Bean pod mottle virus: Spatial and temporal dynamics at different spatial scales and the impact of time of infection on soybean yield and quality

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Bean pod mottle virus: Spatial and temporal dynamics at different spatial scales and the impact of time of infection on soybean yield and quality

by

Emmanuel Byamukama

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Plant Pathology

Program of Study Committee:
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Iowa State University
Ames, Iowa
2008

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DEDICATION

To my late father Mr. Evaristo Nzabarinda, who raised me and sacrificed the little he had to provide for my education, but passed away when I was just six months into this program, and to my wife Agatha and our precious children, Ian, Lisa and Krista.
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ABSTRACT

A comprehensive state-wide soybean disease survey was carried out during the 2005-2007 growing seasons to determine seasonal and geospatial patterns of soybean diseases in Iowa. *Bean pod mottle virus* (BPMV) was one of the viruses assessed in the survey. To quantify BPMV prevalence and incidence at the field and county scales, 30 soybean plants per field were sampled from 8-20 soybean fields/county using a systematic sampling design. The GPS coordinates for each soybean field sampled were recorded. Soybean plants were tested for the presence of BPMV using enzyme linked immunosorbent assay (ELISA). Field- and county-scale BPMV prevalence and incidence data were mapped using ArcGIS (ESRI, Redlands, CA). County-scale prevalence of BPMV was 40.6% in 2005, 90.1% in 2006, and 74.7% in 2007. On the field basis, the prevalence of BPMV in soybean fields was 9.5%, 40.4% and 27.4% for 2005, 2006, and 2007, respectively. BPMV incidence within soybean fields from 2005-2007 was 4.4%, 24.8%, 9.8%, respectively. Moran’s I analysis revealed significant spatial dependence for BPMV incidence among counties, indicating a nonrandom distribution of BPMV among Iowa counties. Based on GIS point maps for the three growing seasons, kriged maps revealed an increased risk for BPMV from the southern border of the state to the northern border of the state. Regression analysis for relationship between BPMV incidence and county latitude indicated a strong linear relationship with BPMV incidence increasing from 0.24 to 2% for every 10 km increase in latitude during the 3-year study. To quantify the temporal and spatial spread of BPMV within soybean fields, 30-cm-long quadrats were established within field plots of the soybean cultivar NE3001 (150 quadrats per plot) in 2006 and 2007. Quadrats were sampled every 8-11 days throughout each growing season, beginning 25 days
after planting. Sap from the youngest, fully-expanded leaflet from each of the four plants per quadrat was group tested for the presence of BPMV using ELISA. To determine the relationship between day of BPMV detection and soybean yield, 35 soybean quadrats representing each BPMV detection time were randomly selected to obtained yield, yield components, and grain quality data. For sampling dates with < 35 quadrats that were BPMV positive, all quadrats were harvested to obtain yield data. Yield, yield components, and grain quality data from quadrats were then plotted with respect to day of year that BPMV was first detected in a quadrat, and linear regression was used to quantify the relationships between time of BPMV detection with yield, yield components (number of pods per plant, seeds per pod, and 100-seed weight), and grain quality (percent mottled seed, protein content and oil content) (y). BPMV was first detected in quadrats as early as the first sampling date (30 May 2006 and 12 June in 2007). The onset of BPMV epidemic (5% BPMV incidence) occurred on 29 June 2006 and on 18 July in 2007. Time to 50% BPMV incidence ranged from day of year 207 (26 July) to 215 (3 August) in 2006. In 2007 time to 50% occurred well past senescence. Final BPMV incidence was between 85.8 and 94.4% in 2006 and between 10.0 and 37.1% in 2007. In 2006, temporal rates of BPMV infection ranged from 0.09 to 0.12 logits/day with R² values ranging from 97.8% to 98.3%, indicating that BPMV incidence within soybean plots doubled every 5.3 to 6.9 days. Rates of BPMV temporal spread in 2007 were significantly slower (0.05 to 0.07 logits/day, R² values ranged from 91.6% to 99.7%), with doubling times ranging from 13.8 to 17.3 days. Plots with the earliest onset of BPMV had the highest BPMV incidence at the end of the growing season and the largest areas under the BPMV progress curves in both 2006 and 2007 (the slope for both years was 1.1 and R² values of 65.4% and 76.9%, respectively). Spatial analyses using ordinary runs revealed that
BPMV-infected quadrats had spatial patterns that were highly aggregated throughout both growing seasons. Time of BPMV detection explained 89.7% and 57.9% of the variation in soybean yield in 2006 and 2007, respectively. The yield damage function (slope) was -0.23 bushels/acre/day in 2006 and -0.12 bushels/acre/day, indicating that for every 4.3 and 8.3 days BPMV detection was delayed, in 2006 and 2007, respectively, soybean yield would increase by one bushel. The linear relationship between number of pods per plant and time of BPMV detection was significant in 2006 (slope = 0.15, $R^2 = 72.8\%$), but not in 2007. The number of seeds per pod was not impacted by time of BPMV detection in either year, and 100-seed weight was impacted only in 2006 (slope = 0.013, $R^2 = 78.5\%$). There was a significant linear relationship between time of BPMV detection and the percentage of mottled seeds in the two years (slope = 0.34, 0.15; $R^2 = 82.8$, 48.3%); earlier BPMV detection was associated with a higher percentage of mottled seeds. Time of BPMV detection in a quadrat did not influence protein and oil content in either year. This research was the first to document the nonrandom distribution of BPMV prevalence and incidence in Iowa, and the first to show that BPMV spread within soybean fields is highly aggregated over time, which has important implications for yield losses and BPMV sampling designs. This project was also the first to quantify the relationship between BPMV date of detection and reduction in yield and impact on yield components and soybean quality.
CHAPTER 1.  
GENERAL INTRODUCTION

Thesis Organization  
This Thesis is divided into five chapters. The first chapter, General Introduction, reviews literature on history, importance, transmission of *Bean pod mottle virus* (BPMV), and justification for this study. The second is on occurrence, distribution and spatial dependence of *Bean pod mottle virus* in Iowa. The third chapter describes the temporal and spatial dynamics of *Bean pod mottle virus*. The fourth chapter quantifies yield losses associated with time of BPMV detection. The last chapter is a summary and general conclusions of this Thesis. The references associated with a chapter are cited at the end of each chapter.

Literature Review  
**History and Occurrence of Bean Pod Mottle Virus**

*Bean pod mottle virus* (BPMV) was first reported in the United States on *Phaseolus vulgaris* cv. Tendergreen in South Carolina in 1945, and was first found to occur on soybean in Arkansas and North Carolina in 1948 (105, 113). To date, BPMV has been reported in most United States soybean-producing states (22, 30, 31, 47, 61, 64, 88, 89, 121). Although BPMV originally was thought to occur primarily in the southern United States, BPMV is now believed to be approaching epidemic levels in the North Central states (106).

Worldwide, BPMV has been reported to occur in Ecuador (122), Peru (25), Brazil (4), Nigeria (112), Canada (73), and more recently Iran (103). It is not known whether the occurrence of BPMV in other countries is due to indigenous strains or if BPMV was
introduced through the movement of BPMV-infected seed (31). It is possible that BPMV may occur in other countries from which it has not yet been reported.

**Importance of BPMV**

**Impact of BPMV on soybean yield and yield components.** *Bean pod mottle virus* has been reported to have a number of negative impacts on soybean yield and yield components. The first study to quantify the effects of BPMV on soybean yield was conducted in 1969 in North Carolina (95). In that study, BPMV was found to cause 13 and 40% yield reductions on cultivars Hill and Lee, respectively. Other studies involved attempts to quantify yield losses due to BPMV, primarily by mechanically inoculating soybeans at various growth stages (46, 78, 95, 110, 118). Soybean yield reductions were much greater (80%) when soybean plants were both mechanically inoculated with SMV and BPMV. A study conducted in Mississippi revealed that BPMV infection was more detrimental to soybean yield than imposed levels of soil water stress (78). Across all water regimes, BPMV-infected plants had significantly less total dry matter and fewer pods per plant compared to healthy soybean plants; however, plant height, number of seeds per pod, and seed weight were not affected by BPMV. Yield losses due to BPMV on four soybean cultivars inoculated at the primary leaf stage (VC) was reported to range from 23 to 44% in Kentucky (110). In Arkansas, Hopkins & Mueller (46) investigated the effect of timing of BPMV inoculations on soybean yield. Subplots with fifty soybean plants were mechanically inoculated at various soybean growth stages from V1 to R6, and the greatest yield loss (52.6%) was recorded on cultivar ‘Bragg’ when soybean plants were inoculated at the V1 growth stage. Time of BPMV inoculation also significantly reduced the number of pods per soybean plot and the
earlier that soybean plants were inoculated, the greater was the reduction in pods per plant. The major criticism of this study was that all soybean plants were mechanically inoculated at a single point in time, whereas such high BPMV incidence (approaching 100%) resulting from BPMV inoculation at one point in time is not likely to occur under natural field conditions (71).

In North Carolina, Ross (97) measured the yield response of three early- and late-planted soybean cultivars in which all plants within a plot were either mechanically-inoculated, screen-caged, or left exposed to natural infection by bean leaf beetles (Cerotoma trifurcarta Foster). He found that BPMV caused yield reductions ranging from 2.3 to 19%. Delay of BPMV infection by caging plants improved soybean yield by 3.4 to 11.6%. However, the effect of caging on reducing light interception (and yield) was not accounted for in these experiments. Moreover, inoculations were once again performed at one point in time (the VC growth stage), which is highly atypical of what actually occurs under natural field conditions.

Windham and Ross (118) found that the incidence of BPMV in early and late-planted (6 weeks apart) soybean cultivars (Ransom and Centennial) was 20% higher in the taller soybean lines (120 cm) located adjacent to relatively short (85 cm) lines at 4 weeks after planting, but BPMV incidences was similar in both short and tall lines during the season. They also attempted to quantify yield compensation from neighboring non-BPMV-infected soybean plants by inoculating soybean plants within rows such that the three adjacent to inoculated plants were left healthy. Individual symptomless Centennial and Ransom soybean plants adjacent to BPMV-infected plants yielded 50% and 16% more than healthy plants growing adjacent to other healthy plants, respectively. Although this is the only published
study focusing on the yield compensation that might result from healthy plants adjacent to BPMV infected plants, it has limitations in that all inoculations were performed at one point in time. Thus, the effect of time of BPMV infection and the potential benefits of yield compensation is still not well documented.

**Impact of BPMV on soybean quality.** Mottling of soybean seed coats has been associated with BPMV infection (43, 125). Hobbs and co-workers (43) reported that soybean plants infected with BPMV alone, or in combination with SMV produced mottled seed. Ziems et al. (126) investigated the response of soybean to BPMV and found that plots that had been inoculated at the VC growth stage had significantly higher mottled seed (%) compared to non-inoculated soybean plots. However, mottled soybean seed does not necessarily mean the seeds are infected with BPMV. Hill (41) tested two seed lots for BPMV infection, one with mottled seed coat and the other without mottled seed coats (normal), and found no differences in the relative antigen levels of BPMV in the two seed lots, indicating that seed coat mottling could result from causes other than BPMV. To further support the possible role of other stresses, a number of environmental (abiotic) stresses have also been reported to cause seed coat mottling (32, 76). Off-colored seed (>10% discoloration) is a seed quality rating factor that can reduce the market grade (and price) of soybean grain (104). One possibility as to the mixed results concerning the presence/absence of BPMV infection and seed coat mottling is the time of inoculation (growth stage) which may be playing a role in the presence/absence of seed coat mottling. The effect of time of BPMV infection on soybean seed mottling, however, has not been investigated.

**Co-infection of BPMV with SMV.** *Bean pod mottle virus* interacts with *Soybean mosaic virus* (SMV) synergistically, resulting in more severe symptoms and greater yield
losses than the losses due to either virus infection alone (3, 14). Studies have shown that the
titer of BPMV is enhanced by SMV co-infection, irrespective of time of inoculation,
inoculation sequence, and/or method of virus inoculation (14, 95). Enhanced symptom
severity of BPMV by SMV has been linked to the helper component protein (HC-Pro) of
SMV. Furthermore, BPMV symptoms were was most severe in the transgenic line expressing
the highest level of HC-Pro and mildest in a non-transformed soybean line (63).

**Other diseases associated with BPMV.** *Phomopsis* seed infection has been
associated with BPMV infection, as evidenced by *Phomopsis* infection being five times
higher on BPMV-inoculated plants than on non BPMV-inoculated plants (110). A delay in
the dry-down period of BPMV-infected soybean plants has also been associated with
increased incidence of *Phomopsis* in BPMV infected plants (1). *Soybean mosaic virus* is also
known to cause increased *Phomopsis* infection (53).

Another effect of BPMV on soybean is known as “soybean green stem disorder”,
With this disorder, soybean stems remain green even after pods and seeds have reached
maturity (33, 44). Due to uneven drying of the soybean plants, green stem disorder interferes
with grain harvest. Schwenk and Nickel (101) sampled plants with green stem disorder
symptoms and tested them for BPMV and found that not all soybean plants that had green
stem disorder tested positive for BPMV. Goh (33) reported that only BPMV inoculation of
soybean with a severe BPMV strain at an early growth stage (V2-V4) could cause green stem
disorder, but this hypothesis has not been tested. However, Hobbes et al. (44) showed that
plants that had BPMV did not necessarily develop green stem disorder symptoms, nor did all
plants with green stem disorder test positive for BPMV. Water stress and green stink bug
*Nezara viridula* (Linnaeus) feeding have also been reported to be associated with green stem
disorder (6, 40). The real cause of green stem disorder is still unknown, but BPMV is speculated to be a contributing factor. Additional studies are needed to determine if time of BPMV infection has an impact on the occurrence of green stem disorder.

**Classification, Genome Organization and Properties of BPMV**

*Bean pod mottle virus* belongs to the genus *Comovirus* in the family *Comoviridae* (93). Virus particles in the *Comoviridae* are icosahedral in symmetry, non-enveloped, and are approximately 28 to 30 nm in diameter (66). *Bean pod mottle virus* has a bipartite positive sense strand genome that consists RNA-1 and RNA-2. The two genome segments are separately encapsidated in 28-nm diameter isomeric particles (35). Separation of segments can be achieved by density gradient centrifugation into three components: top (T), middle (M), and bottom (B). The middle component contains a single RNA1 molecule, whereas the bottom component has RNA2 and the top particle lacks nucleic acid (98). Di et al. (20) (1999) reported that RNA1 has approximately 6000 base pairs (6 kb), whereas RNA2 has 3,600 base pairs (3.6 kb) (69). Both RNAs are polyadenylated and have a small basic protein, and a viral genome-linked protein (VPg), that is covalently linked to their 5’ termini (65, 98). From the 5’ to 3’, RNA-1 codes for five mature proteins that are necessary for replication: protease cofactor (32K), a putative helicase (58K), a viral genome-linked protein (VPg), a protease (24K), and a putative RNA-dependent RNA polymerase (RdRp)(87K) (98).

Proteinase and putative helicase have been reported to be determinants of BPMV symptom development (35). The RNA-2 component codes for putative cell-to-cell movement protein and two coat proteins, large (L) and small (S) (20, 69). *Bean pod mottle virus* is heat stable,
with a temperature inactivation point of 70°C. Its dilution end point in fresh plant extract is 10,000 and its longevity in vitro varies from 62 days at 18°C to 93 days (13, 121).

**Host range of BPMV.** The host range of *Bean pod mottle virus* is restricted to legumes (66). After its discovery on common bean, BPMV was shown to be readily transmitted mechanically to several cultivars of snap and dry beans. In an experimental host range study, 25 species representing 20 plant genera were evaluated for susceptibility to BPMV, including soybean (*Glycine max*) (121). While a number of host species have been found to be susceptible to BPMV by mechanical inoculation, only four have been found to be naturally-infected with BPMV: *Desmodium canadense* (47), *D. paniculatum* (L.) (75) *Glycine max* (L.) (105), and *Phaseolus vulgaris* L. (121). Krell et al. (54) tested 23 plant species for their ability to serve as alternative hosts of BPMV in Iowa, but only one, *D. canadense*, was re-confirmed to be a naturally-occurring alternative host of BPMV. Another species, *Desmodium illinoense* (Gray), was recently reported to also be an alternative host of BPMV in Iowa (11). Other BPMV alternative hosts reported elsewhere in the U.S. when mechanically-inoculated with BPMV include *Glycine soja, Lespedeza cuneata, L. striata, L. stipulacea, Phaseolus acutifolius, P. lunatua, P. vulagris*, and *Stizolobium deerunguiculata* (27, 54).

