12-9-2011

Diversity And Ecology Of Bean Pod Mottle Virus In Mississippi

Ronald Christian Stephenson

DIVERSITY AND ECOLOGY OF BEAN POD MOTTLE VIRUS IN MISSISSIPPI

By

Ronald Christian Stephenson

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Plant Pathology
in the Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology

Mississippi State, Mississippi

December 2011

DIVERSITY AND ECOLOGY OF BEAN POD MOTTLE VIRUS IN MISSISSIPPI

By

Ronald Christian Stephenson

Approved:

Sead Sabanadzovic Associate Professor of Plant Pathology (Major Professor)	Shien Lu Associate Professor of Plant Pathology (Committee Member)
Fred R. Musser Associate Professor of Entomology (Committee Member)	Alan Henn Extension Professor of Plant Pathology (Committee Member)
Michael R. McLaughlin Research Plant Pathologist, USDA-ARS (Committee Member)	Michael A. Caprio Professor of Entomology (Graduate Coordinator)

George Hopper
Dean of the College of Agriculture and Life Sciences

Name: Ronald Christian Stephenson

Date of Degree: December 9, 2011

Institution: Mississippi State University

Major Field: Plant Pathology

Major Professor: Sead Sabanadzovic

Title of Study: DIVERSITY AND ECOLOGY OF BEAN POD MOTTLE VIRUS IN MISSISSIPPI

Pages in Study: 64

Candidate for Degree of Master of Science

Sequences of 33 *Bean pod mottle virus* (BPMV) isolates collected from Mississippi were obtained from four regions of the viral genome. Sequences were compared mutually and to subgroups I and II type isolates. Mississippi isolates collected have greater similarity to subgroup II. Helicase sequences obtained from Mississippi isolates showed characteristics of isolates producing mild or moderate symptoms. Biological comparison of isolates from Mississippi confirmed predictions from molecular data.

Plant species were tested for susceptibility to BPMV. Seven of 52 species tested positive. *Desmodium perplexum* was infected by beetle feeding and served as an inoculum source for transmission of BPMV to soybeans. Beetle species collected from soybean and clover were tested to determine if they serve as BPMV vectors. Of eight species tested, *Hypera postica* transmitted BPMV to 1 of 14 test plants. Beetle overwintering transmission of BPMV was tested, but of 187 beetles collected, none transmitted the virus.

DEDICATION

This work is dedicated to my father, Ronald H. Stephenson, for his endless support and encouragement throughout my education.

ACKNOWLEDGEMENTS

I would like to thank my major professor, Dr. Sead Sabanadzovic, for his patience and assistance throughout my program, as well as the members of my committee for their advice and efforts on my behalf. I would like to thank Drs. Billy Moore and Tom Allen for their assistance in making collections for this study as well as Ms. Kathy Knighten and her field staff for their help collecting insects and constructing cages. I would like to thank Dr. Richard Brown and the staff of the Mississippi Entomological Museum for their help with insect identification and Mr. Chris Dofitt of the MSU Herbarium for his help with identifying plant specimens. I would also like to thank the staff of the R.R. Foil Plant Science Research Center and North Mississippi Experiment Station for their help. Lastly, my thanks to all the faculty, staff, and students of the Department of Entomology and Plant Pathology with whom I have spent the past years. Your help and friendship has been much appreciated.

TABLE OF CONTENTS

Page

DEDICATION ii

ACKNOWLEDGEMENTS iii

LIST OF TABLES vi

LIST OF FIGURES vii

CHAPTER

I. INTRODUCTION 1

History and Importance of Soybean Production 1
Bean pod mottle virus 3
Bean pod mottle disease symptoms 6
Beetle transmission of plant viruses 8
Transmission of *Bean pod mottle virus* 13
Bean pod mottle virus host range 16
Management of *Bean pod mottle virus* 17
General conclusions 18
References Cited 19

II. DIVERSITY OF BEAN POD MOTTLE VIRUS IN MISSISSIPPI 25

Abstract 25
Introduction 26
Materials and Methods 29
Results and Discussion 32
Conclusion 39
References Cited 41

III. ECOLOGY OF BEAN POD MOTTLE VIRUS IN MISSISSIPPI 43

Abstract 43
Introduction 44
Materials and Methods 47
Alternative Plant Hosts for *Bean pod mottle virus* 47

Alternative Vectors for *Bean pod mottle virus* 51
Transmission of *Bean pod mottle virus* by Overwintered Bean Leaf Beetles.....52
Results and Discussion 54
Conclusion 60
References Cited 62

LIST OF TABLES

TABLE Page

1 Nucleotide sequence of primers used in this study with length of generated RT-PCR products.....33

2 List of plant species collected from edges of soybean fields in MS and tested for BPMV. Sample denotes a set of twelve individual plants. 48

3 List of species/accessions for mechanical transmission study. Seeds were obtained from National Seed Storage Lab.....49

4 Results of mechanical and vector transmission tests for species found to be positive for BPMV after mechanical inoculation. 55

5 Transmission of BPMV by potential vector species collected from soybean and clover fields in Noxubee and Oktibbeha county Mississippi. Number of samples tested refers to individual plants with each being exposed to three beetles.....58

LIST OF FIGURES

FIGURE Page

1 Transmission electron microscope image of negatively stained partially purified particles of *Bean pod mottle virus*. Note the presence of both apparently intact and empty particles (penetrated by stain). Bar is reported for reference.....4

2 Diagrammatic representation of BPMV genome organization with key nucleotide coordinates. Nucleotide sequences the strain "KY G-7" used as model for this figure (NCBI Refseq # NC_003495 and NC_003496) (MacFarlane et al., 1991; Di et al., 1999). Boxes represent ORFs coding for large polyproteins and lines non-translated regions at the genome extremes. Functions of mature proteins are indicated: co-PRO = co-protease factor, HEL = helicase, VPg = viral protein genome-linked, PRO = protease, RdRp = RNA-dependent RNA polymerase, MP = movement protein, CPL and CPS = large and small coat proteins. The ovals at the 5' end represent the VPg and "polyA" at the 3'end indicate the presence of a poly A tail. 5

3 Symptoms of *Bean pod mottle virus* on soybean showing characteristic mottling (A) and blistering (B).....7

4 Bean leaf beetle *Cerotoma trifurcata* (Förster) (Coleoptera: Chrysomelidae) adult feeding on soybean leaves. 14

5 Map of Mississippi showing counties from which isolates have been collected and used in this work. 30

6 Agarose gel electrophoresis of RT-PCR products generated by different sets of primers used in the diversity study. Primer set names derive from the target genome region: Hel = helicase, Pol = RNA-dependent RNA polymerase, MP = movement protein, CP = coat protein. Reference DNA marker (1kbPlus DNA ladder, Invitrogen, Carlsbad, CA) is in lane M and BPMV-free sample is in lane "Heal". Nucleotide sequences of each primer set and the exact size of PCR products are reported in Table 1. 33

7 Phylogenetic tree inferred with the Neighbor-Joining method (Saitou and Nei 1987) based on combined partial amino acid sequences of the BPMV RNA polymerase and Helicase regions of 20 isolates from Mississippi. Reference isolates for BPMV subgroups I and II (K-Graves and K-Hancock, respectively. The bootstrap consensus tree is inferred from 1000 replicates (Felsenstein 1985). The phylogenetic tree was linearized assuming equal evolutionary rates in all lineages (Takezake et al. 2004). Evolutionary distances were computed using the Poisson correction method (Zuckerland and Pauling 1965). 36

8 Phylogenetic tree inferred with the Neighbor-Joining method (Saitou and Nei 1987) based on combined partial amino acid sequences of the BPMV movement protein and coat protein regions of 20 isolates from Mississippi. Reference isolates for BPMV subgroups I and II (K-Graves and K-Hancock, respectively. The bootstrap consensus tree is inferred from 1000 replicates (Felsenstein 1985). The phylogenetic tree was linearized assuming equal evolutionary rates in all lineages (Takezake et al. 2004). Evolutionary distances were computed using the Poisson correction method (Zuckerland and Pauling 1965). 37

9 Comparison of amino acids surrounding positions 359 and 408 (arrows) of the polyprotein encoded by RNA-1 (helicase domain) and linked to BPMV symptom severity. No isolates from Mississippi showed the amino acid substitutions linked to severe symptoms. Isolates marked by "(I)" (Subgroup I) and "(II)" (Subgroup II) are reference sequences obtained from NCBI. 38

10 BPMV symptoms induced in AG4903 soybean plants by Mississippi isolate Monroe compared with type isolates of Subgroup I (K-Graves) showing mild symptoms, Subgroup II (K-Hancock) showing moderate symptoms, and an isolate diploid for BPMV RNA-1 (K-Hopkins) showing severe symptoms. 39

CHAPTER I

INTRODUCTION

History and Importance of Soybean Production

Soybean was first domesticated in northern China in the eleventh century B.C. Seeds of wild soybean, native to Southeast Asia, were small, hard, and black to dark brown and were more prone to shattering than modern cultivated soybean. Wild soybean seed had very different oil and protein composition (9.8% and 46 %, respectively) in comparison to modern cultivated varieties (21 % and 46 %) (Hymowitz 1970). Domestication of wild soybean has also resulted in a more upright growth as this facilitates the use of modern mechanized harvesting techniques (Hymowitz 1976).

Several biological factors affect soybean production. These include photoperiod, maturity group, and determinacy. Soybean flowering and pod development is controlled by day length. Since day length varies according to latitude, most soybean varieties are grown in a narrow north to south band. To the north of this band, day length will shorten too early, resulting in soybean maturing later than desired. South of this band, plants will mature earlier than desired (Hymowitz 1970). Soybean varieties have been grouped into thirteen maturity groups, from '000' to 'X' according to the photoperiod at which the plants mature. Earlier maturity groups enter reproductive stages earlier in the growing season in response to longer day lengths (Fehr and Caviness 1977). As a rule, soybeans are mature and ready for harvest 90-100 days after planting. This may, however, vary significantly, and very early varieties may mature in as few as 75 days while very late

varieties that take as much as 200 days to reach maturity. Soybean cultivation in the Southeastern United States has shifted from planting soybean in late May using Maturity Groups V and VI to planting Maturity Groups IV and V in mid to late April minimizing the impact of late season drought stress (Heatherly 1999). Soybean varieties grown in the Southern United States are generally of the determinate type, meaning that they cease vegetative growth prior to beginning reproductive development. Indeterminate soybeans, which continue vegetative growth after reproductive development are generally grown in the Northern United States and Canada (Hymowitz 1976).

The first documented introduction of soybeans into the United States occurred in 1765. Early soybean production in the U.S. was as a forage crop rather than having been harvested for seed. No estimate of soybean acreage was given until 1924, when only 1.8 million acres of soybean were cultivated. In order to replace oil imports, soybean production in the United States expanded rapidly during World War II. Soybean was successful as a crop as its production was similar to that of other crops already produced and there was an immediate need for oil and meal production. The United States dominated world production of soybean from the 1950's through the 1970's and remains a major world producer of soybean. Soybean production is a major industry in the United States. In 2010, the United States soybean crop had an estimated value of $38.9 billion from production on 77.4 million acres, representing 35 % of world production. (www.soystats.com, www.nass.usda.gov). Iowa is the leading soybean producer in the United States, followed by Illinois and Minnesota. The State of Mississippi ranks as the 14th of 31 soybean producing States in the U.S., with a production value of $821 million in 2010. Soybean is the third largest industry in the state behind poultry and forestry, and soybeans make up 41 % of the field crop acreage in the state (www.soystats.com).

Bean pod mottle virus

Bean pod mottle virus (BPMV) is a member of the genus *Comovirus,* family *Secoviridae*,. As with other comoviruses, the BPMV genome consists of two molecules of positive sense, single-stranded, monocistronic RNA. This two-part genome consists of RNA-1 and RNA-2 encapsidated separately in 28nm isometric particles (Hull 2002, Šutić et al. 1999). An electron micrograph of a negatively stained partially purified preparation of BPMV is shown in Figure 1. Density gradient separation of virions results in top, middle, and bottom components. No nucleic acid is contained within particles in the top component. The bottom component contains a single molecule of RNA-1 and the middle component a single molecule of RNA-2 (Giesler et al. 2002). Each of the three components share identical protein components, which consist of sixty copies each of two coat proteins. Viral genomic RNAs are polyadenylated at the 3' end, with a viral genome-linked protein bound to the 5' terminus of each molecule. The BPMV genome is expressed through synthesis and cleavage of a large polyprotein precursor. The two genomic RNAs for BPMV have been sequenced and the proteins coded for by each component have been identified. RNA-1 encodes five proteins required for transcription. RNA–1 has previously been shown to have the ability to replicate in the absence of RNA–2 (Hull 2002). The proteins encoded by BPMV RNA-1 are (in 5'-3' direction): a protease cofactor, a helicase, a viral genome-linked protein, a protease, and an RNA-dependent RNA polymerase. The two coat proteins which make up the capsid and a cell-to-cell movement protein are encoded on RNA-2 (MacFarlane et al. 1991, Di et al. 1999, Hull 2002) (Figure 2).

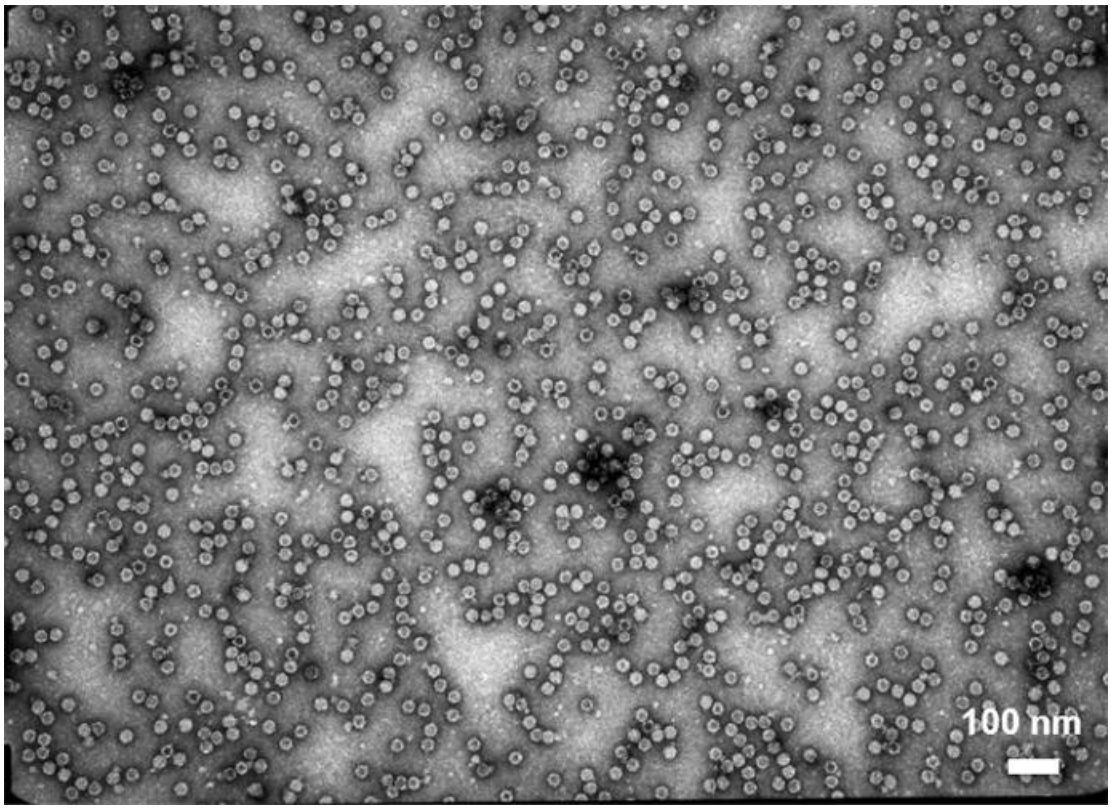

Figure 1 Transmission electron microscope image of negatively stained partially purified particles of *Bean pod mottle virus*. Note the presence of both apparently intact and empty particles (penetrated by stain). Bar is reported for reference.

