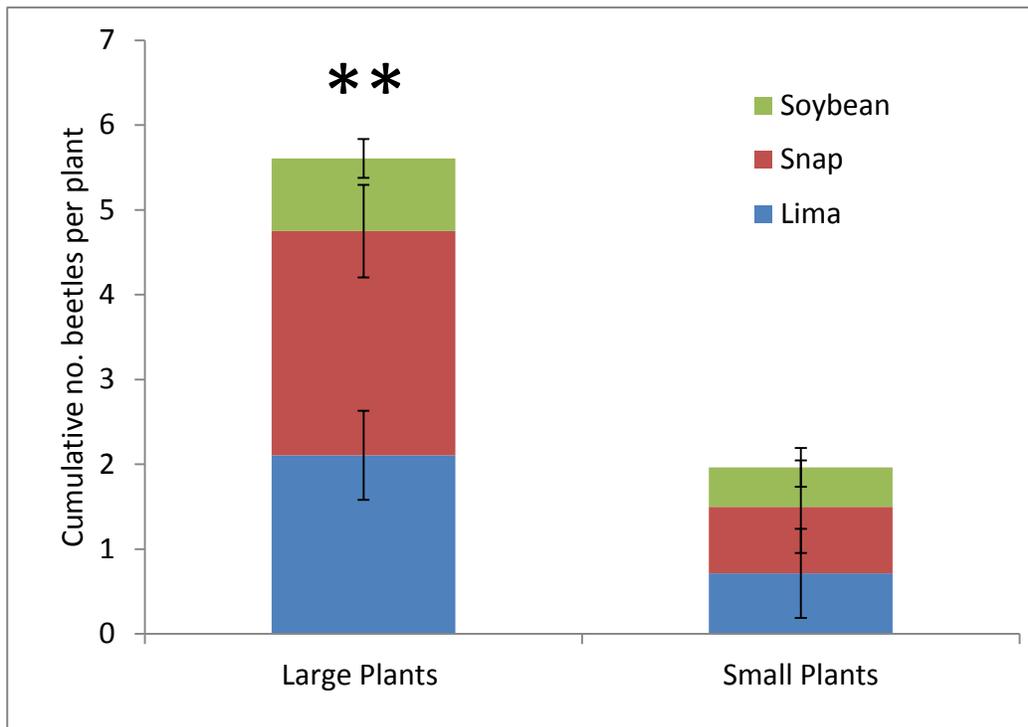


Fig. 3.3. Mean \pm SE cumulative counts of bean leaf beetle adults on two growth stages of snap beans, lima beans, and soybeans during a host plant choice field experiment conducted in June 2007 in Painter, VA. “Large plants” were at V4 stage and “Small Plants” were at V1 stage at the beginning of beetle sampling.

Chapter 4

Bean pod mottle virus prevalence and incidence in Eastern Virginia

Abstract

A search for *Bean pod mottle virus* (BPMV) in Virginia was carried out during the 2008 through 2010 seasons. Both ELISA and tissue blot immunoassay (TBIA) methods were used for detecting BPMV in bean leaf beetles (BLB), *Cerotoma trifurcata*, and soybeans, *Glycine max* (L.) Merrill. A targeted sampling showed that BPMV is prevalent on the Eastern Shore and Northern Neck of Virginia where BPMV was detected in both soybean foliage and bean leaf beetles in 2009 and 2010. On the Eastern Shore, BPMV prevalence and incidence in the fields varied year to year, suggesting there was no epicenter of virus. Beetle extracts were positive for BPMV by ELISA and were infective to soybean seedlings.

Introduction

Bean pod mottle virus (BPMV; Comovirus; Comovirinae; Secoviridae) is widespread across the U.S. in soybean, *Glycine max* (L.) Merrill, production areas, and has the potential to cause a significant impact on yield. BPMV causes a disease that affects both pod production and seed quality (Horn et al. 1973, Hobbs et al. 2003). Yield loss can be up to 52% when BPMV infection is severe (Gergerich 1999), while specific reports estimate a yield loss of between 10 and 40% (Ross 1968, Hartman et al. 1999). Plant symptoms in response to the virus may be mild or severe depending on what plant growth stage was infected by the virus. Mild symptoms in soybean include slight chlorotic mottling of the foliage to a severe mosaic with wrinkled leaves and patches of yellow foliage (Giesler et al. 2002). BPMV may cause terminal necrosis and even death of the infected plant if

the infection is severe. This virus may also cause delayed maturity of the plant leading to “green stem” disorder (Schwenk and Nickell 1980).

BPMV was first reported in bean, *Phaseolus vulgaris* var. Tendergreen in Charleston, South Carolina (Zaumeyer and Thomas 1948). The first reported isolation of BPMV from soybean was from Arkansas (Walters 1958). The virus was then reported in North Carolina and Virginia (Skotland 1958). The virus ranges geographically from the southeastern U.S. as far north as Kentucky and Illinois (Ghabrial et al. 1977, Milbrath et al. 1975). Ross and Butler (1985) surveyed 56 soybean fields in eastern North Carolina and reported that 37% were BPMV-positive. Surveys conducted from 1985-1987 showed that 254 of 382 fields from all 28 counties in western Kentucky had virus-infected soybeans and viruliferous beetles when tested by ELISA (Ghabrial et al. 1990). Observations 10 years later revealed the virus had moved eastward to infect plants in fields in all of Kentucky (Gu et al. 2002). The virus continued to spread across the U.S. to South Dakota (Langham et al. 1999) and was documented in Canada in 2002 (Michelutti et al. 2002). Mabry et al. (2003) detected BPMV in soybean plants from 38 of 46 counties across Illinois in 2000. Krell et al. (2003) surveyed Iowa and found that all 84 counties were BPMV-positive. BPMV was most recently reported from soybeans in Alabama in 2003 (Sikora and Murphy 2005). In Virginia, BPMV was found in 1973 in eastern Virginia (Tolin and Roane 1974) and again in work summarized by Mackasmiel (2004).

The bean leaf beetle (BLB), *Cerotoma trifurcata*, (Coleoptera: Chrysomelidae) causes economic damage to soybeans by feeding on leaves, stems and by scarring pods (Eddy and Nettles 1930, Smelser and Pedigo 1992). The beetle causes further damage to soybeans by reducing crop yield as well as seed-quality by transmitting pathogens such as BPMV (BPMV) and *Alternaria tenuissima*, a fungal pathogen (Horn et al 1973). Virus particles are spread by

beetles regurgitating infected leaf material along with gastric juices onto uninfected soybeans. Beetles lack salivary glands and the regurgitant acts as lubrication for beetle mouthparts (Snodgrass 1935). Beetle feeding mechanically wounds the plant leaves and the virus particles in the regurgitant enter through these wounds (Ross 1963).

The market value of this crop can decline dramatically due to poor seed quality caused by BPMV. Poor seed quality due to this virus infection manifests itself as an irregular black pattern or streaking of the hilum (Tolin and Lacy 2004). The highest instance of yield reduction was seen in plants that were infected early on in development (Ross 1986).

Virginia harvested 570,000 acres of soybeans with a crop value of 160 million dollars in 2008. The Eastern Shore of Virginia produced 2 million bushels of soybeans in 2008 with a value of 17 million dollars. Both Accomack and Northampton County are in the top five counties in Virginia for the production of soybeans (NASS 2008). As a activity of the Legume ipmPIPE project in 2007, soybean rust sentinel plots at the Eastern Shore Agricultural Research and Extension Center (ESAREC) and at the Tidewater AREC were sampled and tested for viruses. In the ESAREC plot, 80% of the 45 soybeans sampled were positive for BPMV (Tolin and Langham 2010). Because of this high BPMV incidence at the ESAREC, beetles were collected in spring of 2008 for assay, and 80% were found to be positive for BPMV. The high percentage of BPMV-positive beetles at the research station led to the hypothesis that this location was a possible epicenter of the virus. Targeted surveys of soybean fields were designed and performed to test this hypothesis. The study also included determination of virus presence and quantitative load in beetles collected in soybean fields in several counties in Virginia and the Delmarva Peninsula. Obtaining information on the incidence of

BPMV in Virginia is expected to help farmers develop integrated pest management tactics for dealing with the viral vector control and disease reduction in the field.

Materials and Methods

Soybean and bean leaf beetle collection.

Beetles and soybean leaf material were collected from 28 commercial fields in the two counties on the Eastern shore, Accomack and Northampton, on the initial collection dates of August 3-7, 2009. After analyzing initial results with these samples, a second collection from 16 fields was taken October 5-7, 2009. Fields were located using the Eastern Shore Agriculture Research and Extension Center (ESAREC) as a point of origin from which to select fields for sampling at 2, 5, 10 and 15 miles from the ESAREC in each cardinal direction. Samples were collected from four fields at each distance and direction and at the station. Because of the elongated nature of the shore, no land existed at some distances east and west of the station, permitting collection at distances only North and South of the station. Fields were coded for identification by assigning a field number, cardinal direction, and distance from the AREC. Forty-five leaf samples were collected from each field from five consecutive plants at nine transect sites within each field (Tolin and Langham 2010). A total of 25 beetles, if available, were collected with sweep nets in each field. Foliage samples were placed in plastic bags labeled for each site and field that were placed on ice in a cooler for transport to the laboratory for processing.

Fields on the Delmarva Peninsula were subjected to a second sampling in October. An attempt was made to resample the same fields, however this was possible with only six fields since some fields had been harvested and others lacked viable foliage to sample. In these cases, fields in close proximity to original

fields were sampled. The sample area was expanded to include the southeastern part of Maryland, where six fields were sampled on 6 October. This location was 40 miles north of ESAREC.

Soybean fields testing positive for BPMV in 2009 were resampled in August and September 2010. In case of crop rotation from soybean to corn, adjacent soybean fields were sampled. Beetles and leaf material were collected 31 August and 1 September into labeled plastic bags and placed on ice in a cooler for transport as above.

Fields in the northern neck of Virginia, a major soybean-growing area, were sampled twice for BPMV in 2009. Three counties; Essex, Richmond and Gloucester, were sampled for virus on 17 September. Because fields were found to have a high incidence of BPMV, an additional collection was made on 7 October to sample five additional fields in Essex and Richmond Counties. Two additional fields in Middlesex County and one field from Gloucester County were also sampled. Soybean foliage as well as beetles, when present, were collected for analysis as described above. BPMV-positive fields were resampled in 2010. It was possible to sample from the same fields in this area since crop rotation was not practiced as frequently as in the Eastern Shore counties.

Two fields in the City of Suffolk on the Tidewater AREC and one field in Hanover County were sampled in 2009. These fields were to act as a control since beetle populations are low and BPMV had not been detected in 2007 (Tolin and Langham 2010). These fields were sampled twice, once early in the growing season and once when the foliage was mature. There were no beetles present at these fields at the two sampling times. These fields were not resampled in 2010.

In 2010 a soybean leaf sample from Appomattox Co. that was submitted to the Plant Disease Clinic tested positive for BPMV. Samples were later collected from six near-by fields that displayed similar foliar symptoms.

Isolation of Bean pod mottle virus cultures

From each region of Virginia where soybean plants were sampled a BPMV-positive sample was mechanically inoculated to cv. Hutcheson soybeans in the greenhouse. Severities of symptoms were compared from each sample location. These and known BPMV cultures were propagated on soybean cv. Hutcheson or Essex and maintained through sequential transfer every 3-4 weeks as positive controls. Leaves of each culture were also stored at -20° C for future studies.

BPMV detection by immunoassay. Soybeans and beetles were assayed for the presence of BPMV. Field collected soybeans leaves were stored in plastic bags and kept cool on ice or at 4°C prior to immunoassays. Beetles were either ground shortly after collection as described below, or held at 4°C or -20°C until tested.

Beetles were tested by double antibody sandwich ELISA using procedures modified from Ghabrial and Schultz (1983). Reagents were from Agdia, Inc. (Elkhart, IN) unless otherwise specified. Single beetles were placed in a 2 ml microcentrifuge tube containing 0.5 ml of general extraction buffer (GEB with Tween-20), then ground with microcentrifuge pestles. Beetle extracts were then held at 4° C. Infected and non-infected soybean leaves were ground in mesh bags at 1:25 (w:v) or greater. Nunc MicroWell™ (Maxisorp) 96 well plates were loaded with 100 µl of anti-BPMV antibody (1:1,000) in carbonate coating buffer (Agdia) and incubated at 30° C for 2 hr. Antibody to BPMV utilized for coating and trapping was kindly provided by M. Redinbaugh, Wooster, OH. Plates were then rinsed 3 times each for 3 minutes with PBS-Tween and then loaded with 200 µl of test beetle GEB extract, as well as positive (BPMV infected soybean leaf tissue EST-3, 116-8) and negative (non-infected soybean leaf tissue) controls. The EST-3 and 116-8 samples were used as positive controls because they had been

collected from the Eastern Shore of Virginia from the PIPE program and had given consistent positive results in previous tests (Tolin and Langham, 2010).

