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Bean pod mottle virus ecology and management in Iowa

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Bean pod mottle virus ecology and management in Iowa

by

Rayda Kathryn Krell

A dissertation submitted to the graduate faculty
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For the Major Program
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ABSTRACT

Bean pod mottle virus (BPMV) is a new disease problem for North Central states soybean (Glycine max (L.)) growers. The bean leaf beetle, Cerotoma trifurcata (Forster) (Coleoptera: Chrysomelidae), is the primary BPMV vector. The main objectives of this research were to understand BPMV ecology and explore possibilities for management. Bean leaf beetle, Cerotoma trifurcata (Forster) (Coleoptera: Chrysomelidae), flight capacity was measured and seventy-one percent of beetles flew <51 m and 11.76% flew >301 m. The mean of flights <51 m was 11 m. The farthest flight made by an individual beetle was 4.9 km. A survey of Iowa counties confirmed BPMV was present throughout the state. In 2002, populations of the bean leaf beetle reached the highest abundance recorded in 14 years. Three BPMV primary inoculum sources in Iowa were confirmed. Seed transmission of BPMV was 0.037%, 1.5% of overwintered bean leaf beetles transmitted BPMV, and Desmodium canadense (L.) was identified as a naturally occurring host. Application of lambda-cyhalothrin (Warrior®) (Syngenta, Wilmington, DE) after soybean emergence and again as first-generation bean leaf beetles emerged, reduced beetle densities for four and seven weeks, reduced BPMV field incidence by 26% and 27%, and reduced seed coat mottling by 10% and 19%, at locations in central and northwest Iowa respectively. Yield was 440.48 kg/ha (6.5 bu/acre) higher in the early followed by mid-season insecticide treatment at the northwest site. Four planting dates and two soybean cultivars were examined in relation to BPMV incidence. In 2000, the lowest BPMV incidence occurred in the third planting date, but the incidence was not significantly different than in the other planting dates. For all years, yield was occasionally highest (although not significantly) in the third planting date and lowest in the last planting date. In 2000, seed coat mottling was
lowest in the third planting date. This research provides new information on BPMV ecology and experimental evidence to support management recommendations for growers.
CHAPTER 1. GENERAL INTRODUCTION

Dissertation Organization

This dissertation is organized in six chapters. The first chapter is a general introduction, which includes the objectives of this dissertation and a review of literature on the bean leaf beetle (*Cerotoma trifurcata* Forster) and *Bean pod mottle virus* (BPMV). Chapters two through five are written for submission to scientific journals. Chapter two reports the findings of a study characterizing bean leaf beetle flight capacity. In chapter three, the results of a study to elucidate the primary sources of BPMV inoculum are presented. Chapter four presents the results of an experiment to evaluate a management tactic using insecticides for bean leaf beetle and BPMV management. Chapter five reports the results of research examining the effect of soybean planting date on incidence of BPMV. Chapter six describes the general conclusions from this dissertation, followed by acknowledgements.

Introduction

The relationship between a virus, its host, and the environment is extremely complex. For plant viruses that are primarily vectored by insects, the relationship is even more complex because abiotic factors act on the vector in addition to the host plant. Pest management of a virus must be based on an understanding of these interactions. To dissect the relationship between virus, vector, and host plant, it is useful to begin by investigating what is known about the cycle of these interactions. If the cycle can be understood, then finding ways to break the cycle can become the focus of management.

A virus that has recently become a concern in Iowa is *Bean pod mottle virus* (BPMV). This virus has been prevalent in the southern United States, but is becoming more
widespread in the North Central states (Marking 2000, Giesler et al. 2002). There are sporadic reports of the virus in the North Central states, including a report of “widespread” disease in Kansas in 1979 (Schwenk and Nickell 1980) (Table 1). Yield losses from bean pod mottle virus have been documented as high as 52% (Hopkins and Mueller 1984) and the seed coat mottling caused by the virus can reduce seed value.

The bean leaf beetle, Cerotoma trifurcata (Forster) (Coleoptera: Chrysomelidae), is considered the primary vector of this virus (Newsom et al. 1980, Hopkins and Mueller 1983, Pitre 1989). Populations of the bean leaf beetle have reached the highest levels recorded in the past 14 years (Fig. 1). Large populations of the bean leaf beetle in the North Central states may be contributing to the increased incidence of BPMV.

Two factors that are known to affect bean leaf beetle densities, winter temperature and planting date, have been favorable in recent years for encouraging large populations. Recently, winter temperatures in Iowa have been above normal (www.iowa-counties.com/weather/). At warmer overwintering temperatures more beetles survive for a longer period of time (Lam 1999). The other main factor thought to encourage large beetle populations is planting date. Early planting of soybeans favors early colonization of plants by beetles. Early colonization of soybean fields is beneficial for beetles because fecundity and survivorship are greater, the longer they feed on soybeans (Zeiss and Pedigo 1996). Despite this knowledge, many farmers are planting soybeans early to increase yield and avoid wet fields in April and May (Whigham 2000). The increase in early-planted soybean fields promotes large and fecund bean leaf beetle populations.

High bean leaf beetle densities can cause economic damage to soybeans through feeding. Beetle feeding in combination with BPMV can produce even greater yield and seed
quality reductions. No study has examined the BPMV-bean leaf beetle relationship in Iowa. The following literature review describes what is known about the virus-beetle relationship and discusses areas for research into breaking the disease cycle.

**Objectives**

This research focused on accomplishing two major objectives: 1) identify sources of bean pod mottle virus inoculum in Iowa, and 2) develop management recommendations for bean pod mottle virus to reduce its impact on soybean quality and yield.

1. Source of BPMV Inoculum

- **Determine the extent of BPMV in Iowa.** To better understand the potential impact of BPMV a map of its distribution is needed.

- **Determine whether BPMV is seed transmitted.** Previous studies have shown that BPMV can be seed transmitted, but no study has examined seed transmission in soybean from Iowa. Seed should be evaluated to determine whether it can serve as a primary inoculum source.

- **Determine whether overwintered beetles can transmit virus.** There is limited information on whether overwintered bean leaf beetles can transmit BPMV. This information is needed to determine how early in the season soybean plants may be exposed to BPMV by beetles in Iowa. It is possible that different strains of the virus may exist in different regions, and the ability of different strains to survive in overwintering beetles may be variable. Additionally, it is possible that different beetle biotypes exist in different regions and may differ in their ability to harbor BPMV through the winter.
• **Identify potential BPMV host plants.** *Desmodium paniculatum* is a perennial legume native to the U.S. that is also present in Iowa and known to serve as a reservoir for BPMV. It is possible that other perennial legumes could also serve as hosts.

• **Quantify bean leaf beetle movement.** An important question to answer that is related to the spread of BPMV is the potential of beetles to introduce the virus to uninfected areas. The flight capacity of bean leaf beetles needs to be quantified.

### 2. Strategies for Management

• **Evaluate the use of insecticides for managing bean leaf beetles and reducing incidence of BPMV.** It has been suggested that early season insecticide applications to reduce bean leaf beetle populations should be considered for areas with high incidence of BPMV. This strategy needs to be tested before it is recommended to farmers.

• **Evaluate the effect of planting date on BPMV incidence.** It is known that delaying soybean planting will delay colonization of fields by bean leaf beetles. The effect of this strategy on BPMV incidence should be determined.

### Literature Review

**Bean Leaf Beetle**

The bean leaf beetle was first described by Forster in 1771 (Eddy and Nettles 1930). The first record of the bean leaf beetle as a pest was in 1875 when it was observed damaging dwarf beans in Kansas (Eddy and Nettles 1930). Until recently, there were not many major soybean-insect pests in the North Central states (Ignoffo et al. 1976, Newsom et al. 1980). In
1980, 66,000 ha of soybean were sprayed for bean leaf beetles in Illinois, which sparked investigation into the bean leaf beetle as a North Central soybean problem (Jeffords et al. 1983). Now, the bean leaf beetle is one of the primary soybean-insect pests in Iowa.

Smelser and Pedigo (1991) described the phenology of the bean leaf beetle in Iowa. The bean leaf beetle has two generations and it is typically the second generation that causes economic damage by feeding on soybean pods (Smelser and Pedigo 1992). Pod feeding also causes seed quality losses from pod desiccation and wounds through which fungi enter pods (Shortt et al. 1982). Bean leaf beetle larval stages feed on soybean roots and nodules, but there are no published studies reporting the effect of this feeding on yield. The effect of larval feeding is assumed minimal.

Bean leaf beetles overwinter as adults. In Iowa, about 80% of bean leaf beetles overwinter in the leaf litter of wooded areas, about 20% overwinter in soybean leaf litter, and only a few (<1%) are found in corn and alfalfa litter (Lam 1999). In early spring, beetles feed on native legumes and alfalfa (Isley 1930, Kogan et al. 1980, Smelser and Pedigo 1991). As soon as soybean emerge, beetles move to these fields to feed because soybean are abundant and preferred over many other host plants (Henn 1989). The first generation usually peaks in mid to late July, and the second generation peaks in late August or early September. As soybean plants senesce, beetles will return to alfalfa and other host legumes to feed and eventually migrate to overwintering sites (Smelser and Pedigo 1991).

**Bean Pod Mottle Virus**

Bean pod mottle virus is a member of the comovirus group. Between 12 and 15 viruses have been classified in this group (Stace-Smith 1981). Most comoviruses are specific to the Fabaceae, but some viruses also infect crucifers, curcurbits, and potatoes (Stace-Smith...
Comoviruses are 25–30 nm in diameter, polyhedral, RNA viruses that tend to have narrow host ranges. They are easily transmitted mechanically and are considered highly stable and antigenic (Fulton et al. 1987).

Bean pod mottle virus has been identified in several states and countries (Table 1). It was first identified in the United States in 1948 from North Carolina (Zaumeyer and Thomas 1948). Most reports of BPMV have been from southern states; however, it was reported in the North Central states as early as 1968 from naturally infected plants in Ottumwa, Iowa (Quiniones and Dunleavy 1971). Other occurrences of the virus in the North Central states have since been reported (Table 1). The virus has also been described from Canada (Michelutti et al. 2002), Ecuador (Zettler et al. 1989), and Brazil (Anjos et al. 1999). Strain diversity among BPMV isolates has been demonstrated, and two major subgroups were identified, however, there is no relationship between subgroup and virus effect on yield (Gu et al. 2002).

Once inside the host plant, both nodules and leaves are sites of BPMV replication (Orellana et al. 1987). The timing of soybean inoculation with virus is an important issue in considering management of the vector to achieve disease control. The earlier plants were inoculated with virus, the greater the transmission efficiency (Hopkins and Mueller 1984).

and filiform outgrowths (Quiniones and Dunleavy 1971). Phenotypic BPMV symptoms can vary depending on environment, soybean cultivar, and virus strain (Stace-Smith 1981, Horn et al. 1973, Walters 1970). For example, some studies recorded plant stunting in relation to BPMV infection (Skotland 1958, Orellana et al. 1987), whereas another study found no height differences between infected and virus-free plants (Myhre et al. 1973).

In addition to BPMV phenotypic plant symptoms, effects on seed quality also have been investigated. Some BPMV infected plants produced mottled seeds (Lin and Hill 1983, Stace-Smith 1981). In other studies no effect on seed quality from BPMV inoculated plants was found (Ross 1963a). Bean pod mottle virus also was found to predispose soybean seed to *Phomopsis* spp. (Stuckey et al. 1982, Abney and Ploper 1994). There was no effect of BPMV on protein or oil content of seed (Ross 1986b), and no differences have been reported in seed emergence from BPMV infected plants (Hopkins and Mueller 1984).

Although the effects of BPMV on seed quality can be variable, the effect on yield is consistent. Early infection of soybeans results in greater yield loss than late infection (Hopkins and Mueller 1984, Ross 1969, Walters 1970, Ragsdale 1984). Environmental effects can act synergistically with BPMV to reduce yield. Bean pod mottle virus reduced yield by 29% alone, but in combination with drought stress, yield was reduced by 45% (Myhre et al. 1973). Significant reductions in yield have been detected at infection levels of at least 40% (Horn et al. 1973, Ragsdale 1984). Bean pod mottle virus infected plants were also found to have lower yields than plants infected with soybean mosaic virus (SMV) (Calvart and Ghabrial 1983).

The actual cause of the yield reductions from BPMV has been attributed to reduction in plant dry matter, fruiting sites, and pods per plant (Myhre et al. 1973, Hopkins and Mueller
Walters (1970) also cited decreased seed size as a contributor to lower yields from BPMV infected plants. Windham and Ross (1985) found some yield compensation in plants adjacent to BPMV infected plants, suggesting that a few virus-infected plants within a field would not result in yield loss.

Relationship between Virus, Vector, and Host Plant

Coleoptera as Virus Vectors

Beetles can be extremely efficient virus vectors. Some beetles have been shown to transmit virus with a single bite on a host plant (Fulton et al. 1987). The mechanism of beetle virus transmission is not fully understood. Several hypotheses for beetle transmission of viruses have been proposed, including through feces and through reflexive bleeding at joint articulations (Fulton et al. 1987). To date, feeding has been the only method identified by which beetles can transmit a virus. Non-beetle transmissible viruses are inhibited by beetle regurgitant, whereas beetle transmissible viruses are not (Gergerich et al. 1983).

Beetle regurgitant can be a selective determinant of beetle virus transmission (Gergerich and Scott 1991); however, there is no evidence for direct inactivation by regurgitant of nonbeetle transmissible viruses (Gergerich and Scott 1991). Gergerich and Scott (1991) speculated beetle regurgitant functions by affecting the host or the interaction of the virus with the host.

Bean Leaf Beetle as a Virus Vector

The bean leaf beetle was first reported as a virus vector in 1924 by Smith for cowpea mosaic disease (Eddy and Nettles 1930). The bean leaf beetle is the primary vector for BPMV (Pitre 1989, Hopkins and Mueller 1983). It can transmit BPMV quickly to host plants within as little as four hours after a 24-h acquisition-feeding period (Patel and Pitre
The bean leaf beetle also transmits other viruses including yellow cowpea mosaic (Jansen and Staples 1971), cowpea chlorotic mottle (Walters and Dodds 1969), southern bean mosaic (Walters 1964), cowpea strain of southern bean mosaic (Fulton and Scott 1974), severe bean mosaic (Walters 1969), and blackgram mottle virus (Scott and Phatak 1979). The efficiency of the beetle as a vector varies, depending on the virus. Twelve percent of bean leaf beetles transmitted cowpea chlorotic mottle virus following acquisition feeding on infected bean plants (Hobbs and Fulton 1979), whereas beetles could transmit cowpea mosaic virus (Sanderlin 1973) or southern bean mosaic virus (Walters 1964) at levels near 100%. Transmission efficiency of the bean leaf beetle for BPMV is not known, but it is assumed to be high.

Field collected bean leaf beetles were found to contain 500–1,500 ng BPMV per beetle, which was a higher titer than that found in lab infected beetles (Ghabrial and Schultz 1983). It is not known if the virus reproduces within the beetle (Fulton et al. 1980). In one study, the BPMV titer was higher in beetles than in the infected plants on which they had fed for three days (Ghabrial and Schultz 1983). This finding suggests that BPMV can replicate in beetles. However, in the same study, the BPMV titer in infected beetles decreased after a six-day feeding period on virus-free plants, suggesting that no virus replication occurs after the virus is in the beetle’s body. It is possible that a higher titer of BPMV was found in beetles than in plants because the virus is selectively accumulated in the beetle’s body.

Some plant viruses (cowpea strain of tobacco ringspot virus and southern bean mosaic virus) have been found in bean leaf beetle hemolymph (Wang et al. 1992). However, there are conflicting reports as to whether BPMV is found in bean leaf beetle hemolymph. Bean pod mottle virus is transmitted in regurgitant and, according to some sources, has not
been found in the hemolymph of bean leaf beetles or other beetles that transmit the virus (Wang et al. 1992). According to another author, BPMV has been identified from bean leaf beetle hemolymph (Fulton and Scott 1974). An inhibiting factor was found in beetle hemolymph that prevented plant virus survival unless the hemolymph was diluted by 200 fold (Fulton et al. 1980), further supporting that the virus does not replicate in bean leaf beetles.

Only one study has examined the potential of larvae to vector BPMV (Hopkins 1983). In the study, BPMV virus particles were found in larvae after feeding on diet sprayed with BPMV, but larvae did not transmit the virus to soybean seedlings. Mechanical inoculation of the virus through roots was possible but at a low level.

There are several aspects of the BPMV–bean leaf beetle interaction that have not been investigated. The full potential of larvae to transmit the virus is not known. There are no reports on larvae becoming viruliferous through feeding on soybean roots in the field or by transovarial transmission. Information also is lacking on the effect of environment on the ability of beetles to transmit virus. Temperature was an important factor in bean leaf beetle transmission of cowpea mosaic virus where the greatest transmission occurred at 25–28°C (Fulton et al. 1980).

**Primary BPMV Inoculum Source**

The primary inoculum source of a virus infecting a commercially important crop is a source that can serve as an initial focus for infection. From these primary sources, spread can occur through mechanical means or vectors. There are three potential primary inoculum sources of BPMV for soybeans: perennial weeds, various overwintered insect vectors, and infected seed. There is evidence that all three can serve as reservoirs for BPMV through the
winter, but the impact of each on initial field primary incidence is unknown. Bean leaf beetles are believed to be the primary cause of virus spread within soybean fields during the growing season (Hopkins and Mueller 1983, Pitre 1989, Walters and Lee 1969).

Wild host plants for viruses are considered common (Heathcote 1973) and wild hosts are known for other beetle transmitted viruses (Lima and Nelson 1977, Valverde 1978). Bean pod mottle virus can infect several host plants and has been identified from field collections in the native weed Desmodium paniculatum (Moore et al. 1969). Desmodium spp. are perennial legumes, usually found in, or at the edge of, wooded areas. It has been shown that bean leaf beetles could transmit the virus from D. paniculatum to soybean (Walters and Lee 1969). The bean leaf beetle has been observed to feed on other naturally occurring Desmodium spp. like D. canescens (McLaughlin et al. 1978), and bean leaf beetle eggs were found near the roots of D. illinoense and D. cuspidatum (Waldbauer and Kogan 1976). Beetles were found to feed on D. canescens in mid-May in Illinois (McLaughlin et al. 1978) and on Desmodium canadense in Iowa (R. Krell personal observation 2000). Feeding on Desmodium spp. occurs early enough in the growing season to serve as a source of virus for overwintered beetles. In Iowa, D. paniculatum only is known to occur in southern parts of the state (Eilers and Roosa 1994). So far, major occurrences of BPMV have been primarily reported from western and central Iowa. Another native Desmodium spp., D. canadense, is known to be common in northwest Iowa. In addition to Desmodium spp., several other plants have been identified as hosts for BPMV, and the bean leaf beetle is known to feed on some of these plants (Table 2). Recently, several new host plants were identified in Brazil from mechanical inoculation (Anjos et al. 1999). There are several plants the bean leaf beetle is known to feed on that are not reported as BPMV host plants (Isely 1930) and
some are non-leguminous plants (Helm et al. 1983). The potential for some of these other beetle host plants to serve as BPMV hosts has not been explored.