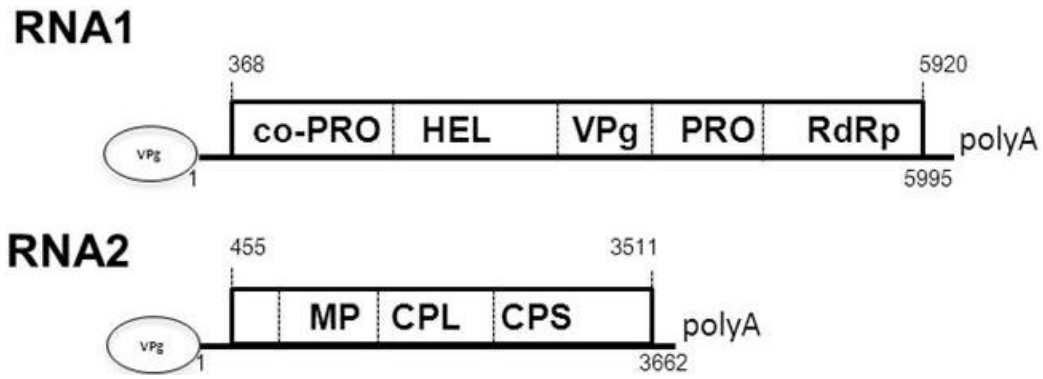

Figure 2 Diagrammatic representation of BPMV genome organization with key nucleotide coordinates. Nucleotide sequences the strain "KY G-7" used as model for this figure (NCBI Refseq # NC_003495 and NC_003496) (MacFarlane et al., 1991; Di et al., 1999). Boxes represent ORFs coding for large polyproteins and lines non-translated regions at the genome extremes. Functions of mature proteins are indicated: co-PRO = co-protease factor, HEL = helicase, VPg = viral protein genome-linked, PRO = protease, RdRp = RNA-dependent RNA polymerase, MP = movement protein, CPL and CPS = large and small coat proteins. The ovals at the 5' end represent the VPg and "polyA" at the 3'end indicate the presence of a poly A tail.

Two distinct subgroups of BPMV have been shown to exist in nature according to both molecular and biological properties. In addition, natural reassortants between the two subgroups have been identified and may be linked to the recent rise in incidence of BPMV in the North Central states (Gu et al. 2002). Interestingly, infection of soybeans by a diploid isolate, containing RNA-1 of both subgroups, induces extremely severe symptoms. (Gu et al. 2007).

Severity of BPMV symptoms has been found to be linked to the specific amino acid content of RNA–1-encoded polyprotein. Symptom determinants have been shown to be located in the C-terminal half of the helicase region and in the protease co-factor region of RNA-1. In particular, severe symptoms have been associated with the presence of the amino acids asparagine (N) and phenylalanine (F) at positions 359 and 408, respectively, on the C-terminal portion of the helicase domain. On the contrary, serine at

position 359 and either leucine (L) or valine (V) at position 408 has been associated with isolates inducing mild or moderate symptoms (Gu and Ghabrial 2005).

BPMV was first reported as a pathogen of *Phaseolus vulgaris* L. var. Tendergreen by Zaumeyer and Thomas in 1948. At that time, BPMV was also reported on several varieties of snap and dry beans, and was shown to be readily mechanically transmissible. The first report of BPMV as a problem in soybean cultivation was in Arkansas in 1958 (Walters 1958). Since that time, BPMV has been reported in the majority of soybean growing areas of the United States including South Carolina, North Carolina, Arkansas, Virginia, Louisiana, Illinois, Kentucky, Nebraska, and Mississippi (Hopkins and Mueller 1984). BPMV was reported to occur in 88 % of surveyed fields in North Carolina and 100 % of surveyed fields in Arkansas (Hopkins and Mueller 1984). According to a recently conducted survey of plant viruses in Mississippi, BPMV is by far the most common virus of soybean in the state (Sabanadzovic unpublished).

Bean pod mottle disease symptoms

In soybean, BPMV causes leaf mottling and puckering of the leaves as well as mottling of pods and seed coats (Stace-Smith 1981) (Figure 3). As with other plant viruses, symptom expression varies according to soybean variety, viral isolate and environment. The stage at which soybean stage is inoculated with the virus may also have a major impact on symptom development (Walters 1970, Scott et al. 1974, Windham and Ross 1985, Hill et al. 2007).

Figure 3 Symptoms of *Bean pod mottle virus* on soybean showing characteristic mottling (A) and blistering (B).

Yield reduction due to BPMV may be as great as 36-52 % (Hopkins and Mueller 1984). This reduction in yield is caused by reduced seed size and pod set (Walters 1970) and is most severe when soybeans are infected as seedlings (Demski and Kuhn 1989). Research conducted in Louisiana determined that the level of infection needed to significantly reduce yield was between 20 and 40 %. Research in North Carolina concluded that BPMV infection occurring before the V6 stage of development negatively affected yield of soybean plants. BPMV may also adversely affect seed quality (Giesler et al. 2002). Soybeans infected with BPMV may produce seed with mottled seed coats. This mottling is referred to as "bleeding hilum", as the color of the hilum appears to spread across the seed coat. Seed infection either does not occur (Skotland 1958, Schwenk and Nickell 1980) or occurs at very low rates (0.037 %) (Lin and Hill 1983, Krell et al. 2003), with the virus usually present in the seed coat (Schwenk and Nickell 1980).

Co-infection of plants with BPMV and the potyvirus *Soybean mosaic virus* (SMV) has been shown to have a synergistic relationship on symptoms and damage (Ross 1963, Walters 1970, Calvert and Ghabrial 1983, Demski and Kuhn 1989). BPMV concentration in plants co-infected with SMV may be increased by two to seven times that of plants infected with BPMV only (Calvet and Ghabrial 1983). Dually infected plants also show a reduction in root nodulation (Tu et al. 1970), and greater yield reduction than soybean infected only with BPMV. Yield may be reduced as much as 80 % in plants infected by both viruses (Hopkins and Mueller 1984). The mechanism of this synergism between BPMV and SMV is not fully understood, but may be related to expression of potyvirus helper component protease interfering with the general plant antiviral response (Anandalakshmi et al. 1998). Interestingly, BPMV does not show a synergistic relationship with other potyviruses (Anjos et al. 1992).

Infection by BPMV may delay senescence of soybean plants resulting in an increase in seed-borne fungi such as *Cercospora* spp. and *Phomopsis* spp. (Demski and Kuhn 1989). In particular, soybean susceptibility to *Phomopsis* seed decay (Ross 1977, Stuckey et al. 1982, Koning et al. 2001), an important disease complex of soybean, may be increased by BPMV infection. High levels of rain, temperature, and relative humidity are also linked to greater incidence of this fungal disease (Kmetz et al. 1979).

Beetle transmission of plant viruses

Although the majority of insect vectors of plant viruses are in the order Hemiptera, forty-two viral species are known to be transmitted by beetles. Viruses transmitted by beetles are within the genera *Comovirus*, *Tymovirus*, *Sobemovirus*, *Bromovirus*, *Machlomovirus*, and *Carmovirus* (Mello et al. 2009). These genera consist

of viruses with a single stranded RNA genome and an icosohedral capsid 20-30 nm in diameter. In addition, viruses in these groups are stable, easily mechanically transmissible, have narrow host ranges, and occur at high concentration in their plant hosts.

Beetle vectors of plant viruses are leaf feeders within the families Chrysomelidae, Curculionidae, Meloidae, (Fulton et al. 1987) Apionidae (Hull 2002), and Scarabaeidae (Wickizer and Gergerich 2007). Some beetle species have been shown to transmit plant viruses as both larvae and adults (Fulton and Scott 1974, Jansen and Staples 1970).

Virus transmission by beetles was originally thought to be through simple mechanical deposition during feeding (Smith 1924). There is, however, a significant degree of specificity in virus-vector-host relationships for beetle transmissible viruses and some viruses transmitted by beetles are maintained by their vector for extended periods of time. Furthermore, many stable sap transmissible viruses, such as *Tobacco mosaic virus* (TMV), are not transmitted by beetle feeding (Fulton et al. 1987). These factors indicate that the transmission of plant viruses by beetles is a more complex biological process.

Beetles may acquire virus as quickly as after one bite on an infected plant and active beetles generally remain viruliferous for one to ten days. The longest reported retention of virus by active beetles was seventeen days for *Squash mosaic virus* transmitted by the striped cucumber beetle, *Acalymma vittatum* (Fabricius) (Freitag 1956). Duration of virus retention varies by species and is closely related to the feeding habits of the beetle. Feeding on healthy plants following virus acquisition has been shown to reduce retention time. Virus may be maintained for months in dormant beetles for overwintering (Wang et al. 1994). It has been proposed that viruses are maintained

for long periods of time in the hemolymph, however, BPMV, as well as some other plant viruses, is not able to cross into the hemocoel and has been shown to be transmitted by overwintered beetles (Wang et al. 1992).

There is no evidence for a latent period before beetles may transmit virus (Fulton et al. 1987). Though prolonged feeding increases efficiency, virus may be transmitted by a single bite (Nault 1997). Transmission of virus may be erratic, with a viruliferous beetle transmitting on one day but not another or skipping transmission to a single plant while infecting the surrounding plants (Hull 2002). Some beetles have shown increased feeding on virus infected plant tissue and larvae fed on infected roots weigh more than those feeding on healthy plants. This may represent a mutually beneficial relationship between virus and vector, with the virus gaining transmission and the vector gaining increased fitness of the larval growth stage (Musser et al. 2003). Although it has been proposed that beetles may also transmit virus by deposition of feces or through reflexive bleeding at the leg joints, no evidence for transmission aside from through feeding injury has been shown (Scott and Fulton 1978).

When beetles feed on infected leaf tissue, they take up both beetle transmissible and non-beetle transmissible viruses. Some viruses have been shown to cross the lumen of the midgut into the hemocoel, and hemolymph may serve as a reservoir for virus. However, not all viruses which have been detected in beetle hemolymph are transmitted by the beetles. Furthermore, virus movement into the hemocoel is not a prerequisite for transmission as BPMV, which is transmitted by a number of beetle species, is not found in the hemolymph of its vectors (Wang et al. 1992). No virus has been shown to propagate in beetles, and virus level decreases gradually during test feedings on healthy plants (Ghabrial and Schultz 1983). As beetles lack salivary glands, they regurgitate

when feeding, bathing their mouthparts in plant sap and virus particles. Both transmissible and non-transmissible viruses may be found in this regurgitant (Slack and Fulton 1971).

Beetle regurgitant has been shown to contain large amounts of virus particles, and is the likely the source of infectious virus particles for beetle transmissible viruses. However, beetle regurgitant also has a high level of ribonuclease (RNase) activity which has been implicated in determination of whether or not a virus may be transmitted though beetle feeding. The mode of action of these RNases is likely dependent on viral species. Methods may include direct inactivation of the virus, inactivation in plant cells, or acting in some way on the plant cell to make them unsuitable for infection. Notably, virus recovered from beetle regurgitant may regain infectivity (Gergerich et al. 1985). This indicates that inactivation occurs at some early stage of the infection process, most likely during uncoating. RNases may block some early event in virus infection of plant cells by digesting viral RNA. Beetle transmissible viruses must then differ in some key way in how they infect plants. Movement away from the infection site and the presence of RNase activity in order to infect non-wounded cells has been shown to be a means by which beetle viruses avoid inactivation. Non-beetle transmissible viruses are either unable to move away from the wound site or are unable to infect non-wounded cells (Fulton et al. 1987).

Immunofloresence has shown that two to three days after inoculation by beetle feeding, non-transmissible viruses were found only at the wound site on the outside of the wound closing layer. In contrast, beetle transmissible viruses were found in veins surrounding the wound site and in mesophyll cells associated with those veins. This suggests either that non-transmissible viruses were unable to be transported into veins

after feeding, or that they were degraded in the leaf after translocation. It may be that non-beetle transmissible viruses are unstable in the xylem or are unable to pass from the xylem to other tissues (Field et al. 1994). Movement of virus into vascular tissue may occur by a separate mechanism from that used in cell to cell movement (Silva et al. 2002). Research has shown that beetle transmissible viruses were able to infect cells away from the wound area both when stems were steam killed and when treated with sodium azide to kill cells around the area of wounding (Field et al. 1994, Gergerich and Scott 1988). The ability of beetle transmissible viruses to move in the xylem may allow them to move to cells away from the activity of RNases. Movement of beetle transmissible viruses in plants may be mediated by the coat protein. A non beetle transmissible virus (*Cucumber mosaic virus*, CMV) was made to be transmissible after altering it to have the coat protein of a beetle transmissible virus, *Cowpea chlorotic mottle virus*, CCMV (Mello et al. 2009).

RNase activity in beetle regurgitant has also been shown to stimulate virus resistance in plants. Beetle feeding increases systemic resistance for BPMV in black valentine beans. Plants which were wounded and treated with RNase derived from Mexican bean beetle regurgitant showed stimulated virus resistance. This stimulation of plant pathogen defenses may be an adaptation by plants to serve as a second line of defense against beetle-transmitted viruses (Musser et al. 2003).