Following incubation at 30° C for 2 hr or 4° C overnight, plates were rinsed as before and 100 µl of the anti-BPMV enzyme conjugate were added to each well and incubated for 2 hr at 30° C. The enzyme conjugate was used at a 1:250 dilution (40 µl in 10 ml) of ECI buffer. Plates were rinsed as before and 200 µl of enzyme substrate, p-nitrophenyl phosphate (SigmaFAST™ pNPP tablets, Sigma-Aldrich®, St. Louis, MO) was added..

Absorbance was measured at 405 nm using a Spectramax Plus (Molecular Devices, Sunnyvale, CA) at 15-minute intervals during a 1 hour period.

Absorbance values of greater than 0.03 were scored as positive for BPMV for beetles. This value was established through the use of histogram analysis of the data (not shown). Positives were also established by values being twice the mean absorbance values of the lowest beetle extracts.

Plant samples were evaluated for virus presence and identity using a tissue blot immunoassay (TBIA) protocol modified from Srinivasan and Tolin (1992) and Chang et al. (2011). Plant sap from freshly torn leaves was blotted onto nitropure nitrocellulose membranes (NCM) and held at room temperature for at least 1 hr. The dried membranes were then placed in potassium phosphate buffered saline (KPS) (0.02 M K₂HPO₄, 0.15 M NaCl, pH 7.4) containing 5% Triton X-100 plus 5% non-fat dry milk (Nestle Carnation, Nestle USA Inc., Solon OH) and 0.5% bovine serum albumin (BSA) (Sigma-Aldrich®) for 30 min. This removed plant debris and green color and blocked to ensure non-antigen specific binding. The membranes were incubated for 90 min in a combined primary antibody specific for BPMV and a secondary enzyme-labeled anti-animal antibody in KPS. The anti-antibodies were from Agdia, Inc. and at a 1:10,000 dilution. After antibody incubation membranes were rinsed then placed in BCIP / NBT (Moss INC.

Pasadena, MD; or SIGMAFAST™ BCIP®/NBT tablets, Sigma-Aldrich®).

Membranes were then rinsed thoroughly in de-ionized water and allowed to air dry on paper towels. The presence of BPMV antigen was observed as a purple spot.

Beetles from the ESAREC in 2008 beetles were collected individually by hand. Extracts of beetles were done by removing the heads of five beetles and macerating the heads in 50 µl deionized H₂O. A pipet tip was dabbed into the beetle mixture to draw up 3-5 µl and then touched to the nitrocellulose paper. The blots were processed following the same protocol as the soybean blots.

Biological assay and virus culture. During the 2009 and 2010 five fields from the Eastern Shore were selected as good representations of virus specimens and were kept cultured in the greenhouse. An additional 5 fields were selected from the Northern Neck region. The 2009 collection samples were inoculated to Hutcheson soybeans in October and kept until symptoms were noted. The inoculated soybeans were then tested by TBIA for the presences of both BPMV and SMV because some symptoms were so severe that a co-infection of both viruses was thought to have occurred. Soybean plants were kept in the greenhouse as reference plants for future immunoassay tests, while other infected plant samples were frozen at - 20°C for future BPMV studies.

Results

In 2007 the Legume ipmPIPE survey of soybean sentinel plots detected a high incidence of BPMV at the Eastern Shore AREC but not at the Tidewater AREC (Tolin and Langham, 2010). Studies of virus prevalence were performed in 2008 on the ESAREC by collecting BLB and determining the percentage of the beetle population that was serologically positive for BPMV. There were 32 samples of five beetle heads each which were tested for BPMV by TBIA. Of these, 26 samples were BPMV-positive, indicating that 80% of the beetles sampled

were BPMV-positive. This led to the hypothesis that the epicenter of BPMV soybeans on the Eastern Shore was the AREC.

Counties sampled in 2009 and 2010 represented the predominant soybean growing areas in Virginia. In 2009, both beetles and soybean foliage were collected in three counties on the Delmarva Peninsula. In 2009, 55 fields were sampled throughout Virginia, and 6 fields from Worcester Co., MD. In 2010, 31 fields in Virginia were sampled with no fields sampled in Maryland (Table 4.1).

Analysis of soybean fields located at selected distances from the ESAREC showed that virus was distributed throughout the length of Virginia's Eastern Shore (Accomack and Northampton counties) in 2009 (Table 4.2). Soybeans or beetles from 16 of the 28 fields were BPMV-positive on the in Accomack and Northampton counties in August (Table 4.2). In fields that were 15 miles north of the ESAREC, BPMV was detected in 3 of 4 fields. Fields 15 miles south of the AREC also had 3 of the 4 fields BPMV-positive. Fields 10 miles north of the ESAREC had detectable BPMV in 1 of 4 fields. Fields 10 miles south of the AREC all four fields had detectable levels of BPMV. None of the fields 5 miles north of the station detected BPMV, while 2 of the 4 fields south of the ESAREC were BPMV-positive. The fields closest (< 2 miles) to the ESAREC had detectable BPMV in 3 of the 4 fields. Of the 3 positive fields no virus was detected in the plants, but beetles were the source of the BPMV-positive samples (Table 4.2). Fields sampled in August had less prevalence of BPMV in soybeans than in beetles.

Incidence was determined by the number of positive leaves sampled. An example of a BPMV-positive tissue blot example is seen in Figure 4.1. In the left panel, each of the 9 transect sites has at least one positive sample, with other in-field sites having 2, 3, or 5 positive plants. In the right panel, the incidence is lower as only 5/9 sites have positive plants with no more than two positives at any site.

Accomack County soybeans had a lower incidence of infected plants than did Northampton County in the early 2009 sampling. Accomack County also had less prevalence within beetles sampled (Fig. 4.1). In Accomack Co. only one of 16 fields sampled had BPMV-positive plants while 7 of the 16 had BPMV-positive beetles. In Northampton Co. 5 of the 12 fields had BPMV-positive plants and 9 of the 19 had BPMV-positive beetles. This indicated that virus incidence within the field was too low for the sampling design to detect at least one infected plant. The resampling done in October gave time for infection level to increase and be detected at this sampling level. Alternatively, even though viruliferous beetles were present at the earlier time, plants may have just been inoculated and virus had not yet spread to the leaves that were sampled. Four of the 6 fields sampled in Worcester Co., MD were BPMV-positive when both plants and beetles were analyzed. The two fields that were negative no beetles were sampled.

Fields resampled in October in Accomack Co. detected BPMV in 6 of the 9 fields in both foliage and beetles. Two of the three fields that were 5 miles north of the AREC were BPMV-positive. All three of the fields < 2 miles from the AREC were BPMV-positive. Three new fields (A3-A6) were sampled due to soybeans being mature or harvested and both BPMV-positive foliage and beetles were detected in one field, A6. The Northampton Co. sites had all been harvested, so 7 new fields were selected. BPMV was detected in foliage from one field and in beetles from three fields, but beetle sampling numbers were low. Fields in Maryland were not resampled.

An example of BPMV-positive tissue blot is seen in Figure 4.1. In panel A, each of the 9 transect sites has at least one positive sample, with other sites having 2, 3, or 5 positive plants. In panel B, the incidence is lower as only 5/9 sites have positive plants with no more than two positives at any site.

Of the 41 fields where BLB were collected from the Delmarva Peninsula in 2009, 29 tested positive for BPMV in beetle extracts by ELISA. A total of 544 beetles were tested from the 41 fields and 222 were BPMV positive (Table 4.2.) ELISA values were used to estimate virus load with each beetle. Beetles were then separated into four groups based on virus load within the beetle. An ELISA value of < 0.029 was negative for BPMV, and $0.03 - 0.49$, $0.5 - 1.59$, and $1.6 - 4.0$ were a low, moderate, or high virus load within the beetle, respectively. Northampton Co. had a higher prevalence of fields with BPMV and higher incidence of BPMV within beetles. There was a correlation of 0.94 when comparing the presence of positive beetles to positive leaf tissue. Beetle extracts that were ELISA-positive (Table 4.2) were confirmed in the greenhouse by successful transfer to soybean by mechanical inoculation. Beetles from fields 14, 17, 19, 20, and 22 from the Eastern Shore had the highest virus loads as indicated by high ELISA values (Table 4.3). The extracts from 2-3 beetles with high virus were combined to give sufficient inoculum volume for application to three plants. The inoculated plants were monitored for symptoms for 3-4 weeks, and BPMV presence confirmed by TBIA. The mechanical inoculation of beetle sap was also performed with extracts from single beetles, but was not successful. Beetle extracts from all fields where inoculum was combined transferred successfully. Fields 20 and 22 became known as beetle isolate 1 and 2 and were selected for maintenance in soybeans in the greenhouse since these soybeans exhibited the most severe symptomology.

Fields on the Eastern Shore that were BPMV positive by testing either beetles or foliage were revisited in the summer of 2010 (Table 4.4). This resampling of BPMV-positive fields was to see if virus reoccurred in the same field year to year, or if a BPMV-positive field had a greater chance of virus prevalence the following year based on previous BPMV status. Due to crop rotation it was not possible to sample the same fields on the Eastern Shore in 2010,

so a soybean field adjacent to the previous location was sampled. Of the fields sampled over the two counties in 2010, only 8 of the 21 had BPMV positive soybean plants (Table 4.4). Thus the virus prevalence was much lower in 2010 than in 2009. Virus incidence was also low with no more than 5/45 soybeans tested being BPMV-positive (11%). Of the 11 fields sampled in Accomack County in 2010 only three were positive for BPMV. In Northampton County five fields that were sampled had 5 of 10 fields sampled be BPMV positive foliage. Virus incidence within the fields for both counties was less than the previous year with a maximum of 5 of 45 plants testing positive for a single field. The number of fields testing positive overall was also less than the previous year. No beetles were present for sampling.

Eastern Virginia fields tested positive for BPMV in the Northern Neck and Tidewater areas in 2009. All fields tested in the counties of Gloucester, Essex and Richmond tested positive for BPMV using TBIA (Table 4.5). There was a high incidence of BPMV-positive soybeans in Essex Co. with 187 of 205 plants testing positive. Sixty four of the 73 beetles tested were BPMV-positive. All but one field in Essex Co. lacked BPMV-positive beetles and all non-infected plants were from this field (Table 4.5). There was also a high incidence of BPMV in Richmond Co. in all six fields sampled, BPMV-positive foliage and beetles were detected. BPMV was detected in 101 / 230 plants tested and was also detected in 51 / 55 beetles. One field in Gloucester Co. was BPMV-positive in both foliage and beetles. In 2009, soybeans collected in Suffolk, Middlesex and Hanover counties (Table 4.5) were negative for BPMV on two sampling dates. Beetles were not present in these fields on the sample dates.

Fields in Richmond and Essex Co. were resampled in 2010 for soybean foliage testing, but no beetles were collected. Virus prevalence within Essex Co. declined with only 2 / 5 fields testing BPMV-positive. Incidence within fields was

also less with BPMV detected in 19 / 50 plants in one field and BPMV in 7 / 45 plants in the other field. In Richmond Co. 3 / 5 fields had BPMV-positive soybean foliage. Field incidence had also decreased from the previous year with 11, 5, and 8 plants out of 45 testing BPMV-positive, respectively (Table 4.5).

In the summer of 2010 an outbreak of BPMV was seen in Appomattox County. The virus had not been detected previously in this low acreage soybean-growing region of Virginia. All six fields tested for virus had BPMV-positive soybean foliage. The incidence within the field was high in all six fields. In two of the fields BPMV infection was detected in all 50 soybean plants tested. The remaining four fields had a BPMV incidence of 30 – 42% of plants sampled. No beetles were collected from these sites. For all six fields where foliage was BPMV-positive, samples were inoculated to Hutcheson soybeans. Plant symptoms ranged from mild mosaic to severe mosaic and mottling of foliage. These plant samples were maintained in the greenhouse and samples were also frozen for preservation and future BPMV studies.