Overwintered beetles also may serve as a reservoir for virus during winter, but only one study has documented the ability of naturally overwintered beetles to transmit virus to soybeans (Walters et al. 1972). Beetles removed from overwintering sites were tested for ability to transmit virus. The percentage beetles able to transmit virus in any one month varied from 0 to 3%, with the exception of February 1970 when 17% of overwintered beetles collected were able to transmit virus to plants (Walters et al. 1972). In another study, overwintered bean leaf beetles were collected from an alfalfa field on 1 April, and 8.3% of beetles were found to contain virus (Mueller and Haddock 1980). Finding beetles so early in the season with virus further supports that possibility that the virus can survive in overwintering beetles. In contrast, another study collected naturally overwintered beetles but did not find that any transmitted the virus, even though beetle regurgitant tested positive for BPMV (Anjos 1991). For BPMV-carrying beetles that were artificially overwintered at 4°C, 6.6% were able to transmit the virus to uninfected soybean plants (Anjos 1991). Other plant viruses have been found to overwinter in chrysomelid beetles (Freitag 1956). No other BPMV vectors have been tested for the ability to serve as overwintering reservoirs.

In addition to the two virus sources mentioned, infected seed also can serve as an initial source of virus. Two studies have documented seed transmission of BPMV. In both studies, transmission occurred in less than one percent of seed from infected plants (Lin and Hill 1983, Ross 1986b). This is not surprising because typically comoviruses are known to have very low rates of seed transmission (Stace-Smith 1981). In other studies, no BPMV seed transmission was detected (Schwenk and Nickell 1980, Skotland 1958). Although the
rates of seed transmission of BPMV identified have been extremely low, Ross (1986b) suggested that in combination with a high vector population, a small source of inoculum could be enough to cause significant yield and quality losses.

Spread of Virus

The most common and economically important mechanism of virus spread is through insect vectors (Matthews 1991, Agrios 1997). In Iowa, populations of the primary BPMV vector have reached historically high abundance (Fig. 1), and there is concern about spread of the virus. Several vectors of BPMV have been identified, but the bean leaf beetle is considered the most important vector (Pitre 1989, Hopkins and Mueller 1983) and its ability to transmit virus was first demonstrated in 1963 (Ross 1963b). Other insect vectors found to transmit BPMV include southern corn rootworm *Diabrotica undecimpunctata howardi* Barber (Milbrath et al. 1975, Ghabrial and Schultz 1983), striped blister beetle *Epicauta vittata* (Fabricius) (Patel and Pitre 1971), Mexican bean beetle *Epilachna varivestis* Mulsant (Fulton and Scott 1974), *Cerotoma facialis maculata* Erickson (Zettler et al. 1991), *Cerotoma arcuata* Olivier (Anjos et al. 1999), *Odontota horni* Smith (Werner et al. 2002), and *Diabrotica virgifera virgifera* LeConte (J. Spencer, pers. comm.).

One reason the bean leaf beetle is considered a more efficient BPMV vector than other insects studied is the long retention time of virus in the beetle. The bean leaf beetle was found to transmit virus up to eight days after acquisition feeding, while the Mexican bean beetle rarely transmitted virus beyond the third day after acquisition feeding (Fulton and Scott 1974). Transmission of BPMV by the striped blister beetle also was low (Patel and Pitre 1971).
Because of the greater importance attributed to the bean leaf beetle as a BPMV vector, most studies examining BPMV vectors have focused on this beetle. The bean leaf beetle has several color morphs varying from beige to red. One study tested the efficiencies of BPMV transmission of the two color morphs but did not find any significant difference between the two (Pitre 1989).

Locations with more viruliferous beetles had higher incidences of BPMV (Mueller and Haddox 1980, Ghabrial and Schultz 1983, Ghabrial et al. 1990). In addition, Walters (1970) found that areas with more bean leaf beetles had the most diseased plants; however, Walters did not test beetles for virus. Bean pod mottle virus in fields was found to be randomly distributed at infection levels below 10%, aggregated between 10 and 40%, and approaching uniformity at infection levels greater than 40% (Hopkins and Mueller 1983). It is likely that virus dispersion is related to beetle dispersion.

Once the beetle has acquired the virus, retention time decreases as the beetle feeds on uninfected plants (Sanderlin 1973, Ghabrial and Schultz 1983) and increases the longer beetles feed on infected plants (Fulton et al. 1987). Bean leaf beetles have been shown to demonstrate long retention times with other viruses. Bean leaf beetles carrying southern bean mosaic virus stayed viruliferous for 4 weeks after a single acquisition feeding (Wang et al. 1994).

Early transmission of virus is important because early inoculation results in higher incidence levels (Patel and Pitre 1976, Hopkins and Mueller 1984) and greater yield reduction (Ross 1969, Walters 1970, Hopkins and Mueller 1984). The later a plant is inoculated, the lower its infectivity (Calvart and Ghabrial 1983).
Although early season virus transmission may cause the greatest yield reductions, most virus is spread later in the growing season. In a study examining the percentage virus infected beetles throughout the growing season, the most viruliferous beetles (16%) were collected on R3 plants (Hopkins and Mueller 1983). Also, incidence in fields increased over time, so that the highest number of infected plants were found late in the season (Hopkins and Mueller 1983, Pitre 1989, Walters and Lee 1969). Virus incidence increased from 11 to 25% in soybean 76–89 days after planting (Calvart and Ghabrial 1983).

BPMV-SMV Interaction

Another soybean virus that is prevalent in Iowa soybean fields is Soybean mosaic virus (SMV). Single plants may be infected with both BPMV and SMV. When plants were doubly infected with both viruses, yields were reduced more than by either virus alone (Calvart and Ghabrial 1983, Ross 1963a, Ross 1969, Quiniones et al. 1971). In one study, the yield reduction was more than twice the yield reduction from single infections of either virus. As with single BPMV inoculation, the early inoculation with both viruses results in greater yield reduction (Ross 1969). Symptoms of doubly infected plants included stunting, mottled leaves, distorted leaves, leaf tissue necrosis (Calvart and Ghabrial 1983), and reduced number of nodules (Tu et al. 1970). Stunting was attributed to shorter internodal length and not slower growth rate (Calvart and Ghabrial 1983). Also, seed quality was lowest in plants infected with both viruses as compared with infection by either virus alone (Ross 1969, Quiniones et al. 1971). Other effects found in doubly infected plants included higher titers of BPMV, (Calvart and Ghabrial 1983), reduced germination (Quiniones et al. 1971), and reduction (Ross 1969) or increase (Quiniones et al. 1971) in seed transmission of SMV from plants also infected with BPMV.
The bean leaf beetle is not a vector of SMV (Ghabrial and Schultz 1983). In addition, after feeding on plants infected with both BPMV and SMV, only BPMV was detected in beetles by using ELISA tests (Ghabrial and Schultz 1983). It is anticipated that the incidence of SMV could increase with the introduction of an exotic soybean pest, the soybean aphid (*Aphis glycine* Matsumura), into the North Central states. The soybean aphid is a known vector of SMV (Hill et al. 2001). Unlike native aphids that are transient and occasionally probe soybeans, the soybean aphid colonizes soybeans throughout the season, and it is suspected that this behavior could increase the incidence of SMV. Soybean aphids and bean leaf beetles could occur in the same fields, increasing the likelihood of SMV and BPMV being vectored to soybeans in the same field. The potential of these two viruses occurring together presents a new threat to growing soybeans in the North Central states and heightens the need to learn about management possibilities for both of these viruses.

**Current BPMV Management Recommendations**

In the past, bean leaf beetle management has focused on targeting second generation beetles, primarily through monitoring soybean fields and spraying insecticide when economic thresholds for pod-feeding are exceeded (Pedigo 1994). However, the pest status of the bean leaf beetle changes in the presence of BPMV, and management of the beetle as a vector becomes important earlier in the season.

Targeting BPMV directly with an antiviral agent, dimethyl sulfoxide, was tried but not effective for reducing BPMV infection (Pitre et al. 1972). Most suggestions for managing BPMV have been targeted at managing the vector. Ross (1986b) suggested applying an insecticide to manage beetles early in the season before the V6 stage because early BPMV infection causes the greatest yield loss. Ghabrial et al. (1990) suggested a
similar approach, recommending that areas with high BPMV occurrence should be sprayed prophylactically for bean leaf beetles early in the season. Hopkins and Mueller (1984) stress that it is most important to prevent early field infection and suggest monitoring BPMV levels and spraying the beetle population to reduce impact on yield.

Another possibility for BPMV management that was suggested is using a trap crop to attract bean leaf beetles early in the season (Newsom and Herzog 1977). Newsom and Herzog (1977) suggested planting a small acreage of soybeans 10 to 14 days earlier than the rest of the soybean crop to attract beetles. They found most beetles stayed in the trap crop area and suggested that spraying only would be required after the first generation beetles started to emerge. They suggested that the trap crop could contribute to suppression of BPMV as well if vectors also were suppressed; however, this method has not been tested.

A cultural management tactic that has shown some success in the tropics is growing beans in a polyculture with maize. The beetle populations were lower in a polyculture compared to beans grown in a monoculture (Risch 1980). In Central America, beetle-transmitted viruses make up the highest percentage of insect-transmitted legume viruses (Gamez 1980), and it was suggested the use of polycultures might help to reduce virus incidence. The beetles observed in polycultures tended to avoid feeding on plants shaded by maize and emigrated more rapidly from host plants (Risch 1980). The effect of shade on beetle presence on host plants was also tested experimentally and data showed that beetles preferred sunny areas (Risch 1981). The use of polycultures for conventional production agriculture probably is not practical; however, it could be an option for smaller producers or organic growers.
Walters (1970) suggested work should be done to find soybean lines resistant to BPMV, and some progress has been made in this area. Scott et al. (1974) tested 169 Glycine max commercial varieties and 123 plant introduction lines and varieties and found that none were resistant to BPMV. Skotland (1958) tested 16 varieties, and all were susceptible. Susceptibility also was found in 36 cultivars tested in Kansas, although the degree of symptom severity varied (Schwenk and Nickell 1980). Ross (1986a) developed four soybean lines with some resistance to BPMV, but no line showed immunity. Other Glycine spp. have been investigated, and immunity was found in some species (Scott et al. 1974). In addition to investigations of natural resistance, transgenic resistance to BPMV has been demonstrated (Di et al. 1996) and shown transferable to the T2 generation (Reddy et al. 2001). Host plant resistance is an ideal solution for BPMV management, but the process for developing resistant varieties will take many years. Farmers need proven tactics that can be immediately implemented to protect soybeans until more sustainable tactics are developed.
Literature Cited


Hopkins, J. D. 1983. The bean leaf beetle, Cerotoma trifurcata (Forster), and its relationship with bean pod mottle virus in Arkansas soybean. Ph.D. dissertation, University of Arkansas, Fayetteville, Arkansas.


Table 1. First recorded reports of bean pod mottle virus.

<table>
<thead>
<tr>
<th>State</th>
<th>Date</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Carolina</td>
<td>1945</td>
<td>Zaumeyer and Thomas 1948</td>
</tr>
<tr>
<td>Virginia</td>
<td>1954</td>
<td>Skotland 1958</td>
</tr>
<tr>
<td>Arkansas</td>
<td>1956</td>
<td>Walters 1970</td>
</tr>
<tr>
<td>North Carolina</td>
<td>1955</td>
<td>Skotland 1958</td>
</tr>
<tr>
<td>Iowa</td>
<td>1968</td>
<td>Quiniones and Dunleavy 1971</td>
</tr>
<tr>
<td>Louisiana</td>
<td>1973</td>
<td>Horn et al. 1973</td>
</tr>
<tr>
<td>Illinois</td>
<td>1974</td>
<td>Milbrath et al. 1975</td>
</tr>
<tr>
<td>Kentucky</td>
<td>1977</td>
<td>Ghabrial et al. 1977</td>
</tr>
<tr>
<td>Kansas</td>
<td>1979</td>
<td>Schwenk and Nickell 1980</td>
</tr>
<tr>
<td>Mississippi</td>
<td>1979</td>
<td>Pitre et al. 1979</td>
</tr>
<tr>
<td>Nebraska</td>
<td>1983</td>
<td>Lin and Hill 1983</td>
</tr>
<tr>
<td>Ecuador, South America</td>
<td>1988</td>
<td>Zettler et al. 1989</td>
</tr>
<tr>
<td>Brazil, South America</td>
<td>1998</td>
<td>Anjos et al. 1999</td>
</tr>
<tr>
<td>South Dakota</td>
<td>1999</td>
<td>Langham et al. 1999</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>1999</td>
<td>Doughty et al. 2001</td>
</tr>
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<td>Ohio</td>
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</tr>
<tr>
<td>Ontario, Canada</td>
<td>2001</td>
<td>Michelutti et al. 2002</td>
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<td>Indiana</td>
<td>2002</td>
<td>Giesler et al. 2002</td>
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Table 2 Bean pod mottle virus host plants and relationship with bean leaf beetle. Citations for each record are indicated by superscripts.

<table>
<thead>
<tr>
<th>BPMV Host Plants</th>
<th>Plant Common Name</th>
<th>Bean Leaf Beetle Host Plant?</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Desmodium canadense</em></td>
<td>showy tick trefoil</td>
<td>Yes, on <em>Desmodium spp.</em></td>
</tr>
<tr>
<td><em>Desmodium paniculatum</em></td>
<td>panicked tick trefoil</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Glycine max</em></td>
<td>soybean</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Glycine soja</em></td>
<td>wild soybean</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Lespedeza cuneata</em></td>
<td>silky bush clover</td>
<td>Yes, on <em>Lespedeza spp.</em></td>
</tr>
<tr>
<td><em>Lespedeza striata</em></td>
<td>common bush clover</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Lespedeza stipulacea</em></td>
<td>Korean bush clover</td>
<td>Yes, on <em>Lespedeza spp.</em></td>
</tr>
<tr>
<td><em>Phaseolus acutifolius</em></td>
<td>tepiary bean</td>
<td>Yes on other <em>Phaseolus spp.</em></td>
</tr>
<tr>
<td><em>Phaseolus lunatus</em></td>
<td>lima bean</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Phaseolus vulgaris</em></td>
<td>common bean</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Stizolobium deeringianum</em></td>
<td>velvet bean</td>
<td>not reported</td>
</tr>
<tr>
<td><em>Trifolium incarnatum</em></td>
<td>crimson clover</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Vigna sinensis</em></td>
<td>cowpea</td>
<td>Yes</td>
</tr>
<tr>
<td><em>Vigna unguiculata</em></td>
<td>cowpea (African)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

1Chittenden 1897                       5Turner and Kogan 1978
2Skotland 1958                         6Kogan et al. 1980
3Walters and Lee 1969                  7Henn 1989
4Joplin 1974                           8Gergerich 1999
Fig. 1  Average second generation bean leaf beetle population per 50 sweeps. Beetles collected from Johnson Farm, Iowa State University, Ames, IA. First 10 years of figure originally published by Lam et al. (2001).
CHAPTER 2. CHARACTERIZATION OF BEAN LEAF BEETLE (COLEOPTERA: CHRYSOMELIDAE) FLIGHT CAPACITY

A paper accepted in the Journal of the Kansas Entomological Society

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Department of Entomology, Iowa State University,

Ames, IA 50011

ABSTRACT

Bean leaf beetle, Cerotoma trifurcata (Forster) (Coleoptera: Chrysomelidae), flight capacity was measured using a computer-monitored tethered-flight mill. Beetles were collected from soybean (Glycine max (L.)(Fabaceae)) and alfalfa (Medicago sativa L.(Fabaceae)) fields in 2000 and 2001. The number of individual flights, flight duration, and flight distance were recorded for a 24-h period. Eighty-nine percent of beetles made at least one flight, and the mean number of individual flights per beetle was 25.59 ± 5.88 (mean ± SE). The mean total-flight duration was >9 min, and the longest duration of a single flight was >38 min. Seventy-one percent of beetles flew <51 m and 11.76% flew >301 m. The farthest flight made by an individual beetle was 4.9 km. The mean flight distance of all beetles flown was 166.01 ± 45.38 m. Beetles collected in August flew farther than beetles collected in any other month. There was a strong positive relationship between flight distance and flight duration but not between flight distance and the number of individual flights. The presence of ectoparasitic mites and ovarian stage did not affect flight capacity.

INTRODUCTION

There are several types of insect flight. Kennedy (1975) defined migratory flights as those between habitats, and trivial flights as those within the primary habitat. Flights also
have been characterized as sustained if the duration of a single flight is >30 min, and trivial if the flight is <30 min (Dingle, 1965; Rankin and Rankin, 1980; Coats et al., 1986). Trivial flights are made to find mates, oviposition sites, or food (Pedigo, 2002).

Insect flight can be measured using several methods including in-field trapping, mark and recapture, wind tunnels, and tethered-flight studies using flight mills. Tethered insect flight is not directly comparable to flight in a natural environment (Cooter and Armes, 1993; Van Dam et al., 2000), but tethered-flight studies can provide a "good first approximation" (Taylor et al., 1992) of insect flight capacity and the potential for dispersal (Beerwinkle et al., 1995).

Tethered-flight mills work well for insects that are sporadic fliers, such as most Coleoptera (Rankin and Singer, 1984), and for categorizing long- and short-distance fliers for subsequent characterization of morphological or physiological differences between insects in these flight categories. Additionally, flight-mill studies can provide quantitative support for field observations of insect flight (Moriya and Hiroyoshi, 1998; Van Dam et al., 2000).