While much of this discussion has focused on the role of RNases in regurgitant as their effect on transmission has been extensively studied, regurgitant also contains proteases, DNases, and cellulases which may have an effect on virus transmission. Exposure to proteases in beetle regurgitant has been shown to have an effect on virus particles. This affects the surface charge of comovirus virions, which may be observed

by electrophoresis. Beetle regurgitant was shown to contain only the altered form of the virus despite beetles feeding on plant material containing both forms. Only the converted form initiates infection during beetle transmission of comoviruses, though both forms are infectious by mechanical inoculation (Langham et al. 1990).

Transmission of *Bean pod mottle virus*

The bean leaf beetle, *Cerotoma trifurcata* (Förster), is the primary vector of BPMV. Transmission of the virus by bean leaf beetles was first reported in 1963 (Ross 1963). Bean leaf beetle, native to the eastern United States, is a member of the family Chrysomelidae. Adult beetles are approximately 1 cm in length with variable color and markings that may be classified into five categories (Herzog 1968). Beige is the most common color of adult beetles, followed by pink, salmon, orange, and crimson. Bean leaf beetle adults may or may not have four distinct black markings on the elytra (Figure 4). Different beetle color morphs have shown some differences in physiology and behavior (Herzog 1968). Coloration of the adult beetle was shown to have no effect on transmission of BPMV (Pitre 1989).

Figure 4 Bean leaf beetle *Cerotoma trifurcata* (Förster) (Coleoptera: Chrysomelidae) adult feeding on soybean leaves.

Bean leaf beetle larvae feed on the roots and nodules of the soybean plant; however, most damage to soybeans is attributed to adult feeding (Hunt et al. 1995). Adult beetles feed primarily on the leaves, leaving distinctive circular holes. Adult leaf feeding can result in significant yield loss as development of critical canopy leaf area is delayed. Adult feeding may also result in destruction of the cotyledon which may lead to as much as a 36 % reduction in leaf area by the V3 growth stage (Hunt et al. 1995). Adults can also feed on stems and pods in later stages of plant development. Beetle pod feeding consumes the outer layers of plant tissue leaving behind only the endocarp. Although feeding damage to pods is considered to be the most important source of damage by the insect and can prevent seed development or reduce seed quality, adult

beetles vector fungal, bacterial and viral plant pathogens (Kaiser and Vakiliv1978, Funderburk et al. 1999). In addition to BPMV, bean leaf beetles may serve as the vector for *Cowpea chlorotic mottle virus* and *Southern bean mosaic virus* (Funderburk et al. 1999). Fungal pathogens may be introduced at feeding sites. *Alternaria tenuissima* (Kunze; Fries) Whiltshire can be isolated from the head and abdomen of bean leaf beetle adults, and the beetles have been shown to increase the dissemination of the pathogen (Pedigo & Zeiss 1996).

Bean leaf beetle is an occasional pest of soybean. Determination of whether beetle management is required is conducted by estimating defoliation levels or sampling for beetle population levels by sweep net or ground cloth (Funderburk et al. 1999). The economic threshold for bean leaf beetle on soybeans is two adults per sweep after pod. Defoliation levels of 20 % or pod injury to 50 % of plants constitute an economic threshold for adult beetles where plant damage is measured (Catchot 2011).

Bean leaf beetles may be effectively controlled by the establishment of a trap crop (Funderburk et al. 1999). Adult beetles emerging from overwintering are attracted to a soybean plot planted 10 - 21 days earlier than the main planting the early-planted soybeans and can be controlled by applying insecticide within this smaller area. The early season production system has resulted in large areas of early planted soybean and has reduced the efficacy of trap crops as means to control bean leaf beetle populations (Baur et al. 2000).

Although the primary vector of *Bean pod mottle virus* is the bean leaf beetle, other beetles can transmit the virus. These vectors include the banded cucumber beetle (*Diabrotica balteata*), spotted cucumber beetle (*Diabrotica undecimpunctata*), striped blister beetle (*Epicauta vittata*), grape colapsis, (*Colaspis brunnea*) (Hopkins & Mueller

1984), Japanese beetle (*Popillia japonica)* (Wickizer and Gergerich 2007) and Soybean leafminer (*Odontota horni* Smith) (Giesler et al. 2002). Beetle vectors discovered to date are members of the families Chrysomelidae (Horn et al. 1973, Mabry et al. 2003, Werner et al. 2003), Meloidae (Patel and Pitre 1971), Coccinellidae (Fulton and Scott 1974), and Scarabaeidae (Wickizer and Gergerich 2007).

Transmission of BPMV by seed was not shown in several studies (Skotland 1958, Schwenk and Nickell 1980). However, a very low rate of seed transmission was observed in two studies. A study conducted in Iowa showed that 0.037 % (3/8067) of seed transmitted BPMV (Krell et al. 2003). This is in agreement with the rate of 0.10 % seed transmission (7/6976) observed in Nebraska (Lin and Hill 1983). Very low seed transmission rates suggest that the virus is associated with the seed coat rather than being harbored within the seed itself (Schwenk and Nickell 1980).

Transmission of BPMV by overwintered bean leaf beetles has also been demonstrated. A study conducted in Iowa determined that 1.6 % (1/64) of bean leaf beetles transmitted BPMV after overwintering (Krell et al. 2003). An earlier study conducted in Arkansas showed 3 % transmission (Walters 1964). Although these results have been interpreted to indicate that BPMV overwinters in bean leaf beetles, neither study ruled out that beetles may acquire virus from feeding on underground parts of dormant plants during the course of the winter (Giesler et al. 2002).

Bean pod mottle virus **host range**

Plant species in the Apocynaceae, Chenopodiaceae,and Fabaceae have been shown to be susceptible to BPMV via mechanical inoculation. Non-susceptible plant hosts may be found in the Compositae, Cruciferae, Cucurbitaceae, Solanaceae, and

Fabaceae (http://biology.anu.edu.au/Groups/MES/vide/). The range of naturally occurring hosts of BPMV is unknown. Though perennial host plants are thought to be an important inoculum source for BPMV (Moore et al. 1969, Horn et al. 1973, Stace-Smith 1981, Krell et al. 2003), at present, only *Desmodium paniculatum* (L.) and *Desmodium canadense* (L.) have been shown to be naturally infected by BPMV (Waldbauer and Kogan 1976, Krell et al. 2003). Distribution of these host plants does not fully explain the appearance and distribution of the virus (Krell et al. 2004). As alternative host plants may represent a means for the virus to overwinter, knowledge of the natural hosts for the virus may be important in determining the primary BPMV inoculum sources.

Management of *Bean pod mottle virus*

Control of vector populations may have an effect on the population of the pathogens they transmit (Perring et al. 1999). Carefully timed chemical application may reduce BPMV incidence and this is a current component of BPMV management recommendations in some areas (Krell et al. 2004, Rice et al. 2007). Planting practices including planting date and trap crops have also been considered as techniques to assist in BPMV management (Newsom and Herzog 1977).

The ideal approach to BPMV management is through host plant genetics. Currently, no BPMV resistant soybean cultivars are commercially available. Examination of other species within the genus *Glycine* has shown the existence of resistance which may potentially be introduced to soybean. The absence of resistance in commercially available soybean cultivars has led to research on introducing pathogen derived resistance. Pathogen derived resistance involves expression of viral genes by the

plant causing disruption of virus processes. Resistance to BPMV has been generated in soybeans which express portions of the viral coat proteins (Di et al. 1996).

General conclusions

Numerous questions remain concerning the biological diversity and ecology of *Bean pod mottle virus*. These questions include basic biological concerns including the methods and efficiencies of transmission, the extent and impact of biological diversity, and the ecology of the virus in terms of its hosts, vectors, and methods of overwintering. The present study is intended to address these questions, in part, for the population of the virus present in Mississippi.

References Cited

Anandalakshmi, R., G. J. Pruss, X. Ge, R. Marathe, A. Mallory, T. H. Smith, and V. B. Vance. 1998. A viral suppressor of gene silencing in plants. Proceedings of the National Academy of Science USA. 95:13079-13084.

Anjos, J. R., U. Jarlfors, and S. A. Ghabrial. 1992. Soybean mosaic potyvirus enhances the titer of two comoviruses in dually infected soybean plants. Phytopathology 82:1022-1027.

Baur, M. E., D. J. Boethel, M. L. Boyd, G. R. Bowers, M. O. Way, L. G. Heatherly, J. Rabb & L. Ashlock. 2000. Arthropod populations in early soybean production systems in the mid-South. Environmental Entomology. 29:312-328

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.

Catchot, A. 2011. 2011 insect control guide for agronomic crops. Mississippi State University Extension Service. 2471.

Demski, J. W., and C. W. Kuhn. 1989. Bean pod mottle. *In* J. B. Sinclair and P. A.Backman (eds.). Compendium of soybean diseases, 3rd ed. APS Press, St. Paul, MN.

Di, R., V. Purcell, G. B. Collins, and S. A. Ghabrial. 1996. Production of transgenic soybean lines expressing the bean pod mottle virus coat protein precursor gene. Plant Cell Reports. 15:746-750.

Di, R., 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 Genes. 18:129-137.

Fehr, W. R., and C. E. Caviness. 1977. Stages of soybean development. Iowa Cooperative Extension Service Special Report. 80.

Field, T. K., C. A. Patterson, R. C. Gergerich, and K. S. Kim. 1994. Fate of virus in bean leaves after deposition by *Epilachna varivestis*, a beetle vector of plant viruses. Phytopathology. 84:1346-1350.

Freitag, J. H. 1956. Beetle transmission, host range, and properties of squash mosaic virus. Phytopathology. 46:73-81.

Fulton, J. P., and H. A. Scott. 1974. Virus vectoring efficiencies of two species of leaf feeding beetles. Proceedings of the American Phytopathological Society. 1:159.

Fulton, J. P., R. C. Gergerich, and H. A. Scott. 1987. Beetle transmission of plant viruses. Annual Review of Phytopathology. 25:111-123.

Funderburk, J., R. McPhearson, and D. Buntin. 1999. Soybean insectmanagement. pp. 273-90. *In* L. G. Heatherly & H. F. Hodges [eds.], Soybean Production in the Mid-South. CRC Press. Boca Raton, FL.

Ghabrial, S. A. and F. J. Schultz. 1983. Serological detection of bean pod mottle virus in bean leaf beetles. Phytopathology. 73:480-483.

Gergerich R. C., H. A. Scott, and S. L. Wickizer. 1985. Evidence that ribonuclease in beetle regurgitant determines the transmission of plant viruses. Journal of General Virology. 67:367-370.

Gergerich R. C. and H. A. Scott. 1988. Evidence that virus translocation and virus infection of non-wound cells are associated with transmissibility by leaf-feeding beetles. Journal of General Virology. 69:2935-2938.

Giesler, L., S. Ghabrial, T. Hunt, and J. Hill. 2002. *Bean pod mottle virus*: a threat to U.S. Soybean Production. Plant Disease.86: 1280-1289.

Gu, H., A. J. Clark, P. B. de Sa, T. W. Pfeiffer, S. Tolin, and S. A. Ghabrial. 2002. Genetic diversity among isolates of *Bean pod mottle virus*. Phytopathology. 92:446-452.

Gu, H., C. Zhang, and S. A. Ghabrial. 2005. The *Bean pod mottle virus* proteinase cofactor and putative helicase are symptom severity determinants. Virology. 333:271-283.

Gu, H., C. Zhang, and S. A. Ghabrial. 2007. Novel naturally occurring *Bean pod mottle virus* reassortants with mixed heterologous RNA1 genomes. Phytopathology. 97:79-86.

Heatherly, L. G. 1999. Early season production system. pp. 103-18. *In* L. G. Heatherly and H. F. Hodges [eds.], Soybean Production in the Mid-South. CRC Press. Boca Raton, FL.

Herzog, D. C. 1968. Seasonal, locational, and sex variation in the color pattern of the bean leaf beetle, *Cerotoma trifurcata* Forst., in Louisiana. M.S. Thesis. Louisiana State University, Baton Rouge, LA. 176 pp.

Hill, J. H., N. C. Koval, J. M. Gaska, and C. R. Grau. 2007. Identification of fieldtolerance to bean pod mottle and soybean mosaic viruses in soybean. Crop Science. 47:212-218.

Hopkins, J. D., and A. J. Mueller. 1984. Effect of bean pod mottle virus on soybean yield. Journal of Economic Entomology. 77:943-947.

Horn, N. S., L. D. Newsom, and R. L. Jensen. 1973. Economic injury thresholds of bean pod mottle and tobacco ringspot virus infection of soybeans. Plant Disease Reports. 57:811-813.

Hull, R. 2002. Matthew's Plant Virology. Academic Press, San Diego, CA. 1001pp.

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. Agronomy Journal. 87:183-188.

Hymowitz, T. 1970. On domestication of soybean. Economic Botany. 24:408-421.

Hymowitz, T. 1976. Soybeans. pp. 159-162. *In* N. W. Simmonds [ed.], Evolution of Crop Plants. Longman Group, Ltd., London.

Jansen, W. P. and R. Staples. 1970. Transmission of cowpea mosaic virus by the mexican bean beetle. Journal of Economic Entomology. 65:1719-1720.

Kaiser, W. J., and N. G. Vakili. 1978. Insect transmission of plant pathogenic xanthomonads to bean and cowpea in Puerto Rico. Phytopathology 68:1057-1063.

Koning, G., D. M. TeKrony, T. W. Pfeiffer, and S. A. Ghabrial. 2001. Infection of soybean with *Soybean mosaic virus* increases susceptibility to *Phomopsis* spp. seed infection. Crop Science. 41:1850-1856.

Kmetz, K., C. W. Ellett, and A. F. Schmitthenner. 1979. Soybean seed decay:sources of inoculum and nature of infection. Phytopathology 69:798-801.

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 Disease. 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. Journal of Economic Entomology 97:192-202.

Langham, M. A. C., R. C. Gergerich, and H. A. Scott. 1990. Conversion of comovirus elctrophoretic forms by leaf-feeding beetles. Phytopathology. 80:900-906.

Lin, M. T., and J. H. Hill. 1983. Bean pod mottle virus: Occurrence in Nebraska and seed transmission in soybeans. Plant Disease. 67:230-233.

Mabry, T. R., H. A. Hobbs, T. A. Steinlage, B. B. Johnson, W. L. Pedersen, 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. P1ant Disease. 87:1221-1225.

MacFarlane, S. A., M. Shanks, J. W. Davies, A. Zlotnick, and G. P. Lomonossoff. 1991. Analysis of the nucleotide sequence of bean pod mottle virus middle component RNA. Virology. 183:405-409.

Mello, A. F. S., A. J. Clark, and K. L. Perry. 2009. Capsid protein of *Cowpea chlorotic mottle virus* is a determinant for vector transmission by a beetle. Journal of General Virology. 91:545-551.