**BPMV symptoms.** Symptoms elicited by BPMV vary from species to species and from virus strain to virus strain. On *Phaseolus vulgaris* BPMV causes a mild-to-severe mottling, and malformed leaves and pods, whereas on soybean, BPMV causes mottling that may lead to leaf rugosity as well as mottling symptoms on seed coats. On soybean, BPMV symptoms are most conspicuous on young leaves. While BPMV causes pod mottling on
snap beans, mottled pods are not common on soybean (31). On *Desmodium paniculatum*, BPMV causes primarily leaf mottling (13, 66, 97).

**Diversity of BPMV isolates.** Diversity among strains of BPMV has been reported. Working with BPMV field isolates from four states Gu et al. (34), found two genetically distinct BPMV subgroups (categorized as subgroups I and II). This classification of BPMV strains was based on nucleic acid hybridization analysis using cloned cDNA probes to RNA-1 from BPMV strains K-G7 and K-Ha1 (34). Giesler et al. (31) proposed that isolation of BPMV reassortants (mixing of RNA 1 or 2 from one subgroup to another) coincided with the recent increase of BPMV incidence and may be responsible for the severe BPMV symptoms that have not been observed more recently. Gu et al. (36) isolated and characterized naturally-occurring partial diploid BPMV reassortant strains (i.e., containing RNA1 from both subgroups I and II) that were diploid for RNA1 and haploid for RNA2. In 43 BPMV isolates representing field isolates from six states (Arkansas, Illinois, Indiana, Kentucky, Mississippi and Virginia), 10 were found to be partial diploid reassortants that caused severe symptoms on soybean plants under both field and greenhouse conditions (36). Furthermore, Zang et al. (123) reported detecting RNA1 recombinants from soybean plants infected with the partial diploid reassortant strain IL-Cb1. In their study, recombinant RNA1 molecules with similar structures to the naturally-occurring recombinant RNA1s were generated in soybean after four inoculations passes, following inoculation with RNA1 reconstructs. The discovery of BPMV reassortants revealed that the enhancement of symptom severity is due to the presence of two distinct RNA1s in the same plant cells. Such occurrences may result from mixed infections brought about by vectors that acquired the mixed strains prior to feeding on healthy plants (29). In Iowa, a naturally-occurring BPMV
reassortant was found on BPMV-infected *Desmodium illinoense* Gray (designated I-Di1), with RNA1 belonging to subgroup I and RNA2 belonging to subgroup II (12). However, the new reassortant did not cause more severe BPMV symptoms on three soybean cultivars (Clark, Essex and Williams). Further molecular characterization and phylogenic analysis of BPMV isolates collected from a soybean field adjacent to the location of *Desmodium illinoense* did not reveal any similarities with this new reassortant (12). However, all BPMV diversity work has shown that subgroup II BPMV strains produce mild symptoms on soybean and these strains are the most prevalent BPMV strains in soybean, and hence, most adapted to this host, which may be a reason that most soybean cultivars mask BPMV symptoms (34, 36, 123).

**Transmission of BPMV and Sources of Initial Inoculum**

*Seed transmission.* Although it’s not known whether BPMV is transmitted to the embryo of soybean seeds (41), the seed coat can harbor BPMV (42). Therefore, seed-to-seedling transmission is thought to take place by entry through damage to the cotyledons and plumule during the early stages of seed germination (31). Several studies have investigated seed-to-seedling transmission using grow-out tests, followed by testing seedlings for the presence of BPMV by ELISA (49, 64). Seed-to-seedling BPMV transmission up to 0.1% levels has been reported (54). For one acre soybean field containing 250,000 soybean seedlings, even a seed-to-seedling transmission level as low as 0.01% can translate into an initial incidence of approximately 25 BPMV-infected plants per acre. Moreover, these 25 infected soybean seedlings would be randomly distributed throughout each acre of soybean field. This level of BPMV incidence would be sufficient to initiate a BPMV epidemic,
especially when bean leaf beetle populations are not limiting virus spread within soybean fields. For other soybean viruses that are seed transmitted, the earlier the plant is infected with a plant virus, the higher the percentage of virus-infected seed for example, Tobacco ring spot virus and Tobacco streak virus (30, 49).

Alternative BPMV hosts. Alternative hosts can serve as reservoir for plant viruses in the absence of a major plant host. Cultivated crops that can server as alternative hosts for BPMV include Phaseolus spp. and Vigna unguiculata cultivars (27). Leguminous weed hosts have also been reported to be reservoirs for BPMV (75). For an alternative host to be an epidemiologically-important source of initial inoculum for BPMV, the alternative host needs also to be a preferred alternative host of the insect vector, as well as a reservoir for the virus (11). Krell et al. (54) sampled and tested 23 potential BPMV alternative hosts and found that only one out of 23 hosts (Desmodium canadense) tested positive for naturally-occurring BPMV. Bradshaw et al. (2007) recently reported that Desmodium illinoinse L. was infected with BPMV and was a preferred host of bean leaf beetles. There is a potential for several alternative hosts of BPMV to be possible sources of BPMV initial inoculum in Iowa. (5).

Insect vector transmission. Acquisition and transmission of BPMV by insect vectors is the predominant means by which BPMV is disseminated within a soybean crop (31). The bean leaf beetle (Ceratoma trinfurcarta Foster) was the first insect vector that was proven to transmit BPMV (94). Patel and Pitre (85) reported striped blister beetle (Epicauta vittata) could acquire and transmit BPMV to soybean. Other beetles reported to acquire and transmit BPMV include the grape colaspis beetle (Colaspis brunnea (Fabricius)) Colaspis lata Shaeffer, the banded cucumber beetle (Diabrotica balteata LeConte), the spotted cucumber beetle (D. undercimpunctata howardi Barber), the striped blister beetle (Epicauta vittata
(Fabricius) (27, 85), the western corn root worm beetle (*Epilachna virgifera* LeConte), (68), the soybean leaf miner (*Odontota horni* [*Xenochalepus horni*]) (116), and the Japanese beetle (*Popillia japonica*) (117). Nevertheless, the bean leaf beetle is considered to be the most important insect vector for BPMV because of its abundance and its efficiency in acquiring and transmitting this virus (31, 45).

**The bean leaf beetle.** The bean leaf beetle (BLB) belongs to the family *Chrysomelidae*, order *Coleoptera*. This insect was first described by Forster in 1771 (23). Bean leaf beetles are native to North America, but were reported to be abundant primarily in the southern part of the U.S. (87). A recent peak in bean leaf beetle populations in Iowa was recorded in Iowa in 2002 (54), with populations as high as 201 beetles per 50 sweeps with a sweep net. Bean leaf beetle populations can damage emerging seedlings, defoliate leaves, and damage pods, thereby providing an infection court for other pathogens to enter. Bean leaf beetle larvae can also burrow through roots, which adversely affects water and nutrient uptake (87).

Other beetles in the *Chrysomelidae* family have been reported to acquire and transmit BPMV (26, 38). Despite the apparent diversity of beetle-vectored viruses, they do share a lot in common. For example, all beetle-vectored viruses have single-stranded RNA, are easily transmitted mechanically. Viruses in this group are also relatively stable, highly antigenic and they often occur in high concentrations within plant tissues (26).

The bean leaf beetle has been reported to be a highly efficient vector, since it can acquire the virus after a single bite from an infected plant. Transmission efficiency, however, has been shown to increases as the acquisition feeding period on diseased plants is increased (26). Wang et al. (114) reported that bean leaf beetles lost BPMV titer over a 4-week period
if they fed on healthy plants post-BPMV acquisition. No latent period has been reported in
the vector for viruses transmitted by bean leaf beetles (26, 114), and virus retention time
were found to increase with the amount of BPMV-infected leaf tissue consumed (18). This
suggests that the virus does not replicate in the beetle (29). Moreover, bean leaf beetles do
not have salivary glands, but a regurgitant factor is thought to be responsible for successful
transmission of beetle transmitted viruses (28). Using a wounding technique, when bean leaf
beetle feeding was simulated by gross wounding of leaf tissue accompanied by inoculation
with purified virus, the transmission efficiencies of both beetle-transmissible and non-beetle
transmissible viruses was achieved (28). When virus inoculum was mixed with beetle
regurgitant, however, only beetle-transmissible viruses had high transmission efficiencies.
The regurgitant factor has recently been reported to be associated with ribonuclease activity
that facilitates virus transmission (77). No reports exist on BPMV transmission by the bean
leaf beetle larvae (45)

**Biology of bean leaf beetles.** Bean leaf beetle adults are small beetles, approximately
5 mm long, and are sub-oval in shape. Beetle color ranges from yellow to red (52). The
most distinguishing character is the presence of four black spots and a prominent black
triangle behind the prothorax (9, 60). Bean leaf beetle eggs are orange, spindle-shaped, and
are approximately 0.8 mm long. Bean leaf beetle eggs are laid in the soil, primarily within
soybean fields. A female beetle is capable of laying 350 eggs a month and eggs hatch after
just 4-5 days (62). The whitish larvae live in soil and feed on the roots of soybean (87, 107).
After three molts, larvae pupate for 7 days before emerging as adult beetles. It takes between
30 to 45 days from egg laying to adult emergence, depending on soil temperature (52). The
number of bean leaf beetle generations in a year varies from region-to-region, with just one
generation per year in Minnesota (67), two generations per year in Iowa (107), Illinois (51) and Nebraska (74), and three generations per year in Arkansas (48) and South Carolina (23, 29).

In Iowa, bean leaf beetles overwinter as adults, primarily in wooded areas (80%), followed by soybean fields (19%), and then corn, alfalfa and grasslands (1%) (60). Overwintering adults migrate to alfalfa and other wild legumes early in the spring (52, 107). Bean leaf beetles eventually migrate to newly emerged soybean seedlings and begin feeding on soybean before laying eggs and then dying in late June (92). The first summer generation of adult bean leaf beetles emerge between late June and mid- to-late July (60). The second summer generation is present from early August until soybean maturation and it is this generation of bean leaf beetles that becomes the overwintering generation (60, 107). Bean leaf beetles return to alfalfa and other legume hosts as soybean plants senesce, and then gradually migrate to the overwintering sites listed above (107).

**Damage caused by bean leaf beetles.** Bean leaf beetles typically consume leaf tissue, resulting in round-to-oval holes in the cotyledon and unifoliate leaves of emerging soybean seedlings (92). Feeding may begin below soil cracks around the emerging seedling, and continue to include the emerged cotyledons, creating small pock marks on the top and/or bottom sides of cotyledons. Fresh feeding sites (less than 12 hours old) are still green and are moist, whereas old feeding sites (more than 2 days old) have feeding scars that are often brown, calloused, and dry (92). Beetles often feed on the edges of young leaves, but small round or oval holes in the middle of a leaf (“bullet holes”) are the most common. The first summer bean leaf beetle generation feeds on trifoliate leaves and does not often cause economic damage, since soybeans are known to have a remarkable capacity to compensate
for early insect injury (39). However, the first summer generation can cause economic
damage if they acquire BPMV. The second summer generation feeds primarily on leaves and
pods and can cause direct economic damage (59, 108) as well as indirect damage resulting
from BPMV transmission.

**Occurrence of bean leaf beetles in Iowa.** Bean leaf beetles were considered as
insignificant pests in the North Central states until 1996 (10, 60). From 1989 to 1996, bean
leaf beetle populations in Iowa were extremely low (91) but beginning in 1997, however,
bean leaf beetle populations in Central Iowa increased steadily each year (54).

One hypothesis for the increase in bean leaf beetle population densities is the mild
winters that occurred during 1997-2002 period. Krell et al. (55) proposed that subfreezing air
temperatures during the winter seasons, could be used as a predictor of early spring bean leaf
population densities prior to spring planting. Lam and Pedigo (58) developed a winter
survival prediction model in which soil surface temperatures from -5\(^0\) to -10\(^0\) C resulted in
significant bean leaf beetle mortality. However, this model was found to underestimate
winter mortality for bean leaf beetles in Minnesota by 34-59\% (15). A revised model
developed by Carrilo et al.(15) incorporated a supercooling critical mortality point, defined
as the temperature at which spontaneous freezing of the bean leaf beetle occurred (120).
Their model was found to predict bean leaf beetle mortality more accurately than the
previous model. This revised model, however, has not been evaluated for its accuracy in
predicting bean leaf beetle winter survival in Iowa.

A second possible reason for increased risk of BPMV in soybeans is related to time of
planting, although inconsistent results have been reported (56). Early planting (and
emergence) of soybeans allows the overwintering bean leaf beetle generation a longer period
of time to acquire and transmit BPMV from BPMV-infected seedlings, and/or alternative hosts. In addition, BPMV-infested bean leaf beetles that survive Iowa winters would also have more time to acquire and transmit BPMV within soybean fields (92). Soybeans are currently being planted approximately 3 weeks earlier by today’s farmers compared to the time of planting soybeans 15 years ago.

Management of Bean Leaf Beetles and BPMV

Despite early reports of BPMV in Iowa (1971) and elsewhere in the North Central region, little has been done in devising effective disease management strategies. In other regions, BPMV research conducted during the 1960s to 1980s concentrated on the evaluation of soybean cultivars for resistance to BPMV (31, 96, 102).

High numbers of bean leaf beetles can cause economic yield loss. Rice (91) estimated two bean leaf beetles per plant at the VC stage as the critical economic injury threshold for overwintering bean beetle leaf populations. This threshold was defined to be between 9 to 48 beetles per 20 sweeps with a sweep net for the first summer generation of bean leaf beetles, depending on the market price for soybean grain.

Foliar insecticide application. Foliar insecticides have been shown to significantly reduce bean leaf beetle population densities (95). Insecticide applications that target the overwintering and first summer bean leaf beetle generations were found to effectively reduce bean leaf beetle population densities (8, 55). However, chemical control of BPMV vectors did not necessarily result in reduced BPMV incidence. For instance, Pedersen et al. (86) found that the application of Warrior insecticide was ineffective in reducing the incidence of BPMV; in fact, plots that were treated with insecticide had higher relative levels of BPMV
antigen in seed (a measure found to be correlated with field incidence of BPMV) than soybean plots that had not been treated with insecticide. Although the in-furrow application of carbofuran 15 G (1.12 kg a.i/ha) at planting significantly reduced the number of bean leaf beetles per plot, seed yield was not significantly different from the non-treated control (74). Bradshaw et al. (8) evaluated the insecticide management program currently recommended for both bean leaf beetles and BPMV in Iowa, including insecticide seed treatments alone or in combination with foliar insecticides that primarily targeted the overwintering generation of bean leaf beetles and/or the first summer generation of bean leaf beetles. He found that although vector populations were reduced, BPMV incidence and BPMV antigen levels in seed were not impacted. To date, the recommended management of BPMV has been solely based on vector population dynamics, and not on BPMV disease dynamics, partly because the temporal dynamics within soybean fields has not been quantified.

Seed treatment. Systemic insecticide seed treatments using imidacloprid (Gaucho®) and or thiamethoxam (Cruiser®) have been found to effectively control bean leaf beetles on snap beans in Minnesota (50), but their impact on BPMV incidence has not been assessed. A limitation of seed treatment for BPMV management is that insecticide is deployed prior to the colonization of soybean fields by bean leaf beetles, in some years.

Planting date. Altering the date of planting date to manage bean leaf beetles was reported to be effectively suppress the overwintering generation of bean leaf beetles because beetles had insufficient time to lay eggs before adult beetles died (56). Early-planted soybeans in Nebraska were reported to have higher overwintering bean leaf beetle population densities than late-planted soybeans (74). This practice has potential disadvantages, including
increased risk for other pests and diseases, poor synchrony with spring rain, and decreasing yield potentials as planting date is delayed (56)

**Host resistance.** Use of host resistance is one of the least expensive and most sustainable means to manage plant diseases (81). Two soybean germplasm lines, HC95-15MB and HC95-24MB, have been reported to reduce defoliation by bean leaf beetles, Japanese beetles, and western corn rootworm, although the population densities of these beetles on soybean lines was not significantly reduced over time (37). Plant resistance to insects is achieved primarily through defensive structures that limit herbivore activity, e.g., insect probing (109). Soybean cultivars that are densely pubescent (trichome density) have been found to have less pod feeding injury by bean leaf beetles in preference feeding tests (59). Moreover, the effectiveness of cultivar resistance to bean leaf beetle feeding as part of BMPV management program is questionable, because just a single bite by a bean leaf beetle can result in successful acquisition/transmission of the virus (114). Therefore, resistance to insect feeding must be extremely effective if included as part of a virus management program (26).

