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Interactions among viruses, insect vectors and the Phomopsis complex in soybean, and effects of integrated management strategies

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Interactions among viruses, insect vectors and the *Phomopsis* complex in soybean, and effects of integrated management strategies

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ABSTRACT

Bean pod mottle virus (BPMV), *Soybean mosaic virus* (SMV), bean leaf beetles (*Cerotoma trifurcata*), soybean aphids (*Aphis glycines*) and *Phomopsis* spp. all affect soybean seed quality in addition to causing yield losses. However, interactions among these pests and pathogens, and the effects of combined management practices, are not well understood. Infection of soybean plants by BPMV and SMV has been reported to increase their susceptibility to seed infection by *Phomopsis* spp., but the mechanism of predisposition is unclear. The overall goal of this research was to better understand the interactions between these soybean viruses and *Phomopsis* spp., and to assess the impact of virus vector management practices on infection of soybean plants by *Phomopsis* spp. Effects of SMV and BPMV were studied in separate greenhouse experiments. Two soybean cultivars, Colfax (tolerant to SMV but not to BPMV) and Spansoy 201 (tolerant to BPMV but not to SMV) were mechanically inoculated with either SMV or BPMV. Cultivar 92M02 was inoculated with BPMV. Virus inoculations were followed by inoculation with *P. longicolla* at stages R3 or R5. Neither virus significantly increased susceptibility to stem infection by *P. longicolla*. In the *BPMV-Phomopsis* experiments, inoculation with BPMV significantly increased susceptibility of Spansoy 201 to seed infection by *P. longicolla* at growth stage R5, without affecting plant maturity. Susceptibility of 92M02 to *P. longicolla* at growth stages R3 and R5 was increased by BPMV. Plants of 92M02 displayed typical BPMV foliar symptoms, seed coat mottling and a delay in maturity. In the *SMV-Phomopsis* experiments, inoculation with the SMV-G2 strain did not increase the incidence of *P. longicolla* seed infection in either of the soybean cultivars (Colfax and Spansoy 201). These results confirm BPMV-induced

predisposition to *P. longicolla* seed infection and indicate that the mechanism of predisposition is not due solely to prolonging seed maturation. The previously reported SMV- *Phomopsis* spp. relationship was not confirmed, but this affect may be cultivar- and strain-dependent.

To evaluate the effects of management strategies, four experiments were established in 6 locations in Iowa during 2008 and 2009. The impacts of bean leaf beetle management strategies on infection of seedborne BPMV and *Phomopsis* spp. infection were evaluated in field trials. In 2008, treatments included two soybean cultivars (BPMV tolerant and BPMV susceptible) and two insecticide treatments (treated and untreated). In 2009 treatments consisted of insecticide applications towards different bean leaf beetle generations combined with fungicide applications at growth stage R5 to control *Phomopsis* spp. infection. Insecticide applications reduced beetle feeding injury of leaves and pods in both years, and *Phomopsis* spp. stem infection in 2008. BPMV incidence was significantly reduced when a virus-tolerant genotype and insecticide applications were combined. To assess the impact of soybean aphid management tactics on seedborne SMV and *Phomopsis* spp. infection, stems and seeds were collected from a soybean aphid management study conducted in 2008 and 2009. None of the insecticide treatments reduced *Phomopsis* spp. incidence. There was no evidence of a relationship between aphid attack and *Phomopsis* infection. Fungicides pyraclostrobin (strobilurin) and tebuconazole (triazole) were applied at growth stages R3, R5 or R3+R5, to evaluate the effect on stem and seed infection by *Phomopsis* spp. Late applications of pyraclostrobin were more effective for reducing *Phomopsis* spp. infection of stems. In 2009, treatments including a late application of pyraclostrobin or two applications of tebuconazole (R3 and R5) were more effective for reducing *Phomopsis* spp. infection of

seed. However, none of the treatments had a significant effect on yield, or seed quality determined by warm and cold germination tests. To assess the effects of foliar applications of fungicides and insecticides on infection by *Phomopsis* spp., BPMV and SMV, soybean stems and seeds were collected from field trials conducted over two years in five regions of Iowa. Treatments consisted of an untreated control and foliar applications of fungicides, insecticides or combinations at growth stage R3. In some locations fungicide applications reduced stem and seed infection, but none of the treatments reduced both stem and seed infection. Insecticide applications reduced aphid populations, and infection of seeds by SMV, *Phomopsis* spp. and BPMV, but in an inconsistent manner. Only the combination treatments increased yield in some locations. Results suggested that R3 applications targeted against soybean aphid and foliar diseases can have an added benefit by reducing SMV and *Phomopsis* spp. infection. Overall, virus incidence and beetle populations were very low in both years, and seed mottling was not observed. Although *Phomopsis* spp. infection of seeds was also low, it affected seed germination in some experiments. This project was the first to evaluate the effect of SMV and BPMV on susceptibility to *P. longicolla* infection on soybean plants under controlled conditions. This research was also the first to investigate benefits of insect management tactics for reduction of *Phomopsis* infection. In addition, it was shown that R3 and R5 fungicide applications targeted to control foliar and stem diseases can have some benefits on seed quality by reducing *Phomopsis* infection.

CHAPTER 1

INTRODUCTION AND JUSTIFICATION

Thesis organization

This thesis is organized into 6 chapters. The first chapter contains the introduction, literature review, and research justification. The second chapter will detail results of greenhouse experiments evaluating interactions between *Bean pod mottle virus* (BPMV) and *Soybean mosaic virus* (SMV) with *Phomopsis longicolla*. The third chapter describes field research on impact of BPMV and SMV insect vector management on infection of soybeans by *Phomopsis* spp. The fourth chapter is a summary of a field study assessing impact of foliar fungicides for reduction of *Phomopsis* spp. infection of soybean stems and seeds in Iowa. The fifth chapter will report the findings of a collaborative experiment conducted with Dr. Alison Robertson and Dr. Matt O'Neal evaluating the effects of fungicide and insecticide applications on infection of soybeans by *Phomopsis* spp., *Bean pod mottle virus* and *Soybean mosaic virus*. The sixth chapter is a summary and general conclusion of this Thesis. The references are listed at the end of each chapter.

Literature review

Phomopsis-Diaporthe complex

The *Diaporthe-Phomopsis* disease complex is one of the most serious seed diseases of soybean (*Glycine max* (L.) Merr.) (130, 131) and overall, causes more losses in soybean than any other single fungal disease (131). This complex consists of seed decay caused by *Phomopsis longicolla* T. W. Hobbs (telomorph unknown), pod and stem blight caused by *Diaporthe*

phaseolorum var. *sojae* (Lehman) Wehrmeyer [anamorph *P. phaseoli* (Desmaz.) Sacc. Synonym *P. sojae* Lehman], northern stem canker caused by *D. phaseolorum* (Cook and Ellis) Sacc. var. *caulivora* Athow and Caldwell; and southern stem canker caused by *D. phaseolorum* var. *meridionalis* Morgan-Jones (56, 63, 65, 99).

In addition, fungi in the *Diaporthe-Phomopsis* complex have been associated with damping-off of seedlings and the soybean root rot complex (90, 131), along with soybean top dieback (162, 163, 164) that is characterized by foliar symptoms and premature senescence during soybean reproductive stages. Even though the occurrence of top dieback has been repeatedly observed in Iowa, the disease has not been well studied and little information exists about the etiology or disease management (162, 163, 164).

This complex is widely distributed throughout most of the soybean producing areas in North America (85, 94, 130, 131). Worldwide, members of this complex have been reported to occur in some countries like Argentina (111), Brazil (141), Canada (159), Croatia (144), Ghana (4), South Africa (61), Taiwan (23) and Yugoslavia (102).

Besides soybeans, these fungi may also colonize other legumes and vegetables, such as lima beans (*Phaseolus lunatus*), rooibos (*Aspalathus linearis*), cowpeas (*Vigna unguiculata*), garlic (*Allium sativum*), peanuts (*Arachis hypogaea*), peppers (*Capsicum frutescens*) and tomatoes (*Lycopersicon esculentum*), and several common weeds including velvetleaf (*Abutilon theophrasti*), cocklebur (*Xanthium strumarium*) and wildbean (*Strophostyles helvola*) can also be infected (61,74, 130, 144, 159).

Historically, it has been difficult to identify and classify members of the *Diaporthe-Phomopsis* complex. The separation and taxonomy of these fungi have been based on

morphological and physiological characters, symptomology and virulence on soybean (31, 80, 92, 165, 167).

Colonies of *P. longicolla* on potato dextrose agar (PDA) are floccose, dense and white with occasional greenish yellow areas. Conidiomata are pycnidial, black, stromatic, solitary or aggregate with prominent necks. Conidiophores are hyaline, branched and septate, with alpha conidia and rarely with beta conidia, with no production of perithecia on PDA. Colonies of *P. longicolla* and *P. phaseoli* are morphologically similar (74, 92).

Typically, isolates of *Diaporthe phaseolorum* var. *sojae* on PDA are floccose, turning to brown with age. Pycnidia are black, stromatic, solitary or aggregated, unilocular, with no beak. Conidiophores are simple phialides, with two types of conidia (alpha and beta); beta is the more common type. Solitary perithecia are formed in old cultures and are spherical and slightly flattened at the base with long beaks. Asci are elongate and clavate. Ascospores are similar in shape to the alpha conidia (74, 92).

Diaporthe phaseolorum var. *caulivora* produces white colonies with cottony tufts of mycelium on PDA. Perithecia are black and globose with long protruding beaks, formed in irregular clusters in small stromata. Ascospores are hyaline, elongate and two- celled (32, 92). *D. phaseolorum* var. *meridionalis* is closely related to *D. phaseolorum* var. *caulivora*, but differs in morphology and other characteristics (5,167). Colonies on PDA are lanose and whitish when young and becomes light to dark brown with age, and specific isolates produce perithecia in stromata (32, 92).

Morphological and physiological characters could vary between isolates of the same species, and some are not exclusive to a particular variety (31, 99). Moreover, is often difficult to determine just by morphological characteristics if the isolated colonies correspond to the

anamorph or the telomorph form. The development of new techniques in molecular biology has facilitated differentiation of closely related organisms, based on molecular genetic differences. Differences among *Phomopsis* spp. from different hosts have been studied by sequencing the amplified internal transcribed spacer (ITS) (116, 166), and species and varieties of *Diaporthe* and *Phomopsis* have been identified from soybean using Random Amplified Polymorphic DNA (RAPD) analysis (165, 166). Serological assays like ELISA have also been used for detecting *D. phaseolorum* and *P. longicolla* in plants and seeds (17, 142, 165). However, in seed health testing, seed plating and blotter test are often used following official procedures approved by International Seed Testing Association (ISTA) (http://www.seedtest.org/en/tcom-shc.html) and National Seed Health System (NSHS) standards (http://www.seedhealth.org/).

Pod and stem blight, mainly caused by *Diaporthe phaseolorum* var. *sojae*, can produce symptoms in stems, petioles or pods after the fungus infects the host early in the growing season; however, the infection may remain latent and appear late in the season when the plant matures (74). Numerous black pycnidia are characteristically produced in rows in dry tissues such as petioles of abscised leaves and stems. Infected dry pods may or may not produce pycnidia, but if so they are not produced in rows (74). This disease also affects the production of seed throughout the growing season; early pod infection induces pod abortion and late infection reduces pod-fill and causes pod flattening (90).

Wrather et al. (156) reported that in Canada in 2006, the delay in harvest due to adverse weather contributed to an increase in Phomopsis seed decay and pod and stem blight and these diseases lowered soybean seed quality. It has also been reported that in rainy areas of Brazil *Diaporthe phaseolorum* var. *sojae*, have frequently been responsible for severe losses (156). In Iowa, pod and stem blight also caused significant yield losses during 1999-2002 (158). In

another study, Slater et al. (132) reported reduction in yield due to a premature plant death and a shortened seed-filling period caused by this disease. However, these are also characteristic symptoms of the soybean top dieback that is also caused by fungi members of the *Diaporthe-Phomopsis* complex (162, 163, 164); the connection between stem blight and symptoms referred to as top dieback is not clear. Moreover, some researchers have disputed evidence that the stem phase of this disease causes significant economic damage (65, 72) besides its role as a secondary cause of Phomopsis seed decay. However, in a recent study, Pedersen and Grau (107) documented that infection of basal stems and upper taproots by *D. phaseolorum* var. *sojae* results in significant soybean yield loss.

Stem canker has been widely recognized as an important soybean disease, since the 1950s when a dramatic epidemic occasioned mainly by the widespread use of two susceptible cultivars caused severe yield losses (32, 72). Differences in pathogenicity, morphological characteristics between populations of the pathogen and disease symptomology, resulted in the distinction of northern stem canker and southern stem canker (5, 32, 72). Molecular and phylogenetic analysis proposed that the causal organisms of both diseases are two varieties of *Diaporthe phaseolorum*, var. *caulivora* causes northern stem canker and var. *meridionalis* causes southern stem canker (31, 32, 85, 167). In general, Fernandez et al. (32) described the typical symptoms of both diseases as sunken, dark-brown cankers that may eventually cause plant wilting and death, along with foliar symptoms, such as interveinal chlorosis and necrosis.

Major crop losses as high as 50 to 80% have been reported to occur in the United States, Canada and Paraguay due to stem canker (32, 72, 156). Pedersen and Grau (107) reported that stem canker pathogens are more aggressive than *D. phaseolorum* var. *sojae*, which is consistent with other studies reporting estimated worldwide yield losses in 1994 for approximately 1.9

million metric tons caused by *D. phaseolorum* var. *caulivora* and *meridionalis* (32), while losses caused by *D. phaseolorum* var. *sojae* were estimated at 265, 400 metric tons (74).

Recently, stem canker has declined in importance due to planting of resistant and tolerant cultivars (32, 85, 92, 156). Lu et al. (85) using data collected from over 3,400 soybean fields sampled in Iowa over a 3-year period, reported that stem canker was detected just in 2005 and only 14 isolates were identified as *D. phaseolorum* var. *caulivora* (northern stem canker) out of 63 isolates from stem canker symptoms. They concluded that stem canker is a minor disease of soybean in Iowa. On the other hand, in the same study *P. longicolla* was the predominant fungus isolated from stem canker lesions, which is consistent with previous reports about the higher recovery frequency of *P. longicolla* compared with the other members of the *Diaporthe-Phomopsis* complex (85, 131,159).

Despite the importance of *D. phaseolorum* var. *sojae*, *D. phaseolorum* var. *caulivora* and *D. phaseolorum* var. *meridionalis* in causing respectively pod and stem blight and northern and southern stem canker, they are not as important as *Phomopsis* spp. in seed decay (63, 65). Kmetz et al. (63) recovered a *Phomopsis* isolate from soybean that was different from both *D. phaseolorum* var. *sojae* and var. *caulivora*, which formed only pycnidia in culture and was highly pathogenic, damaging up to 85% of inoculated seeds. In consequence, it was proposed that the seed phase of the disease should be named Phomopsis seed decay (65), and in 1985 the unknown *Phomopsis* species reported by Kmetz et al. (63) was described as a new species, *Phomopsis longicolla* (56).

Today it is well known that although all members of the *Diaporthe-Phomopsis* complex are seedborne (2, 166), *P. longicolla* is the principal causal organism of Phomopsis seed decay of soybeans (94, 95,126, 157, 167). Sinclair (131) reported that *P. longicolla* is the most frequently

recovered fungus of this complex, followed respectively by *D. phaseolorum* var. *sojae* and either *D. phaseolorum* var. *caulivora* or *D. phaseolorum* var. *meridionalis*. Consistently, in recent studies conducted in Ontario, Canada (159) and Iowa, United States (85) *P. longicolla* was the predominant species isolated from diseased plants. In contrast, in a study conducted in southwestern Indiana, Baird et al. (6) found that the primary pathogenic species identified from the *Diaporthe/Phomopsis* complex were *D. phaseolorum* var. *caulivora* and var. *sojae*, and *P. longicolla* was identified from only 1% of the total samples.

Phomopsis spp. can penetrate the seed through openings in the seed coat such as pores and cracks or directly through pod walls, and then start the colonization of the seed coat, cotyledons and eventually the radicle and plumule (74, 130). Heavily infected seeds are elongated, shriveled, severely cracked, flattened, and may be partly or completely covered with white mycelium; however, seeds can be infected without showing symptoms (74, 92).

Phomopsis seed decay reduces seed quality by reducing viability, germination, and emergence (45, 65, 92, 126, 165) and causing physical damage (45, 72). Seed infection may also lower commercial seed grade by increasing the number of moldy and splitting seeds and decreasing volume and density (90, 98). Moreover, severe incidence of seed decay can also affect quality of seeds used for processing, causing an alteration in protein content (97) and reduction of flour and oil quality (45, 158). It has been reported that production of mycotoxins by these fungi can result in damage to animals (27, 75, 98). Kung et al. (75) observed that a toxin produced by *Phomopsis* spp. on soybeans caused a necrosis of liver and hemorrhage on chicks. In another study, Cole et al. (27) identified two mycotoxins from *Phomopsis sojae* that were toxic to chickens and also showed plant growth inhibition.

Pods are the main pathway for infection of seeds, and although pods can be infected at any time after they are formed, major seed infection will not occur before physiological maturity (72, 91, 127) and only under specific conditions of humidity and temperature (9, 127, 134). For this reason the movement of *P. longicolla* from infected pods into seeds is higher when harvest is delayed, and plants are exposed to prolonged wet periods and warm weather during pod development and maturation (126, 127, 130).

Moreover, it has been reported that seeds tend to be more susceptible to seed decay when plants are deficient in potassium, heavily attacked by insects, and infected with one or more viruses (35, 72, 130, 131). Wounds, insect injuries and pod wall cracks caused by changes in environmental conditions also allow secondary infection by fungal pathogens such *Phomopsis* spp., resulting in greater number of infected seeds (8, 93, 104, 133).

Infected seeds are the main means of long distance dissemination and the major portal of entry of the pathogen into noninfested areas (74, 159). On the other hand, short distance dissemination is by sexual or asexual spores that are released and spread to plants by wind and rain (34, 130, 159). These spores are formed in fruiting bodies produced on overwintered plant debris or infected plants, and represent the primary source of inoculum for infection of stems, petioles and pods (34, 64, 127, 130, 159).

Due to the ability of these fungi to survive and sporulate on crop residues and produce inoculum for subsequent season, tillage and crop rotation practices can influence disease intensity (7). Different studies have reported higher colonization of soybean stems and pods by members of the *Diaporthe-Phomopsis* complex, and lower yields in continuous soybean cropping under minimal tillage (6, 59, 107, 124, 125).

Other management strategies that have been reported are the use of late maturity cultivars or delaying the planting of early and mid season cultivars to reduce seed infection (65, 92, 139, 158). In different studies, early plantings have resulted in higher disease frequency and lower seed quality and germination (95, 96). These findings do not support the use in the midsouthern US of the early soybean production systems (ESPS) that includes planting of early maturity cultivars (MG III, IV and V) from late March through April (94, 95). Despite the higher risk of seed infection with *Phomopsis* spp., some advantages of the ESPS have been observed, and the avoidance of the late season drought and harvest under dry conditions are the main benefits of this system (94, 95, 155, 158). It has been suggested that the use of *Phomopsis* spp.-resistance cultivars could increase the usefulness of the ESPS (158).

Lower incidence of *Phomopsis* spp. has been observed with resistant soybean lines, such as SS 93-6012 and SS 93-6181 (106, 157, 158). Previous to the release of these lines, Minor et al. (98), using a plant introduction source of genetic resistance in breeding programs (PI417479), observed seed infection of 0-3%, compared with 25-59% of the susceptible cultivar. However, development of high yielding *Phomopsis* spp.-resistance cultivars is still needed (157, 158).

The effectiveness of late season (R6) fungicide applications to prevent the movement of *Phomopsis* spp. from pods to seeds has been reported previously (9, 91, 94, 98, 158). However, some of the effective products are no longer labeled for use on soybean, and other products have shown mixed results, sometimes resulting in increased infection compared to the untreated control (9, 91, 94, 98, 138, 158).

As mentioned above, there is some evidence that virus infection and insect damage predispose soybean plants to *Phomopsis* infection (35, 74, 130). Therefore, there is a potential for optimizing management practices that involve insects and seedborne pathogens.

Soybean viruses- *Phomopsis* spp. interactions

Published literature suggests that seeds from plants infected by either *Soybean mosaic virus* (SMV) or *Bean pod mottle virus* (BPMV) may be more susceptible to infection by *Phomopsis* spp. (1, 45, 68, 69, 137). One of the reasons of the increased susceptibility in virus-infected plants could be that these two viruses extend the length of the late-season growth stage intervals, and therefore, prolonged the exposure of pods and seeds to infection by *Phomopsis* spp. (1, 68, 69).

Previous studies have reported that under specific conditions mixed infection by BPMV and SMV increased the incidence of *Phomopsis* spp. Stuckey et al. (137) found a consistent significant increase in *Phomopsis* spp. seed infection only in plants that were co-infected with SMV and BPMV. Other workers have observed significant differences in *Phomopsis* spp. seed infection in the presence of SMV infection when plants have been inoculated with both SMV and *Phomopsis* spp. under controlled conditions (45, 68, 69). In field studies, Abney and Ploper (1) demonstrated that infection of pods by *Phomopsis* spp. was increased by BPMV inoculations and seed infection was increased only if the virus infection delayed the rate of seed maturation. These data are from field trials under natural *Phomopsis* spp. infection and vector populations therefore, it was not possible to separate confounding effects of beetle activity from a direct BPMV/*Phomopsis* interaction.

In Iowa, *Phomopsis* spp., BPMV and SMV were all prevalent in some areas in 1998 (160), and recently the frequent detection of *Phomopsis longicolla* in stems collected during the Iowa Soybean Disease Survey conducted from 2005-2007 (85) coincided with a resurgence in bean leaf beetle (*Cerotoma trifurcata* Förster), populations and BPMV symptoms (53).

Bean pod mottle virus

Bean pod mottle virus (BPMV) is a major virus disease found throughout most of the soybean producing areas in Eastern, Southern and Midwestern states of the United States (35, 72). In North Central states it was first reported to occur on soybeans in Iowa in 1968 (72) and recently has become an increasing problem in this area (36, 71).

BPMV belongs in the genus *Comovirus* in the family *Comoviridae* (39) and has a bipartite positive sense strand genome that consists of RNA-1 and RNA-2 that are separately encapsidated in 28-nm diameter isomeric particles (39). Genetic determinants for BPMV symptom severity are found on RNA–1 which also synthesizes proteins important for replication (36, 40), while RNA–2 synthesizes the large and small coat proteins, 58k RNA–2 replication co-factor and cell-to-cell movement protein (36, 88).

In a study on strain diversity among BPMV isolates, Gu et al. (39) reported that representative isolates of BPMV collected from soybean fields in four states (Kentucky, Virginia, Arkansas, and Iowa) were classified into two distinct subgroups. This study, based on nucleic acid hybridization analysis and nucleotide sequencing data, found the occurrence in nature of both subgroups I and II, as well as reassortants between the two subgroups (39, 41). These reassortants are diploid, containing RNA-1 or RNA-2 from both subgroup I and II, and they can be generated in either the hosts or insect vectors as a consequence of mixed infections (41).

Although subgroup II BPMV strains are generally thought to be the most common BPMV strains in soybean, mild symptoms are associated with this subgroup; likely due to an adaptation process that involves masking the symptoms by soybean plants (39, 41, 168). Moreover, BPMV reassortants containing the two different RNA-1 have been associated with the occurrence of very severe symptoms (39, 168). However, Gu et al. (39) found no significant

differences in yield reductions caused by the different strain subgroups (I, II, and the reassortants I/II).

Recently, Bradshaw et al. (15) discovered a naturally-occurring BPMV reassortant on *Desmodium illinoense* Gray with a RNA-1 and RNA-2 from subgroup I and II, respectively, which caused mild to moderate symptoms on three representative soybean cultivars. In the same study, BPMV isolates were collected from a soybean field adjacent to the location of the original *D. illinoense*, and the co-existence of two BPMV subgroups of RNA–1 and genetic diversity among isolates was reported (15).

In addition to the virus strain, symptoms of infection on soybean by BPMV vary depending on environment and soybean cultivar, tending to be more evident during periods of cool weather and when infection occurs early in the growing season (35, 51, 60). Foliar symptoms ranged from chlorotic mottling to a severe mosaic in young leaves in the upper canopy (35), and infected plants may have green stems after maturation and may retain petioles after leaf blades have abscised. This delay in maturation has been associated with the soybean green stem disorder (128), which is characterized by the lack of stem senescence even when pods and seeds have already matured (55). BPMV was first reported as the causal agent of this disorder in 1980 by Schwenk and Nickell (128), however, in that study not all the BPMV infected plants developed green stem symptoms. Similar results were obtained by Hobbs et al. (55), finding BPMV infection did not increase green stem disorder incidence in comparison to controls, and typical symptoms can develop in absence of BPMV infection. In addition to BPMV, green stem disorder has been associated with southern green stinkbug (*Nezara viridula*) feeding and fungicide applications (46, 55), however the cause of the green stem disorder remains unknown.

Other studies have reported that the delay in senescence caused by BPMV may increase susceptibility to Phomopsis seed decay, by extending the dry down period which may result in an increase of *Phomopsis* spp. seed infection (1, 35, 36). Nevertheless, the main impact of BPMV in soybean production is reducing seed quality and causing yield losses.

Yield is mainly impacted by reduction in seed size and pod set by as much as 40%, and yield losses could range from and 3 to 52% (35). Recently, Ziems et al. (170) evaluated the response of different soybean cultivars available in the north-central region of the United States to BPMV infection, demonstrating that substantial yield losses occurred due to BPMV infection. Previous studies have also shown that yield reduction could vary across soybean cultivars (60, 123).