The bean leaf beetle, Cerotoma trifurcata (Forster) (Coleoptera: Chrysomelidae), is a soybean (Glycine max (L.) (Fabaceae)) pest that has two generations per year in the Midwest (Smelser and Pedigo, 1991) and at least two known periods of migratory flight (Jeffords et al., 1983). In spring, overwintered adults exit leaf litter from wooded areas (Lam and Pedigo, 2000) and migrate to soybeans as soon as the first seedlings emerge (Smelser and Pedigo, 1991). These beetles produce two generations during the summer, and in autumn, adults make a second migratory flight to overwintering habitats (Jeffords et al., 1983).

Recently, there is increased interest in bean leaf beetle movement because the beetle is the primary vector for Bean pod mottle virus (BPMV) (Comoviridae) (Pitre, 1989), a
disease of soybean. For insects that are vectors of plant disease, the epidemiology of the disease is often directly related to insect movement. In locations with more viruliferous beetles, higher incidences of BPMV have been recorded (Ghabrial and Schultz, 1983; Ghabrial et al., 1990; Mueller and Haddox, 1980), however no study has considered beetle flight in relation to spread of the disease. Before disease epidemiology is studied, more data on bean leaf beetle flight capacity are needed and there has been only limited study of bean leaf beetle flight characteristics (Boiteau et al., 1979a). According to Boiteau et al. (1979a, 1980), adults make trivial within-season flights in addition to the two periods of migratory flight activity. Boiteau et al. (1979a) used in-field beetle trap catches and determined that 92% of beetle flights were made at heights of ≤2.4 m, probably reflecting trivial movement. Beetles also have been collected at heights of 61 m (Glick, 1939), probably representing migratory movements.

Data gathered from flight mills only represent insect flight capacity within the flight boundary layer. For insects such as the bean leaf beetle that mostly make low-level flights (Boiteau et al., 1979a), flight mill data can be representative of natural flights in the field. This study characterizes bean leaf beetle flight through quantification of number of individual flights, flight distance, and duration. The objectives of this study were to 1) characterize bean leaf beetle flight capacity, and 2) attempt to explain differences in flight capacity by examining seasonal flight variation and beetle ovarian development.

**MATERIALS AND METHODS**

**Study organisms.** Bean leaf beetles were collected from field locations near Ames, IA from May to August in 2000 and 2001. Beetles placed on flight mills on 24–25 May 2000 and 21–29 May 2001 were collected from alfalfa (*Medicago sativa* L. (Fabaceae)). All
other beetles were collected from soybean. After collection, beetles were placed in a cage with the same plants from which they had been collected (alfalfa or soybean). Cages were housed in a greenhouse and beetles were held for 1–7 d until placed on flight mills. In total, 171 female beetles were tethered to flight mills and 16 beetles could be flown for each 24-h test period. Only females were flown because location of oviposition sites is considered the primary force causing beetle dispersion (Boiteau et al., 1979b). Beetle age could not be determined because all insects were field collected.

All beetles were dissected after flight to check for ectoparasitic mites (Peterson et al., 1992), and to rate ovarian stage. If present, ectoparasitic mites are found under the wings attached between dorsal sclerites; therefore, the presence of mites potentially could affect bean leaf beetle flight ability. Ovarian development was rated because preovipositional insects are known to fly farther (Dingle, 1972; Coats et al., 1986). A rating scale with three stages of ovarian development was created: 1) no egg development, 2) egg development visible, but eggs not fully formed; and 3) at least one fully developed egg. Boiteau et al. (1979c) used an 8-stage rating scale for ovarian development. Our scale is similar to Boiteau et al. (1979c) so that: stage 1 = stages 1 and 2; stage 2 = stages 3, 4, 5 and 6; and stage 3 = stages 7 and 8.

Flight mills. The flight mills were conceptually similar to those used by Coats et al. (1986) and were housed in a closed room with no air currents. The temperature was 26°C, relative humidity was 75–80%, and lighting was a 14:10 light:dark period. Light was provided by 12 incandescent lamps (40 watt) on the ceiling in the middle of the room. To simulate dawn, lights were programmed to come on sequentially at 4-min intervals. To simulate dusk, the lights were programmed to turn off at 4-min intervals. The thorax of each
beetle was attached to an aluminum foil point (1 cm x 2 mm) on the aluminum flight mill arms with sticky wax (Whip Mix Corp., Louisville, KY). Beetles remained suspended for a 24-h test period. The circumference of one flight was equal to 1 m. An infrared sensor was attached to a post below each flight mill arm and each pass of the arm over the sensor recorded a single 1-m flight. Each mill was enclosed in a clear vinyl cover to restrict airflow. A moistened paper towel was placed at the base of each post to maintain humidity. Each mill was connected to a Vectra VL Series 4 microcomputer (Hewlett Packard, Palo Alto, CA) that operated Flight Mill System software (Clarke et al., 1984; Beerwinkle et al., 1995). Flight distance and duration were recorded at 10-s intervals.

**Statistical analysis.** Three parameters (number of individual flights, flight duration, and flight distance) were measured to characterize bean leaf beetle flight. Beetles that made at least one flight were included in statistical analyses. Beetles that did not fly were not included and many of these beetles died during the test period. Number of flights, flight duration, and distance did not follow a normal distribution, and no transformation adequately normalized the data. Thus, the equality of medians for each of number of flights, flight duration, and flight distance by month were compared using the Kruskal-Wallis chi-square test in a one-way analysis of variance context. Flight characteristics were compared by month because differences in bean leaf beetle seasonal flights have been recorded (Jeffords et al., 1983). If the overall test for differences in flight characteristics by month was significant at the 5% level, pairwise comparisons between months were conducted with each being tested at the 5% level. Flight distance was regressed on the other flight characteristics by using a first order linear regression model. (REG) (SAS Institute, 1999) ($P < 0.05$).
RESULTS

There were no significant differences in number of flights ($\chi^2 = 0.22$, df = 1, $P = 0.6377$), flight duration ($\chi^2 = 1.47$, df = 1, $P = 0.2258$), or distance ($\chi^2 = 2.05$, df = 1, $P = 0.1519$) between beetles collected from alfalfa or soybean in May. Because there were no differences, beetles from both host plants were grouped and included in all data summaries and analyses.

Flight parameters. A high percentage of beetles (89.47%) made at least one flight during the test period. Beetles made $25.59 \pm 5.88$ (mean ± SE) separate flights during the 24-h test period. Only one beetle made a single flight that qualified as "sustained" (>30 min) (Dingle, 1965), and thus represented the maximum consecutive flight duration (Table 1). Ten beetles (6.54%) made individual flights of cumulative duration lasting >30 min (Table 2). Eight of these beetles were collected in June, one in July, and one in August. The longest cumulative flight for a single beetle was 2 h 49 min (Table 1).

The mean distance flown by all beetles was $166.01 \pm 45.38$ m (Table 3). The minimum flight distance was 1 m and the maximum was 4.88 km. Based on the histogram of beetles making flights of various distances (Fig. 1), a short flight is defined as less than 50 m and a long flight is one more than 301 m. Seventy-one percent of beetles made flights <50 m, and only 11.76% made flights >301 m. The mean distance of the short flights was $11.75 \pm 1.15$ m, whereas the mean distance of the long flights was $1180.89 \pm 294.55$ m (Table 3).

Significant differences were detected between median flight duration ($\chi^2 = 10.21$, df = 3, $P = 0.0168$) and distance ($\chi^2 = 10.63$, df = 3, $P = 0.0139$) by month (Fig. 2). August flights were significantly longer in duration and distance than flights made by beetles collected in other months. May flights were the shortest in duration and distance, and the
June and July flights were not significantly different in duration or distance. There were no significant differences in the number of flights made by beetles ($\chi^2 = 5.90, \text{df} = 3, P = 0.1166$) by month (Fig. 2). There was a significant positive relationship between flight distance and duration ($R^2 = 0.79, P < 0.0001$) (Fig. 3a) and between flight distance and the number of individual flights ($R^2 = 0.11, P < 0.0001$) (Fig. 3b).

Ectoparasitic mites were found on 11 beetles; however, the presence of the mites did not seem to affect flight because one beetle with mites flew >776 m. No mites found on beetles were engorged. There were no significant differences in ovarian development related to number of flights ($\chi^2 = 1.87, \text{df} = 2, P = 0.3933$), flight duration ($\chi^2 = 3.20, \text{df} = 2, P = 0.2017$), or distance ($\chi^2 = 3.05, \text{df} = 2, P = 0.2173$) (Fig. 4).

**DISCUSSION**

Most bean leaf beetle flights were short in both duration and distance. The majority of flights could not be considered sustained because beetles flew for less than 30 min, thus the flights of most beetles would qualify as trivial. Most flights may have been trivial because beetles were collected from adequate host plants, and there was no stimulus to initiate migratory flight. Movement in relation to host quality has been identified in bean leaf beetles from soybean planting date studies. When early-planted soybean begin to senesce, beetles move to later-planted soybean, which remains green later into the season (Pedigo and Zeiss, 1996). Another study noted no movement by overwintered bean leaf beetles from an early-planted soybean area until the first generation was produced (Newsom and Herzog, 1977).

Despite the majority of short flights, a few beetles did have a cumulative flight duration >30 min. Only one beetle made a single flight >30 min in duration, but nine others
made flights of cumulative duration >30 min that were comparable in distance. Many of these cumulative long duration flights were >1 km. If these flights were unidirectional, it is possible that over a several day period beetles could travel between regions in a state. However, in tethered flight studies, no information on flight direction can be assumed and it cannot be determined whether cumulatively long flights would represent many flights within a field or longer distance unidirectional flights.

Most beetle flights were <51 m, which corresponds with field observations of beetle flights of <30 m (Boiteau et al., 1979a) and other studies reporting that observed flight distances were "short" (Newsom and Herzog, 1977). Compared with two other chrysomelids, the mean distance of bean leaf beetle trivial flights is similar. The distance of most Colorado potato beetle, Leptinotarsa decemlineata (Say) (Coleoptera: Chrysomelidae), short flights was ≤10 m (Weber et al. 1993), and the mean distance of western corn rootworm, Diabrotica virgifera virgifera LeConte (Coleoptera: Chrysomelidae), trivial flights was 68 m (Coats et al., 1986).

The mean distance traveled by the long (>301 m) fliers was >1000 m. If the period of bean leaf beetle migratory activity had lasted several days, beetles could travel several kilometers. Such distances would allow for movement between adjacent fields. Because so few beetles made long flights, it seems that most beetles are not making migratory flights once they colonize adequate habitats. This idea is supported by findings that another chrysomelid, the Colorado potato beetle, flew longer distances if individuals were unfed (Ferro et al., 1991). It is reasonable that a small proportion of the population would make aseasonal migratory flights to spread the population should one location be negatively affected by adverse environmental conditions (Kennedy, 1961; Coats et al., 1986; Voss and
Ferro, 1990b). The distances recorded in this study could be magnified in the field due to wind. Records of bean leaf beetles at heights of 50 m (Boiteau et al., 1979a) and 61 m (Glick, 1939) are probably outside of the boundary layer and indicate beetles might use air currents for migratory flight.

The differences detected in distance of beetle flights by month show that flights toward the end of the season, in August, were the longest. Of the time periods examined, this was the most likely time for detecting long flights because it is late in the season. Boiteau et al. (1979a) recorded beetles at high altitudes only in September, when beetles would be making long-distance flights to overwintering habitats. The idea that beetles might be preparing for overwintering is supported by the beetle dissections because upon dissection, it was noticed that the August beetles had the most fat relative to beetles dissected from other months. It is possible that late-season populations, which usually are larger than at other times in the season, also might provide incentive for some beetles to disperse to avoid crowding.

There was a significant \( P < 0.0001 \) positive relationship between flight distance and duration, which suggests that flight speed was consistent across flight distances. There was no indication that beetles flying longer distances were flying faster, which would have been indicated by a shorter duration for longer flying beetles. There was a very weak, but significant \( P < 0.0001 \), positive relationship between the number of flights made by an individual beetle and flight distance. Apparently, beetles flying further were making longer duration flights, rather than frequent flights, supporting the idea that migratory flights are typically sustained flights lasting for \( \geq 30 \) min.
The presence of ectoparasitic mites did not seem to affect beetle flight; however, if more beetles had been flown that were carrying engorged mites (Peterson et al., 1992) an effect on flight capacity might have been detected. There were no significant differences between ovarian development and flight capacity, but overall, number of flights, flight duration, and flight distance were lowest for beetles that carried at least one fully developed egg. In addition, none of the beetles classified as long fliers had fully developed eggs. Boiteau et al. (1979c) found ovarian development was less in flying beetles. It is possible that ovarian development may affect flight, but more individuals need to be tested.

Only the flight capacity of females was evaluated. Little is known about bean leaf beetle mating behavior, but if males search for females, some aspects of beetle flight were not detected in this study. For other beetles, males make more flights than females in mid-season when searching for mates (Voss and Ferro, 1990a). To fully understand bean leaf beetle flight capacity, male bean leaf beetles also should be tested.

To determine whether collecting beetles from adequate host plants affected flight capacity, beetles could be trapped as they emerge from overwintering habitats and placed on flight mills before they fly to host plants. Also, more beetles from July and August should be flown to supplement evidence for flight patterns detected at these times.

Some insects' capacity for movement, in part, is what makes them efficient pests (Giles and Jutsum, 1989), and understanding this capacity is helpful for understanding population dynamics. Insect movement can serve as the primary cause of rapid increases or decreases in insect populations (Pedigo, 2002). These factors become important for pest management because they can ensure that tactics are applied at times when the insect is the most vulnerable, and they help in making predictions about insect population dynamics.
The results of this study quantify what has been observed anecdotally in the field for bean leaf beetle flight capacity. Knowing that most overwintered beetles do not make migratory flights after they have colonized a field supports management tactics that target beetles during the field season. It also supports work suggesting that delaying planting to deter colonization by beetles is an effective management strategy.

The need for knowledge on bean leaf beetle flight capacity has recently been heightened because this beetle is primary vector of BPMV (Newsom et al., 1980; Hopkins and Mueller, 1983; Pitre, 1989). Overwintered beetles are primary inoculum sources of BPMV, but virus transmission is low (Walters et al., 1972; Krell et al, in prep). The most important role beetles play in virus ecology is in spreading virus within fields during the growing season (Ross and Butler, 1985; Hopkins and Mueller, 1983; Pitre, 1989; Walters and Lee, 1969). The trivial flights recorded in this study probably represent the mechanism behind within-field BPMV epidemiology. The results also suggest that spread of BPMV through a region, such as the Midwest, is not the result of long-distance bean leaf beetle migration throughout the season. Regional spread of BPMV is likely the result of primary inoculum sources (e.g. soybean seed and other host plants) (Krell et al., in prep.) from which the virus is subsequently spread by localized bean leaf beetle populations.
ACKNOWLEDGEMENTS

We thank the Iowa Soybean Promotion Board and the North Central Soybean Research Association for supporting this research. Additionally, we thank J. H. Hill and J. L. Todd for helpful suggestions on this manuscript, R. D. Landes for statistical consultation, and L. G. Higley and an anonymous reviewer for reviewing this manuscript. This is Journal Paper No. 19889 of the Iowa Agricultural Experiment Station, Ames, IA, Project No. 2428.
LITERATURE CITED


Table 1. Summary bean leaf beetle flight duration data ($n = 153$).

<table>
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<tr>
<th>Flight duration / 24 h</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum continuous duration</td>
<td>10 sec</td>
</tr>
<tr>
<td>Maximum continuous duration</td>
<td>38 min 20 sec</td>
</tr>
<tr>
<td>Mean total flight duration</td>
<td>9 min 14 sec ± 1 min 59 sec SE</td>
</tr>
<tr>
<td>Maximum total flight duration</td>
<td>2 h 49 min 10 sec</td>
</tr>
</tbody>
</table>
Table 2. Flight distance and duration for bean leaf beetles making cumulative flights of >30 min (year 2000).

<table>
<thead>
<tr>
<th>Date</th>
<th>Cumulative flight duration (min)</th>
<th>Total flight distance (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 - June</td>
<td>31.67</td>
<td>283</td>
</tr>
<tr>
<td>7 - June</td>
<td>46.83</td>
<td>1,959</td>
</tr>
<tr>
<td>12 - June</td>
<td>89.83</td>
<td>1,088</td>
</tr>
<tr>
<td>14 - June</td>
<td>34.67</td>
<td>222</td>
</tr>
<tr>
<td></td>
<td>111.50</td>
<td>676</td>
</tr>
<tr>
<td></td>
<td>169.17</td>
<td>4,877</td>
</tr>
<tr>
<td>28 - June</td>
<td>54.33</td>
<td>776</td>
</tr>
<tr>
<td></td>
<td>143.50</td>
<td>3,484</td>
</tr>
<tr>
<td>6 - July</td>
<td>94.17</td>
<td>566</td>
</tr>
<tr>
<td>14 - August</td>
<td>102.00</td>
<td>2,434</td>
</tr>
</tbody>
</table>
Table 3. Summary of bean leaf beetle flight distance data ($n = 153$).