Field trials to evaluate BPMV resistance in soybean cultivars have routinely been conducted (19, 90, 96, 102, 115, 124), however, effective resistance to BPMV has yet to be found and incorporated into commercial soybean cultivars. Scott et al. (102) evaluated over 169 commercial cultivars and 123 *Glycine max* plant introductions in Arkansas, and all were found to be susceptible to BPMV. The same researchers tested 169 *Glycine* spp. and found only 8 to be immune. Ziems et al. (124) also reported that 30 cultivars evaluated had significant yield losses in response to BPMV infection when mechanically-inoculated at different soybean growth stages. In another study, soybeans expressing the capsid
polyprotein to BPMV were found to be resistant up to the T₂ progeny (21). This approach however, necessitates further development probably via the use of other transformation strategies, such as post-transcriptional gene silencing (PTGS), to develop soybean cultivars with acceptable and durable resistance.

Tolerant soybean cultivars were first identified by Ross (97), after mechanically-inoculating soybean germplasm lines and observing symptoms severity. Wang et al. (115) evaluated 52 North American ancestral soybean lines and all were found to be susceptible to BPMV. Zheng et al. (124) evaluated 117 G. soja and 198 G. tomentella plant introduction (PI) lines for BPMV tolerance and just 12 and 37 PI lines exhibited tolerance (ability to produce relatively high yields in the presence of same level of disease intensity (100) ). They suggested the use of these lines in breeding programs to develop BPMV resistant cultivars. Hill et al. (42) identified soybean cultivars with BPMV tolerance by comparing virus antigen levels in the seed, coupled with the percentage of mottled seed. This method revealed four PI lines that were tolerant to BPMV and eight lines that were tolerant to both BPMV and SMV.

**Justification**

*Bean pod mottle virus* prevalence and incidence have reached near epidemic proportions in the North Central region of U.S. and significant yield losses due to BPMV now occur annually (106). Apparently, the increased abundance of the predominant vector, the bean leaf beetle, is responsible for the increase in BPMV disease risk (31). Current BPMV management recommendations target the insect vectors primarily, with no consideration of BPMV dynamics. Krell et al. (54) found that while the number of bean leaf beetles significantly declined in response to the application of foliar insecticides, BPMV
incidence did not necessarily decline. Similar inconsistent findings have been recently reported by Bradshaw et al. (8). Although delayed planting can reduce BPMV disease risk by avoiding overwintering insect vector populations, its effectiveness has been inconsistent and delayed planting has been found to adversely reduce soybean yield potential (56). These tactics have limited benefits, moreover, none alone is presently reliable to manage BPMV.

Existing BPMV management practices do not account for spatial or temporal changes in BPMV incidence, even though vector management practices must be synchronized with BPMV disease dynamics to achieve optimum management benefits (119). Quantifying the temporal changes in a pathogen population over time can reveal important epidemiological events, such as time of disease onset, the rate of change in disease intensity over time, and when the highest disease intensity is reached (24, 70, 80, 84). Knowledge concerning changes in the spatial patterns of diseased plants over time can help to pinpoint potential sources of initial inoculum, pathogen dispersal mechanisms, and the direction and distance of pathogen dispersal (70, 84).

To date, no quantitative information is available concerning the temporal and spatial dynamics of BPMV epidemics within and among soybean fields. There is a need to better understand the temporal and spatial dynamics of BPMV to answer such questions as: do BPMV sources of inoculum originate from within and/or from outside soybean fields? There is also a lack of quantitative information concerning the potential sources of initial inoculum and the primary mechanisms for BPMV dissemination at different spatial scales. Investigating the temporal and spatial dynamics of BPMV throughout the soybean growing season at different spatial scales may provide insights as to the importance and sources of initial inoculum, thereby helping to design more effective management tactics. Moreover, appropriate sampling designs to
estimate the incidence of BPMV within soybean fields have not been developed, and quantitative data concerning the spatial pattern of BPMV incidence within soybean fields over time should provide the spatial data needed to design biologically sound sampling protocols for this pathosystem.

Yield loss estimates due to BPMV vary among cultivars and by time of BPMV infection (14, 16, 46, 57, 99, 108). To date, all studies quantifying yield losses due to BPMV have utilized mechanical inoculations of all soybean plants within soybean rows or plots at various stages of soybean development (14, 16, 46, 78). Since all plants were mechanically inoculated at the same time (resulting BPMV incidence levels approaching 100% at one point in time), such studies are likely to have overestimated yield losses due to BPMV. This is because such inoculation methods do not accurately represent the natural situation, because all soybean plants are seldom infected all at once. Such studies have not considered the benefits of yield compensation from neighboring non-infected (healthy) plants. Accurate yield loss information is vital in evaluating the impact of new BPMV resistant cultivars, new integrated BPMV management programs, and/or risk factors or new government policies that might impact disease risk and soybean yield (70-72, 83, 99).

A common goal in epidemiological studies is to identify factors that correlate strongly with disease/pathogen risk and that can be used to predict future disease risk in terms of the occurrence, prevalence, and level of a disease/pathogen incidence within a defined host population (111). The prevalence and incidence of a pathogen/disease at higher spatial scales (county, state, region etc.) may reveal important area-specific disease/pathogen risk factors that operate at spatial scales above the field scale (2, 111). Disease/pathogen mapping using global positioning systems (GPS) and geographical information systems
GIS) technologies has been used in plant pathology to geospatially define disease risk and risk factors that operate at higher spatial scales (7, 79), for example report by Coelho Netteo and Nutter (17, 82) revealed a large-scale risk factor (periodic flooding) that impacted the risk of moko disease of banana in the Amazon River Basin, which led to effective disease management tactics to successfully mitigate disease risk. Currently, there is no quantitative information concerning the distribution of BPMV within and among soybean fields and within or among Iowa counties, yet such information could set the stage for identifying large scale risk factors associated with areas of high BPMV incidence (risk).

Based on the above knowledge gaps, the objectives of this research were to:

1. Quantify the temporal and spatial dynamics of BPMV within soybean fields.
2. Quantify and map the prevalence and incidence of BPMV within Iowa counties and soybean fields over three soybean growing seasons.
3. Quantify the effect of time of BPMV detection (infection) on soybean yield, yield components, and grain quality.
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CHAPTER 2.
QUANTIFYING THE WITHIN-FIELD TEMPORAL AND SPATIAL DYNAMICS
OF BEAN POD MOTTLE VIRUS IN SOYBEAN

Manuscript prepared for submission to Phytopathology

ABSTRACT

A quadrat-based sampling method was developed to quantify the within-field temporal and spatial dynamics of Bean pod mottle virus (BPMV) in soybean in 2006 and 2007. Twenty-five, 30-cm-long quadrats were established within each row of soybean (Glycine max) in field plots that were 6 rows wide by 7.5 m long. Ten days after soybean emergence, quadrats were thinned to four soybean plants/quadrat. Four treatments with three replications per treatment (12 plots) were used to quantify treatment effects on the temporal and spatial dynamics of BPMV epidemics in soybean. Treatments were: (i) establishing a BPMV inoculum point source within plots (2 center quadrats were mechanically inoculated at V4 in 2006 and V1 in 2007), (ii) foliar insecticide (Warrior) applications at the V1 and R2 growth stages, (iii) establishing a BPMV-inoculated point source, plus two foliar applications of insecticide, and (iv) a non-treated control. Sampling of soybeans to detect BPMV began 25 days after planting and continued at 8- to 11-day intervals until crop senescence. All quadrats in each plot were sampled (census) by selecting the youngest fully-expanded leaflet from each of four plants within quadrats. Leaf sap was then extracted from each 4-leaflet (bulked) sample (quadrat), and the extracted sap was tested for the presence of BPMV by ELISA
Quadrat position (plot, row, and quadrat number) and the date of sampling on which each quadrat first tested positive for BPMV were recorded and mapped. *Bean pod mottle virus* was detected within field plots as early as the first sampling date (30 May 2006 and 12 June in 2007). The rate of BPMV infection in 2006 ranged from 0.09 to 0.17 logits/day. In 2007, BPMV infection rates were significantly slower, ranging from 0.05 to 0.07 logits/day. Based on these rates, BPMV incidence was doubling every 5.3 to 6.9 days in 2006 and every 9.9 to 13.9 days in 2007. Plots that had the earliest BPMV detection had the highest BPMV incidence at the end of the growing season ($R^2 = 66.5\%$ and $R^2 = 70.4\%$ for 2006 and 2007, respectively) and also the largest areas under the BPMV progress curves ($R^2 = 80.9\%$ and $R^2 = 75.6\%$ for 2006 and 2007, respectively), indicating that early-season BPMV infection had a large impact on final BPMV incidence. Spatial analyses using ordinary runs revealed that BPMV-infected quadrats had a spatial pattern that was highly aggregated throughout both growing seasons. The implications of rates within-field temporal spread indicate that BPMV incidence can double in as a short time as 5.3 days and that spatial pattern of BPMV being clustered indicate a systematic sampling design, and higher yield loss due limited yield compensation. To our knowledge, this is the first study to quantify the spatio-temporal dynamics of the within-field spread of BPMV in soybean.

**INTRODUCTION**

*Bean pod mottle virus* (BPMV) in soybeans has been reported to be increasing in prevalence in the North Central region of the United States, with disease intensities approaching epidemic levels (14). The first report of BPMV in the North Central region of
the United States occurred in Iowa in 1968 (43). Since then, a number of other states in the North Central region have also reported presence of BPMV in soybean fields (9, 33, 34).

Soybean yield losses due to BPMV have been reported to range from 3 to 52% (20, 28, 38, 46). Yield components potentially affected by BPMV include the number of pods per plant, the number of seeds per pod, and 100-seed weight (19, 38). In addition to direct yield losses, BPMV has been reported to cause soybean seed discoloration in the harvested grain (18, 23), whereas others have found no cause and effect relationship between BPMV incidence in the field and seed discoloration (16). Plants infected with BPMV in the field have also been reported to significantly increase the risk of seed infection by Phomopsis spp. (1, 53).

*Bean pod mottle virus* is in the family *Comoviridae* and belongs to the genus *Comovirus*. This virus is bipartite, with a positive-strand RNA genome comprised of separately encapsidated RNA1 and RNA2. Virus particles are isomeric and are approximately 28 nm in diameter (8, 47). *Bean pod mottle virus* is disseminated primarily by insect vectors, of which the bean leaf beetle (*Cerotoma trifucarta* Foster) is the predominant vector. Population densities of the bean leaf beetle within soybean crops can be extremely high, especially following mild winters, when the probability of bean leaf beetle winter survival is high (11, 12, 25). The increase in BPMV prevalence in the North Central United States has been associated with increased winter survival of bean leaf beetle populations (21, 29). The highest population density of bean leaf beetles ever recorded occurred in Iowa in 2002 (201 beetles/50 sweeps) (14, 26, 45).

Three primary sources of BPMV initial inoculum have been reported: (i) BPMV-infected seed that leads to seed-to-seedling BPMV infection (usually <0.1%), (ii) BPMV-
infested bean leaf beetles that survive the winter (usually <0.5%), and the intercrop survival of BPMV within alternative weed hosts (mostly plants in the genus *Desmodium*) (24).

Although BPMV-infected seed, infested bean leaf beetles, and alternative weed hosts appear to represent a very low risk for initial BPMV incidence in soybean fields, a high rate of plant-to-plant spread of BPMV by insect vectors could account for high end-of-season levels of BPMV incidence (14, 24). A critical initial step in disease management is to obtain quantitative information on disease risk (3, 35, 39). To date, there is a paucity of quantitative information concerning the rate of BPMV spread within soybean fields. Knowledge of the rate of temporal spread of a pathogen is important in risk assessment and risk management (36, 40, 41, 48). Such knowledge may lead to the development of timely management programs that effectively reduce infection rates (39).

The spatial pattern and temporal spread of BPMV at the field scale are presently unknown. This information is needed to develop appropriate sampling designs to quantify BPMV incidence over time, as well as to quantify the impact of the spatial spread of BPMV on soybean grain yield and yield components (40, 42, 52). This information is needed because, in theory, random spatial patterns of spread will result in less yield loss than aggregated patterns (42). In addition, quantitative knowledge concerning the spatial pattern of a plant pathogen over time can provide valuable information on the relative importance of potential sources of primary inoculum, mechanisms of pathogen dispersal, and directional pattern of pathogen spread (59).

Therefore, the objectives of this study were to: (i) quantify the temporal rate of BPMV infection in soybean, and (ii) determine and quantify the spatial patterns of BPMV soybean over time.
MATERIALS AND METHODS

Field plots. Field plots were established at Iowa State University’s Curtiss Research Farm in Ames, Iowa. The soybean cultivar NB 3001, which is susceptible to BPMV but has some resistance to SMV (17), was planted on 5 May 2006 and 18 May 2007. Each plot consisted of eight rows, 7.6 m in length, with a row spacing of 0.76 m. The two outermost rows and an additional 1.5 m of row on both ends of each plot were used as borders to minimize edge effects. Each plot was established at least 15.2 m from other experimental plots. All land area between plots was planted with soybean (cv. NB3001). The six center rows of each plot were partitioned into 150, 30 cm-long quadrats (6 rows x 25 quadrats/row = 150 quadrats per plot) using 30 cm white wooden stakes. Stakes were placed within rows at a 45° angle to delineate quadrats. Soybean plants within quadrats were thinned to four plants per quadrat on 24 May 2006, and 12 June 2007.

Treatments. In order to induce differential temporal and spatial dynamics of BPMV epidemics within soybean plots, four treatments were used. The four treatments were: (i) the establishment of a BPMV-inoculated point source within plots by mechanically inoculating two quadrats with BPMV); (ii) two foliar applications of lambda-cyhalothrin (Warrior™ 1EC, Syngenta Crop Protection, Greenville, NC); (iii) establishment of a BPMV-inoculated point source and two foliar applications of lambda-cyhalothrin, (Warrior™), and (iv) a non-treated control. For treatments that included BPMV-inoculated point sources (treatments (i) and (iii)), soybean plants located in the 13th quadrat position of the center two rows (3rd and 4th rows) were mechanically inoculated with sap extracted from BPMV-infected soybean plants that were maintained in a greenhouse on 30 June 2006 and 13 June, 2007. Mechanical
inoculations were performed by lightly dusting carborundum (600-mesh) on the topmost fully-expanded leaf, and then rubbing the leaf surface lightly with the index finger dipped into BPMV-positive soybean leaf sap plus extraction buffer (1 g leaf tissue: 3 ml extraction buffer).

Treatments (ii) and (iii) received two foliar applications of the insecticide lambda-cyhalothrin at a rate of 6.9 fl oz /ha \textit{ai} using a CO$_2$- powered sprayer at 40 Pa at the first primary leaf (VC) and early reproductive (R2) soybean growth stages (25).

**Data collection.** To test individual quadrats for the presence of BPMV, soybean plants within each 30 cm quadrat were sampled every 8 to 11 days, beginning 25 days after planting. Each quadrat within each plot (6 rows x 25 quadrats = 150 quadrats) was sampled by removing a single leaflet from the youngest fully expanded trifoliate leaf of each soybean plant within the quadrat. All four leaflets from a quadrat were sealed in a sandwich-size plastic bag (bulked sample), that were pre-labeled with plot number, row, and quadrat position, and stored at 4º C until sap extraction.

**Sap extraction.** Sap was extracted from each four-leaflet sample using a leaf press (Ravenel Specialties Corp., Seneca, SC). Plant leaflets were fed into the extractor rollers and approximately 1 ml of general extraction buffer (pH 7.4) was added between the metal rollers using a squirt bottle. The extracted sap was collected into paper portion cups (Instaoffice, Kennesaw, GA); approximately 1 ml of the extract was poured into pre-labeled, 1.5-ml eppendorf tubes and stored at -20º C until testing.