In addition, some effect of plant height has been observed, with higher incidence of BPMV associated with taller soybean plants (153). However, the higher infection levels may have been due to a higher exposure of taller plants to the virus vector (153). Redinbaugh et al. (114) also found that BPMV incidence was lower in a determinate, semidwarf genotype Troll than in standard indeterminate cultivars, regardless of the resistance level to insect-feeding. It has also been suggested that yield compensation effects can occur in infected fields, where symptomless plants adjacent to infected plants yielded up to 50% more than healthy plants adjacent to other healthy plants (153).

BPMV infection also impacts seed quality due to the production of seeds with mottled seed coats by infected plants (36, 84). This mottling consists of dark streaks originating at the hilum, and has been called the “bleeding hilum” symptom by some authors (36, 54). Seed coat discoloration has a negative impact on the marketability of seeds and food-grade soybeans, compromising the quality standards and causing rejection of seeds, which translate into financial

losses (54, 72, 114). Even though no effect in seed germination and vigor has been found from infected plants (19, 60), other components of seed quality could be affected by BPMV. In contrast with previous studies (123), Ziems, et al. (170) demonstrated that under certain conditions BPMV infection can also affect oil and protein content.

The intensity of seed coat mottling depends on virus strain, host genotype, time of inoculation and environmental conditions and mixed infection with other viruses (36, 54, 71, 170). The effect of BPMV causing discolored seed coat mottling or discoloration has been well reported. Hobbs et al. (54) found that BPMV and SMV, alone or in combination, produced more seed coat mottling, compared with the noninoculated plants. Daniels (29) reported that BPMV was consistently recovered at a higher rate from symptomatic seeds than in symptomless seeds after harvest. Recently, in a study conducted in Nebraska and Ohio, BPMV inoculation increased seed mottling in the inoculated plots compared with the noninoculated plots (170). However, other studies have not reported a relationship between seed coat mottling and BPMV infection, and therefore symptom appearance does not imply seed infection (36, 48, 112).

Plant stage at infection also has a significant effect on incidence and severity of the diseases, for instance the earlier the plants get infected the higher the reduction in yield (21, 60, 115, 170). Ziems et al. (170) reported that the impact in yield and seed quality was higher when plants were inoculated at early growth stages (VC-V4) than in inoculations at growth stages R6 to R7.

Yield reduction up to 44% has also been observed after BPMV inoculations at growth stage V1 (60). Symptom expression and yield loss could also be enhanced in mixed infections of BPMV and *Soybean mosaic virus* (SMV) due to synergistic interactions (21, 112, 170). Quiniones et al. (112) reported higher percentages of seed coat mottling by mixed infection

(96%) than that caused by SMV alone (92%). In other studies, yield losses up to 80% have been attributed to dual infection of BPMV and SMV (170).

Occurrence of BPMV in soybean fields early in the growing season has a larger effect on yield and seed coat mottling than later season infections. Previous studies have shown that the earlier a plant is inoculated, the higher its infectivity, resulting in more seed mottling, foliar symptoms and greater yield reduction (21, 115, 60). The early BPMV infection is strongly dependent on the prevalence of the virus inoculum and vector abundance.

Krell et al. (71) identified three potential sources of inoculum for BPMV in Iowa: perennial host plants, infected seed, and overwintered insect vectors. In all three sources the virus can survive through the winter (70).

Several BPMV alternative hosts have been identified (35), and these are particularly important as inoculum sources early in the season when beetles emerge and feed on them in the absence of soybean plants (133). Giesler et al. (36) reported that in the north central United States rather than cultivated crops, perennial legumes such as *Desmodium* spp. can serve as alternative hosts for BPMV. Walters and Lee (1969) cited by Krell (70) demonstrated that BPMV could be transmitted from *D. paniculatum* to soybeans by bean leaf beetles. In recent studies conducted in Iowa, Krell et al. (71) established that out of 23 naturally occurring plants species, only *D. canadense* tested positive for BPMV (71). Also Bradshaw et al. (15) found that *D. illinoinse* L. was infected with BPMV and was a preferred host of bean leaf beetles.

It has been demonstrated that BPMV can be transmitted through seed, but at a very low rate (<1%) (71, 84). Moreover, BPMV can survive in overwintered beetles (71, 72, 100, 145), and be transmitted after they emerge the next spring in a low percentage (71, 145). Although the rate transmission of BPMV by seed and hibernating beetles is low, it may be sufficient for the

virus to be established in the field representing an important primary inoculum source when vector populations are high (71, 145). BPMV is mainly transmitted by bean leaf beetle, although other insects can also act as vectors (35, 86).

Bean leaf beetle (*Cerotoma trifurcata* Förster)

The bean leaf beetle, *Cerotoma trifurcata (*Förster) (Coleoptera, Chysomelidae) is one of the primary insect pests that can reduce soybean yield (42, 70, 133). Throughout most of the Midwest, the bean leaf beetle develops two generations per year (43, 66, 133, 154). Although the larval stage feeds on root nodules, this damage is not as significant as the injury on the aboveground parts, such as leaves, stems and pods (43, 133). The first generation feeds on soybean during vegetative and early reproductive growth stages in early summer; the next generation emerges in late summer and feeds mostly on stems and pods (43, 62, 114). It is typically the second generation that causes economic damage affecting yield-bearing tissue (70). Pod injury also causes seed quality losses from pod desiccation and wounds that allow secondary infection by fungal pathogens such as *Alternaria tenuissima* and *Phomopsis* spp. (104, 129, 133).

The second-generation beetles overwinter as adults at the end of the field season. In Iowa, it has been observed that beetles overwinter in leaf litter of field crops or woodlands (76, 78). After overwintering, bean leaf beetles emerge in early spring and feed on native perennial legumes (133), until soybeans emerge and they move into these fields (15, 70).

The bean leaf beetle is considered the most important vector of BPMV (35, 86), because of its abundance and vectoring efficiency (19, 36), along with a longer retention time of virus in the beetle compared with other insects (70). Previous studies have confirmed that BPMV incidence is positively correlated with bean leaf beetle populations (100).

It has been reported that beetles can acquired a virus from an infected plant and transmit it on a susceptible plant after a single bite, but increased feeding improves the efficiency of acquisition or transmission (33). It has also been reported that BPMV is retained in the digestive tract and transmitted in regurgitant, however, it is not clear whether the virus is found in the beetle hemolymph. Fulton and Scott (1974) cited by Krell (70), reported that BPMV was identified from bean leaf beetle hemolymph. However, these results are not supported by Wang et al. (147), who found plant viruses such as tobacco ringspot virus and southern bean mosaic virus in bean leaf beetle hemolymph, but not BPMV.

Spring and early summer BPMV infection have a strong impact later in the season, causing stronger symptoms and greater yield reduction (21, 60, 115). However, it has been reported that most BPMV infection occurs after the first-generation beetle density peaks in mid to late July (13, 21, 60, 70).

Even though bean leaf beetle is native to North America, in Iowa its populations remained very low during the 90's (117). However, *C. trifurcata* have experienced large population fluctuations during the past decade. These changes have been associated with abiotic factors that might impact survival, such as the occurrence of mild winters (13, 19, 36, 71). In order to integrate this information about environmental conditions and bean leaf beetle population survival into the designing of management techniques, prediction models have been developed mainly based on subfreezing air and soil surface temperatures during the winter seasons (72, 77). In Iowa, population outbreaks of *C. trifurcata* have been reported in previous years *(*14, 53), and this population increase has also coincided with an increase of BPMV incidence in the field (53, 160).

Lately, Iowa has experienced extremely severe winters and predictive models have suggested the occurrence of massive bean leaf beetle winter mortality (57, 58). Byamukama et al. (20) designed a model to predict the risk of BPMV based on winter temperature and insect vector mortality. Thus, the fewer days with temperatures below freezing during the winter, the higher the survival rate for bean leaf beetles and the greater the risk of BPMV. Therefore, low risk for BPMV incidence has been predicted in 2009 and 2010 (20).

Management strategies to control bean leaf beetles traditionally have been focused on late season control for suppressing beetle populations at a time when pods are susceptible to feeding (70, 114). However, because early BPMV infection causes more damage on soybeans, the management of the bean leaf beetle as the main vector of BPMV becomes more important earlier in the season (118). In previous studies, it has been reported that seed or early foliar-applied insecticides aimed to control overwintering and first generation of bean leaf beetles, reduce both *C. trifurcata* populations and incidence of BPMV, and improve yield and protect seed quality (16, 29, 72). However, the outcome of insecticide applications is not always the reduction of BPMV incidence. In fact, Pedersen et al. (108) reported that foliar insecticide applications timed to suppress soybean aphid populations increased incidence of BPMV. In this case, it was suggested that insecticide applications may encouraged the movement of bean leaf beetle, resulting in enhancement of disease caused by BPMV.

Delayed soybean planting is known to reduce bean leaf beetle feeding injury in soybean (109, 154), mainly because soybeans are preferred host plants and they will not be available by the time that overwintered beetles are moving into soybeans to feed and lay eggs after they emerge from overwintering habitats beetles habitats (109).

It has been reported there is a positive relationship between plant age and susceptibility to BPMV (36), and BPMV outbreaks have been associated with early planted soybean (161). In Iowa, however, delayed soybean planting inconsistently reduced the incidence of BPMV (73). Even though there is a potential for using planting date to reduce bean leaf beetle and BPMV, later planted fields could produce lower yields, and may be more vulnerable to other pest such as aphids or late season beetles which move to younger plants later in the season to feed (73).

Other cultural practices for management of bean leaf beetles that have been suggested are the use of trap crops to attract beetles early in the season, or barrier crops and polycultures to keep the beetles away from the main crop (13, 70). However the use of these techniques might be impractical in intensive agricultural production.

Insect injury on soybean can also be reduced by host plant resistance; however, the effectiveness of plant resistance to insect attack may be different depending on insect feeding behaviors. Lam and Pedigo (78) demonstrated that densely pubescent soybean cultivars have the potential to resist bean leaf beetle feeding on pods. Moreover, Hammond et al. (43) demonstrated that the insect-resistant lines HC95-24MB and HC95-15MB presented lower levels of defoliation by bean leaf beetles; however, population densities and pod feeding were not significantly reduced. In a recent study, Redinbaugh et al (114) found that the insect feeding resistance was insufficient to reduce the incidence and spread of BPMV in Ohio, suggesting that resistance mechanisms should be combined with other strategies such as chemical treatments in vector-virus management programs (114).

Even though all these management strategies aim to lower bean leaf beetle populations and reduce their feeding in turn to reduce BPMV incidence, host resistance is thought to be the most effective long term approach to control viral diseases (36, 51). Although, resistance to

BPMV has not yet been incorporated into commercial soybean cultivars (72, 169), several studies have been conducted to evaluate BPMV resistance in soybean and identified plant material to use in breeding programs. Wang et al. (151) tested 52 soybean accessions that represent the major ancestral lines of North America cultivars and found that all were susceptible to BPMV. Zheng et al. (169) screened 115 *Glycine max* accessions for resistance to BPMV but all became systemically infected after inoculations. However, in the same study, some accessions of the wild *Glycine* species, such as *G. tomentella* and *G. soja* were respectively resistant and tolerant to BPMV, suggesting that they may be useful for development of resistant commercial soybean cultivars. However, the feasibility of this technique has been questioned because of the difficulty of making interspecific crosses (51). Recently, Hill et al. (51) evaluated 33 soybean accessions for field response to virus infection, based on measurement of relative level of virus antigen in seed and mottling of soybean seed coats. It was found that four accessions were tolerant to BPMV, while eight accessions were tolerant to SMV and, three were tolerant to both SMV and BPMV.

Soybean mosaic virus

Soybean mosaic virus (SMV) is one of the most common viral diseases of soybean, and Worldwide has been reported to occur in Korea, China, Canada, Georgia and Brazil (47, 82, 92, 135). The virus was likely introduced into the United States in shipments of soybean seeds from Asia in 1915 (135), but it was not described until 1921 when it was named as soybean mosaic virus by Gardner and Kendrick (146). SMV belongs in the genus *Potyvirus* in the family *Potyviridae*, and has a single strand, positive sense RNA which is enclosed by repeating coat protein monomeric subunits (47). In the USA nine strains of the virus have been identified based on their symptom expression on a set of eight different soybean cultivars (25, 47). The nine

strains currently known are G1-G7, G7a and C14; however, additional strains have been reported in other countries such as Brazil, Japan and China (25, 47, 83, 92). Recently, four new SMV isolates were found in Southern China, which differ from other isolates previously reported in Northeast China (82).

SMV infection causes significant reductions in yield and quality (47, 103 122); however symptoms vary with soybean genotype, virus strain, plant age at infection and environment (47). Early-infected plants tend to be stunted, with chlorotic curled leaves and fewer pods and seeds (47). Seedlings derived from infected seeds are in general severely stunted with rugose and mottled unifoliate leaves (92).

Seed quality deterioration and yield loss of about 35 to 50% have been observed under natural field conditions (122). In field inoculation experiments yield losses could range from 8 to 35%, but can be as high as 86 to 94% (38, 47, 92). In a study using different transformed lines, Steinlage et al. (136) found that soybean lines with the lowest infection rates presented higher yields.

Early plant infection by SMV reduces pod set, increases seed coat mottling, reduces seed size, oil content and nodulation (47), and also reduces seed germination and vigor (47, 69).

The effect of SMV inducing seed coat mottling has been well documented (68, 69 152), and the occurrence of a positive linear relationship between infection rates and seed coat mottling has been reported (50, 68, 136). This discoloration has been described as the accumulation of anthocyanins or leucoanthocyanins in irregular bands of black and brown pigments in the seed coat (67, 140).

Koning et al. (69) reported that SMV antigen can be detected in almost all (>99%) the seeds from infected plants. Therefore seed coat infection is a good indicator of SMV infection

and could be useful to estimate SMV incidence in the field. In contrast, seed mottling does not always indicate SMV infection, since not all seeds from infected plants are infected and uninfected plants may also produce some mottled seeds (47, 105). The intensity of seed coat mottling is strongly influenced by virus strain, host genotype, time of inoculation and environmental conditions and mixed infection with other viruses (12, 67, 69).

It has been reported that resistance to seed coat mottling is conferred by a single dominant gene (*Im*) while color and distribution of the pigment in the seed is controlled by five independent loci (*I, R W, O, T*) (28, 67). The lack of the resistance gene in susceptible cultivars has been associated with the occurrence of seed coat mottling, while cultivars containing this gene have been shown to be immune to SMV-induced seed coat mottling, independently of the environment (67).

Early plant infection by SMV negatively impacts seed quality, increasing seed coat mottling (47). Konning et al. (69) found that seed coat mottling was observed in up to 91% of the seeds from plants infected with SMV before floral development. However, in field inoculation studies the greater incidence of seed coat mottling has been noticed when plants are infected at reproductive growth stages. For instance, Bryant et al. (18) observed that more seed coat mottling in plants inoculated at growth stage R2 than at V1, although no differences in levels of SMV antigen were found.

The expression of seed coat mottling in seeds from SMV infected plants is also influenced by temperature. Ross (121) demonstrated that seed coat mottling occurred in inoculated plants exposed at 20 C degree during flowering, while it was not observed in seeds from plants exposed to higher temperatures.

Negative impacts of SMV reducing yield and seed quality on soybeans can be intensified by co-infection with other viruses. It has been reported that combined infection of potyviruses, such as SMV with other plant viruses causes disease synergism and elevation in the level of the nonpotyviruses involved (54, 89). This increase has been attributed to suppression of gene silencing activity induced by helpercomponent proteinase (HC-Pro) of potyviruses (36, 89).

Ross (120) showed that mixed infection with BPMV and SMV reduced yield up to 85%. Others have also reported that co-infection with this two viruses resulted in more severe symptoms and greater yield losses than infection with either virus alone. (3, 21). Quiniones et al. (112) reported higher percentages of seed coat mottling by mixed infection (96%) than that caused by SMV alone (92%). Furthermore, it has been reported that these two viruses can increase susceptibility to *Phomopsis* spp. seed infection (1, 68, 69, 74).

In addition to BPMV, SMV can establish synergistic interaction with other viruses, such as *Cowpea mosaic virus* (CPMV) and *Alfalfa mosaic virus* (AMV) (3, 89). Malapi-Nelson et al. (89) reported that co-infection of soybean with AMV and SMV resulted in severe symptoms in doubly infected plants, suggesting the occurrence of synergistic interaction of AMV with SMV. This interaction has become important due to the introduction in the US of the soybean colonizing aphid (*Aphis glycines*) which is capable of transmitting both viruses (26). It has been suggested that AMV has the potential to become a serious viral disease of soybean in the future, due to the combined effect of the disease synergism and the establishment of the major vector (89).

Plants infected with SMV may lack noticeable symptoms in some cultivars while symptoms are quite severe in other cultivars (24), which has been associated with genetic variability for resistance to SMV. Three resistance genes *Rsv1*, *Rsv3* and *Rsv4* have been

reported to confer resistance to SMV (47). Even though the sources of resistance have been identified from different soybean lines and cultivars (24, 25, 47, 83), not all are effective against all SMV stains. Wang et al. (150) evaluated resistance to SMV strains G1 and G5 of 850 commercial and precommercial soybean cultivars, and found that just 1.5% and 6.7% were resistant to SMV-G1 and SMV-G5, respectively, while no cultivars were resistant to both strains. These findings are consistent with one study reporting that resistance to SMV-G5 was more common than resistance to G1 in soybean ancestral lines (151), although G5 has been also described as a highly virulent SMV strain (25).

Although some SMV resistant soybean cultivars are available, the use and effectiveness of this approach is restricted by the lack of a greater number of resistance genes. One disadvantage of using single resistance genes is that resistance-breaking SMV strains may emerge (136). For instance, some SMV strains overcome resistance induced by the *Rsv1* gene and can induce severe symptoms often leading to the death of the plant (47). It has been shown that soybean lines containing the *Rsv1* gene could be infected by strains to which they are normally resistant, after inoculations with some pairs of virus strains that might present a complementation effect that allows infection to occur (47).

Furthermore, Gu et al. (39) reported that *Rsv*1 and *Rsv*4 do not provide any protection against BPMV; thus, this strategy might not be sufficient to reduce the negative impact caused by mixed infections. Recently, Hill et al. (51) reported field tolerance to either BPMV or SMV in soybean accessions, suggesting that subsequent incorporation of SMV resistance genes into BPMV field tolerant cultivars could reduce the synergism between the two viruses. In the same study, three accessions were identified as tolerant to both SMV and BPMV, which could be used in breeding programs to develop resistant cultivars.

SMV is effectively transmitted through infected seeds and insect vectors, and can also be transmitted by mechanical means and grafting *(47).* In the Midwest, SMV infected seeds are the main means of long distance dissemination and the major portal of entry of the pathogen into noninfested areas (50, 52). Secondary spread within and among soybean fields is carried out by different species of aphids (47, 136).

The primary inoculum source of SMV is thought to originate from SMV-infected seed, since overwintering hosts are not found in northern United States (30, 52). In a recent study conducted in Nebraska, SMV was not detected in any field in the first year of the experiment, but it was detected in 31% of fields in the second year, suggesting that the virus was introduced and spread thought seed (37).

Under greenhouse conditions in the absence of other inoculum sources, the rate of seed transmission can be as high as 76% (146). In a field environment, the rate of SMV seed transmission varies from 0 to 68% but is most often close to 10%, depending of genotype, virus strain and time of infection (69). Bowers and Goodman (11) reported a reduction in virus transmission through seed from 16 to 3% if plants were inoculated with SMV before or after the onset of flowering, respectively. Transmission of SMV can occur from both mottled and nonmottled seeds, although the rate tends to be higher for mottled seeds (92).

It is generally thought that virus in seeds remains viable for a long period of time, although, a slightly reduction in seed transmission has been observed after storage for 6 months at 14° C (11). In addition, Domier et al. (30) found that SMV isolates that are transmitted poorly through seed also were transmitted poorly by the Asian soybean aphid.

SMV is transmitted in a non persistent manner by more than 30 different species of noncolonizing aphids (47, 136), along with the colonizing soybean aphid *Aphis glycines* Matsumara (26, 49).

The virus is initially acquired by aphids after probing an infected plant and the virus can be transmitted to other host plants after a brief probe. Wang and Ghabrial (148) demonstrated that *A. glycines* could efficiently transmit SMV after 1-min acquisition probe. However, the density of trichomes in the leaves could interfere with probing and affect the transmission efficiency (92). Additionally, insecticide sprays have been shown to alter aphid behavior by agitating aphids and inducing them to take flight prematurely after just few initial probes, which sometimes are not enough to acquire the virus. Nevertheless, if virus was successfully acquired, the increase in movement could result in a faster spread within and among field (135).

It has been shown that transmissibility of isolates of the pathogen differs among vector species (92). Wang et al. (149) reported that *A. glycines* transmitted SMV from soybean to soybean more efficiently than *Myzus persicae*. In addition, Domier et al. (30) reported high transmission efficiency of several North American and Asian SMV isolates by *A. glycines*.

Soybean aphid (*Aphys glycines*)

Soybean aphid has rapidly spread throughout the principal soybean growing areas in the North Central states since it was found in Wisconsin in 2000 (143, 148).

This is the only aphid that colonizes soybeans (49, 81 108) and it can be controlled through insecticide applications (108, 113). However, *A. glycines* has the ability to rapidly increase population densities and recover from early insecticide applications (101); therefore, applications should be based on insect population thresholds (108, 113). Unnecessary

applications can result in over-application of insecticides, or ineffective control of soybean aphid (62, 119).

Even in absence of the virus, aphid feeding injury can induce plant physiological stresses (22, 87), reduce crop biomass, soybean yield, and seed quality (10, 22). *A. glycines* can cause yield losses up 50%, by directly inducing plant stresses, or indirectly reducing seed quality and transmitting soybean viruses, such as *Soybean mosaic virus* (SMV) (22, 26, 81).

Infection of soybean plants by *Phomopsis* spp. is also enhanced by plant stresses, including insect injury (74). Therefore it is feasible that soybean aphid control may have an impact on *Phomopsis* infection, independent of SMV/*Phomopsis* interactions.

Justification

Trends toward special-use soybeans will require high-quality beans with specific compositional characteristics, and these diseases are a threat to the economic benefits of price premiums for these quality traits. The results of this project should be useful for integrating the management of these interacting pests and diseases. This information will help guide decisions about the appropriate level of management input for controlling *Phomopsis* diseases. The objectives of this research project were to:

1. Assess the effects of *Bean pod mottle virus* and *Soybean mosaic virus* infection on susceptibility of soybean plants to infection by *Phomopsis* spp.
2. Determine the impact of bean leaf beetle and soybean aphid management on infection of soybeans by *Phomopsis* spp.
3. Evaluate impact of foliar fungicides for reduction of *Phomopsis* spp. infection of stems and seeds.

4. Assess the integration of insecticide and fungicide application programs on infection by *Phomopsis* spp., BPMV and SMV.

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CHAPTER 2

EFFECTS OF *BEAN POD MOTTLE VIRUS* AND *SOYBEAN MOSAIC VIRUS* INFECTION ON SUSCEPTIBILITY OF SOYBEAN PLANTS TO INFECTION BY *PHOMOPSIS LONGICOLLA*

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ABSTRACT

Infection of soybean plants by *Bean pod mottle virus* (BPMV) and/or *Soybean mosaic virus* (SMV) has been reported to increase their susceptibility to seed infection by *Phomopsis* spp., but the mechanism of predisposition is unclear. These effects of SMV and BPMV were studied in separate greenhouse experiments. Two soybean cultivars, Colfax (tolerant to SMV but not to BPMV) and Spansoy 201 (tolerant to BPMV but not to SMV) were mechanically inoculated with either SMV (G2 strain) or BPMV (subgroup II) at growth stage V2-V3. Cultivar 92M02 was inoculated with BPMV at the same growth stage. Virus inoculations were followed by inoculation with *P. longicolla* at growth stages R3 or R5. Virus infection was confirmed by ELISA, while stem and seed infection by *P. longicolla* were evaluated by culturing stem sections and seeds. Neither virus increased susceptibility to stem infection by *P. longicolla*. Inoculation with BPMV significantly increased susceptibility of Spansoy 201 to seed infection by *P. longicolla* at growth stage R5, without affecting plant maturity. Susceptibility of 92M02 to *P.*

longicolla at growth stages R3 and R5 was increased by BPMV. Plants of 92M02 displayed typical BPMV foliar symptoms, seed coat mottling and a delay in maturity. In the *SMV-Phomopsis* experiments, inoculation with the SMV-G2 strain did not increase the incidence of *P. longicolla* seed infection in either of the soybean cultivars (Colfax and Spansoy 201). These results confirm BPMV-induced predisposition to seed infection by *P. longicolla* and indicate that the mechanism of predisposition is not due solely to prolonging seed maturation. The previously reported SMV- *Phomopsis* spp. relationship was not confirmed, but this effect may be cultivar and strain dependent.

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is affected by several seed-borne pathogens that reduce seed quality (22, 29), affecting both marketability (13, 24) and germination potential (28, 29). One of the most common seedborne diseases is Phomopsis seed decay, primarily caused by *Phomopsis longicolla* T. W. Hobbs (14, 17), a member of the *Diaporthe-Phomopsis* complex (30). Members of this complex are widespread throughout most of the soybean producing areas around the world (21, 22) and together cause more losses in soybean than any other single fungal disease (30). Seed infection by members of this complex reduces quality of seeds used for planting by causing physical damage (9, 30) and reducing germination (8, 20). Moreover, severe incidence of seed decay affects the quality of soybean used for processing, by altering protein content (22) and reducing oil quality (36).

Phomopsis longicolla moves from infected pods into seeds, and although pods can be infected at any time after they are formed, major seed infection will not occur before physiological maturity (17, 19). Higher incidence of seed decay occurs when there is warm weather during pod development and maturation and harvest is delayed (27, 29). Seeds tend to

be more susceptible to infection by *P. longicolla* within deteriorated pods, or when plants are deficient in potassium, heavily attacked by insects, and infected with one or more viruses (17, 29).