Discussion

The survey of fields on the eastern part of Virginia in 2009 and 2010 showed that BLB are present in areas where soybeans are commonly grown. High incidences of BPMV-positive BLB and soybean foliage were observed on Delmarva Peninsula, Appomattox Co., and in Richmond and Essex counties on the Northern Neck of Virginia. No beetles were collected in some areas due to a gap between beetle generations on sampling dates.

On the Delmarva Peninsula 37 fields had BPMV-positive beetles in both years with incidence ranging from < 10 to 100%. Fields sampled on the Delmarva Peninsula had a high incidence of positive beetles in 2009, but in 2010 the incidence had decreased to < 12% of beetles testing positive for BPMV. Beetle

populations as well as BPMV prevalence is not consistent and shows no patterns on the Delmarva Peninsula and the ESAREC is not the epicenter of the virus. This inconsistent BPMV infection of plants and incidence within beetles was also seen in all other counties sampled for both years. Prediction of BPMV infection rates and areas may not be possible based on these results. However, there is a high correlation between viruliferous beetles and virus in plants within fields. Table 4.3 demonstrates the virus load within beetles and can be directly related to high incidence within soybeans fields when compared to Table 4.2.

A method was developed for testing single beetles by ELISA that determined the relative incidence of viruliferous beetles in a field and also gave a relative content of BPMV within each beetle. This method was a modification of that used by Ghabrial and Schultz (1982) who tested groups of five beetles for BPMV and quantified the amounts of virus relative to with BPMV purified virus plus BPMV-negative beetles. By doing these comparisons, they demonstrated that there were no interfering chemicals within beetles that would distort the ELISA values. Their work further justified the quantitative aspect of ELISA for detecting BPMV load in beetles by assessing the amount of virus in each beetle and then adding the same amount (ng /ml) of purified virus to virus-free beetles, performing ELISA, and obtaining similar results. This validated the procedure, showing that for testing beetles for BPMV by ELISA was consistent and accurate.

Bean leaf beetles were able to be collected in both counties and both years on the Eastern Shore of Virginia. The distribution of beetles and population numbers varied for both sampling years. In 2009, the southern area in Northampton County had a greater population of BPMV-positive beetles in August, but when fields were resampled in October more fields in Accomack had BPMV-positive beetles. This is likely due to the maturation of soybeans in Northampton Co. and beetles moving off to find younger foliage. Another

possibility is that they had moved onto snap beans that had been planted in neighboring fields. The fall snap bean crop is planted in August and September, so beetles likely moved into those fields. The increase in BPMV-positive beetles corresponds with an increase in BPMV-positive soybean foliage. The greatest increase of BPMV-infected plants was in Accomack County from soybeans that were sampled in October. This increase is likely due to beetles spreading the virus from plant to plant during the growing season. Beetles collected and analyzed by ELISA had a high virus load quantitatively in August and had a high probability of transferring the virus to plants. These results are similar to those reported in Arkansas, in which late collected beetles had a higher percentage of BPMV-positive beetles than in those collected in the spring (Hopkins and Mueller 1983). Beetles were collected and were assayed by placing them on soybean plants for inoculation feeding, resulting in virus transmission by the beetles. Later collected beetles during the second generation had a higher transmission rate than those collected early in the spring belonging to the first generation (Hopkins and Mueller 1983). Beetles in October are the overwintering generation in Virginia and have been feeding on mature soybean foliage that may have been infected with BPMV, so the chances for finding BPMV-positive beetles would increase as the growing season progressed. In fields with a high incidence of virus in plants, nearly all of the beetles were ELISA positive and had a high virus loads.

Looking for the source of beetles for the Eastern Shore, surrounded on three sides by water, beetles had a greater chance of migrating south from Maryland than by crossing the Chesapeake Bay. The Southern Tidewater area was free of BPMV, but sampling was limited and occurred early in the growing season. Data collected by Mackasmiel et al. (2001) sampled soybeans in Suffolk and found no BPMV-positive foliage. Similar results were also seen when the legume PIPE surveyed the tidewater area in 2007 and found no BPMV in the sentinel soybeans (Tolin and

Langham 2010). There were four BPMV-positive fields in southeastern Maryland, which suggests that the beetles and BPMV probably migrated to the Eastern Shore of Virginia from the North. This hypothesis is further strengthened by detecting no BPMV in beetles or foliage detected in the Tidewater area. Bean leaf beetles are capable flyers, but the 17 miles needed to cross from the Tidewater area to the Eastern Shore is too vast a distance. Krell et al (2003b) characterized beetle flight as being short flights between food sources of less than one kilometer. Another observation by Krell (2003b) was that flights were less than 30 mins long with a mean flight distance of 166 meters. Boiteau et al (1979) also observed flights of BLB and recorded flight time as being < 30 mins. It is more likely that positive beetles made their way from fields in Maryland and then slowly flew field to field until they populated the length of the Eastern Shore and then were unable to fly across the Bay to the Tidewater region of Virginia.

To test the seasonal variability of BPMV between counties, resampling of BPMV-positive fields was done in 2010 to determine whether fields that were positive in previous years are more likely to have BPMV the following year. The rationale for doing this was to examine whether re-occurrence might be associated with a local source of virus, either perennial plants or locally overwintering beetles. The lack of a year to year relationship suggests that the source might be infected seed or migrating beetles.

On the Eastern Shore crop rotation from soybean to corn prevented the resampling of every field from one year to the next. Even though fields do not have soybeans continuously the beetles have no problem finding alternative host plants such as clovers and vetch since these are used as winter cover crops and are endemic to the area. These beetles also fly from field to field, so if corn is planted in the area the beetles emerge then they simply fly to the adjacent fields containing soybeans (Krell 2003b). Beetles may also fly from older soybean foliage to

younger foliage acting as vectors of BPMV from infected fields to non-infected fields. BPMV prevention in fields is difficult due to the prevalence of the beetle on the Shore as well as the beetles' mobility.

Another aspect of cropping systems with soybeans on the Eastern part of Virginia is that soybeans are usually planted in double-crop system with winter wheat. Soybeans are direct seeded into the ground after the winter wheat has been harvested. By extending the planting date of BPMV host plants viruliferous beetles may choose to feed upon snap beans that may be located next to soybean fields. By feeding on non-host plants of BPMV beetles may cleanse themselves of virus. This preference for snap beans is demonstrated in chapter 3. Alternatively, emerging beetles may have been foraging on alternative host plants that may have been infected with BPMV while the soybeans have been germinating. Upon soybean seedling emergence the potentially viruliferous beetles then move onto young soybeans. The potential for yield loss from BPMV infection is greatest when seedlings are infected by BPMV in the vegetative stages (Ross 1986). Fields where beetles were positive in August early in the growing season also had BPMV-positive soybean foliage. There was a correlation of 0.94 between positive beetles early in the season and finding positive soybean foliage in that same field later in the growing season for double crop soybeans. This correlation can be used as a monitoring tool where beetles can be tested early in the growing season to ascertain if a field has a high or low probability for having a BPMV outbreak. If there is a high percentage of BPMV-positive beetles additional sprays may be needed to decrease vector populations. By decreasing vector populations virus spread within the field will be decreased. This study supports work done by Ghabrial and Hershman (1990) that beetles can be used as an indicator of the virus incidence in the field. There was only one field in Essex Co. in 2009 that had soybeans that were BPMV-positive, but there were no positive beetles collected.

Another factor influencing BLB populations is soil type. The soil in the Tidewater area is sandy loam and does not hold moisture (WSS 2011). This may play a role in the lack of BLB present in the area, which suggests that the incidence of BPMV would be low as well. BPMV was detected for the first time in Appomattox County in August of 2010 where virus incidence was extremely high. The mild winters and organic soils in these areas contribute to the BLB survival and reproductive rates. However, no beetles could be collected from this area for analysis.

The Northern Neck of Virginia does not have soil suitable for corn, thus crop rotation out of soybean is not as common as on the Eastern Shore. This meant that samples from the same fields could be obtained year-to-year. In this area of sampling, due to lack of crop rotation, it was found that fields that had virus in the previous year also had BPMV infected soybeans the next season. The soil here is Rumford type with loamy sand for the first 13 inches where BLB larvae and pupa would be found (WSS 2011). Soil type and a lack of crop rotation make this a prime BLB habitat. The fields sampled from 2009 and 2010 had the same incidence of BPMV in each year. Areas that have BLB and soybeans present also have a high incidence of virus-infected soybeans within fields in that area. Cropping systems and soil type may be two factors influencing the prevalence of BPMV in certain areas of Virginia.

Knowledge of BPMV distribution in Virginia is necessary to monitor virus progression and develop possible control tactics. It may be possible to limit the spread of BPMV by controlling vector populations or by eliminating BPMV host plants from the soybean fields. The disease cycle of BPMV is complex and involves several variables that fluctuate year to year as does the cropping systems in soybean growing areas. Continued monitoring of the prevalence of BPMV in Virginia is needed to fully understand the epidemiology of this disease.

References

- Boiteau, G., J.R. Bradley, Jr., and J.W. Van Duyn. 1979. Bean leaf beetle: Flight and dispersal behavior. *Ann. Entomol. Soc. Am.* 72: 298-302.
- Bradshaw, J.D., L.P. Pedigo, J.H. Hill, and M.E. Rice. 2008. Evaluation of management strategies for bean leaf beetles (Coleoptera: Chrysomelidae) and bean pod mottle virus (Comoviridae) in soybeans. *J. Econ. Entomol.* 101: 1211-1227.
- Byamukama, E., A.E. Robertson, and F.W. Nutter, Jr. 2011. Quantifying the within-field temporal and spatial dynamics of *Bean pod mottle virus* in soybean. *Plant Dis.* 95:126-136.
- Chang, P.-G., W. McLaughlin, and S.A. Tolin. 2011. Tissue blot immunoassay and direct RT-PCR of cucumoviruses and potyviruses from the same NitroPure nitrocellulose membrane. *J. Virol. Methods.* 117: 345-351.
- Capinera, J.L. 2001. *Handbook of Vegetable Pests.* Academic Press, New York. 93 pp
- Eddy, C. O., and W. C. Nettles. 1930. The bean leaf beetle. *S.C. Agric. Exp. Stn. Bull.* 265: 1-25.
- Gergerich, R.C., H.A. Scott, and J.P. Fulton. 1986. Evidence that ribonuclease in beetle regurgitant determines the transmission of plant viruses. *J. Gen. Virol.* 67: 367- 370.
- Gergerich, R.C. 1999. Comovirus: Bean pod mottle virus Comovirus. Pages 61-62 in *Compendium of Soybean Diseases*, 4th ed. G.L. Hartman, J.B. Sinclair, and J.C. Rupe, eds. American Phytopathological Society, St. Paul, MN.
- Ghabrial, S.A., C.M. Pickard, and R.E. Stuckey. 1977. Identification and distribution of virus diseases of soybean in Kentucky. *Plant Dis. Rep.* 61: 690-694.
- Ghabrial, S.A., and F.J. Schultz. 1982. Serological detection of bean pod mottle

- virus in bean leaf beetles. *Phytopathology* 73: 480-483.
- Ghabrial, S.A., D.E. Hershman, D.W. Johnson, and D. Yan. 1990. Distribution of bean pod mottle virus in soybeans in Kentucky. *Plant Dis.* 74: 132-134.
- Giesler, L.J., S.A. Ghabrial, T.E. Hunt, and J.H. Hill. 2002. Bean pod mottle virus: A threat to U.S. soybean production. *Plant Dis.* 86: 1280-1289.
- Gu, H., A.J. Clarke, P.B. de Sa, T.W. Pfeiffer, S. Tolin, and S.A. Ghabrial. 2002. Diversity among isolates of *Bean pod mottle virus*. *Phytopathology* 92: 446-452.
- Hill, C.B., G.L. Hartman, R. Esgar, and H.A. Hobbs. 2006. Field evaluation of green stem disorder in soybean cultivars. *Crop Sci.* 46: 879-885.
- Hobbs, H.A., G.L. Hartman, Y. Wang, C.B. Hill, R.L. Bernard, W.L. Pedersen, and L.L. Domier. 2003. Occurrence of seed coat mottling in soybean plants inoculated with bean pod mottle virus and soybean mosaic virus. *Plant Dis.* 87: 1333-1336.
- Hopkins, J.D., and A.J. Mueller. 1983. Distribution of bean pod mottle virus in Arkansas as related to the bean leaf beetles, *Cerotoma trifurcata* (Coleoptera: Chrysomelidae) population. *Environ. Entomol.* 12: 1564-1567.
- Horn, N.L., L.D. Newsom, and R.L. Jensen. 1973. Economic injury thresholds of bean pod mottle virus and tobacco ringspot virus infection of soybeans. *Plant Dis. Rep.* 57: 811-813.
- Krell, R.K., L.P. Pedigo, J.H. Hill, and M.E. Rice. 2003a. Potential primary inoculum sources of bean pod mottle virus in Iowa. *Plant Dis.* 87: 1416-1422.
- Krell, R.K., L.P. Pedigo, J.H. Hill, and M.E. Rice. 2003b. Characterization of bean leaf beetle (Coleoptera: Chrysomelidae) flight capacity. *J. Kans. Entomol. Soc.* 76: 405-416.
- Krell, R.K., L.P. Pedigo, J.H. Hill, and M.E. Rice. 2004. Bean leaf beetle