Moore, B. J., H. A. Scott, and H. J. Walter. 1969. *Desmodium paniculatum,* a perennial host of bean pod mottle virus in nature. Plant Disease Reporter 53:154-155.

Musser, R.O., S. M. Hum-Musser, G. W. Felton, and R. C. Gergerich. 2003. Increased larval growth and preference for virus-infected leaves by the mexican bean beetle, *Epilachna varivestis* Mulsant, a plant virus vector. Journal of Insect Behavior. 16:247-256.

Nault, L. R. 1997. Arthropod transmission of plant viruses: a new synthesis. Annals of the Entomological Society of America. 90:521-541.

Newsom, L. D., and D. C. Herzog. 1977. Trap crops for control of soybean pests. Louisiana Agriculture. 20:14-15.

Patel, V. C., and H. N. Pitre. 1971. Transmission of bean pod mottle virus to soybean by the striped blister beetle, *Epicauta vittata*. Plant Disease Reporter 55:628–629.

Pedigo, L. P., and M. R. Zeiss. 1996. Effect of soybean planting date on bean leaf beetle (Coleoptera: Chrysomelidae) abundance and pod injury. Journal of Economic Entomology. 89:183-188.

Perring, T. M., N. M. Gruenhagen, and C. A. Farrar. 1999. Management of plant viral diseases through chemical control of insect vectors. Annual Review of Entomology. 44:457-481.

Pitre, H. N., and V. C. Patel. 1975. Characteristics of bean pod mottle virus transmission by *Cerotoma trifurcata* (Forster). Journal of the Mississippi Academy of Science. 19:186

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. Journal of Entomological Science. 24:582-587.

Rice, M. E., J. D. Bradshaw, and J. H. Hill. 2007. Revisiting an integrated approach to bean leaf beetle and bean pod mottle virus management. Integrated Crop Management. 498(3):87-88.

Ross, J. P. 1963. Transmission of bean pod mottle virus in soybeans by beetles. Plant Disease Reporter 47:1049-1050.

Ross, J. P. 1977. Effect of aphid-transmitted soybean mosaic virus on yields of closely related resistant and susceptible soybean lines. Crop Science 17:869-872.

Schwenk, F. W., and C. D. Nickell. 1980. Soybean green stem caused by bean pod mottle virus. Plant Disease. 64:863-865.

Scott, H. A., J. V. Van Scyoc, and C. E. Van Scyoc. 1974. Reactions of *Glycine* spp. to bean pod mottle virus. Plant Disease Reporter 58:191-192.

Scott, H. A., and J. P. Fulton. 1978. Comparison of the relationships of *Southern bean mosaic virus* and the cowpea strain of tobacco mosaic virus with the bean leaf beetle. Virology 84:207-209.

Silva, M. S., J. Wellink, R. W. Goldbach, and J. W. M. van Lent. 2002. Phloem loading and unloading of *Cowpea mosaic virus* in *Vigna unguiculata*. Journal of General Virology. 83:1493-1504.

Skotland, C. B. 1958. Bean pod mottle virus of soybeans. Plant Disease Reporter. 42:1155-1156.

Slack, S. A., and J. P. Fulton. 1971. Some plant virus-beetle vector relations. Virology. 43:728-729.

Smith, C. E. 1924. Transmission of cowpea mosaic by bean-leaf beetles. Science. 60:268.

Stace-Smith, R. 1981. Comoviruses, pp. 171-194. *In* Kurstak, E. [ed.], Handbook of Plant Virus Infections and Comparative Diagnosis. Elsevier/North-Holland Biomedical Press, New York, NY.

Stuckey, R. E., S. A. Ghabrial, and D. A. Reicosky. 1982. Increased incidence of *Phomopsis* spp. in seeds from soybean infected with bean pod mottle virus. Plant Disease. 66:826-829.

Šutić, D. D., R. E. Ford, and M. T. Tošić. 1999. Handbook of plant virus diseases, CRC Press, Boca Raton, FL. 584pp.

Tu, J. C., R. E. Ford, and S. S. Quiniones. 1970. Effects of soybean mosaic virus and/or bean pod mottle virus infection on soybean nodulation. Phytopathology 60:518-523.

Waldbauer, G. P., and M. Kogan. 1976. Bean leaf beetle: phenological relationship with soybean in Illinois. Environmental Entomology. 5:35-44.

Walters, H. J. 1958. A virus disease complex in soybeans in Arkansas. (Abstr.) Phytopathology. 48:346.

Walters, H. J. 1964. Transmission of bean pod mottle virus by bean leaf beetles (Abstr.) Phytopathology 54:240.

Walters, H. J. 1970. Bean pod mottle virus disease of soybeans. Arkansas Farm Research. 19:8.

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

Wang, R. Y., R. C. Gergerich, and K. S. Kim. 1994. The relationship between feeding and virus retention time in beetle transmission of plant viruses. Phytopathology 84:995-998.

Werner, B. J., R. K .Krell, L. P. Pedigo, and J. H. Hill. 2003. The soybean leaf miner (Coleoptera: Chrysomelidae) as a vector of bean pod mottle virus. Journal of the Kansas Entomological Society. 76:643-644.

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 Disease. 91 (5):637.

Windham, M.T., and J.P. Ross. 1985. Phenotypic response of six soybean cultivars to bean pod mottle virus infection. Phytopathology. 75:305-309.

Zaumeyer, W. J., and H. R. Thomas. 1948. Pod mottle, a virus disease of beans. Journal of Agricultural Research. 77:81-96.

CHAPTER II

DIVERSITY OF BEAN POD MOTTLE VIRUS IN MISSISSIPPI

Abstract

The principle viral pathogen responsible for yield loss and reduced seed quality of soybean in Mississippi is *Bean pod mottle virus* (BPMV). BPMV symptom severity has been linked to amino acid substitutions on the C-terminal half of the putative helicase and protease cofactor regions of RNA-1. Naturally occurring reassortants between BPMV isolates of subgroups I and II and RNA-1 diploidy have been shown to dramatically increase symptom severity. Soybean samples showing symptoms of BPMV were collected from production and research fields throughout Mississippi. Partial sequences of 33 isolates were obtained from four regions of the viral genome coding for RNA-dependent RNA polymerase, helicase, movement protein, and coat protein. Sequence analysis was conducted using Lasergene software and Clustal W for multiple sequence alignment. Phylogenetic trees were generated using MEGA4. Helicase sequences were compared to sequences from isolates of known symptom severity, paying particular attention to regions noted as being determinants of symptom severity. All helicase sequences obtained from Mississippi isolates showed similarity to isolates producing mild or moderate symptoms. Sequences obtained from all genomic regions were compared to the type isolates of subgroups I and II. Mississippi isolates were shown to have greater similarity to subgroup II. Biological comparison of BPMV isolates from Mississippi was conducted using type isolates of subgroup I and II as well as a

reassortant isolate which produces severe symptoms. This comparison showed Mississippi isolates to produce mild/moderate symptoms consistent with the results of genomic sequence analyses.

Introduction

Bean pod mottle virus (BPMV), a member of the genus *Comovirus*, family *Secoviridae*, was first described from *Phaseolus vulgaris* L. var. Tendergreen by Zaumeyer and Thomas in 1948. BPMV was first reported as a problem in cultivated soybean in Arkansas (Walters 1958), and has since been reported in the majority of soybean growing areas of the United States (Hopkins and Mueller 1984).

As with other comoviruses, the BPMV genome consists of bipartite positive sense single stranded monocistronic RNA. The genomic RNAs of BPMV are polyadenylated at the 3' end and viral genome-linked protein is bound to the 5' termini. The genome is expressed by the synthesis and cleavage of large polyprotein precursors (Giesler et al. 2002). The two genomic RNAs for BPMV have been sequenced and the proteins coded for by each component identified. Five mature proteins are encoded on the BPMV RNA-1 component. These are, from 5' to 3', protease co-factor, helicase, viral genome-linked protein, a protease, and RNA dependent RNA polymerase. BPMV RNA-2 codes for a cell-to-cell movement protein and for two capsid coat proteins (Di et al. 1999).

Two distinct subgroups of BPMV exist in nature. They were initially distinguished using Northern hybridization analysis of the type isolate of BPMV, K-Graves, in relation to other isolates (Gu et al. 2002). K-Graves was designated the prototype of Subgroup I and an isolate showing no hybridization (K-Hancock) was selected as a representative of Subgroup II. These subgroups may be also be

distinguished by their molecular properties (Gu et al. 2002). Sequence identities between the above mentioned isolates were 85.5% for RNA-1 and 86.9% for RNA-2. Sequences of other members of these subgroups show greater differences between subgroups than within them (Gu et al. 2002).

Natural reassortants of these two subgroups, possessing RNA-1 of Subgroup I and RNA-2 of Subgroup II, were identified in the same study and have been indicated as a potential cause for the increase in symptom severity in BPMV infection recently observed in the North Central United States. High populations of the primary vector of BPMV, *Cerotoma trifurcata* (Förster) (Family Chrysomelidae) (Ross 1963), is associated with greater incidence of BPMV as well as increased symptom expression, probably because of increased opportunity for recombination and reassortment of the BPMV genome (Gu et al. 2002).

In soybean, BPMV induces symptoms varying from mild chlorotic mottling to severe mosaic, leaf crinkling and deformation with younger leaves exhibiting the most obvious symptoms. Determinants for symptom severity are located in the C-terminal half of the putative helicase and protease cofactor regions of BPMV RNA-1. In particular, the presence of the amino acids asparagine and phenylalanine at positions 359 and 408, respectively, in the helicase region are characteristic of isolates inducing severe symptoms, whereas the presence of serine at position 359 and either leucine or valine at position 408 are correlated with isolates inducing mild or moderate symptoms (Gu and Ghabrial 2005). The protease co-factor is involved in adhering replication proteins to the endoplasmic reticulum (ER) of the plant cell. The protease co-factor of Subgroup I causes a proliferation of ER and membrane vesicles in plant cells, resulting in an increase in the area available for viral replication (Gu et al. 2002). In addition, natural partial

diploid recombinants between subgroups have been described which are diploid for RNA-1 and haploid for RNA-2. These partial diploid recombinants produce very severe symptoms in soybean (Gu et al. 2007).

Mixed infections with two BPMV strains may be the cause of diploid reassortment. As a plant is protected from cross-infection once it is infected with the first BPMV strain, plants must be simultaneously infected with different strains. Accumulation of different strains in the vector prior to feeding on healthy plants is the likely source for mixed infections. Thus, these mixed infections become more common with large beetle populations and greater incidence of BPMV. Reassortants are potentially stable in natural populations and were shown to occur at relatively high frequency in north central states. Partial diploid reassortants may have an impact on new strains of haploid virus and this may play an important role in the epidemiology and evolution of the virus (Gu et al. 2007).

The State of Mississippi ranks as the 14th of 31 soybean producing states in the U.S., with a production value of $821 million in 2010. Soybean is the third largest industry in the state of Mississippi and represents 41 % of the field crop acreage in the state (www.nass.usda.gov/ms, www.soystats.com). A recent survey conducted in Mississippi indicated that *Bean pod mottle virus* (BPMV) was by far the most common virus of soybean in the State (Sabanadzovic, unpublished). BPMV causes reduced seed size and pod set in soybean, and may lead to yield reductions as great as 52% when soybeans are infected early in the season (Walters 1970, Hopkins and Mueller 1984, Demski and Kuhn 1989, Horn et al. 1973). A synergistic relationship in terms of symptoms and damage has been shown for plants dually infected with BPMV and the potyvirus *Soybean mosaic virus* leading to yield reductions as high as 80%. (Ross 1963,

Walters 1970, Calvert and Ghabrial 1983, Demski and Kuhn 1989, Hopkins and Mueller 1984).

This research examines the BPMV population in Mississippi in terms of its genetic diversity. Particular attention is paid to the relationship if isolates collected from the major soybean growing areas of the state to type isolates of Subgroups I and II as well as to known determinants of symptom severity in soybean.

Materials and Methods

Symptomatic soybean samples, consisting of single whole plants, were collected from the main soybean growing areas of Mississippi (Figure 5). Areas sampled consisted of both research and production fields. Symptomatic plant samples were stored at 4°C and tested by DAS-ELISA for *Bean pod mottle virus* infection (Agdia, Elkhart, IN). Leaf tissue (0.15 g) from selected healthy and infected samples was stored at -20°C for RNA extraction. Additional leaf tissue from BPMV-infected soybean plants was preserved for further use in mechanical inoculation.

Figure 5 Map of Mississippi showing counties from which isolates have been collected and used in this work.

Sample leaf tissue (0.15 g) was macerated in 1.5 ml of sample extraction buffer. In order to prevent RNA degradation and tissue oxydation, 2-mercaptoethanol was added to the buffer to a final concentration of 1% (v/v). RNA was extracted using the RNeasy plant minikit (Qiagen, Valencia, CA) protocol for plant tissue. Total RNA extracted from plant tissue was stored at -70°C. Complementary DNA (cDNA) was synthesized for use in of polymerase chain reaction. Total RNA (5.0 µl) was added to 1 µl random primers (500 ng/µl) and 4 µl nanopure water. This was incubated at 70ºC for five minutes. After incubation, 20 µl of mix comprised of 4.5 µl nanopure water, 6 µl 5X M-MLV buffer, 3

µl 0.1 M DTT, 3 µl 0.3 M mercaptoethanol, 2 µl dNTPs (100 ng/µl of each NTP), 1 µl M-MLV reverse transcriptase (200 units; Promega, Madison, WI), and 0.5 µl RNase inhibitor (Promega, Madison, WI) were added and the sample was incubated at 39ºC for one hour. The resulting cDNA was stored at -70ºC.

cDNA was amplified by polymerase chain reaction (PCR) at the following running conditions: initial denaturation at 94ºC for two minutes followed by forty cycles of: 94ºC for 30 seconds, 52 ºC for 35 seconds, 72ºC for 1 minute 15 seconds, and final extension at 72ºC for 15 minutes. PCR was run with 4 µl cDNA in 27.5 µl nanopure water, 10 µl 5X GoTaq FlexiBuffer, 4 µl 25 mM $MgCl_2$, 1.5 µl forward and resverse primers (100 ng/µl), 1.5 µl dNTPs and 0.25 µl Taq polymerase (Promega). Results of PCR were analyzed by agarose gel electrophoresis. PCR products were ligated in pGEM-T Easy vector (Promega, Madison, WI) and transferred in DH5α competent cells.

Cells for transformation were maintained in LB medium at 4ºC. Ligation mix (5 µl) was added to 100 µl competent cells. Quality of transformations was determined by plasmid extraction from selected colonies. DNA was extracted from cells with a QIAprep Spin Miniprep Kit according to the procedure detailed for a microcentrifuge (Qiagen, Valencia, CA). Selected plasmids were submitted to the custom sequencing service of MWG-Biotech (Huntsville, AL) to obtain the sequence of the amplified target regions of the viral genome.