Bean pod mottle virus (BPMV) and *Soybean mosaic virus* (SMV) are the most common viral diseases of soybean (7, 10); both reduce yield and impact seed quality (7, 10). Both viruses can cause seedcoat mottling (7, 10, 13); this discoloration has a negative impact on the marketability of seeds and food-grade soybean (13). Moreover, SMV infection also reduces seed germination and vigor (10, 16), and under certain conditions, BPMV and SMV can also affect oil and protein content, nodulation and nitrogen fixation (10, 39).

In Iowa, *Phomopsis* spp., BPMV and SMV were all prevalent in some areas in 1998 (37), and recently the frequent detection of *P. longicolla* in stems collected during the Iowa soybean disease survey conducted from 2005-2007 (18) coincided with a resurgence in bean leaf beetle (*Cerotoma trifurcata* Förster) populations (3) and BPMV symptoms (12).

Several reports suggest that seed from plants infected by either SMV or BPMV are more susceptible to infection by *Phomopsis* spp. (1, 8, 16, 32). The mechanism for this predisposition is unclear. One proposed mechanism for the increased susceptibility in virus-infected plants is that these two viruses extend the length of the late-season growth stage intervals, and therefore, prolong the exposure of pods and seeds to infection by *Phomopsis* spp. (1, 16). Although this mechanism has been proposed, none of the published studies has directly tested this hypothesis.

Stuckey et al. (32) found a consistent significant increase in seed infection by *Phomopsis* spp. in plants that were infected with BPMV or doubly infected with SMV and BPMV, but not in plants inoculated with SMV only. Other workers have observed significant differences in seed

infection by *Phomopsis* spp. when plants have been inoculated with SMV and *Phomopsis* spp. (8, 15, 16).

In field studies, Abney and Ploper (1) demonstrated that infection of pods by *Phomopsis* spp. was increased by BPMV inoculations and seed infection was increased only if the virus infection delayed the rate of seed maturation. However, it has also been reported that pod injury caused by *C. trifurcata*, allow secondary infection by fungal pathogens such as *Alternaria tenuissima* and *Phomopsis* spp. (23, 28, 31). Therefore, it is not possible to separate confounding effects of beetle activity from a direct BPMV/*Phomopsis* interaction from experiments conducted under natural field conditions.

Host resistance has been reported as the most effective approach to control viral diseases (11, 24). However, the effect of virus resistance on infection by *Phomopsis* spp. is not completely understood. Konning et al. (15) observed lower incidence of infection of seeds by *Phomopsis* spp. in SMV resistant plants, but this effect was more associated with the lack of SMV infection rather than a direct effect of the SMV resistance alleles. This relationship has not been investigated for the recently identified varieties tolerant to BPMV infection and symptoms (11).

The effects of changes in duration of late-season growth stage intervals on *P. longicolla* infection established before pod and seed maturation has not been studied yet. Also previous studies have not considered the effects of either BPMV or SMV infection on stem infection by *P. longicolla*. A better understanding of the relationship between virus infection and susceptibility to Phomopsis seed decay will facilitate the development of integrated management practices that impact all three diseases. The objective of this study was to assess the effects of *Bean pod mottle*

virus and *Soybean mosaic virus* infection on susceptibility of soybean plants to infection by *P. longicolla* at different growth stages.

MATERIALS AND METHODS

Inoculation procedures. A preliminary experiment was performed in order to identify an aggressive *Phomopsis* isolate to be used in further experiments. Eleven isolates were obtained from soybean seeds harvested from field trials conducted in 2006 and 2007. These isolates were transferred to potato dextrose broth (PDB) in 1.5-ml tubes and kept for 3 day on a shaker at room temperature to promote colony growth; and then, tubes were then stored at 4°C until needed. All isolates were identified as *P. longicolla* by conventional PCR as described by Zhang et al. (38). Based on the results obtained from this preliminary experiment (data not shown), isolate Ph#3 from Mahaska County, IA, was selected to use in subsequent experiments.

In order to perform *P. longicolla* inoculations, the chosen isolate was transferred from PDB tubes to antibiotic-amended potato dextrose agar (PDA) (200 mg streptomycin sulfate, 50 mg chlortetracycline hydrochloride, 120 mg neomycin sulfate, 39 g Difco PDA per liter) and allowed to grow for 16-36 days in 9 cm diameter Petri dishes. *Phomopsis longicolla* conidial suspension was prepared as described by Rupe and Ferris (27) with modifications. Each *P. longicolla* culture was flooded with 5 ml of sterile deionized water and the culture surface was rubbed with a sterile glass spreader to dislodge conidia. Then, it was filtered through four layers of sterile cheesecloth and placed on a stir plate for 5-10 min to enhance conidial release from pycnidia. Finally the suspension was diluted with sterile deionized water to give a final concentration of 1×10^6 conidia ml^{-1}.

Conidial suspensions were applied onto soybean plants at specific plant growth stages using a hand-held sprayer to apply the suspension to flowers, pods, and stems. Plants in

treatments that did not include *P. longicolla* inoculation were mock-inoculated with sterile deionized water. In order to keep a humid environment to enhance conditions for infection, plants were covered with plastic bags for 48-72 hours following inoculation (to promote pod and stem infection) and again when they reached R7 growth stage (to induce movement of the fungus from pods to seeds). The staging of soybean plants was defined as previously described (5), and plants were visually evaluated and specific vegetative or reproductive stages were determined when 50% or more of the plants were in that stage.

Virus inoculum was maintained by continuous greenhouse transfers using mechanical inoculations and frozen and stored infected leaves. A BPMV subgroup II isolate was obtained from symptomatic plants collected from the field in Iowa. A non-aphid-transmissible isolate of SMV-G2 was kindly provided by Dr. A. Eggenberger, Iowa State University. Inoculum was prepared by grinding infected leaves with sterilized pestles and mortars in chilled Agdia extraction buffer (Agdia, Inc., Elkhart, IN). To inoculate plants, the inoculum was rubbed with the index finger onto carborandum-dusted leaf surface of plants at growth stage V2-V3. Virus symptoms were observed 25-30 days after inoculation. Plants of treatments that did not include virus inoculation were mock-inoculated with Agdia general extraction buffer at the same growth stage.

Greenhouse studies. Experiments were conducted in 2009 and 2010 at the Plant Pathology greenhouse facilities at the main campus of Iowa State University, in Ames, Iowa. Treatments were in a 2 x 2 x 3 factorial, with two soybean cultivars, two virus treatments and three *P. longicolla* treatments. The two soybean cultivars were Colfax (Univ. of Nebraska, Lincoln, NE) and Spansoy 201 (Spangler Seed Tech, Inc., Jefferson, WI). The soybean cultivar Colfax is tolerant to SMV but not to BPMV; the cultivar Spansoy 201 is tolerant to BPMV but

not to SMV (11). The two virus treatments were inoculated and non-inoculated (mock inoculated), and the three *P. longicolla* treatments consisted of inoculation at growth stage R3, at growth stage R5, and non-inoculated (mock inoculated). Plants were mechanically inoculated with either SMV or BPMV in separate experiments, and then inoculated with *P. longicolla* conidial suspensions at growth stages R3 or R5. Each experiment was conducted twice and all were planted in 2009, the BPMV-*Phomopsis* in 11 Mar and 3 Aug, while the SMV-*Phomopsis* in 19 May and 1 Oct. The experimental design was a randomized complete block with five replications of two plants.

When plants reached the reproductive growth stages (R1-R2), they were assayed to confirm the virus infection. The middle leaflet of the topmost fully-expanded leaf from each soybean plant was sampled, placed in a plastic bag and stored in a cooler for transportation. Sap was extracted using a leaf press (Ravenel Crop Specialties, Seneca, SC) as described by Byamukama (4). Leaflets were individually placed between metal rollers and Agdia extraction buffer was added while plant sap was collected into 5-ml portion cups (Instaoffice, Kennesaw, GA). Extracted sap was transferred to 1.5-ml tubes which were then frozen and stored at -20^{o}C for subsequent testing by double antibody sandwich enzyme-linked immunosorbent assay (DAS ELISA), using Agdia antibodies and protocol (Agdia, Inc., Elkhart, IN).

Each sample was tested for the presence of SMV or BPMV, according to the respective experiment. For each sample 100 µl of leaf sap extract was placed in wells on the pre-coated ELISA plate. Two positive and negative controls were prepared and placed in each plate. The plates were evaluated with a PowerWave^{TM} Microplate Spectophotometer (Biotek$^{®}$Instruments, Inc., Winooski, VT) set at 405 nm wavelength. Sample wells were considered positive if absorbance values were higher than twice the values for the negative controls. For each treatment

an extra set of plants were kept under the same conditions in order to assure the availability of infected plants by the time of the fungal inoculations. These plants were also tested for virus infection by ELISA. In case that any virus-inoculated plant tested negative to the respective virus, they were replaced with plants that tested positive.

Plants were inoculated with *P. longicolla* when virus non-inoculated plants reached growth stages R3 and R5. In the BPMV-*Phomopsis* experiment, R3 inoculations were carried out on 21 May and 25 Sep, and R5 inoculations on 12 Jun and 20 Oct, 2009. In the SMV-*Phomopsis* experiment, R3 inoculations were carried out on 31 Jul and 18 Dec, 2009, and R5 inoculations on 25 Aug, 2009, and 26 Jan, 2010.

After inoculations, plants were kept in the greenhouse and were hand harvested when virus non-inoculated plants reached growth stage R8 (95% of the pods and stems were brown mature color and all the leaves had fallen). Stems were aseptically cut at the base of the plant, placed in a paper bag and stored in the laboratory. In these experiments, except for the second replication of the SMV-*Phomopsis* experiment, the two cultivars were harvested at different times because of differing cultivar maturities. Plants from BPMV-*Phomopsis* experiment were harvested in 2009 on the following dates: 22 Jul and 19 Nov (Spansoy 201), and 1 Jul and 3 Dec 2009 (Colfax). Plants from SMV-*Phomopsis* experiment were harvested on 25 Sep, and 26 Feb, 2010 (Spansoy), and 5 Oct 2009, and 26 Feb, 2010 (Colfax). Seeds were separated by hand from plant tissue and visually assessed for Phomopsis seed decay. Seeds from each replicate were placed in paper envelopes and stored in the laboratory.

For each treatment 10 plants (2 plants/replicate) and 100 seeds (20 seeds/replicate) were evaluated for *P. longicolla* infection. Morphological characteristics of the colonies and spores

were used to identify *P. longicolla* colonies growing from stems and seeds as described by Kulik and Sinclair (17) and McGee (20).

In order to evaluate stem infection by *P. longicolla*, a stem plating test was performed following the procedure of Garzonio and McGee (6) with modifications. Stems were cut into sections (approximately 3 cm long), surface sterilized in 1% sodium hypochlorite for 1 min and rinsed in sterile-distilled water for 30 sec. Under a laminar flow hood using aseptic technique, stem sections were then partitioned, and ten pieces (approximately 1 cm each) from each plant were arbitrarily selected and five pieces per plate were placed on antibiotic-amended potato dextrose agar in 9 cm diameter Petri dishes. After 5-7 days plates were inspected for colonies of *P. longicolla*. If *Phomopsis* was observed from any section of a stem, that stem was counted as infected, and the percentage of infected stems per replicate was calculated.

To evaluate seed infection by *P. longicolla*, a seed planting test was performed as described by Walcott (33). Twenty seeds from each replicate were arbitrarily selected, surface sterilized in 1% sodium hypochlorite for 30 sec, and rinsed in sterile distilled water for 30 sec. Under a laminar flow hood and using aseptic technique, five seeds per plate were placed on antibiotic-amended potato dextrose agar in 9 cm diameter Petri dishes. After 5-7 days plates were inspected for infection of seeds by *P. longicolla*.

Further BPMV-*Phomopsis* experiments were conducted under the same conditions, using soybean cultivar 92M02 (Pioneer Hi-Bred Int., Inc., Des Moines, IA), and following the same procedures as described above. The cultivar 92M02 is susceptible to BPMV. These experiments were planted on 1 Oct and 3 Dec, 2009; inoculated with *P. longicolla* at R3 on 15 Nov, 2009 and 22 Feb, 2010, and inoculated at R5 on 4 Dec, 2009, and 8 March, 2010. Plants were harvested on 21 Jan and 07 April, 2010.

Data analysis. Each experiment was conducted twice and treatment-by-repetition interactions were not significant except for stem infection in the experiments SMV-*Phomopsis,* and BPMV-*Phomopsis* experiment with cultivar 92M02. Therefore, the data from both repetitions of each experiment were pooled together and treatment effects on percentage of infection of stems and seeds by *P. longicolla* were tested in a combined analysis using PROC GLM of SAS, version 9.2. Statistical effects of treatments were estimated based on analysis of variance (ANOVA); means were separated using Tukey's test and considered significantly different if $P \leq 0.05$. The normal and homogeneous distribution of residuals was examined using SAS PROC PLOT.

RESULTS

In experiments BPMV-*Phomopsis* with cultivars Spansoy and Colfax, treatments that included *Phomopsis longicolla* inoculations showed higher infection of stems by *P. longicolla* no matter the cultivar or virus inoculation (Table 1 and Figure 1). Regardless of the growth stage in which inoculations were carried out, both *P. longicolla* inoculations resulted in similar incidence in stems, and were significantly different from the *P. longicolla* non-inoculated plants. For seed infection, there were significant differences between cultivars, and significant main effects of BPMV inoculation and plant growth stage at inoculation with *P. longicolla*. Moreover, there were significant interactions among the effects of cultivar, BPMV inoculation, and *P. longicolla* inoculation (Tables 1 and Figure 1).

In these experiments foliar symptoms were exhibited in BPMV inoculated plants of both cultivars; but seed coat mottling of seeds was not observed in either cultivar. Although differences in plant maturity were observed between Spansoy 201 and Colfax, BPMV-inoculated

and BPMV non-inoculated plants from each cultivar tended to senesce at the same time and therefore this effect cannot be attributed to infection by BPMV.

Incidence of infected seeds by *P. longicolla* was higher in Colfax than Spansoy 201 (Figure 1). Plants of both cultivars inoculated with BPMV and *P. longicolla* at any of the two growth stages presented higher stem and seed infection by *P. longicolla* compared with the BPMV non-inoculated plants (Figure 1), however this effect was not always statistically significant. In addition, when *P. longicolla* was inoculated at growth stage R5, more seed infection by *P. longicolla* occurred compared with the seed harvested from plants inoculated at R3 and the *P. longicolla* non-inoculated plants (Figure 1). However, the virus effect was significant only in the cultivar Spansoy 201, and the BPMV inoculated plants presented higher seed infection by *P. longicolla*, compared with the BPMV non-inoculated plants, when both were inoculated with the fungus at R5 (Figure 1).

In SMV-*Phomopsis* experiments with cultivars Spansoy 201 and Colfax, there were significant differences in stem infection by *P. longicolla* between cultivars and the plant growth stage when *P. longicolla* inoculations were performed (Table 2). In both cultivars, infection of stems and seeds by *P. longicolla* tended to be higher when plants were inoculated at growth stage R5, compared with inoculations at R3 and the *P. longicolla* non-inoculated plants (Figure 2). However, SMV inoculations did not increase stem or seed infection by *P. longicolla* compared with the SMV non-inoculated plants (Figure 2). Even though SMV inoculated plants tested positive for virus infection, foliar symptoms were inconsistently observed, and seed coat mottling of seeds was not observed. As in experiments BPMV-*Phomopsis* with cultivars Spansoy 201 and Colfax, there were differences in cultivar maturities, but these differences were independent of the virus inoculation treatment.

In experiments BPMV-*Phomopsis* with cultivar 92M02, seed infection by *P. longicolla* was significantly higher in treatments that included BPMV inoculation or *P. longicolla* inoculation. Neither stem nor seed infection were affected by the growth stage in which *P. longicolla* inoculations were carried out. However, there was a significant interaction between the effects of BPMV inoculation and *P. longicolla* inoculation on seed infection (Table 3). Compared with the BPMV non-inoculated plants, BPMV infected plants were more susceptible to seed infection by *P. longicolla*, when plants were inoculated with the fungus at growth stages R3 and R5 (Figure 3).

Moreover, in this experiment all the BPMV inoculated plants displayed typical BPMV foliar symptoms; seed coat mottling of seeds and a delay in maturity (Figure 4). In addition, the number of seeds was slightly lower in these plants compared with BPMV non-inoculated plants; however, these differences were not significant (data not shown).

DISCUSSION

To our knowledge, this study is the first to evaluate the effect of SMV and BPMV on susceptibility to *P. longicolla* infection on soybean plants under controlled conditions.

Soybean stems were susceptible to infection by *P. longicolla* when plants were inoculated at either growth stage R3 or R5, no matter the soybean cultivar or the virus inoculation treatment. These results suggest that under the conditions of this study, neither SMV nor BPMV significantly increased susceptibility to stem infection by *P. longicolla*.

The effect of BPMV infection on susceptibility to *P. longicolla* differed among cultivars. In cultivar Spansoy 201 inoculation with BPMV significantly increased susceptibility to seed infection by *P. longicolla* only in plants inoculated with *P. longicolla* at growth stage R5.

However, there was not an effect on the Colfax cultivar. In cultivar 92M02, BPMV-inoculated plants were more susceptible to seed infection by *P. longicolla* at both growth stages R3 and R5.

The results with Spansoy 201 and 92M02 are consistent with previous studies reporting the increased incidence of *Phomopsis* spp. in seeds from BPMV infected plants (1). Unlike the previous studies, the effect of BPMV on seed infection by *P. longicolla* observed in this study was independent from the effects that beetle vectors of BPMV can have in pod and seed infection by *Phomopsis* spp. (23, 28, 31).

Our data suggest that BPMV-induced predisposition to *P. longicolla* and it is not due solely to prolonging seed maturation. In this study differences in maturity between virus treatments within cultivars was observed only in cultivar 92M02, in which BPMV-inoculated plants took longer to senesce (Figure 4). Consistently, Abney and Ploper (1) observed a significant effect of BPMV increasing seed infection by *Phomopsis* spp. only if the virus infection delayed the rate of seed maturation. However, in our study, seed infection by *P. longicolla* of Spansoy 201 was increased even in the absence of any effect on plant maturity. In 92M02, BPMV infection enhanced seed infection by *P. longicolla*, even when the fungus was inoculated as early as R3. This suggests that BPMV increases pod susceptibility, as reported by Abney and Ploper (1), and higher seed infection might simply be due to a higher proportion of pods being infected. Koning et al. (16) speculated on the mechanism by which prolonged maturity predisposed virus-infected to *Phomopsis* spp. They argued that the effect might be due to longer exposure of the pods and seeds to weather conditions favorable for seed infection. Abney and Ploper (1) speculated that the mechanism was longer moisture retention in seeds of BPMV infected plants. In our study, neither hypothesis would explain the increased susceptibility because plants were exposed to high humidity (plastic bag covering) for only one

brief period during seed maturation. It is unlikely that pod-to-seed movement of *P. longicolla* occurred after that period, because specific environmental conditions are required for pod-to-seed movement (2, 26). Koning et al. (16) also noted that virus-induced predisposition occurred in some years when there was no effect of virus infection on plant maturity.

It has been suggested that the effect of BPMV on soybean maturity is caused by an inhibition of pod formation and to increase pod abortion that result in plants remaining green after uninfected plants have matured (1, 35). In BPMV-*Phomopsis* experiments with cultivar 92M02, the number of seeds per plant was reduced in BPMV inoculated plants (mean=61) compared with the BPMV non-inoculated plants (mean=82), however, these differences were not statistically significant.

Inoculation with SMV did not increase the incidence of seed infection by *P. longicolla*. Similar results were reported by Stuckey et al. (32) who reported that SMV had no effect on seed infection by *Phomopsis* spp. and found a significant increase in *Phomopsis* spp. only in plants co-infected with SMV and BPMV. These results are not consistent with those of other investigators (8, 15, 16), who found that SMV infection increases seed infection by *Phomopsis* spp. These disagreements in results might be due to differences in the SMV strain used, or because of the soybean cultivars chosen for the study. In previous studies only highly virulent SMV isolates caused an increase in seed infection by *Phomopsis* spp., and the effect differed among cultivars (8, 15, 16).

Koning et al. (16) observed a significant increase in seed infection by *Phomopsis* spp. due to inoculations with a SMV-G2 strain, which was likely to cause an extension of the seed development interval in SMV susceptible cultivars. In the current study, neither an increased in seed infection nor delayed in senescence were observed in the cultivars Spansoy 201 or Colfax

as a response to infection by the SMV-G2 strain used. Therefore differences in virulence between SMV isolates may have caused the inconsistency in results. Moreover, this study evaluated the effect of SMV on infection by *P. longicolla*, which differs from previous studies that have reported the effect of SMV on infection by either *Phomopsis* spp. (15, 16, 32) or *P. sojae* (8, 25).

Even though it has been reported (11) that soybean cultivars Spansoy 201 and Colfax are tolerant to BPMV and SMV, respectively, in this study both cultivars were infected by both viruses when plants were mechanically inoculated at early growth stages (V1-V2). This is not surprising because the previously reported tolerance was based on parameters that were not considered in this experiment, such as relative level of virus antigen in seed and mottling of soybean seed coats. In addition, Wang et al. (34) reported that Colfax was resistant to SMV-G1 but susceptible to SMV-G5, and it is possible that it is also susceptible to the SMV-G2 strain used in this study.

The incidence of seed infection by *P. longicolla* in cultivars Spansoy 201 and Colfax tended to be higher when plants were inoculated at R5, while seed infection in cultivar 92M02 was not affected by the growth stage at inoculation. In fact in all three cultivars seed infection occurred when plants were inoculated at both growth stages. In this study plants were covered with a plastic bag to keep a humid environment after each inoculation with *P. longicolla* to enhanced pod infection and again at beginning of maturity to enhance conditions for infection of seeds. Previous studies have reported that under field conditions major seed infection will not occur before physiological maturity (17, 19, 26) and only under particular conditions of humidity and temperature (2, 26).

Although the predisposing effect of plants infected with either SMV or BPMV to seed infection by *Phomopsis* spp. has been well documented, the mechanisms underlying these interactions are still not completely understood. However, our data suggest that delayed plant maturity is not the sole mechanism. Future studies should focus on the effects of *P. longicolla* inoculations at later growth stages (after R5) on plants infected with either SMV or BPMV, and co-infected plants.

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Tables

Table 1. Analysis of variance (ANOVA) for effect of soybean cultivar, *Bean pod mottle virus* (BPMV) and *Phomopsis longicolla* inoculation treatment on infection of stems and seeds by *P. longicolla*. Data are from two repetitions of a greenhouse experiment.

Source	DF	*P* value	
		Stems	Seeds
Repetition	1	0.7222	0.3277
Block	4	0.9695	0.0851
Cultivar*	1	0.1569	<.0001
BPMV[+]	1	0.1842	0.0005
P. longicolla [&]	2	<.0001	<.0001
Cultivar × BPMV	1	0.1328	0.5928
BPMV × *P. longicolla*	2	0.4398	0.0061
Cultivar × BPMV× *P. longicolla*	5	0.1553	<.0001

*Cultivar: two cultivars tested; Spansoy 201 and Colfax.

[+]BPMV treatments: inoculated or non-inoculated.

[&]*P. longicolla* treatments: Inoculations at growth stages R3 or R5 and non-inoculated.

Table 2. Analysis of variance (ANOVA) for effect of soybean cultivar, *Soybean mosaic virus* (SMV) and *Phomopsis longicolla* inoculation treatment on infection of stems and seeds by *P. longicolla*. Data are from two repetitions of a greenhouse experiment.

Source	DF	*P* value	
		Stems	Seeds
Repetition	1	0.0003	0.0764
Block	4	0.8309	0.9948
Cultivar*	1	0.0429	0.4867
SMV[+]	1	0.2088	0.5279
P. longicolla[&]	2	<.0001	<.0001
Cultivar × SMV	1	0.4092	0.1537
SMV× *P. longicolla*	2	0.6124	0.3390
Cultivar × SMV× *P. longicolla*	5	0.2628	0.0932

*Cultivar: two cultivars tested; Spansoy 201 and Colfax.

[+]SMV treatments: inoculated or non-inoculated.

[&]*P. longicolla* treatments: Inoculations at growth stages R3 or R5 and non-inoculated.

Table 3. Analysis of variance (ANOVA) for effect of *Bean pod mottle virus* (BPMV) and *Phomopsis longicolla* inoculation treatment on infection of stems and seeds by *P. longicolla* of soybean cultivar 92M02. Data are from two repetitions of a greenhouse experiment.

Source	DF	*P* value	
		Stems	Seeds
Repetition	1	<.0001	0.3251
Block	4	0.9178	0.9667
BPMV[+]	1	0.2137	0.0006
P. longicolla[&]	2	<.0001	<.0001
BPMV× *P. longicolla*	2	0.6825	0.0418

[+]BPMV treatments: inoculated or non-inoculated.

[&]*P. longicolla* treatments: Inoculations at growth stages R3 or R5 and non-inoculated.