- (Coleoptera: Chrysomelidae) management for reduction of bean pod mottle virus. *J. Econ. Entomol.* 97: 192-202.
- Lam, W.F., and L.P. Pedigo. 2000. A predictive model for survival of overwintering bean leaf beetles (Coleoptera: Chrysomelidae). *Environ. Entomol.* 29: 800-806.
- Langham, M.A.C., D.C. Doxtader, J.D. Smolik, and R.A. Scott. 1999. Outbreak of a viral disease affecting soybeans (*Glycine max* (L.) Merrill) in South Dakota. *Phytopathology* 89: S43 (Abstr.).
- Mabry, T.R., H.A. Hobbs, T.A. Steinlage, B.B. Johnson, W.L. Penderson, J.L. Spencer, E. Levine, S.A. Isard, L.L. Domier, and G.L. Hartman. 2003. Distribution of leaf-feeding beetles and bean pod mottle virus (BPMV) in Illinois and transmission of BPMV in soybeans. *Plant Dis.* 87: 1221-1225.
- Mackasmiel, L.A. 2004. Assessing the Distribution and Impact of *Bean pod mottle virus* (BPMV) as a Re-emerging Virus, and *Soybean mosaic virus* (SMV) in Soybean Grown in Virginia. Ph.D. dissertation, Virginia Polytechnic Institute and State University, Blacksburg, VA.
- Marrone, P.G. and R.E. Stinner. 1983a. Effects of soil physical factors on egg survival of the bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae). *Environ. Entomol.* 12: 673-679.
- Marrone, P.G. and R.E. Stinner. 1983b. Bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae): Physical factors affecting larval movement in soil. *Environ. Entomol.* 12: 1283-1285.
- Marrone, P.G. and R.E. Stinner. 1984. Influence of soil physical factors on survival and development of the larvae and pupae of the bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae). *Can. Entomol.* 116: 1015-1023.
- Michelutti, R., J.C. Tu, D.W.A. Hunt, D. Gagnier, T.R. Anderson, T.W. Welacky,

- and A.U. Tenuta. 2002. First report of *Bean pod mottle virus* in soybean in Canada. *Plant Dis.* 86:330.
- Milbrath, G.M., M.R. McLaughlin, and R.M. Goodman. 1975. Identification of bean pod mottle virus from naturally infected soybeans in Illinois. *Plant Dis. Rep.* 59: 982-983.
- United States Department of Agriculture [USDA]: National Agricultural Statistics Service (NASS). 2008. (http://www.nass.usda.gov/Data_and_Statistics/index.asp). Accessed May 12, 2009.
- Ross, J.P. 1963. Transmission of bean pod mottle virus in soybeans by beetles. *Plant Dis. Rep.* 47: 1049-1050.
- Ross, J.P. 1968. Effect of single and double infections of soybean mosaic and bean pod mottle viruses on soybean yields and seed characteristics. *Plant Dis. Rep.* 52: 344-348.
- Ross, J.P. 1986. Response of early- and late-planted soybeans to natural infection by bean pod mottle virus. *Plant Dis.* 70: 222-224.
- Ross, J.P. and A.K. Butler. 1985. Distribution of bean pod mottle virus in soybeans in North Carolina. *Plant Dis.* 69: 101-103.
- Schwartz, H.F., Langham, M.A.C., Golod, J., Tolin, S.A., LaForest, J., and K.F. Cardwell. 2009. Legume ipmPIPE: The next evolution of web-based interactive tools for disease management and extension outreach. *APSnet Feature* May 2009 (www.apsnet.org/).
- Schwenk, F.W., and C.D. Nickell. 1980. Soybean green stem caused by bean pod mottle virus. *Plant Dis.* 64: 863-865.
- Sikora, E.J., and J.F. Murphy. 2005. First report of *Bean pod mottle virus* in soybean in Alabama. *Plant Dis.* 89: 108.
- Skotland, C.B. 1958. Bean pod mottle virus of soybeans. *Plant Dis. Rep.* 42: 1155-

1156.

- Smelser, R.B. and L. Pedigo. 1992. Bean leaf beetle (Coleoptera: Chrysomelidae) herbivory on leaf, stem, and pod components of soybean. *J. Econ. Entomol.* 85: 2408-2412.
- Snodgrass, R.E. 1935. *Principles of Insect Morphology*. pp. 154-155. McGraw-Hill, New York.
- Srinivasan, I., and S. Tolin. 1992. Detection of three viruses of clovers by direct tissue immunoblotting. *Phytopathology* 82:721.
- Sukkariyah, B., G. Evanylo, and L. Zelazny. 2007. Distribution of copper, zinc, and phosphorus in coastal plain soils receiving repeated liquid biosolids applications. *J. Environ. Qual.* 6: 1618-1626.
- Tolin, S. A. and G.H. Lacy. 2004. Viral, bacterial, and phytoplasmal diseases of soybean. *Soybeans: Improvement, Production, and Uses*, 3rd ed, Agronomy Monograph no. 16. pp. 765-819.
- Tolin, S.A. and C.W. Roane. 1975. Identification and distribution of soybean viruses in Virginia. *Proc. Amer. Phytopath. Soc.* 2:129.
- Tolin, S.A. and Langham, M.A.C. 2010. Virus surveillance in beans using tissue blot immunoassay: Three years experience of the legume ipm-PIPE. Report of the Bean Improvement Conf. 53:52-53.
(http://www.css.msu.edu/bic/PDF/Reports/BIC_2010_Volume_53.pdf)
- United States Department of Agriculture [USDA]: National Agricultural Statistics Service (NASS). 2008.
(http://www.nass.usda.gov/Data_and_Statistics/index.asp). Accessed April 10, 2009.
- United States Department of Agriculture [USDA]: National Agricultural Statistics

Service (NASS). 2009.

(http://www.nass.usda.gov/Data_and_Statistics/index.asp). Accessed Dec 10, 2009.

United States Department of Agriculture [USDA]: Web Soil Survey (WSS). 2011.

(<http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx>). Accessed January 5, 2011.

Walters, H.J. 1958. A virus disease complex in soybeans in Arkansas. (Abstr.)

Phytopathology 48: 346.

Table 4.1. Counties in Virginia surveyed for Bean pod mottle virus in 2009 and 2010.

County	Number of Fields Tested for BPMV in 2009	Number of Fields Tested for BPMV in 2010
<u>Eastern Shore</u>		
Worcester (Maryland)	6	0
Accomack	19	11
Northampton	19	10
<u>Northern Neck</u>		
Richmond	6	5
Essex	5	5
Middlesex	2	0
Gloucester	1	0
<u>Tidewater</u>		
Suffolk	2	0
Hanover	1	0
<u>Piedmont</u>		
Appomattox	0	6

Table 4.2. Bean pod mottle virus (BPMV) incidence in fields from the Delmarva Peninsula in 2009. Soybean foliage and beetles were sampled for BPMV from fields surveyed at distances from the Eastern Shore Agriculture Research and Extension Center (ESAREC).

Location	Field ID ^a	August 3-7		October 5	
		Plants +ve/n ^b	Beetles +v/n ^c	Plants +ve/n ^b	Beetles +ve/n ^c
Accomack	1-N-15	0/50	6/8		
	2-N-15	0/50	14/25		
	3-N-15	2/50	25/25		
	4-N-15	0/50	0/25		
	5-N-10	0/50	0/25		
	6-N-10	0/50	0/25		
	7-N-10	0/50	0/25		
	8-N-10	0/50	6/18		
	9-N-5	0/50	0/19		
	10-5-5	0/50	0/24	15/50	7/9
	11-N-5	0/50	0/25	0/45	-
	12-N-5	0/50	0/25	5/45	3/9
	A-N-2	0/50	6/34	6/25	5/5
	B-N-2	0/50	2/26	32/45	10/10
	C-N-2	0/50	0/25		
	D-N-2	0/50	4/24	27/45	9/9
	A3			0/45	-
	A5			0/45	-
	A6			6/25	5/5
	Northampton	13-S-5	0/50	1/25	
14-S-5		2/50	19 / 25		
15-S-5		0/50	0 / 15		
16-S-5		0/50	0/19		
17-S-10		0/50	25/25		
18-S-10		0/50	18/20		
19-S-10		3/50	20/20		
20-S-10		1/50	23/25		
21-S-15		2/50	13/25		
22-S-15		38/50	25/25		
23-S-15		0/50	7/19		
24-S-15		0/50	0/3		
N1				15/35	4/8
N2				0/40	4/6
N3			0/40	2/3	
N4			0/40	-	
N5			0/40	-	
N6			0/40	-	
N7			0/40	-	
Worcester, MD	W1			0/40	-
	W2			0/40	-
	W3			1/40	1/9
	W4			7/40	5/9
	W5			1/40	1/9
	W6			2/43	1/9

^a = field number or letter sampled, N = North of ESAREC, S = South of ESAREC; 2, 5, 10, 15 = miles from ESAREC. Fields with only a letter and one number were sampled in October. A = Accomack Co., N = Northampton Co., W = Worcester, MD. Number is the sequence fields were sampled.

+ve/n^b = combined positive soybean plants for BPMV / all samples tested.

+ve/n^c = combined positive bean leaf beetles for BPMV / all samples tested.

- = not collected.

Table 4.3. Relative Bean pod mottle load in single bean leaf beetles collected from fields on the Eastern Shore of Virginia 3-7 August 2009. ELISA values grouped into low, medium and high based on a histogram of values of beetles in Table 4.2.

Field ID	Negative ^a	Low ^b	Moderate ^c	High ^d
1-N-15	2	0	2	4
2-N-15	11	13	0	1
3-N-15	0	18	2	6
8-N-10	12	5	0	1
A-N-2	28	4	2	0
B-N-2	24	0	0	2
D-N-2	20	2	1	1
13-S-5	24	0	1	0
14-S-5	6	5	3	11
17-S-10	0	4	1	20
18-S-10	2	9	2	7
19-S-10	0	4	2	14
20-S-10	0	0	1	22
21-S-15	9	11	0	2
22-S-15	0	1	1	24
23-S-15	12	3	1	3

^a = ELISA values < 0.029

^b = ELISA values 0.03 – 0.49

^c = ELISA values 0.5 – 1.59

^d = ELISA values 1.6 – 4.0

Table 4.4. Incidence of *Bean pod mottle virus* in soybean foliage collected from the Eastern Shore of Virginia in 31 August and 1 September 2010. Virus confirmation was determined by TBIA.

Location	Fields	Plants ^a
Accomack	1	0/45
	2	0/45
	3	0/45
	4	0/45
	5	5/45
	6	0/45
	7	0/45
	8	0/45
	9	5/45
	10	0/45
	11	5/45
Northampton	1	0/41
	2	0/45
	3	0/45
	4	1/48
	5	5/40
	6	3/45
	7	5/45
	8	3/45
	9	0/45
	10	0/45

^a = combined positive soybean plants for BPMV / all samples tested.