<table>
<thead>
<tr>
<th>Flight distance / 24 h</th>
<th>Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>1.00 m</td>
</tr>
<tr>
<td>Maximum</td>
<td>4.88 km</td>
</tr>
<tr>
<td>Mean</td>
<td>166.01 m ± 45.38 SE</td>
</tr>
<tr>
<td>Mean of flights &lt;51 m</td>
<td>11.75 m ± 1.15 SE</td>
</tr>
<tr>
<td>Mean of flights ≥301 m</td>
<td>1.18 km ± 294.55 m SE</td>
</tr>
</tbody>
</table>
Fig. 1  Cumulative flight distances flown by bean leaf beetles ($n = 153$).
Fig. 2. Medians plus first and third quartiles for (A) number of flights, (B) flight duration, and (C) flight distance. Significant differences ($P < 0.05$) are represented by different letters. May, $n =$ 27; June, $n =$ 107; July, $n =$ 7; and August, $n =$ 12.
$y = 0.0387x + 2.8036$

$R^2 = 0.789$

$y = 0.0427x + 18.666$

$R^2 = 0.1089$

Fig. 3. (A) Relationship between bean leaf beetle cumulative flight distance and duration. (B) Relationship between bean leaf beetle cumulative flight distance and number of individual flights ($n = 153$).
Fig. 4 Median plus first and third quartiles for (A) number of flights, (B) flight duration, and (C) flight distance at each stage of ovarian development. 1, n = 63; 2, n = 59; 3, n = 25.
CHAPTER 3. PRIMARY INOCULUM SOURCES OF BEAN POD MOTTLE VIRUS (BPMV) IN IOWA

A paper to be submitted to Plant Disease

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ABSTRACT

A survey of Bean pod mottle virus (BPMV) in Iowa counties was conducted and the virus was present throughout the state. A long-term monitoring study (1989–2002) of the main BPMV vector, the bean leaf beetle, Cerotoma trifurcata (Forster), indicated that populations reached the highest abundance recorded in 14 years. To determine whether an early season primary inoculum source could be found, three potential sources were tested: 1) soybean, Glycine max (L.), seed, 2) overwintering bean leaf beetles, and 3) alternate BPMV-host plants. Examination of 5,804 and 8,064 soybean seedlings of two cultivars yielded zero and three seedlings, respectively, infected with BPMV. BPMV was detected in mottled and non-mottled soybean seeds. Some mottled seeds did not contain BPMV, indicating that soybean seed coat mottling is an unreliable indicator for presence of the virus in seed. Of 182 naturally overwintered bean leaf beetles, only one transmitted BPMV to soybean. BPMV was detected serologically only in one alternate host, Desmodium canadense (L.), out of 23 naturally occurring plant species. The three inoculum sources discovered in this study could be important primary sources when vector populations are high.
INTRODUCTION

*Bean pod mottle virus* (BPMV) is a new pathogen of concern in soybean (*Glycine max* (L.)) in the North Central states (Giesler et al. 2002). BPMV causes reductions in yield (Myhre et al. 1973) and seed quality (Stace-Smith 1981) and no resistance is known in conventional soybean varieties (Skotland 1958, Scott et al. 1974, Schwenk and Nickell 1980). The primary vector for BPMV transmission to soybean is the bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae), (Newsom et al. 1980, Hopkins and Mueller 1983, Pitre 1989), and it is the main agent of virus spread during the growing season (Walters and Lee 1969, Hopkins and Mueller 1983, Ross and Butler 1985, Pitre 1989).

A primary inoculum source is one that causes the original infection at the beginning of the disease cycle (Agrios 1997). The success of plant-virus management relies on identification of primary sources of virus inoculum and understanding virus ecology (Broadbent 1969). There are at least three potential BPMV primary inoculum sources in soybean: infected seed, overwintered insect vectors, and perennial host plants. Two studies have documented low levels (<1%) of BPMV seed transmission (Lin and Hill 1983, Ross 1986) and comoviruses, such as BPMV, have very low rates of seed transmission (Stace-Smith 1981). In other studies, no seed transmission of BPMV was detected (Schwenk and Nickell 1980, Skotland 1958).

Overwintered bean leaf beetles also may serve as a reservoir for BPMV during winter, but transmission by these beetles is reportedly low. In Arkansas, 0 to 17% of bean leaf beetles collected from overwintering sites transmitted BPMV to soybean (Walters et al. 1972). In another Arkansas study, 13.5% of bean leaf beetles collected from overwintering...
sites transmitted BPMV, and 8.3% of beetles collected from an alfalfa (*Medicago sativa* L.) field on 1 April transmitted the virus (Mueller and Haddox 1980). Finding viruliferous beetles early in spring, shortly after beetle emergence from overwintering sites, suggests that transmissible virus may survive in beetles during winter.

In contrast, Anjos (1991) demonstrated that naturally overwintered beetles did not transmit BPMV, even though beetle regurgitant tested positive serologically. For BPMV-carrying beetles that were artificially overwintered at 4°C, 6.6% transmitted the virus to soybean and a small percentage (0.02%) collected from alfalfa transmitted BPMV (Anjos 1991). Other chrysomelid vectors for BPMV are known (Ross 1963, Horn et al. 1970, Werner et al. 2002), but none have been tested as overwintering reservoirs for BPMV.

Horn et al. (1970) and Stace-Smith (1981) suggested that the principle source of primary inoculum is BPMV-infected perennial host plants. Bean pod mottle virus is known to occur in several plants in the family Fabaceae (Milbrath et al. 1975), but more information is needed on the relative occurrence and importance of these hosts in virus ecology.

The objective of this study was to identify and assess potential sources of primary inoculum for BPMV in Iowa. Results from this study are applicable to the widespread occurrence of BPMV in the North Central states and may assist in development of virus management tactics.

**MATERIALS AND METHODS**

**Virus detection. Immunological assays.** The biotin-avidin double antibody sandwich enzyme-linked immunosorbent assay (ELISA) used was similar to that for *Soybean mosaic virus* (SMV) (Steinlage et al. 2002), except that wells of Immulon 1B polystyrene microtiter plates (Dynex Technologies Inc., Chantilly, VA) were coated with BPMV
polyclonal antibody (1.0 \mu g/ml ) prepared to the I-JH1 BPMV isolate (Gu et al. 2002).

Biotinylated polyclonal BPMV antibody was used at 0.5 \mu g/ml and alkaline phosphatase conjugated-Extravidin (1:40,000) (Sigma-Aldrich, St. Louis, MO) was followed by \( p \)-nitrophenyl phosphate (1.0 mg/ml). Plates were rinsed six times in phosphate buffered saline (PBS) (0.05 M sodium phosphate, pH 7.0, with 0.15 M NaCl ) containing 0.1% Tween 20 (PBST) between each step. Samples were considered positive if the absorbance value of duplicate wells was greater than twice the standard deviation plus the mean of the negative control (sap from noninoculated soybean leaves). For assays of virus in soybean seeds, seeds were extracted as described by Bryant et al. (1982) and tested by ELISA for SMV and BPMV. Tests for SMV were performed because it can cause seed coat mottling similar to BPMV (Hill 1999). Methods for SMV assay by ELISA were previously described by Steinlage et al. (2002).

Plants tested by ELISA for BPMV were additionally tested by dot blot assay (Hibi and Saito 1985). Samples (100 \mu l) were spotted on a supported nitrocellulose membrane (0.45-\mu m pore size) (Schleicher & Schuell, Keene, NH) secured to a Bio-Dot microfiltration apparatus (Bio-Rad, Hercules, CA). The membrane was blocked with 5% nonfat dried milk. After blocking, primary antibody, was added at 1.0 \mu g/ml. After incubation with goat anti-rabbit IgG-alkaline phosphatase conjugate (1:10,000) (Sigma-Aldrich), the membrane was incubated in NBT/BCIP (50 mg/ml nitroblue tetrazolium, in \( N,N \)-dimethylformamide, 50 mg/ml 5-bromo-4 chloro-3-indolyl-phosphate in \( N,N \)-dimethylformamide in alkaline phosphatase buffer [12.1 g of Tris base, 5.8 g of NaCl, and 1.02 g of MgCl\(_2\) ] pH 9.5) (Promega, Madison, WI). Immunoreagents were in PBST and incubation times were 60 min except for the final reaction, which was approximately 20 min.
All samples that tested positive for BPMV by ELISA or dot blot were further tested by Western blot following a procedure similar to Anjos et al. (1992). The samples were concentrated before testing by placing 200 μl of each sample in a 1.5-ml microcentrifuge tube with dialysis membrane (12,000–14,000 MW) covering the lid (Overall 1997). The sample was inverted in a test tube and centrifuged at 1570 × g (International Equipment Co., Needham, MA) until the sample volume had been decreased to approximately 50 μl. A 20-μl sample aliquot was mixed with 40 μl of Laemmli buffer (Laemmli 1970) (0.5 M Tris, pH 6.8, 19% glycerol, 3.8% SDS, and 9.5% 2-mercaptoethanol), heated at 95°C for 4 min, and 35 μl was loaded in each well of a 12% SDS-polyacrylamide gel. Samples were electrophoresed at 200 V for approximately 45 min. BPMV-infected soybean sap was used as a positive control, and sap from noninoculated soybean served as a negative control. Proteins were transferred overnight to a supported nitrocellulose membrane (Schleicher & Schuell) by using a transblot cell (Bio-Rad, Richmond, California) at 30 V in Tris/192 mM glycine buffer, pH 8.3, containing 20% methanol (Towbin et al. 1979). After transfer, the nitrocellulose membrane was blocked in 5% nonfat dried milk. Subsequent procedures were as described for dot blots except that the primary and secondary antibodies were used at 30.0 μg/ml and 1:1,500, respectively, and incubation times were increased to 2.5 h.

**Biological assay.** Transmissibility of BPMV was confirmed by local lesion assay, performed on pinto bean (*Phaseolus vulgaris* L.) (Zaumeyer and Thomas 1948), for soybean tissue samples on which overwintered bean leaf beetles had fed.

**BPMV distribution and bean leaf beetle abundance.** To assess the relative importance of BPMV and the bean leaf beetle in Iowa, BPMV distribution in the state and bean leaf beetle abundance by year were evaluated. The distribution of BPMV was
evaluated in August and September 2000. In 84 of Iowa's 99 counties at least 20 live bean leaf beetles per county were collected from farmer's fields using a sweep-net (38 cm) and mailed to the laboratory at Iowa State University, Ames, IA. Beetles were immediately frozen (-20°C) and later macerated individually in a 1.5-ml microcentrifuge tube with 1 ml of PBS. Macerated samples were frozen until analysis by ELISA.

Bean leaf beetle second-generation population abundance was monitored at the Iowa State University Johnson Farm over a 14-year period. Sampling methods and field design for 1989–1998 were described by Lam et al. (2001). From 1999 to 2002, methods were similar, except only three fields were sampled. For each year, means were calculated from all three plots for each week of sampling. A mean for the entire second-generation population by year was calculated from the mean of the three or four weeks that the second-generation population was sampled.

Seed transmission. To examine potential seed transmission, 5,804 and 8,064 seeds of two commercially produced cultivars (A and B), respectively, in December–February in a greenhouse. Plants, grown under supplemental light (14:10 light:dark, 400 W sodium lamps), were evaluated at the V2 growth stage (Fehr et al. 1971). Sap samples were extracted in PBS from leaves of individual plants that showed abnormal phenotypic symptoms (e.g. leaf mottling or rugosity), by using a sap extractor (Ravenel Specialties Corp., Seneca, SC). Samples were stored frozen at -20°C until analysis by ELISA.

Three, 100-seed samples of each cultivar were visually evaluated for seed coat mottling. Any seed with seed coat discoloration was considered mottled. After counting, the three samples of each cultivar were combined, and one 100-seed sample from each cultivar was tested for SMV and BPMV by ELISA. Seeds of an additional 100-seed sample from
each cultivar were individually assessed for presence of seed coat mottling and placed in microcentrifuge tubes. Each seed was macerated in 1 ml of PBS and tested for BPMV by ELISA.

**Overwintered bean leaf beetles.** Leaf litter was collected in February and March 2000 from wooded areas near Correctionville and Ames, IA soybean fields. In March 2001 leaf litter was collected from Ames only. Leaf litter was placed in 5.3-m³ cages covered with screen (1 x 1-mm mesh). As beetles naturally emerged from leaf litter in spring, they were removed and on V1-V3 stage (Fehr et al. 1971) soybeans in a greenhouse placed at a density of one beetle per plant. Seedlings were grown from seed harvested from virus-free ‘Williams 82’ soybeans that had been grown in a greenhouse. In 2000 and 2001, 154 and 46 beetles, respectively, were collected. Cylindrical, clear plastic cages (62.23 x 47.5 cm) covered on one end with white elastic hose (95% nylon, 5% spandex) were placed over each plant in a pot. After an inoculation access time of 3 weeks, each beetle was macerated in 1 ml of PBS and frozen until testing by ELISA. Plants were grown for an additional 3 weeks, after which three trifoliolate leaves were harvested from each plant and extracted individually in PBS for ELISA. Final confirmation of ELISA-positive samples was determined by Western blot and local lesion assay.

**Potential BPMV host plants.** Twenty-two fabaceous, and one solanaceous species were collected from sites with high BPMV incidence, in central and northwestern Iowa, including forage trials at Iowa State University research farms, prairies, and roadside areas. Some plants evaluated had previously been reported as hosts for BPMV, but not from naturally occurring plants in Iowa. Samples, collected on various dates from April through
September 2000 and 2001, were extracted in PBS and tested by ELISA and dot blot assay. Any ELISA- or dot blot-positive sample was further tested by Western blot.

RESULTS AND DISCUSSION

**BPMV distribution and bean leaf beetle abundance.** Previous work showed that presence of BPMV in bean leaf beetles reliably predicted virus field incidence (Ghabrial et al. 1990); therefore, presence of BPMV in a county was determined by identification of at least one serologically positive beetle sample. Beetles examined from all 84 counties were positive for BPMV, indicating that the virus distribution was widespread throughout the state (Fig. 1). The percentage of tested counties that were positive for BPMV (100%) was similar to percentages determined from surveys conducted in Mississippi (91%) (Pitre et al. 1979), North Carolina (90%) (Ross and Butler 1985), and Kentucky (100%) (Ghabrial et al. 1990). However, in this study more counties were tested than in the other states reporting BPMV distribution and the distribution in Iowa is more widespread than that of any other state surveyed.

From 1997 to 2002, the second-generation bean leaf beetle population increased almost 8 times and the population in 2002 reached the highest abundance recorded in 14 years (Fig. 2). It is likely that the recent increase in BPMV incidence is related to the increase in bean leaf beetle abundance.

**Seed transmission.** Sixty-five percent and eighty-seven percent mottled seeds were determined from cultivar A and B respectively. Seed transmission of BPMV was not detected from cultivar A and was detected in three (0.037%) seedlings of cultivar B. By ELISA, combined 100-seed samples were positive for BPMV and negative for SMV. Both viruses are known to cause seed coat mottling (Bowers and Goodman 1979, Stace-Smith...
1981), and analysis of 100 individual seeds of both cultivars showed that some mottled seeds were positive for BPMV, but not for SMV (Table 1). Additionally, some nonmottled seeds were positive for BPMV (Table 1), indicating seed coat mottling is an unreliable indicator for virus presence in a single seed. Because the seed lots tested negative for SMV, it is less likely that this virus was the cause of mottled seed.

Bean pod mottle virus seed transmission was low, which agrees with previous studies (Lin and Hill 1983, Ross 1986). Despite this low level, it may be sufficiently high for seed to serve as an important primary inoculum source (Stace-Smith 1981) when vector populations are high. For example, soybean seed is usually planted at rates varying from 328,600 to 543,600 seeds per hectare (Whigham 2002). If seeds were planted at the common rate of 395,350 per hectare and 0.037% were infected, then 146 infected plants per hectare could occur. This level of seed infection alone probably would not be sufficient to cause economic losses from BPMV because 40% infected plants was calculated to result in economic loss (Horn et al. 1973). Ross (1986) made a similar calculation based on the rate of seed transmission (0.013%) detected in his study. Ross (1986) suggested that a high vector population with a small inoculum source could be enough for beetles to spread BPMV throughout a field early in plant development, thus maximizing yield and quality losses. Field incidence levels of BPMV at soybean growth stage VC (Fehr et al. 1971) were recorded as high as 34% (R. Krell, unpublished data), which could be caused by the combination of BPMV seed transmission and subsequent spread by bean leaf beetles. Seed transmission may be an important early season inoculum source.

**Overwintered bean leaf beetles.** One of 66 (1.5%) overwintered beetles from Correctionville transmitted BPMV to a soybean plant in 2000 (Table 2). The presence of
BPMV coat protein in the soybean sample was confirmed by Western blot (Fig. 3A) and biologically by local lesion assay. No beetles transmitted BPMV in 2001. Despite the low percentage of transmission to soybean, several beetles were positive as carriers of BPMV when macerated and tested at the end of the study (Table 2). Twenty-three percent of the beetles in 2000, and 46% in 2001 were BPMV-positive from Ames, and 24% of the beetles from Correctionville were BPMV-positive in 2000.

The beetle transmission rate is lower than that reported by Walters et al. (1972) and Mueller and Haddox (1980). The Walters et al. (1972) study did not report the method used to assay plants for BPMV; therefore, it is possible that false positives were recorded and transmission rates were inflated. Anjos (1991) did not detect transmission by naturally overwintered beetles; however, he tested only 15 beetles from emergence cages, and 65 beetles from cages placed outdoors under natural overwintering conditions were tested. If the rate of one transmission per 66 beetles is representative, as suggested in this study, it is possible too few beetles were tested. In contrast, Walters et al. (1972) tested 777 beetles over two winters, and approximately 3% transmitted BPMV. Both studies reported using soybean seedlings for BPMV transmission attempts, but the growth stage of seedlings was not given.

A strong positive relationship has been well-documented between early inoculation and greater plant susceptibility to BPMV (Ross 1969, Walters 1970, Hopkins and Mueller 1984). Because the growth stage of seedlings in the Walters et al. (1972) and Anjos (1991) studies was unclear, it is possible that transmission experiments were conducted when plants were not optimally susceptible. Future studies examining BPMV inoculum sources might consider examining BPMV transmission by overwintered beetles by using VE or VC (Fehr et al. 1971) soybean developmental stages.
The discrepancy between the low rate of beetle BPMV transmission and relatively high number of beetles that tested positive for BPMV may be because of virus aging (Lomonossoff and Ghabrial 2001). Proteolysis of the small coat-protein subunit that occurs at the C terminus with aging may make the virus nontransmissible by beetles (Anjos 1991). Another possible explanation is that the conditions for successful BPMV transmission after beetle overwintering may be specific, such that only a small percentage of beetles would experience those conditions during overwintering. Overwintered beetles that transmit BPMV may need to acquire the virus during overwintering by feeding on shoots or roots of infected host plants below the leaf litter (Walters et al. 1972). Overwintering beetles have been found with food in the gut (Boiteau et al. 1979), and this condition could enable the virus to remain intact longer in plant tissue that is not entirely digested. Another factor that may affect overwintering of transmissible virus in beetles is that beetles feed on alternate host plants after soybean senescence and before overwintering (Smelser and Pedigo 1991). Several bean leaf beetle host plants are also hosts for BPMV (Table 3). Perhaps pre-overwintering food choice affects BPMV overwintering in beetles if host species differentially affect virus aging and hence, potential for transmission. Transmission of BPMV by overwintered beetles may be further complicated because there are several virus strains (Gu et al. 2002) and, presumably, beetle biotypes that may interact differently under various overwintering conditions. Although the method of BPMV overwintering in beetles remains unresolved, its apparent occurrence at low levels in the beetle population provides one potential explanation for early detection of infected plants at the VC growth stage (R. Krell, unpublished data).