The process described above was conducted for four regions of the viral genome encoding for different proteins, RNA dependent RNA polymerase (Pol), helicase (Hel), movement protein (MP), and coat protein (CP). Primer sequences and target sequence lengths are reported in Table 1. Pairwise comparison between corresponding regions of different isolates was conducted with Clustal W software (Thompson et al. 1994).

Genomic data from the same viral RNA molecule (RNA-1 or RNA-2) were combined and analyzed in order to further investigate the relationship of isolates from Mississippi. Phylogenetic trees were constructed using the Neighbor Joining method in MEGA4 (Tamura et al. 2007). Analyses always included genomic data of reference isolates (Gu et al. 2002) deposited in the NCBI/GenBank.

In order to study biological properties of BPMV, an isolate from Mississippi (MS-Monroe1) was mechanically inoculated onto V1 stage AG4903 soybean plants (Asgrow, St. Louis, MO) grown under greenhouse conditions along with type isolates for subgroups I and II (K-Graves and K-Hancock, respectively), and a reassortant isolate of Subgroup I RNA-1 and Subgroup II RNA-2 (K-Hopkins) obtained from S. Ghabrial used as references for comparison. Plants were maintained in the greenhouse and symptoms were observed daily in order to compare the symptoms induced by isolates. Symptoms expressed by plants inoculated with type isolates were considered standards for mild (K-Hancock), moderate (K-Graves), and severe (K-Hopkins) symptoms.

Results and Discussion

Primers designed for the four target genomic regions were applied on 33 isolates from 20 counties in Mississippi (Figure 5). Each primer set generated a single band of predicted size (Figure 6) under PCR conditions described in Materials & Methods.

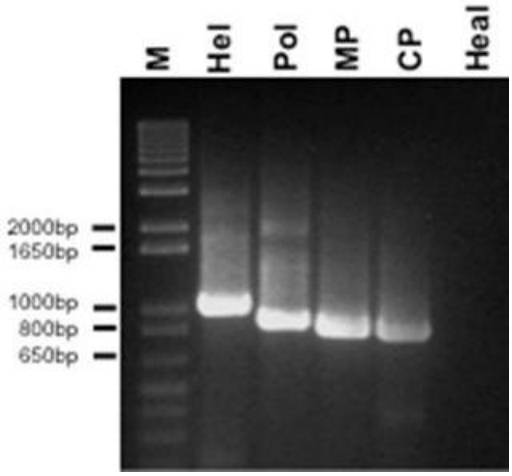

Figure 6 Agarose gel electrophoresis of RT-PCR products generated by different sets of primers used in the diversity study. Primer set names derive from the target genome region: Hel = helicase, Pol = RNA-dependent RNA polymerase, MP = movement protein, CP = coat protein. Reference DNA marker (1kbPlus DNA ladder, Invitrogen, Carlsbad, CA) is in lane M and BPMV-free sample is in lane "Heal". Nucleotide sequences of each primer set and the exact size of PCR products are reported in Table 1.

Table 1 Nucleotide sequence of primers used in this study with length of generated RT-PCR products.

Target genome region	**Primer name**	**Nucleotide sequences (5'-3')**	**RT-PCR product size (bp)**
Helicase	Hel-F	CCATTAATTTCAAGTGCTCCATAC	**1040**
	Hel-R	ACAAAAACTCCCCCATTACCAG	
RdRp	Rep-F	ACCTAAGACGCGCTGCTTC	**899**
	Rep-R	TGCTATGGTAGGCACATCAGC	
Movement protein	MP-F	GGGCGTTGGTGCAAATGTTTG	**883**
	MP-R	CTGGTTCAATCTGCACAATGG	
Coat protein	CP-F	AAAATGGTGGCCACAGTTGG	**884**
	CP-R	CGTGAGCCTGAAATACCAGG	

The viral polymerase showed differences in nucleotide sequences up to 4.9% between isolates from Marshall and Talahatchie Counties. The average nucleotide

sequence distance between tested isolates was 2.1%. A maximum of 2.4% amino acid differences was observed between the two most divergent isolates. The deduced amino acid content of the viral polymerase region of isolates from Mississippi showed greater similarity to the type isolate of Subgroup II (K-Hancock) than to that of Subgroup I (K-Graves).

The helicase appears to be the most conserved region of the genome with a maximum nucleotide sequence distance of 3.6% between isolates from Marshall and Coahoma Counties, and an average of 1.7% difference in nucleotide sequences among all tested Mississippi isolates. These differences translate to a maximum difference of 2.9% of amino acids. As with the polymerase region, the amino acid content of helicase of isolates from Mississippi shared greater similarity to Subgroup II.

The viral movement protein showed a maximum nucleotide sequence distance of 4.7% between isolates from Holmes County and Verona, MS. This translated to a maximum difference of 3.5% in amino acids. As with both tested regions of BPMV RNA-1, the movement protein region showed a distinctly greater similarity to Subgroup II than Subgroup I type isolates.

The coat protein region showed a maximum nucleotide difference of 4.1% between isolates from Oktibbeha and Chickasaw Counties. However, in terms of amino acids, the coat protein was highly conserved, with a maximum difference of only 2.8%. The difference between subgroup I and II BPMV seemed less significant in relation to the coat protein region of BPMV as compared to other regions analyzed. Gu et al. (2002) observed that the amino acid sequence of the coat protein is highly conserved among isolates of BPMV regardless of Subgroup. This was also confirmed by reaction of various BPMV isolates in Western Blot. All BPMV isolates reacted strongly to

antiserum designed against K-Graves regardless of being Subgroup I or II (Gu et al. 2002).

Isolates from Monroe and Marshall Counties formed a separate clade from other Mississippi isolates in terms of their RNA-1. Notably, the difference between these isolates and others collected from Mississippi as well as K-Hancock, the type isolate of Subgroup II (<5%) was markedly less than the difference between these isolates and K-Graves, the type isolate of Subgroup I (~15%) (Figure 7).The separation of isolates from Marshall and Monroe counties into a separate clade was limited to regions of RNA-1 sequenced, and was not observed for genomic regions of RNA-2 (movement protein and coat protein) (Figure 8).

Analyses of the helicase region of isolates from Mississippi have shown characteristics associated with mild or moderate strains of BPMV. Previous research by Gu and Ghabrial (2005) showed an association of severe symptoms with specific amino acids at positions 359 and 408 in the putative helicase. In severe strains sequenced, an asparagine and phenylalanine are present in these two positions, respectively. In contrast, all 33 Mississippi isolates contain a serine at position 359 and a valine at position 408. This amino acid combination has been associated with mild/moderate symptoms (Figure 9).

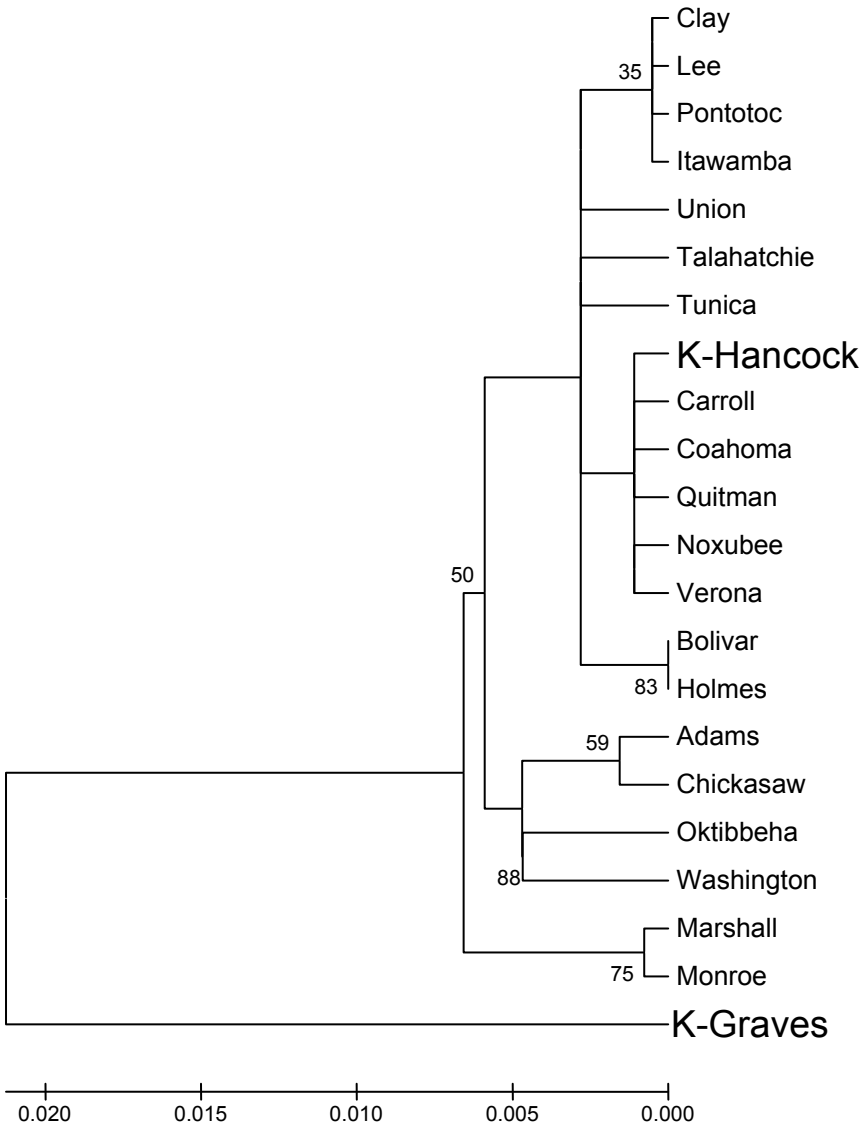

Figure 7 Phylogenetic tree inferred with the Neighbor-Joining method (Saitou and Nei 1987) based on combined partial amino acid sequences of the BPMV RNA polymerase and Helicase regions of 20 isolates from Mississippi. Reference isolates for BPMV subgroups I and II (K-Graves and K-Hancock, respectively. The bootstrap consensus tree is inferred from 1000 replicates (Felsenstein 1985). The phylogenetic tree was linearized assuming equal evolutionary rates in all lineages (Takezake et al. 2004). Evolutionary distances were computed using the Poisson correction method (Zuckerland and Pauling 1965).

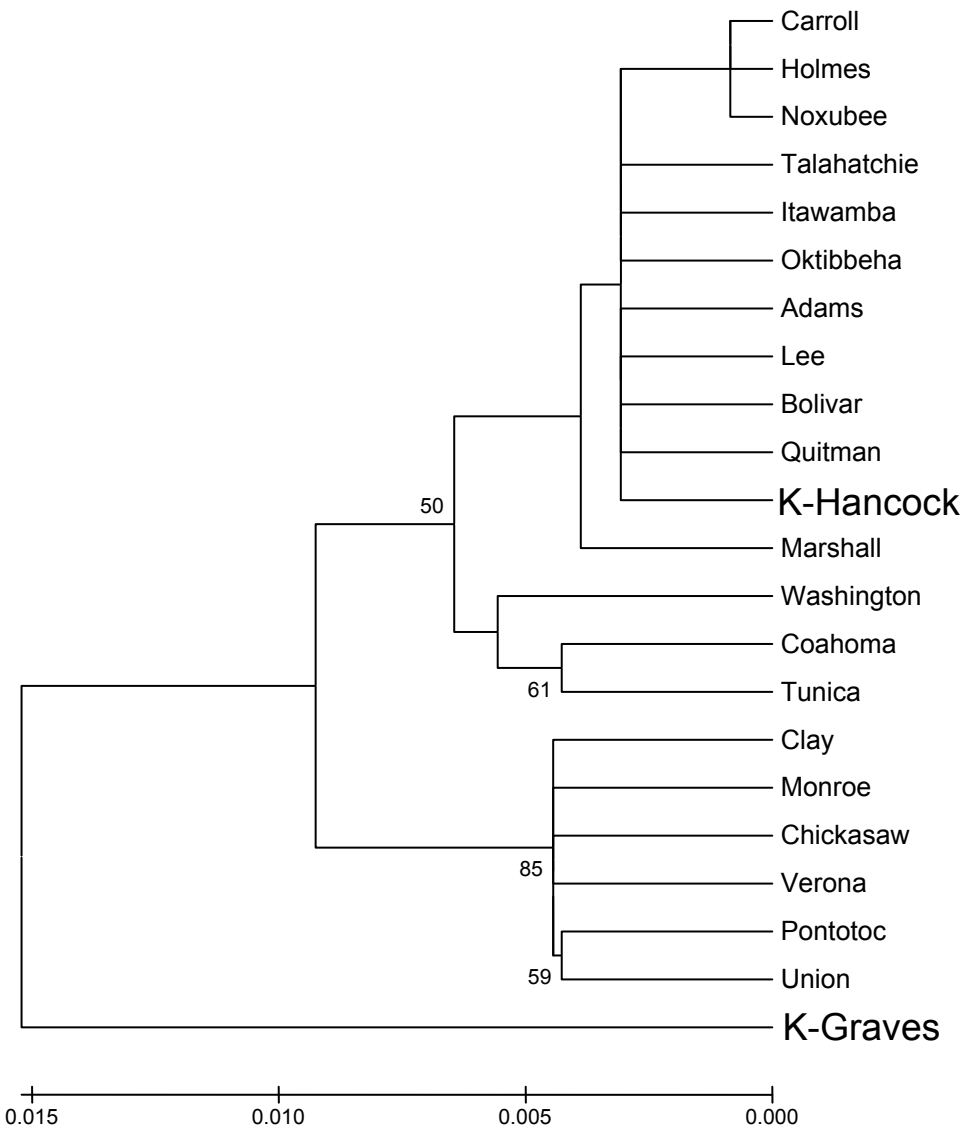

Figure 8 Phylogenetic tree inferred with the Neighbor-Joining method (Saitou and Nei 1987) based on combined partial amino acid sequences of the BPMV movement protein and coat protein regions of 20 isolates from Mississippi. Reference isolates for BPMV subgroups I and II (K-Graves and K-Hancock, respectively. The bootstrap consensus tree is inferred from 1000 replicates (Felsenstein 1985). The phylogenetic tree was linearized assuming equal evolutionary rates in all lineages (Takezake et al. 2004). Evolutionary distances were computed using the Poisson correction method (Zuckerland and Pauling 1965).

Figure 9 Comparison of amino acids surrounding positions 359 and 408 (arrows) of the polyprotein encoded by RNA-1 (helicase domain) and linked to BPMV symptom severity. No isolates from Mississippi showed the amino acid substitutions linked to severe symptoms. Isolates marked by "(I)" (Subgroup I) and "(II)" (Subgroup II) are reference sequences obtained from NCBI.

