Research Article

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Assessing the value and pest management window provided by neonicotinoid seed treatments for management of soybean aphid (*Aphis glycines* Matsumura) in the Upper Midwestern United States

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Abstract

BACKGROUND: A 2-year, multi-state study was conducted to assess the benefits of using soybean seed treated with the neonicotinoid thiamethoxam to manage soybean aphid in the upper Midwestern USA and compare this approach with an integrated pest management (IPM) approach that included monitoring soybean aphids and treating with foliar-applied insecticide only when the economic threshold was reached. Concentrations of thiamethoxam in soybean foliage were also quantified throughout the growing season to estimate the pest management window afforded by insecticidal seed treatments.

RESULTS: Both the IPM treatment and thiamethoxam-treated seed resulted in significant reductions in cumulative aphid days when soybean aphid populations reached threshold levels. However, only the IPM treatment resulted in significant yield increases. Analysis of soybean foliage from thiamethoxam-treated seeds indicated that tissue concentrations of thiamethoxam were statistically similar to plants grown from untreated seeds beginning at the V2 growth stage, indicating that the period of pest suppression for soybean aphid is likely to be relatively short.

CONCLUSION: These data demonstrate that an IPM approach, combining scouting and foliar-applied insecticide where necessary, remains the best option for treatment of soybean aphids, both in terms of protecting the yield potential of the crop and of break-even probability for producers. Furthermore, we found that thiamethoxam concentrations in foliage are unlikely to effectively manage soybean aphids for most of the pests' activity period across the region.

Keywords: *Aphis glycines*; soybean aphid; neonicotinoid; seed treatment; thiamethoxam; economic analysis

1 INTRODUCTION

Neonicotinoid insecticides were first marketed commercially in the 1990s and have since become the dominant class of insecticides used in the USA.[1] Approximately 60% of applications of neonicotinoid insecticides are delivered via soil or seed treatments,[2] often in combination with protectant fungicides. It is estimated that neonicotinoids account for one-third of the world insecticide market.[3] In the case of many of the principal agronomic crops grown worldwide (e.g., maize, soybeans, wheat, canola and cotton), neonicotinoids are routinely applied to seeds to guard against early-season insect pests. In North America alone, these crops represent approximately 115 million ha of production annually (94.5 million ha in the USA and 21.5 million ha in Canada).[4,5] In many cases, this rapid adoption has occurred in the absence of any documented increase in pest threats,[6] although the onset of widespread neonicotinoid use on soybean seed was only slightly after the introduction of soybean aphid as a major pest.[7]

The use of neonicotinoids as seed treatments began with the registration of imidacloprid in 1994.[3] The predominant neonicotinoids used in seed treatment formulations for grain and oilseed crops are thiamethoxam, clothianidin (a metabolite of thiamethoxam) and imidacloprid. When applied as a seed treatment, the high water solubility of neonicotinoids permits translocation via xylem flow, moving the insecticide systemically throughout the growing plant.[1,8] Seed treatments have the potential to offer a more targeted and precise delivery of insecticides when compared with conventional alternatives (i.e., foliar-applied organophosphates and pyrethroids).[2] However, the approach of

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prophylactically treating seeds with neonicotinoids in the absence of monitoring for pests is not without pitfalls: recent research has revealed a host of non-target effects upon the fitness of natural enemies and pollinators in agro-ecosystems following exposure to these systemic insecticides,[9–12] with sublethal effects being of particular concern.[13] High water solubility presents advantages for moving neonicotinoid insecticides throughout the plant,[14] but also leads to the potential for translocation beyond the planted field.[15–18] While these formulations can provide crop protection, particularly from aphids and other phloem-feeding insects,[19,20] economic benefits associated with their use have not always been readily documented in the crops where they are widely used, including soybeans.[21–25]

Soybean production in the Upper Midwest and Canada benefits from a fairly limited suite of pest insects. These include bean leaf beetles [Chrysomelidae: *Ceratoma trifurcata* (Forster)), lepidopteran larvae, spider mites (Tetranychidae: *Tertranychus urticae* (Koch)] and the invasive soybean aphid (Aphididae: *Aphis glycines* Matsumura).[26] The initial report of soybean aphid in North America in 2000[7] was quickly followed by economically damaging levels of this new pest in many areas of the Midwest. Currently, the soybean aphid remains the most important soybean pest to US farmers, according to recent survey data.[27] Initial infestations were managed primarily using foliar applications of pyrethroid insecticides. However, complementary management techniques were sought for this species, as it demonstrated the capacity to rapidly reach economically damaging population levels.[28] These methodologies have included the development of an economic threshold,[29] coupled with foliar sprays of pyrethroids and organophosphates and, to a limited extent, the use of aphid-resistant soybean varieties.[30–32] Neonicotinoid seed treatments were marketed to soybean producers as a novel approach for treatment of this key pest shortly after its introduction, and use in soybeans has been steadily increasing since this time.[1,6] Before the efficacy of seed treatment for soybean aphids can be evaluated, an important consideration is the longevity of the insecticide in plant tissues targeted by pest insects. Earlier reports indicate that the window of control offered by seed treatments is relatively narrow – an estimated 3 weeks after planting.[14,21] Further quantification of neonicotinoid concentrations in the plant tissues targeted by soybean aphid is a secondary focus of the work we report here.