Unlike many insect vectors, the bean leaf beetle plays dual roles in BPMV ecology during the growing season. Initially, it can serve as a source of primary inoculum, although
at presumably low levels, and subsequently, it serves as the apparent agent of within-season viral spread, which suggests the need for targeting the insect both early and mid-season.

**Alternate BPMV host plants.** Sixteen of the 23 naturally occurring plant species tested positive for BPMV by ELISA, and 12 of these species were positive by dot blot assay (Table 4). Only extracts of *D. canadense* revealed protein bands that occurred coincidentally with those from soybean tissue infected with BPMV in Western blots (Fig. 3B). Therefore, *D. canadense* was confirmed as the only naturally occurring alternate host species for BPMV of those tested. This BPMV-positive sample was collected from northwestern Iowa, where this plant is known to be common (Eilers and Roosa 1994), on 11 June 2000. The identification of BPMV from *D. canadense* at this early date increases its potential to serve as a primary inoculum source for BPMV because bean leaf beetles have been observed feeding on this plant species before soybeans emerge (16 May 2000, R. Krell, personal observation). Although *D. canadense* has been identified as a natural host of BPMV in Louisiana (Horn et al. 1970), naturally occurring BPMV-infected *D. canadense* from the North Central states had not been documented. There are no reports of BPMV transmission from *D. canadense* to soybean by bean leaf beetles; however, transmission from *D. paniculatum* (L.) to soybean was shown (Walters and Lee 1969). *Desmodium paniculatum* is the only BPMV alternate host for which transmission by bean leaf beetles to soybean has been demonstrated. Also, bean leaf beetles were observed feeding on *D. canescens* (L.) in Illinois (McLaughlin et al. 1978) and bean leaf beetle eggs were found near the roots of *D. illinoense* Gray and *D. cuspidatum* (Muhl. ex Willd.) (Waldbauer and Kogan 1976); however, BPMV has not been detected in these species. Other BPMV vectors have been observed on *Desmodium* spp. such as the grape colaspis, *Colaspis brunnea* (F.); southern
corn rootworm, *Diabrotica undecimpunctata* (Barber) (Tugwell et al. 1973); and soybean leafminer, *Odontota horni* (Smith) (Kogan and Kogan 1979), but their importance in BPMV ecology is unknown.

Most plants tested had not been previously reported as BPMV hosts; however, two documented mechanically inoculated hosts, *Lespedeza striata* L. and *Trifolium incarnatum* L. (Table 3), were not confirmed as naturally occurring hosts (Table 4). It is possible too few plants of these species were sampled. Alternatively, it is possible that some mechanically inoculated hosts may not occur naturally. Most BPMV host plants have been determined by mechanical inoculation only (Table 3). Although not shown for BPMV, determination of a BPMV host species based on mechanical inoculation may be unimportant in virus ecology, depending on vector preference (Table 3), phenology, or inhibition of transmission. Beetle regurgitant can be a selective determinant of beetle virus transmission (Gergerich and Scott 1991); however, there is no evidence for direct inactivation by regurgitant of nonbeetle transmissible viruses (Gergerich and Scott 1991). Gergerich and Scott (1991) speculated beetle regurgitant functions by affecting the host or the interaction of the virus with the host. Determination of host plants by mechanical BPMV inoculation can be used to identify potential naturally occurring host plants; however, these data should be followed by field identification of these plants as hosts before conclusions are made about their relationship to virus ecology. Efforts are in progress to assess additional host plant species from the field.

The host plant data confirm the prior demonstration (McLaughlin et al. 1984) that forage legume sap can yield unreliable ELISA results (Table 4). McLaughlin et al. (1984) emphasized that plant and sample buffer combinations should be optimized through experimentation to ensure reliable ELISA results with forage legumes. In extensive
screening studies, such as the one reported herein, optimizing sample conditions would be laborious. However, if only data from ELISA tests had been used, many false new naturally occurring hosts of BPMV would have been reported (Table 4). Because of the ease and speed of ELISA, it was used for initial screening of field-collected plant samples. Subsequently, all ELISA-positive samples were analyzed by dot blot assay. Samples regarded as positive in ELISA or dot blot assay were concentrated and tested by Western blot. The data demonstrate that both ELISA and dot blot assay, although useful for initial screening of forage legumes, may not always provide conclusive results. Future studies with ELISA to screen forage legumes for viruses may consider using sample buffers shown to optimize ELISA results, or performing mechanical inoculations to a single host, such as soybean, and testing the inoculated host tissue for virus. Alternatively, tests of naturally occurring alternate virus hosts could be performed by Western blot.

This study documents three sources of primary inoculum for BPMV in Iowa. Insufficient information is available to suggest that one inoculum source is the primary contributor to early season BPMV infection. Although the percentage of beetles transmitting BPMV after overwintering was low, the total beetles transmitting BPMV could be high when beetle populations are large. BPMV seed transmission may be more important than previously thought. Farmers who have detected BPMV in their soybean fields may consider not using the soybean harvested from such fields for seed in subsequent years as a precaution. It is possible that the combined effect of multiple inoculum sources contributes to the high level of early-season infection that has been documented. It may not be possible to break the disease cycle by directly targeting a single primary inoculum source. The most
promising management tactics presently, are those that focus on inhibiting the most important mechanism for virus spread, the bean leaf beetle (Giesler et al. 2002).
ACKNOWLEDGEMENTS

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REFERENCES CITED


Table 1. Presence of *Bean pod mottle virus* (BPMV) in 100 individual soybean seeds of two soybean cultivars.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Mottled seed</th>
<th>Nonmottled seed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BPMV positive</td>
<td>BPMV negative</td>
</tr>
<tr>
<td>A</td>
<td>47</td>
<td>18</td>
</tr>
<tr>
<td>B</td>
<td>79</td>
<td>8</td>
</tr>
</tbody>
</table>

\(^{1}\)Individual seedlings were tested for BPMV by ELISA.
Table 2. *Bean pod mottle virus* (BPMV) transmission to soybean seedlings by overwintered bean leaf beetles collected in Iowa.

<table>
<thead>
<tr>
<th>Year</th>
<th>Location collected</th>
<th>Total bean leaf beetles tested for BPMV transmission</th>
<th>Total bean leaf beetles positive for BPMV by ELISA</th>
<th>Total soybeans positive for BPMV by Western blot</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>Ames</td>
<td>90</td>
<td>21&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0</td>
</tr>
<tr>
<td>2000</td>
<td>Correctionville</td>
<td>66</td>
<td>16&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>2001</td>
<td>Ames</td>
<td>46</td>
<td>21&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>1</sup>Five beetles could not be tested because they could not be found in cages.
<sup>2</sup>Six beetles could not be tested because they could not be found in cages.
<sup>3</sup>Local lesion assay and macerated bean leaf beetle from this plant were also positive.
<sup>4</sup>Nine beetles could not be tested because they could not be found in cages.
Table 3. Known hosts of *Bean pod mottle virus* (BPMV) and the bean leaf beetle in North America (citations for each record are indicated by superscripts).

<table>
<thead>
<tr>
<th>Method of BPMV host plant identification</th>
<th>BPMV Host plants</th>
<th>Bean leaf beetle host plant?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural occurrence</td>
<td><em>Desmodium canadense</em> (L.)(^8) yes(^14)</td>
<td>yes(^14)</td>
</tr>
<tr>
<td></td>
<td><em>Desmodium paniculatum</em> (L.)(^6) yes(^7)</td>
<td>yes(^7)</td>
</tr>
<tr>
<td></td>
<td><em>Glycine max</em> (L.)(^4) yes(^13)</td>
<td>yes(^13)</td>
</tr>
<tr>
<td></td>
<td><em>Phaseolus vulgaris</em> L.(^2) yes(^12)</td>
<td>yes(^12)</td>
</tr>
<tr>
<td>Mechanical inoculation</td>
<td><em>Glycine</em> spp.(^10)</td>
<td>yes(^14)</td>
</tr>
<tr>
<td></td>
<td><em>Lespedeza cuneata</em> G.(^3) not reported</td>
<td>not reported</td>
</tr>
<tr>
<td></td>
<td><em>Lespedeza striata</em> L.(^3) yes(^13)</td>
<td>yes(^13)</td>
</tr>
<tr>
<td></td>
<td><em>Lespedeza stipulacea</em> Maxim.(^3) not reported</td>
<td>not reported</td>
</tr>
<tr>
<td></td>
<td><em>Phaseolus lunatus</em> L.(^5) yes(^1)</td>
<td>yes(^1)</td>
</tr>
<tr>
<td></td>
<td><em>Pisum sativum</em> L.(^11) not reported</td>
<td>not reported</td>
</tr>
<tr>
<td></td>
<td><em>Stizolobium deeringianum</em> Bort.(^3) not reported</td>
<td>not reported</td>
</tr>
<tr>
<td></td>
<td><em>Trifolium incarnatum</em> L.(^3) yes(^13)</td>
<td>yes(^13)</td>
</tr>
<tr>
<td></td>
<td><em>Vigna sinensis</em> (L.)(^3) yes(^12)</td>
<td>yes(^12)</td>
</tr>
<tr>
<td></td>
<td><em>Vigna unguiculata</em> (L.)(^11) yes(^9)</td>
<td>yes(^9)</td>
</tr>
</tbody>
</table>

---

\(^1\)Chittenden (1897)  
\(^2\)Zaumeyer and Thomas (1948)  
\(^3\)Skotland (1958)  
\(^4\)Walters (1958)  
\(^5\)Thornberry (1966)  
\(^6\)Moore et al. (1969)  
\(^7\)Walters and Lee (1969)  
\(^8\)Horn et al. (1970)  
\(^9\)Joplin (1974)  
\(^10\)Scott et al. (1974)  
\(^11\)Hampton et al. (1978)  
\(^12\)Turner and Kogan (1978)  
\(^13\)Kogan et al. (1980)  
\(^14\)Henn (1989)  
\(^15\)R. Krell, personal observation
Table 4. Results of testing naturally occurring plants for *Bean pod mottle virus* (BPMV) by three different methods.

<table>
<thead>
<tr>
<th>Host plant</th>
<th>Test method</th>
<th>ELISA</th>
<th>Dot blot</th>
<th>Western blot</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amorpha canescens</em> Pursh.</td>
<td></td>
<td>1/1(^1)</td>
<td>1/1</td>
<td>0/1</td>
</tr>
<tr>
<td><em>Astragalus cicer</em> L.</td>
<td></td>
<td>0/1</td>
<td>0/1</td>
<td>—(^2)</td>
</tr>
<tr>
<td><em>Baptisia lactea</em> Raf.</td>
<td></td>
<td>1/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td><em>Caragana arborescens</em> Lam.</td>
<td></td>
<td>1/1</td>
<td>1/1</td>
<td>0/1</td>
</tr>
<tr>
<td><em>Cladrastis lutea</em> Michx.</td>
<td></td>
<td>1/2</td>
<td>1/2</td>
<td>0/1</td>
</tr>
<tr>
<td><em>Coronilla varia</em> L.</td>
<td></td>
<td>1/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td><em>Dalea purpurea</em> Vent.</td>
<td></td>
<td>1/1</td>
<td>1/1</td>
<td>0/1</td>
</tr>
<tr>
<td><em>Desmodium canadense</em> (L.)</td>
<td></td>
<td>2/2</td>
<td>2/2</td>
<td>1/2</td>
</tr>
<tr>
<td><em>Desmodium</em> spp.</td>
<td></td>
<td>3/3</td>
<td>2/3</td>
<td>0/3</td>
</tr>
<tr>
<td><em>Gleditsia triacanthos</em> L.</td>
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<td>1/1</td>
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<td>0/1</td>
</tr>
<tr>
<td><em>Lespedeza capitata</em> Michx.</td>
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<td>3/3</td>
<td>1/3</td>
<td>0/2</td>
</tr>
<tr>
<td><em>Lespedeza striata</em> L.</td>
<td></td>
<td>1/1</td>
<td>0/1</td>
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<tr>
<td><em>Lotus corniculatus</em> L.</td>
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<td>2/6</td>
<td>0/2</td>
</tr>
<tr>
<td><em>Medicago sativa</em> L.</td>
<td></td>
<td>3/26</td>
<td>5/26</td>
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</tr>
<tr>
<td><em>Melilotus alba</em> Medik.</td>
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<td>5/22</td>
<td>0/4</td>
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<tr>
<td><em>Melilotus officinalis</em> (L.)</td>
<td></td>
<td>0/1</td>
<td>0/1</td>
<td>—</td>
</tr>
<tr>
<td><em>Psoralea argophylla</em> Pursch.</td>
<td></td>
<td>0/1</td>
<td>1/1</td>
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<tr>
<td><em>Robinia pseudoacacia</em> L.</td>
<td></td>
<td>2/2</td>
<td>2/2</td>
<td>0/2</td>
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<tr>
<td><em>Solanum carolinense</em> L.(^3)</td>
<td></td>
<td>1/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td><em>Trifolium ambiguum</em> L.</td>
<td></td>
<td>0/1</td>
<td>0/1</td>
<td>—</td>
</tr>
<tr>
<td><em>Trifolium hybridum</em> L.</td>
<td></td>
<td>0/1</td>
<td>0/1</td>
<td>—</td>
</tr>
<tr>
<td><em>Trifolium incarnatum</em> L.</td>
<td></td>
<td>0/1</td>
<td>0/1</td>
<td>—</td>
</tr>
<tr>
<td><em>Trifolium pratense</em> L.</td>
<td></td>
<td>17/31</td>
<td>4/31</td>
<td>0/5</td>
</tr>
<tr>
<td><em>Vicia villosa</em> Roth.</td>
<td></td>
<td>0/1</td>
<td>0/1</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^1\)Numerator represents number of positive samples, and denominator is total number of samples tested.

\(^2\)Test not conducted.

\(^3\)Family = Solanaceae, all others are Fabaceae.
Fig. 1 Iowa counties in which *Bean pod mottle virus* (BPMV) was documented.
Fig. 2. Bean leaf beetle second-generation population abundance at Iowa State University Johnson Farm, Ames, IA. First 10 years of figure originally published in Lam et al. (2001).
Fig. 3 Detection of *Bean pod mottle virus* (BPMV) by Western blotting. A) Potential for overwintered bean leaf beetles to transmit BPMV. Samples from soybean leaf tissue mechanically inoculated with BPMV (lane 1), noninoculated soybean leaf tissue (lane 2), leaf tissue from soybean plants used for a 3-week inoculation access by overwintered bean leaf beetles (lanes 3 and 4). B) Detection of BPMV in naturally-occurring host plants. Samples from soybean leaf tissue mechanically inoculated with BPMV (lane 1) noninoculated soybean leaf tissue (lane 2), leaf tissue collected from field-collected *Desmodium canadense* (lane 3). Molecular weights of markers are shown to the left of each panel. Arrows to the left of each panel designate BPMV capsid proteins. The two bands indicated by the bottom arrow in A, represent the slow- and fast-migrating forms of the BPMV small coat protein, respectively (Lomonossof and Ghabrial 2001).
CHAPTER 4. BEAN LEAF BEETLE (COLEOPTERA: CHRYSOMELIDAE) MANAGEMENT FOR REDUCTION OF BEAN POD MOTTLE VIRUS

A paper to be submitted to the *Journal of Economic Entomology*

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ABSTRACT

Bean pod mottle virus (BPMV), a new problem for soybean (*Glycine max* (L.)) producers in the North Central states, is transmitted by the bean leaf beetle, (*Cerotoma trifurcata* (Forster)) and causes significant yield losses and reduction in seed quality.

Application of lambda-cyhalothrin (Warrior®) (Syngenta, Wilmington, DE) after soybean emergence and again as first-generation bean leaf beetles emerged in July, reduced beetle densities for four and seven weeks, BPMV field incidence by 26% and 27%, and seed coat mottling by 10% and 19%, at locations in central and northwest Iowa respectively. Yield was increased by 440.48 kg/ha (6.5 bu/acre) at the northwest site. Results were compared with a single insecticide application once after soybean emergence, and an insecticide application after soybean emergence and again approximately 10 d later. The data demonstrate that virus vector management is one viable option to reduce BPMV incidence. Also, the study identifies two critical periods of soybean susceptibility (VE and R2) when management tactics to reduce vector populations result in lowered BPMV incidence.
Introduction

In 1945, *Bean pod mottle virus* (BPMV) was first recorded in *Phaseolus vulgaris* L. from South Carolina (Zaumeyer and Thomas 1948). BPMV is currently considered a major soybean (*Glycine max* (L.)) virus and has been described as widespread in Mississippi (Pitre et al. 1979), North Carolina (Ross and Butler 1985), Kentucky (Ghabrial et al. 1990), and Iowa (Krell et al. 2002b). In Arkansas, BPMV was described as the most important and widespread soybean virus (Hopkins and Mueller 1984) and as the most “consistently prevalent” soybean virus in Louisiana (Horn et al. 1973). BPMV was not reported in the North Central states until 1968 from Iowa (Quiniones and Dunleavy 1971); however, recently it has been recognized as widespread in the North Central states (Rice et al. 2000, Giesler et al. 2002, Krell et al. 2002b). BPMV is a concern for soybean growers because it can cause yield losses over 50% (Hopkins and Mueller 1984) and seed coat mottling (Lin and Hill 1983, Stace-Smith 1981), which can result in financial loss. Additionally, BPMV can occur with *Soybean mosaic virus* (SMV), and the combined effect of both viruses is synergistic (Calvert and Ghabrial 1983, Ross 1963, Ross 1969) causing yield losses over 80% (Ross 1963). The potential for overlap of these two viruses is heightened because an exotic pest, the soybean aphid (*Aphis glycines* Matsumura), transmits SMV and has been abundant in some North Central states (Rice 2000b). BPMV also is associated with increased incidence of the fungus, *Phomopsis* spp. (Stuckey et al. 1982, Abney and Ploper 1994), which is considered one of the most important seed diseases of soybean.

