Proceedings of the Integrated Crop Management Conference

Proceedings of the 22nd Annual Integrated Crop Management Conference

Dec 1st, 12:00 AM

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Soto-Arias, J. P.; Robertson, Alison; O'Neal, Matthew E.; and Munkvold, Gary, "Virus-Phomopsis interactions on soybean and the effects of insect and disease management practices" (2010). *Proceedings of the Integrated Crop Management Conference*. 21.
https://lib.dr.iastate.edu/icm/2010/proceedings/21

Virus – Phomopsis interactions on soybean and the effects of insect and disease management practices

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Introduction

Soybean (*Glycine max* (L.) Merr.) is affected by several seed-borne pathogens that reduce seed quality, affecting both marketability (Gergerich, 1999; Koning et al. 2001) and germination (Sinclair, 1999). One of the most common seedborne diseases is *Phomopsis* seed decay, primarily caused by *Phomopsis longicolla* T. W. Hobbs, a member of the *Diaporthe-Phomopsis* complex (Sinclair, 1999). Fungi of this complex are widespread throughout most of the soybean producing areas around the world, and the biggest impacts on seed and grain quality are physical damage, reduction in germination, alteration in protein content and reduction of oil quality (Meriles et al., 2004; Sinclair, 1999).

Bean pod mottle virus (BPMV) and Soybean mosaic virus (SMV) are the most common viral diseases of soybean, and both can cause seed coat mottling, that has a negative impact on the marketability of seeds and food-grade soybean (Gergerich, 1999; Hill, 1999). Both viruses can be transmitted through seed, and also can be transmitted by different insect vectors (Gergerich, 1999; Hill, 1999). Bean leaf beetle (*Cerotoma trifurcata* Förster) is the main vector of BPMV (Gergerich, 1999), and can also cause feeding damage, which leads to damage by molds, including *Phomopsis* spp. Soybean aphid (*Aphis glycines* Matsumura) is one of the vectors of SMV(Hill 1999), and also cause plant stress by removing photosynthates and reducing photosynthesis.

In Iowa, *Phomopsis* spp., BPMV and SMV have been prevalent in some years, depending on weather conditions during key periods (Yang, 1999). Recently the frequent detection of *P. longicolla* in stems collected during the Iowa soybean disease survey conducted from 2005-2007 (Lu et al., 2010) coincided with high soybean aphid densities, a resurgence in bean leaf beetle populations (Bradshaw et al., 2006) and BPMV symptoms (Hill et al., 2006).

Several reports suggest that seeds from plants infected by either SMV or BPMV are more susceptible to infection by *Phomopsis* spp. (Abney and Ploper, 1994; Hepperly et al., 1979; Koning et al., 2001). However, the mechanism for this predisposition is unclear. One proposed mechanism is that virus infection prolongs plant maturity, and therefore, extends the exposure of pods and seeds to infection by *Phomopsis* spp. (Abney and Ploper, 1994; Koning et al., 2001). Although this mechanism has been proposed, none of the published studies has directly tested this hypothesis.

It has been shown that the *Phomopsis* seed decay can be controlled by late season fungicide applications (Wrather et al., 2004); and well-timed insecticide applications can considerably reduce population densities of the *C. trifurcata* (Bradshaw et al., 2008; Krell et al., 2004) and *A. glycines* (Ohnesorg et al., 2009). However, the effectiveness of these strategies to control virus infection has been inconsistent (Bradshaw et al., 2008; Krell et al., 2004), and the effect of these management tactics on infection by *Phomopsis* spp. has not yet been studied. The objectives of this study were to assess the effects of virus infection on susceptibility of soybean plants to *Phomopsis longicolla*, and the impacts of combined management practices currently used on soybean production.