Figures

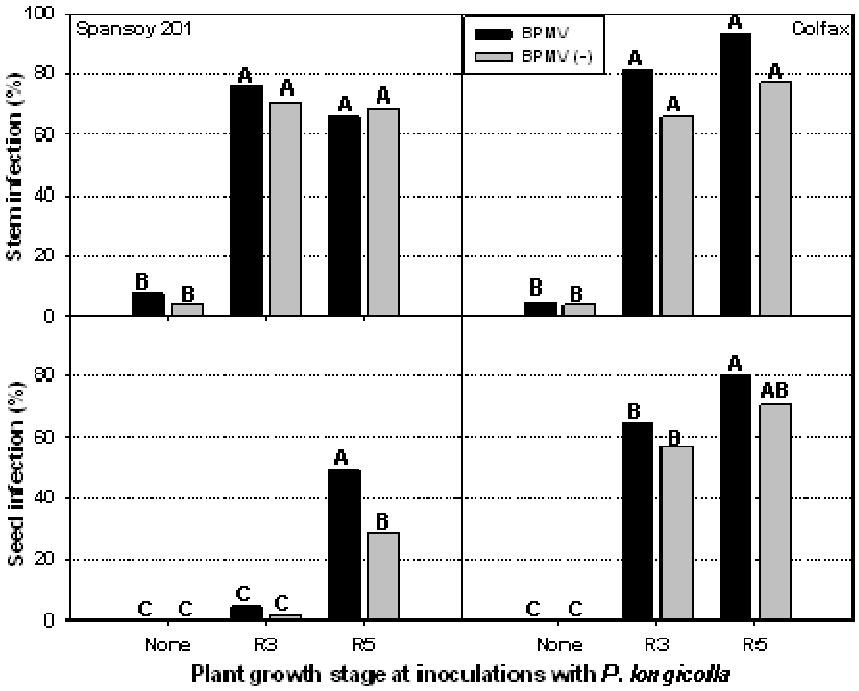

Figure 1. Effects of *Bean pod mottle virus* (BPMV) and *Phomopsis longicolla* inoculation treatment on infection of stems (top) and seeds (bottom) by *P. longicolla* of two soybean cultivars, BPMV tolerant-Spansoy 201 (left) and BPMV susceptible-Colfax (right) at different plant growth stages. Means labeled with the same letter were not significantly different according to Tukey's test considered significantly different at $P \leq 0.05$. Data are from two repetitions of a greenhouse experiment.

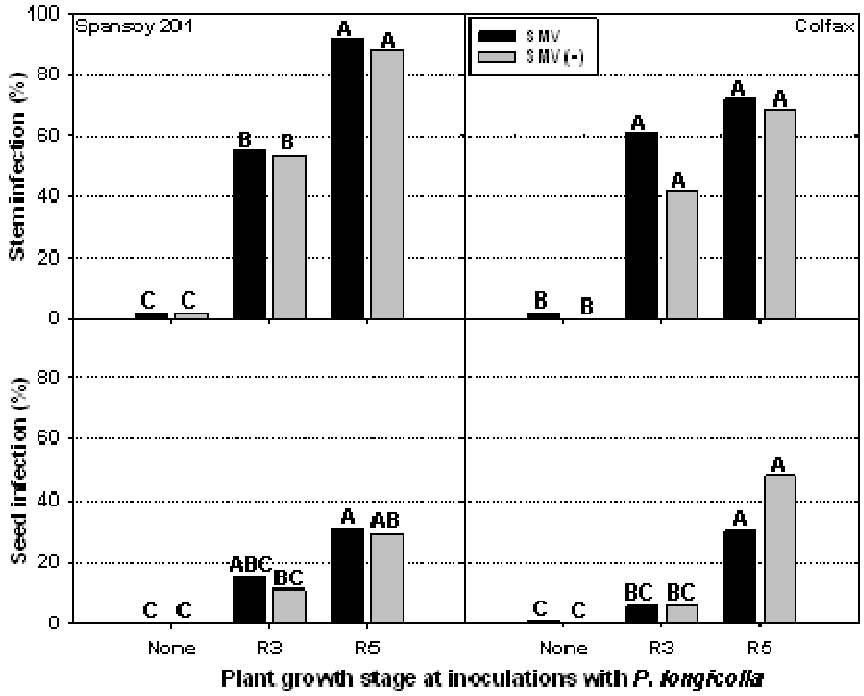

Figure 2. Effects of *Soybean mosaic virus* (SMV) and *Phomopsis longicolla* inoculation treatment on infection of stems (top) and seeds (bottom) by *P. longicolla* of two soybean cultivars, SMV susceptible-Spansoy 201 (left) and SMV tolerant-Colfax (right) at different plant growth stages. Means labeled with the same letter were not significantly different according to Tukey's test considered significantly different at $P \leq 0.05$. Data are from two repetitions of a greenhouse experiment.

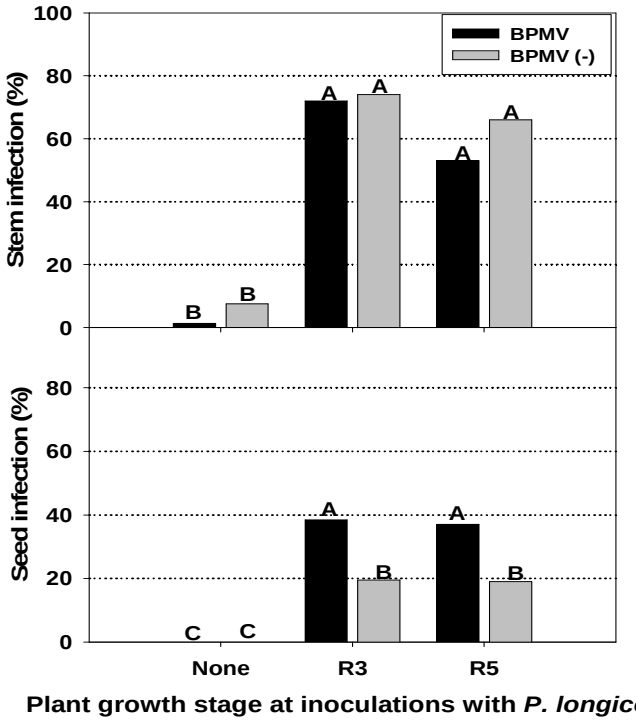

Figure 3. Effects of *Bean pod mottle virus* (BPMV) and *Phomopsis longicolla* inoculation treatment on infection of stems (top) and seeds (bottom) by *P. longicolla* of soybean cultivar 92M02 at different plant growth stages. Means labeled with the same letter were not significantly different according to Tukey's test considered significantly different at $P \leq 0.05$. Data are from two repetitions of a greenhouse experiment.

Figure 4. Effect of *Bean pod mottle virus* (BPMV) treatments on soybean cultivar 92M02 (Pioneer Hi-Bred Int., Inc., Des Moines, IA). *Top:* Delay in senescence caused in BPMV inoculated plants (A) compared with BPMV non- inoculated plants (B). *Bottom:* Seeds from BPMV inoculated plants presenting seed coat mottling (left) compared with seeds from BPMV non-inoculated plants (right) (C). Foliar symptoms observed in BPMV inoculated plants (D).

CHAPTER 3

IMPACTS OF BEAN LEAF BEETLE AND SOYBEAN APHID MANAGEMENT ON INFECTION OF SOYBEAN BY *PHOMOPSIS* SPP. AND SEEDBORNE VIRUSES

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ABSTRACT

Bean pod mottle virus (BPMV), *Soybean mosaic virus* (SMV), bean leaf beetles (*Cerotoma trifurcata*), soybean aphids (*Aphis glycines*) and *Phomopsis* spp. can affect soybean seed quality in addition to causing yield losses. However, the effects of management practices on interactions among these pests and pathogens are not well understood. To evaluate the effect of *C. trifurcata* management strategies on infection of soybean by *Phomopsis* spp., field studies were conducted in Iowa at one location in 2008 and two locations in 2009. Insecticide applications reduced stem infection by *Phomopsis* spp. and *C. trifurcata* feeding injury of leaves in one year, and feeding injury of pods in both years. BPMV incidence was reduced when a virus-tolerant genotype and insecticide applications were combined. In 2009, fungicide application treatments also were included and reduced stem and seed infection by *Phomopsis* spp. in one location. In 2009, soybean yield was impacted individually by either fungicide or insecticide depending on the location. Low *C. trifurcata* populations in both years may have limited the impact of insect management tactics on interactions with *Phomopsis* spp. To assess the impact of *A. glycines*

control on infection by *Phomopsis* spp., soybean stems and seeds were collected from an *A. glycines* management study conducted in 2008 and 2009 in Iowa. Consistently, foliar insecticide applications significantly reduced plant exposure to *A. glycines*, but none of the treatments resulted in a significant reduction of incidence of SMV or *Phomopsis* spp. There was no evidence of a relationship between *A. glycines* and *Phomopsis* infection. Overall, results indicated some benefits of *C. trifurcata* management tactics for reduction of *Phomopsis* infection, but not consistently.

INTRODUCTION

Bean pod mottle virus (BPMV) and *Soybean mosaic virus* (SMV) are the most common viral diseases in the majority of soybean production areas; including the north central US (18). These viruses have different structural and epidemiological characteristics and belong in separate families, BPMV in *Comoviridae* and SMV in *Potyviridae* (13, 16). However, they can induce similar phenotypic symptoms such as chlorotic mottling of leaves, stunting growth and seed coat discoloration (13, 16). Yield losses caused by these viruses could range from 8 to 35% for SMV (16) and 3 to 52% for BPMV (13). The impact of virus infection on yield depends on plant developmental stage at infection (14), strain of the virus, soybean cultivar, and environmental conditions that influence the epidemiology of the disease (44). Moreover, symptom expression and yield loss can be greater in mixed infections of BPMV and SMV due to synergistic interactions (7, 42).

In addition to yield reduction, viral infection may also impact seed quality. Hobbs et al. (19) showed that both viruses can cause seedcoat mottling in inoculated plants. Seed discoloration has a negative impact on the marketability of seeds and food-grade soybean. This market, in particular, has high quality standards that are based on visual appearance and

uniformity of the seeds, therefore seeds with dark mottling, including those with virus symptoms, are often rejected (19, 44).

Moreover, other components of seed quality can be affected by these viruses. Koning et al. (24) found that germination and vigor was lower in seeds harvested from SMV-inoculated plants than in seeds from non-inoculated plants. However, this may have been due to a combined infection of SMV and *Phomopsis* spp. Ziems et al. (57) demonstrated that under certain conditions BPMV infection can also affect oil and protein content. It also has been reported that these two viruses may affect susceptibility to *Phomopsis* spp. seed infection (1, 24).

Both viruses can be transmitted through seed (16, 30), and also can be transmitted by different insect vectors (41). *Bean pod mottle virus* is mainly transmitted by bean leaf beetle (*Cerotoma trifurcata* Förster), although other insects can act as vectors (13, 31). *Soybean mosaic virus* is transmitted by several species of aphids, including the colonizing soybean aphid (*Aphis glycines* Matsumura) and more than 30 different species of non-colonizing aphids (16).

Cerotoma trifurcata can reduce soybean yield regardless of its effect as a virus vector (48). Although the larval stage feeds on root nodules, this damage is not as significant as the injury on the aboveground parts, such as leaves and pods (15, 48). Throughout most of the Midwest, *C. trifurcata* develops two generations per year and overwinters in the adult stage at the end of the field season (15, 23, 48, 53). The first generation (F_1) feeds on soybean during vegetative and mid reproductive growth stages in early summer, while the second generation (F_2) emerges in late summer and feeds mostly on stems and pods (15, 23). Pod feeding injury also allows secondary infection by fungal pathogens such as *Alternaria tenuissima* and *Phomopsis* spp. (39, 48). The overwintered beetles (F_0) are presumed to serve as a reservoir of BPMV to be transmitted after they emerge the next spring and feed on soybean seedlings and other hosts (25).

Spring and early summer infection have a stronger impact on yield than infections occurring later in the season (21). Also, the early infected plants will represent a source of inoculum during the entire field season.

In addition to different species of migratory aphids, the colonizing soybean aphid *A. glycines* can efficiently transmit SMV among soybean plants (9, 17). Even in absence of the virus, aphid feeding injury can induce plant physiological stresses (8, 32), reduce crop biomass, soybean yield, and seed quality (4, 8). Populations of *A. glycines* can rapidly increase in favorable environments (38). It was first reported in the US in 2000 (51), and currently it has spread to most of the soybean growing regions in North America (29, 51).

Population outbreaks of *C. trifurcata* and *A. glycines* have threatened production of soybean in recent years, encouraging growers to apply insecticides to prevent yield losses. It has been reported that well-timed insecticide applications can considerably reduce population densities of the *C. trifurcata* (6, 26) and *A. glycines* (40, 43). However, the effectiveness of these strategies to control virus infection has been inconsistent (6, 26, 29, 41). Pedersen et al. (41) concluded that due to different phenologies of the insect vectors, BPMV and SMV management cannot be integrated through application of insecticide treatments.

Host resistance would be the most effective approach to control viral diseases of soybean (18). Although SMV resistant soybean cultivars are currently available, not all are effective against all SMV strains (16) and resistance to BPMV has not yet been incorporated into commercial soybean cultivars (26). Also, Redinbaugh et al. (44) found that insect feeding resistance was insufficient to reduce the incidence and spread of BPMV in Ohio, suggesting that resistance should be combined with chemical treatments in vector-virus management programs (44).

As mentioned above, SMV and BPMV (1, 24, 28), and *C. trifurcata* (39), all can have an additional impact on soybean quality increasing susceptibility to seed infection by *Phomopsis* spp. Moreover, it is feasible that aphid induced plant stresses and feeding injury have some impact on *Phomopsis* infection as well.

The *Diaporthe-Phomopsis* complex consists of seed decay, pod and stem blight and stem canker, caused mainly by *Phomopsis longicolla* T. W. Hobbs, *Diaporthe phaseolorum* var. *sojae* (Lehman) Wehrmeyer, *D. phaseolorum* (Cook and Ellis) Sacc. var. *caulivora* Athow and Caldwell; and *D. phaseolorum* var. *meridionalis* Morgan-Jones, respectively (47). This complex is widely distributed throughout most of the soybean producing areas in North America, and causes losses in yield and seed quality (35, 36, 55).

It has been reported that the use of resistant cultivars (37) and late season fungicide applications could reduce seed infection by preventing the movement of *P. longicolla* from pods to seeds (3, 54). However, the effect of insect-virus management strategies on infection by *Phomopsis* spp. has not yet been studied. The objectives of this study were to: i) determine the impact of *C. trifurcata* management and fungicides applications at growth stage R5 on infection of soybean by *Phomopsis* spp. and BPMV, and ii) determine the impact of *A. glycines* management on infection of soybean by *Phomopsis* spp. and SMV.

MATERIALS AND METHODS

Two experiments were conducted in 2008 and 2009 in Iowa. The first experiment was designed to evaluate the impact of *C. trifurcata* management practices on the BPMV-*Phomopsis* interaction. The second experiment was designed to assess the impact of *A. glycines* management tactics on the SMV-*Phomopsis* interaction. Subsequently these experiments will be referred as *C. trifurcata* and *A. glycines* management trials, respectively.

Experiment 1: *C. trifurcata* management trials

Experimental design. In 2008 a field study was carried out at Iowa State University (ISU) Johnson Farm near Ames (Story County, IA) to evaluate the effect of insecticide treatments and soybean cultivar on BPMV-*Phomopsis* infection. Two soybean cultivars, BPMV tolerant (Spansoy 201, Spangler Seed Tech, Inc., Jefferson, WI) (18), and BPMV susceptible (92M02, Pioneer Hi-Bred Int., Inc., Des Moines, IA), were planted on 20 Jun 2008. Late planting was due to heavy early season rain.

The experimental design was a randomized complete block with four replications. Individual plots were 6.1 m long and 4.6 m wide with four rows. Treatments were in a 2 x 2 factorial with two soybean cultivars (BPMV tolerant and BPMV susceptible) and two insecticide treatments (treated and untreated). Insecticide applications were timed to prevent foliar and pod feeding injury by the F_0 and F_2 generations of *C. trifurcata,* respectively.

The Spansoy 201 seeds were kindly provided by Dr. Craig Grau (University of Wisconsin-Madison). Although the two cultivars were similar in maturity rating (early MG II), their development and agronomic performance differed widely. Cultivar 92M02 developed normally, but Spansoy 201 did not appear to be well adapted for central Iowa and its growth, development, and yield were poor. It also appeared to be strongly affected by environmental conditions and other diseases such as brown stem rot (*Phialophora gregata*). Therefore, it was not used in further experiments. Because adapted commercial cultivars with BPMV tolerance were not available, we were unable to assess the impact of BPMV tolerance on *Phomopsis* spp. infection of seeds.

In 2009 a field study was conducted at two locations: the ISU Southeast Research and Demonstration Farm near Crawfordsville (Washington County, IA) and ISU Hinds Farm near

Ames (Story County, IA) using soybean cultivar 92M76 (Pioneer Hi-Bred Int., Inc., Des Moines, IA). In both locations, the field was divided into four blocks, and all treatments were tested in each block. Within each block, half of the area was mechanically bulk-planted with insecticide-treated seed, and half was planted without insecticidal seed treatment. All seeds were treated with a fungicide combination. Then, foliar fungicide and insecticide treatments were randomly assigned within each sub-block. Experimental units were plots measuring 12.1 m long and 6.1 m wide, with 8 rows. These were planted on 21 and 31 May, at Crawfordsville and Ames respectively.

In this year (2009) treatments consisted of seed and foliar insecticide applications timed to prevent feeding by different *C. trifurcata* generations combined with fungicide applications at growth stage R5 to control infection by *Phomopsis* spp. Insecticide treatments were: 1) no insecticide, 2) seed and foliar applied insecticide to control F_0 and F_1 generations of *C. trifurcata*, 3) seed and foliar applied insecticide to control F_0, F_1 and F_2 generations of *C. trifurcata* and 4) foliar applied insecticide to control F_1 and F_2 generations of *C. trifurcata*. Fungicide treatments were: 1) no fungicide, 2) pyraclostrobin application at growth stage R5 and 3) tebuconazole application at growth stage R5.

In 2009, to promote pod infection, each plot was inoculated with a spore suspension of *Phomopsis longicolla*. The fungal isolate (Ph#3), obtained from soybean seeds from field trials conducted in 2007 (Mahaska County, IA), was identified as *P. longicolla* by conventional PCR as described by Zhang et al. (56). The isolate was transferred to potato dextrose broth (PDB) in 1.5-ml tubes and kept for 3 day on a shaker at room temperature to promote colony growth. Tubes were then stored at 4°C until needed. Then, isolate was transferred from PDB tubes to antibiotic-amended potato dextrose agar (PDA) (200 mg streptomycin sulfate, 50 mg

chlortetracycline hydrochloride, 120 mg neomycin sulfate, 39 g Difco PDA per liter) and allowed to grow for 16-36 days in 9 cm diameter Petri dishes. *Phomopsis longicolla* conidial suspensions were prepared as described by Rupe and Ferris (46) with modifications. Each *P. longicolla* culture was flooded with 5 ml of sterile deionized water and the culture surface was rubbed with a sterile glass spreader to dislodge picnidia and conidia. The conidial suspension was filtered through four layers of sterile cheesecloth and placed on a stir plate for 5-10 min to enhance conidial release from pycnidia. Finally the suspension was diluted with sterile deionized water to give a final concentration of 1×10^6 conidia ml^{-1}.

Inoculum was applied onto soybean plants at R6 growth stage, on 2 and Sept, 2009 in Crawfordsville and Ames, respectively. Spore suspensions were sprayed from both sides of the middle four rows of each plot to cover as much of the stem and pods, using a backpack sprayer with a single hand-held nozzle (*Solo*®, Newport News, VA), calibrated to deliver 180 l ha^{-1} at a pressure of 0.2 MPa. In the field, inoculations were made in the evening to take advantage of overnight dew.

For each plot, the center two (2008) or four (2009) rows were machine harvested at maturity, recording the weight corrected to a moisture content of 130 g kg^{-1}. Seeds were separated into subsamples and stored at 10^oC until the subsequent tests were performed.

In all the experiments the staging of soybean plants was defined as previously described (11). Plots were visually evaluated and a specific vegetative or reproductive stage was determined when 50% or more of the plants in the field were in that stage.

Pesticide applications. In 2008, insecticide treatment consisted of seed treatment of the neonicotinoid insecticide thiamethoxam (Cruiser 5FS, Syngenta Crop Protection, Greensboro, NC) at a rate of 0.5 g a.i. per kg of seed, and a foliar application of the pyrethroid insecticide

lambda-cyhalothrin (Warrior, Syngenta Crop Protection, Greensboro, NC) at a rate of 0.020 kg a.i. ha^{-1}. In addition, all seeds were treated with a fungicide combination of fludioxonil and mefenoxam (Apron Maxx, Syngenta Crop Protection, Greensboro, NC) at a rate of 0.025 g fludioxonil and 0.01 g mefenoxam per kg of seed. Seed treatment was carried out on 14 May, 2008, using a laboratory batch seed treater (Wintersteiger, Austria). Lambda-cyhalothrin was applied onto soybean plants at R5-R6 growth stage, on 28 Aug, using a backpack sprayer with a single hand-held nozzle (*Solo*®, Newport News, VA), calibrated to deliver 180 l ha^{-1} at a pressure of 0.2 MPa.

In 2009, insecticide products were the same as in the 2008 experiment. Seeds were treated on 6 May, 2009, and lambda-cyhalothrin was applied on 7 Jul and 14 Aug, 2009, in Crawfordsville and 10 Jul and 16 Aug, 2009 in Ames. Fungicide treatments consisted of a foliar application of triazole fungicide tebuconazole (Folicur 3.6F, Bayer CropScience, NC) at 0.11 kg a.i. ha^{-1} (3.5 fl oz $acre^{-1}$), and strobilurin fungicide pyraclostrobin (Headline, BASF, NC) at a rate of 0.14 kg a.i. ha^{-1} (7.5 fl oz acre-1), amended with nonionic surfactant (Wet Sol 99, Schaeffer Manufacturing Co, St Louis, MO) at 0.125% of spray volume.). Both fungicides were applied onto soybean plants at R5 growth stage in Crawfordsville and Ames respectively, on 18 and 20 Aug 2009, using a backpack battery sprayer (*Solo*®, Newport News, VA) with a single standard flat jet nozzle, calibrated to deliver 180 l ha^{-1} at 0.2 MPa.

Rating insect feeding injury. Foliar and pod injury, mainly caused by *C. trifurcata*, was assessed as previously described (10, 45, 48). These symptoms consisted of small symmetrical round holes between major leaflet veins and scarred pods. Field scouting and insect sweeps were also used to confirm that the *C. trifurcata* were the predominant insects present in the plots (data

not shown). Based on early season scouting, in 2008 and 2009, beetle populations ranged from 1 to 3 and 8 to 12 beetles per 20 sweeps, respectively.

Leaf injury was assessed on twenty five arbitrarily chosen plants per replicate plot at growth stage V2-V3. Plants were rated using an ordinal scale as described by Daniels (10) with modifications, where 0=no injury (0 holes), 1=minor injury (1-5 holes), 2=medium injury (6-10 holes), 3=severe injury (11-20 holes). An average score was calculated over each replicate, and was used for statistical analysis. This assessment was done only in 2009, because in 2008 plots were planted late, bean leaf beetle populations were too low to be measured, and injury was negligible.

In 2008 and 2009 experiments, pod injury was assessed as previously described by Wilson (52), evaluating all pods on ten arbitrarily chosen plants per replicate at R7-R8 growth stage. On each plant the number of total pods and the number of injured pods were counted, and percentage of injured pods was calculated.

Stem and seed analyses. Stem infection by *Phomopsis* spp. was assessed at late R6 growth stage (when plants had green stems, and pods with green seeds that filled the pod cavity at one of the four-uppermost nodes with a fully developed trifoliate leaf) (11). Five plants were arbitrarily sampled from each plot, and stem plating was performed following the procedure of Garzonio and McGee (12) with modifications. Stems from each plant were cut into sections (approximately 3 cm long), surface sterilized in 1% sodium hypochlorite for 1 min and rinsed in sterile-distilled water for 30 sec. Under a laminar flow hood using aseptic technique, stem sections were partitioned, and five pieces (approximately 1 cm each) from each plant were arbitrarily selected and plated on antibiotic-amended PDA in 9 cm diameter Petri dishes. After 5-

7 days plates were inspected for colonies of *Phomopsis* spp. based on morphological characteristics of the fungi (28, 33). The percentage of infected stems was recorded for each plot.

From each plot, 400 seeds were tested by a blotter test for *Phomopsis* spp. as previously described (34). Seeds were surface sterilized in 1% sodium hypochlorite for 30 sec, and rinsed in sterile-distilled water. Using aseptic techniques, seeds were placed on two layers of sterile blotters in plastic boxes measuring approximately 28 x 17 x 4 cm (Melmat Inc, Huntington Beach, CA). Blotters were previously moistened with dicloran at 500 μg ml^{-1} (Botran 75W, Gowan, Yuma, AZ), to suppress the growth of *Rhizopus* spp. Four boxes containing 100 seeds each were prepared for each seed sample form individual plots. These were incubated at 25°C in the dark for 7 days, and seeds were inspected for *Phomopsis* spp., based on morphological characteristics of the fungi (28, 33). The percentage of infected seeds was recorded for each plot.

An additional sample of one hundred seeds was tested for *Bean pod mottle virus* infection by double antibody sandwich enzyme-linked immunosorbent assay (DAS ELISA), using Agdia antibodies and protocol (Agdia, Inc., Elkhart, IN) and modifications of previously described protocols (27). Seeds from each replicate were divided into twenty groups of five seeds and placed in a plastic mesh bag (Agdia, Inc., Elkhart, IN). Agdia general extraction buffer was added to each bag based on seed weight in 1:10 ratio (weight of the sample:volume of buffer added) and these were incubated overnight at 4ºC. Soaked seeds were macerated with a pestle, and the extract was transferred to 1.5-ml-tubes and stored in the freezer. For each sample, 100 μl of seed sap extract was placed in wells on the pre-coated ELISA plate. Two positive and two negative controls were prepared and placed in each plate. The plates were evaluated with a PowerWave™ Microplate Spectophotometer (Biotek®Instruments, Inc., Winooski, VT) set at 405 nm wavelength. Sample wells were considered positive if absorbance values were higher

than twice the values for the negative controls. In 2009, only seeds harvested from treatments that included insecticide applications and the untreated control were tested for BPMV. The estimated incidence of infected seeds by BPMV in seeds harvested from each plot was determined by using statistical methods for seed group testing as described by Block et al. (5). The result of positive and negative groups (5 seeds each) out of 20 was recorded and the overall proportion of infected seeds was calculated for each replicated. At least one positive sample (group) was required to estimate the proportion of infected seeds for each replicate of each treatment. Seed infection percentages were obtained by multiplying this proportion by 100.