Despite the history of BPMV in the United States and its potential impact, there are no proven management recommendations for reducing incidence in soybean. The ideal BPMV-management tactic would be soybean resistance to the virus. Soybean BPMV
resistance has been explored, but no resistance is known in conventional soybean varieties (Skotland 1958, Scott et al. 1974, Schwenk and Nickell 1980). There has been success in generating transgenic soybeans resistant to BPMV (Di et al. 1996, Reddy et al. 2001); however, this trait has not been incorporated into any commercially available varieties.

One BPMV-management option for soybean growers is to reduce the population density of the primary vector, the bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae) (Hopkins and Mueller 1983, Pitre 1989). This beetle is common on soybean in the North Central states, and in central Iowa its population has increased to the greatest abundance recorded in 14 years (Krell et al. 2002b). Before BPMV became a concern in the North Central states, the bean leaf beetle was primarily managed late in the season when populations exceeded economic thresholds for pod feeding. When high populations of the bean leaf beetle occur in the presence of BPMV, early-season vector suppression may be important; therefore, it is critical to understand how the phenology of the bean leaf beetle relates to BPMV epidemiology before implementing any management tactic.

The bean leaf beetle overwinters as an adult (Isely 1930) in wooded areas (Lam and Pedigo 2000). In early spring it leaves overwintering sites and moves to feed on naturally-occurring legumes and alfalfa (Isley 1930, Kogan et al. 1980, Smelser and Pedigo 1991). At least one native legume, *Desmodium canadense* (L.), has been identified as a naturally-occurring host of BPMV (Krell et al. 2002b) and bean leaf beetles are known to feed on this species (R. Krell, pers. obs.). As soon as soybeans emerge, beetles move to soybean plants to feed (Smelser and Pedigo 1991). BPMV can be seed transmitted (Lin and Hill 1983, Krell et al. 2002b) and transmitted by overwintered bean leaf beetles (Krell et al. 2002b); therefore, early season inoculum sources are available for beetles to acquire and spread BPMV. The
beetles produce two generations (Smelser and Pedigo 1991) and each generation takes
approximately one month to develop from egg to adult (Isely 1930).

Application of insecticides after soybean emergence to reduce early-season
inoculation and spread of BPMV by bean leaf beetles has been suggested (Hopkins and
Mueller 1984, Ross 1986, Ghabrial et al. 1990); however, evaluation of this concept has not
been reported. The emphasis on early-season management is suggested because bean leaf
beetles feed on soybean as soon as cotyledons emerge, and there is a strong positive
relationship between plant age at infection and yield reduction (Ross 1969, Walters 1970,

Alternatively, Hopkins and Mueller (1983), based on finding the greatest increase in
BPMV incidence following the peak of the first-generation beetle population and recording
the most viruliferous beetles (16%) at mid-season, suggested application of insecticides in
the middle of the soybean-growing season. Calvert and Ghabrial (1983) also recorded the
greatest increase of BPMV titer in soybeans 76–89 days after planting, which would
correspond to the timing of the increase in field incidence reported by Hopkins and Mueller
(1983).

Based on knowledge of the bean leaf beetle life cycle and virus epidemiology, this
study tested three insecticide management regimes for effect on bean leaf beetle density and
subsequent BPMV incidence. Objectives were to develop a management recommendation
for BPMV that would be readily available to growers and to identify key times in the season
when reducing vector populations would result in reduced BPMV incidence.
Materials and Methods

Field sites. The experiment was performed in 2000 and 2001 at two Iowa field locations, known to have had large bean leaf beetle populations and apparent symptoms of BPMV. In 2000, the central site was located in Ames at the Iowa State University Ross farm and at the Accola farm in 2001. The northwest site was located on the Linn farm in Correctionville in 2000 and on Iowa State University’s Allee research farm in Newell, 2001. A different variety was planted at each location. A food-grade soybean (Vinton 81) was planted at the central site. At the northwest site, a commodity soybean variety (MRK 9823) was planted in 2000, and in 2001, the most similar variety containing a trait for resistance to soybean cyst nematode (SCN) (Heterodera glycines Ichinohe) (MRK 9923CTA) was planted because SCN was known to occur in the field. The central site was planted on 15 May in 2000 and 11 June in 2001 and the northwest field was planted on 3 May in 2000 and 15 May in 2001. All fields were planted in 76.2-cm (30-inch) row spacings. Weed control was implemented, as needed, following standard agronomic practices.

Treatments. Each treatment plot was at least 152.4 m by 36.8 m (48 rows wide) and plots were arranged in a randomized complete block design with four replicates. Each plot was large (at least 0.56 ha) to reduce potential beetle movement between sample areas in each plot because beetles are known to make trivial flights of 11 m (Krell et al. 2002a). Only the middle twenty rows of each plot were sampled.

The insecticide regimes tested used lambda-cyhalothrin (Warrior®) (Syngenta, Wilmington, DE) because it provides relatively long adult bean leaf beetle density suppression (Hammond 1996). Treatments were 1) a single early-season application of lambda-cyhalothrin ((0.023 kg [AI]/ha (2.5 oz./acre)) at the VE-VC (Fehr et al. 1971)
soybean stage, 2) one application each of lambda-cyhalothrin (0.023 kg [AI]/ha (2.5 oz/acre)) at the VE-VC stage and the second made 9–13 days later, and 3) an early-season lambda-cyhalothrin application at the same rate and time as the single early season application, followed by a mid-season application (0.028 kg [AI]/ha (3.2 oz/acre)) at approximately the R2 plant stage (near 15 July) (Fehr et al. 1971), and 4) an untreated control. The mid-season insecticide was applied when the first teneral bean leaf beetles were identified from the field indicating the beginning of the first generation. The early-season application was designed to prevent initial BPMV transmission, and the mid-season application was designed to reduce virus spread by first-generation beetles.

**Immunological Assay.** For all immunological assays, a biotin-avidin double antibody sandwich enzyme-linked immnosorbent assay (ELISA) was used. The ELISA was similar to that described for soybean mosaic virus (Diaco et al. 1985), except that wells of Immulon 1B polystyrene microtiter plates (Dynex Technologies Inc., Chantilly, VA) were coated with anti-BPMV polyclonal antibody prepared to the I-JH1 BPMV isolate (Gu et al., 2002) (1.0 µg/ml) and biotinylated polyclonal anti-BPMV was used at 0.5 µg/ml. Alkaline phosphatase conjugated Extravidin (1:40,000) (Sigma Chemical Co., St. Louis, MO) was followed by p-nitrophenyl phosphate (1 mg/ml). Samples were positive if the absorbance value of duplicate wells was greater than twice the standard deviation plus the mean of the negative control (sap from healthy soybean leaves of plants grown in the greenhouse).

**Insecticide Efficacy.** To evaluate the efficacy of the insecticide treatments bean leaf beetles were sampled weekly, beginning the first week of June at the central site and the third week of May at the northwest site. At plant stages VE-V4, *in situ* counts were made in each plot by examining every plant in 5-m of row. Following the V4 stage, 50-sweep samples
within a single row were performed using a sweep net (38-cm diameter) and performed
parallel in each row; and a different row was sampled each week. Each sample was placed in
a plastic bag and frozen (-20°C) until beetles could be counted.

**BPMV Incidence.** Leaf samples were taken at plant stages VC, R3-4, R6, and R7
(Fehr et al. 1971) to determine BPMV incidence. Samples were taken at the VC plant stage
to determine whether BPMV was present early in soybean development. For VC samples,
single unifoliate leaves were removed from different plants at equal intervals within a 5-m
length of row. At the central site, 5 unifoliates were collected per treatment in both years,
and at the northwest site, 2 unifoliates in 2000, and 5 unifoliates in 2001 were collected from
each treatment. In 2000, unifoliate samples were taken two days after the insecticide
application at the central site and only 10 days after the application at the northwest site. In
2000, there were no significant differences in BPMV incidence detected from unifoliate
samples following the first insecticide application at both sites; therefore, data were
combined across all treatments to determine BPMV incidence. In 2001, unifoliate samples
were taken before the first insecticide application and were combined across all treatments to
determine BPMV incidence. At other plant stages, 10 trifoliolate leaves were blindly chosen
from a single middle row at 5-step intervals until 10 were collected. Sap was extracted
individually from each leaf sample in phosphate buffered saline (PBS) (0.05 M sodium
phosphate, pH 7.0, containing 0.15 M NaCl) using a sap extractor (Ravenel Specialties
Corp., Seneca, SC). Samples were frozen (-20°C) until testing by ELISA.

On the same sample dates when trifoliolates were collected for BPMV tests, bean leaf
beetles were saved from sweep-net samples to evaluate the percentage virus-carrying beetles
by treatment. Beetles were frozen (-20°C) and macerated individually in 1.5 ml
microcentrifuge tubes with 1 ml of PBS. Macerated samples were frozen until analysis with ELISA. Additionally, a one-hundred seed samples from each treatment were analyzed for BPMV antigen content as described by Steinlage et al. (2002).

**Yield and Seed Quality.** Seed was harvested from the middle 16 rows of each treatment to determine yield and seed quality. A sample (≈3000 cm$^3$) was randomly collected from each treatment as the seed was harvested for agronomic and seed quality assessment.

Two evaluations of seed coat mottling were made. A 1-kg seed sample from each treatment was sent to a seed quality analysis facility (Eastern Iowa Grain Inspection and Weighing Services, Inc, Davenport, IA) for analysis of seed coat mottling and seed damage. In the grain quality assay, any single seed with >50% seed discoloration was counted as mottled. Any other seed shape or color abnormalities were incorporated into an estimate of damaged seed. As a second measure of seed coat mottling, three 100-seed samples from each field were evaluated, and any seed showing >0% seed coat discoloration was counted as mottled.

As an additional measure of seed quality, three 100-seed samples from each treatment were planted in trays in a greenhouse and rated for percentage emergence after 10 days. In 2000, the treatments showing the lowest and highest emergence were sent to the Iowa State University Seed Testing Lab to test for presence of *Phomopsis* spp.

**Data analyses.** For all analyses, data were combined, by location, from both years of the study and significant differences were designated at $P<0.05$. Equality of treatment means for bean leaf beetle density, yield, percentage BPMV field incidence, percentage seed coat mottling, percentage seed damage, and percentage emergence were tested using analysis of
variance with sources of variation being year, replications within a year, and treatment. If differences were found, mean separations were performed by comparing the least significant difference (LSD) between treatment means. To determine whether there were differences in the proportion of bean leaf beetles carrying BPMV by treatment; Fisher's Exact test (SAS 1999) was used. If differences in the proportions of BPMV-carrying beetles were found, Fisher's Exact test was used to perform orthogonal contrasts to examine treatment associations. Correlation analyses (SAS 1999) were performed between percentage BPMV field incidence, seed coat mottling (>0%), yield, and BPMV antigen content of seed to compare relationships between response variables.

Disease progress curves were created from BPMV field incidence data obtained from trifoliolates collected at stages R3-R4, R6, and R7 to examine the change in disease incidence over time \((dy/dt)\). Values for percentage disease incidence from treatment replicates were used to determine the best model for describing disease progress for each treatment. Five models, including Gompertz, exponential, logistic, monomolecular, and linear were compared using the EPIMODEL (Nutter and Parker 1996) program for best fit to the data. Appropriate data transformations were made for each model to linearize the data. The \(F\) statistic, coefficient of determination \((R^2)\), and root mean square error (MSE) were compared to determine the most appropriate model. Once the best model was identified for disease progress curves, slopes of disease progress within location, by treatment, were compared using analysis of covariance (SAS 1999).

**Results and Discussion**

**Insecticide efficacy.** Throughout the season the bean leaf beetle population at each location was usually highest in the unsprayed control and, following the mid-season
application, lowest in the plots receiving a single early and mid-season application (Fig. 1).

Following the single early-season application, beetle density was significantly lower than in the unsprayed control for three weeks at the central site (Table 1; week 2: \( F = 6.82; df = 3, 9; P = 0.0108 \); week 3: \( F = 3.23; df = 3, 24; P = 0.0402 \); week 4: \( F = 6.82; df = 3, 9; P = 0.0108 \)) and for five weeks at the northwest site (Table 1; week 1: \( F = 12.28; df = 3, 24; P = 0.0001 \); week 2: \( F = 7.02; df = 3, 24; P = 0.0015 \); week 3: \( F = 5.55; df = 3, 24; P = 0.0049 \); week 4: \( F = 3.90; df = 3, 24; P = 0.0210 \); week 8: \( F = 6.75; df = 3, 9; P = 0.0111 \)). Following the two early season treatments, beetle density remained lower than the control for 3 weeks at the central site (Table 1; week 2, week 3, week 4) and for 7 weeks at the northwest site (Table 1; week 1, week 2, week 3, week 4, week 8; week 10: \( F = 3.90; df = 3, 24; P = 0.0211 \); week 11: \( F = 4.86; df = 3, 24; P = 0.0088 \)). In the treatments receiving a single early application followed by a mid-season application, beetle density was significantly lower than the control for 4 weeks at the central location (Table 1; week 2-4; week 6: \( F = 3.42; df = 3, 24; P = 0.0335 \)) and for 7 weeks at the northwest location (Table 1; week 1-4, 8, 10-11).

The insecticide treatments reduced beetle populations to levels significantly lower than the control, indicating that treatments successfully reduced the vector population. The differences in week 8 at the northwest site were likely because of reductions in the overwintered bean leaf beetle population, which led to continued suppression when the first-generation adults initially emerged. At the central site, only the one early, one-mid-season application provided control late enough in the season to suppress first generation bean leaf beetles. However, at the northwest site, the twice-early application and the one early, one mid-season application provided two weeks of first-generation population suppression.
Overall, spraying once early and once mid-season seemed to be the best treatment for reducing bean leaf beetle densities for the longest time at both sites.

In addition to suppression of bean leaf beetle densities for BPMV reduction, the twice early and one early, one mid-season treatments at the northwest site kept beetle densities below late-season thresholds for pod feeding. The economic threshold for beetle pod feeding would have been approximately 225 bean leaf beetles per 50-sweeps (Rice 2000a). The density suppression in the treatment receiving one early and one mid-season spray suggests that reducing beetles earlier in the season may reduce second-generation densities to levels low enough to avoid late season insecticide application for pod feeding. Future studies on bean leaf beetle management could attempt model development to predict whether second-generation populations would exceed economic thresholds. Based on this prediction, well timed early or mid-season insecticide applications could be tested as tactics to suppress overwintering or first-generation bean leaf beetle populations to reduce second generation densities. The potential to suppress populations early in the season would be desirable for growers because often late-season insecticide applications are complicated by pre-harvest intervals and equipment damage to tall plants during insecticide application.

**BPMV incidence.** In VC unifoliate samples from the central site, 57.5% and 87.65% were positive for BPMV in 2000 and 2001, respectively. In the VC samples from the northwest site, 18.75% and 54.25% were positive in 2000 and 2001, respectively. Overall, a higher than expected BPMV incidence was detected early in the season, reinforcing the supposition that early season beetle suppression is important for BPMV reduction because sources of inoculum are abundant.
At the R6 plant stage, the percentage BPMV infected plants was significantly lower in the treatments receiving two early-season insecticide applications, or one early, and one mid-season application at the central site (Fig 2.; $F = 5.04; \text{df} = 3, 21; P = 0.0087$). At the northwest site, the incidence was significantly lower in the treatment receiving one early and one mid-season application only (Fig 2.; $F = 4.23; \text{df} = 3, 21; P = 0.0174$). The percentage BPMV incidence in the one early, one-mid season application treatment at the northwest site was 39%, indicating that BPMV incidence did not exceed the economic threshold (40%) reported for BPMV (Horn et al. 1973).

There were significant differences in the percentage bean leaf beetles carrying BPMV at the central site at all three sample times (Table 2). In samples taken at the R3-R4 soybean stage, there were significantly more BPMV-carrying beetles in the unsprayed control. The treatments receiving two early, or one early and one-mid season application had the lowest percentage BPMV-carrying beetles. At the R6 stage, the control had significantly fewer BPMV-carrying beetles compared to the treatment receiving one early application, however, overall incidences were over 90% in all treatments. At the R7 stage, the treatments receiving two-early, or one early, and one mid-season spray had significantly fewer BPMV-carrying beetles. At the northwest site, the percentage BPMV-carrying bean leaf beetles was lowest ($P=0.0544$) in the one early and one mid-season treatment at the R3-R4 plant stage (Table 2). There were no significant differences in the percentages of BPMV-carrying beetles from the other sample times at the northwest site. The incidence of BPMV detected from bean leaf beetles mirrors the results obtained for BPMV incidence from the soybean trifoliolate samples, which supports the conclusion that virus suppression was achieved in some of the treatments.
A single model did not consistently describe all data for disease progress curves; however, the logistic model fit most of the data well and is a standard model that has fewer assumptions and more direct interpretation than other models (Epstein et al. 1997). Therefore, the logistic model was chosen as the best description of the data. BPMV incidence increased over time at both locations, however the rates of increase differed, although not significantly, by treatment (Fig. 3, Table 3). The rates of disease progress over time were highest in the unsprayed control and lowest in the treatment receiving one early, and one mid-season application at both locations (Fig. 3, Table 3); however, the slope was not significantly different than that of the other treatments (Table 3). The $R^2$ values for any line were not high, but most predicted lines were significant (Table 3). Future studies of BPMV progress over time should take samples on more sample dates to derive a better prediction of disease progress.

**Yield and seed quality.** There were no significant differences in yield at the central location (Fig. 4; $F = 0.43; \text{df} = 3, 21; P = 0.7359$). The food-grade soybeans used at the central site are known to be a relatively low-yielding variety (Fehr 2001). At the northwest location, yield was highest in the treatment receiving one early and one mid-season application (Fig. 4; $F = 2.99; \text{df} = 3, 21; P = 0.0543$). The yield protection conferred at the northwest site in the one-early, one mid-season application was enough to pay for the cost of the insecticide treatment. Applying once early and once mid-season would have cost approximately $35/ha ($14/acre) and the average price of soybeans during the study years was $0.17/kg ($4.50/bu). Therefore, the gain threshold was 209.18 kg/ha (3.11 bu/acre), and the protection conferred exceeded that minimum because yield was 440.48 kg/ha (6.5 bu/acre) greater than the control. Additionally, the actual value of the soybean in the one
early followed by one mid-season application may have been even greater (e.g. if sold for seed) because seed quality was higher (less mottling and less damage). One-hundred seed-weights were lowest in the control at both locations, but there were no significant differences at either location (Fig. 5).