Materials and methods

Viruses-Phomopsis interactions

Greenhouse studies were established to determine the effects of virus infection on susceptibility of soybean plants to infection by *Phomopsis longicolla* at different growth stages. Three Maturity Group II soybean cultivars (Spansoy 201, Colfax and 92M02) were inoculated with BPMV at growth stage V2-V3, and with P. longicolla at R3, R5, or R7. The effect of SMV inoculations on infection by *P. longicolla* was studied in a separate experiment, using cultivars Spansoy 201 and Colfax. Virus infection was confirmed by ELISA, while stem and seed infection by *P. longicolla* were evaluated by culturing stem sections and seeds.

To evaluate the effects of management strategies, experiments were established in 6 locations in Iowa during 2008 and 2009. These experiments were designed to evaluate the impact of fungicide applications and integrated insect-disease management strategies to control foliar diseases and *A. glycines* populations, on infection of soybeans by *Phomopsis* spp., BPMV and SMV.

Fungicide trials

In order to assess the impacts of fungicide application timing for reduction of *Phomopsis* infection, field trials were established in 2008 at ISU Curtis Farm (Ames, IA) and in 2009 at ISU Northeast Research and Demonstration Farm near Nashua. The treatments consisted of foliar fungicide applications of the triazole fungicide tebuconazole (Folicur 3.6F, 3.11 kg a.i. ha^{-1}, Bayer CropScience, NC), and strobilurin fungicide pyraclostrobin (Headline, 0.14 kg a.i. ha^{-1}, BASF, NC) applied at stages R3 and R5. Stem and seed infection were evaluated along with soybean yields, compared against non treated controls.

Integrated insect-disease management trials

In order to evaluate the impact of bean leaf beetle management techniques on BPMV-*Phomopsis* interaction, three field experiments were established in Iowa in the last two years. Foliar and pod feeding injury, mainly caused by *C. trifurcata*, was assessed, along with stem and seed infection by *Phomopsis* spp., seed infection by BPMV, germination and yield.

In 2008 a field trial was established at ISU Johnson Farm (Ames, IA). Two soybean cultivars 92M02 (BPMV susceptible) and Spansoy 201 (BPMV tolerant) and insecticidal treatment were tested. Insecticide treatments consisted of seed-applied thiamethoxam (Cruiser 5FS, 0.5 g a.i. per kg of seed), and foliar-applied lambda-cyhalothrin (Warrior, 0.020 kg a.i. ha^{-1})

In 2009 two field trials were conducted at the ISU Southeast Research and Demonstration Farm (Crawfordsville) and Hinds Farm (Ames), with one soybean cultivar (92M76). 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*. Insecticide products were the same as in the 2008 experiment. Fungicide treatments were: 1) no fungicide, 2) pyraclostrobin (Headline, 0.14 kg a.i. ha^{-1}) application at growth stage R5 and 3) tebuconazole (Folicur 3.6F, 0.11 kg a.i. ha^{-1}) application at growth stage R5.

The effects of applications of fungicides, insecticides or combinations at growth stage R3 were assessed in different field trials conducted in same years in five regions of Iowa. Insecticide applications in these trials were focused on soybean aphid management. Stem and seed infection by *Phomopsis* spp. and seed infection by BPMV and SMV were evaluated, along with soybean aphid populations, germination and yield.

In 2008 treatments were: 1) untreated control, 2) trifloxystrobin + prothioconazole (Stratego Pro, Bayer CropScience, NC) at a rate of 0.036 kg of each a.i. ha^{-1}, 3) pyraclostrobin (Headline, BASF, NC) at a rate of 0.11 kg a.i. ha^{-1}, 4) imidacloprid + 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, and 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, Dupont, Crop Protection, Wilmington, DE) at a rate of 0.056 kg a.i. ha^{-1} (9.6 oz $acre^{-1}$) and 7) combination of pyraclostrobin with esfenvalerate (Headline + Asana XL).

Results and discussion

Viruses-Phomopsis interactions

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 seed infection by *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 (Fig. 1). 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 growth stages R3, R5 and R7 (Fig. 2).

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 (Abney and Ploper, 1994). 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. (Smelser and Pedigo, 1992).