For each replicate of each treatment, germination was evaluated by warm and cold tests, conducted at the ISU seed testing laboratory according to the Association of Official Seed Analysts (AOSA) rules (2).

Experiment 2: *A. glycines* management trials

Experimental design and treatments. To assess the impact of *A. glycines* management tactics on infection by *Phomopsis* spp., soybean stems and seeds were collected from a study conducted in 2008 and 2009 at the ISU Northeast Research and Demonstration Farm near Nashua (Floyd County, IA). Experimental units were plots measuring 15.2 m long and 4.6 m wide, with 6 rows.

This study included 32 different treatments that included applications of insecticides alone or in combination to control populations of *A. glycines*. However, not all the treatments were sampled for this study. Seeds from three treatments were collected in 2008: 1) untreated control, 2) zero aphid control (40), and 3) insecticide at the economic threshold (43). The zero aphid treatment received a foliar application of the pyrethroid insecticide lambda-cyhalothrin (Warrior, Syngenta Crop Protection, Greensboro, NC) at a low rate (0.017 kg a.i. ha^{-1}), and the

organophosphate insecticide chlorpyrifos (Lorsban 4E, Dow AgroSciences, Indianapolis, IN) at a rate of 1.17 kg a.i. ha^{-1}, whenever aphids were detected (more than one aphid per plant). In the treatment corresponding of insecticide at the economic threshold, lambda-cyhalothrin was foliar applied when the mean *A. glycines* population density reached approximately 250 aphids per plant. In 2009, the zero aphid treatment was modified to a higher rate of lambda-cyhalothrin (0.028 kg a.i. ha^{-1}). In addition, a seed treatment of the neonicotinoid insecticide thiamethoxam (Cruiser Maxx, Syngenta Crop Protection, Greensboro, NC) at a rate of 0.5 g a.i. per kg of seed, with a fungicide combination of benzodioxalcarbonitrile (fludioxonil) and phenylamide (mefenoxam) at a rate of 0.025 g a.i. per kg of seed, was added to the list of tested treatments.

For each plot, the middle four rows were machine at harvested maturity and yields were adjusted to a moisture content of 130 g kg^{-1}. Seeds were separated into subsamples and stored at 10ºC until the subsequent tests were performed. For each replicate of each treatment, germination was evaluated by warm and cold tests, conducted at the ISU seed testing laboratory according to the Association of Official Seed Analysts (AOSA) rules (2).

Stem and seed analyses. Methods described in Experiment 1 to assess stem and seed infection by *Phomopsis* spp. were also used in this experiment. In 2008, seed samples were tested for SMV infection following same procedure as described above for BPMV. However, this evaluation was not repeated in 2009 due to the low levels of infection by SMV present in the untreated control (data not shown).

Cumulative aphid days. Seasonal exposure of *A. glycines* was calculated as cumulative aphid days (CAD). This estimate is based on the number of aphids present on soybean plants between two sampling dates, as described by Johnson et al. (22). Populations of *A. glycines* were assessed weekly, beginning in early summer and until plants senesced or until the aphid

populations decreased for two consecutive weeks. Depending on the severity of the infestation, soybean aphids were counted on five to twenty consecutive plants at randomly selected locations in each plot. All aphids were counted on each plant.

Data analyses. Data collected from *C. trifurcata* management trials were analyzed separately by year. Due to the modifications in the objective from 2008 to 2009, different parameters were analyzed in each year. In 2008, treatment effects on percentages of stems and seeds infection by *Phomopsis* spp., seed infection by BPMV, *C. trifurcata* feeding injury of pods, percentages of seed germination and yield were tested using PROC GLM of SAS, version 9.2. Statistical effects of treatments were estimated based on analysis of variance (ANOVA); means were separated using Tukey's test and considered significantly different if $P \leq 0.05$. To test relationships among all the variables, correlations were estimated using Pearson's linear correlation analysis (SAS PROC CORR).

In 2009, treatment effects on percentages of stems and seeds infection by *Phomopsis* spp., seed infection by BPMV, *C. trifurcata* feeding injury of leaves and pods, percentages of seed germination and yield were tested using SAS PROC MIXED. These experiments were analyzed as split plots due to the restriction in randomization of the experimental design (sub-blocks). Data from each location were analyzed separately because of a highly significant treatment by location interaction. Least square means (LSMean) were also separated using Tukey's test and considered significantly different if $P \leq 0.05$.

For *A. glycines* management trials, the same analysis used for 2008 *C. trifurcata* experiment was used to test treatment effect in the respective measurements. Data were analyzed separately by year, due to an unbalanced data set, containing three treatments in 2008 and four treatments in 2009, in addition to different measurements.

The normal and homogeneous distribution of residuals was examined using SAS PROC PLOT.

RESULTS

Preliminary data were collected by assessing infection of seeds by *Phomopsis* spp. and BPMV harvested from seed treatment trials conducted in 2006 and 2007, which included ten locations in Iowa and two insecticide treatments. Insecticide treatment consisted of seed treatment of the neonicotinoid insecticide thiamethoxam (Cruiser Maxx at a rate of 0.5 g a.i. per kg of seed, with a fungicide combination of benzodioxalcarbonitrile (fludioxonil) and phenylamide (mefenoxam) at a rate of 0.025 g a.i. per kg of seed), and an untreated control. These seeds were tested for infection by *Phomopsis* spp. and BPMV as previously described. In these trials, insecticide seed treatments did not have a significant effect on infection of seeds by BPMV or *Phomopsis* spp. (Appendix 1, 2).

In the 2008 *C. trifurcata* management experiment there were differences between cultivars for all the parameters evaluated, except infection of seeds by BPMV (Table 1). Insecticide treatment significantly reduced *C. trifurcata* feeding injury of pods and infection of stems by *Phomopsis* spp. in both cultivars (Table 1 and Figure 1). It also reduced infection of seeds by BPMV in the BPMV tolerant cultivar (Spansoy 201) (Table 1 and Figure 1). These differences between the insecticide treatment and the untreated plants were not reflected in yield, infection of seeds by *Phomopsis* spp. or germination (Table 1).

Infection of seeds by *Phomopsis* spp. was negatively correlated with yield, warm germination, and cold germination. On the other hand, infection of stems by *Phomopsis* spp. was positively correlated with *C. trifurcata* feeding injury of pods (Table 2).

In 2009 experiments, treatments presented significant differences in some of the responses evaluated, however many of these effects were not consistent between the locations (Table 3). The effect of insecticide treatments on *C. trifurcata* feeding injury was consistent for both locations. However, insect activity and *Phomopsis* infection were very low at the Ames location, and this may have limited our ability to observe differences in infection by *Phomopsis* spp. amongst the treatments, and consistency between both locations.

At Crawfordsville, insecticide and fungicide impacted both stem infection by *Phomopsis* spp. and *C. trifurcata* feeding of pods. Also, while insecticide caused an impact on foliar feeding injury, fungicide had an effect on infection of seeds by *Phomopsis* spp. and yield (Table 3). At Ames, just the effect of the insecticide was significant, specifically on yield and *C. trifurcata* feeding of leaves and pods. None of the treatments significantly impacted germination or infection of seeds by BPMV (Table 3 and Table 4).

At both locations seed treatment and foliar insecticide applications reduced insect feeding injury of leaves and pods, respectively (Table 3 and 4). Treatments that included insecticidal seed treatment to control F_0 populations of *C. trifurcata* reduced foliar feeding injury at both locations. At the Crawfordsville location, treatments that included a foliar insecticide application to control F_2 populations of *C. trifurcata* reduced pod feeding injury compared with other treatments. Moreover, the combination of early *C. trifucata* control strategies with an application of the pyraclostrobin fungicide had the same effect reducing pod feeding injury as the strategies to control F_2 populations of *C. trifucata*. At the Ames location, all insecticide treatments reduced injured pods compared with the control, but there were no significant differences among insecticide applications aimed to control different generations of *C. trifucata* (Table 4).

Insecticide treatment aimed to control F_0 and F_1 populations of *C. trifucata* combined with either of the fungicides, and the single pyraclostrobin application, reduced infection of seeds by *Phomopsis* spp. compared with the untreated control (Table 4).

Soybean yield was impacted individually by either fungicide or insecticide depending on the location (Table 3). Treatments that included applications of pyraclostrobin ($P = 0.01$) at Crawfordsville, and seed and foliar applied insecticide to control all generations of *C. trifurcata* ($P = 0.04$) at Ames, significantly increased yield compared with the untreated control (data not shown).

Overall, data from *A. glycines* management trials indicated that aphid control did not significantly reduce infection of stems or seeds by *Phomopsis* spp. (Table 5). Insecticide treatments significantly reduced *A. glycines* populations in both years, and increased yield in 2009. The highest *A. glycines* populations were always observed in the untreated control, while the lowest populations were observed in the zero aphid treatment in both years. In 2009, the application of lambda-cyhalothrin based on economic threshold (250 aphids/plant) significantly reduced *A. glycines* populations as well (Table 5).

In 2008, except for *A. glycines* populations, none of the treatments had an effect on any of the responses evaluated. In 2009, foliar insecticide applications significantly increased yield, but the zero aphid treatment presented higher infection of seeds by *Phomopsis* spp. and lower warm and cold germination of seeds, compared to the untreated control. Warm germination was also reduced in the economic threshold-based insecticide treatment. Seed-applied insecticide only had an effect reducing *A. glycines* populations. However, this treatment was not as effective as the foliar insecticide applications.

None of the treatments resulted in significant differences in infection of stems by *Phomopsis* spp. or seed infection by SMV (Table 5). Correlation analysis did not show significant relationship between any of the responses evaluated.

DISCUSSION

Separately, fungicide and insecticide treatments have been reported to control infection by *Phomopsis* spp. and populations of *C. trifurcata* and *A. glycines*, respectively (6, 26, 40, 43, 54). However, the effect of integrated strategies to control soybean viruses and vectors on infection by *Phomopsis* spp. has not been reported yet.

Consistent with previous studies (6, 10), in 2009 seed-applied insecticide reduced foliar feeding injury caused by F_0 populations of *C. trifurcata* early in the season. Injured pods were reduced by late season foliar insecticide applications aimed to control F_2 populations of *C. trifurcata* in 2008 and 2009. However, the combination of insecticide treatments targeted to reduced F_0 and F_1 populations of *C. trifurcata* with an application of pyraclostrobin also reduced injured pods, but there is no explanation for this treatment effect.

Data obtained in this study suggest that in addition to the known effect that feeding injury of pods has reducing seed quality (15, 48), *C. trifurcata* may also increase secondary stem infection by fungi such as *Phomopsis* spp. In both years, insecticide treatments reduced injured pods, and in 2008, also reduced stem infection by *Phomopsis* spp., and there was a significant positive correlation between these two variables. It suggests the possibility that control of *C. trifurcata* with insecticides may have added benefits for reducing infection of stems by *Phomopsis* spp. The effect of insecticide treatments reducing stem infection by *Phomopsis* spp. has never been reported. To understand the effect of *C. trifurcata* and its management strategies on stem infection by *Phomopsis* spp., more studies are needed. In future studies, it will be

necessary to pay more attention to feeding behaviors of *C. trifurcata* during pod setting and seed-filling growth stages, and to the relationship between *Phomopsis* infection of pods and stems.

On the other hand, this study found no relationship between *C. trifurcata* feeding injury of pods and seed infection by *Phomopsis* spp. However, the experiments were conducted under conditions of low *C. trifurcata* populations and low incidence of *Phomopsis* infection. Overall, more data are needed to better understand the value of *C. trifurcata* management in relation to infection of both stems and seeds by *Phomopsis* spp.

Even though *C. trifurcata* populations were low in both years and seedcoat mottling was not observed, samples from all treatments tested positive for BPMV at a low incidence level. Previous studies have reported that seed-applied or early foliar-applied insecticides reduce both *C. trifurcata* populations and incidence of BPMV (6, 26). In 2008, the use of a field tolerant cultivar to BPMV or insecticide treatments alone was ineffective for reducing seed infection by BPMV compared with controls. However, when these strategies were combined, BPMV incidence was significantly reduced, suggesting that resistance mechanisms should be combined with chemical treatments in vector-virus management programs to enhance individual control effects (44). Although the BPMV-tolerant cultivar used in 2008 had reduced BPMV incidence, its agronomic performance was poor. This emphasizes the need for incorporating virus resistance traits into high-yielding adapted cultivars (18).

Previous studies have reported that well timed insecticide applications have resulted in an improvement of both yield and seed coat color (6, 26); but in this study none of these effects were consistently observed. Only multiple insecticide applications to control F_0, F_1 and F_2 populations of *C. trifurcata*, increased yield in one location (Ames) in 2009 compared with the untreated control.

In 2009, seed and foliar applied insecticides were combined with fungicides. At Crawfordsville, the effect of fungicide treatments on infection by *Phomopsis* spp. was enhanced when combined with an insecticide treatment. A reduction in stem infection by *Phomopsis* spp. was observed in the treatment that included applications of pyraclostrobin and insecticides to control F_0 and F_1 populations of *C. trifurcata*. However, mixed results were obtained in terms of infection of seeds by *Phomopsis* spp. At the same location, applications of either tebuconazole or pyraclostrobin reduced infection of seeds by *Phomopsis* spp. when they were combined with insecticide treatment to control F_0 and F_1 populations of *C. trifurcata*. However, the same effect was observed when pyraclostrobin was applied alone.

In addition, compared with the treatments that did not receive an application of fungicide, the combination insecticide treatments with an application of pyraclostrobin tended to present lower infection by *Phomopsis* spp., *C. trifurcata* pod feeding injury and higher yield. However, these effects were not always statistically significant and were not observed in the combination of insecticide treatment to control F_0, F_1 and F_2 populations of *C. trifurcata* with pyraclostrobin.

In 2009, *Phomopsis* incidence was significantly different between locations, and fewer treatment effects were observed at Ames which was likely related to a lower disease incidence observed. Similar results were reported by Swoboda and Pedersen (50) under low disease pressure systems. In this study (50), few differences were observed among the treatments and foliar fungicides applied in the absence of foliar disease did not produce non-fungicidal physiological effect or associated yield improvement.

On the other hand, at Crawfordsville, warm and cold germination of seeds were significantly lower compared with Ames, suggesting that there was an effect of higher incidence of *Phomopsis* spp. reducing seed quality, as in previous studies (35, 49).

It is important to emphasize that late planting and harsh winter in both years reduced *C. trifurcata* densities, which likely affected our results. Delayed planting is known to reduce *C. trifurcata* feeding injury, primarily because plants will not be available at the time that newly emerged adults (F_0) are moving from overwintering habitats into soybean to feed and lay eggs (27, 53). Moreover, high *C. trifurcata* winter mortality has been predicted in recent years in Iowa based on accumulating subfreezing degrees (20).

In *A. glycines* management trials foliar insecticide applications based on aphid density significantly reduced plant exposure to *A. glycines* and increased yield compared with the preventive seed treatment and the untreated control. Yield and *A. glycines* populations in the economic threshold-based treatment were not significantly different than in the zero aphid treatment. These results are consistent with previous studies (40). Our findings also agree with Pedersen et al. (41) and indicate that foliar application of the lambda-cyhalothrin or chlorpyrifos insecticides aimed to reduce *A. glycines* populations did not reduce SMV incidence.

Additionally, these treatments did not reduce infection of stems or seeds by *Phomopsis* spp. In one year, foliar applications of insecticides had a negative effect on germination of seeds, and multiple insecticide applications (zero aphid) also increased infection of seeds by *Phomopsis* spp. However, as in the *C. trifurcata* management experiment, *Phomopsis* infection was very low in both years, and it may has masked treatment effect on infection by *Phomopsis* spp. In this study we did not observe any evidence that *A. glycines* colonization of soybean increases susceptibility to *Phomopsis* infection, or that management strategies to control *A. glycines* populations have any added benefit related to *Phomopsis* spp.

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Tables

Table 1. Analysis of variance for cultivar and insecticide treatment effect on stems and seeds infected by *Phomopsis* spp., seeds infected by *Bean pod mottle virus* (BPMV), pods injured by bean leaf beetle (*Cerotoma trifurcata*), yield and seed germination in a field experiment in Story Co., IA, during 2008.

	P value			
Response evaluated	Block	Cultivar*	Insecticide[+]	Cultivar × Insecticide
Phomopsis infected stems	0.9258	0.0002	<.0001	0.0030
Phomopsis infected seeds	0.4373	<.0001	0.2757	0.7225
BPMV infected seeds	0.5038	0.7361	0.0230	0.0042
Injured pods	0.1866	0.0365	<.0001	0.0810
Yield	0.0526	<.0001	0.8816	0.1718
Germination-warm test	0.2662	<.0001	0.7753	0.7753
Germination-cold test	0.1214	<.0001	0.9160	0.2254

*Cultivars were Spansoy 201 (Spangler Seed Tech, Inc., Jefferson, WI) and 92M02 (Pioneer Hi-Bred Int., Inc., Des Moines, IA).
[+]Insecticide: seed applied thiamethoxam (Cruiser 5FS, 0.5 g a.i. kg of seed^{-1}) and fludioxonil and mefenoxam (Apron Maxx, 0.025 g a.i. and 0.01 g a.i. kg of seed^{-1}, respectively), and foliar application of lambda-cyhalothrin (Warrior, 0.020 kg a.i. ha^{-1}).

Table 2. Correlations among stems and seeds infected by *Phomopsis* spp., seeds infected by *Bean pod mottle virus* (BPMV), pods injured by bean leaf beetle (*Cerotoma trifurcata*) and yield and seed germination in a field experiment in Story Co., IA, during 2008[+ *].

	Phomopsis spp. infected		BPMV infected	Injured		Germination	
Variable	Stems	Seeds	Seed	pods	Yield	Warm	Cold
		-0.39	0.12	0.50	0.43	0.48	0.43
Phomopsis spp. infected stems	-	0.13	0.66	0.05	0.10	0.06	0.09
			0.05	0.33	-0.86	-0.91	-0.84
Phomopsis spp. infected seeds	-	-	0.86	0.21	<.0001	<.0001	<.0001
				0.44	0.04	0.04	0.15
BPMV infected seeds	-	-	-	0.09	0.87	0.88	0.58
					-0.17	-0.18	-0.24
Injured pods	-	-	-	-	0.53	0.49	0.38
						0.91	0.80
Yield	-	-	-	-	-	<.0001	0.0002
							0.89
Germination-Warm	-	-	-	-	-	-	<.0001

[+]Top value is correlation coefficient; bottom is *P* value (SAS PROC CORR).
*Based on 16 observations.

Table 3. Overall tests of significance for insecticide and fungicide treatment effect on stems and seeds infected by *Phomopsis* spp., seeds infected by *Bean pod mottle virus* (BPMV), leaves and pods injured by bean leaf beetle (*Cerotoma trifurcata*), yield and seed germination in two locations in Iowa in 2009[+]

			P value**		
Location*	Evaluated response	Block	Insecticide	Fungicide	Insecticide × fungicide
Crawfordsville	*Phomopsis* infected stems	0.0707	0.0462	0.0236	0.1644
	Phomopsis infected seeds	0.1900	0.2533	0.0025	0.2678
	BPMV infected seeds	0.1292	0.6362	-	-
	Injured leaves	0.8155	0.0233	0.9549	0.9992
	Injured pods	0.0471	<.0001	0.0396	0.0543
	Yield	0.0771	0.1621	0.0207	0.5894
	Germination-warm	0.7987	0.2940	0.0935	0.5685
	Germination-cold	0.3869	0.6549	0.0549	0.6008
Ames	*Phomopsis* infected stems	0.0300	0.1148	0.3529	0.9568
	Phomopsis infected seeds	0.1378	0.3541	0.3118	0.1502
	BPMV infected seeds	0.1203	0.5518	-	-
	Injured leaves	0.4907	0.0055	0.6954	0.4896
	Injured pods	0.5188	<.0001	0.9527	0.5438
	Yield	0.0396	0.0079	0.2343	0.2784
	Germination-warm	0.2267	0.0785	0.2943	0.2619
	Germination-cold	0.0705	0.4823	0.2273	0.8963

[+]Treatments consisted of insecticide applications to control different *C. trifurcata* generations, combined with fungicide applications at R5 growth stage to control *Phomopsis* spp. infection. Insecticide applications consisted of seed applied thiamethoxam (Cruiser 5FS, 0.5 g a.i. kg of seed^{-1}) and fludioxonil and mefenoxam (Apron Maxx, 0.025 g a.i. and 0.01 g a.i. kg of seed^{-1}, respectively), and foliar application of lambda-cyhalothrin (Warrior, 0.020 kg a.i. ha^{-1}). Fungicide treatments consisted of foliar application tebuconazole (Folicur 3.6F, 0.11 kg a.i. ha^{-1}) and pyraclostrobin (Headline, 0.14 kg a.i. ha^{-1}).

*Two locations in Iowa: Crawfordsville (Washington County), Ames (Story County).

** Based on mixed model ANOVA (SAS PROC MIXED).

(-) Data were not collected for specific treatments.

Table 4. Insecticide and fungicide treatment effects on stems and seeds infected by *Phomopsis* spp., seeds infected by *Bean pod mottle virus* (BPMV), leaves and pods injured by bean leaf beetle (*Cerotoma trifurcata*), yield and seed germination in two locations in Iowa in 2009.

			Phomopsis spp. infection		BPMV	Feeding injury		Yield	Germination seeds	
	Treatment**		Stems	Seeds	Seeds	Leaves	Pods		Warm	Cold
Location*	Insecticide	Fungicide	(%)	(%)	(%)	(score***)	(%)	(kg/ha)	(%)	(%)
1	None	None	60 a	11.8 a	5.6 a	2.4 a	11.7 a	4552 ab	82.0 a	87.0 a
		Tebucon	33 ab	7.4 ab	.	2.4 a	7.9 ab	4792 ab	80.8 a	84.3 a
		Pyraclost	35 ab	5.1 b	.	2.4 a	9.7 a	5040 a	78.8 a	81.8 a
	F0 + F1	None	36 ab	8.6 ab	5.3 a	1.2 b	8 ab	4421 ab	85.0 a	86.5 a
		Tebucon	34 ab	5.6 b	.	1.3 b	9.1 a	4452 ab	80.3 a	84.5 a
		Pyraclost	21 b	4.3 b	.	1.2 b	2.6 bc	4580 ab	80.5 a	84.5 a
	F0 + F1 + F2	None	43 ab	8.8 ab	7.3 a	1.2 b	1.8 c	3861 b	83.8 a	84.5 a
		Tebucon	45 ab	7.2 ab	.	1.3 b	2.3 bc	4647 ab	84.0 a	86.5 a
		Pyraclost	48 ab	7.7 ab	.	1.3 b	0.6 c	4429 ab	82.8 a	84.0 a
	F1 + F2	None	50 a	7.3 ab	7.5 a	2.3 a	1.7 c	4325 ab	82.5 a	84.3 a
		Tebucon	35 ab	7.7 ab	.	2.3 a	1.6 c	4479 ab	83.8 a	85.5 a
		Pyraclost	38 ab	6.1 ab	.	2.3 a	1.1 c	4773 ab	81.3 a	79.0 a
2	None	None	27 a	0.4 a	2.7 a	1.6 ab	5.1 a	2931 a	99.0 a	97.5 a
		Tebucon	20 a	0.3 a	.	2.1 a	4.9 a	2676 a	98.0 a	97.0 a
		Pyraclost	24 a	0.2 a	.	2.0 a	4.9 a	3093 a	98.5 a	97.0 a
	F0 + F1	None	32 a	0.8a	4.5 a	0.4 bc	0.8 b	3248 a	97.8 a	97.8 a
		Tebucon	27 a	0.3 a	.	0.1 c	0.1 b	2961 a	98.3 a	95.3 a
		Pyraclost	24 a	0.1 a	.	0.6 bc	1.0 b	3577 a	98.0 a	95.5 a
	F0 + F1 + F2	None	32 a	0.4 a	3.3 a	0.6 bc	0.6 b	3421 a	98.8 a	97.8 a
		Tebucon	29 a	0.1 a	.	0.3 bc	0.4 b	3573 a	97.5 a	96.8 a
		Pyraclost	26 a	1.0 a	.	0.3 bc	0.5 b	3151 a	97.5 a	96.0 a
	F1 + F2	None	24 a	0.9 a	2.1 a	2.1 a	0.2 b	2891 a	98.3 a	96.5 a
		Tebucon	24 a	0.9 a	.	1.6 ab	1.0 b	2836 a	98.3 a	96.5 a
		Pyraclost	24 a	0.2 a	.	1.8 ab	0.1 b	3241 a	98.5 a	96.3 a

*Two locations in Iowa: 1=Crawfordsville (Washington County), 2=Ames (Story County).
**Treatments consisted of insecticide applications to control different *C. trifurcata* generations (F_0=overwinter, F_1=first generation and F_2=second generation), combined with fungicide applications at R5 growth stage to control *Phomopsis* spp. infection.
Insecticide applications consisted of seed applied thiamethoxam (Cruiser 5FS, 0.5 g a.i. kg of seed^{-1}) and fludioxonil and mefenoxam (Apron Maxx, 0.025 g a.i. and 0.01 g a.i. kg of seed^{-1}, respectively), and foliar application of lambda-cyhalothrin (Warrior, 0.020 kg a.i. ha^{-1}).
Fungicide treatments consisted of foliar application tebuconazole (Folicur 3.6F, 0.11 kg a.i. ha^{-1}) and pyraclostrobin (Headline, 0.14 kg a.i. ha^{-1}).
***Average score of injured leaves is based on foliar feeding injury scale (0=no injury, 1=minor injury, 2=medium injury, 3=severe injury).
(-) Data were not collected for specific treatments.

Table 5. Insecticide treatment effect on stems and seeds infected by *Phomopsis* spp., seeds infected by *Soybean mosaic virus* (SMV), soybean aphid (*Aphis glycines*) populations, yield and seed germination in Iowa in 2008 and 2009[+].