Evaluation of the biological properties of isolates from Mississippi confirmed molecular results. Symptoms of the Monroe and Oktibbeha isolates inoculated onto AS4903 soybeans and kept under greenhouse conditions were comparable to those of inoculated with the K-Hancock type isolate of Subgroup II BPMV. Symptom development on soybean kept under identical conditions with the K-Graves type isolate of Subgroup I BPMV and K-Hopkins, an isolate diploid for BPMV RNA-1, was both faster and more severe than for the Mississippi isolates (Figure 10).

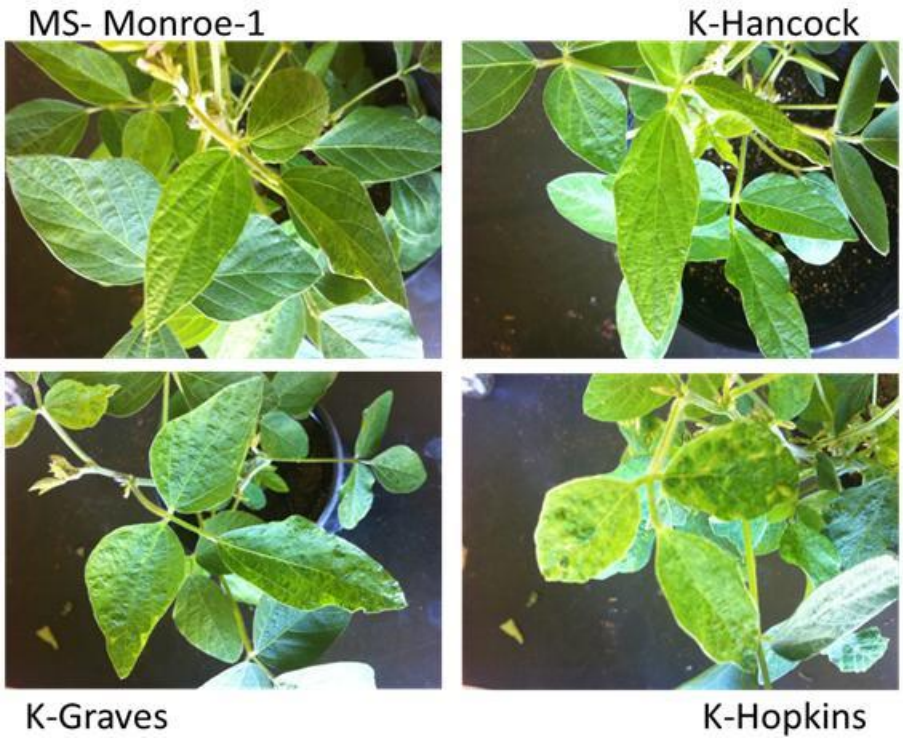

Figure 10 BPMV symptoms induced in AG4903 soybean plants by Mississippi isolate Monroe compared with type isolates of Subgroup I (K-Graves) showing mild symptoms, Subgroup II (K-Hancock) showing moderate symptoms, and an isolate diploid for BPMV RNA-1 (K-Hopkins) showing severe symptoms.

Conclusion

All isolates collected in Mississippi in this study, in terms of both their genomic RNA-1 and RNA-2 sequences, were more closely related to subgroup II than with Subgroup I (Figures 7, 8). The molecular data generated from the local isolates suggest that the BPMV population in Mississippi is both relatively uniform and biologically mild in comparison with some strains recently described in the North Central United States (Gu and Ghabrial 2005).

Except for the highly conserved coat protein region, all regions of the genome considered showed greater similarity to Subgroup II than to Subgroup I isolates of

BPMV. This is significant, as symptoms induced by Subgroup II isolates are predicted to be milder than those of Subgroup I isolates. Furthermore, examination of the amino acids of the helicase region showed that all Mississippi isolates possessed characteristics related to mild or moderate symptom expression. This is supported by comparison of symptoms on plants inoculated with the type isolates of the two Subgroups, a reassortant isolate and representative isolates from Mississippi.

Severe BPMV symptoms have been related to reassortant strains which possess Subgroup I RNA-1 and Subgroup II RNA-2 (Gu et al. 2002). Additionally, isolates which are diploid for RNA-1 and haploid for RNA-2 have been shown to generate severe symptoms (Gu et al. 2007). Neither reassortants nor partial recombinants were observed amongst the isolates collected from Mississippi.

Although the molecular characteristics discussed are reliable indicators of symptoms induced by an isolate of BPMV, the effect of these factors on yield is not yet fully understood. In a study conducted in Kentucky, all tested isolates of BPMV were shown to reduce soybean yields compared to the non-inoculated control, however, no significant differences in amount of yield reduction were observed between Subgroups and reassortant strains. Though not significant, greatest impact on yield was observed in the reassortant strain K-Hopkins which generates severe symptoms (Gu et al. 2002). Significant differences in yield reduction were noted between isolates within Subgroup II. One isolate (K-D1) evidenced mild symptoms under greenhouse conditions, but induced more severe symptoms in the field and reduced yield significantly more than the K-Hancock isolate (Gu et al. 2002). Further research is needed to clarify the relationship between the molecular characteristics of an isolate of BPMV and yield loss in soybean.

References Cited

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

Demski, J. W., and C. W. Kuhn. 1989. Bean pod mottle. *In* J. B. Sinclair and P. A. Backman (eds.). Compendium of soybean diseases, 3rd ed. APS Press, St. Paul, MN.

Di, R., 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 Genes. 18:129-137.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution. 39:783-791.

Giesler, L., S. Ghabrial, T. Hunt, and J. Hill. 2002. *Bean pod mottle virus*: a threat to U.S. soybean production. Plant Disease. 86:1280-1289.

Gu, H., A. J. Clark, P. B. de Sa, T. W. Pfeiffer, S. Tolin, and S. A. Ghabrial. 2002. Genetic diversity among isolates of *Bean pod mottle virus*. Phytopathology. 92:446-452.

Gu, H. C., and S. A. Ghabrial. 2005. The *Bean pod mottle virus* proteinase cofactor and putative helicase are symptom severity determinants. Virology. 333:271-283.

Gu, H., Zhang, C., and S. A. Ghabrial. 2007. Novel naturally occurring *Bean pod mottle virus* reassortants with mixed heterologous RNA1 genomes. Phytopathology. 97:79-86.

Hopkins, J. D., and A. J. Mueller. 1984. Effect of bean pod mottle virus on soybean yield. Journal of Economic Entomology. 77:943-947.

Horn, N. S., L. D. Newsom, and R. L. Jensen. 1973. Economic injury thresholds of bean pod mottle and tobacco ringspot virus infection of soybeans. Plant Disease Reporter. 57:811-813.

Ross, J. P. 1963. Transmission of bean pod mottle virus in soybeans by beetles. Plant Disease Reporter 47:1049-1050.

Saitou N., and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 4:406-425.

Takezaki N, A. Rzhetsky, and M. Nei. 2004. Phylogenetic test of the molecular clock and linearized trees. Molecular Biology and Evolution 12:823-833.

Tamura K, J. Dudley. M. Nei, and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution. 24:1596-1599.

Thompson J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research. 22:4673-4680.

Walters, H. J. 1958. A virus disease complex in soybeans in Arkansas. Phytopathology. 48:346.

Walters, H. J. 1970. Bean pod mottle virus disease of soybeans. Arkansas Farm Research. 19:8.

Zaumeyer, W. J., and H. R. Thomas. 1948. Pod mottle, a virus disease of beans. Journal of Agricultural Research. 77:81-96.

Zuckerandl, E., and L. Pauling. 1965. Evolutionary divergence and convergence inproteins.pp. 97-166 *In* V. Bryson and H.J. Vogel [eds.], Evolving Genes and Proteins. Academic Press, New York, NY.

CHAPTER III

ECOLOGY OF BEAN POD MOTTLE VIRUS IN MISSISSIPPI

Abstract

Bean pod mottle virus (BPMV) is the principle viral pathogen of soybean in Mississippi. Infection results in yield loss and a reduction in seed quality. Plant species from areas surrounding soybean fields were collected and tested for infection by BPMV. In addition, selected plant species belonging to the family Fabaceae and native to Mississippi were obtained and mechanically inoculated with BPMV in order to determine their susceptibility to the virus. Inoculated plants were tested by DAS-ELISA to determine the success of mechanical inoculation. Positive ELISA results were confirmed by PCR. Feeding by bean leaf beetles on species testing positive by ELISA was determined. Seven species previously not reported as hosts tested positive by ELISA and RT-PCR after mechanical inoculation. Among them, one species, *Desmodium perplexum* was shown to be susceptible to BPMV by both mechanical and beetle inoculation. *D. perplexum* was additionally shown to serve as an inoculum source for transmission of BPMV to soybean by bean leaf beetle. Eight beetle species collected from clover were tested for the ability to transmit BPMV from soybean to soybean. Only one of these species, *Hypera postica*, transmitted the virus to 1 of 14 test plants. Bean leaf beetle overwintering transmission of BPMV was tested, but of 187 beetles collected, none transmitted the virus.

Introduction

Soybean production is the third largest industry in the state of Mississippi, accounting for 41% of the field crop acreage in the state and a production value of $821 million in 2010 (www.soystats.com). A recent survey of viruses in Mississippi showed that *Bean pod mottle virus* (BPMV), a member of genus *Comovirus* in the family *Secoviridae*, is by far the most common virus of soybean in the state (Sabanadzovic, unpublished).

BPMV in soybean in was first reported from Arkansas by Walters in 1958, and has since been reported in the other soybean growing areas of the United States (Hopkins and Mueller 1984). Symptoms of BPMV on soybean include mild chlorotic mottling to severe mosaic, leaf crinkling and deformation, with the most obvious symptoms being present on young leaves. Yield reduction due to BPMV may range from 3 to 52 % (Giesler et al. 2002) with estimates of the economic threshold for BPMV in soybean fields calculated at 20-40% infection (Horn et al. 1973).

The primary vector of BPMV is the bean leaf beetle (*Cerotoma trifurcata* (Förster), a member of the family Chrysomelidae native to the eastern United States. Transmission of the virus by bean leaf beetles was first reported in 1963 (Ross, 1963). Adult beetles are approximately 1 cm in length with variable color and markings (Herzog 1968). Most commonly, adult beetles are beige; however, they may also be pink, salmon, orange, and crimson. Bean leaf beetle adults may or may not have four distinct black markings on the elytra. Although different beetle color morphs have shown some differences in physiology and behavior (Herzog 1968), color was not shown to influence transmission of BPMV (Pitre 1989). Higher bean leaf beetle populations have been

linked to an increase in BPMV incidence and symptom severity, perhaps due to greater opportunity for recombination and reassortment of the BPMV genome (Gu et al. 2002).

Other known vectors of BPMV include other members of the Chrysomelidae family (Horn et al. 1970, Mabry et al. 2003, Werner et al. 2003), and members of the Meloidae (Patel and Pitre 1971), Coccinellidae (Fulton and Scott 1974), and Scarabaeidae (Wickizer and Gergerich 2007). In particular, the banded cucumber beetle (*Diabrotica balteata*), spotted cucumber beetle (*Diabrotica undecimpunctata*), striped blister beetle (*Epicauta vittata*), grape colapsis, (*Colaspis brunnea*) (Hopkins & Mueller 1984), Japanese beetle (*Popillia japonica*) (Wickizer and Gergerich 2007) and soybean leafminer (*Odontota horni* Smith) (Giesler et al. 2002) have been identified as vectors of the virus.

Though perennial host plants are thought to be an important inoculum source for BPMV (Moore et al. 1969, Horn et al. 1970, Stace-Smith 1981, Krell et al. 2003), the known natural host range for BPMV to date includes only four species: soybean (*Glycine max*), bean (*Phaseolus vulgarius*), panicled-leaf tick trefoil (*Desmodium paniculatum*) and showy tick trefoil (*Desmodium canadense*) (Krell et al. 2003). The distribution of these host plants does not fully explain the appearance and distribution of the virus (Krell et al. 2004). Plant species in the Apocynaceae, Chenopodiaceae, and Fabaceae, including *Trifolium incarnatum*, *Stizolobium deeringianum,* and *Lespedeza* spp. have been shown to be susceptible to BPMV via mechanical inoculation. Non-susceptible plant hosts, such as *Datura stramonum*, *Vicia faba*, *Capsicum frutescensm*, and *Pisum sativum* are found in the Compositae, Cruciferae, Cucurbitaceae, Solanaceae, and Fabaceae (http://biology.anu.edu.au/Groups/MES/vide/). The full range of naturally occurring hosts of BPMV is unknown. As alternative host plants may represent a means for the

virus to overwinter, knowledge of the natural hosts for the virus may be important in determining the primary BPMV inoculum sources.

Transmission of BPMV by seed was not shown in several studies (Skotland 1958, Schwenk and Nickell 1980). However, a very low rate of seed transmission was observed in two studies. A study conducted in Iowa showed that 0.037 % (3/8067) of seed transmitted BPMV. This number is conservative, however, as only symptomatic seedlings were tested (Krell et al. 2003). This is in agreement with the rate of 0.10 % seed transmission (7/6976) observed in Nebraska (Lin and Hill 1983). Very low seed transmission rates suggest that the virus is associated with the seed coat rather than being harbored within the seed itself (Schwenk and Nickell 1980).

Transmission of BPMV by overwintered bean leaf beetles has been demonstrated. A study conducted in Iowa determined that 1.6 % (1/64) of bean leaf beetles transmitted BPMV after overwintering (Krell et al. 2003). An earlier study conducted in Arkansas showed 3 % transmission (Walters 1964). Although these results have been interpreted to indicate that BPMV overwinters in bean leaf beetles, neither study ruled out that beetles may acquire virus from feeding on underground parts of dormant plants during the course of the winter (Giesler et al. 2002).

Low rates of transmission by both seed and overwintering beetles may be of importance in establishing BPMV in the field. High vector populations may allow for the spread of the virus early in the season. Although there is limited information on the incidence of BPMV early in the season, a survey conducted in Iowa indicated 54 and 56% incidences of infected plants in two research fields at the VC stage of soybean development, suggesting that early inoculum sources are important for spread of the virus (Krell et al. 2003).

Materials and Methods

Alternative Plant Hosts for *Bean pod mottle virus*

In order to investigate potential alternative host plants for *Bean pod mottle virus* (BPMV) in Mississippi, plants from areas surrounding soybean fields throughout the state were sampled and tested for BPMV infection. Sampling for alternative plant hosts was conducted in early season (April-May) and late season (August-September) in 2007-2009. Where possible, plants were collected from the borders of fields with an established history of BPMV infection. Plants showing potential beetle feeding damage or viral symptoms were preferentially collected, however, some plants collected showed neither feeding damage nor symptoms. Each sample collection consisted of twelve individual plants of each species within a location. Where possible, sample species were collected from multiple locations in order to more broadly test for the presence of the virus.