**ELISA assay for BPMV.** A double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) commercial kit for BPMV was used to test for the presence of BPMV in each soybean quadrat sap sample (Agdia Reagent Set, Alkaline
phosphatase label; Agdia, Inc., Elkhart, IN). All ELISA steps were followed as recommended by the manufacturer. Briefly, micro-titer high-binding plates (Agdia Inc. Elkhart, IN) were first coated with BPMV capture antibody diluted 1:200 in a coating buffer and incubated overnight at 4º C. A 100-µl aliquot of plant sap was then loaded into duplicate wells and incubated overnight at 4 ºC. Four known BPMV-positive and -negative samples were loaded into each plate as controls. The second antibody (BPMV alkaline phosphatase enzyme diluted 1:200 in enzyme conjugate immunoassay buffer) was added and plates were incubated at room temperature for 2 hours. Between each step, plates were thoroughly washed with phosphate buffer saline-Tween 20 (PBST) using a micro-plate washer model (ELx405, Biotec Inc., Winooski, VT). After the final washing, color development substrate (p-Nitrophenol, PNP) was added and plates were kept in the dark for 30 minutes before obtaining absorbance readings (at 405 nm) using a plate reader model Elx800 (Biotek Inc., Winooski, VT). Quadrat sap samples were considered positive for BPMV if the absorbance value of a quadrat sample was greater than twice the value of the mean absorbance of the negative controls. Incidence of infected plants (pathogen incidence) within a soybean plot (%) was determined at each sampling date by dividing the number of quadrats testing positive for BPMV by the total number of quadrats (n = 150), then multiplying by 100.

**Monitoring of bean leaf beetle population density.** Bean leaf beetle population density was monitored by randomly selecting 16 quadrats from each soybean plot and counting the number of bean leaf beetles per quadrat each week. The total number of bean leaf beetles from the 16 quadrats within each plot was plotted versus day of year. Treatment effects on bean leaf beetle population densities were tested using the GLM procedure and a Tukey option for mean separation (SAS Institute, NC).
**Data analyses.** To obtain BPMV pathogen progress curves for each treatment, the percentage of soybean quadrat samples testing positive for BPMV within soybean plots were plotted against sampling date (day of year) (40). In addition to BPMV progress curves, the rate of change in BPMV incidence versus time was also plotted to help determine the most appropriate population growth models for quantifying temporal rates of BPMV spread. Based on the shapes of these curves, exponential, logistic, and Gompertz models appeared to be likely fits. To select the most appropriate population growth model and use it to obtain parameter estimates for slopes (rate of BPMV temporal spread) and intercepts, BPMV incidence data were transformed using these three population growth models and transformed incidence values were regressed against time using simple linear regression (35, 42). The model(s) that best fit transformed BPMV incidence over time were evaluated based upon the following model criteria: the F-statistic, coefficients of determination ($R^2$), standard errors of the estimate for pathogen incidence ($y$), and subjective evaluation of standardized residuals versus predicted values of $y$ (40, 42, 52). The model that gave the best fit for the data from all treatments and years was used to obtain and compare parameter estimates. The slope parameter obtained from regression equations was taken as a quantitative measure of the rate of BPMV spread among quadrats (temporal infection rate). Mean separations for intercepts, slopes, area under pathogen progress curve (AUPPC), time to BPMV onset (5%), time to 50% BPMV incidence were used to compare treatment effects on BPMV epidemics by ANOVA using a Tukey option ($P \leq 0.05$) for mean separations (Statistical Analysis System, SAS Institute, Cary NC). The relationship between time (day of year) that BPMV was first detected in a plot and final BPMV incidence, and relative areas under the BPMV progress curve were quantified using linear regression (42).
To determine if BPMV incidence within plots was random or aggregated, individual quadrats testing positive for BPMV were mapped, and the resulting spatial patterns were analyzed using ordinary runs analysis (42, 52). Briefly, a run is defined as a series of like events. In this case, a run is a series of BPMV-infected quadrats, or a series of non BPMV-infected soybean quadrats. To test for aggregation (or lack of it, i.e., random), the number of observed runs was statistically compared to the number of expected runs that would occur by random chance for a given level of BPMV incidence (42). This was done by calculating a z-score for a one-sided z test given by:

\[ z = \frac{O - E}{s(O)} \]

where \( O \) is the number of observed runs, \( E \) is the expected number of runs, and \( s(O) \) is the standard deviation of observed runs. Under the null hypothesis that BPMV-infected quadrats occur in a random spatial pattern within soybean plots, a z-score of less than \(-1.64\) indicates a spatial pattern that is aggregated (rejection of the null hypothesis), and a score greater than \(-1.64\) indicates the presence of a random spatial pattern of BPMV-infected quadrats (acceptance of null hypothesis, at \( P = 0.05 \) level of significance).

**RESULTS**

The day of year that BPMV was first detected in soybean plots varied among treatments, with the non treated control having the earliest onset in 2006 (30 May, DOY 150). In 2007, the soybean plots having earliest BPMV detection occurred in the BPMV-inoculated plot (12 June, DOY 162). The onset of BPMV epidemics (defined as the day of year when BPMV incidence reached 5%), occurred on 29 June in 2006 (DOY 180) and on 18 July 2007 (DOY 199) (Table 1).
In both years, the change in BPMV incidence over time in all treatments was best described by the logistic model based upon model evaluation criteria, which included the shapes of BPMV progress curves (Figure 1), BPMV rate curves (not shown), F-statistics, and coefficients of determination ($R^2$) values. F-statistics for the logistic model ranged from 97.8 to 263.7 and all were highly significant ($P<0.001$). $R^2$ –values for the logistic model ranged from 91.6 to 99.1%, indicating that day of year explained 91.6 to 99.1% of the variation in logit BPMV incidence (Figure 2). The logistic model also provided lowest standard errors of the estimate for $y$ (SEEy) compared to other models and ranged from 0.005 to 0.01 in 2006 and from 0.004 to 0.01 in 2007 (Table 2, Figure 2).

Although there were no significant differences among slopes (BPMV infection rates) among the four treatments in either year, rates of change in BPMV incidence were significantly faster ($P<0.001$) in 2006 compared to 2007 (Table 2). The rate of BPMV spread within soybean plots in 2006 ranged from 0.11 to 0.13 logit/day and from 0.05 to 0.70 logit/day in 2007 (Table 2). Based upon these rates of BPMV infection, BPMV incidence was doubling every 5.3 to 6.3 days in 2006 and every 9.9 to 13.9 days in 2007.

Treatments in 2006 did not affect the time to 50% BPMV incidence, which ranged from day of year 207 (26 July) to 215 (3 August) in 2006 (Table 3). The incidence of BPMV in 2007 did not reach 50% in any treatments and the predicted time (day of year) to reach 50% BPMV incidence occurred well after crop senescence. Predicted time to 50% incidence ranged from day of year 256 (24 September) to 306 (3 November) (Table 3).

The incidence of BPMV infected quadrats was higher at the end of the season in 2006 (85.8 to 94.4%) than 2007 (10.0 to 37.1%) (Figure 1, Table 3). Treatments had no significant effects on the end-of-season (final) BPMV incidence in 2006, plots that received two foliar
applications of Warrior insecticide had statistically the same final BPMV incidence as the non-sprayed plots \((P > 0.05)\). However, in 2007, final BPMV incidence was significantly affected by treatment, inoculated plots had nearly three times the incidence level in non-inoculated plots \((P<0.01)\) (Table 3).

The area under the pathogen progress curves (AUPPC) differed significantly between years; with AUPPC values being significantly higher in 2006 than in 2007, indicating that the season-long stress from BPMV epidemics exerted on soybeans was much greater in 2006 than in 2007 (Table 3). There were no significant differences in AUPPC values among treatments in 2006. However, the treatments that had the two center quadrats inoculated with BPMV in 2007 had significantly higher AUPPC values, indicating that if initial inoculum was not introduced early in the growing season, BPMV epidemic onset would be delayed by 12 to 20 days compared to the non-treated control.

The date that BPMV was first detected within a plot had a significant (and positive) linear relationship \((P=0.009)\) with the day of year of BPMV epidemic onset (i.e., when BPMV incidence reached 5\%) \((R^2 = 58.2\%)\) (Figure 3). The day of year of epidemic onset in 2006 ranged from 180 to 193 and there were no significant differences among treatments in 2006 (Table 1). In 2007, day of year of epidemic onset statistically differed among treatments and ranged from 199 to 235. Time of first detection could also be used to predict final BPMV incidence \((R^2 = 66.5-70.4\%)\) and the relative areas under pathogen progress curves \((R^2 = 58.0\%)\) (Figure 4 and 5). The earlier BPMV was detected in a soybean plot, the earlier the day of year of BPMV epidemic onset, and the higher the final BPMV incidence within plots (Figure 5). Moreover, the earlier BPMV was detected within soybean plots, the higher was the AUPPC.
Spatial pattern analyses of BPMV-infected quadrats over time (using ordinary runs analysis) (52), revealed that the spatial pattern of all BPMV-infected quadrats were nearly always aggregated within all treatments and nearly all sampling times (Table 4 and 5, Figure 6 and 7). Quadrats that had BPMV infection early in the season tended to be initially random, indicating that BPMV-infected seed and/or overwintering viruliferous adult bean leaf beetles were responsible for initial infections. However, after day of year 170 and 180 for 2006 and 2007, respectively, the pattern of BPMV-infected quadrats was highly aggregated over the remainder of each growing season.

The number of bean leaf beetles (per 16 quadrats per plot) on soybean did not differ significantly among treatments in 2006 and 2007. In 2006, however, there were significantly (P<0.05) more bean leaf beetles per soybean plot than in 2007, based upon the number of bean leaf beetles counted in 16 randomly-selected quadrats within each plot. Insecticide applications did not significantly affect the number of bean leaf beetles observed within soybean plots over time across all treatments. Hence, the mean number of bean leaf beetles within each growing season were pooled across replications and treatments. A graph of the cumulative number mean bean leaf beetles per 16 quadrats in soybean plots versus day of year time indicated fewer numbers of bean leaf beetles early in the growing season (overwintering population) (Figure 8), but after day of year 159 in 2006 and 189 in 2007, bean leaf beetle populations increased steadily, reflecting emergence of the first and second summer generations.

**DISCUSSION**

This is the first comprehensive study to quantify the rate of BPMV epidemics over the course of two soybean growing seasons (9 assessment dates in 2006 and 10 assessment
dates in 2007). Although very few previous studies did publish disease progress curves, these studies had only 3 to 5 points (fewer assessment dates) and did not fit any temporal models to the data to estimate epidemic rates.

Based upon the BPMV epidemic rates that we obtained, BPMV incidence was shown to double within 5.3 days, indicating that BPMV can reach high incidence levels in a relatively short period of time. Because early BPMV detection in soybean field plots best predicted subsequent epidemic development, this suggests that management tactics that delay and reduce the level of initial inoculum should be deployed before the emergence of the first summer generation of bean leaf beetles. Initial inoculum could be reduced by seed testing and/or by producing seed in areas that have low BPMV disease risk. Currently, there are no BPMV-resistant soybean cultivars available, and the BPMV pathosystem would be a good candidate for developing and deploying rate reducing resistance (4, 17, 44). The currently-recommended economic injury threshold for bean leaf beetles was developed based on direct damage to soybeans but does not consider transmission of BPMV insect vectors (45). Thus, there is a need to develop a new economic threshold that incorporates yield losses due to the BPMV transmission that occur prior to R1 soybean growth stage.

We determined that the spatial patterns of BPMV-infected quadrats over time in soybean were highly aggregated. This has important implications in the development of non-biased sampling designs to more accurately estimate BPMV incidence within soybean fields and for modeling yield loss. The clustered nature of BPMV epidemics over time dynamics suggest that sampling for BPMV should be done between the V3 and R3 growth stages and that the sampling design employed to estimate the incidence of BPMV should take clustering into
account (42). Therefore, a systematic sampling design that employs more sampling transects (i.e.,
more sampling arms), is recommended to avoid sampling bias (36, 40, 42).

The delay in epidemic onset (5% pathogen incidence) in non inoculated plots in 2007
indicates the importance of a local (within field) source of inoculum for epidemic
development and suggests that sources of initial inoculum from outside soybean plots is quite
limited in some years. Furthermore, we found that there was a significant linear relationship
between day of year when BPMV was first detected in a plot and the day of year of epidemic
onset, confirming that early BPMV spread from quadrat to quadrat was likely due to the
overwintering generation of bean leaf beetles. This would indicate a very low but early
source of BPMV initial inoculum in soybean that can lead to high levels of BPMV incidence
when the rate of BPMV infection is high (as in 2006).

The temporal progress of BPMV epidemics in all soybean plots was best explained
by logistic model in both years and this is the first study to quantify the rate of BPMV
infection within soybean fields. The logistic population growth model has been reported to
best explain the rate of pathogen spread of plant disease epidemics in which there is plant-to-
plant spread. The rate of infection ($r_L$) early in the season is limited by the proportion (or
percentage) of diseased plants and then once $y = 0.5$ (50%), the rate of epidemic is limited by
the proportion of healthy plants remaining (36, 40, 42, 52). Using the equation for the
absolute rate of logistic model:

$$\frac{dy}{dt} = r_L y (1 - y),$$

$dy/dt$ is the absolute rate of disease increase, $r_L$ is the logistic rate parameter, $y$ is the
proportion of diseased sampling units, and $(1-y)$ is the proportion of healthy sampling units
remaining (BPMV-free quadrats) (35), in 2006 when the population of bean leaf beetles was high and there was early inoculum, this resulted in a higher rate of disease increase. By the time the second summer generation of bean leaf beetles emerged, soybean rows were beginning to close, which facilitated the movement of bean leaf beetles to crawl not just from quadrat-to-quadrat within soybean rows (pawn movement) but also from quadrat to quadrat across the rows (rook movement). As more rows fully closed in, the probability for diagonal spread (bishop movement) would increase, leading to faster spatial and temporal spread of BPMV.

This study demonstrated that the risk of BPMV was greatly affected by time of BPMV detection (day of year that BPMV was found within a plot), in that the earlier a plot was found to have soybean quadrats that tested positive for BPMV, the higher was the final BPMV incidence and area under pathogen progress curve at the end of season. The fastest period of the BPMV infection rate in all treatments coincided with bean leaf beetle population densities on soybean. Similar patterns have been reported on Citrus tristeza virus-aphid vector pathosystems where after canopy closing, within-row transmission was attributed to dispersal behavior of the predominant aphid species Toxoptera citricida which colonize citrus (15).

Our study revealed that quadrat to quadrat spread of BPMV originated from BPMV-infected quadrats within soybean plots, as evidenced by the within-row spread of BPMV early in the season. Moreover, BPMV-infected quadrats were first detected with BPMV were located close to a mechanically-inoculated point source of inoculum, indicating that quadrats in close proximity had a higher probability of being infected with BPMV, and therefore, quadrats did not have equal chance of being infected (i.e., random spread from unknown source of inoculum). Gibson and Austin (13) referred to this as “local transmission”. The
clustered spatial pattern of BPMV-infected quadrats strongly indicates that BPMV spread from quadrat-to-quadrat within soybean plots was due to the limited movement of bean leaf beetles after they had acquired BPMV from infected soybean plants.

Limited spread of BPMV further away from previously BPMV positive quadrats may be attributed to limited bean leaf beetle movement within soybean field. The flight behavior of bean leaf beetles has been found to consist of short trivial flights within a host population (5, 27). Boiteau et al. (5) reported < 30 m flight distance by bean leaf beetles, and Krell and others (27) have reported <51 m potential flight distance using a computer-tethered flight mill. Generally, Chrisomelids have been reported to involve short, trivial flights with a crop unless they are migrating to overwintering sites (55, 57). For instance the trivial flights for Corolado potato beetle (Leptinotarsa decemlineata (Say) (Coleoptera, Chrysomelidae) were reported to be < 10 m (57). Lack of quantifiable BPMV spread by overwintering beetle populations may be due to the fact that overwintering populations have been reported to have minimal flight distance (27).