		Phomopsis spp.		SMV	Cumulative		Germination	
		Stems	Seeds	Seeds	aphids days	Yield	Warm	Cold
Year	Treatment*	(%)	(%)	(%)	(CAD)	(kg/ha)	(%)	(%)
2008	Untreated	-	0.2 a	0.2 a	3600 a	3901 a	98.2 a	95.7 a
	Zero aphid	-	0.4 a	0.5 a	135 c	3960 a	98.3 a	95.7 a
	Insecticide at 250 aphids	-	0.5 a	1.0 a	1721 b	4039 a	98.5 a	95.8 a
2009	Untreated	34 a	7.3 b	-	10151 a	3846 c	91.3 a	85.8 ab
	Zero aphid	22 a	14.1 a	-	51 c	4225 ab	85.7 b	83.2 b
	Insecticide at 250 aphids	23 a	6.9 b	-	821 c	4309 a	85.3 b	83.8 ab
	Seed treatment	19 a	8.5 b		5714 b	3929 bc	90.2 ab	88.0 a

[+]Means followed by the same letter are not statistically different at $P \leq 0.05$.
*Treatments consisted of: untreated control, zero aphid= multiple applications of lambda-cyhalothrin (Warrior, 0.017 kg a.i. ha^{-1}) and chlorpyrifos (Lorsban 4E, 1.17 kg a.i. ha^{-1}), insecticide at 250 aphids= application of lambda-cyhalothrin (Warrior, 0.017 kg i.a ha^{-1}) based on aphid population threshold (250 aphids per plant). In 2009, zero aphid= multiple applications of lambda-cyhalothrin (Warrior, 0.028 kg a.i. ha^{-1}) and chlorpyrifos (Lorsban 4E, 1.17 kg a.i. ha^{-1}, seed treatment= seed applied thiamethoxam (Cruiser Maxx, 0.5 g a.i. per kg of seed) and fludioxonil and mefenoxam (Apron Maxx, 0.025 g a.i. and 0.5 g a.i. kg of $seed^{-1}$, respectively).
(-) Data were not collected for specific treatments.

Figures

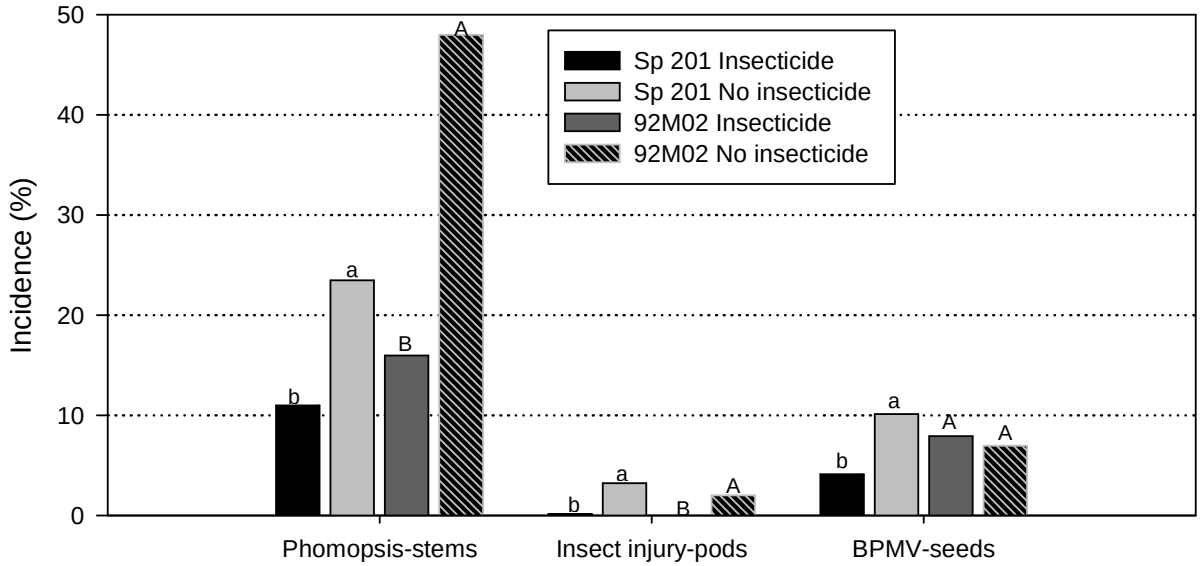

Figure 1. Percentage of infected stems by *Phomopsis* spp., infected seeds by *Bean pod mottle virus* (BPMV) and pods injured by bean leaf beetle (*Cerotoma trifurcata*), of two soybean cultivars, BPMV tolerant (Spansoy 201) and BPMV susceptible (92M02), with and without insecticide applications. Insecticide treatment: seed applied thiamethoxam (Cruiser 5FS, 0.5 g a.i. kg of seed) and fludioxonil and mefenoxam (Apron Maxx, 0.025 g a.i. and 0.01 g a.i. kg of seed^{-1}, respectively), and foliar application of lambda-cyhalothrin (Warrior, 0.020 kg a.i. ha^{-1}). Means based on 16 observations.

CHAPTER 4

IMPACTS OF FOLIAR FUNGICIDES ON INFECTION OF SOYBEAN BY *PHOMOPSIS* SPP. IN IOWA

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ABSTRACT

Fungicides pyraclostrobin (strobilurin) and tebuconazole (triazole) were applied to soybean [*Glycine max* (L.) Merr.] at growth stages R3, R5 or R3+R5, in 2008 and 2009 at two locations in Iowa. Incidence of infection of stems and seeds by *Phomopsis* spp. was evaluated, along with yield and seed quality. Stem infection by *Phomopsis* spp. was reduced in both years by pyraclostrobin applied at R3+R5, and in 2008 by pyraclostrobin at R5, compared to the untreated control. In 2009, treatments including applications of tebuconazole at R3 and pyraclostrobin at R5 significantly reduced infection of seed by *Phomopsis* spp., compared to the untreated control. Only the application of pyraclostrobin at R3+R5 reduced both stem and seed infection by *Phomopsis* spp. in 2009. None of the treatments had a significant effect on yield, or seed quality. Seed infection by *Phomopsis* spp. was negatively correlated with seed quality. Fungicides applied at these growth stages can have an impact on infection by *Phomopsis* spp., but their effectiveness varies according to weather conditions and disease intensity.

INTRODUCTION

The application of foliar fungicides on soybean has increased recently in the United States because of the arrival of the soybean rust pathogen (*Phakopsora pachyrhizi* Sydow) in North America (Schneider et al., 2005). In the Midwestern U.S. there is a trend toward the use of fungicides in management strategies for soybean rust prevention and to control several other foliar and stem diseases.

Two classes of fungicides, triazoles and strobilurins, have become the most commonly used products because of their broad spectra of activity and other characteristics (Munkvold, 2009). Additionally, it has been reported that strobilurin fungicides may have plant health benefits that increase yield and tolerance to environmental stresses (Bartlett et al., 2002). However, these benefits under stressful conditions have been questioned (Nason et al., 2007) and recent studies have reported no significant increase in yield following fungicide applications with different products at various timings (Hanna et al., 2008; Swoboda and Pedersen, 2009).

Pod and stem blight and Phomopsis seed decay, caused by members of the *Diaporthe-Phomopsis* complex (Kulik and Sinclair, 1999), are common throughout most of the soybean producing areas in North America, and cause significant losses in yield and seed quality (Kulik and Sinclair, 1999; Wrather et al., 2010). In 2006 pod and stem blight was ranked 13th and Phomopsis seed decay 17th out of 25 diseases that cause significant yield reductions in the United States with losses of 208 and 122 thousand metric tons, respectively (Wrather et al., 2010).

In addition, members of the *Diaporthe-Phomopsis* complex have been associated with soybean top dieback (Yang and Robertson, 2007), which causes foliar symptoms and premature plant senescence. This early maturation can result in yield losses if it occurs before the pod filling is completed; but also can increase stem and seed infection by *Phomopsis* spp., because

plants often senesce under optimal conditions for disease development, such as warm and wet periods (Balduchi and McGee, 1987; Kulik and Sinclair, 1999). Although the top dieback has been repeatedly observed in Iowa, the disease has not been well studied and little information exists about its etiology or management (Yang and Robertson, 2007). However, it is feasible that management strategies that have an effect on stem infection by *Phomopsis* spp. could also have some impact on top dieback.

Chemical control tactics are not always necessary or effective in management of this pathogen complex, because of the variability among years and locations caused by the strong influence of weather conditions on disease severity (Balduchi and McGee, 1987; Kulik and Sinclair, 1999). The effectiveness of late season fungicide applications to prevent the movement of *Phomopsis* spp. from pods to seeds has been reported previously (Tekrony et al., 1985; Wrather et al., 2004). However, some of the effective products are no longer labeled for use on soybean, and other products have shown mixed results, sometimes resulting in increased infection compared to the untreated control (Tekrony et al., 1985; Wrather et al., 2004). Late season applications are not often implemented solely for control of *Phomopsis* spp., but there may be some seed quality benefits associated with current practices involving fungicide applications at growth stage R3 or R5 recommended to control foliar and stem diseases. The objective of this study was to evaluate the effects of two fungicides (tebuconazole and pyraclostrobin) at different timings on infection of stems and seeds by *Phomopsis* spp. and on soybean yield in Iowa.

MATERIALS AND METHODS

Field trials were established at Iowa State University (ISU) Curtis Farm (Story Co., IA) in 2008 and at Iowa State University Northeast Research and Demonstration Farm (Floyd Co.,

IA) in 2009. Both fields had been planted with soybean in the prior year. The experimental design in both experiments was a randomized complete block with four replications. Plots were 14 m long and 4 m wide with six rows spaced approximately 76 cm apart. Fields were planted on 23 June and 22 May in 2008 and 2009, respectively.

Treatments consisted of applications of two fungicides at either growth stage R3 (beginning pod), R5 (beginning seed), or R3+R5, and an untreated control. Chemical products evaluated were triazole fungicide tebuconazole (Folicur 3.6F, Bayer CropScience, NC) at 0.11 kg a.i. ha^{-1} (3.5 fl oz $acre^{-1}$), and strobilurin fungicide pyraclostrobin (Headline, BASF, NC) at a rate of 0.14 kg a.i. ha^{-1} (7.5 fl oz acre-1), amended with nonionic surfactant (Wet Sol 99, Schaeffer Manufacturing Co, St Louis, MO) at 0.125% of spray volume. Foliar fungicides were applied in water using a CO_2 pressurized backpack sprayer and TeeJet (Springfield, IL) XR nozzles (XR 8003-VS), calibrated to deliver 180 l ha^{-1} at a pressure of 0.2 MPa. The hand boom consisted of 6 nozzles spaced 50 cm apart covering 3 m (4 - 76 cm rows). Applications were made to the center-four rows to minimize interplot interference between experimental units.

Applications at R3 were carried out on 20 August 2008 and 3 August 2009, and at R5 on 12 September 2008 and 24 August 2009. Plots were visually evaluated and reproductive stages were determined when 50% or more plants were in the stage for treatment (Fehr et al., 1971). In both years, the center-four rows were machine harvested at maturity on 29 October 2008 and 11 October 2009. Yields were adjusted to a moisture content of 130 g kg^{-1}. Seeds were separated into subsamples and stored at 10^{o} C until the subsequent tests were performed. For each replicate of a treatment, germination was evaluated by warm and cold tests, conducted at the ISU seed testing laboratory according to rules of the Association of Official Seed Analysts (1998).

Stem infection by *Phomopsis* spp. was assessed at growth stage R6 (Fehr et al., 1971). Five plants were arbitrarily sampled from each plot, and stem plating was performed following the procedure of Garzonio and McGee (1983) with modifications. Stems were cut into sections (approximately 3 cm long), surface sterilized in 1% sodium hypochlorite for 1 min and rinsed in sterile-distilled water for 30 sec. Under a laminar flow hood using aseptic technique, stem sections were partitioned, and five pieces (approximately 1 cm each) from each plant were arbitrarily selected and plated on antibiotic-amended potato dextrose agar (200 mg streptomycin sulfate, 50 mg chlortetracycline hydrochloride, 120 mg neomycin sulfate, 39 g Difco PDA per liter) in 9-cm-diameter Petri dishes. After 5-7 days plates were inspected for colonies of *Phomopsis* spp.

From each plot, 400 seeds were tested by a blotter test for *Phomopsis* spp. (McGee et al., 1980). Seeds were surface sterilized in 1% sodium hypochlorite for 30 sec, and rinsed in sterile-distilled water. Using aseptic techniques, seeds were placed on two layers of sterile blotters in plastic boxes measuring approximately 28 x 17 x 4 cm (Melmat Inc, Huntington Beach, CA). Blotters were previously moistened with dicloran at 500 μg ml^{-1} (Botran 75W, Gowan, Yuma, AZ), to suppress the growth of *Rhizopus* spp. Four boxes containing 100 seeds each were prepared for each seed sample. These were incubated at 25 ^{o}C in the dark for 7 days, and seeds were inspected for *Phomopsis* spp., based on morphological characteristics of the fungi (Kulik and Sinclair, 1999; McGee, 1992).

Treatment effects on percentages of stems and seeds infected by *Phomopsis* spp., percentages of seed germination and yield were tested in a combined analysis using PROC GLM of SAS, ver. 9.2. There was a significant year x treatment interaction for seed infection, therefore the data were analyzed and presented separately by year. Statistical effects of treatments were

estimated based on analysis of variance (ANOVA) and means were separated using Tukey's test and considered significantly different if $P \leq 0.05$. The normal and homogeneous distribution of residuals was examined using SAS PROC PLOT. To test relationships among all the variables, correlations were estimated using Pearson's linear correlation analysis (SAS PROC CORR).

RESULTS AND DISCUSSION

Fungicide applications at R3 have been reported to reduce severity of several foliar and stem diseases and increased yield in the Midwest (Dorrance et al., 2010; Robertson et al., 2009); however, in this study, R3 applications did not consistently impact stem infection by *Phomopsis* spp. In 2008, both treatments that included pyraclostrobin at R5 significantly reduced stem infection by *Phomopsis* spp. compared to the untreated control (Fig. 1). In 2009, the same effect was observed only with pyraclostrobin at R3+R5.

In 2008, the incidence of seed infection was very low and quantifiable differences between treatments were not observed. Low disease pressure could have been a consequence of late planting because of heavy early season rain, which caused the period of maximum susceptibility of seeds to occur later when dry and cool conditions prevailed (Fig. 1). Long dry periods have also reduced infection levels of *P. sojae* Lehm. in previous studies (Kulik, 1984).

Seed infection by *Phomopsis* spp. was approximately nine-fold higher in the untreated control in 2009, compared to the previous year (Fig. 1), allowing observation of treatment effects. In the 2009 trial, both treatments that included application of tebuconazole at R3 or application of pyraclostrobin at R5, significantly reduced seed infection by *Phomopsis* spp., compared with the untreated control (Fig. 1). Pyraclostrobin at R3+R5 resulted in the greatest reduction in seed infection, and was significantly different from tebuconazole at R3. Only the

application of pyraclostrobin at R3+R5 had a dual effect, reducing stem and seed infection by *Phomopsis* spp. in 2009.

These results were consistent with previous studies reporting that fungicides applied from mid-late flowering to the late maturity stages can effectively reduce Phomopsis seed decay (Kulik and Sinclair, 1999; Tekrony et al., 1985; Wrather et al., 2004). However, Wrather et al. (2004) reported that applications of a strobilurin fungicide (azoxystrobin) at R3+R5 resulted in greater seed infection by *Phomopsis* spp. compared to the control. Previous studies did not report results for fungicide effects on stem infection. In the present study none of the fungicide applications increased infection by *Phomopsis* spp.

Yields were slightly higher in 2009 than in 2008, but none of the treatments had a significant effect on yield (data not shown). Tekrony et al. (1985) found little relationship between seed infection by *Phomopsis* spp. and yield, and no yield increase after fungicide treatments. Other studies conducted in Iowa and Indiana provided evidence that prophylactic fungicide applications do not necessarily provide consistent economic returns when conditions were not conducive for disease development (Hanna et al., 2008; Swoboda and Pedersen, 2009).

There were no significant differences among treatments in seed quality according germination tests, possibly resulting from low levels of infection by *Phomopsis* spp., especially in 2008. Consistent with previous studies (Tekrony et al., 1985), *Phomopsis* spp. infection of seeds and seed quality were correlated. Incidence of seed infection was negatively correlated with germination percentages from warm ($P < 0.05$) and cold ($P < 0.05$) tests (Table 1).

This study provides evidence that these two fungicides currently registered for use on soybean, differ in their ability to control stem and seed infection by *Phomopsis* spp., based on the growth stage at which they are applied. Moreover, there was no evidence for plant health

benefits resulting from applications of these products, and fungicide treatments did not significantly affect yield. Appropriate timing of fungicide applications, weather conditions and inoculum pressure, have important roles in the effectiveness of these disease management techniques. The effect of pyraclostrobin at R3+R5 on reducing *Phomopsis* spp. of both stems and seeds, suggests that this management practice could have value such as improving seed quality and possibly yield in some varieties, locations and years.

RESEARCH HIGHLIGHTS

Current soybean production practices often include fungicide applications at growth stages R3 or R5 to control foliar and stem diseases that can impact soybean yield. These applications are not directed at the control of *Phomopsis* spp., but we hypothesized that they could contribute to reducing stem and seed infeciton by *Phomopsis* spp. In field experiments in Iowa in 2008 and 2009, we found that single applications of pyraclostrobin or tebuconazole could reduce either stem or seed infection, but only two applications of pyraclostrobin reduced both stem and seed infection. These results indicate that there can be some added value to R3 or R5 fungicide applications in terms of reducing infection by *Phomopsis* spp.

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Tables

Table 1. Linear correlations for infection of seeds and stems by *Phomopsis* spp., warm and cold germination tests and yield*.

	Phomopsis spp. infected stems	Warm germination	Cold germination	Yield
Phomopsis spp.		0.141	0.159	-0.191
infected stems		0.2984	0.2418	0.1580
Phomopsis spp.	0.246	-0.704	-0.451	0.487
infected seeds	0.0680	<0.0001	0.0005	0.0001

*Top value is Pearson's linear correlation coefficient; bottom is *P* value (SAS PROC CORR). *n* = 56

Figures

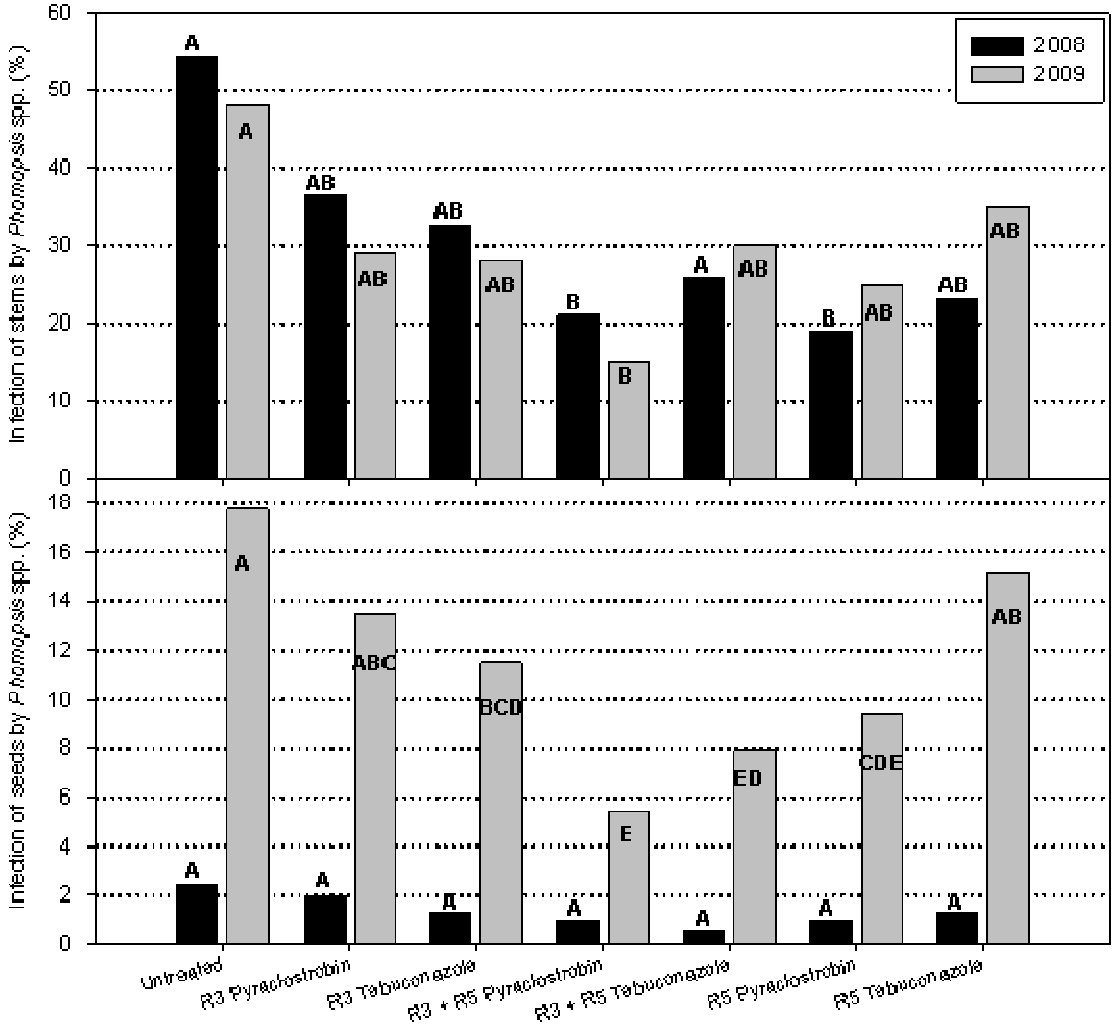

Figure 1. Effects of pyraclostrobin at 0.14 kg a.i. ha^{-1} (Headline, 7.5 fl oz $acre^{-1}$) and tebuconazole at 0.11 kg a.i. ha^{-1} (Folicur 3.6, 3.5 fl oz $acre^{-1}$) applications at different growth stages on infection of soybean stems (top) and seeds (bottom) by *Phomopsis* spp. Within each year, means labeled with the same letter were not significantly different ($P > 0.05$) according to Tukey's test.

CHAPTER 5

IMPACT OF FUNGICIDE AND INSECTICIDE APPLICATIONS ON INFECTION OF SOYBEANS BY *PHOMOPSIS* SPP., *BEAN POD MOTTLE VIRUS* AND *SOYBEAN MOSAIC VIRUS* IN IOWA

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ABSTRACT

Foliar applications of fungicides and insecticides are implemented in soybeans to control foliar diseases, bean leaf beetle (*Cerotoma trifurcata*), and soybean aphid (*Aphis glycines*). The target insects are vectors of *Bean pod mottle virus* (BPMV) and *Soybean mosaic virus (*SMV), and could possibly interact in other ways to influence infection of soybeans by *Phomopsis* spp. There may be opportunities to integrate management of these interacting pests and pathogens; therefore, the effects of pesticide applications at R3 were assessed in field trials conducted over two years in five regions of Iowa. In 2008 treatments were: 1) untreated control, 2) trifloxystrobin + prothioconazole (Stratego Pro), 3) pyraclostrobin (Headline), 4) imidacloprid + cyfluthrin (Leverage 2.7), 5) combination of trifloxystrobin + prothioconazole with imidacloprid + cyfluthrin (Stratego Pro + Leverage 2.7). In 2009, the same treatments were used in addition to 6) esfenvalerate (Asana XL) and 7) combination of pyraclostrobin with esfenvalerate (Headline

+ Asana XL). Stem and seed infection by *Phomopsis* spp. and seed infection by BPMV and SMV were evaluated, along with soybean aphid populations, germination and yield. Fungicide applications inconsistently reduced infection by *Phomopsis* spp. in both years, but none of the treatments had a dual effect in reducing both stem and seed infection. Insecticide applications reduce soybean aphid populations, and seed infection by *Phomopsis* spp., SMV and BPMV, but in an inconsistent manner. Only the combination treatments increased yield in some locations. Results suggested that R3 applications targeted against soybean aphid and foliar diseases can have an added benefit by reducing infection by SMV and *Phomopsis* spp.

INTRODUCTION

Traditionally, the production of soybean (*Glycine max* Merr. (L.)) in the North central United States had been characterized by minimal control of pest and pathogens. However, this has changed in the last decade, as consequence of population outbreaks of native pests such as bean leaf beetle (*Cerotoma trifurcata* Förster) (17), and the introduction of two exotic pests, soybean aphids (*Aphis glycines* Matsumura) (29) and soybean rust (*Phakopsora pachyrhizi*) (32). Even though soybean rust is not yet an economic problem in the upper Midwest, annual yield losses have been predicted to reach 10 percent (8).

The use of fungicides on soybeans has been on the increase in the region, for implementation of management strategies to prevent the onset and spread of the soybean rust pathogen, and to control other several foliar and stem diseases (33). In Iowa, fungicide applications at growth stage R3 reduced severity of brown spot and anthracnose stem blight and increased yields compared to earlier applications (31). However, fungicide applications do not always translate into yield benefits (12, 33), and their effectiveness and profitability are highly dependent on weather conditions and disease pressure (8, 9).

Throughout the principal soybean growing areas in the North Central states, *A. glycines* has rapidly spread since it was found in Wisconsin in 2000 (29). This is the only aphid that colonizes soybeans (14) and it can be controlled through insecticide applications (26, 30). However, *A. glycines* has the ability to rapidly increase population densities and recover from early insecticide applications (25). In fact, Johnson et al. (15) reported that based on spatial-temporal variation of the density and distribution of *A. glycines*, preventative insecticide treatments are ineffective to economically manage *A. glycines* populations. Therefore, applications should be based on insect population thresholds (30).