Samples were individually bagged for transport to the laboratory and storage. Plant samples were stored at 4ºC until tested for presence of BPMV. Whole plants were stored for later identification using a plant press. Plant species identification was accomplished by Mr. Chris Doffitt of the Mississippi State University Herbarium. A list of species sampled in this survey is presented in Table 2. While the majority of samples were identified to species, some identification was not possible due to lack of necessary taxonomic structures at the time of collection. Samples that could not be identified were tested for BPMV, but were not further considered for this study.

Plant samples were tested for the presence of BPMV by alkaline phosphatase double antibody sandwich ELISA (Agdia, Ekhart, IN). Samples for testing consisted of a 0.20 g composite sample of four individual plants. ELISA results were evaluated by

visual comparison to the positive control. For samples showing positive results, the individual plants making up the composite were tested by DAS-ELISA to determine which samples may be positive for BPMV. Single plant samples which tested positive in DAS-ELISA were tested for *Bean pod mottle virus* by RT-PCR using the primers and cycling conditions described in the previous chapter. Samples were submitted to RT-PCR using primers for two separate genomic regions in order to prevent false negatives due to variation of the genome.

Table 2 List of plant species collected from edges of soybean fields in MS and tested for BPMV. Sample denotes a set of twelve individual plants.

Species	**Samples**	**Common Name**	**Species**	**Samples**	**Common Name**
Amaranthus sp.	4	Pigweed	*Pueria lobata*	2	Kudzu
Ambrosia trifida	3	Great ragweed	*Ranunculus sardous*	3	Hairy buttercup
Ampelopsis arborea	1	Peppervine	*Rubus argatus*	2	Sawtooth blackberry
Boehmeria cylindrical	2	False nettle	*Rumex crispus*	2	Curlydock
Brunnichia ovate	4	Redvine	*Salvia lyrata*	2	Lyre-leaf sage
Campsis radicans	3	Trumpet creeper	*Sambucus canadensis*	2	Elderberry
Eupatorium maculatum	3	Joe-Pye weed	*Sesbaia exaltata*	3	Coffeeweed
Euphorbia maculate	2	Eyebane	*Sida spinosa*	1	Pickly sida
Ipomoea sp.	5	Morning glory	*Solanum carolinensis*	2	Horse nettle
Iva annua	5	Annual marsh elder	*Solidago canadensis*	3	Goldenrod
Lathyrus hirsutis	2	Caley pea	*Trifolium incarnatum*	3	Crimson clover
Mollugo verticillata	2	Green carpetweed	*Trifolium pratense*	3	Red clover
Oenothera speciosa	2	Pink evening primrose	*Trifolium repens*	2	White clover
Passiflora incarnate	2	Passionflower	*Trifolium resupinatum*	3	Persian clover
Physalis angulata	2	Cutleaf groundcherry	*Verbena brasiliensis*	2	Vervain
Polygonum lapathifolium	3	Curlytop knotweed	*Vicia villosa*	1	Hairy vetch
Polygonum pennylvanicum	2	Pink knotweed	*Vitis rotundifolia*	2	Muscidine

A list of the Fabaceae recorded as present in the state of Mississippi was compiled from information available online through the University of Mississippi Pullen Herbarium (www.herbarium.olemiss.edu). Seeds of species which were available from the National Seed Storage Lab were obtained and plants were grown under greenhouse conditions. A total of 51 species, (59 accessions) were tested in this study (Table 3).

Table 3 List of species/accessions for mechanical transmission study. Seeds were obtained from National Seed Storage Lab.

	Species/Accession		Species/Accession
1	*Aeschynomene indica*	**24**	*Medicago orbicularis*
2	*Chaemaecrista fasciculata*	**25**	*Medicago polymorpha*
3	*Chaemaecrista nicticans*	**26**	*Medicago sativa sativa (US)*
4	*Crotolaia spectabilis*	**27**	*Pueraria montana var. lobata*
5	*Crotolaria ochroleuca*	**28**	*Pueraria montana var. montana*
6	*Crotolaria pallida*	**29**	*Senna alata*
7	*Crotolaria retusa*	**30**	*Senna corymbosa*
8	*Desmodium cuspidatum*	**31**	*Senna marilandica*
9	*Desmodium glabellum*	**32**	*Trifolium arvense*
10	*Desmodium obtusum*	**33**	*Trifolium campestre*
11	*Desmodium paniculatum*	**34**	*Trifolium carolinanum*
12	*Desmodium perplexum*	**35**	*Trifolium dubium*
13	*Desmodium tortuosum* 2751089	**36**	*Trifolium hybridum*
	Desmodium tortuosum 317058	**37**	*Trifolium incarnatum*
	Desmodium tortuosum 647846	**38**	*Trifolium lappaceum*
14	*Kummerowia stipulacea* 286454	**39**	*Trifolium pratense*
	Kummerowia stipulacea 593053	**40**	*Trifolium reflexum*
15	*Kummerowia striata*	**41**	*Trifolium resupinatum*
16	*Lathyrus aphaca*	**42**	*Trifolium subterraneum*
17	*Lathyrus hirsutus*	**43**	*Trifolium vesiculosum*
18	*Lathyrus latifolius (Netherlands)*	**44**	*Vicia disperma*
	Lathyrus latifolius (US)	**45**	*Vicia grandiflora*
19	*Lathyrus sylvestris*	**46**	*Vicia hirsuta*
20	*Lespedeza capitata* 215217	**47**	*Vicia lathyroides*
	Lespedeza capitata 653751	**48**	*Vicia sativa sativa*
21	*Lespedeza cuneata* 186171	**49**	*Vicia tetrasperma*
	Lespedeza cuneata 613537	**50**	*Vicia villosa*
22	*Medicago arabica*	**51**	*Vigna luteola*
23	*Medicago lupulina (Canada)*	**52**	*Vigna unguiculata* supsp. *unguiculata*
	Medicago lupulina (US)		

Plants were inoculated using soybean leaf tissue infected with a known isolate of BPMV (Monroe-1). Mechanical inoculation was conducted by grinding leaf tissue in 0.1 M phosphate buffer (pH 7.2) and rubbing it gently onto celite-dusted leaves of the test plants. Plants were maintained for four weeks following inoculation and then individually tested for BPMV via DAS-ELISA as described above. ELISA was conducted a second time in order to provide further confirmation of previous results. In the case of unclear or borderline results, a third ELISA was run for clarification. Plants testing positive in ELISA were further tested by molecular methods in order to confirm ELISA results. Total RNA was extracted using a Qiagen RNeasy kit and cDNA was synthesized. RT-PCR was conducted using specific primers as described in the previous chapter.

Plants testing positive by both ELISA and RT-PCR were tested to determine whether they were also a host for the principle vector species, bean leaf beetle. Beetles were field collected from soybean using a 20-cm sweep net and maintained in 105mm Petri dishes for 48 hours prior to being given access to test plants. This fasting time was intended to promote beetle feeding on test plants and also allowed for weak beetles to be removed. After this, five beetles were placed on individual leaves of the test plants in Petri dishes. A moistened cotton ball was included and the Petri dish was sealed with parafilm to prevent desiccation. Beetles were kept under test conditions for 48 hours and plant tissue was observed for feeding daily. Three repetitions of this test were conducted.

Plants testing positive for both mechanical inoculation and beetle feeding were grown under greenhouse conditions in order to determine whether BPMV could be transmitted to them by bean leaf beetles. Bean leaf beetles were collected by sweepnet in soybean fields at the R.R. Foil Plant Science Research Center (FPSRC), Mississippi State

University as described above. Beetles were given access to BPMV infected soybean leaf tissue for a period of 48 hours before being caged on test plants. Beetle cages were constructed by cutting off the top and bottom of a 2 liter plastic soda bottle. The top of the bottle was replaced by a fine mesh, while the bottom of the cage was driven into the soil in which plants were grown. A total of 15 viruliferous beetles were placed on each test plant: three groups of five beetles each were successively placed on each plant to be tested at intervals of two days for a total of six days. Soybean plants were used as positive controls. Plants were maintained for four weeks following initial beetle feeding and then tested by DAS-ELISA for infection by BPMV as described above. Plants testing positive by ELISA were further tested by RT-PCR as described previously.

Alternative Vectors for *Bean pod mottle virus*

Beetles of various species were collected from soybean fields in Noxubee County and soybean and clover fields located in Oktibbeha County for determination of their potential as vectors of BPMV. Beetle collection was conducted by sweepnetting as previously described. Beetles collected from clover were taxonomically identified by the curator of the Mississippi Entomological Museum.

In order to determine if beetles collected from clover would feed on soybean leaf tissue, collected beetles were maintained and tested following the same procedure described previously. Beetles were kept under test conditions for 48 hours and plant tissue was observed for feeding daily. Three repetitions of this test were conducted to ensure feeding occurred readily.

Beetles collected from soybean fields, or otherwise tested positive for feeding on soybean plants were given access to BPMV infected leaf tissue in order to determine their

ability to acquire and transmit the virus. Three beetles/species were placed in 105mm Petri dishes along with a leaflet of infected soybean. The number of dishes used per species varied from 6 for lesser clover leaf weevil and Fuller rose weevil to 14 for alfalfa weevil and clover head weevil. Variation in the number of beetles of each species tested in this study reflects the ease with which they could be collected. Beetles were maintained on infected tissue for 48 hours and feeding was observed daily. After acquisition feeding, beetles were starved for 24 hours before being placed on test plants. Fifteen (alfalfa weevil, clover head weevil) to 30 % (Fuller rose weevil, lesser clover leaf weevil) of the total number of beetles of each species collected were tested by DAS-ELISA after acquisition feeding in order to determine whether the virus was acquired and maintained in the beetle.

Virus transmission to soybean was tested by placing three beetles per plant on V1 soybean plants grown under greenhouse conditions. Cages were constructed as previously described. The number of test plants per species was determined by the number of beetles that could be collected. Beetles were maintained on test plants for one week. Plants were observed daily for feeding injury. Plants showing no injury after one week were not further considered in this study. Plants were maintained for a period of four weeks after beetle feeding was initiated. Plants were them tested by DAS-ELISA for infection by BPMV. Plants testing positive for BPMV by ELISA were tested by RT-PCR as described above in order to confirm results.

Transmission of *Bean pod mottle virus* by Overwintered Bean Leaf Beetles

In order to determine the ability of bean leaf beetles to transmit BPMV after overwintering, beetles were collected from soybean at the FPSRC, Mississippi State

University by sweepnet as described above. Beetle collection was conducted from August to September in each of three study years, 2007-2009. Beetles collected were maintained on BPMV positive soybean leaf tissue for a minimum of 48 hours.

Cages were erected in soybean fields located at the, FPSRC, Mississippi State University and North Mississippi Experiment Station in Verona, MS. Cages consisted of 2m X 2m frames covered with heavy mesh. Bottoms of cages were covered with soil in order to seal them. Soybean plants within the area of the cage were removed and replaced with soybean plants grown under greenhouse condition which had been mechanically inoculated and tested positive for BPMV. Four BPMV-infected plants were included per cage. This was done in order to insure that beetles had access to feeding material and were viruliferous prior to entering diapause. Leaf litter from forested field borders was collected, and a minimum of 3 inches depth of litter was added to each cage. Two cages were erected in each location in 2007 and 2008. Four cages were erected in each location in 2009. Due to variation in numbers of beetles, different numbers of beetles were placed in cages in each study year. In 2007 and 2009, 500 beetles were placed in each cage for a total of 2000 beetles in 2007 and 4000 beetles in 2009. Three hundred beetles were placed in each cage in 2008 for a total of 1200.

Leaf litter and soil was collected from cages in the following months. Leaf litter from one cage from each location (2 in 2009) was collected in mid-February, and remaining cages were collected in early March. Beetles were collected from leaf litter by hand sorting and passing material through a fine sieve. Beetles collected were maintained in plastic containers for 24 hours to ensure that they were alive and healthy. After 24 hours, beetles were placed on V1 soybean plants grown under greenhouse conditions. Cages used in this study were constructed as previously described. Beetles

were maintained on test plants for one week. Plants were observed daily for beetle feeding, and those not showing feeding damage were excluded from further consideration in this study. After beetle feeding, plants were maintained in the greenhouse for four weeks. Plants were tested by DAS-ELISA for infection by BPMV as previously described.

Results and Discussion

In a survey of potential alternative host plants surrounding soybean fields, a total of 1020 plants belonging to 34 botanical species were collected from the edges of soybean fields and tested for infection by BPMV by DAS-ELISA. None of these samples tested positive for BPMV. ELISA of horse nettle (*Solanum carolinensis*) and Lyre-leaf sage, (*Salvia lyrata*) samples gave a positive result. However, RT-PCR tests of all positive samples from these species were negative, indicating that results in ELISA were false. False positives in ELISA for *Solanum carolinensis* were also noted from samples collected in Iowa by Krell et al (2003).

Mechanical inoculation of soybean and *Fabaceae* native to Mississippi resulted in eight species positive by both ELISA and RT-PCR (Table 4). Species found to be positive after mechanical inoculation included three species in the genus *Desmodium*, (*D. paniculatum*, *D. perplexum* and *D. cuspidatum*). Members of this genus have been found be natural hosts of BPMV (*D. paniculatum* and *D. canadense*) (Pitre 1970, Krell 2003). Two clover species, *Trifolium reflexum* and *T. lappaceum*, were also found to be susceptible to BPMV when mechanically inoculated. *Crotolaria ochraleuca* (slender leaf rattlebox), *Aeschynomene indica* (Indian joint vetch), and *Vicia lathyroides* (spring vetch) were also positive for BPMV after mechanical inoculation. These species were further

tested to determine if they could be infected by feeding of viruliferous beetles and if they could serve as a source for beetles to acquire the virus for transmission to soybean and/or other hosts.

Table 4 Results of mechanical and vector transmission tests for species found to be positive for BPMV after mechanical inoculation.