The flight behavior of insect vectors coupled with feeding behavior and virus retention characteristics can greatly influence the spatial pattern of infected plants in insect-vectored pathosystems. While working with Southern bean mosaic virus (SBMV), a Comovirus, Wang et al. (56) found that bean leaf beetles became non-viruliferous if they continued to feed on healthy tissue. This may further help to explain the clustering of infected plants from a point source of initial inoculum, and the non-random spread from point inoculum sources. In addition, bean leaf beetles transmit BPMV in a non-circulative manner; that is, the virus does not multiply inside the insect (56). Hence, BPMV titer in the bean leaf beetle may be lowered over time as the vector transmission efficiency of BPMV by bean leaf beetles unless virus titer is recharged by bean leaf beetles feeding on BPMV-
infected plants (56). In further evidence that the clustering of BPMV-infected quadrats was due to the bean leaf beetle movement and feeding behavior, overwintering adult bean leaf beetle populations have been found to be clustered within soybean crops and overwintering sites (22, 32, 51). Larvae and pupae aggregation on soybean roots has also been reported (32).

The fact that there were no significant effects of insecticide spraying on BPMV incidence, confirms the effect of foliar insecticide sprays on reducing BPMV incidence remains inconsistent, as Krell et al. (25) reported a reduction in BPMV incidence due to insecticide sprays for a single year. In addition, recent studies by Bradshaw et al. (6) showed that foliar insecticide treatments had similar BPMV incidence levels compared to non-treated plots, despite the significant reduction in bean leaf beetle numbers by insecticide application.

ACKNOWLEDGEMENTS

We thank the Iowa Soybean Association, the USDA-NRI Plant Biosecurity Grants Program, and the Iowa State University Institute for Food Safety and Security for supporting this study. Help from graduate students Lu Liu and Xin Lu, summer interns, and undergraduate students who worked on the project is greatly appreciated.


inoculated with *Bean pod mottle virus* and *Soybean mosaic virus*. Plant Dis. 87:1333-1336.


TABLE 1. Treatment effects on the day of year that BPMV was first detected and the time to BPMV epidemic onset (time to 5% BPMV incidence) in soybean cv. NE3001 planted at the Iowa State University Curtiss Research farm in 2006 and 2007.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day of year BPMV was first detection</th>
<th>Date of epidemic onset (day of year when BPMV incidence = 5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2006&lt;sup&gt;z&lt;/sup&gt;</td>
<td>2007&lt;sup&gt;z&lt;/sup&gt;</td>
</tr>
<tr>
<td>BPMV-inoculated</td>
<td>177 a</td>
<td>172 b</td>
</tr>
<tr>
<td>Two foliar sprays</td>
<td>163 a</td>
<td>216 a</td>
</tr>
<tr>
<td>BPMV-inoculated and two foliar sprays</td>
<td>184 a</td>
<td>169 b</td>
</tr>
<tr>
<td>Non-treated control</td>
<td>170 a</td>
<td>209 a</td>
</tr>
</tbody>
</table>

<sup>z</sup> Treatments followed by same letter within the same column are not significantly different (<i>P</i>&lt;0.05) using ANOVA and a Tukey test for mean separations.
TABLE 2. Logistic model parameters and statistics describing the temporal progress of *Bean pod mottle virus* (BPMV) epidemics within soybean cv. NE 3001 plots planted at the Iowa State University Curtiss Research Farm near Ames in 2006 and 2007.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Logistic model parameters and statistics</th>
<th>2006</th>
<th>Doubling time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPMV-inoculated</td>
<td></td>
<td>-28.7 a</td>
<td>0.13 a</td>
</tr>
<tr>
<td>Two foliar sprays</td>
<td></td>
<td>-22.7 a</td>
<td>0.11 a</td>
</tr>
<tr>
<td>BPMV-inoculated and two foliar sprays</td>
<td></td>
<td>-28.8 a</td>
<td>0.13 a</td>
</tr>
<tr>
<td>Non treated control</td>
<td></td>
<td>-24.1 a</td>
<td>0.12 a</td>
</tr>
<tr>
<td>2007</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPMV-inoculated</td>
<td></td>
<td>-15.6 a</td>
<td>0.05 a</td>
</tr>
<tr>
<td>Two foliar sprays</td>
<td></td>
<td>-16.0 a</td>
<td>0.06 a</td>
</tr>
<tr>
<td>BPMV-inoculated and two foliar sprays</td>
<td></td>
<td>-12.9 a</td>
<td>0.05 a</td>
</tr>
<tr>
<td>Non treated control</td>
<td></td>
<td>-19.0 a</td>
<td>0.07 a</td>
</tr>
</tbody>
</table>

*Treatments followed by same letter within the same column are not significantly different (\(P<0.05\)) using ANOVA and a Tukey test for mean separations.*
TABLE 3. Treatment effects on final BPMV incidence and area under the *Bean pod mottle virus* (BPMV) pathogen progress curve (AUPPC) in soybean cv. NE 3001 quadrats planted at the Iowa State University Curtiss Research Farm in 2006 and 2007.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time to 50% BPMV incidence</th>
<th>Final BPMV incidence (%)</th>
<th>AUPPC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2006 ‡</td>
<td>2007 ‡, ‡</td>
<td></td>
</tr>
<tr>
<td>BPMV-inoculated</td>
<td>215 a</td>
<td>256 a</td>
<td>91.6 a</td>
</tr>
<tr>
<td>Two foliar sprays</td>
<td>207 a</td>
<td>284 a</td>
<td>94.4 a</td>
</tr>
<tr>
<td>BPMV-inoculated &amp; two foliar sprays</td>
<td>215 a</td>
<td>260 a</td>
<td>90.0 a</td>
</tr>
<tr>
<td>Non treated control</td>
<td>206 a</td>
<td>306 a</td>
<td>85.8 a</td>
</tr>
</tbody>
</table>

Predicted time to 50% BPMV incidence in 2007 occurred after crop senescence.

Treatments followed by same letter within the same column are not significantly different ($P<0.05$), using ANOVA and a Tukey test for mean separations.
TABLE 4. Ordinary runs analysis of z-score values for spatial patterns of *Bean pod mottle virus* (BPMV) infected quadrats within soybean plots (cv. NE 3001) at the Iowa State University Curtiss Research Farm in 2006.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Replication</th>
<th>Day of year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>150</td>
</tr>
<tr>
<td>BPMV-inoculated</td>
<td>1</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.116</td>
</tr>
<tr>
<td>Two insecticide applications</td>
<td>1</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.116</td>
</tr>
<tr>
<td>BPMV-inoculated &amp;</td>
<td>1</td>
<td>0.116</td>
</tr>
<tr>
<td>Two insecticide applications</td>
<td>2</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.116</td>
</tr>
<tr>
<td>Non-treated control</td>
<td>1</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.116</td>
</tr>
</tbody>
</table>

* value less than −1.64, indicates a nonrandomness (i.e., the spatial pattern at that sampling time was aggregated).
TABLE 5. Ordinary runs analysis of $z$-score values for spatial patterns of *Bean pod mottle virus* (BPMV) infected quadrats within soybean plots (cv. NE 3001) at the Iowa State University Curtiss Research Farm in 2007.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Replication</th>
<th>Day of year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>163</td>
</tr>
<tr>
<td>BPMV-inoculated</td>
<td>1</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.116</td>
</tr>
<tr>
<td>Two insecticide applications</td>
<td>1</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.116</td>
</tr>
<tr>
<td>Two insecticide applications</td>
<td>2</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.116</td>
</tr>
<tr>
<td>Non-treated control</td>
<td>1</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.116</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.116</td>
</tr>
</tbody>
</table>

*value less than −1.64, indicates a nonrandomness (i.e., the spatial pattern at that sampling time was aggregated).
Fig. 1. Incidence of Bean pod mottle virus (BPMV) in soybean quadrats (cv. NE3001) planted at Iowa State University Curtiss Research Farm in (A) 2006 and (B) 2007.

Fig. 2. Relationship between logit Bean pod mottle virus (BPMV) incidence and day of year quadrats were sampled and tested for the presence of BPMV in soybean (cv. NE3001) quadrats planted at Iowa State University Curtiss Research Farm during 2006 (A) and 2007 (B).

Fig. 3. Relationship between day of year that Bean pod mottle virus was first detected in a plot versus day of year of BPMV onset (5% incidence) in soybean plots (cv. NE3001) planted at Iowa State University Curtiss Research Farm (2006 and 2007 combined).

Fig. 4. Relationship between day of year that Bean pod mottle virus was first detected in soybean plots versus final BPMV incidence corresponding soybean plots (cv. NE3001) planted at Iowa State University Curtiss Research Farm in 2006 (solid circles), and 2007 (open circles).

Fig. 5. Relationship between day of year that Bean pod mottle virus was first detected in soybean plots versus the relative area under the BPMV pathogen progress curves in corresponding soybean plots (cv. NE3001)
**Fig. 6.** Maps depicting the spatial spread of BPMV over time in soybean field plots (cv. NE3001) at the Iowa State University Curtiss Research Farm in 2006. The orange boxes (quadrats) depict quadrats testing positive for BPMV, whereas the green boxes depict healthy quadrats. The numbers within boxes indicate the sampling period in which BPMV was first detected within a quadrat.

**Fig. 7.** Maps depicting the spatial spread of BPMV over time in soybean field plots (cv. NE3001) at the Iowa State University Curtiss Research Farm in 2007. The orange boxes (quadrats) depict quadrats testing positive for BPMV, whereas the green boxes depict healthy quadrats. The numbers within boxes indicate the sampling period in which BPMV was first detected within a quadrat.

**Fig. 8.** (A) The average number of bean leaf beetles per 16 quadrats per soybean plot (cv. NE3001), and (B) the cumulative number of bean leaf beetles per 16 quadrats per plot of soybean cv. NE 3001 planted at the Iowa State University Curtiss Research Farm in 2006 (solid circle) and 2007 (open circles).
Day of year when BPMV was first detected in quadrats

BPMV incidence (%)

BPMV-inoculated
Two foliar insecticide sprays
BPMV-inoculated and sprayed
Non treated control

Fig. 1.
Fig. 2.
Fig. 3.

The scatter plot shows the relationship between the day of year when BPMV was first detected in quadrats and the day of year for BPMV onset (5% incidence). The regression line is given by the equation $y = 56.7 + 0.79x$, with $R^2 = 58.2\%$. The data points are plotted on a graph with the x-axis representing the day of year when BPMV was first detected in quadrats, and the y-axis representing the day of year for BPMV onset (5% incidence).
Fig. 4.
Fig. 5.
Fig. 6.
Fig. 7.
Fig. 8.
CHAPTER 3.

OCCURRENCE, INCIDENCE, DISTRIBUTION AND SPATIAL DEPENDENCE OF

BEAN POD MOTTLE VIRUS ON SOYBEAN IN IOWA

Manuscript prepared for submission to Phytopathology

ABSTRACT

A survey was carried out from 2005 through 2007 to map, analyze, and quantify the temporal dynamics, and test spatial for spatial dependence of Bean pod mottle virus (BPMV) prevalence and incidence in soybeans at the field and county scales in Iowa. Eight to 20 fields were arbitrarily or randomly selected in each county of 96 counties in 2005 and 99 counties in 2006 and 2007, sampled and tested for the presence of BPMV. Thirty soybean plants were systematically sampled from each soybean field and tested for BPMV using ELISA. Prior to sampling, the GPS coordinates of each selected field were recorded. Field- and county-scale BPMV prevalence and incidence were mapped using ArcGIS. The 2006 growing season had the highest BPMV prevalence both at field (35.9%) and county scale (90.1%); whereas prevalence of BPMV at the county scale was 39/96 (40.1%) in 2005 and 74/99 counties (74.7%) in 2007. Field scale (within-county) BPMV incidence ranged from 0 to 100%. Moran’s I analysis revealed significant spatial dependence (clustering) at the county scale, indicating that counties with high BPMV incidence tended to be neighbored by other counties that also had high incidence, and counties with low BPMV incidence tended to be neighbored by counties with low BPMV incidence. Kriged maps and regression analysis revealed BPMV incidence was higher in the southern half of the state during each of the
three years. The use of GPS, GIS, and geostatistics provided new information at large spatial scales to better understand the season-to-season and geographical distribution of BPMV disease risk in Iowa.

INTRODUCTION

Iowa leads the nation in soybean (*Glycine max* L.) production, accounting for approximately 17% of the soybeans produced in the United States (17). Among the most important yield-limiting pathogens is the soybean cyst nematode (28), along with stem and foliar bacterial and fungal diseases (29, 31), and plant viruses (6). Of the 45 viruses known to infect soybean (3), *Bean pod mottle virus* (BPMV) is the most common and widespread (6).

*Bean pod mottle virus* has been reported to cause up to 52% yield loss (10), and adversely affects seed quality by discoloring the seed coat (9). When soybean plants are co-infected with both *Soybean mosaic virus* (SMV) and BPMV, symptom expression and yield loss is greater than when either virus infects alone (2, 4). For example, Quinones et al. (23) reported yield reductions in the cultivars Lee and Hill due to BPMV alone were 26% and 14%, respectively, and 10% and 18% respectively, due to SMV alone. However, when these cultivars were co-infected with both viruses, yield losses were as high as 66%. Previous reports state that BPMV was also found to predispose soybean plants to *Phomopsis* blight infection in soybean seed, resulting in seed deterioration, poor seed quality, and poor germination (1).

Although BPMV was first reported in Iowa in 1966 (23), it only recently became a major concern to soybean growers in the North Central region and Northern Great Plains of the United States (6). This virus has been reported in most of the in the North Central region...
of United States as well as in Canada (15, 16, 32). The incidence of BPMV in the North Central region has been reported to be near-epidemic levels (4), and is now viewed as an emerging threat to the soybean industry (4). The increased BPMV prevalence and incidence (risk) is thought to be related to an increase in the winter survival of bean leaf beetle (Cerotoma trifurcata (Forster)), the primary vector (6, 12, 15). Despite early reports and importance of this virus in Iowa, large knowledge gaps exist concerning its geographical distribution. Although Krell et al. (13) tested bean leaf beetles for the presence of BPMV that were collected from 84 Iowa counties, the same study indicated that bean leaf beetles that tested positive for BPMV by ELISA did not necessarily transmit BPMV to soybean seedlings. Thus, at present, there is very little reliable information on the incidence of BPMV in Iowa soybeans. Information concerning the geographical distribution of BPMV in Iowa could indicate whether all soybean fields and every Iowa county has an equal risk of BPMV occurrence, and if not, whether some fields and counties are at greater risk than others. The development of risk maps for BPMV among Iowa counties may lead to identification of large scale risk factors that can be used to predict risk within well-defined geographical zones prior to spring planting. This has implications on threshold levels for different regions and the timing of management tactics.

One of the tools that can be used to reveal the presence of spatial dependence (patterns) at large geographical scales involves the use of Global Positioning System (GPS) and Geographical Information System (GIS) technologies. Integration of GPS and GIS technologies has helped to identify, quantify and display spatial patterns of risk factors affecting diseases and/or pathogen populations over time and space (14, 18, 19). The spatial
question that is important to address is to test the hypothesis that all soybean fields and counties in Iowa are at a random risk for BPMV.

The objectives of this study were to (i) quantify the prevalence and incidence of BPMV at field and county scales in Iowa over time, and (ii) determine the spatial dependence of BPMV at field and county scales in Iowa.

**MATERIALS AND METHODS**

**Sample collection.** A state-wide soybean disease survey (The Iowa Soybean Disease Survey) was carried out in Iowa during the 2005, 2006, and 2007 growing seasons. Eight to twenty fields per county were sampled at growth stages V2-V3, R1-R2, R4-R5, and R6-R7 from 96 counties in 2005, and from all 99 counties in 2006 and 2007.