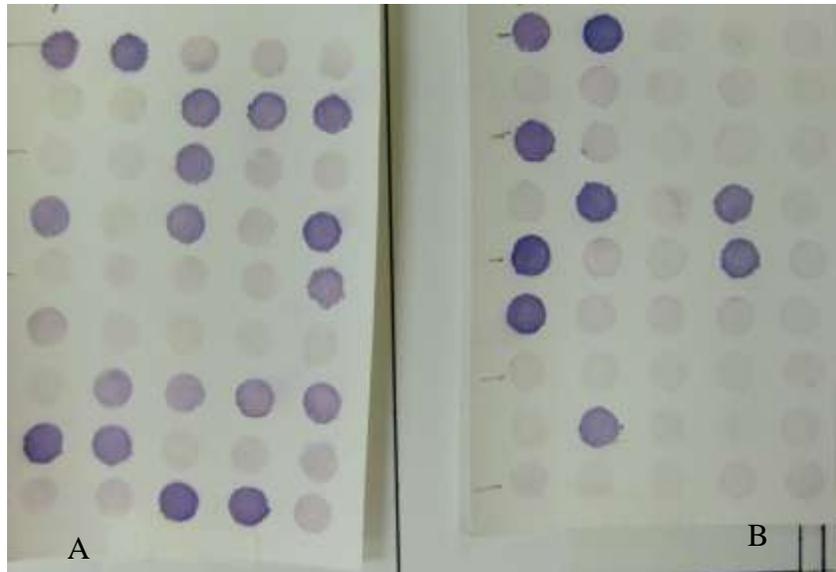


Figure 4.1. Tissue blot immunoassay results for fields 5 (A) and 6 (B) sampled from Richmond Co, VA. Samples of 5 consecutive soybean plants in 9 different locations in each field for a total of 45 plants sampled. Purple spots indicate a Bean pod mottle positive soybean sample.

Table 4.5. Incidence of BPMV from soybean foliage and bean leaf beetles sampled from fields in eastern Virginia in fall of 2009 and 2010, analyzed by TBIA and ELISA, respectively.

Location	Field ID No.	September and October 2009		September 2010
		Plants +ve/n ^a	Beetles +ve/n ^b	Plants +ve/n ^a
Essex Co.	1	50/50	2/2	19/50
	2	50/50	32/32	7/45
	3	50/50	23/23	0/45
	4	35/35	7/7	0/45
	5	2/25	0/9	0/45
Gloucester Co.	1	9/30	5/6	
Middlesex	1	0/40	-	
	2	0/40	-	
Richmond Co.	1	8/20	13/13	
	2	6/30	4/6	0/45
	3	20/45	9/9	0/45
	4	30/45	9/9	11/45
	5	22/45	9/9	5/45
	6	11/45	7/9	8/45
Suffolk*	1	0/50	-	
	2	0/50	-	
	1	0/50	-	
	2	0/50	-	
Hanover*	1	0/50	-	
	1	0/50	-	

^an = positive soybean plants for BPMV / all samples collected.

^bn = positive bean leaf beetles for BPMV / all samples collected.

* = Samples collected on September 9 and July 2 of 2009.

- = Not collected

Chapter 5

Potential inoculum source of BPMV in eastern Virginia

Abstract

Experiments were conducted in eastern Virginia to find the source of *Bean pod mottle virus* (BPMV). Overwintering bean leaf beetles did not seem to be the source of BPMV on the Eastern Shore because the level of virus in beetles was low at the beginning of the season. Virus load within the beetle and the proportion carrying virus increased with time after emergence, indicating that virus acquisition most likely occurred during post-emergence feeding. Perennial and winter annual weeds were collected in and near soybean fields that were positive for BPMV the previous season. Some specimens of yellow wood sorrel, *Oxalis stricta*, white clover, *Trifolium repens*, red clover, *Trifolium pretense*, hairy vetch, *Vicia villosa*, and red sorrel, *Rumex acetosella*, were positive for the virus by tissue blot immunoassay (TBIA). Certain samples of these plant species were also weakly positive by ELISA, except for white clover. However, mechanical transmission from weeds to soybeans could not be demonstrated.

Introduction

Bean pod mottle virus (BPMV) was first identified in South Carolina in 1947 in green beans, *Phaseolus vulgaris* (L.), and has become a concern for soybean growers in recent years (Giesler et al. 2002). The primary vector of BPMV is the bean leaf beetle, *Cerotoma trifurcata* (Forster), which is endemic in regions of the U.S where soybeans, *Glycine max* (L.) Merrill, are commonly grown (Isley 1930). BPMV can decrease overall yield as well as seed quality due to seed coat discoloration (Hill et al. 2007). To understand the epidemiology of this disease, it is paramount to identify the original source of this obligate pathogen

after a non-crop period. If the beginning of the disease cycle can be determined, then plant-virus management strategies could be implemented to decrease the virus prevalence in soybean growing areas. The occurrence of a plant disease depends upon the interaction of virulent pathogens, susceptible hosts, and competent vectors or other means of dissemination (Agrios 1997).

Three potential sources of BPMV include, seed transmission, overwintered viruliferous beetles, and an alternative host plant. Because there are no cultivars that are resistant to BPMV, it is critical to further investigate the source of this virus to explore potential control tactics (Scott et al. 1974). The importance of seed transmission as a source of BPMV of epidemiological significance is inconclusive with mixed results from several sources over several years. Some studies demonstrate low transmission rates, while others show no transmission at all. Lin and Hill (1983) tested 1,626 soybean seedlings from BPMV-infected parent plants and all were negative. Hartman et al. (1999) reported that seeds from BPMV-infected soybean gave rise to infected seedlings at a rate of 0.1%. Ross (1986) also reported a seed transmission rate of 0.1% based on finding seven seedlings out of 6,976 seeds that were BPMV-positive. Later studies by Krell et al. (2003) reported that only three infected seedlings for every 11,864 seedlings tested over a two-year period were BPMV-positive. Even if BPMV is seed transmitted it is uncertain if these reported levels are high enough to start the disease cycle each year. With the aphid-transmitted *Soybean mosaic virus*, the level of seed transmission critical to initiating epidemics is dependent upon the level and timing of aphid vectors (Irwin et al. 2000). Nonetheless, it is likely that the role of seed transmission is minor in the epidemiology of BPMV.

The transmission by overwintered beetles has been demonstrated. The BLB transmits BPMV in a non-persistent or semi-persistent manner (Wang et al. 1992). The virus is inoculated to plants through the gross wounding of plant foliage by

beetle feeding. The beetle then regurgitates onto the plant leaf to help lubricate mouthparts for feeding (Snodgrass 1935). The beetle may feed upon BPMV-infected plant tissue before entering winter diapause. Upon emerging the following spring, the beetle may then transmit the virus to suitable host plants, thus continuing the disease cycle. The virus is able to survive the winter within the beetle, but transmission rates have been variable. Mueller and Haddock (1980) determined that 13.5% of BLB collected from overwintering sites in Arkansas transmitted BPMV. In Iowa, Krell et al. (2003) demonstrated that of 64 collected overwintered BLB from Correctionville Co., only 1 (1.6%) transmitted BPMV to a soybean assay plant. When beetles from Ames Co. were placed on seedling soybeans none of the 88 beetles transmitted the virus. When beetles from Correctionville Co. were retested in 2001, none of 42 beetles tested transmitted the virus to soybean seedlings. Thus, because both seeds and beetles have such low transmission rates of BPMV in early spring, it has been speculated that there may be a living host plant or infected soybean seedling acting as a reservoir on which the BLB feeds each spring to acquire the virus (Giesler et al. 2002).

Among cultivated crops, BPMV has a narrow natural host range and is known to infect only two plant species, *Glycine max* (L.) Merr., and *Phaseolus vulgaris* (L.) (Moore et al. 1969). Among weed species, *Desmodium* sp. have been shown to be confirmed hosts of BPMV. Moore et al. (1969) identified *D. paniculatum* as a perennial host of BPMV, and Walters and Lee (1969) confirmed BLB transmission of BPMV from *D. paniculatum* to soybeans. Studies were conducted in Iowa that determined *D. canadense* as a definitive inoculum source for BPMV. This species had also been recognized as a host of BPMV in Louisiana (Horn et al. 1970). Moreover, Bradshaw et al. (2008) confirmed BPMV-positive sap extract from *D. illinoense* by ELISA and western blot. In Virginia, although *Desmodium* sp. occur in the landscape (Harvill et al. 1992), they are not considered

common weeds in the soybean growing areas. Other weed species could be playing a role in BPMV epidemiology in Virginia. Krell et al. (2003) was able to mechanically inoculate BPMV to several other BLB host plants including: *Lespedeza striata* (L.), *Phaseolus lunatus* (L.), *Trifolium incarnatum* (L.), *Vigna sinensis* (L.), and *V. unguicata* (L.). Inoculation to non-host plants of the BLB was also successful and included: *Lespedeza cuneata* (G.), *L. stipulacea* (Maxim.), *Stizolobium deeringianum* (Bort.), and *Pisum sativum* (L.) (Krell et al. 2003). Other leguminous plants such as clovers and vetches are abundant and may be the source of BPMV in Virginia, but have not been identified as host plants of BPMV. Bradshaw et al. (2008) listed crimson clover, *Trifolium incarnatum*, as a possible host plant of BPMV. Krell et al. (2003) tested 31 samples of red clover, *Trifolium pratense*, and were not able to detect BPMV in leaf sap by ELISA. The objective of this study was to identify and assess potential weeds as sources of primary inoculum for BPMV in Virginia.

Materials and Methods

Virus detection.

Immunoassays.

The methodology used for testing plant samples by ELISA and tissue blot immunoassay (TBIA) techniques is described in Chapter 4. Leaves were blotted on nitrocellulose membranes for TBIA. Plant samples were also analyzed by ELISA to confirm BPMV presence quantitatively. Individual BLB were tested by ELISA as described in Chapter 4.

A 25 gm leaf sample each was subjected to purification to concentrate any virus present. The frozen leaves were homogenized in 0.10 M sodium phosphate buffer, pH 7.0, containing 0.01M NaDIECA and 0.02M sodium thioglycollate, then

strained through cheesecloth and centrifuged at 10,000 rpm for 10 min. A portion of the supernatant was centrifuged at 40,000 rpm for 10 minutes, and resulting pellets were resuspended in a volume of 0.05 NaPhosphate, pH 7.0, to give a 7-10 x (wt/vol) concentration. The virus was also concentrated by adding 4% polyethylene glycol and 0.5M NaCl. The supernatant was treated for 2 hr, and then centrifuged at 10,000 rpm for 10 mins. Pellets were re-suspended to give a 5X concentration. After viral purification steps, all samples were analyzed by ELISA as described in Chapter 4.

Samples were considered BPMV-positive if the ELISA value was two times the standard deviation of the negative control samples. The ELISA values of the same weed species were also compared to observe any variances between wells, so a species negative value could be assigned. The control samples were BPMV-negative 'Hutcheson' soybeans. Positive controls were BPMV-positive 'Hutcheson' soybeans that had been mechanically inoculated in the greenhouse.

Biological assay.

The TBIA-positive samples were also tested by inoculation to cv. Hutcheson soybean. Suspected positive weed tissue was ground in a chilled mortar and pestle with 10 ml 0.01 M sodium phosphate buffer, pH 7.0. The inoculum was applied by dipping the pestle into the mixture and then gently rubbing unifoliate leaves previously dusted with 600 mesh carborundum (Buehler, Lake Bluff, IL). Leaves were then rinsed with tap water. The weed sap inoculated soybeans were maintained in the greenhouse and observed for symptoms over 3-4 weeks. To confirm BPMV transfer from weeds to soybeans, the weed sap inoculated plants were analyzed by TBIA and ELISA. Two separate attempts to inoculate BPMV-positive weed sap to soybean foliage were made using 14 'Hutcheson' soybean plants. A second inoculation technique was also used to try and transfer BPMV-

positive weed sap to soybeans that involved another buffer solution. After weed samples had been placed into the pestle, a solution of 0.02% sodium sulfite was used as the grinding buffer. This buffer was used to try and eliminate interfering inhibitors in weed sap and facilitate virus transfer to soybeans. Five soybean plants in one pot were used in each trial. White clover and hairy vetch samples concentrated by purification were also inoculated to greenhouse soybeans to detect virus presence.

Beetle collection

Beetles were collected from various locations within each field. Sweep nets were used to collect either 25 or 50 beetles per field. Beetles were then placed into plastic bags and held in a cooler until they could be taken back to the lab and processed for ELISA. In the lab, beetles were placed in individual tubes and ground with a microcentrifuge pestle in general extract buffer (Agdia, Inc., Elkhart, IN). Beetle extracts were then held at 4°C until an ELISA could be performed. Beetles were also stored whole in refrigerator at 4°C or frozen at -20°C until processed for use in ELISA.