Nationally, the highest load rates (>0.4978 lbs per square mile or 0.0002818 kg m^{-2}) of thiamethoxam (the primary neonicotinoid used in soybeans) are applied to agricultural land in the Midwestern USA.[33] Despite this widespread adoption, there are few independent studies in the key soybean-producing areas of the Midwest that assess the efficacy of neonicotinoid seed treatments. Here we use a key – albeit sporadic in terms of economic injury – pest of soybean, the soybean aphid (*Aphis glycines* Matsumura) to assess the economic viability of neonicotinoids in comparison with an integrated pest management (IPM) approach. The IPM approach includes scouting fields to assess soybean aphid populations and applying a foliar insecticide, often a synthetic pyrethroid, when the 250 aphid/plant economic threshold is reached.[29] We describe the results of a multi-year, multi-state study that include quantification of seed treatment insecticides in the foliage of soybean plants over time, and an economic assessment that compares the value of a scouting-based, IPM approach with the use of a prophylactic, neonicotinoid seed treatment approach to management of the key pest of soybeans in the region. Because neonicotinoid-treated soybean seeds are typically also treated with fungicides, we include an assessment of a fungicide-only treatment to disentangle the effects of the insecticidal and fungicidal components of seed treatments.

2 EXPERIMENTAL METHODS

2.1 Field study locations and sampling protocol

The following protocol was implemented at seven sites in 2012 (Table 1) and eight sites in 2013 (Table 2). The following locations participated in the 2012 experiment: Indiana, Iowa, Minnesota, North Dakota (two locations), South Dakota and Wisconsin. In 2013, an additional location in Kansas was added to the experiment.

The experiment consisted of a randomized complete block design (RCBD) with four treatments, replicated four times, for a total of 16 plots per location (i.e., $n = 4$). Plots at each location varied slightly in width and length (Tables 1 and 2) and were planted with a soybean variety and maturity group appropriate to that location. The treatments were as follows: (1) soybean with CruiserMaxx® seed treatment containing both thiamethoxam and two fungicides; (2) soybean with ApronMaxx® seed treatment (two fungicides); (3) soybean with untreated seed; and (4) soybean with untreated seed and a foliar application of pyrethroid insecticide applied at the soybean aphid threshold of 250 soybean aphids per plant,[29] i.e., the IPM treatment. Specific details regarding formulations, rates and methods of insecticide application are shown in Table 2. Treatments were applied at the following rates, in accordance with labels for respective compounds: CruiserMaxx® (3.75 g mefenoxam fungicide, 2.5 g fludioxonil fungicide and 50 g thiamethoxam insecticide 100 kg^{-1} seed) and ApronMaxx® (3.75 g mefenoxam and 2.5 g fludioxonil 100 kg^{-1} seed).

In each plot, weekly aphid counts were taken using whole-plant visual searches beginning prior to typical aphid arrival dates (late June or mid-July, depending on locality), and continued until soybean full seed (reproductive stage R6). For each weekly count, five plants per plot were randomly selected and searched thoroughly for aphids. The average number of aphids per plant was calculated for each plot and used to determine the timing of foliar insecticide application in the IPM treatment and for statistical analyses. Cumulative aphid days (CAD) were calculated for each location and treatment.[29]

2.2 Leaf sample collection and analysis

In order to quantify insecticide concentrations in plant tissues, leaf samples were collected from plots located at Throckmorton Purdue Agriculture Center (TPAC; Tippecanoe County, IN, USA). Plots were planted on 6 June 2011 and were placed in fields that were part of a long-term corn–soybean rotation. Plots ($n = 8$) were 24.38 m wide by 16.76 m long. Aphid-susceptible seed type 76R (var. SD01-76R; maturity group II) and resistant type Rag1 (var. LD (05)-16060, maturity group II) were each treated with one of two treatments: (1) 76R genotype ApronMaxx ® (3.75 g mefenoxam, 2.5 g fludioxonil 100 kg^{-1} seed); or (2) a fungicide–insecticide combination of CruiserMaxx® (3.75 g mefenoxam fungicide, 2.5 g fludioxonil fungicide, and 50 g thiamethoxam insecticide 100 kg^{-1} seed) (Syngenta AG, Greensboro, NC, USA).