Our data suggest that BPMV-induced predisposition to *P. longicolla* 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. Consistently, Abney and Ploper (1994) 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. This suggests that BPMV increases pod susceptibility, as previously reported (Abney and Ploper, 1994), and higher seed infection might simply be due to a higher proportion of pods being infected.

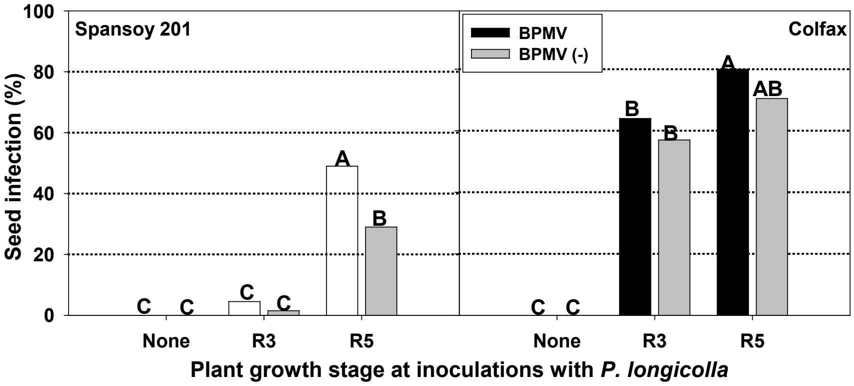

Figure 1. Effects of Bean pod mottle virus (BPMV) and *Phomopsis longicolla* inoculation treatment on infection of seeds by *P. longicolla* of two soybean cultivars, BPMV tolerant-Spansoy 201 (left) and BPMV susceptible-Colfax (right) at different plant growth stages.

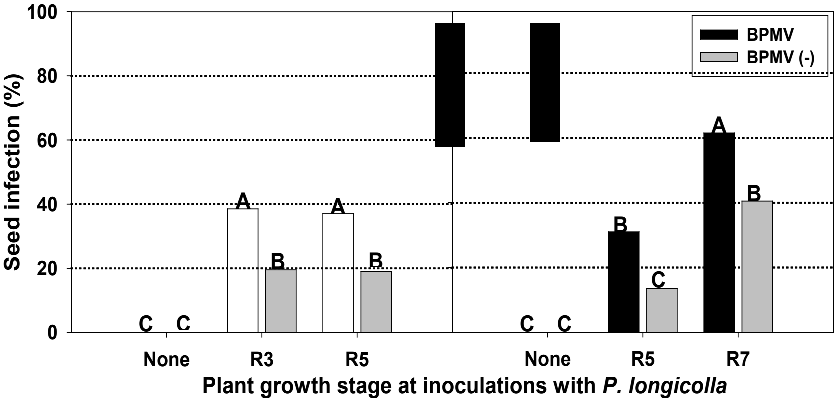

Figure 2. Effects of Bean pod mottle virus (BPMV) and *Phomopsis longicolla* inoculation treatment on infection of seeds by *P. longicolla* of soybean cultivar 92M02 at different plant growth stages.

SMV inoculations did not increase stem or seed infection by *P. longicolla* compared with the SMV non-inoculated plants (data not shown). In previous studies only highly virulent SMV isolates caused an increase in seed infection by *Phomopsis* spp., and the effect differed among cultivars (Hepperly et al., 1979; Koning et al., 2001). Therefore, it is suggested that the SMV-*Phomopsis* spp. relationship may be cultivar and strain dependent.

Fungicide trials

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 (Fig. 3).

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.

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.