Potential source plant collection

In May 2008, at the Virginia Tech Eastern Shore Agricultural Research and Extension Center (ESAREC) near Painter, VA, foliar samples of white clover, *Trifolium repens*, red clover, *Trifolium pretense*, snap bean, *Phaseolus vulgaris*, alfalfa, *Medicago sativa*, soybean, *Glycine max*, lima bean, *Phaseolus lunatus*, black locust, *Robinia pseudoacacia*, and hairy vetch, *Vicia hirsuta*, were sampled and tested by TBIA. Fifty foliar samples of each species were analyzed.

In 2009, vetch and white clover samples, used as overwinter cover crops, were collected from the Eastern Shore AREC and placed in bulk plastic bags and frozen

at -20°C until processed by purification. These processed samples were then analyzed by ELISA. In addition, soybean fields from Accomack, Northampton, Essex, and Richmond counties that were BPMV-positive in the fall of 2009 (see Chapter 4) were selected as likely locations to find a natural source of BPMV the next spring. Locations were based on the high percentage of BPMV-positive foliage and beetles. Both leguminous and non-leguminous weeds were collected (Table 5.1). Selection of plants to collect from fields was based on the plant family and dominance in the landscape. Ten weed samples from each field were collected using a golf hole cutter or trowel and replanted into flats or pots in the greenhouse in Blacksburg, Virginia. Weeds were identified visually (Richard et al. 1997) and confirmed by visual inspection by weed scientists at Virginia Tech (Blacksburg, VA).

Results

Beetles. Emerging overwintered beetles collected in spring of 2009 were monitored for BPMV. The virus load was low from the newly emerged beetles, however as the weeks progressed, the virus load in the beetles increased (Fig. 5.1). The first beetle was collected on 7 May (JD=127) and beetles were monitored through 23 June (JD=174). Results from immunoassay of BLB for BPMV by ELISA strongly suggest that beetles were acquiring the virus after emergence by feeding on a BPMV-infected source.

Potential source plants. All leguminous host plants on the ESAREC collected in 2008 tested negative for BPMV using to TBIA results. In 2009, sample numbers were increased and sampling was confined to white clover and hairy vetch. All 10 samples collected of clover were negative, but one sample out of 10 of the hairy vetch was BPMV-positive from the ESAREC using ELISA. Bulk samples of white clover and vetch from the ESAREC were concentrated and tested for BPMV

by ELISA and bioassay. All seven concentrated samples of vetch and white clover from the purification were negative. The 5X concentration purified from hairy vetch was then mechanically inoculated to soybeans. The concentrated vetch extracts induced virus-like symptoms in one soybean plant, but tests for the virus were negative. TBIA sampling of legumes on the ESAREC detected BPMV in some samples, while others were negative. All 97 hairy vetch samples were negative for BPMV using TBIA, four of the 65 red clover samples were positive, two of the 75 samples of white clover were BPMV-positive by TBIA. None of these samples were inoculated to soybeans.

In 2010, weeds were collected from soybean fields from which BPMV-positive soybean foliage or beetles had been detected in 2009. Among non-leguminous weeds, yellow wood sorrel, *Oxalis stricta* and red sorrel, *Rumex acetosella*, showed BPMV-positive tissue blots in some samples, but many were negative (Fig 5.3). Seven collections of yellow wood sorrel tested BPMV-positive by TBIA from four different fields; three from fields in Accomack Co. and one from a field in Richmond Co. A BPMV-positive yellow wood sorrel plant can be seen in Figure 5.2. Two of the red sorrel samples were also TBIA-positive. The red sorrel plants were collected from the same field located in Accomack Co. on the Eastern Shore (Table 5.1). When ELISA was performed to test for the virus presence in the leaves, some weakly positive results were observed. Of the 25 species tested, four species were ELISA-positive. These were yellow wood sorrel, red sorrel, red clover and hairy vetch (Table 5.1). Positive TBIA blots can be seen in Figure 5.2 with the positive red clover coordinates; 1A, 1B and the negative coordinates 3B, 4B. The yellow wood sorrel positive blot coordinates are 2A-2E and negative blot coordinates located at 3A, 3C, 3D, 3E, 4A, and 4D. The positive coordinate for red sorrel is 1C and the negative is 4C. Only one white clover was

tested and was negative and is located at coordinate 1E. The hairy vetch sample from the ESAREC in 2009 had one out of 10 samples test BPMV-positive. The positive sample had an ELISA value of 0.066 with a healthy control mean of 0.029; all other vetch samples had a mean ELISA value of -0.05. The single hairy vetch sample from Richmond Co. was weakly positive with an ELISA value of 0.010. One out of three red clover had a value of 0.016, the two negative clover values were -0.005 and 0.003. The healthy control had a mean ELISA value measuring -0.0005. The red sorrel from Richmond Co. had a single weakly positive value of 0.045 out of three samples tested. The two negative red sorrel samples had ELISA values of -0.008 and 0.000. The yellow wood sorrel had a single positive out of eight tested and an ELISA value of 0.045. The mean ELISA values of the negative yellow wood sorrel samples was 0.0006. The healthy control samples had a value of 0.025. The BPMV-positive controls all had value of 3.00 or above at the 60 min reaction time.

Biological transfer of virus from immunopositive plants by inoculation to soybeans was unsuccessful. All inoculated plants were TBIA-negative, even though a few plants exhibited virus-like symptoms. Yellow wood sorrel was mechanically inoculated on two separate occasions to soybeans with 4 source plant samples each. These samples were from the same source plants that had tested BPMV-positive using the tissue blot. Red sorrel, red clover and white clover were also tested twice with four source samples each, but none could be transferred to soybeans. Yellow wood sorrel and red sorrel are not recognized as host plants of the BLB or BPMV, but these plants are often found near clover and vetch.

Discussion

A temporal progression of BPMV positive foliage and beetles in Accomack County can be seen (Fig. 5.1). My observations of low BPMV-positive beetles and foliage incidence demonstrated that virus incidence is low early in the growing season when beetle populations are low and the soybeans are young. This seasonal pattern was also seen by Mueller and Haddox (1980), when the percentage of viruliferous beetles emerging from overwintering sites was low. Additionally, when Mueller and Haddox (1980) placed newly emerged beetles on soybeans, virus transmission rate was also low. Increase of virus load in the emerging beetle generation suggests that the beetles are acquiring BPMV from host plants feed upon after emerging. This result was also found by Krell et al. (2003) who showed that BPMV transmission by overwintered BLB is $< 1\%$. Beetle transmission to soybeans would also decrease if beetles had fed upon non-BPMV host plants and were then placed onto susceptible plants. For example, snap beans are typically planted in April and May on the Delmarva Peninsula prior to soybeans, and newly emerged seedlings are available for beetles to feed upon after emergence. Since snap bean varieties that are commonly grown in this area are not susceptible to BPMV (S. Tolin, unpublished), beetles could be feeding upon them and cleansing themselves of the virus before moving over to later-planted soybeans. This scenario would decrease the virus load within the beetles and lower transmission rates to soybeans.

While eastern Virginia has a relatively low occurrence of *Desmodium sp.* near soybean fields, my surveys indicate that other plant species may also harbor BPMV. The TBIA and ELISA-positive foliage of red and white clovers, as well as red sorrel, yellow wood sorrel, and vetch suggests that these plants could play a role in BPMV epidemiology. To my knowledge, this is the first report of BPMV

identified in yellow wood sorrel and red sorrel in the U.S. However, Krell et al. (2003) also reported BPMV-positive dot blot assays from mechanically-inoculated plants, but was unable to get confirmation by Western blots. This inability to obtain BPMV-positive results by Western blot suggests that false positives for some weed species can occur. However, in the TBIA analysis for BPMV-positive weeds, I was able to obtain both positive and negative results. These results were obtained from different plants and from different fields, so the occurrence of false positives is unlikely. If false positives had been responsible for purple color development, then every sample would have been positive using this technique. BPMV-positive plant samples were confirmed by ELISA. Foliage of BPMV-positive yellow wood sorrel plants has a yellowing of the upper leaves, which is a symptom also seen in BPMV-positive soybean foliage. The visual symptomology, positive tissue blots and ELISA strongly suggests that yellow wood sorrel, red sorrel, hairy vetch, and red clover are potential sources of BPMV in Virginia.

Mechanical transfer of BPMV from these TBIA-positive weeds to soybeans could not be demonstrated. This failure could be because the virus concentration in leaves was not high enough to ensure transfer by standard inoculation techniques. The ELISA values were weakly positive for BPMV, which suggests a low virus load within the naturally-infected potential source plants.

Bean leaf beetles have not been observed feeding on these potential host plants, however their proximity to leguminous plants and their prevalence in the landscape may lend themselves to accidental or test feeding by beetles. Even though recovery of BPMV from weeds to soybeans was unsuccessful by mechanical inoculation, the beetle / virus and plant interactions may be more complex and not capable of being mimicked in the greenhouse. Gergerich et al. (1983) speculated that beetle regurgitant may affect the host plant or the interaction of the virus with the host plant. The inability to mechanically transfer virus from

immuno-positive leaf tissue to soybeans by standard techniques demonstrates a specificity of beetle and virus interactions with a host plant or beetle regurgitant, mainly the ability of beetle-transmitted viruses to escape ribonuclease activity (Gergerich and Scott 1988a). Another study by Gergerich and Scott (1988b) showed that it is the behavior of the virus within the plant following mechanical inoculation by the beetle that determines whether a susceptible plant becomes infected with the virus. The primary inoculum search on the Eastern Shore of Virginia demonstrates that even though a BPMV-positive plant is found, bioassay to soybeans may not be possible because of low virus concentrations, inhibitors, or specific interactions between the beetle, virus and plant serving as source or recipient of virus.

This study evaluates the potential of weed species as the primary inoculum source of BPMV in Eastern Virginia and provides a strong justification for future studies of these BPMV-positive weeds. There was no single weed species identified as an inoculum source of the virus. However, due to the abundance of the BPMV-positive weeds in proximity to soybean fields and the prevalence of these plants in the landscape, all must be considered as potential sources of BPMV each season. This prevalence around soybean fields where beetles are emerging each spring and the relative lack of other potential host plants may force beetles to feed on non-leguminous species to survive until cultivated legumes become available. This scenario is demonstrated by the increase of BPMV load within the beetles when cultivated legumes are not available. Continued monitoring for virus within the beetles, and persistent weed sampling needs to be conducted to further understand the epidemiology of BPMV in eastern Virginia.

References

- Agrios, G.N. 1997. *Plant Pathology*, 4th edition. Academic Press, New York. pp. 635.
- Bradshaw, J.D., L.P. Pedigo, J.H. Hill, and M.E. Rice. 2008. Evaluation of management strategies for bean leaf beetles (Coleoptera: Chrysomelidae) and bean pod mottle virus (Comoviridae) in soybeans. *J. Econ. Entomol.* 101: 1211-1227.
- Gergerich, R.C., and H.A. Scott. 1988a. The enzymatic function of ribonuclease determines plant virus transmission by leaf-feeding beetles. *Phytopathology* 78: 270-272.
- Gergerich, R.C., and H.A. Scott. 1988b. Evidence that virus translocation and virus infection of non-wounded cells are associated with transmissibility by leaf-feeding beetles. *J. Gen. Virol.* 69: 2935-2938.
- Gergerich, R.C., R.A. Scott, and J.P. Fulton. 1983. Regurgitant as a determinant of specificity in the transmission of viruses by beetles. *Phytopathology* 73: 936-938.
- Ghabrial, S.A., and F.J. Schultz. 1982. Serological detection of bean pod mottle virus in bean leaf beetles. *Phytopathology* 73: 480-483.
- Giesler, L.J., S.A. Ghabrial, T.E. Hunt, and J.H. Hill. 2002. Bean pod mottle virus: a threat to U.S. soybean production. *Plant Dis.* 86: 1280-1289.
- Hartman, G.L., J.B. Sinclair, and J.C. Rupe (eds.) 1999. *Compendium of soybean disease*, (4th edition). Amer. Phytopathol. Soc., APS Press. pp. 100.
- Harvill, Jr., A.M., T.R. Bradley, C.E. Stevens, T.F. Wieboldt, D.M.E. Ware, D.W. Ogle, G.W. Ramsey, and G.P. Flemming. 1992. *Atlas of the Virginia Flora III*. Burkeville, Virginia.
- Irwin, M.E., W.G. Ruesink, S.A. Isard, and G.E. Kampmeier. 2000. Mitigating