The percentage seed with >0% seed coat mottling was significantly lower in the treatment receiving one early and one mid-season application at the central site, and in all insecticide treatments at the northwest site (Fig. 6a; central: \( F = 5.11; \text{df} = 3, 85; P = 0.0027 \); northwest: \( F = 37.63; \text{df} = 3,85; P = 0.0001 \)). The percentage seed with seed coat mottling >50% was not significantly different at the central site, but was significantly lower in all insecticide treated plots at the northwest site (Fig. 6b; central: \( F = 2.39; \text{df} = 3, 21; P = 0.0972 \); northwest: \( F = 7.31; \text{df} = 3, 21; P = 0.0015 \)).

Overall, the percentage damaged seed was low. There were no significant differences in the percentage damaged seed at the central location, but the percentage damaged seed was significantly lower in the twice early, and one early followed by one mid-season application at the northwest site (Fig. 7; central: \( F = 2.92; \text{df} = 3, 21; P = 0.0580 \); northwest: \( F = 6.22; \text{df} = 3, 21; P = 0.0034 \)). The reduction in seed damage is probably related to the reduction in bean leaf beetle densities because pod feeding at late season is known to result in seed quality reduction (Shortt et al. 1982, Smelser and Pedigo 1992). There were no significant differences in the percentage emerged seed from any treatment (Fig. 8; central: \( F = 1.74; \text{df} = 3, 21; P = 0.1891 \); northwest: \( F = 1.42; \text{df} = 3, 21; P = 0.2644 \)); however, emergence recorded from the central site was less than 80% from all treatments, which is less than the 85% that is typically considered an acceptable minimum. Seed tested for *Phomopsis* spp. from the control and from the one early followed by one-mid season application tested
negative. Therefore, there was no association between *Phomopsis* spp. and BPMV detected in this study as has been shown in previous studies (Stuckey et al. 1982, Abney and Ploper 1994).

In addition to the differences reported, there were significant relationships between some response variables (Table 4). There was a significant negative correlation between BPMV incidence and yield at the northwest location. This correlation supports the assertion that suppression of BPMV was helpful in protecting yield. There was a positive correlation between seed coat mottling (>0%) and BPMV incidence at both locations, suggesting that seed coat mottling could, at least in part, be attributed to BPMV. There was a negative relationship between seed coat mottling and yield at the northwest site suggesting that mottling and yield were affected by similar factors, presumably BPMV. Last, the percentage virus antigen content in the seed was significantly positively correlated with seed coat mottling at both locations. This suggests that, at least for the varieties tested, seed coat mottling could be used to predict presence of BPMV antigen content in seed. However, it should be noted that other soybean varieties may respond differently and BPMV in seed cannot be equated with seed-transmissible virus (Krell et al. 2002b).

The results indicate that carefully timed insecticide applications can reduce vector populations, resulting in decreased BPMV. The success of the one early followed by one mid-season regime was built on a strong understanding of bean leaf beetle phenology and BPMV from prior research. The large plot size of the each treatment (0.56 ha) was probably important in the detection of treatment differences because beetles could make trivial flights of average distance (11.75 m) (Krell et al. 2002a) and still remain within a single treatment. No previous study has suggested or tested an early and mid-season
application management regime for BPMV reduction. The tactics tested herein should be considered a short-term, transitional option while long-term, sustainable options for BPMV suppression are found. An important result is the discovery that reducing vector populations, before peak levels during soybean vegetative and early reproductive stages, can keep BPMV below economic levels (<40%) (Horn et al. 1973). Other tactics intended to target vector populations for BPMV management should be tested for efficacy at these periods in bean leaf beetle phenology.
Acknowledgements

The authors thank the Iowa Soybean Promotion Board and the North Central Soybean Research Program for funding this research. We thank L. Henn, D. Linn, and L. Rossiter for collaborating on this project and providing agronomic expertise at each farm. Additionally, we thank X. B. Yang for assistance with disease progress curves and R. D. Landes for providing statistical consultation.
References Cited


Table 1. Mean bean leaf beetle counts for two years combined (2000-2001) from two Iowa locations. For sample weeks 1-4 means were calculated from in situ counts of 5-m of soybean row. For other sample weeks means were calculated from 50-sweep samples. Data correspond to Fig. 1.

<table>
<thead>
<tr>
<th>Location and treatment</th>
<th>1&lt;sup&gt;b&lt;/sup&gt;</th>
<th>2&lt;sup&gt;c&lt;/sup&gt;</th>
<th>3&lt;sup&gt;d&lt;/sup&gt;</th>
<th>4</th>
<th>5</th>
<th>6&lt;sup&gt;e&lt;/sup&gt;</th>
<th>7</th>
<th>8</th>
<th>9&lt;sup&gt;f&lt;/sup&gt;</th>
<th>10</th>
<th>11&lt;sup&gt;g&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Iowa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>one early</td>
<td>0.13</td>
<td>0.00b</td>
<td>0.00b</td>
<td>0.00b</td>
<td>16.75</td>
<td>26.13a</td>
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<td>35.00</td>
<td>36.75</td>
<td>20.25</td>
<td>76.63</td>
</tr>
<tr>
<td>two early</td>
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<td>0.00b</td>
<td>0.13b</td>
<td>0.00b</td>
<td>18.25</td>
<td>14.88ab</td>
<td>24.00</td>
<td>50.13</td>
<td>28.00</td>
<td>25.38</td>
<td>71.38</td>
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<td>0.00b</td>
<td>0.25b</td>
<td>0.00b</td>
<td>16.25</td>
<td>1.13b</td>
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<td>35.75</td>
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<td>1.25a</td>
<td>19.00</td>
<td>30.63a</td>
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<td>62.75</td>
<td>42.38</td>
<td>48.13</td>
<td>68.00</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>1.25b</td>
<td>0.50b</td>
<td>0.13b</td>
<td>2.00</td>
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<td>0.00</td>
<td>2.25b</td>
<td>15.13</td>
<td>10.75b</td>
<td>8.25bc</td>
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<td>0.75b</td>
<td>0.75b</td>
<td>0.25b</td>
<td>2.13</td>
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<td>28.75a</td>
<td>17.50</td>
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<td>25.13a</td>
</tr>
</tbody>
</table>

<sup>a</sup>Means followed by the same letter within locations are not significantly different (P < 0.05).
<sup>b</sup>First insecticide application made before this sample date for both locations.
<sup>c</sup>Second insecticide application performed before this sample date for the northwest location.
<sup>d</sup>Second insecticide spray performed before this sample date for the central location.
<sup>e</sup>Mid-season insecticide spray performed before this sample date for the central location.
<sup>f</sup>Mid-season insecticide spray performed before this sample date for the northwest location.
<sup>g</sup>Mean densities sampled following week 11 were not significantly different between treatments; therefore, they are not included in the table.
Table 2. Percentage bean leaf beetles carrying BPMV for two years (2000-2001) combined data.

<table>
<thead>
<tr>
<th>Plant Stage Sampled and Treatment</th>
<th>% bean leaf beetles carrying BPMV&lt;sup&gt;1&lt;/sup&gt;</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td><strong>Central Iowa Location</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3-R4</td>
<td></td>
<td>1.369&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
<tr>
<td>one early</td>
<td>57.78b</td>
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</tr>
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<td>two early</td>
<td>44.62c</td>
<td></td>
</tr>
<tr>
<td>one early, one mid-season</td>
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<td></td>
</tr>
<tr>
<td>control</td>
<td>82.00a</td>
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</tr>
<tr>
<td>R6</td>
<td></td>
<td>0.0490</td>
</tr>
<tr>
<td>one early</td>
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<td></td>
</tr>
<tr>
<td>two early</td>
<td>96.40ab</td>
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</tr>
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<td>98.43ab</td>
<td></td>
</tr>
<tr>
<td>control</td>
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</tr>
<tr>
<td>R7</td>
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</tr>
<tr>
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</tr>
<tr>
<td>two early</td>
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<td>one early, one mid-season</td>
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<tr>
<td>control</td>
<td>94.38a</td>
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<td>0.0544</td>
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<td>one early</td>
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<td>one early, one mid-season</td>
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<tr>
<td>control</td>
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<tr>
<td>one early</td>
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<tr>
<td>two early</td>
<td>75.68</td>
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<tr>
<td>one early, one mid-season</td>
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<tr>
<td>control</td>
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<tr>
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<tr>
<td>control</td>
<td>89.83</td>
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<sup>1</sup>Means followed by different letters indicate significant differences (P<0.05).
Table 3. Logistic model parameters and statistics describing the progress of *Bean pod mottle virus* (BPMV) for two years combined (2000-2001). Slopes were not significantly different ($P<0.05$) between treatments by location.

<table>
<thead>
<tr>
<th>Location and treatment</th>
<th>Intercept</th>
<th>Slope</th>
<th>$R^2$</th>
<th>SEE$_y^1$</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td>Central Iowa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>one early</td>
<td>-2.73</td>
<td>0.013</td>
<td>0.05</td>
<td>0.01</td>
<td>0.3650</td>
</tr>
<tr>
<td>two early</td>
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<td>0.21</td>
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<td>0.0417</td>
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<td>0.03</td>
<td>0.01</td>
<td>0.4796</td>
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<tr>
<td>unsprayed control</td>
<td>-8.72</td>
<td>0.043</td>
<td>0.36</td>
<td>0.02</td>
<td>0.0186</td>
</tr>
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<tr>
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<tr>
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<tr>
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<td>0.49</td>
<td>0.01</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

$^1$Standard error for the estimate of $y$. 
Table 4. Correlations between measured response variables at two locations. Data from four treatments were combined (n=32) by location and years (2000-2001).

<table>
<thead>
<tr>
<th>Location</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>% BPMV field incidence vs. yield (bu/acre)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Iowa</td>
<td>-0.33</td>
<td>0.0648</td>
</tr>
<tr>
<td>Northwest Iowa</td>
<td>-0.62</td>
<td>0.0002</td>
</tr>
<tr>
<td>% BPMV field incidence vs. % seed coat mottling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Iowa</td>
<td>0.48</td>
<td>0.0085</td>
</tr>
<tr>
<td>Northwest Iowa</td>
<td>0.39</td>
<td>0.0291</td>
</tr>
<tr>
<td>% seed coat mottling vs. yield (bu/acre)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Iowa</td>
<td>-0.09</td>
<td>0.6262</td>
</tr>
<tr>
<td>Northwest Iowa</td>
<td>-0.41</td>
<td>0.0185</td>
</tr>
<tr>
<td>% BPMV antigen content of seed vs. % seed coat mottling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central Iowa</td>
<td>0.47</td>
<td>0.0072</td>
</tr>
<tr>
<td>Northwest Iowa</td>
<td>0.36</td>
<td>0.0452</td>
</tr>
</tbody>
</table>
Fig. 1. Bean leaf beetle population trend for combined years (2000-2001) from A) central and B) northwest Iowa. For sample weeks 1-4 means are based on in situ sample counts of 5-m of soybean row. For all other sample weeks means are calculated from 50-sweep samples. Significant differences by sample week are presented in Table 1.
Fig. 2. Mean ± SE percentage field incidence of BPMV-infected soybean plants at stage R6. Data were analyzed separately by location for two years (2000-2001) combined. Means not followed by the same letter are significantly different ($P<0.05$).
Fig. 3. Disease progress curves derived from logit transformation of disease incidence by treatment. Incidence data reported are means and model parameters were estimated from replicate data where \(0 < y < 1\). A) Central Iowa. B) Northwest Iowa.
Fig. 4. Mean±SE yield of soybeans in experiments to reduce disease caused by BPMV. Yield was standardized to 13% moisture. Data were analyzed separately by location for two years combined. Means not followed by the same letter are significantly different ($P<0.05$) at the central location. Means are different at the northwest location at $P<0.0543$. 
Fig. 5 Mean±SE 100-seed weight (g) of seeds harvested from experiments to reduce disease caused by BPMV. Data were analyzed separately by location for two years combined.
Fig. 6. Mean ±SE percentage mottled soybean seeds from soybeans in experiments to reduce disease caused by BPMV. A) Each treatment mean was calculated from three one-hundred seed counts from each replicate. Seed discoloration >0% was considered mottled. B) Seed mottling evaluated from 1 kg seed sample. Seed with >50% seed coat discoloration was counted as mottled. Data were analyzed separately by location for two years (2000-2001) combined. Means not followed by the same letter are significantly different (P<0.05).
Fig. 7. Mean ±SE percentage damaged seed harvested from soybeans in experiments to reduce disease caused by BPMV. Damage was considered any seed abnormality other than seed coat mottling. Means not followed by the same letters are significantly different (P<0.05).
Fig. 8. Mean ± SE percentage seed emergence of soybeans harvested experiment to reduce disease caused by BPMV. Three, one-hundred seed samples harvested from each treatment were planted and evaluated for emergence in a greenhouse. Data from each location were combined from two years (2000-2001).
CHAPTER 5. SOYBEAN PLANTING DATE AS A POTENTIAL MANAGEMENT TACTIC FOR BEAN POD MOTTLE VIRUS

A paper to be submitted *Plant Health Progress*

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ABSTRACT

Four planting dates ranging from mid-March to mid-June and two soybean (*Glycine max* (L.)) cultivars were examined in relation to *Bean pod mottle virus* (BPMV) incidence at a field in central Iowa for three years (2000-2002). Delayed soybean planting is known to reduce populations of the primary BPMV-vector, the bean leaf beetle (*Cerotoma trifurcata* (Forster)). In 2002, beetle density was highest in the earliest planting date at an early plant stage. In 2000, the lowest BPMV incidence occurred in the third planting date, but the incidence was not significantly different than in the other planting dates. For all years, yield was occasionally highest (although not significantly) in the third planting date and lowest in the last planting date. In 2001 and for one cultivar in 2002, 100-seed weight was lowest in the first planting date. In 2000, seed coat mottling was lowest in the third planting date treatment. Generally, stem lengths were longest in the third planting date and number of pods was greatest in the first planting date. Because the earliest planting date had the highest beetle density when plants were at an early growth stage, it is possible plants in this treatment were most susceptible to early-season infection. However, any protection from BPMV
conferred was not evident at the end of the growing season. Delaying soybean planting may protect plants from early-season bean leaf beetle BPMV transmission; however, future studies should test this tactic by using larger plot sizes.

**Introduction**

*Bean pod mottle virus* (BPMV) has become an increased disease concern for soybean (*Glycine max* (L.)) growers in the North Central states (Giesler et al. 2002, Krell et al. 2002c). BPMV can cause yield losses over 50% (Hopkins and Mueller 1984) and seed coat mottling (Lin and Hill 1983, Stace-Smith 1981), resulting in financial penalties. Additionally, BPMV can occur with *Soybean mosaic virus* (SMV), and the combined effect of both viruses is synergistic (Calvert and Ghabrial 1983, Ross 1963, Ross 1969) causing yield losses over 80% (Ross 1963). The potential for overlap of these two viruses may be greater because an exotic pest, the soybean aphid (*Aphis glycines* Matsumura), transmits SMV and has been abundant in some North Central states (Rice 2000b). Also, BPMV is associated with increased incidence of the fungi, *Phomopsis* spp. (Stuckey et al. 1982, Abney and Ploper 1994), which cause one of the most important seed diseases of soybean (Sinclair 1999).

The increased incidence of BPMV in the North Central states is likely related to the increase in populations (Krell et al. 2002c) of the main vector, the bean leaf beetle, *Cerotoma trifurcata* (Forster) (Coleoptera: Chrysomelidae), (Hopkins and Mueller 1983, Pitre 1989) and possibly an increase in the number of soybean acres that are planted in early spring. BPMV outbreaks have been reported as associated with early-planted soybean (Yang 2001). A few soybean growers have planted as early as March (Whigham 2000), and recommendations for soybean planting vary from April to mid-May (Whigham 2002). Bean
leaf beetles emerge from overwintering sites in April (Jeffords et al. 1983, Smelser and Pedigo 1991), and emergence continues through late May, with peak emergence typically occurring in mid-May (Smelser and Pedigo 1991). If soybean are planted when beetles are beginning to colonize fields, these fields typically have higher populations (Pedigo and Zeiss 1996), and may be more susceptible to BPMV transmission.

There is no known resistance to BPMV in conventional soybean varieties (Skotland 1958, Scott et al. 1974, Schwenk and Nickell 1980). One of the few management options for BPMV is to reduce densities of the bean leaf beetle by applying insecticides (Krell et al. 2002a); however, any management tactic that could alter virus ecology should be considered as an option for virus management. Heathcote (1973) suggested, "... simple changes in farming practice or in the choice of plant variety may give economic control of a virus disease." Maezler (1986) suggested that delaying crop-planting date could provide an option for plant virus management. Because the bean leaf beetle life cycle is well understood (Isley 1930, Smelser and Pedigo 1991), there is potential for cultural tactics such as those suggested by Heathcote (1973) and Maezler (1986) to be effective for BPMV management.

Early season beetle populations can be reduced by delaying soybean planting until after overwintered bean leaf beetles have emerged (Pedigo and Zeiss 1996). Bean leaf beetles overwinter as adults in wooded areas (Lam and Pedigo 2000), and they are strongly attracted to soybean as soon as plants emerge (Waldbauer and Kogan 1976, Witkowski and Echtenkamp 1996). Soybean are preferred host plants compared to other legumes (Henn 1989), however if soybean are not available, bean leaf beetles will feed on other host plants. Alfalfa is a common host, but it is known to shorten female life span and egg-laying (Zeiss and Pedigo 1996), thus reducing populations throughout the season. Delaying soybean
planting until at least mid-May is already recommended as a method for reducing bean leaf beetle populations in soybean (Krell and Pedigo 1999); however, the relationship between planting date and BPMV incidence has not been fully explored. The effects of BPMV on yield and seed quality are known to be more severe the earlier plants are infected (Ross 1969, Walters 1970, Hopkins and Mueller 1984, Ragsdale 1984). The longer vectors can be prevented from colonizing soybean and potentially spreading BPMV, the greater the possibility early infection can be avoided.

The objective of this study was to test a range of soybean planting dates to determine an alternative management strategy to insecticides for reducing bean leaf beetle densities and resulting BPMV incidence.