The GPS coordinates, soybean cultivar, previous year’s crop, presence or absence of bean leaf beetles, and growth stage were recorded before each soybean field was sampled. A systematic sampling design was used to collect 30 soybean plants from each field. Soybean plants were collected by either Iowa State Field Agronomists from May through September each year using a modified cross sampling pattern, or by National Agricultural Statistical Services (NASS) staff during July through August using a modified W sampling pattern with 10 arms. The selected plants were uprooted, bagged and overnight mailed to Iowa State University, where they were kept at 4°C until they were assessed. The middle leaflet of the topmost fully-expanded leaf from each plant was removed; thus, 30 leaflets were tested from each field. After the 30-leaflet samples were stratified into five six-leaflet sub-samples, the sub-samples were labeled and stored in plastic zip-lock bags at 4°C until leaflet sap was extracted.
**Sap extraction.** Sap from each sub-sample was extracted using a leaf press (Ravenel Crop Specialties, Seneca, SC). Leaflets were individually placed between metal rollers and 3-4 ml of general extraction buffer (a solution containing 3% sodium sulphite, 46% polyvinylpyrolidone, 4.5% egg albumin, 46% Tween 20 and 0.5% sodium azide, dissolved in phosphate buffer saline-Tween 20, pH 7.4) was added between rollers as plant sap was collected into 5-ml portion cups (Instaoffice, Kennesaw, GA). A 1.5-ml aliquot from each sub-sample was pipetted into each of three 1.7-ml eppendorf tubes, which were then frozen and stored at -20 °C for subsequent testing by ELISA.

**Detection of BPMV.** Presence or absence of BPMV in a sub-sample was determined using a double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) commercial kit for BPMV (Agdia Reagent Set, alkaline phosphatase label; Agdia, Inc., Elkhart, IN). Briefly, micro-titer high-binding plates (Agdia Inc. Elkhart, IN) were first coated with BPMV antibody that was diluted 1:200 in a coating buffer and kept at 4º C overnight. After thawing, 100 µl of plant sap was loaded into duplicate wells and plates were kept overnight at 4º C. Four known BPMV-positive and four negative control samples were loaded onto each plate as checks. The second antibody, BPMV alkaline phosphatase enzyme, diluted 1:200 in enzyme conjugate immunoassay (ECI) buffer, was added to each well and plates incubated at room temperature for 2 hours. Between each step, plates were washed with phosphate buffer saline-Tween 20 (PBST) using a microplate washer (ELx405 Biotec Inc. Winooski, VT). After the final washing, color development solution (p-Nitrophenol phosphate, PNP) was added and plates were kept in the dark for 30 minutes before reaction products were read at 405 nm absorbance using plate reader model ELx 800 (Biotek Inc.,
A sub-sample was considered positive for BPMV if the absorbance value was greater than twice the average absorbance of the negative controls.

**Temporal data analysis.** Prevalence of BPMV at the county scale was calculated as the number of counties that had at least one sub-sample testing positive for BPMV, divided by the total number of counties tested for BPMV, multiplied by 100. Prevalence (%) at the field scale was defined as the number of soybean fields with at least one of the five sub-samples testing positive for BPMV, divided by the total number of soybean fields tested for BPMV, multiplied by 100. Cumulative prevalence data were plotted against day of year of sampling to determine the seasonal pattern and rate of increase in the percentage of soybean fields testing positive for BPMV.

Incidence at the county scale was obtained by calculating the number of BPMV-positive sub-samples within a county, divided by the total sub-samples tested in that county, multiplied by 100. To estimate incidence (%) at the field scale, the number of BPMV-positive sub-samples from a soybean field was divided by the total number of sub-samples tested per field, multiplied by 100. Cumulative prevalence and incidence data at the field scale were plotted against day of year of sampling to obtain cumulative BPMV prevalence and incidence curves. The progress curves were subjected to population growth models, and the most appropriate model was selected based on the following criteria: F-statistic for each model, coefficients of determination ($R^2$), standard errors of the estimate for pathogen incidence ($\hat{\gamma}$), and on the subjective evaluation of plots of standardized residuals versus predicted values (7, 20, 21, 25).

**Spatial data analysis.** Prevalence data at county and field scales were mapped using ArcGIS (ESRI, Redlands, CA). County prevalence and incidence were mapped as polygon
data. Boundary shape files for Iowa counties were obtained from a freely-available US Census website (http://www.census.gov/geo/www/cob/cs2000.html#shp). Field prevalence and incidence were mapped as point data using GPS coordinates for each soybean field using Universal Transverse Mercator datum (UTM) 1983 zone 15. To detect spatial dependence for county (polygon) data, Moran’s Index was used to obtain a weighted correlation coefficient. The index ranges from -1 to 1, with values close to one indicating a strong spatial dependence (departure from randomness) where polygons (counties) with high values are neighbored by polygons with also high values and vice-versa, depending on the neighborhood structure. Neighborhood structure in this case was created using the inverse distance squared option. To determine if BPMV risk was related to south-north or east-west geographical location, county centroid latitude and longitude location (x-and y-coordinate, obtained from the county centroid) was graphed with respect to county BPMV incidence used to detect if county location had a linear relationship with county BPMV incidence, using linear regression analysis.

In order to estimate the field incidence for non-sampled soybean fields within Iowa, the ordinally kriging method was used (14, 19). Based on the minimum mean squared errors and lack of patterns in residual plots, the model that best fit BPMV incidence data was used to obtain semi-variograms. Field BPMV incidence data were subjected to trend analysis and anisotropy before selecting the appropriate kriging model.

RESULTS

Prevalence of BPMV in Iowa. In 2005, BPMV was first detected in soybean fields on day of year 167 (16 June) Washington county. In 2006, BPMV was first detected on day of year 165 (14 June) in Union county, and on day of year 171 (20 June) in Marshall county
in 2007 (Table 1, Figures 5-7). Prevalence of BPMV in Iowa counties varied among years, with 2006 having the highest BPMV prevalence (Table 2, Figures 5-7). In 2005, BPMV was found in 39 of the 96 counties sampled (41%), 90 of 99 counties (90%) in 2006. BPMV was not detected in any of the samples collected over the entire soybean growing season in 9 counties in 2006. Of the 99 counties sampled and tested in 2007, BPMV was detected in 74 counties (75%). Counties in which BPMV was detected in the previous year also had BPMV detected in the following year, with just a few exceptions in 2007. In all three years, the number of counties when BPMV was detected peaked in July and August (Figures 5-7).

Cumulative progress curves for BPMV prevalence for the 2005, 2006, and 2007 soybean growing seasons are shown in Figure 1. Field BPMV prevalence varied among the three soybean growing seasons with 2006 having the highest field prevalence (35.49%). In 2005, field prevalence was 10.29% and 20.16% in 2007 (Table 1). The relationship between field prevalence and day of year of sampling was best described by linear model based on model evaluation criteria (on the F-value, R^2 value, SEEy and residual plots). Slope values ranged from 0.11 to 0.47% fields/day (R^2 = 89.5%, 94.6% and 92.2% for 2005, 2006, and 2007, respectively), indicating that for every 10 days of sampling, BPMV was detected in 1.1 to 4.7% of the field sampled (Figure 1).

**Incidence of BPMV in Iowa.** Incidence of BPMV at the county scale varied across years and among counties (Table 2, Figure 7). In 2005, the highest incidence (31%) occurred in only one county. The majority of counties had between 1 and 5% BPMV-positive fields in 2005. In 2006, the highest BPMV incidence (>60%) occurred in 11 counties; at least 44 counties had BPMV incidence levels ranging from 15 to 60%. Although there were many
counties with BPMV in 2007, incidence was relatively low in many counties; the highest incidence (>60%) was found in only one county.

Incidence of BPMV within fields also varied across years (Figure 2). The highest level of BPMV incidence was reached on day of year 260 (17 September) in 2005, day of year 250 (7 September) in 2006 and day of year 267 (24 September) in 2007 (Table 1, Figure 2, 8). The highest BPMV incidence (24.85%) occurred in 2006 (Table 2) where 160 fields that had all samples collected in each field testing positive for BPMV. In 2005 and 2007, very few soybean fields had 50% or more BPMV-positive samples. The fastest rate of increase of new BPMV-infected soybean fields occurred on day of year 206 (24 July), 211 (30 July) and 207 (25 July) for 2005, 2006 and 2007, respectively (Figures 3 and 4). The linear model best explained the relationship between BPMV incidence and day of year for the three years (Figure 4). Coefficients of determination ranged from 91.8 to 97.8%, indicating that day of year (time) explained 91.8 to 97.8% of the variation in BPMV incidence.

The relationship between BPMV prevalence and incidence at the field scale is shown in Figure 9. Coefficients of determination ranged from 99.4 to 99.6% indicating that 99.4% of variation in BPMV incidence was explained by BPMV prevalence. Higher prevalence was always associated with higher BPMV incidence.

**Spatial Dependence Analysis for BPMV Incidence.** There was a significant spatial dependence for county scale BPMV incidence using Moran’s I measure of spatial aggregation in all years, meaning that counties that had high BPMV incidence were neighbored by counties that had also high BPMV incidence (Table 3). The strongest spatial dependence, 0.68 was recorded in 2006. Initial detection of BPMV occurred in the southern
counties of the state in all three years. In fact, for 2005 and 2006, the first two counties to be found with BPMV were within similar neighborhoods (Figures 5 & 6). Kriged maps showing estimations for non-sampled areas using semivariograms for the three years also indicated higher incidence of BPMV in the southern than the northern half of the state (Figure 10). There was a significant linear relationship between county BPMV incidence and county latitude distance for the three years. The strongest relationship occurred in 2006; slope of –0.20% per km, $R^2 = 57.9\%$ indicated that for every 10 km increase in latitude distance, BPMV incidence decreased by 2% in 2006 (Figure 11). In 2005 and 2007, there was a weak ($R^2 = 10.4$, 17.4% for 2005 and 2007, respectively) but significant linear relationship between county BPMV incidence and latitude.

**DISCUSSION**

This is the first comprehensive survey to map the distribution of BPMV in Iowa at different spatial scales. The coupling of GPS and GIS technologies with detailed disease survey data provided a platform to depict and quantify temporal and spatial patterns at different spatial scales. Our results revealed that BPMV incidence in Iowa counties was spatially dependent. One of the most important finding of this study was the rejection of the null hypothesis that BPMV prevalence and incidence occurs at a random within and among Iowa counties. We demonstrated that Iowa counties have differing levels of BPMV risk and that biotic and abiotic risk factors may be specific to different areas within the state. Disease gradients for BPMV incidence were for the first time shown to be present in Iowa, with the risk of BPMV decreasing from South to North direction in all the three years of the survey.

The rate of change in BPMV incidence was fastest late July-early August, which coincided with the peak in bean leaf beetle population. Increased beetle numbers, following
high bean leaf beetle winter survival has been linked to increased BPMV incidence in the North Central region (6). Smesler and Pedigo (24) showed that the first generation of bean leaf beetles occurs in late July and early August and peaks by the end of August. This period corresponded with the high BPMV incidence in all the three years. Limited BPMV detection in the months of June and July indicated that there is limited initial inoculum and BPMV spread occurs due to high bean leaf beetle numbers, as opposed to high initial inoculum levels.

A previous study assessing inoculum sources of BPMV in Iowa found that overwintering bean leaf beetles collected from 84 counties tested positive for BPMV (13). However, the same report indicated that although bean leaf beetles may test positive for BPMV, these beetles may not necessarily transmit the virus to healthy plants. Limited or non-BPMV transmission by overwintered bean leaf beetle population, coupled with year to year variation in bean leaf beetle winter survival within and among Iowa counties may explain (at least in part) the spatial aggregation of BPMV incidence in Iowa.

The consistently high BPMV incidence in the southern part of the state may likely indicate area-specific risk factors associated with BPMV in this region. Spatial dependence analysis at county and field scales indicated clustering of disease intensity in all three years. A number of reports including other pathosystems have been published concerning association of high disease incidence with area-specific disease risk factors (5, 26, 27, 30). For incidence, Nelson et al. (19) reported that the risk of a virus disease complex on tomatoes in Del Fuerte Valley was spatially dependent and they identified “conducive landscapes” that were associated with high incidence levels of the tomato virus complex. Our results indicate
unequal risk of BPMV in Iowa, which may be due to different biotic and abiotic risk factors at play.

Early planting dates have been associated with higher BPMV incidence, though this was inconsistent (13). Counties in the southern part of the state may be planting earlier in response to warmer spring temperatures. This may result in a longer period of time for bean leaf beetles to interact with emerging soybean plants resulting in higher levels of BPMV incidence earlier in the growing season leading to greater BPMV risk. There is a need to further investigate the importance of other risk factors that may be at play in this pathosystem, such as the role of winter mean temperature in beetle survival, the presence or absence of BPMV the previous season, snow depth, number of snow days, aspect, topography, and abundance of alternative weed hosts.

ACKNOWLEDGMENTS

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TABLE 1. Temporal variables describing progress of *Bean pod mottle virus* in Iowa counties in 2005 through 2007

<table>
<thead>
<tr>
<th>Year</th>
<th>Day of year when BPMV was first detected</th>
<th>Day of year highest when BPMV incidence was reached</th>
<th>Day of year that the rate of BPMV incidence (dy/dt) was highest</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>167</td>
<td>260</td>
<td>206</td>
</tr>
<tr>
<td>2006</td>
<td>165</td>
<td>250</td>
<td>211</td>
</tr>
<tr>
<td>2007</td>
<td>171</td>
<td>267</td>
<td>207</td>
</tr>
</tbody>
</table>
TABLE 2. Final *Bean pod mottle virus* prevalence and incidence at county and field scales during the 2005, 2006, and 2007 soybean growing seasons in Iowa.

<table>
<thead>
<tr>
<th>Year</th>
<th>Final county BPMV prevalence (%)</th>
<th>Final field BPMV prevalence (%)</th>
<th>Highest county BPMV incidence reached</th>
<th>Final field BPMV incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>40.6</td>
<td>10.29</td>
<td>31.0</td>
<td>4.36</td>
</tr>
<tr>
<td>2006</td>
<td>90.1</td>
<td>35.49</td>
<td>100</td>
<td>24.85</td>
</tr>
<tr>
<td>2007</td>
<td>75.4</td>
<td>20.16</td>
<td>50.0</td>
<td>9.80</td>
</tr>
</tbody>
</table>
TABLE 3. Spatial dependence of *Bean pod mottle virus* incidence in Iowa counties using Moran’s I, for 2005, 2006 and 2007

<table>
<thead>
<tr>
<th>Year</th>
<th>Final BPMV</th>
<th>Moran’s I</th>
<th>z-score</th>
<th>P-value</th>
<th>pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>incidence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>40.6</td>
<td>0.27</td>
<td>3.59</td>
<td>0.01</td>
<td>Clustered</td>
</tr>
<tr>
<td>2006</td>
<td>90.1</td>
<td>0.68</td>
<td>8.59</td>
<td>0.01</td>
<td>Clustered</td>
</tr>
<tr>
<td>2007</td>
<td>74.7</td>
<td>0.2</td>
<td>2.94</td>
<td>0.01</td>
<td>Clustered</td>
</tr>
</tbody>
</table>
**Fig. 1.** (A) Cumulative prevalence of BPMV in soybean fields in Iowa in 2005, 2006 and 2007 soybean growing seasons, (B) Linear relationship between cumulative field BPMV prevalence (%) and day of year of sampling during the 2005, 2006, and 2007 soybean growing seasons in Iowa.

**Fig. 2.** Cumulative BPMV incidence (%) plotted against day of year during the 2005, 2006, and 2007 soybean growing seasons.

**Fig. 3.** Rate of change in BPMV field incidence versus time (dy/dt) BPMV was detected by ELISA in Iowa soybean fields in 2005, 2006 and 2007.

**Fig. 4.** Linear relationship between cumulative incidence (%) of BPMV (y) and day of year (x) during the 2005, 2006, and 2007 soybean growing seasons in Iowa.

**Fig. 5.** Prevalence of BPMV in Iowa counties during the months of (A) June, (B) July, (C) August, and (D) September, in 2005.

**Fig. 5** Prevalence of BPMV in commercial soybean fields within Iowa counties during the months of (A) June, (B) July, (C) August, and (D) September, in 2006.

**Fig. 7.** Prevalence of BPMV in commercial soybean fields within Iowa counties during the months of (A) June, (B) July, (C) August, and (D) September, in 2007.
**Fig. 8.** Incidence of BPMV in commercial soybean fields among the Iowa counties in (A) 2005, (B) 2006, and (C) 2007.

**Fig. 9.** Relationship between field Bean pod mottle virus incidence and prevalence for 2005 through 2007 in Iowa.

**Fig. 10.** Kriged maps showing the estimated Bean pod mottle virus incidence in Iowa during (A) 2005, (B) 2006, and (C) 2007.