- epidemics caused by non-persistently transmitted aphid-borne viruses: The role of the plant environment. *Virus Res.* 71: 185-211.
- Horn, N.L., L.D. Newsom, and R.L. Jensen. 1970. Effects of virus diseases on soybean in Louisiana. *La. Agric.* 13: 12.
- Isley, D. 1930. The biology of the bean leaf beetle. *Arkansas Agr. Exp. Sta. Bull.* 248.
- Krell, R.K., L.P. Pedigo, J.H. Hill, and M.E. Rice. 2003. Potential primary inoculum sources of bean pod mottle virus in Iowa. *Plant Dis.* 87: 1416-1422.
- Lin, M.T., and J.H. Hill. 1983. Bean pod mottle virus: occurrence in Nebraska and seed transmission in soybeans. *Plant Dis.* 67: 230-233.
- Moore, B.J., H.A. Scott, and H.J. Walters. 1969. *Desmodium paniculatum*, a perennial host of bean pod mottle virus in nature. *Plant Dis. Rep.* 53: 154-155.
- Mueller, A.J., and A.W. Haddox. 1980. Observations of seasonal development of bean leaf beetle, *Cerotoma trifurcata* (Forster) and incidence of bean pod mottle virus in Arkansas' soybeans. *J. Ga. Entomol. Soc.* 15: 398-403.
- Richard H.U., J.C. Neal, and J.M. DiTomaso. Weeds of the Northeast. Cornell University Press. Comstock Publishing Associates. 1997.
- Ross, J.P. 1986. Response of early-and late-planted soybeans to natural infection by bean pod mottle virus. *Plant Dis.* 70: 222-224.
- Scott, H.A., J.V. Van Scyoc, and C.E. Van Scyoc. 1974. Reactions of *Glycine* spp. to bean pod mottle virus. *Plant Dis. Rep.* 58: 191-192.
- Snodgrass, R.E. 1935. *Principles of Insect Morphology*. pp. 154-155. McGraw-Hill, New York.
- Walters, H.J., and F.N. Lee. 1969. Transmission of Bean pod mottle virus from

Desmodium paniculatum to soybean by the bean leaf beetle. Plant Dis. Rep. 53: 411.

Wang, R.Y., R.C. Gergerich, and K.S. Kim. 1992. Noncirculative transmission of plant viruses by leaf-feeding beetles. Phytopathology 82: 946-950.

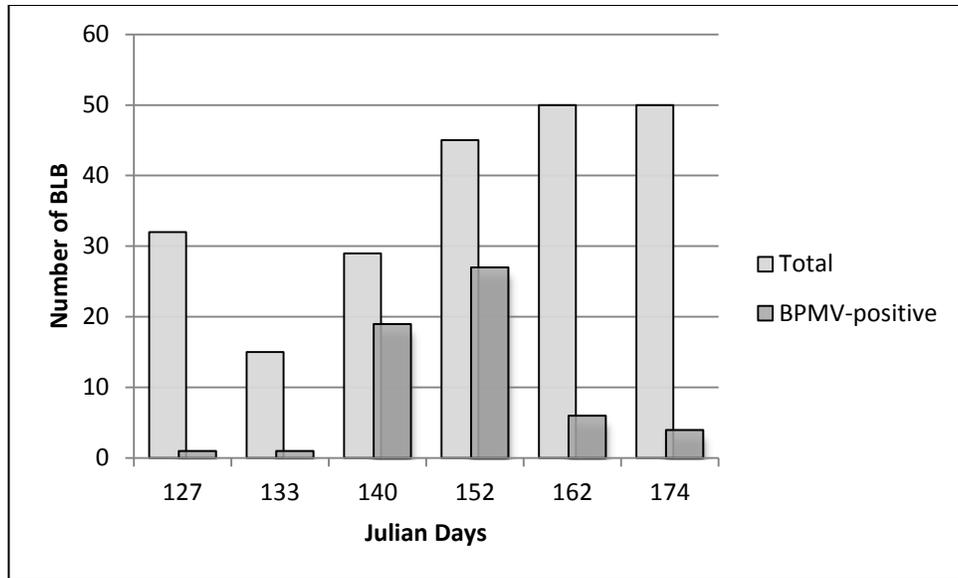


Figure 5.1. Progression of Bean pod mottle virus-positive bean leaf beetles represented as part of the total number tested by ELISA or TBIA in Painter, VA during the spring of 2009.

Table 5.1. Plants collected in soybean fields on the Eastern part of Virginia tested for Bean pod mottle virus by TBIA and ELISA as potential overwintering virus hosts.

<u>Plant Family</u>	<u>Scientific name</u>	<u>Common Name</u>	<u>Collection Location**</u>	<u>Number of Samples</u>	<u>Positive for BPMV by TBIA</u>	<u>Positive for BPMV by ELISA</u>
Asteraceae	<i>Achillea millefolium</i>	Common Yarrow	N	1	-	-
Asteraceae	<i>Conyza bonariensis</i>	Horseweed	A	1	-	-
Asteraceae	<i>Gnaphalium polycephalum</i>	Gnaphalium	A, N	2	-	-
Asteraceae	<i>Gnaphalium purpureum</i>	Purple Cudweed	A	4	-	-
Asteraceae	<i>Solidago virgaurea</i>	Rough Golden Rod	A, N, R	4	-	-
Brassicaceae	<i>Thlaspi arvense</i>	Field Pennycress	N	1	-	-
Caprifoliaceae	<i>Lonicera Japicona</i>	Honeysuckle	A, N	4	-	-
Caryophyllaceae	<i>Scleranthus annuus</i>	Knawel	A, N	2	-	-
Chenopodiaceae	<i>Chenopodium album</i>	Common Lambs Quarter	A	1	-	-
Convolvulaceae	<i>Ipomoea indica</i>	Morning Glory	A	1	-	-
Euphorbiaceae	<i>Euphorbia maculata</i>	Spotted Spurge	N	1	-	-
Fabaceae	<i>Medicago sativa</i>	Alfalfa	A	56	-	-
Fabaceae	<i>Glycine max</i>	Soybean	A, N, R	60	-	-
Fabaceae	<i>Robinia pseudoacacia</i>	Black Locust	A	50	-	-
Fabaceae	<i>Trifolium pratense</i>	Red Clover	A, N, R	65	yes* (field R 1)	+
Fabaceae	<i>Trifolium repens</i>	White Clover	A, N, R	75	yes*(field ES 21, 22)	-
Fabaceae	<i>Vicia villosa</i>	Hairy Vetch	A, N, R	97	-	+
Geraniaceae	<i>Geranium carolinianum</i>	Carolina Geranium	A	1	-	-
Oxalidaceae	<i>Oxalis stricta</i>	Yellow wood sorrel	A, R	8	yes* (fields ES 7, 18, 4; R 4)	+
Poaceae	<i>Digitaria insularis</i>	Sourgrass	A, N	4	-	-
Poaceae	<i>Muhlenbergia schreberi</i>	Nimblewill	A	1	-	-
Polygonaceae	<i>Rumex acetosella</i>	Red sorrel	A, N, R	5	yes* (field ES 20)	+
Rubiaceae	<i>Asperugo procumbens</i>	Catch Weed	A, N	2	-	-
Schrophulariaceae	<i>Verbascum thapsus</i>	Common Mullein	A	1	-	-
Schrophulariaceae	<i>Veronica persica</i>	Speedwell	A, N	2	-	-

**N = Northampton Co., R = Richmond Co., A = Accomack Co.

*Field numbers correspond to those in chapter 4, Table 4.2

- = negative for BPMV

+ = positive for BPMV



Figure 5.2. Yellow wood sorrel (*Oxalis stricta*) sampled from a field on the Delmarva Peninsula. Showing yellow mottling on young leaflets. Photo taken by Dr. Sue Tolin, April 2010.

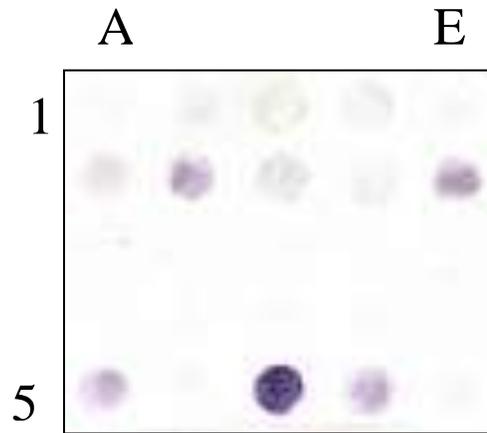


Figure 5.3. Bean pod mottle virus-positive tissue blots. Bean pod mottle positive control was 116-7 (C5) and EST-3 (D5) and negative controls were healthy ‘Bronco’ snap bean (E5) and a SMV infected soybean (E4). Yellow wood sorrel blots locations: D1, A2, B2, C2, D2, E2, A3, C3, D3, E3, A4, and D4. White clover blot location: E1. Red clover blots locations: A1, B1, B3, and B4. Red sorrel blots locations: C1 and C4. Bean leaf beetle extract blots locations: A1 and B1.

Chapter 6

Residual effect of thiamethoxam insecticide seed treatment on bean leaf beetle mortality

Abstract

The efficacy of thiamethoxam (Cruiser®) insecticidal seed treatment was evaluated for controlling bean leaf beetle, *Cerotoma trifurcata* (Forster), (Coleoptera: Chrysomelidae) on soybeans, *Glycines max* (L., Merr.) in Painter, Virginia in 2009. Leaves from soybean plants that were treated with a thiamethoxam seed coating were collected weekly (from VC – V3 growth stages) and exposed to beetles in Petri dishes. Beetles were allowed to feed for 24 hours and the amount of leaf area consumed as well as beetle mortality was recorded. The thiamethoxam seed treatment significantly reduced beetle feeding and caused significant beetle mortality for ~ three weeks after planting up to the V-3 stage of growth.

Introduction

Bean leaf beetle (BLB), *Cerotoma trifurcata*, (Coleoptera: Chrysomelidae) is distributed throughout the Midwest and eastern U.S. (Kogan et al. 1980), and can cause economic damage to soybeans by defoliating seedlings, feeding directly on pods later in the crop development, and by transmitting Bean pod mottle virus (Smelser and Pedigo 1992, Hunt et al. 1995, Krell et al. 2004).

It The beetle is multivoltine in Virginia and adults emerge in spring and begin feeding upon leguminous hosts (Isley 1930). Controlling BLB adult populations early in the crop development is important to minimize the effects of defoliation as well as incidence of BPMV in the field (Hunt et al. 1995, Krell et al. 2004, Bradshaw et al. 2008). Insecticidal sprays are

effective at killing adult BLB (Bradshaw et al. 2008), but are subject to weather conditions at the time of application and have limited residual efficacy, particularly as plants continue to grow. Seeds pretreated with systemic insecticides are not dependent on weather and are immediately available for absorption by newly germinated seeds, and may offer a more convenient and effective alternative for beetle suppression.

Thiamethoxam is a chloronicotinyl insecticide within the neonicotinoid class. The insecticide is available as a seed treatment product (Cruiser®, Syngenta Crop Protection, Inc., Greensboro, NC), which has been shown to control BLB in snap beans and soybeans (Koch et al. 2005, Bradshaw et al. 2008). Based on research in snap beans and sweet corn, the insecticide, when absorbed by the plant, can provide foliar protection for weeks after planting (Kuhar et al. 2002, Nault et al. 2004). The objectives of this study were to assess the residual efficacy of thiamethoxam seed treatments on BLB adults on soybean. This information will be useful in knowing how long thiamethoxam seed treatments on soybeans remain effective in the field.

Materials and Methods

In 2009, at the Eastern Shore Agricultural Research and Extension Center (ESAREC) soybean seeds (cultivar SS RT4440N) treated and not treated with thiamethoxam insecticidal seed treatment (Cruiser®, Syngenta Crop Protection, Inc., Greensboro, NC), at 1.28 fl oz / cwt, were planted in 10-cm diameter pots in the greenhouse. Upon plant emergence, the unifoliate leaf was removed and placed on a 9-cm diameter water agar Petri dish. Beetles (unsexed) that had been collected the day before from soybeans at the ESAREC and starved for 24 hours were placed on the plate

(one beetle per dish). Beetles were allowed to feed on the excised leaf for 24 hours, after which the leaf was removed and a photocopy was made. A cutout of the leaf was run through a LI-COR model no. LI-3100 leaf area meter (LI-COR Inc., Lincoln, NE) and beginning leaf area was calculated. The fed upon portion and holes were then cut from the photocopy of the leaf. The area consumed by the beetle was calculated by subtracting the starting leaf area from the fed upon leaf area. Beetles were then observed daily for 72 hours to check for beetle mortality. This was done for 4 weeks testing each newly unfurled trifoliolate. A total of one unifoliolate (VC) and three trifoliate (V1-V3) were tested. A sample size of 25 leaves and beetles was used for each leaf stage. A t-test was used to analyze treatment differences compared with an untreated control. The same experiment was attempted in 2010, but problems with a drought killed many of the soybean plants, and human error in conducting the bioassays prevented usable data from being collected.