**Materials and Methods**

**Experimental design.** Research was conducted for three years (2000–2002) at the Iowa State University Sorenson Research farm in Ames, IA. Two locally adapted cultivars (A and B) and four planting dates were tested. Planting dates were determined based on growing degree days (GDD) and were planted at 34, 78, 217, and 387 base 10°C GDD, hereafter referred to as the first through fourth planting dates. Soybean were planted at 439,838 to 469,490 seeds per ha in a randomized split-block design. In 2000, each sub-plot was eight 76.2 cm-rows wide (6.1 m) by 12.2 m long. In 2000, a single insecticide application of carbaryl (0.28 kg [AI] / ha) (Sevin) was applied to the first and second planting dates on 16 May because bean leaf beetle feeding was intense. In 2001, each sub-plot was four 76.2-cm rows wide (3.05 m) by 13.7 m, and each planting date block was separated by 4 rows. In 2002, each sub-plot was four 76.2-cm rows wide by 15.2 m long and each planting date plot was separated by 8 rows. No insecticides were applied in 2001 or 2002.
Bean leaf beetle density. Insect sampling was conducted only in August of 2000 and periodically throughout the season in 2001 and 2002. Beetle densities were evaluated by in situ counts for plant samples up to stage V4 (Fehr et al. 1971). A 3-m section of a middle row of each sub-plot was evaluated. For each sample after plant stage V4, 10-sweep samples were taken in each sub-plot in a center row. Samples were placed in bags and taken back to the lab and frozen (-20°C) until beetles could be counted.

Immunological assay. For all immunological assays, a biotin-avidin double antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used. The ELISA was similar to that described for SMV (Diaco et al. 1985), except that wells of Immulon 1B polystyrene microtiter plates (Dynex Technologies Inc., Chantilly, VA) were coated with BPMV polyclonal antibody prepared to the I-JH1 BPMV isolate (Gu et al., 2002) (1.0 µg/ml) and biotinylated polyclonal BPMV antibody was used at 0.5 µg/ml. Presence of virus antigen was quantified by color development using alkaline phosphatase conjugated Extravidin (1:40,000) (Sigma Chemical Co., St. Louis, MO) was followed by p-nitrophenyl phosphate (1 mg/ml). Samples were positive if the absorbance value of duplicate wells was greater than twice the standard deviation plus the mean of the negative controls (sap from healthy soybean leaves).

BPMV incidence. Leaves were sampled on 25 August in 2000, on 15 June, 31 July, and 3 September in 2001, and 2 September in 2002. For each treatment, 10 trifoliolate leaves were blindly chosen from a single middle row at 3-step intervals until 10 were collected. Sap was extracted individually from each trifoliolate in phosphate buffered saline (PBS) (0.05 M sodium phosphate, pH 7.0, containing 0.15 M NaCl) using a sap extractor (Ravenel Specialties Corp., Seneca, SC). Samples were frozen (-20°C) until testing with ELISA.
Additionally, a 100-seed sample from each treatment were analyzed for BPMV antigen content as described by Steinlage et al. (2002).

**Grain yield and seed mottling.** Grain yield was harvested from the middle four rows of each treatment in 2000, all four rows were harvested in 2001, and the middle two rows were harvested in 2002. One-hundred-seed weight measurements were taken from harvested seed samples. Stem lengths and pod number were evaluated for each treatment. Five stems were cut just above the soil surface at 5-step intervals from a single row of each sub-plot and taken to the lab for measuring. All pods containing at least one fully developed seed were counted. Each stem was evaluated separately and subsequently combined to calculate a mean for each treatment. Three 100-seed samples from each sub-plot were evaluated, and any seed showing any seed coat discoloration was counted as mottled. The three counts were used to calculate a mean percentage discolored seed in each treatment.

**Data analyses.** Differences between beetle densities, virus incidence, yield, seed coat mottling, stem length, and pod number were analyzed using the general linear model procedure (GLM) (SAS 1999). Differences generating a significant $F$-value differences were further compared by examining the least significant difference (LSD). Additionally, correlation analyses (SAS 1999) were performed to identify associations between response variables.

**Results and Discussion**

**Bean leaf beetle density.** In 2000, there were significant differences in beetle density on the 26 August sample date for both cultivars (Figs. 1a and 2a). There were significantly more bean leaf beetles in the latest planting date than in other treatments (cultivar A: $F = 9.04; df = 3, 9; P = 0.0044$; cultivar B: $F = 15.33; df = 3, 9; P = 0.0007$),
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probably because plants in other treatments were senescing and beetles moved to younger plants in the late treatment (Pedigo and Zeiss 1996). Insecticide applied to the two earliest planting dates, did not affect beetle populations late in the season because densities were not significantly lower in these plots. In 2001, there were no significant differences in beetle densities on any sample date for either cultivar (Figs. 1b and 2b), which may have been because plot size was reduced in 2001 to 4 rows (vs. 8 rows in 2000), and there was more beetle movement between treatments.

In 2002, there were more beetles on the 15 May sample date in the earliest planting date for both cultivars (Figs. 1c and 2c), but the difference only was significant for cultivar A ($F = 34.35; df = 3, 7; P = 0.0099$). For cultivar A there were no significant differences in beetle densities for any other sample date. For cultivar B, there were significantly more beetles in third planting date compared to the last planting date on 28 June ($F = 5.12; df = 3, 7; P = 0.0348$) (Fig. 2c). The significant differences observed on 15 May in cultivar A and 28 June for cultivar B show that the early-planted plots generally had the highest beetle populations early in the season. A large vector population in early season increases the potential for BPMV to cause yield and seed quality losses because of the positive association with infection at early soybean growth stages (Ross 1969, Walters 1970, Hopkins and Mueller 1984, Ragsdale 1984). For cultivar B, on 30 July, there were significantly more beetles on the latest planting date compared to the earliest planting date (Fig. 2c). Finding more beetles in the last planting date late in the season follows what has been shown that beetles move to younger plants as older plants senesce (Waldbauer and Kogan 1976, Pedigo and Zeiss 1996). Overall, it is likely greater differences between planting dates would have
been detected if plot sizes had been larger. Beetles are known to make mean trivial flights of 11 m (Krell et al. 2002b), therefore it is likely that movement occurred between plots.

**BPMV incidence.** Bean pod mottle virus incidence was not significantly different on any of the planting dates sampled in any year (Table 1). However, in 2000, the late-season BPMV incidence was lowest in the third planting date for both cultivars, indicating the potential for virus reduction with later planting.

**Grain yield and seed mottling.** In 2000, soybean yield was significantly highest in the third planting date for cultivar A ($F = 11.60; \text{df} = 3, 9; P = 0.0019$) (Table 2). For cultivar B, yield was highest in the third planting date, but the difference only was significantly higher than the fourth planting date ($F = 19.11; \text{df} = 3, 9; P = 0.0003$) (Table 2). There were no significant differences in one-hundred seed weights for cultivar A, but cultivar B had significantly lower seed weights in the fourth planting date ($F = 9.80; \text{df} = 3, 9; P = 0.0034$) (Table 2). The percentage seed mottling was lowest in the third planting date for both cultivars. The only significant difference in seed coat mottling was in cultivar B where the latest planting date had the highest percentage mottled seed ($F = 12.54; \text{df} = 3, 9; P = 0.0014$).

In 2001, there were no significant differences in yield or seed coat mottling for either cultivar, however seed weights were lowest in the earliest planting date (Table 2). In 2002, yield was significantly lower in the latest planting date for both cultivars (cultivar A: $F = 11.06; \text{df} = 3, 9; P = 0.0022$; cultivar B: $F = 14.75; \text{df} = 3, 9; P = 0.0008$) (Table 2). Additionally, 100-seed weights were lowest in the early planting date for cultivar A and were not significantly different for cultivar B (Table 2).
There were significant differences in mean stem lengths and number of pods for both cultivars in all three years (Table 3, Figs. 3 and 4). Generally, stems were longest in the third planting date, and there were the most pods in the earliest planting date. Additionally, there was a positive correlation between stem length and number of pods in 2001 ($P=0.0580$) and in 2002 for cultivar B ($P=0.0316$) (Table 4). The stem length data suggest that early beetle-feeding or BPMV transmission may have affected height, but it is likely that environmental factors like temperature and day length had a greater effect on growth.

Generally, there were few associations between response variables (Table 5); however, for cultivar B in 2000 there was a significant positive relationship between yield and 100-seed weight, and there were significant negative relationships between yield and seed coat mottling, 100-seed weight and seed coat mottling, and seed coat mottling and BPMV antigen content of seed. The results suggest that yield and seed weight were affected by similar factors. Additionally, the results highlight the specificity of virus-plant interactions, because with cultivar B, a higher percentage of mottled seeds was associated with lower BPMV antigen content. A previous study showed a positive relationship between BPMV antigen in seed and seed coat mottling in two different cultivars (Krell et al. 2002a). This variation among cultivars indicates that seed coat mottling is not a reliable indicator of BPMV-antigen content in seed.

The data on early-season bean leaf beetle populations in 2002, BPMV incidence in 2000, and grain yield and seed mottling in 2000 suggest there is potential for using planting date to avoid soybean infection with BPMV. It is not be a highly reliable method; however, delaying planting until the peak overwintered bean leaf beetle emergence could reduce early season BPMV infection. In some years the phenology between soybean and bean leaf beetle
could be more dependent on early-season weather conditions (e.g. precipitation, temperature) rather than planting date. The results of this study showed that planting date (mid-May) could provide a good starting point for reducing initial BPMV-transmission. However, fields should be closely monitored and if bean leaf beetles are common in fields, an insecticide application should be considered (Krell et al. 2002a). Insecticide application to suppress early and mid-season bean leaf beetle populations was optimal for reducing BPMV incidence and protecting yield and seed quality (Krell et al. 2002a). Therefore, even if delayed planting reduced early-season bean leaf beetle populations, first generation populations should still be monitored at mid-season and an insecticide application considered. A difficulty in this study is determining the relative impact of the combined effects of environment and planting date, compared with the potential effect of BPMV, on soybean yield.

Alternatives to insecticide management for BPMV are needed. Currently, organic soybean growers have no adequate BPMV management options and mottled seed coats can result in rejection of an entire seed lot. Additionally, widespread insecticide use may result in resistance developing in bean leaf beetle populations and growers would lose a valuable management tool. No adverse effects of later soybean planting were detected in this study. Therefore, late planting should be considered a management option that carries few risks and several potential benefits including reduced bean leaf beetle populations and, potentially, reduction in BPMV incidence.
Acknowledgements

We thank the Iowa Soybean Promotion Board and the North Central Soybean Research Program for funding portions of this research. Additionally, we thank John Lundvall for his field assistance.
Literature Cited


Table 1. Mean ± SE *Bean pod mottle virus* (BPMV) incidence.

<table>
<thead>
<tr>
<th>BPMV Sample Date and Planting Date</th>
<th>Cultivar A</th>
<th>P</th>
<th>Cultivar B</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% BPMV</td>
<td></td>
<td>% BPMV</td>
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<td>0.0632</td>
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<td>90.00±10.00</td>
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<tr>
<td>May 16</td>
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<td>100.00±0.00</td>
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1 No sample taken because soybean had not emerged.
2 Not applicable.
Table 2. Yield, 100-seed weight, and seed mottling for two soybean cultivars by year and treatment.

<table>
<thead>
<tr>
<th>Year and Planting Date</th>
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<th>Cultivar B</th>
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<td></td>
<td>Yield (kg/ha)</td>
<td>100 seed weight (g)</td>
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<td>March 27</td>
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1Data not available.
Table 3. General linear model statistics for comparisons of stem length and pod number by planting date (df = 3, 73). Statistics correspond to Figs. 3 and 4.

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<th>Year</th>
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<td>Stem Length (cm)</td>
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Table 4. Correlations between stem length and pod number \((n = 80)\) for three years (2000–2002) and two cultivars.

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<tr>
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Table 5. Correlations between measured response variables in two years (2000–2001) for two cultivars. (n = 16).

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<td>yield (kg/ha) vs. 100-seed wt (g)</td>
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<td>% seed coat mottling vs. antigen in seed</td>
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Fig. 1. Bean leaf beetle density in cultivar A sub-plots. A) 2000, B) 2001, C) 2002. *Indicates no sample was taken on date because plants had not emerged. Data analyzed separately for each sample date and means followed by the same letter are not significantly different (P>0.05).
Fig. 2. Bean leaf beetle density for cultivar B sub-plots. A) 2000, B) 2001, C) 2002. *Indicates no sample was taken on sample date because plants had not emerged. Data were analyzed separately by location and means followed by different letters are not significantly different ($P>0.05$).
Fig. 3. Mean±SE stem length for two cultivars and three years. A) 2000, B) 2001, C) 2002. Data analyzed separately by cultivar and means followed by the same letter are not significantly different ($P>0.05$).
Fig. 4. Mean±SE pod counts for three years and two cultivars. A)2000, B)2001, C)2002. Data analyzed separately by cultivar and means followed by the same letter are not significantly different ($P>0.05$).
CHAPTER 6.
GENERAL CONCLUSIONS

The main objectives of this research were to increase understanding of Bean pod mottle virus (BPMV) ecology and explore possibilities for management. Progress towards both of these objectives was achieved. BPMV continues to be a problem in Iowa and throughout the North Central states and this work makes valuable contributions towards helping soybean growers.

The research on BPMV ecology focused on areas where information was inconclusive or absent. There were no published studies examining bean leaf beetle, Cerotoma trifurcata (Forster) (Coleoptera: Chrysomelidae), flight capacity and this information is considered an important foundation for understanding virus epidemiology. Most beetles made trivial flights of a mean 11-m distance. The flight distance information could be helpful to researchers in designing future studies because plot sizes of at least 11 m² could help to reduce beetle movement between plots. A few beetles made long-distance flights, and the farthest flight was 4.9 km. The length of the longest flights indicates that long-distance transport of BPMV by bean leaf beetles is possible, but not a primary concern. Beetles collected in August flew farther than beetles collected in any other month, suggesting that migratory beetle flights occur at the end of the season.

The information on beetle flight is important for understanding the potential for BPMV spread. To understand the distribution of BPMV in Iowa, a survey of counties demonstrated that the virus was present throughout the state. Additionally, continued monitoring of bean leaf beetle populations showed that in 2002, the beetle population reached the highest level recorded in 14 years.
The discovery of three primary inoculum sources in Iowa provides critical information for understanding the disease cycle. Seed transmission occurred from 0.037% seeds, 1.5% of overwintered beetles transmitted BPMV, and BPMV was found in Desmodium canadense (L.). Because all three inoculum sources occurred at low levels, it is possible that their combined presence is contributing to abundant early season inoculum sources. However, the high levels of BPMV detected by the end of the summer are probably related to the extremely high populations of the bean leaf beetle.

In addition to progress on understanding BPMV ecology, two management tactics were tested. One option for BPMV management that was successful for suppressing vector populations and virus transmission was application of insecticide after soybean emergence and again as first-generation bean leaf beetles emerged. This tactic suppressed vector populations, reduced BPMV incidence, and protected yield and seed quality. The data demonstrate that vector management is one viable option to reduce BPMV incidence. Also, the study identified two critical periods of soybean susceptibility (VE and R2) when management tactics to reduce vector populations resulted in lower BPMV incidence.

As an alternative to insecticide sprays for BPMV management, four planting dates and two soybean cultivars were examined in relation to BPMV incidence and seed quality. Because the earliest planting date had the highest beetle density when plants were at an early growth stage, it is possible plants in this treatment were most susceptible to early-season infection. However, any protection from BPMV that may have been conferred was not evident at the end of the growing season. A problem with the study was that plot sizes were small and beetles likely moved between plots, which may have masked some results. Future planting date studies examining BPMV management should consider using larger plot sizes.
Delaying soybean planting date may be a useful tactic for bean leaf beetle and BPMV management, but it could exacerbate problems related to other soybean insect and virus complexes like aphids and SMV. Aphid populations usually increase at mid-season, therefore if soybean are planted later they may be more vulnerable (i.e., earlier growth stage) when aphids are abundant. Additionally, later planted fields may be more vulnerable to late-season bean leaf beetle feeding because beetles move to younger plants later in the season to feed. Therefore, in making any cultural management decisions, it is important for growers to monitor the insects in their fields to understand the pest complex requiring management.

The BPMV management studies provide information on which management recommendations for growers can be based. The two tactics were tested separately, but it is possible that a combination of late planting and a later season insecticide application could be effective and future studies may explore this option. Before this research, no tested management tactics were available for growers. The recommendations should be considered interim options while long-term options, such as host plant resistance to BPMV, are developed.

This research contributes to understanding BPMV ecology and finding solutions to BPMV management for growers; however, more research is needed. Integrated pest management is dynamic and must change as the pest complex changes. The insecticide management tactic is a start, but it is not a sustainable option for BPMV management. An alternative strategy using insecticide to target the vector that has been suggested, but never tested, is to plant a trap crop of early planted soybeans to attract beetles early in the season and spray the crop with an insecticide (Newsom and Herzog 1977). Based on bean leaf beetle phenology, this strategy holds promise and could reduce the total area sprayed with
insecticides, while still providing early-season control. Other management strategies could include exploring oils, antifeedants and repellents as alternatives to conventional insecticides. Success with these methods has been reported for other insect-virus complexes (Maelzer 1986).

For management of other viruses a crop-free period for a region has been tried (Broadbent 1969). In this method, crops are not planted when vectors are present. For soybean, this could mean delaying planting until after peak emergence of vectors. At present, it is not likely this method would be accepted by growers in Iowa, but in exploring the maximum number of management options it should be considered.

Research is still needed on the interaction of environment and BPMV. Virus transmission by some insects is inhibited under certain environmental conditions (Harpaz 1982). If specific conditions (e.g. temperature, humidity) are required for bean leaf beetle transmission of BPMV, then predictions about the efficiency of transmission under different environmental conditions could be made, and the potential for high virus incidence could be better anticipated.

Economic thresholds for insects that vector disease are difficult to determine, but it should be a long-term goal (Heinrichs 1979). A problem in developing such thresholds is that it is difficult to determine the percentage insects that are carrying virus. It is possible that preliminary models could be created to determine a working threshold and this threshold could be modified as more biological information is gained. BPMV and the bean leaf beetle will continue to cause problems for Iowa soybean growers, but research is ongoing to understand the disease cycle, improve management options, and ultimately, find varieties with resistance to the virus.
Literature Cited


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I have truly enjoyed my dissertation research experience. There were certainly challenges in the process including, living apart from my husband and the usual logistical frustrations, but in the end I can say that overall I enjoyed the ride.

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