**Fig. 11.** Relationship between the Bean pod mottle virus incidence at county scale and the county centroid latitude (y-coordinate) during (A) 2005, (B) 2006, and (C) 2007.
Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.
Fig. 5.
Fig. 6.
Fig. 7.

A

B

C

D

BPMV Prevalence
(# of counties)

- Green: BPMV not detected (7)
- Orange: BPMV detected (5)
- Gray: Not sampled (21)

BPMV Prevalence
(# of counties)

- Green: BPMV not detected (58)
- Orange: BPMV detected (39)
- Gray: Not sampled (2)

BPMV Prevalence
(# of counties)

- Green: BPMV not detected (30)
- Orange: BPMV detected (69)

BPMV Prevalence
(# of counties)

- Green: BPMV not detected (25)
- Orange: BPMV detected (74)
Fig. 8.
Fig. 9.
Fig. 10.
Distance of Iowa county centroids (km)

BPMV incidence (%) in Iowa counties

Fig. 11.
CHAPTER 4.
QUANTIFICATION OF THE EFFECT OF BEAN POD MOTTLE VIRUS TIME OF INFECTION ON SOYBEAN YIELD, YIELD COMPONENTS, AND QUALITY

Manuscript to be submitted to *Phytopathology*

ABSTRACT

The impact of time of *Bean pod mottle virus* (BPMV) detection (related to time of infection) on soybean yield, yield components and grain quality was quantified during the 2006 and 2007 soybean growing seasons. Soybean quadrats (30cm in length, four plants) were established within soybean plots (cv. NE3001), that were 6 rows and 7.6 m long. Quadrats were sampled every 8-11 days beginning 25 days after planting. There were 9 sampling dates in 2006 and 10 sampling dates in 2007. Leaflet samples from each quadrat were returned to the laboratory and sap from each quadrat (of leaflets) was extracted and then tested for presence/absence of BPMV by ELISA. The day of year when BPMV was first detected within a quadrat was recorded. To determine the effect of time of BPMV detection on soybean yield, yield components and grain quality, all or up to 35 quadrats were randomly selected from the cohort population of soybean quadrats that had the same sampling date when BPMV was first detected and harvested by hand. Soybean yield, number of pods per plant, number of seeds per plant, and 100-seed weight for each quadrat were determined. The relationship between time of BPMV detection (infection) and grain yield, yield components, and grain quality was quantified using linear regression. Time of BPMV detection explained
57.9% and 89.7% of the variation in soybean grain yield in 2006 and 2007, respectively. The yield damage function (slope) was -15.5 kg/ha/day in 2006 and -7.9 kg/ha/day, indicating that for each day that BPMV detection was delayed, soybean yield increased by 15.5 and 7.9 kg/ha in 2006 and 2007, respectively. There was a significant linear relationship between number of pods per plant and time of BPMV detection in 2006, with slope of 0.15, indicating that pod numbers per plant increased by 0.15 pods for every day that BPMV detection was delayed (R² = 72.8%). The number of seeds per pod was not impacted by time of BPMV detection in either year. The 100-seed weight had a significant linear relationship with day of year when BPMV was detected in a quadrat with a slope of 0.013 (R² = 34.7%), meaning that 100-seed weight was increasing by 0.013g for each day BPMV detection was delayed. Linear regression analysis showed the percentage of mottled seeds in both years decreased by 0.34% and 0.15% (R² = 82.8%, 48.3%) for each day that BPMV detection was delayed in 2006 and 2007, respectively. Protein and oil content were not impacted by time of BPMV detection in a quadrat in both years, however, protein and oil content tended to be impacted inversely (slope = -0.67, R² = 98.2%), indicating quadrats that had early BPMV tended to have high protein content and low oil content. This study demonstrated that time of BPMV detection can be used to quantify reductions in soybean yield, yield components and grain quality.

INTRODUCTION

Bean pod mottle virus (BPMV) (genus Comovirus, family Comoviridae) is increasingly becoming a threat to soybean production in the U.S. (7). To date, BPMV has been reported to occur in the major soybean producing states in the U.S. (7). Increasing levels of BPMV in soybean have been attributed to increases in bean leaf beetle (Cerotorma trifurca Foster).
Bean pod mottle virus has been reported to adversely affect both soybean grain quantity and quality (11, 13, 34).

A number of reports on yield loss caused by BPMV (4, 5, 13, 15, 17, 18, 24, 27, 32), indicate various levels of yield reduction. The first study on the effects of BPMV on soybean yield was done in 1969 in North Carolina (27). In this study BPMV infection of the cultivars ‘Hill’ and ‘Lee’ resulted in yield reductions of 13 and 40%, respectively. In another study, the effect of BPMV infection on soybean yield components found that BPMV infection was more detrimental to yield than yield reductions that occurred in response to imposed soil water stress (24). Yield loss due to BPMV on four cultivars inoculated at primary leaf growth stage (VC) in Kentucky ranged from 23 to 44% (30). Hopkins & Mueller (13) found that time of BPMV inoculation influenced soybean. In their study, two soybean cultivars ‘Bragg’ and ‘Lee 74’ were mechanically inoculated at growth stages ranging from V1 to R6. The greatest yield loss (52.6%) was recorded for ‘Bragg’ plants that were inoculated at the earliest growth stage (V1). Hopkins and Muller also reported that time of inoculation also affected the number of pods per plot. Ross (27) in North Carolina measured the response of three early and late planted soybeans cultivars to infection by BPMV from plots that were either mechanically-inoculated, screen caged, or left unprotected (to allow natural infection by viruliferous bean leaf beetles). BPMV infection caused yield reduction ranging from 2.3 to 19%. Delay of BPMV infection through caging improved soybean yield by 3.4 to 11.6%, and late planted soybeans had higher yields than did early planted soybeans. However the effect of caging on reduction of light interception was not accounted for in Ross’s study, and inoculation of all soybean plants in a plot (100% infection) was done at the VC soybean growth stage. Ziems et al. (34) evaluating 11 major soybean cultivars grown in the North
Central region of the U.S, reported varying levels of yield loss when plants were mechanically inoculated at different soybean growth stages.

In all of the above yield loss studies, all soybean plants in a treatment were mechanically inoculated at one point in time. This scenario is highly unlikely to occur under natural field epidemics (20, 25). Moreover, mechanical inoculation of all plants does not allow for yield compensation by neighboring healthy plants (32), which likely results in overestimation of the true impact of BPMV on yield and yield components. In addition, mechanical damage to inoculated leaves may affect the physiology of the plant and provide entry for other pathogens. For example, yields were found to be more severely reduced in by artificially inoculated induced plots than in naturally-infected plots with the same level of Barley yellow dwarf virus incidence (12).

Quantitative information regarding the effect of time of BPMV detection on soybean yield under natural field BPMV epidemics remains largely unknown, yet such knowledge is important in justifying the need for development of management tactics needed to maintain soybean yield potentials, as well as determining when specific management strategies will deliver economic benefits (i.e., cost-effective).

In addition to causing direct yield loss, BPMV has been associated with soybean seed coat mottling (11, 33) and off-colored seed (>10% discoloration) is a primary-rating factor that reduces the market grade of soybean (29). Hobbs et al. (11) reported that plants co-inoculated with BPMV and SMV did not always exhibit higher percentages of mottled seed than did plants inoculated with BPMV or SMV alone. Moreover, Hill (9) tested two seed lots for the presence BPMV, one with colored seed coats and the other without seed coat color (normal). Both seedlots had comparable BPMV levels, indicating that the measure of seed
coat mottling was not best indicator of BPMV infection. Although other environmental stresses have been reported to cause soybean seed coat mottling (8, 14, 23), the interaction between time of BPMV infection, relative to soybean growth stage, and soybean seed mottling has not been investigated. For example, a seed lot infected by BPMV late in the season may contain fewer or no mottled seeds, but may still test positive for BPMV.

The objective of this study was to quantify the relationship between time of BPMV infection (or first detection) and soybean yield, yield components and grain quality.

**MATERIALS AND METHODS**

**Field plots.** Soybean field plots were established at the Iowa State University Curtiss Research Farm in Ames, Iowa. The soybean cultivar NE3001, which is susceptible to BPMV but tolerant to SMV (10), was planted on 5 May in 2006 and on 18 May in 2007. Each soybean plot consisted of eight rows, 7.6 m in length, with a row spacing of 0.76 m. The two outermost rows and an additional 1.5 m of row on both ends of each plot served as borders to minimize edge effects. Each soybean plot was located at least 15.2 m from other experimental plots. The six center rows (7.6 m in length) were partitioned into 150, 30 cm-long quadrats (6 rows x 25 quadrats/row =150 quadrats per plot). White wooden stakes (22.8 cm) were placed at 45° to ground within rows to delineate quadrats. Soybean plants were thinned to four plants per quadrat on 24 May in 2006, and 12 June in 2007.

**Treatments.** Four treatments were used to differentially affect time of BPMV detection (related to time of infection) within soybean plots. The treatments were: (i) establishing a BPMV-inoculated point source (2 quadrats per plot mechanically inoculated with BPMV); (ii) two applications of lambda-cyhalothrin (Warrior™ 1EC, Syngenta Crop
Protection, Greenville, NC); (iii) the establishment of a BPMV-inoculated point source and two applications of lambda-cyhalothrin, and (iv) a non-treated control. For the BPMV-inoculated point source treatments (treatments (i) and (iii)), soybean plants located in the 13th quadrat positions of the center two rows (3rd and 4th rows) were mechanically inoculated with sap extracted from a BPMV-infected soybean plant. The BPMV point sources were inoculated on 30 June in 2006 and on 13 June 2007. Treatments (ii) and (iii) received two applications of the insecticide lambda-cyhalothrin when soybean plants reached the primary leaf growth stage (V1) and the early reproductive growth stage (R2) (14). Insecticide was applied at a rate of 6.9 fl oz ai/ha using a CO2-powered sprayer at 40 Pa.

**Data collection.** To determine when individual quadrats first tested positive for the presence of BPMV, soybean plants in each 30-cm quadrat in each plot (6 rows x 25 quadrats, i.e., 150 quadrats) was sampled by removing a single leaflet from the youngest, fully-expanded trifoliate leaf from each of the four plants within a quadrat. Quadrats were sampled every 8-11 days, beginning 25 days after planting. The four leaflets from each quadrat were placed together in pre-labeled (plot number, row number, quadrat number, and date of sampling) plastic sandwich-size bag (bulk sample), and stored at 4 ºC until sap extraction.

**Sap extraction.** Sap was extracted from the four leaflets sampled from each quadrat using a leaf press (Ravenel Specialties Corp., Seneca, SC). Plant sap was diluted by adding approximately 1.75 ml of general extraction buffer (pH 7.4), and extracted sap was stored in 1.5-ml eppendorf tubes at -20º C until testing.

**ELISA assay for BPMV.** A double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) for BPMV was used to test for the presence of BPMV in each soybean quadrat sap sample (Agdia Reagent Set, Alkaline phosphatase label; Agdia,
Inc., Elkhart, IN). All ELISA steps were followed as recommended by the manufacturer. Briefly, micro-titer high-binding plates (Agdia Inc. Elkhart, IN) were first coated with BPMV capture antibody diluted 1:200 in a coating buffer and incubated overnight at 4ºC. A 100-µl aliquot of plant sap was loaded into duplicate plate wells and incubated overnight at 4 ºC. Four known BPMV-positive and negative control samples were loaded onto each plate as a check. The second antibody, (BPMV alkaline phosphatase enzyme diluted 1:200 in enzyme conjugate immunoassay buffer), was added, and plates were incubated at room temperature for 2 hours. Between each ELISA step, plates were washed with phosphate buffer saline-Tween 20 (PBST) using a micro-titer plate washer (ELx405 Biotec Inc. Winooski, VT). After the final washing, color development solution (p-Nitrophenol phosphate, PNP) was added, and plates were kept in the dark for 30 minutes before reaction products were read at 405 nm absorbance using a Biotek ELx 800 plate reader (Biotek Inc., Winooski, VT). Quadrat sap samples were considered positive for BPMV if the absorbance value of a quadrat sample was greater than twice the value of the mean absorbance of the negative controls.

**Field yield data.** Soybean quadrats were considered healthy (non-infected) if BPMV was not detected by the end of the soybean growing season. The position and date when a quadrat first tested positive for BPMV was recorded and mapped. After soybeans had reached maturity, all or up to 35 soybean quadrats from a cohort of soybean quadrats for each detection period numbered 1 to n were randomly selected (using a random number generator) (Figure 1). The four plants from a quadrat were harvested by hand and placed in cotton bags with pre-labeled tag identifying the plot number, row number, quadrat number, and date when BPMV was first detected. Yield and yield components were determined for each.
harvested quadrat. The number of pods per plant within quadrats as affected by time that BPMV was detected on the four plants per quadrat were counted and recorded. The pods were then shattered, and the seed cleaned by hand. The number of seeds per pod, grain weight and 100-seed weight were determined for each harvested soybean quadrat.

The yield gap due to time of BPMV detection was obtained by subtracting the average grain weight of soybean quadrats for each BPMV detection time from the average grain weight of healthy quadrats (BPMV negative throughout the entire growing season) (21). Simulated (theoretical) yield for each plot was obtained by averaging yield from each detection time multiplied by the number of quadrats with the same detection date in a plot and totaling up the yield for all detection times in a given plot. The percentage of mottled seed for each quadrat was determined by counting seeds that had any seed coat discoloration, and then dividing by the total number of seeds per quadrat x 100. Seeds from each harvested quadrat were tested for protein, oil and fiber content using near infrared spectroscopy (Infratec-1225, Eden Prairie, MN).

Data analysis. To obtain BPMV progress curves, percentage of soybean quadrats testing positive for BPMV each sampling time (BPMV incidence) was plotted against time of sampling (day of year). The variables of seed weight, number of pods per plant, number of seeds per pod, and 100-seed weight were subjected to analysis of variance using GLM procedure of SAS (SAS Institute Inc, Cary, NC) to determine effect of treatment on yield and yield parameters. When no significant interaction was found among treatments and blocks for yield or yield components, quadrat data were grouped together across blocks and treatments according to time of BPMV detection and an average value for each detection period plotted against time (day of year) when BPMV was first detected and subjected to
linear regression analysis. In 2006, there were no significant treatment or block differences in slopes and intercepts, so variables were pooled across treatments. In 2007, there was heavy aphid infestation, therefore only foliar insecticide treated plots were considered for yield and yield components analysis.

**Greenhouse component.** A greenhouse experiment was conducted to determine the effect of time of BPMV inoculation on soybean yield and to compare greenhouse data to results from field experiments. Soybean cultivar NE3001 was planted in 10-cm clay pots (¾ filled with potting mixture; 1 peat: 2 perlite: 2 bark mix) on 1 March, 2007, and on 6 March, 2008. The greenhouse room was kept at 29°C and was given 14 hours of light and 10 hours darkness. Pots were thinned to one plant per pot after emergence. There were four plants for each treatment. Treatments consisted of nine different inoculation dates with three replications arranged in a completely randomized design. The first inoculation was done at V1 (first trifoliate leaf growth stage), and subsequent inoculations were performed at 10-day intervals. Inoculations were performed mechanically by light dusting of carborundum (600-mesh) on the topmost, fully-expanded leaf, and then rubbing the leaf surface lightly with the index finger dipped into BPMV-positive soybean leaf sap plus extraction buffer (1 g leaf tissue: 3 ml extraction buffer).

**Greenhouse yield data.** Each soybean plant was harvested after plant senescence (17 September, 2007, and 24 September 2008), placed in a large envelope and dried to 13% moisture content. The number of soybean pods, number of seeds per pod, and 100-seed weight were determined for each plant. Data from four plants for each treatment were averaged to obtain a treatment value for number of pods per plant, number of seeds per plant, number of seeds per pod, seed weight per plant, and 100-seed weight for each replicate. The
above variables were subjected to regression analysis to determine the linear relationship between the time of BPMV inoculation on soybean yield and yield components. The effect of time of BPMV inoculation on protein and oil content was determined using linear regression.

For both field and greenhouse data, yield variables that had statistically similar slopes and intercepts, the two year data were combined into one data set for regression analysis (6).