Yield losses of up to 50% can be caused by *A. glycines*, by directly inducing plant stresses, or indirectly reducing seed quality and transmitting soybean viruses, such as *Soybean mosaic virus* (SMV) and *Alfalfa mosaic virus* (AMV) (7, 14, 19). In addition to *A. glycines*, SMV can be transmitted by more than 30 different species of migratory aphids and through seeds (13).

Negative impacts of SMV on soybeans can be intensified by co-infection with *Bean pod mottle virus* (BPMV), reducing yield and seed quality (5, 13). It has been reported that these two viruses can increase susceptibility to seed infection by *Phomopsis* spp. (1, 16). However, no information is available on the impact of virus infection on stem infection by *Phomopsis* spp.

Infection of soybean plants by *Phomopsis* spp. is also enhanced by plant stresses, including insect injury (18). Therefore, it is feasible that *A. glycines* control may have an impact on *Phomopsis* incidence, independent of SMV/*Phomopsis* interactions.

Pod and stem blight (caused by *Diaporthe phaseolorum* var. *sojae* (anamorph *P. phaseoli*) and Phomopsis seed decay (caused by *P. longicolla* T. W. Hobbs) are members of the *Diaporthe-Phomopsis* complex (22). These fungi are widespread throughout most of the soybean

producing areas in North America, and cause significant losses in yield and seed quality (22, 23, 35).

In previous studies lower incidence of seed infection by *Phomopsis* spp. was observed in cultivars with resistance to Phomopsis seed decay (24, 27) and SMV (16). However, in the case of SMV resistance, reduction of seed infection by *Phomopsis* spp. was more associated with the lack of SMV infection rather than a direct effect of SMV resistance (16).

It also has been shown that the Phomopsis seed decay can be controlled by late season fungicide applications, which prevent the movement of the fungi from pods to seeds (3, 34). Nevertheless, the timing of these applications is later than recommended for control of soybean rust and other foliar diseases (8, 9, 31). In addition, mixed results for insecticide effect on incidence of SMV have been reported (19, 28). Currently, applications of both fungicides and insecticides are used by soybean growers as integrated crop management techniques (31). However, the effect of these management tactics on infection by *Phomopsis* spp. has not yet been studied. The objective of this study was to assess the impact of fungicide and insecticide applications to control foliar diseases and *A. glycines* populations on infection of soybeans by *Phomopsis* spp., BPMV and SMV.

MATERIALS AND METHODS

Soybean seeds analyzed in this study were sampled from a pest management study conducted in 2008 and 2009, at six Iowa State University (ISU) research farms around Iowa. Soybean growth stage-based applications of fungicides (R1 or R3) were compared with applications of pesticides based on insect thresholds, to explain yield responses, foliar disease severity and aphid populations (31). Treatments evaluated in this study for their effect on infection by *Phomopsis* spp., BPMV and SMV, consisted of an untreated control and foliar

applications of fungicides, insecticides or combinations at growth stage R3, when pods were 5 mm long at one of the four uppermost nodes with a fully expanded trifoliate leaf (10).

In 2008, experiments were carried out at ISU Northwest Research and Demonstration Farm near Sutherland (O'Brien County, IA), ISU Southeast Research Farm near Crawfordsville (Washington County, IA), ISU Southwest Research and Demonstration Farm near Lewis (Pottawattamie County, IA) and ISU Agronomy Research Farm near Boone (Boone County, IA) in central Iowa. In 2009, experimental plots were established in the same northwest and southeast locations, in addition to the ISU Northeast Research and Demonstration Farm near Nashua (Floyd County, IA), ISU Neely-Kinyon Research and Demonstration Farm near Greenfield (Adair County, IA) and ISU Curtis farm near Ames (Story County, IA) in southwest and central Iowa, respectively. Subsequently these locations will be referred as the respective geographic area of Iowa in which the experiments were conducted: northwest, northeast, southwest, southeast and central Iowa.

A randomized complete block design with five to six replications was used at each location. Individual plots were 10.7 m long and 3.48 m wide with four rows in 2008, and 13 m long and 4.6 m wide with six rows in 2009. Among locations planting dates ranged from 12 to 22 May (2008), and 14 to 22 May (2009).

Treatments. Chemical products evaluated in 2008 and 2009 field trials were: strobilurin fungicide trifloxystrobin and triazole fungicide prothioconazole (Stratego Pro, Bayer CropScience, NC) at a rate of 0.036 kg of each a.i. ha^{-1} (4 oz $acre^{-1}$), strobilurin fungicide pyraclostrobin (Headline, BASF, NC) at a rate of 0.11 kg a.i. ha^{-1} (6 oz $acre^{-1}$), and neonicotinoid insecticide imidacloprid and pyrethroid insecticide cyfluthrin (Leverage 2.7, Bayer CropScience, NC) at a rate of 0.052 kg a.i. ha^{-1} imidacloprid and 0.037 kg a.i. ha^{-1} cyfluthrin (3.76 oz $acre^{-1}$).

In 2009, pyrethroid insecticide esfenvalerate (Asana XL, Dupont, Crop Protection, Wilmington, DE) at a rate of 0.056 kg a.i. ha^{-1} (9.6 oz $acre^{-1}$) was also used. In 2008 treatments were: 1) untreated control, 2) trifloxystrobin + prothioconazole (Stratego Pro), 3) pyraclostrobin (Headline), 4) imidacloprid + cyfluthrin (Leverage 2.7), 5) combination of trifloxystrobin + prothioconazole with imidacloprid + cyfluthrin (Stratego Pro + Leverage 2.7). In 2009, the same treatments were used in addition to 6) esfenvalerate (Asana XL) and 7) combination of pyraclostrobin with esfenvalerate (Headline + Asana XL). Other treatments were included in the pest management study but were not sampled for this study. Foliar treatments were applied in water using a CO_2 pressurized backpack sprayer and TeeJet (Springfield, IL) XR nozzles (XR 8003-VS), calibrated to deliver 180 l ha^{-1} at a pressure of 0.2 MPa. Applications were made to the center two (2008) and four (2009) rows, to minimize interplot interference between experimental units.

Yield and seed quality. For each plot, the center two (2008) or four (2009) rows were machine harvested at maturity, and yields were adjusted to a moisture content of 130 g kg^{-1}. For each replicate of each treatment germination was evaluated by warm and cold tests conducted at the ISU seed testing laboratory according to the Association of Official Seed Analysts (AOSA) rules (2). However, cold test results will not be discussed, due to the lack of significant differences between treatments or correlations with other variables.

Isolation of *Phomopsis* spp. from stems and seeds. From one location in 2008 (central Iowa) and three locations in 2009 (northwest, northeast, and southwest), stem infection by *Phomopsis* spp. was assessed at growth stage R6 (green stems, pods containing a green seed that fills the pod cavity at one of the four uppermost nodes with a fully developed trifoliate leaf) (10). Five plants were arbitrarily sampled from each plot, and stem plating was performed following

the procedure of Garzonio and McGee (11) with modifications. Stems were cut into sections (approximately 3 cm long), surface sterilized in 1% sodium hypochlorite for 1 min and rinsed in sterile-distilled water for 30 sec. Under a laminar flow hood using aseptic technique, stem sections were partitioned, and five pieces (approximately 1 cm each) from each plant were arbitrarily selected and plated on antibiotic-amended potato dextrose agar (200 mg streptomycin sulfate, 50 mg chlortetracycline hydrochloride, 120 mg neomycin sulfate, 39 g Difco PDA per liter) in 9-cm-diameter Petri dishes. After 5-7 days plates were inspected for colonies of *Phomopsis* spp. based on morphological characteristics of the fungi (18, 20). The percentage of infected stems was recorded for each plot.

After harvest, 400 seeds from each plot were tested by blotter test for *Phomopsis* spp. as described (21). Seeds were surface sterilized in 1% sodium hypochlorite for 30 sec, and rinsed in sterile-distilled water. Using aseptic techniques, seeds were placed on two layers of sterile blotters in plastic boxes measuring approximately 28 x 17 x 4 cm (Melmat Inc, Huntington Beach, CA). Blotters were previously moistened with dicloran at 500 $\mu g\ ml^{-1}$ (Botran 75W, Gowan, Yuma, AZ), to suppress the growth of *Rhizopus* spp. Four boxes containing 100 seeds each were prepared for each seed sample. These were incubated at $25^{o}C$ in the dark for 7 days, and seeds were inspected for *Phomopsis* spp., based on morphological characteristics of the fungi (18, 20). The percentage of infected seeds was recorded for each plot.

BPMV and SMV incidence. From the treatments that included insecticide applications alone and the untreated control, a sample of one hundred seeds per plot was evaluated for virus incidence. In 2008, seeds from combination of trifloxystrobin and prothioconazole with imidacloprid + cyfluthrin treatment were also tested. Either SMV or BPMV infection was determined by double antibody sandwich enzyme-linked immunosorbent assay (DAS ELISA),

using the respective Agdia antibodies and protocol for each virus (Agdia, Inc., Elkhart, IN) and modifications of previously described protocols (17). Seeds from each plot were divided into twenty groups of five seeds and placed in a plastic mesh bag (Agdia, Inc., Elkhart, IN). Agdia general extraction buffer was added to each bag based on seed weight in 1:10 ratio (weight of the sample:volume of buffer added), and these were incubated overnight at 4ºC. Soaked seeds were macerated with a pestle, and the extract was transferred to 1.5-ml-tubes and stored in the freezer. For each sample, 100 µl of seed sap extract was placed in wells on the pre-coated ELISA plate. Two positive and negative controls were prepared and placed in each plate. The plates were evaluated with a PowerWave™ Microplate Spectophotometer (Biotek®Instruments, Inc., Winooski, VT) set at 405 nm wavelength. Wells in which color develops indicate positive results. Sample wells were considered positive if absorbance values were higher than twice that of the negative controls. The estimated incidence of BPMV or SMV infected seeds in seed harvested from each plot was determined by using statistical methods for group testing as described by Block et al. (4). The result of positive and negative groups (5 seeds each) out of 20 was recorded and the overall proportion of infected seeds was calculated for each replicated. At least one positive sample (group) was required to estimate the proportion of infected seeds. Seed infection percentages were obtained by multiplying this proportion by 100.

Cumulative aphid days. Seasonal exposure of *A. glycines* was calculated as cumulative aphid days (CAD). This estimate is based on the number of aphids present on soybean plants between two sampling dates, as described by Johnson et al. (15).

Populations of *A. glycines* were assessed weekly, beginning in June (16 June, 2008 and 8 June, 2009). Counts were taken until mid-September, when plants senesced or until the aphid populations decreased for two consecutive weeks. Depending on the severity of the infestation,

five to twenty consecutive plants at randomly selected locations were chosen in each plot, and all aphids were counted on each plant.

In 2008 CAD was estimated for all treatments from four locations but southwest and southeast, where plots from the treatment that consisted of an application of pyraclostrobin were not assessed. In 2009 CAD was calculated for all treatments from northwest, northeast and central Iowa locations, and at the southwest and southeast location only the untreated control and the combination of pyraclostrobin with esfenvalerate were assessed.

Data analyses. Data collected from each year were analyzed separately by year due to the modifications in treatments and locations from 2008 to 2009. Treatment effects on percentage of stem and seed infection by *Phomopsis* spp., seed infection by BPMV and SMV, *A. glycines* populations (cumulative aphid days), percentage of seed germination and yield were tested in separate analysis for location due to significant treatment by location interaction (Table 1, 2). Data were analyzed using PROC GLM of SAS, ver. 9.2. Statistical effects of treatments were estimated based on analysis of variance (ANOVA), means were separated using Tukey's test and considered significantly different if $P \leq 0.05$. The normal and homogeneous distribution of residuals was examined using SAS PROC PLOT. To test relationships among all the variables, correlations were estimated using Pearson's linear correlation analysis (SAS PROC CORR).

RESULTS

Data from both years are presented in 5 tables corresponding to the respective geographic area of Iowa in which the experiments were conducted. These geographic areas are: northwest (Sutherland, 2008-2009), northeast (Nashua, 2009), southwest (Crawfordsville, 2008-2009),

southeast (Armstrong, 2008, Greenfield, 2009) and central Iowa (Boone, 2008, Ames, 2009) (Tables 3-7).

In both years, significant differences were observed between treatments for all the responses evaluated except to infection of seeds by SMV in 2008, yield in 2009, and infection of seeds by BPMV in both years (Tables 1, 2).

Overall, the highest incidence of seed infection by *Phomopsis* spp. was observed in northeast and southeast Iowa in 2009, while the highest incidence of stem infection was observed in 2008 in central Iowa. In general stem infection by *Phomopsis* spp. ranged from 2 to 81%, and it was higher than seed infection, which ranged from 0 to 11%. In addition, *A. glycines* populations (CAD) tended to be higher in 2008, whereas yields were higher in 2009 (Tables 3-7).

In northwest Iowa (Table 3), incidence of seed infection by *Phomopsis* spp. was very low in both years. In 2008 no significant differences were observed among treatments, while in 2009 seed infection by *Phomopsis* spp. was observed only in the untreated control and combination of trifloxystrobin + prothioconazole with imidacloprid + cyfluthrin. This treatment was also the only treatment that significantly reduced stem infection compared with the control. Treatments that included insecticide applications increased yield in 2008, reduced infection of seeds by SMV in 2009, and reduced *A. glycines* populations in both years. However, none of the treatments had an effect on infection of seeds by BPMV or germination in either year (Table 3).

In 2009 all treatments, except the application of pyraclostrobin alone, significantly reduced infection of seeds by *Phomopsis* spp. in northeast Iowa (Table 4). Application of trifloxystrobin + prothioconazole reduced stem infection by *Phomopsis* spp. by approximately 50% compared with the untreated control; however this effect was not statistically significant.

The insecticide imidacloprid + cyfluthrin reduced *A. glycines* populations per plant more than the rest of the treatments. There was no treatment effect for stem infection by *Phomopsis* spp., seed infection by SMV nor BPMV, yield or germination (Table 4).

In central Iowa (Table 5), all treatments that included fungicide applications showed significantly lower infection of stems by *Phomopsis* spp. than the untreated plants. In both years, none of the treatments significantly impacted seed infection by *Phomopsis* spp., with the exception of the application of imidacloprid + cyfluthrin in 2008 which increased seed infection. However, applications of this insecticide significantly reduced infection of seeds by SMV (0%) compared with the untreated control (2%). None of the treatments had an effect on infection of seeds by BPMV in either year. Combination of trifloxystrobin + prothioconazole with imidacloprid + cyfluthrin (2008) and applications of imidacloprid + cyfluthrin (2008, 2009) and esfenvalerate (2009), all reduced *A. glycines* densities compared with the untreated control. Yield was significantly increased by the application of imidacloprid + cyfluthrin in 2009, and the combinations of trifloxystrobin + prothioconazole with imidacloprid + cyfluthrin, and pyraclostrobin with esfenvalerate in 2008 and 2009, respectively. None of the treatments affected germination of seeds (Table 5).

In both years, none of the treatments reduced seed infection by *Phomopsis* spp. in southwest Iowa (Table 6), and, in 2009, only trifloxystrobin + prothioconazole reduced the infection of stems by *Phomopsis* spp. Although the highest incidence of BPMV (ranged from 37 to 42%) was observed at this location in 2008, applications of insecticides did not impact seed infection by BPMV in either year. In 2008, treatments had a negative effect increasing infection of seeds by SMV compared with the untreated control. Lower numbers of aphids were observed in 2008 in all treatments that included either applications of insecticide or fungicides. In 2009

populations of *A. glycines* were lower than 2008, and no differences were observed for aphid populations per plant between treatments. In 2009 applications of pyraclostrobin and both combinations of insecticide and fungicide significantly increased yield. In 2008, application of trifloxystrobin + prothioconazole increased germination of seeds, but no differences between treatments were observed in 2009 (Table 6).

In southeast Iowa (Table 7), although incidence of *Phomopsis* spp. was very low in both years, all treatments, except imidacloprid + cyfluthrin in 2008, significantly reduced the infection of seeds by the fungus compared with the untreated control. The application of imidacloprid + cyfluthrin significantly reduced seed infection by BPMV in 2008; however the same effect was not observed when this treatment was combined with an application of the fungicide trifloxystrobin + prothioconazole. Insecticide applications did not reduce seed infection by BPMV in 2009, nor SMV in 2008. Treatments that included insecticide applications significantly reduced *A. glycines* populations in 2008. Compared with the untreated control, in 2009 higher numbers of aphids were observed in the pyraclostrobin with esfenvalerate treatement. In both years, the combination of trifloxystrobin + prothioconazole with imidacloprid + cyfluthrin was the only treatment that significantly increased yield compared with the untreated control. In 2009, applications of pyraclostrobin and, trifloxystrobin + prothioconazole alone or in combination with imidacloprid + cyfluthrin, significantly increased germination of seeds; however, there were no differences among treatments for germination in 2008 (Table 7).

Only seed infection by BPMV and SMV presented a significant positive correlation in both years; other significant negative and positive correlations were inconsistently observed in this study (Table 8). For instance, yield was negatively correlated with CAD in 2008 and with infection of stems by *Phomopsis* spp. in 2009. In 2008, germination was negatively correlated

with BPMV and SMV, while in 2009 negatively correlated with infection of seeds and stems by *Phomopsis* spp. Additionally, in 2008 BPMV was negative correlated with CAD, while in 2009 SMV negatively correlated with infection of seeds by *Phomopsis* spp. Significant positive correlations were observed between CAD and germination in 2008, and between infection of seeds and stems by *Phomopsis* spp., and SMV with germination in 2009 (Table 8).

DISCUSSION

This study is the first to evaluate the impact of integrated management strategies for foliar diseases and *A. glycines* populations on infection of soybeans by seed-borne pathogens in Iowa. Late season fungicide applications have been previously recommended for control of *Phomopsis* spp. (3, 34), based on the fact that extensive infection of seed by *Phomopsis* spp. does not occur before R7 growth stage and only under certain weather conditions (3). In this study fungicide applications at R3 growth stage inconsistently reduced stem and seed infection in both years (Tables 3-7), but no treatments had reduced both stem and seed infection by *Phomopsis* spp.

Selection of the appropriate fungicide is essential to control infection of seeds by *Phomopsis* spp. Wrather et al. (34) reported that under early soybean production systems, foliar applications of azoxystrobin may increase Phomopsis seed decay, because of delayed maturity that prolongs the window for infection by *Phomopsis* spp. A similar mechanism has been proposed for the increase in susceptibility to infection by *Phomopsis* spp. on virus infected plants (1, 16). In the current study none of the fungicides increased infection of stems or seeds by *Phomopsis* spp. However, in one the application of insecticide imidacloprid + cyfluthrin increased seed infection by *Phomopsis* spp. in 2008 (Table 5) , but there is no explanation for this treatment effect.

The use of fungicides with different modes of action and a broad spectrum of activity, such as trifloxystrobin + prothioconazole should be considered while designing management strategies to control multiple diseases. In this study, this fungicide performed slightly better than the pyraclostrobin fungicide, reducing fungal infection of seeds by *Phomopsis* spp., but not necessarily its detrimental effect on seed germination (22). An increment in germination was only observed when trifloxystrobin + prothioconazole and pyraclostrobin reduced respectively stem and seed infection by *Phomopsis* spp. (Tables 6, 7).

On the other hand, pyraclostrobin was the only fungicide that increased yield when it was applied alone (Tables 3, 6). Although infection by *Phomopsis* spp. was negatively correlated with yield, the effect of fungicide increasing yield was not consistent. Recent studies have reported no significant increase in yield under low disease pressure due to fungicide applications (12, 33).

Although, low incidence of seed infection may have masked treatment effects, results obtained in this study suggest that earlier applications of fungicides may reduce seed infection by *Phomopsis* spp., and may also be useful to reduce stem and pod infection. Thus it is suggested that fungicide applications at growth stage R3 could have value for reducing the negative impact of fungi members of the *Diaporthe-Phomopsis* complex in seed quality and yield (22, 23, 35) under higher disease pressure. These applications can also have value for control of soybean top dieback, which causes premature senescence during reproductive stages and has been associated with members of this disease complex (36).

Consistent with earlier findings, insecticide applications reduce *A. glycines* populations (26, 30). A reduction in SMV was inconsistently observed in two locations when these applications also reduced *A. glycines* populations. These results are in agreement with other

researchers reporting inconsistent effects of insecticide treatments to control *A. glycines* on incidence of SMV (19, 28).

Aphis glycines is an effective vector of SMV (7, 19) and induces plant stresses (6, 19) which could enhance infection by *Phomopsis* spp. (18). Consequently, it was hypothesized that infection by *Phomopsis* spp. could be impacted when *A. glycines* populations and their detrimental effects are reduced. In this study, single applications of the fungicides trifloxystrobin + prothioconazole and pyraclostrobin sporadically reduced *A. glycines* populations (Tables 3, 6). However, this effect did not impact seed infection by *Phomopsis* spp. and no evidence that *A. glycines* colonization of soybeans increases susceptibility to *Phomopsis* infection was observed.

Another mechanism, through which insecticide applications could reduce infection of seeds by *Phomopsis* spp., is the reduction of *C. trifurcata* populations and their respective injury of pods that allows secondary infection by fungal pathogens such as *Phomopsis* spp. (17, 18). However, in this study neither feeding injury nor populations of *C. trifurcata* were quantified. None of the treatments included in this study were timed to maximize effects on *C. trifurcata*, because *A. glycines* was the primary target insect, and it has been reported that management strategies to control *C. trifurcata* have limited value for *A. glycines* management (15).

Even though there was a significant positive correlation between SMV and BPMV infections of seeds in both years, incidences were low and no sign of a synergistic effect was observed. The application of imidacloprid + cyfluthrin reduced BPMV infection of seeds but only in one location in one year (Table 7). However, insecticide treatments used in this study were different than that previously reported to reduced BPMV incidence and seed coat mottling (17). In a similar study, Pedersen et al. (28) found that because of different phenologies of the

insect vectors, BPMV and SMV management cannot be integrated through application of insecticide treatments.

Combination treatments tended to have the same effect as the single applications of the respective fungicide or insecticide. For instance in central Iowa, applications of trifloxystrobin + prothioconazole, and imidacloprid + cyfluthrin alone or in combination resulted in reduction of infection of stems by *Phomopsis* spp., seed infection by SMV and *A. glycines* populations. However, an increased in yield was only observed in the combination treatment. It could be suggested that the combined effect on the two pathogens and the aphid populations, in turn resulted in the higher yields (Table 5).

These results suggest that under the conditions of this study, R3 applications targeted against *A. glycines* and foliar diseases can have an added benefit by reducing infection by SMV and *Phomopsis* spp. However, the effectiveness of these applications will vary based on the magnitude of the insect vector density and disease pressure. The profitability of these techniques was not analyzed in this study, but results obtained here will be complemented with data from foliar disease incidence.

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Tables

Table 1. Analysis of variance for location and treatment effect on stems and seeds infected by *Phomopsis* spp., seeds infected by *Soybean mosaic virus* (SMV) and *Bean pod mottle virus* (BPMV), soybean aphid (*Aphis glycines*) populations, yield and seed germination in Iowa in 2008[+]*.

			P value	
Response evaluated	Block	Location	Treatment	Location × Treatment
Phomopsis infected seeds	0.5170	<.0001	<.0001	<.0001
Phomopsis infected stems[&]	0.3320	-	<.0001	-
SMV infected seeds	0.0053	<.0001	0.9620	0.0003
BPMV infected seeds	0.6052	<.0001	0.3560	0.2049
Cumulative aphids days	0.3724	<.0001	<.0001	<.0001
Yield	0.2462	<.0001	<.0001	0.0005
Germination-warm test	0.0389	<.0001	<.0001	0.0047

[+]Locations: ISU Northwest Research and Demonstration Farm near Sutherland (O'Brien County; IA), ISU Southeast Research Farm near Crawfordsville (Washington County, IA), ISU Southwest Research and Demonstration Farm near Lewis (Pottawattamie County, IA) and ISU Agronomy Research Farm near Boone (Boone County, IA).
*Treatment: consisted of an untreated control and foliar applications of fungicides, insecticides or combinations at growth stage R3. Chemical products were: trifloxystrobin + prothioconazole (Stratego Pro, 0.036 kg of each a.i. ha^{-1}), pyraclostrobin (Headline, 0.11 kg a.i. ha^{-1}), imidacloprid + cyfluthrin (Leverage 2.7, 0.052 kg a.i. ha^{-1} and 0.037 kg a.i. ha^{-1}, respectively) and trifloxystrobin + prothioconazole with imidacloprid + cyfluthrin.
[&]Stem infection was only assessed at the central Iowa location.

Table 2. Analysis of variance for location and treatment effect on stems and seeds infected by *Phomopsis* spp., seeds infected by *Soybean mosaic virus* (SMV) and *Bean pod mottle virus* (BPMV), soybean aphid (*Aphis glycines*) populations, yield and seed germination in Iowa in 2009 [+]*.

			P value	
Response evaluated	Block	Location	Treatment	Location × Treatment
Phomopsis infected stems	0.9543	<.0001	<.0001	0.3433
Phomopsis infected seeds	0.5541	<.0001	<.0001	<.0001
SMV infected seeds	0.0866	<.0001	<.0001	0.0088
BPMV infected seeds	0.0740	<.0001	0.0908	0.0322
Cumulative aphids days	0.4873	<.0001	<.0001	0.0577
Yield	0.4921	<.0001	0.0167	0.1039
Germination-warm test	0.0525	<.0001	<.0001	0.0042

[+]Locations: ISU Northwest Research and Demonstration Farm near Sutherland (O'Brien County; IA), ISU Southeast Research Farm near Crawfordsville (Washington County, IA), ISU Northeast Research and Demonstration Farm near Nashua (Floyd County, IA), ISU Neely-Kinyon Research and Demonstration Farm near Greenfield (Adair County, IA) and ISU Curtis farm near Ames (Story County, IA).