Collection of plant samples began on 16 June and ended 31 August 2011: a total of 20 sampling dates in all. Sampling occurred at each growth stage, starting at emergence (VE) and ending at beginning maturity (R7). Foliage samples were taken from the newest trifoliate of each plant. Following collection, the plant was broken at the basal stem to prevent resampling of the plant at a

Table 1. Plot details for 2012 field experiments

County, state	GPS coordinates	Variety/maturity group, row spacing, plot size	Planting date	Harvest date	Foliar insecticide applied?
Tippecanoe Co., IN	40.3013033, −86.7254923	Asgrow 3432/3.4, 76.2 cm, 12.1 m × 30.4 m	22 May 2012	30 Oct 2012	No
Story Co, IA	41.982225, −93.639749	Syngenta NK S20-Y2, 76.2 cm rows, 9.1 m wide × 13.4 m long	3 May 2012	14 Oct 2012	No
Riley Co., KS	39.143831, −96.633759	Pioneer 94Y01, 76.2 cm rows; 9.1 m wide × 13.7 m long	25 May 2012	7 Oct 2012	No
Redwood Co., MN	44.242425 −95.308314	NK 519-A6/1.9, 76.2 cm, 9.1 × 19.8 m	18 May 2012	2 Oct 2012	No
Leonard, ND	46.600221 −97.176631	Peterson Farms 12R06, 76.2 cm, 6.0 × 16.7 m	1 June 2012	25 Sep 2012	No
Mapleton, ND	46.928519 −97.002872	Peterson Farms 12R06, 76.2 cm, 6.0 × 16.7 m	2 June 2012	25 Sep 2012	No
Volga, SD	44.299763 −96.922711	Syngenta S19-A6, 76.2 cm, 12.1 m × 30.4 m	15 May 2012	3 Oct 2012	No

Table 2. Plot details for 2013 field experiments

County, state	GPS coordinates	Variety/maturity group, row spacing, plot size	Planting date	Harvest date	Foliar insecticide applied?
Tippecanoe Co., IN	40.3013033, −86.7254923	Asgrow 3432/3.4, 76.2 cm, 12.1 m × 30.4 m	7 Jun 2013	10 Nov 2013	No
Story Co, IA	41.982225, −93.639749	Syngenta NK S20-Y2, 12.1 × 13.7 m	12 Jun 2013	21 Oct 2013	Yes[a]
Riley Co., KS	39.143923, −96.632886	Pioneer 94Y01, 76.2 cm rows, 9.1 m wide × 13.7 m long	6 Jun 2013	9 Oct 2013	No
Redwood Co., MN	44.240356 −95.308994	NK S19-A6/1.9, 76.2 cm, 9.1 × 19.8 m	3 Jun 2103	30 Oct 2013	Yes[b]
Emerado, ND	47.836174 −97.472553	14R007 6.0 × 16.7 m	19 Jun 2013	9 Oct 2013	Yes[c]
Harwood, ND	47.013136 −96.836685	12R06 6.0 × 16.7 m	29 May 2013	10 Oct 2013	No
Volga, SD	44.30017 −96.92419	Syngenta S19, 76.2 cm, 12.1 m × 30.4 m	5 Jun 2013	28 Oct 2013	Yes[d]
Arlington, WI	43.297950, −89.348524	Syngenta NK S20Y2, 76.2 cm, 12.2 m × 22.9 m	3 Jun 2013	23 Oct2013	No

[a] Warrior II (lambda-cyhalothrin), 116 mL ha^{-1} (1.6 fl. oz/acre), applied 15 Aug 2013.
[b] Tundra (bifenthrin), 291.4 mL ha^{-1} (4 fl. oz./acre), applied 15 Aug 2013.
[c] Warrior II (lambda-cyhalothrin), 116 mL ha^{-1} (1.6 fl oz/acre), applied 16 Aug 2013.
[d] Warrior II (lambda-cyhalothrin), 232 mL ha^{-1} (3.2 fl oz/acre), applied 16 Aug 2013.

later date. Each plot ($n = 8$) was sampled five times at random locations during each sampling period. The samples were placed into zipper seal plastic bags and stored at −80 °C until quantification of insecticide concentrations in tissues (described below) could be performed.