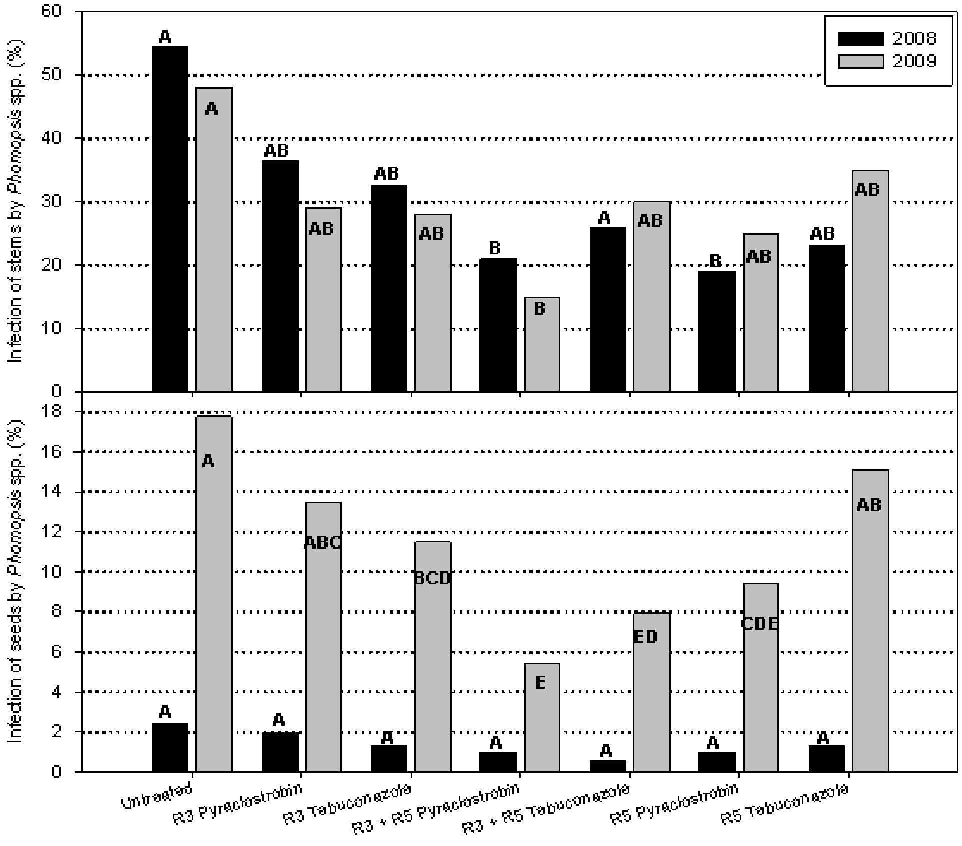

Figure 3. Effects of pyraclostrobin (Headline) and tebuconazole (Folicur 3.6) applications at different growth stages on infection of soybean stems (top) and seeds (bottom) by *Phomopsis* spp.

Integrated insect-disease management trials

Data obtained in this study suggest that in addition to the known effect that feeding injury of pods has reducing seed quality (Smelser and Pedigo, 1992), *C. trifurcata* may also increase secondary stem infection by fungi such as *Phomopsis* spp. In both years, insecticide treatments reduced injured pods (Fig. 4, Table 1), and in 2008, also reduced stem infection by *Phomopsis* spp. (Fig. 4), and there was a significant positive correlation between these two variables (data not shown). It suggests the possibility that control of *C. trifurcata* with insecticides may have added benefits for reducing infection of stems by *Phomopsis* spp. On the other hand, this study found no relationship between *C. trifurcata* feeding injury of pods and seed infection by *Phomopsis* spp.

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 (Fig. 2), suggesting that resistance mechanisms should be combined with chemical treatments in vector-virus management programs to enhance individual control effects. In 2009 (Table 1), seed and foliar applied insecticides were combined with fungicides. 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.

As in previous studies (Spilker et al., 1981), the higher incidence of *Phomopsis* spp. infection might had an effect reducing seed quality at Crawfordsville in 2009, where warm and cold germination of seeds were significantly lower (Table 1). Late planting and harsh winter in both years reduced *C. trifurcata* densities and incidence of *Phomopsis* infection, which likely affected our results, and may have limited the impact of insect management tactics on interactions with *Phomopsis* spp.