*Treatment: consisted of an untreated control and foliar applications of fungicides, insecticides or combinations at growth stage R3. Chemical products used were: trifloxystrobin + prothioconazole (Stratego Pro, 0.036 kg of each a.i. ha^{-1}), pyraclostrobin (Headline, 0.11 kg a.i. ha^{-1}), imidacloprid + cyfluthrin (Leverage 2.7, 0.052 kg a.i. ha^{-1} and 0.037 kg a.i. ha^{-1}, respectively), esfenvalerate (Asana XL, 0.056 kg a.i. ha^{-1}), trifloxystrobin + prothioconazole with imidacloprid + cyfluthrin, and pyraclostrobin with esfenvalerate.

Table 3. Insecticide and fungicide treatment effects on stems and seeds infected by *Phomopsis* spp., seeds infected by *Soybean mosaic virus* (SMV) and *Bean pod mottle virus* (BPMV), soybean aphid (*Aphis glycines*) populations, yield and seed germination in northwest Iowa in 2008 and 2009[+&].

		Phomopsis spp. infection		Seed infection		Cumulative		
Year	Treatment*	Stems (%)	Seeds (%)	SMV (%)	BPMV (%)	aphids days (CAD)	Yield (kg/ha)	Germination (%)
2008	Untreated	-	0.5 a	0.7 a	4.4 a	92281 a	2398 c	98.3 a
	Triflox+prothio	-	0.4 a	-	-	60877 ab	2704 cb	97.8 a
	Pyraclostrobin	-	0.2 a	-	-	52087 b	2887 b	98.3 a
	Imidac+cyflut	-	0.1 a	0.6 a	4.4 a	17472 c	3723 a	96.4 a
	Triflox+prothio + imidac+cyflut	-	0.3 a	0.2 a	4.6 a	9062 c	3879 a	97.2 a
2009	Untreated	22.0 a	0.2 a	7.2 a	5.2 a	17696 a	3749 a	98.0 a
	Triflox+prothio	6.0 ab	0.0 b	-	-	25087 a	3518 a	98.0 a
	Pyraclostrobin	19.0 ab	0.0 b	-	-	16612 a	3924 a	98.5 a
	Imidac+cyflut	16.0 ab	0.0 b	3.5 b	3.2 a	1679 b	4020 a	98.5 a
	Triflox+prothio + imidac+cyflut	3.0 b	0.1 ab	-	-	3660 b	3446 a	98.5 a
	Esfenvalerate	15.0 ab	0.0 b	2.6 b	5.3 a	3091 b	4010 a	97.0 a
	Pyraclostrobin + esfenvalerate	16.0 ab	0.0 b	-	-	4142 b	3888 a	97.5 a

[+]Within each year, means labeled with the same later were not significantly different according to Tukey's test considered significantly different at $P \leq 0.05$.

[&]Experiments were conducted in both years at ISU Northwest Research and Demonstration Farm near Sutherland (O'Brien County; IA).

*Treatment: consisted of an untreated control and foliar applications of fungicides, insecticides or combinations at growth stage R3. Chemical products used were: trifloxystrobin + prothioconazole (Stratego Pro, 0.036 kg of each a.i. ha^{-1}), pyraclostrobin (Headline, 0.11 kg a.i. ha^{-1}), imidacloprid + cyfluthrin (Leverage 2.7, 0.052 kg a.i. ha^{-1} and 0.037 kg a.i. ha^{-1}, respectively), esfenvalerate (Asana XL, 0.056 kg a.i. ha^{-1}), trifloxystrobin + prothioconazole with imidacloprid + cyfluthrin, and pyraclostrobin with esfenvalerate.

(-) Data were not collected for specific treatments.

Table 4. Insecticide and fungicide treatment effects on stems and seeds infected by *Phomopsis* spp., seeds infected by *Soybean mosaic virus* (SMV) and *Bean pod mottle virus* (BPMV), soybean aphid (*Aphis glycines*) populations, yield and seed germination in northeast Iowa in 2009[+&].

		Phomopsis spp. infection		Seed infection		Cumulative		
Year	Treatment*	Stems (%)	Seeds (%)	SMV (%)	BPMV (%)	aphids days (CAD)	Yield (kg/ha)	Germination (%)
2009	Untreated	28.0 a	10.9 a	2.1 a	0.5 a	21895 a	4090 a	94.0 a
	Triflox+prothio	13.0 a	3.9 cd	-	-	20683 a	4340 a	96.0 a
	Pyraclostrobin	36.0 a	8.2 ab	-	-	21186 a	4167 a	93.3 a
	Imidac+cyflut	27.0 a	4.9 bcd	1.0 a	0.8 a	4152 b	3846 a	94.8 a
	Triflox+prothio + imidac+cyflut	25.0 a	2.3 d	-	-	3393 b	4280 a	96.0 a
	Esfenvalerate	33.0 a	6.2 bc	1.0 a	1.0 a	19544 a	4128 a	92.0 a
	Pyraclostrobin + esfenvalerate	18.0 a	6.0 bc	-	-	14597 ab	4335 a	94.0 a

[+]Within each year, means labeled with the same later were not significantly different according to Tukey's test considered significantly different at $P \leq 0.05$.
[&]Experiments were conducted in 2009 at ISU Northeast Research and Demonstration Farm near Nashua (Floyd County, IA).
*Treatment: consisted of an untreated control and foliar applications of fungicides, insecticides or combinations at growth stage R3. Chemical products used were: trifloxystrobin + prothioconazole (Stratego Pro, 0.036 kg of each a.i. ha^{-1}), pyraclostrobin (Headline, 0.11 kg a.i. ha^{-1}), imidacloprid + cyfluthrin (Leverage 2.7, 0.052 kg a.i. ha^{-1} and 0.037 kg a.i. ha^{-1}, respectively), esfenvalerate (Asana XL, 0.056 kg a.i. ha^{-1}), trifloxystrobin + prothioconazole with imidacloprid + cyfluthrin, and pyraclostrobin with esfenvalerate.
(-) Data were not collected for specific treatments.

Table 5. Insecticide and fungicide treatment effects on stems and seeds infected by *Phomopsis* spp., seeds infected by *Soybean mosaic virus* (SMV) and *Bean pod mottle virus* (BPMV), soybean aphid (*Aphis glycines*) populations, yield and seed germination in central Iowa in 2008 and 2009[+&].

		Phomopsis spp. infection		Seed infection		Cumulative		
		Stems	Seeds	SMV	BPMV	aphids days	Yield	Germination
Year	Treatment*	(%)	(%)	(%)	(%)	(CAD)	(kg/ha)	(%)
2008	Untreated	80.8 a	2.7 b	1.7 a	6.7 a	36796 a	3582 b	98.0 a
	Triflox+prothio	29.2 c	1.2 b	-	-	26024 a	4149 ab	98.0 a
	Pyraclostrobin	43.2 b	1.3 b	-	-	28567 a	4252 ab	97.6 a
	Imidac+cyflut	-	4.6 a	0.0 b	7.6 a	4620 b	4439 ab	96.0 a
	Triflox+prothio + imidac+cyflut	26.4 c	2.0 b	0.0 b	9.7 a	1547 b	4846 a	97.3 a
2009	Untreated	-	0.1 a	-	1.8 a	11910 a	3907 b	93.5 a
	Triflox+prothio	-	0.0 a	-	-	22094 a	4044 ab	93.8 a
	Pyraclostrobin	-	0.0 a	-	-	21637 a	4243 ab	92.8 a
	Imidac+cyflut	-	0.0 a	-	1.0 a	6262 b	4533 ab	94.0 a
	Triflox+prothio + imidac+cyflut	-	0.0 a	-	-	8718a b	4718 a	95.3 a
	Esfenvalerate	-	0.0 a	-	1.8 a	2923 b	4440 ab	95.5 a
	Pyraclostrobin + esfenvalerate	-	0.1 a	-	-	8040 ab	4715 a	93.3 a

[+]Within each year, means labeled with the same later were not significantly different according to Tukey's test considered significantly different at $P \leq 0.05$.

[&]Experiments were conducted in 2008 at ISU Agronomy Research Farm near Boone (Boone County, IA) and in 2009 at ISU Curtis farm near Ames (Story County, IA).

*Treatment: consisted of an untreated control and foliar applications of fungicides, insecticides or combinations at growth stage R3. Chemical products used were: trifloxystrobin + prothioconazole (Stratego Pro, 0.036 kg of each a.i. ha^{-1}), pyraclostrobin (Headline, 0.11 kg a.i. ha^{-1}), imidacloprid + cyfluthrin (Leverage 2.7, 0.052 kg a.i. ha^{-1} and 0.037 kg a.i. ha^{-1}, respectively), esfenvalerate (Asana XL, 0.056 kg a.i. ha^{-1}), trifloxystrobin + prothioconazole with imidacloprid + cyfluthrin, and pyraclostrobin with esfenvalerate.

(-) Data were not collected for specific treatments.

Table 6. Insecticide and fungicide treatment effects on stems and seeds infected by *Phomopsis* spp., seeds infected by *Soybean mosaic virus* (SMV) and *Bean pod mottle virus* (BPMV), soybean aphid (*Aphis glycines*) populations, yield and seed germination in southwest Iowa in 2008 and 2009[+&].

		Phomopsis spp. infection		Seed infection		Cumulative		
Year	Treatment*	Stems (%)	Seeds (%)	SMV (%)	BPMV (%)	aphids days (CAD)	Yield (kg/ha)	Germination (%)
2008	Untreated	-	1.5 a	1.7 b	42.4 a	12832 a	3265 a	91.4 bc
	Triflox+prothio	-	1.0 a	-	-	3994 b	3456 a	94.6 a
	Pyraclostrobin	-	1.2 a	-	-	-	3377 a	92.6 ab
	Imidac+cyflut	-	1.7 a	3.7 a	39.1 a	2087 b	3613 a	89.0 c
	Triflox+prothio + imidac+cyflut	-	0.7 a	3.7 a	36.5 a	1217 b	3584 a	93.8 ab
2009	Untreated	23.0 a	1.7 a	-	4.2 a	1052 a	4576 b	94.3 a
	Triflox+prothio	2.0 b	0.3 a	-	-	-	4729 ab	95.3 a
	Pyraclostrobin	16.0 ab	1.1 a	-	-	-	5079 a	94.3 a
	Imidac+cyflut	11.0 ab	0.8 a	-	3.8 a	-	4815 ab	93.5 a
	Triflox+prothio + imidac+cyflut	8.0 ab	0.2 a	-	-	-	5100 a	95.5 a
	Esfenvalerate	14.0 ab	1.3 a	-	9.6 a	-	4801 ab	92.5 a
	Pyraclostrobin + esfenvalerate	17.0 ab	0.7 a	-	-	744 a	5165 a	93.8 a

[+]Within each year, means labeled with the same later were not significantly different according to Tukey's test considered significantly different at $P \leq 0.05$.

[&]Experiments were conducted in 2008 at ISU Southwest Research and Demonstration Farm near Lewis (Pottawattamie County, IA) and in 2009 at ISU Neely-Kinyon Research and Demonstration Farm near Greenfield (Adair County, IA).

*Treatment: consisted of an untreated control and foliar applications of fungicides, insecticides or combinations at growth stage R3. Chemical products used were: trifloxystrobin + prothioconazole (Stratego Pro, 0.036 kg of each a.i. ha^{-1}), pyraclostrobin (Headline, 0.11 kg a.i. ha^{-1}), imidacloprid + cyfluthrin (Leverage 2.7, 0.052 kg a.i. ha^{-1} and 0.037 kg a.i. ha^{-1}, respectively), esfenvalerate (Asana XL, 0.056 kg a.i. ha^{-1}), trifloxystrobin + prothioconazole with imidacloprid + cyfluthrin, and pyraclostrobin with esfenvalerate.

(-) Data were not collected for specific treatments.

Table 7. Insecticide and fungicide treatment effects on seeds infected by *Phomopsis* spp., *Soybean mosaic virus* (SMV) and *Bean pod mottle virus* (BPMV), soybean aphid (*Aphis glycines*) populations, yield and seed germination in southeast Iowa in 2008 and 2009[+&].

		Seed infection			Cumulative		
		Phomopsis spp.	SMV	BPMV	aphids days	Yield	Germination
Year	Treatment*	(%)	(%)	(%)	(CAD)	(kg/ha)	(%)
2008	Untreated	2.2 a	1.7 a	12.4 a	583 a	4459 b	97.2 a
	Triflox+prothio	0.3 b	-	-	412 a	4590 ab	95.8 a
	Pyraclostrobin	0.9 b	-	-	-	4777 ab	97.6 a
	Imidac+cyflut	1.3 ab	1.7 a	6.9 b	156 b	4655 ab	95.0 a
	Triflox+prothio + imidac+cyflut	0.5 b	1.9 a	11.8 a	159 b	4988 a	94.4 a
2009	Untreated	8.9 a	-	3.9 a	32 b	3879 a	85.8 b
	Triflox+prothio	3.3 cd	-	-	-	4129 a	91.0 a
	Pyraclostrobin	5.8 b	-	-	-	3868 a	91.8 a
	Imidac+cyflut	3.8 cbd	-	1.6 a	-	3917 a	88.0 ab
	Triflox+prothio + imidac+cyflut	2.6 d	-	-	-	4017 a	91.7 a
	Esfenvalerate	4.8 cb	-	3.5 a	-	4060 a	86.0 b
	Pyraclostrobin + esfenvalerate	2.3 d	-	-	50 a	4080 a	89.8 ab

[+]Within each year, means labeled with the same later were not significantly different according to Tukey's test considered significantly different at $P \leq 0.05$.

[&]Experiments were conducted in both years at ISU Southeast Research Farm near Crawfordsville (Washington County, IA).

*Treatment: consisted of an untreated control and foliar applications of fungicides, insecticides or combinations at growth stage R3. Chemical products used were: trifloxystrobin + prothioconazole (Stratego Pro, 0.036 kg of each a.i. ha^{-1}), pyraclostrobin (Headline, 0.11 kg a.i. ha^{-1}), imidacloprid + cyfluthrin (Leverage 2.7, 0.052 kg a.i. ha^{-1} and 0.037 kg a.i. ha^{-1}, respectively), esfenvalerate (Asana XL, 0.056 kg a.i. ha^{-1}), trifloxystrobin + prothioconazole with imidacloprid + cyfluthrin, and pyraclostrobin with esfenvalerate.

(-) Data were not collected for specific treatments.

Table 8. Linear correlations among stems and seeds infected by *Phomopsis* spp., seeds infected by *Soybean mosaic virus* (SMV) and *Bean pod mottle virus* (BPMV), soybean aphid (*Aphis glycines*) populations, yield and seed germination in Iowa in 2008 and 2009[+&].

Year	Variable	*Phomopsis* spp. infected stems	SMV	BPMV	CAD*	Yield	Germination
2008	*Phomopsis* spp. infected seeds	-	0.03 0.8295 53	-0.03 0.8519 53	-0.17 0.1121 84	0.18 0.0831 91	-0.07 0.5329 93
	SMV	-	-	0.59 <.0001 53	-0.20 0.1402 56	-0.02 0.8910 52	-0.5 0.0001 53
	BPMV	-	-	-	-0.26 0.0500 56	-0.25 0.0738 52	-0.73 <.0001 53
	CAD	-	-	-	-	-0.70 <.0001 84	0.42 <.0001 84
	Yield	-	-	-	-	-	0.08 0.4544 91
2009	*Phomopsis* spp. infected stems	-	-0.24 0.2430 24	-0.21 0.2207 36	0.12 0.3562 64	-0.07 0.5060 84	-0.34 0.0014 84
	Phomopsis spp. infected seeds	0.52 <.0001 84	-0.47 0.0211 24	-0.2 0.1325 60	0.15 0.1335 100	-0.19 0.0285 140	-0.46 <.0001 140
	SMV	-	-	0.91 <.0001 24	0.03 0.8801 24	-0.23 0.2689 24	0.52 0.0092 24
	BPMV	-	-	-	-0.06 0.7006 44	0.17 0.1984 60	-0.01 0.9037 60
	CAD	-	-	-	-	-0.12 0.2393 100	0.09 0.3538 100
	Yield	-	-	-	-	-	-0.05 0.5358 140

[+]Top value is Pearson's linear correlation coefficient; middle value is *P* value; bottom value is number of observations (SAS PROC CORR).

[&]Experiments conducted at ISU Northwest Research and Demonstration Farm near Sutherland (O'Brien County; IA), ISU Southeast Research Farm near Crawfordsville (Washington County, IA), ISU Southwest Research and Demonstration Farm near Lewis (Pottawattamie County, IA), ISU Neely-Kinyon Research and Demonstration Farm near Greenfield (Adair County, IA), ISU Agronomy Research Farm near Boone (Boone County, IA) and ISU Curtis farm near Ames (Story County, IA) in central Iowa, and ISU Northeast Research and Demonstration Farm near Nashua (Floyd County, IA).

*CAD, cumulative aphid days.

CHAPTER 6
GENERAL CONCLUSIONS

The objectives of this research were to understand interactions between *Phomopsis* spp. and seedborne viruses of soybean and study the role that insect vectors may have increasing *Phomopsis* infection. In Iowa, *Phomopsis* spp., BPMV and SMV have been prevalent in some years, depending on weather conditions during key periods. Recently the frequent detection of *Phomopsis* spp. in stems coincided with a resurgence in bean leaf beetle (*Cerotoma trifurcata* Förster), populations and BPMV symptoms. Results included in this thesis correspond to different studies designed to evaluate the effect of *Bean pod mottle virus* and *Soybean mosaic virus* on susceptibility of soybean plants to infection by *Phomopsis* spp., and the impacts of combined management practices currently used on soybean production.

Data from greenhouse experiments show that BPMV can increase susceptibility to seed infection by *P. longicolla* in plants of two different cultivars, in which virus infection did not induce the same response. Inoculated plants of the cultivar 92M02 displayed typical BPMV foliar symptoms, seed coat mottling and a delay in maturity, while in Spansoy 201 only foliar symptoms were observed. However, BPMV infection enhanced *P. longicolla* seed infection in both cultivars. Therefore, we concluded that BPMV induced predisposition to *P. longicolla* seed infection and this effect is not due solely to prolonging seed maturation. Unlike previous studies, the effect of BPMV on incidence of *P. longicolla* seed infection observed in this study was completely independent from the effects that beetle vectors of BPMV can have in pod and seed infection by *Phomopsis* spp. In the SMV-*Phomopsis* experiments, inoculation with the SMV-G2 strain did not increase the incidence of *P. longicolla* seed infection in either of the soybean

cultivars tested (Colfax and Spansoy 201), which suggested that SMV- *P. longicolla* relationship may be cultivar- and strain-dependent.

During the years when this research was conducted, Iowa experienced extremely severe winters; and predictive models have suggested the occurrence of very high bean leaf beetle winter mortality and low risk for BPMV incidence. Moreover, in 2008 a low incidence of seed infection by *Phomopsis* spp. was observed in some of the experiments. This low disease pressure could have been a consequence of the late planting due to the heavy early season rain, which caused the period of maximum susceptibility of seeds to occur under dry and cool conditions. In general, these situations may have obscured treatment effects.

Results from field experiments show that applications of fungicides and insecticides reduced disease incidence, insect populations and their negative effects on soybean plants. In studies aimed to evaluate the impact of insect vector management strategies on infection of seedborne viruses and *Phomopsis* spp., insecticide applications reduced beetle feeding injury of leaves and pods and plant exposure to soybean aphids. These treatments in combination with other management strategies also reduced seed and stem infection by BPMV and *Phomopsis* spp., respectively. In addition to the known effect that feeding injury of pods has on reducing seed quality, our results suggest that bean leaf beetle may also increase secondary stem infection by *Phomopsis* spp. However, this study found no relationship between insect pod injury and seed infection by *Phomopsis* spp. Low beetle populations associated with the severe winters experienced lately may have limited the impact of insect management strategies on interactions with *Phomopsis* spp. On the other hand, although populations of *C. trifurcata* were low in both years and seedcoat mottling was not observed, samples from all treatments tested positive for BPMV at a low incidence level. The use of virus resistance or insecticide treatments alone was

ineffective for reducing BPMV incidence of seeds compared with controls. However, when these strategies were combined, BPMV incidence was significantly reduced, suggesting that resistance mechanisms should be combined with chemical treatments in vector-virus management programs to enhance individual control effects. The BPMV-tolerant cultivar used in 2008 had reduced BPMV incidence, but its agronomic performance was poor. This emphasizes the need for incorporating virus resistance traits into high-yielding adapted cultivars. In general, management techniques aimed to control soybean aphids did not affect *Phomopsis* spp. infection, and we did not observe any evidence that aphid colonization of soybeans increases susceptibility to *Phomopsis* infection, or that *A. glycines* management strategies have any added benefit related to *Phomopsis* spp.

Even though timing was earlier than that previously recommended for *Phomopsis* control, current practices involving fungicide applications at growth stage R3 or R5 targeted to control foliar and stem diseases, can have some benefits on seed quality by reducing *Phomopsis* infection of stems or seeds. In addition, when this approach was complemented with insecticide applications, yield enhancement was observed. This effect might be related to suppression of aphid populations and infection by *Phomopsis* spp. or other fungal diseases. Therefore, it appears that applications targeted against soybean aphid and foliar diseases can have an added benefit by reducing *Phomopsis* spp. infection.

However, caution should be taken when implementing these results. Compared with untreated controls, applications of fungicides and insecticides reduced disease incidence and insect injury. These effects did not always result in an increase in yield, so the economic justification for these applications may not have been sufficient. The need and effectiveness of these strategies depends on environmental conditions, disease pressure and insect population

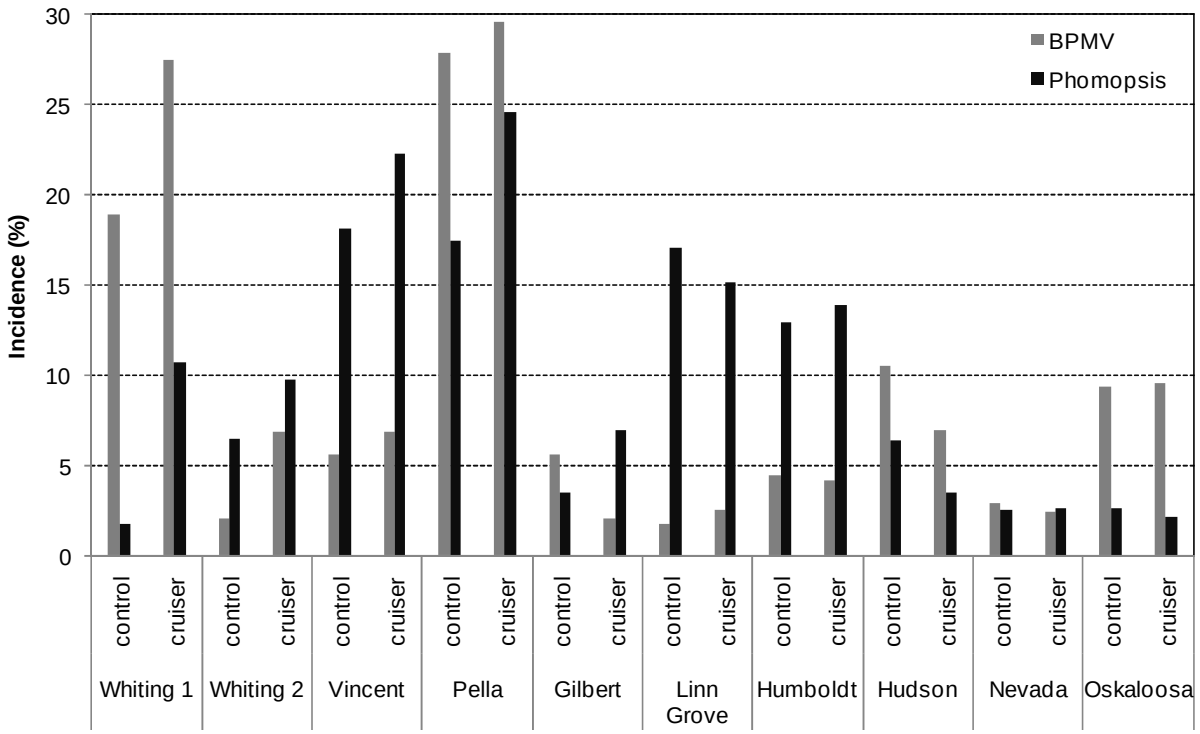
dynamics; therefore, they should be taken into consideration while designing management programs.

APPENDIX

Appendix 1. Analysis of variance for location and insecticide seed treatment effect on *Phomopsis* spp. and *Bean pod mottle virus* (BPMV) infection of seeds in Iowa[&+*].

Response evaluated	*P* value		
	Location	Treatment	Location x Treatment
Phomopsis infection seeds	<.0001	0.3353	0.8834
BPMV infection seeds	<.0001	0.7930	0.7111

[&]Data from insecticide seed treatment trials conducted in 2006 and 2007.
[+]Locations: Whiting (Monona Co.), Vincent (Webster Co.), Pella (Marion Co.), Gilbert (Story Co.), Linn Grove (Buena Vista Co.), Humboldt (Humboldt Co.), Hudson (Black Hawk Co.), Nevada (Story Co.) and Oskaloosa (Mahaska Co.).
*Treatment: insecticide seed treatment of Cruiser 5FS (0.5 gr a.i. per kg of seed) and untreated.



Appendix 2. Percentage of *Phomopsis* spp. and *Bean pod mottle virus* (BPMV) infection of seeds with and without insecticide seed treatment from ten different locations in Iowa (2006-2007) [&+*].

[&]Insecticide treatment consisted of seed treatment of Cruiser 5FS (thiametoxam, 0.5 gr a.i. per kg of seed).
[+]Locations: Whiting (Monona Co.), Vincent (Webster Co.), Pella (Marion Co.), Gilbert (Story Co.), Linn Grove (Buena Vista Co.), Humboldt (Humboldt Co.), Hudson (Black Hawk Co.), Nevada (Story Co.) and Oskaloosa (Mahaska Co.).
*No significant differences between insecticide seed treatment for seed infection by *Phomopsis* spp. or BPMV.

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