Results

Thiamethoxam-treated soybeans resulted in high mortality (69-85%) of BLB adults exposed to unifoliolate, 1st-trifoliolate and 2nd-trifoliolate stage leaf discs, but had no efficacy on BLB by the 3rd-trifoliolate stage (Table 6.1). Also, there was significantly less leaf area consumed at the unifoliolate, 1st, and 2nd trifoliolate stages on thiamethoxam-seed treated soybeans compared with and untreated soybeans ($P \leq 0.05$). The seed treatment provided protection against BLB feeding up to three weeks post planting when the seedling reached the third trifoliolate stage.

Discussion

Excised leaf bioassays demonstrated that thiamethoxam seed treatment effectively controlled beetles and prevented leaf feeding up to the third trifoliate stage (21 days after planting (DAP)) in soybeans. These results were also seen by Koch et al. (2005) who showed that thiamethoxam seed-treated snap bean seedlings had less bean leaf beetle feeding injury than untreated controls. The 21 days of residual activity of the seed treatment is similar to results by Nault et al. (2004), when thiamethoxam seed treated snap bean seedlings were protected against potato leafhopper, *Empoasca fabae* (Harris), injury up to 21-28 DAP. Thus, it could be assumed that in the field, thiamethoxam seed treatments could effectively control beetles for up to three weeks postplanting. Moreover, if beetle populations can be reduced early, then the overall population may remain low throughout the growing season. These results have implications for protecting soybean seedlings from early defoliation as well as suppression of BPMV, which is transmitted by BLB (Krell et al. 2004). In Iowa, Bradshaw et al. (2008) showed that insecticidal seed treatments were successful in reducing BLB numbers in soybeans as well as concomitant incidence of BPMV.

References

- Bradshaw, J.D., L.P. Pedigo, J.H. Hill, and M.E. Rice. 2008. Evaluation of management strategies for bean leaf beetles (Coleoptera: Chrysomelidae) and bean pod mottle virus (Comoviridae) in soybeans. *J. Econ. Entomol.* 101: 1211-1227.
- Hunt, T.E., L.G. Higley, and J.F. Witkowski. 1995. Bean leaf beetle injury to Seedling soybean: consumption, effects of leaf expansion, and economic injury levels. *Agron. J.* 87:183-188.
- Isley, D. 1930. The biology of the bean leaf beetle. *Ark. Agric. Exp. Stn. Bull.* 248.
- Koch, R.L., E.C. Burkness, W.D. Hutchison, and T.L. Rabaey. 2005. Efficacy of systemic insecticide seed treatments for protection of early-growth-stage snap beans from bean leaf beetles (Coleoptera: Chrysomelidae) foliar feeding. *Crop. Prot.* 24: 734-742.
- Kogan, M., G.P. Waldbauer, G. Boiteau, and C. Eastman. 1980. Sampling bean leaf beetles on soybean, pp.201-236. *In* M. Kogan and D.C. Herzog [eds.], *Sampling Methods in Soybean Entomology*. Springer, New York.
- Krell, R.K., L.P. Pedigo, J.H. Hill, and M.E. Rice. 2004. Bean leaf beetle (Coleoptera: Chrysomelidae) management for reduction of bean pod mottle virus. *J. Econ. Entomol.* 97: 192-202.
- Kuhar, T.P., L.J. Stivers-Young, M.P. Hoffman, and A.G. Taylor. 2002. Control of corn flea beetle and Stewart's wilt in sweet corn with imidacloprid and thiamethoxam seed treatments. *Crop Prot.* 21: 25-31.
- Nault, B.A., A.G. Taylor, M. Urwiler, T. Rabaey, and W.D. Hutchison.

2004. Neonicotinoid seed treatments for managing potato leafhopper infestations in snap beans. *Crop Prot.* 23: 147-154.
- Smelser, R.B., and L. Pedigo. 1992. Bean leaf beetle (Coleoptera:Chrysomelidae) herbivory on leaf, stem, and pod components of soybean. *J. Econ. Entomol.* 85: 2408-2412.

Table 6.1. Mean \pm SE area of soybean leaf consumed by a single adult bean leaf beetle on thiamethoxam-treated and untreated soybean leaves in a 24 hour period and the resulting beetle mortality at 72 hours post feeding.

	Treated	Control	Treated	Control	Treated	Control	Treated	Control
Growth Stage	Unifoliolate		1 st trifoliolate		2 nd trifoliolate		3 rd trifoliolate	
% Beetle mortality 72 hr after feeding on leaf	76	0	69	0	85	0	0	0
Leaf area consumed (cm ²)	0.22 \pm 0.14	0.87 \pm 0.59	0.35 \pm 0.17	1.1 \pm 0.36	0.29 \pm 0.15	0.77 \pm 0.11	1.15 \pm 0.81	1.07 \pm 0.18
P-value	< 0.001 ^a		< 0.001 ^a		< 0.001 ^a		P = 0.2	

^a Means are statistically different than the control leaf of the same trifoliolate tested at P < 0.05 level of significance.

Chapter 7

Conclusions

The bean leaf beetle (BLB), *Cerotoma trifurcata*, is a common pest of legumes on the Delmarva Peninsula. Experiments were conducted to assess the impact of BLB leaf feeding on snap bean yield. Based on manual defoliation experiments conducted across a range of conditions, including irrigated versus non-irrigated snap beans, and two defoliation sessions versus four weekly defoliations, the results were similar in that 50% and 75% defoliation resulted in significant yield loss in virtually all of the experiments (Chapter 2). In three of the eight experiments defoliation levels of 25% also resulted in significant yield loss. These results are similar to Greene and Minnick (1967) who reported that early-season defoliation of snap beans can be overcome if the total leaf area removed is less than 25% and does not occur around bloom. My results in snap beans are also comparable to research in soybeans by Todd and Morgan (1972), who reported that early-season insect defoliation would not cause yield losses if levels were kept below 33%. In addition, Hammond (1982) showed that a single manual hole-punch defoliation of 37% during the V1 stage did not cause a reduction in soybean yield.

To assess the effect of BLB density on defoliation and concomitant yield loss in snap beans, field cage experiments were conducted in 2006 and 2007. In 2006, a range of densities from 0 to 1.4 BLB per plant or 0 to 92 beetle-days resulted in >50% defoliation at the highest beetle densities, and a significant correlation between beetle density and defoliation, and also between beetle density and whole plant mass and bean yield. However, in

2007, a greater range of beetle densities from 0 to 2.7 BLB per plant or 130 beetle-days resulted in a maximum defoliation level of only 18%, and no correlation between beetle density and snap bean yield. The differences in results obtained in these two experiments indicate that other variables can impact the beetle density/defoliation/yield loss relationship in snap beans. Although the quantitative relationship needed in order to calculate an economic injury level for BLB on early-stage snap beans may still be unknown, my experiments reiterate the need for BLB and other insect control in order to prevent foliar feeding injury exceeding 25%.

From 2007 to 2009, experiments were conducted in the laboratory and field to determine potential host plant preferences of BLB among snap bean, lima bean, and soybean (Chapter 3). In the lab, paired choice tests with 5-cm² leaf discs of each plant revealed that beetles consumed significantly more leaf tissue of snap bean and lima bean than soybean in both years. In the field, numbers of beetles were recorded from two different growth stages of each of the three bean species arranged in paired combinations. Over a three week sampling period, significantly more beetles were found on larger (V4-stage) plants than smaller (V1-stage) plants, and more cumulative beetles were found on snap bean and lima bean compared with soybean. No significant differences were found between snap bean and lima bean. Thus, in the lab and field, BLB adults preferred snap bean and lima bean to soybean. This information could have implications for beetle movement and concomitant pest management when these crops are grown adjacent to one another.

In addition to feeding on leaves and pods, BLB is also the primary vector of *Bean pod mottle virus* (BPMV, Genus *Comovirus*, Family: Secoviridae: formally Comoviridae). This is a re-emerging virus in Virginia

and has not been previously studied in this area. Field surveys of both beetles and soybean foliage from 2008-2010 showed that the virus is present in all soybean growing areas of Virginia except for the Tidewater region (Chapter 4). BPMV was found throughout the Eastern Shore of Virginia with no one County having a higher prevalence of the virus. In 2009 an outbreak of virus was seen in the Northern Neck of Virginia followed by an outbreak in Appomattox County in 2010. This may be a new occurrence of the virus in these areas or it could have been there for years, just no one was looking for it in these production areas. The presence of BPMV-positive beetles in soybean fields is a good indicator that BPMV-positive soybean foliage will be found as well, which was also noted by Ghabiral et al. (1990) in his study of BPMV distribution in Kentucky.

The primary inoculum source for the BLB in eastern Virginia could be red or white clover as well as red sorrel, yellow wood sorrel and vetch. Bradshaw (2008) listed clover as being a possible host plant in Iowa. Samples of these weed species tested positive for BPMV using a tissue blot immunoassay (TBIA)(Chapter 5). All tested BPMV-positive by ELISA except white clover which further strengthens the argument that these weed species are a source of BPMV. RNA confirmation by PCR would be beneficial to confirm BPMV in weeds in eastern Virginia. Virus confirmation by bioassay from weed tissue to soybeans was not successful. Beetle regurgitant, plant chemical complexes and virus behavior within the plant all play a role in the ability to mechanically transfer a virus from one host to another (Gergerich and Scott 1988a, 1988b). It is possible that the beetle / virus/ plant interactions are too complex to replicate in greenhouse conditions.

An experiment conducted in 2009 showed that soybean seeds treated with the neonicotinoid insecticide thiamethoxam controlled BLB and significantly reduced feeding injury up to the third trifoliate stage of plant growth (approximately 30 days after planting) (Chapter 6). Thiamethoxam seed treatments appear to be a very good tool for early-season protection against BLB, and may also reduce the incidence of BPMV in soybeans (Bradshaw et al. 2008).

In summary, my research has improved our understanding of BLB and its pest potential in Virginia by showing the following: 1) leaf feeding on seedling snap beans could cause significant yield loss if defoliation levels exceed 25%; 2) when given a choice, BLB prefer to feed on snap beans and lima beans over soybeans; 3) *Bean pod mottle virus* is prevalent in eastern Virginia soybeans and BLB plays a significant role in the epidemiology of the disease; 4) weeds including clovers, red sorrel and yellow sorrel may be the source of BPMV early in the year if BLB encounter these weeds before feeding on soybean; and 5) thiamethoxam seed treatment is an effective early-season management tool for BLB in soybeans.

References

- Bradshaw, J. D., L. P. Pedigo, J. H. Hill, and M. E. Rice. 2008. Evaluation of management strategies for bean leaf beetles (Coleoptera: Chrysomelidae) and bean pod mottle virus (Comoviridae) in soybeans. *J. Econ. Entomol.* 101: 1211-1227.
- Gergerich, R. C., and H. A. Scott. 1988a. The enzymatic function of ribonuclease determines plant virus transmission by leaf-feeding beetles. *Phytopathology.* 78: 270-272.
- Gergerich, R.C., and H. A. Scott. 1988b. Evidence that virus translocation and virus infection of non-wounded cells are associated with transmissibility by leaf-feeding beetles. *J. gen. Virol.* 69: 2935-2938.
- Ghabrial, S.A., and D. E. Hershman, D. W. Johnson, and D. Yan. 1990. Distribution of bean pod mottle virus in soybeans in Kentucky. *Plant Dis.* 74: 132-134.
- Smelser, R.B. and L. Pedigo. 1992. Bean leaf beetle (Coleoptera:Chrysomelidae) herbivory on leaf, stem, and pod components of soybean. *J. Econ. Entomol.* 85: 2408-2412.
- Todd, James W. and Loy W. Morgan. 1972. Effects of hand defoliation on yield and seed weight of soybeans. *J. Econ. Entomol.* 65: 567-570.