Plant Species	ELISA 1	ELISA 2	ELISA 3	PCR	Vector Feeding	Vector transmission (cumulative) tested/positive
Glycine max	POS	POS	POS	POS	Y	7/4
Desmodium perplexum	POS	POS	POS	POS	Y	16/1
Desmodium cuspidatum	POS	NEG	POS	POS	Y	18/0
Desmodium paniculatum	POS	POS	N/A	POS	Y	10/0
Crotolaria ochroleuca	POS	POS	N/A	POS	Y	9/0
Vicia lathyroides	POS	POS	POS	POS	Y	9/0
Aeschynomene indica	POS	NEG	POS	POS	Y	17/0

In order to study if these plants could be naturally infected under field conditions, it was first necessary to determine if bean leaf beetles would feed on them. Beetles placed on single leaves/leaflets in a Petri dish were observed feeding on leaf tissue for all species. While feeding on most plants was observed after 24 hours, feeding on *Aeschynome indica* was observed only after 48 hours, potentially indicating that this plant is a less favored host for the beetle.

Beetles caged on plants in the greenhouse were observed feeding on leaf tissue after 24 hours. After being maintained in the greenhouse for four weeks, positive results in DAS-ELISA and PCR were obtained from the soybean plants included as a positive control, *Desmodium cuspidatum* and *Desmodium perplexum* (Table 4). Species within the genus *Desmodium* have been previously shown to be natural hosts of BPMV, and

consideration of the potential for further species in this group to serve as natural hosts is warranted. Unfortunately, it was not possible to find naturally occurring examples of these two *Desmodium* species adjacent to soybean fields, and so testing to determine their role as natural hosts is incomplete. Retesting of all of these species at larger sample sizes may provide positive results as efficiency of transmission may be very low. It may also be warranted to determine if these plant species may be host to other beetle species known to transmit BPMV as well as if virus transmission occurs through their feeding. Notably, other vectors of the virus have been observed on plants of the genera *Desmodium* and *Trifolium* (Giesler et al. 2002), and may have a more important role in virus epidemiology than is currently understood.

Bean leaf beetles fed on infected leaf tissue of the above species were placed on caged soybean plants to determine if they may serve as a source for beetles to acquire the virus. After four weeks, soybean plants were tested by ELISA. It was shown that beetles fed on BPMV-infected soybean, included as a positive control, *Desmodium perplexum*, and *Trifolium reflexum* then removed and caged on healthy soybean plants transmitted BPMV to soybean test plants. The transmission of BPMV obtained from *D. perplexum* is significant as it is further evidence that this species may serve as a natural host for the virus. Transmission of virus obtained from *T. reflexum* would only be important under natural conditions should the plant be shown to be inoculated by beetle feeding.

The growth habit of potential alternative host plants should be considered when evaluating their role as inoculums sources for BPMV. *Aeschynomene indica* and *Crotolaria ochroleuca*, are annual herbaceous species which are present in the fall, though *A. indica* is occasionally perennial (http://database.prota.org, Radford et al. 1968). These plants species have the potential to serve as late season hosts to bean leaf beetles

and BPMV but will not persist to the following spring. In contrast, *Vicia lathyroides* and *Trifolium lappaceum* are present from April to mid-summer (Radford et al. 1968), which may allow them to serve as early season hosts, while not being maintained through the fall. Of more potential interest as a potential source of inoculums, *Trifolium reflexum* is a biennial species (Radford et al. 1968), which may allow infected plants to persist from one season to another. Similarly, both *Desmodium cuspidatum* and *Desmodium perplexum* are perennials (Radford et al. 1968) and plants infected in one season may serve as sources for inoculums in the following year.

Beetles collected from soybean and clover were found to readily feed on soybean leaf tissue when placed on a single leaflet in a Petri dish. Due to variation in success of beetle collections, the same number of samples could not be tested for all beetle species. As identification of white-fringed beetles to species is difficult and highly specialized, they are included in this study solely by the generic name *Naupactus* sp.

Transmission of BPMV was not observed in either Dectes stem borer (*Dectes texanus texanus*) or white-fringed beetle (*Naupactus* sp.) which were collected in production soybean fields in Noxubee County, MS. Transmission of BPMV was observed from the alfalfa weevil, *Hypera postica* (Table 5). Notably, this positive result in ELISA was comparable to positive controls used in the study indicating that titer of the virus in the plant was similar between plants inoculated by feeding by bean leaf beetle and alfalfa weevil. Symptoms were readily apparent on the test plant. Positive results in ELISA were confirmed by RT-PCR. Given the apparent low efficiency of transmission, it would be valuable to expand this study to consider a larger number of individuals of each beetle species. This may reveal that species previously not known as BPMV vectors could contribute to its spread in the field, although likely not at high levels of efficiency.

The alfalfa weevil, *Hypera postica* (Gyllenhal), is a member of the family Curculionidae. It is a 5-6mm long beetle with brown coloration, bearing a dark stripe down the dorsal side of the thorax and abdomen. Although alfalfa weevils aestivate through the majority of the summer months (ipm.ncsu.edu/ag271/forages/alfalfa_weevil.html), their activity early in the soybean growing season and towards its end make it possible that this species plays a role in virus overwintering and establishment. However, as they primarily inhabit clover fields, this would only be likely should a species of the genus *Trifolium* be found as a natural host of the virus. As two species of clover present in Mississippi (*T. reflexum* and *T. lappaceum*) were identified as being positive for BPMV after mechanical inoculation, but not after feeding by bean leaf beetle, the principle vector, it would be valuable to further examine the role of alfalfa weevil as a vector of BPMV on these species.

Table 5 Transmission of BPMV by potential vector species collected from soybean and clover fields in Noxubee and Oktibbeha county Mississippi. Number of samples tested refers to individual plants with each being exposed to three beetles.

Beetle Species	Common Name	Vector Transmission (cumulative) tested/positive
Hypera postica	**Alfalfa weevil**	**14/1**
Dectes texanus texanus	Dectes stem borer	8/0
Naupactus sp	White fringed beetle	8/0
Naupactus cervinus	Fuller rose weevil	6/0
Hypera meles	Clover head weevil	14/0
Hypera nigirostis	Lesser clover leaf weevil	6/0
Sitona lineatus	Pea leaf weevil	13/0
Myochrius denticollis	Southern corn leaf beetle	7/0
Cerotoma trifurcata	**Bean leaf beetle**	**6/2**

In a test of BPMV transmission by overwintered bean leaf beetles, insects were obtained from both test locations and were caged on soybean plants in the greenhouse. A total of 238 beetles were collected from field cages. Of these, 167 beetles (70.2%) fed on test soybean plants. DAS-ELISA of plants on which beetle feeding occurred showed no transmission by overwintering beetles.

Survival of overwintering beetles in the second and third study years was exceptionally poor. Negative weather conditions in the second year, including high rainfall in the fall of 2009 and spring of 2010 led to difficulty in collecting beetles high mortality. Only 14 beetles collected from the four cages erected in that study year. Similarly, beetle mortality in the 2010-2011 overwintering trial was almost complete. Only 6 beetles were collected despite expanding the number of cages to 4 per location. Of the beetles collected in these study years, all successfully fed on caged soybean plants under greenhouse conditions. However, no transmission of BPMV was detected by ELISA of the test plants.

In this study, over the course of three years, a total of 187 overwintered beetles were given access to soybean plants grown under greenhouse conditions. None of these beetles were found to transmit the virus. Previous studies have shown successful transmission of the virus by overwintered beetles. A study in Correctionville, IA indicated that 1.6% of overwintered beetles successfully transmitted BPMV, however, no beetle transmission was observed at several other locations within the same study (Krell et al. 2004). A higher rate of overwintering transmission was reported by Walters et al. in 1972 in a study carried out in Arkansas and presented as a conference paper. However, as no full paper has been published resulting from that study, it is not possible

to determine the methods used to collect beetles or evaluate transmission making this work impossible to repeat.

It has been suggested that virus aging may have an effect on beetle transmission of BPMV. In particular, damage to the small coat protein subunit may make the virus nontransmissible by beetles (Krell et al. 2003). This may account for the very low efficiency of transmission by overwintered bean leaf beetles. It is also possible that specific environmental conditions experienced during overwintering are required to allow virus to be transmissible in the spring. Similarly, it may be necessary that virus be acquired by the beetles by feeding on shoots or roots underneath the leaf litter. Regardless of these factors, it is possible that even a low level of transmission may be significant in the establishment of BPMV field infections (Giesler et al. 2002). Due to these factors, transmission of the virus by overwintering beetles merits further consideration. In particular, it may be interesting to test transmission by other beetle vectors after overwintering or while present in weed plants surrounding soybean fields.

Conclusion

In this study, a survey was conducted of plants surrounding soybean fields but no natural hosts of BPMV were determined. Mechanical inoculation showed 7 previously unreported species to be potential alternative hosts, especially as all of these species were determined to also serve as potential hosts of the principal vector. Of these species, only one, *Desmodium perplexum*, was shown to be positive for BPMV following feeding by viruliferous beetles. Bean leaf beetles fed on infected tissue from *D. perplexum* and *Trifolium reflexum* were shown to successfully transmit BPMV to soybean. Other species in the genus *Desmodium* (*D. paniculatum*, *D. canadense*, and *D. illinoense*) have

been shown to be hosts of BPMV either under laboratory or field condition (Pitre 1970, Krell 2003, Bradshaw et al. 2007). As such, this genus merits particular attention in studies related to the role of wild plant species in the epidemiology of BPMV.

Of the beetle species tested as potential vectors of BPMV, one species, the alfalfa weevil, *Hypera postica*, transmitted the virus to 1 of 14 plants. This beetle was collected from clover, *Trifolium sp*. Species in the genus *Trifolium* were shown to be susceptible to BPMV by mechanical inoculation in this work as well as in previous studies (Skotland 1958). Further testing of the ability of this vector to transmit BPMV to species within the genus *Trifolium* is warranted. This may be of particular interest given the planting of crimson clover along roads in many areas of Mississippi.

Transmission by overwintered bean leaf beetles was not observed in this study. Mortality of captive beetle populations severely limited testing in two of three study years. Given the low reported efficiency of overwintered beetles in transmitting the virus, it is probable that overwintered beetles play a role in virus establishment although that was not observed in this study. The role of other vector species in overwintering of the virus should also be investigated.

References Cited

Bradshaw, J.D., M. E. Rice, J. H. Hill. 2007. No-choice preference of Cerotoma trifurcata (Coleoptera: Chrysomelidae) to potential host plants of Bean pod mottle virus (Comoviridae) in Iowa. Journal of Economic Entomology. 100 (3):808-814.

Fulton, J. P., and H. A. Scott. 1974. Virus vectoring efficiencies of two species of leaf feeding beetles. Proceedings of the American Phytopathological Society. 1:159.

Giesler, L., S. Ghabrial, T. Hunt, and J. Hill. 2002. *Bean pod mottle virus*: a threat to U.S. Soybean Production. Plant Disease. 86:1280-1289.

Gu, H., A. J. Clark, P. B. de Sa, T. W. Pfeiffer, S. Tolin, and S. A. Ghabrial. 2002. Genetic diversity among isolates of *Bean pod mottle virus*. Phytopathology. 92: 446-452.

Herzog, D. C. 1968. Seasonal, locational, and sex variation in the color pattern of the bean leaf beetle, *Cerotoma trifurcata* Forst., in Louisiana. M.S. Thesis. Louisiana State University, Baton Rouge, LA. 176 pp.

Hopkins, J. D., and A. J. Mueller. 1984. Effect of bean pod mottle virus on soybean yield. Journal of Economic Entomology. 77:943-947.

Horn, H. L., L. D. Newsom, R. G. Carver, and R. L. Jensen. 1970. Effects of virus diseases on soybeans in Louisiana. Louisiana Agriculture. 13:12-15.

Horn, N. S., L. D. Newsom, and R. L. Jensen. 1973. Economic injury thresholds of bean pod mottle and tobacco ringspot virus infection of soybeans. Plant Disease Reporter. 57:811-813.

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 Disease. 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. Journal of Economic Entomology. 97:192-202.

Lin, M. T., and J. H. Hill. 1983. Bean pod mottle virus: Occurrence in Nebraska and seed transmission in soybeans. Plant Disease. 67:230-233.

Mabry, T. R., H. A. Hobbs, T. A. Steinlage, B. B. Johnson, W. L. Pedersen, 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. P1ant Disease. 87:1221-1225.

Moore, B. J., H. A. Scott, and H. J. Walter. 1969. *Desmodium paniculatum,* a perennial host of bean pod mottle virus in nature. Plant Disease Reporter. 53:154-155.

Patel, V. C., and H. N. Pitre. 1971. Transmission of bean pod mottle virus to soybean by the striped blister beetle, *Epicauta vittata*. Plant Disease Reporter. 55:628–629.

Perring, T. M., N. M. Gruenhagen, and C. A. Farrar. 1999. Management of plant viral diseases through chemical control of insect vectors. Annual Review of Entomology. 44:457-481.

Pitre, H. N. 1970. Insect and experimental transmission studies in relation to bean pod mottle virus disease of soybeans. Mississippi Farm Research. 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. Journal of Entomological Science. 24:582-587.

Radford, A. E., H. E. Ahles, and C. R. Bell. 1968. Manual of the Vascular Flora of the Carolinas. The University of North Carolina Press. Chapel Hill, NC. 1183pp.

Rice, M. E., J. D. Bradshaw, and J. H. Hill. 2007. Revisiting an integrated approach to bean leaf beetle and bean pod mottle virus management. Integrated Crop Management. 498 (3):87-88.

Ross, J. P. 1963. Transmission of bean pod mottle virus in soybeans by beetles. Plant Disease Reporter. 47:1049-1050.

Schwenk, F. W., and C. D. Nickell. 1980. Soybean green stem caused by bean pod mottle virus. Plant Disease. 64:863-865.

Skotland, C. B. 1958. Bean pod mottle virus of soybeans. Plant Disease Reporter. 42:1155-1156.

Stace-Smith, R. 1981. Comoviruses, pp. 171-194. *In* Kurstak, E. [ed.], Handbook of Plant Virus Infections and Comparative Diagnosis. Elsevier/North-Holland Biomedical Press, New York, NY.

Waldbauer, G. P., and M. Kogan. 1976. Bean leaf beetle: Phenological relationship with soybean in Illinois. Environmental Entomology. 5: 35-44.

Walters, H. J. 1958. A virus disease complex in soybeans in Arkansas. (Abstr.) Phytopathology. 48:346.

Walters, H. J. 1964. Transmission of bean pod mottle virus by bean leaf beetles (Abstr.) Phytopathology 54:240.

Walters, H. J. 1970. Bean pod mottle virus disease of soybeans. Arkansas Farm Research. 19:8.

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.

Werner, B. J., R. K .Krell, L. P. Pedigo, and J. H. Hill. 2003. The soybean leaf miner (Coleoptera: Chrysomelidae) as a vector of bean pod mottle virus. Journal of the Kansas Entomological Society. 76:643-644.

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 Disease. 91 (5):637.

Zaumeyer, W. J., and H. R. Thomas. 1948. Pod mottle, a virus disease of beans. Journal of Agricultural Research. 77:81-96.