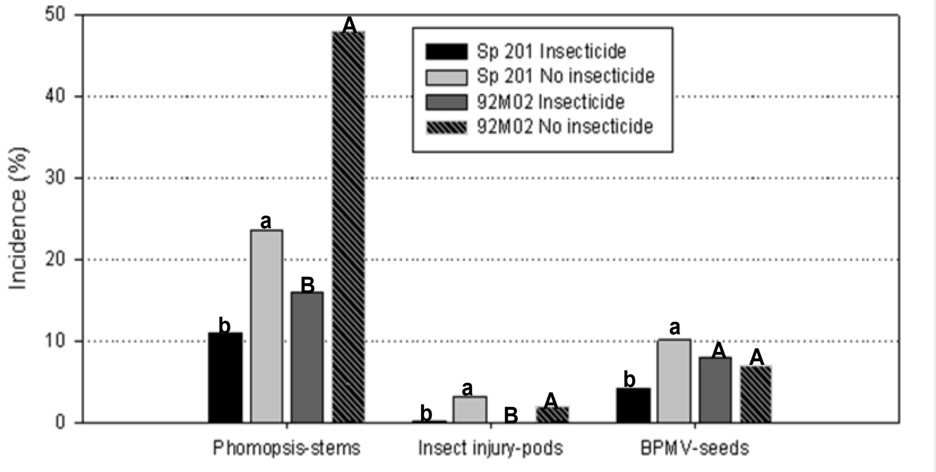

Figure 4. Percentage of infected stems by *Phomopsis* spp., infected seeds by Bean pod mottle virus (BPMV) and pods injured by bean leaf beetle (*Cerotoma trifurcata*), of two soybean cultivars, BPMV tolerant (Spansoy 201) and BPMV susceptible (92M02), with and without insecticide applications.

Table 1. 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.

*** 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.

In the multiple-location trials focusing on soybean aphid, fungicide applications reduced infection by *Phomopsis* spp. in some locations, but none of the treatments had a dual effect in reducing both stem and seed infection (Table 2). Results obtained in this study suggest that R3 applications of fungicides to control foliar diseases may reduce seed infection by *Phomopsis* spp. under some conditions.

Consistent with earlier findings, insecticide applications reduce *A. glycines* populations (Ohnesorg et al., 2009), but also seed infection by *Phomopsis* spp., SMV and BPMV, were reduced in an inconsistent manner. It was hypothesized that infection by *Phomopsis* spp. could be impacted when *A. glycines* populations and their detrimental effects are reduced; however, in this study, there was no evidence that *A. glycines* colonization of soybeans increases susceptibility to *Phomopsis* infection.

An increased in yield was only observed in the fungicide-insecticide 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 2. 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 two locations in Iowa in 2008.

		Phomopsis spp. infection		Seed infection		Cumulative aphids days	Yield	Germination
		Stems	Seeds	SMV	BPMV			
		(%)	(%)	(%)	(%)	(CAD)	(kg/ha)	(%)
Southeast	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
Central	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

[+] 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 at ISU Southeast Research Farm near Crawfordsville (Washington County, IA) (southeast), and ISU Agronomy Research Farm near Boone (Boone County, IA) (Central).

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

(-) Data were not collected for specific treatments.

Acknowledgements

We want to thank Dr. John Hill, Dr. Leonor Leandro, Dr. Alan Eggenberger, Dr. Chunquan Chris Zhang, Dr. Emmanuel Byamukama, Sharon Eggenberger, Dave Volker, Kevin Johnson, John Shriver, Kevin van Dee, Ken Pecinovsky, Dr. Darren Mueller, Rebekah Ritson, Nathan Bestor, Dr. Phillip Dixon, and Adam Pintar. This work was funded by the Iowa Soybean Association and the Iowa Corn and Soybean Initiative; funding and/or crop protection chemicals were provided by Bayer CropScience, Dupont Crop Protection and Valent Agricultural Products and BASF Crop Protection.

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