**RESULTS**

**Impact of time of BPMV detection on soybean yield.** In 2006, BPMV incidence in soybean plots at the end of the growing season ranged from 90.0% to 94.4%. In 2007, the highest BPMV incidence within soybean plots was 37.1% (Figure 2). Less than 10% of soybean quadrats had BPMV detected by the 5\textsuperscript{th} sampling date in both years. Most quadrats tested positive for BPMV after day of year 190 (9 July) in 2006 and after day of year 200 (19 July) in 2007.

Soybean grain weight, number of pods per plant, and 100-seed weight were not influenced significantly by treatments in 2006 and 2007, and therefore, data from soybean quadrats with the same BPMV detection date were pooled across treatments. Time of BPMV detection had significant linear relationship with the yield gap in both years (Figure 3). A single point model relating time of BPMV detection to the corresponding yield gaps (kg/ha) explained 89.7% and 57.9% of the variation in the soybean yield gaps in 2006 and 2007, respectively. The yield damage function (slope) was -15.5 kg/ha/day in 2006 and -7.9 kg/ha/day in 2007, indicating that for each day that BPMV detection was delayed, the soybean yield gap decreased by 15.5 and 7.9 kg/ha in 2006 and 2007, respectively. The area under the BPMV pathogen progress curve had a strong linear relationship with simulated
soybean yield per plot in both years (P<0.0001), with coefficient of determination (R²) values of 94.4 and 96.7% indicating that 94.4 and 96.7% of the variation in simulated yield was explained by area under the pathogen progress curves for 2006 and 2007, respectively (Figure 4).

**Impact of time of BPMV detection on yield components.** Time of BPMV detection in soybean quadrats significantly reduced the number of pods per plant in 2006, with a slope of 0.15 (R² = 72.8%), indicating that there was a gain of 0.15 pods per plant for each day that BPMV detection was delayed (Figure 5). In 2007, there was no linear relationship between number of pods per plant and day of year of BPMV detection, but there was a numerical difference between number of pods per plant in quadrats that had BPMV detected early in the growing season and late in the season. In both years, time of BPMV detection did not influence the number of seeds per pod in cultivar NE3001 (Figure 6). The day of year when BPMV was first detected in a soybean quadrat significantly affected 100-seed weight (combined for two years) with a slope of 0.013g/day (R² = 34.7%), indicating that for every day BPMV detection was delayed, there was a gain of 0.013 g in 100-seed weight (Figure 7).

**Effect of time of BPMV detection on grain quality.** The day of year that BPMV was first detected in a quadrat significantly affected the percentage of mottled seed in both 2006 and 2007 (Figure 8). Day of year of BPMV detection explained between 82.8% and 48.3% of the variation in the percentage of mottled seed in 2006 and 2007, respectively. The slope was -0.34% for 2006 and -0.15% in 2007, indicating that percent mottled seeds decreased by 0.34 and 0.15% for each day that BPMV detection was delayed in 2006 and 2007, respectively. Day of year that BPMV was first detected in a quadrat did not affect the percent protein and oil content in both years (Figure 9), however, there was a numerical
increase (0.4%) in protein content for quadrats that tested positive for BPMV early in the season compared to quadrats in which BPMV was detected later in the season. There was, however, a strong linear relationship between percent protein and percent oil content with a negative slope of 0.67 ($R^2 = 98.2\%$), indicating that quadrats that had high protein content (%) had lower oil content (%) (Figure 10).

**Greenhouse experiments.** There was a significant linear relationship between time of inoculation and the soybean yield (g/plant) in initial and repeated experiment. Time of inoculation explained 45.1% and 41.5% of the variation in yield in initial and repeated experiments, respectively (Figure 11). The damage function (slope) was 0.045 and 0.07g for experiment 1 and 2, respectively, indicating that for every day BPMV inoculation was delayed, grain yield per plant increased 0.045 and 0.07g. Time of BPMV inoculation did not affect the number of pods per plant, 100-seed weight, or the number of seeds per pod in the two greenhouse experiments.

Time of BPMV inoculation in the greenhouse experiments significantly increased the percentage of mottled seed (combined data for the two experiments due to the treatment x year interaction being non significant) with a slope of -0.61, indicating that for every day that BPMV inoculation was delayed, there was a 0.61% reduction in the percentage of mottled seed (Figure 12). Time of BPMV inoculation affected protein content (%) in 2007, with a slope of -0.02 ($R^2 = 80.2\%$), indicating that percent protein content decreased by 0.02% for each day that BPMV inoculation was delayed. Time of BPMV inoculation did not, however, significantly affect protein content in 2008 (Figure 13). Oil content was not affected by time of BPMV inoculation in both greenhouse experiments.
DISCUSSION

Quantification of yield loss is important for assessing economic importance of plant pathogens and justifying the need for management strategies. Moreover, information on yield loss can be used to evaluate different management methods and cultivar resistance or tolerance to plant pathogens (28). In our study, BPMV yield loss values were as high as 27%, which when coupled with reduction in quality due to seed mottling and altered protein and oil content, may lead to further loss in revenue for soybean growers. The results from this study show that time of BPMV detection significantly affected soybean yield and the percentage of mottled seed if BPMV was detected before R2 growth stage. Thus, management strategies that delay the time of BPMV infection beyond R2 growth stage would effectively mitigate yield loss due to BPMV.

The single point model utilizing area under the pathogen progress curve as the independent variable explained up to 96.4% of the variation in simulated soybean yield. Madden et al. (20) reported the area under the curve to be a better predictor of yield loss because it provides a measure of crop stress for the entire epidemic, rather than point variable such as final disease/pathogen incidence, severity/incidence at flowering, or any other given growth stage. The plots that had higher AUPPC also had lowest yield indicating that the more quadrats that were infected with BPMV especially early in the season, the less was yield from such plots.

The highest incidence of BPMV over the two growing seasons was recorded after the R2 growth stage. However, yield losses associated with BPMV detection after the R2 growth stage was limited. Several reports have shown similar finding of limited yield loss due to BPMV after R2 growth stage when artificial inoculation was used (13, 27, 34).
The number of soybean pods per plant was significantly affected by time of BPMV detection only in 2006. Late appearance of the disease may be the reason for limited effect of BPMV infection on number of pods per plant in 2007. Reduced number of pods per plant may be a result of flower abortion caused by BPMV infection. Flower abortion because of plant virus infection has been reported for other viruses (35). This study demonstrated that there was no effect of BPMV infection on number of seeds per pod as evidenced by the lack of linear relationship between number of seeds per pod and time of BPMV detection in both years. This suggests that the cv. NE3001 is tolerant to BPMV in the number of seeds per pod yield component.

Our results indicate that time of BPMV infection significantly affected the percentage mottled seed in the two year experiments. Most of the reports on BPMV effects on mottled seed used one time assessment of mottled seed or from plants inoculated at once (11, 33). The slopes for the linear relationship between time of BPMV detection and percent mottled seed were high, indicating a steep decline in percent mottled seed with delayed BPMV infection. This may result in late infected soybean seed having few or no mottled seeds and yet test positive for BPMV (9, 11). While other factors are associated with soybean mottling, our study confirms that time of BPMV infection determines the level of seed coat discoloration. Although in some pathosystems (e.g., *Tomato spotted wilt tospovirus* pathosystem (22), seed quality is affected irrespective of time of viral infection, BPMV affected soybean seed quality when infection took place before the R2 growth stage.

Protein content was not impacted by time of BPMV detection in soybean quadrants only in both years. The fact that there was still a numerical decrease in protein content in both years, indicates that soybean BPMV infection increases the amount of protein.
Moreover, the negative slope for the relationship between percent protein content and oil content indicates that as protein increased in infected soybean plants, oil content decreased. While other stresses can increase protein content (26), this study suggests that time of BPMV infection may affect the protein content of soybean seeds. This study also showed that cultivar NE3001 is tolerant to BPMV for oil content as delaying time of BPMV had no significant impact on oil content.

Greenhouse experiments confirmed seed weight was linearly related to time of BPMV inoculation in the two years. Other variables were not repeatable. The second year experiments were affected by faulty temperature controls (temperature reached 49° C one weekend). This may have affected the experiment results in 2008.

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**LITERATURE CITED**


symptom severity of *Bean pod mottle virus* and SMV. American Society for Virology Meeting (Abstr).


Fig. 1. An example of one of 12 plots showing cohorts of soybean quadrats for each period of BPMV first detection in soybeans (cv. NE3001) planted at Iowa State University Research Curtiss Farm near Ames in 2006. Numbers in boxes indicate when a quadrat first tested positive for BPMV.

Fig. 2. Incidence of Bean pod mottle virus (BPMV) in soybean quadrats (cv. NE3001) planted at Iowa State University Curtiss Research Farm in (A) 2006 and (B) 2007.

Fig. 3. Relationship between day of year of Bean pod mottle virus detection and yield gap of soybean (cv. NE3001) planted at Iowa State University Research Curtiss Farm near Ames in 2006 (solid circles) and 2007 (open circles).

Fig. 4. Relationship between simulated yield and area under Bean pod mottle virus pathogen progress curve in soybean quadrats (cv. NE3001) planted at Iowa State University Research Curtiss Farm near Ames in 2006 (solid circles) and 2007 (open circles).

Fig. 5. Relationship between number of pods per plant and day of year when Bean pod mottle virus was first detected in soybean quadrats (cv. NE3001) planted at Iowa State University Research Curtiss Farm near Ames in 2006 (solid circles) and 2007 (open circles).

Fig. 6. Relationship between number of seeds per pod and day of year when Bean pod mottle virus was first detected in soybean quadrats (cv. NE3001) planted at Iowa State University Research Curtiss Farm near Ames in 2006 (solid circles) and 2007 (open circles).
**Fig. 7.** Relationship between the 100-seed weight and day of year when *Bean pod mottle virus* was first detected in soybean quadrats (cv. NE3001) planted at Iowa State University Research Curtiss Farm near Ames in 2006 and 2007. Slopes and intercepts for the relationship between 100-seed weight and day of year for 2006 and 2007 were not significantly different, hence data were combined.

**Fig. 8.** Relationship between percent mottled seed and day of year when *Bean pod mottle virus* was first detected in soybean quadrats (cv. NE3001) planted at Iowa State University Research Curtiss Farm near Ames in 2006 (solid circles) and 2007 (open circles).

**Fig. 9.** Relationship between A) percent protein content and B) percent oil content, and the day of year when BPMV was first detected in soybean quadrats (cv. NE3001) planted at Iowa State University Research Curtiss Farm near Ames in 2006 (solid circles) and 2007 (open circles).

**Fig. 10.** Relationship between percent protein content and percent oil content as influenced by *Bean pod mottle virus* in soybean (cv. NE3001) planted at Iowa State University Research Curtiss Farm near Ames in 2006 and 2007. Slopes and intercepts for the relationship between protein content and oil content in 2006 and 2007 were not significantly different (*P* > 0.05) hence data were combined.
**Fig. 11.** Relationship between soybean yield and day of year of *Bean pod mottle virus* inoculation of soybean plants grown in Iowa State University greenhouse in 2007 and 2008.

**Fig. 12.** Relationship between percent mottled seed and day of year of *Bean pod mottle virus* inoculation of soybean plants grown in Iowa State University greenhouse in 2007 and 2008. Slopes and intercepts for the relationship between percent mottled seed and day of year of BPMV inoculation were not significantly different ($P>0.05$), hence data were combined.

**Fig. 13.** Relationship between A) percent protein content and B) percent oil content, and day of year of *Bean pod mottle virus* inoculation of soybean plants grown in Iowa State University greenhouse in 2007 (solid circles) and 2008 (open circles).
Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.
Day of year when BPMV was first detected

Number of pods per plant

0 20 40 60 80

160 180 200 220 240 260

2006 $y = 23.76 + 0.15x$, $R^2 = 72.8\%$

2007 F-statistic not significant, $y = 47.7$

Fig. 5.
Fig. 6
Day of year when BPMV was first detected

100-seed weight

\[ y = 13.6 + 0.01x, \quad R^2 = 34.7 \]

Fig. 7.
Fig. 8.
Fig. 9.
Fig. 10.

\[ y = 41.9 - 0.67x, \quad R^2 = 98.2\% \]
Day of year of BPMV inoculation

Seed weight (g/plant)

2007  \( y = 27.1 + 0.07x \), \( R^2 = 45.10\% \)

2008  \( y = 5.98 + 0.05x \), \( R^2 = 41.50\% \)

Fig. 11.
Day of year of BPMV inoculation

Percent mottled seed

\[ y = 107.3 - 0.61x, \quad R^2 = 69.4\% \]

**Fig. 12.**
Fig. 13.
CHAPTER 5.

GENERAL CONCLUSIONS

The work contained in thesis investigated the prevalence, incidence, and risk of Bean pod mottle virus in Iowa. This was part of the Iowa Soybean Disease survey. Data from the three-year state-wide soybean surveys show that there is a risk of BPMV in Iowa but that this risk is not equal among Iowa counties. The significant spatial dependence found among counties indicated that counties that had high BPMV incidence were neighbored by counties with high BPMV incidence and vice-versa. The incidence of BPMV in counties within the southern part of Iowa was consistently higher than northern part. Thus, this area is at high risk for BPMV epidemics. Initial sources of BPMV inoculum (alternative weed hosts, infected seed and infested overwintering bean leaf beetle population) are responsible for BPMV epidemics. Likely BPMV risk factors that are area specific within counties are alternative weed hosts and overwintering bean leaf population. Previous work has shown that accumulating subfreezing temperatures decreased bean leaf beetle overwintering population survival. Related weather factors that may be influencing BPMV incidence levels among counties could be investigated. These may include snow depth, snow days, snow cover and soil temperature. Biological risk factors that should be examined include previous status of the sampling unit, source of planting seed, and population of overwintering bean leaf beetles.

Our field trials investigated the temporal and spatial dynamics of Bean pod mottle virus in soybean plots. In both 2006 and 2007, the source of inoculum for BPMV spread to new quadrats originated from within the plot. This was evidenced by limited BPMV spread in non inoculated plots. For example in the second season, although there were low bean leaf beetle numbers, BPMV incidence reached only 10% in the non-inoculated plots, compared to
49% in the inoculated plots. Another evidence of limited spread of BPMV to quadrats located further away from initial inoculum was the clustered nature of infected quadrats around quadrats previously infected. Throughout both growing seasons, infected quadrats displayed a clustered pattern. This suggests that incoming bean leaf beetles into the soybean fields were non-viruliferous or the viruliferous bean leaf beetles were very few, however, this needs further investigation. Progress of BPMV within soybean quadrats was limited in the first 4 samplings (approx. 65 days after planting) but later increased with some plots reaching 100% BPMV incidence in 2006. The dynamics of BPMV were related to bean leaf beetle population densities. Higher numbers of bean leaf beetles were associated with higher BPMV incidence. Limited BPMV incidence in the early soybean growth stages was due to fewer vector numbers.

Quantification of yield loss is important in qualifying the mandate for rational disease management programs. Yield loss information is also important when evaluating different management options. We found that yield loss was associated with time of BPMV infection. In both years of field study, soybean quadrats that had early BPMV detection had greater yield losses. This was also demonstrated in the parallel greenhouse experiment. Coupled with BPMV progress in soybean, we found that BPMV incidence varies with growth stages of soybean and that BPMV incidence is dependant on bean leaf beetle population density, as well as the yield loss associated with time of BPMV detection.

This work examined importance of BPMV time of infection on soybean quality. Both the field and greenhouse experiments showed a strong relationship between time of BPMV infection and percentage of mottled seed. Late-infected soybean plants had fewer mottled seeds. Although other factors have been reported to cause soybean seed mottling, our results
show that time of BPMV infection influences the level of mottling. Time of BPMV
detection, however, did not significantly affect protein or oil content consistently in this
cultivar (NE3001), there was a general tendency for protein content to decrease with delayed
BPMV detection; the reverse was true for oil content.

Overall, the research presented in this thesis has advanced our understanding of
BPMV infection over time and the relationship between disease progression and soybean
yield. The soybean disease survey results indicate that BPMV is a potential problem in Iowa.
Future work on BPMV epidemiology should re-examine threshold levels for bean leaf
beetles in relation to risk of BPMV, and evaluate risk factors to develop pre-plant BPMV risk
predictions for different regions in Iowa.
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