The protocol for leaf sample analyses was adapted from Payá *et al.*[34] Foliage samples were separated into leaf and stem tissues; only leaf tissues were used in analyses. Tissues (1.5 g) were placed in15 mL Falcon centrifuge tubes and stored at −80 °C until analysis. Extraction and partitioning followed a modified version of Payá *et al.*[35] Tissue samples were combined with 5 mL acetonitrile and homogenized. A two-speed motorized drive unit (Waring Lab, Odessa, FL, USA) with a stainless steel semi-micro jar (Waring Lab) was used for tissue homogenization. An additional

5 mL acetonitrile was added, followed by a buffer–salt mixture, consisting of sodium chloride (0.5 g), magnesium sulfate (2.5 g) and sodium sesquihydrate (0.25 g). A thiamethoxam internal standard (d-3 thiamethoxam, Sigma-Aldrich, St Louis, MO, USA) was added, followed by 5 mL acetonitrile. Samples were cleaned using a modified version of the QuECHeRs protocol.[36] Following addition of salt, samples were hand shaken for 1 min and placed on a horizontal vortex for 10 min. Samples were placed in a centrifuge at 4000 rpm (2 °C) for a 10 min cycle. After centrifuging, supernatant (hereafter referred to as sample) was removed and added to dSPE (dispersive solid phase extraction) microvials. These were placed in a vortex to ensure sample and vial contents were thoroughly mixed. Samples were centrifuged for 10 min and supernatant was removed and placed in vials with a split-septum screw cap. Finally, samples were analyzed for thiamethoxam concentrations using liquid chromatography–dual mass spectrometry (Agilent 6460 triple-quadrupole mass spectrometer coupled with an Agilent 1200 rapid resolution high-performance liquid chromatograph) at the Bindley Metabolite Processing Facility Laboratory (Purdue Research Park, West Lafayette, IN, USA).

2.3 Statistical and economic analyses

An analysis of variance (ANOVA) was conducted to estimate the effects of four treatment regimes – IPM, a combined seed-applied insecticidal and fungicidal seed treatment, a seed-applied fungicidal seed treatment and an untreated control – on aphid pressure, measured in cumulative aphid-days (CAD), with location as a blocking factor. Data were normalized by dividing each plot yield by the highest yield result for the site–year to incorporate that source of variability and facilitate economic analysis. Thus yields are reported as proportions of the maximum observed plot yield for their respective site in their respective year (Tables 3 and 4).[37]

The estimated effects on the mean and standard deviation were adjusted for the cost of each treatment, and used to estimate the probability that a farmer would at least break even financially with each soybean aphid management approach (IPM and/or CruiserMaxx seed treatment). This break-even probability in soybean insect pest management is based on Esker and Conley[38] and presents an intuitive metric to use for developing grower recommendations for decisions under risk. It is based on economic variables and yield regardless of observed pest pressure, and thus provides a measure of economic return in the face of any soybean insects that might have been present in addition to soybean aphid. We applied the following assumptions for this analysis:

Soybean price (P) \$10.10/bushel or \$371.11/metric ton – price for 2014[5]

Costs associated with seed treatment

CruiserMaxx® cost (C_{CRZ}) \$18.95/ha GfK Kynetec average 2010–2012[39]

Costs associated with IPM

Scouting cost (C_{SCT}) \$18.38/ha Midwest/South average[39]

Application cost (C_{APP}) \$17.79/ha Midwest/South average[39]

Warrior cost (C_{WAR}) \$10.95/ha GfK Kynetec average 2010–2012[39]

First, the treatment cost for IPM was determined from the following equation:

$$\text{Treatment cost}_{IMP} = C_{SCT} + \text{Probability of application}\ \left(C_{WAR} + C_{APP}\right)$$

For management with CruiserMaxx®, the treatment cost was considered the price of the seed treatment (C_{CRZ}). Next, and to account for variability in locations, a location-specific proportional yield was calculated for each plot. The treatment-associated yield benefit for each plot was then calculated by taking the difference between a plot's proportional yield and the location's mean proportional yield of its control plots. Two separate *t*-tests comparing the overall yield benefit of the IPM and CruiserMaxx® treatments to the control were conducted to calculate the mean *treatment effect* on yield as well as its corresponding standard deviation (SD_{Treat}) (Tables 5 and 6). In this manner, a difference in treatment-associated yield can be presented as a percentage change. A *t*-test is appropriate here to provide parameters (treatment effect and SD_{Treat}) upon which the mean net expected return (μ) and standard deviation (σ) in US dollars per hectare can be calculated for each respective management approach. Both μ and σ were calculated as

$$\mu = P \times \text{ Yield } \times \text{ Treatment effect } - \text{ Treatment cost}$$

and

$$\sigma = P \times \text{ Yield } \times \text{ SD}_{treat}$$

and were used to parametrize a probability density function of the form

$$f\left(x;\mu,\sigma^2\right) = \left(1/\sqrt{2\pi\sigma^2}\right) e^{-0.5}\left(x - \mu/\sigma\right)^2$$

Table 3. Summary of normalized yield by state and treatment, 2012

State	Treatment	Mean	SD	*N*
Indiana	All	0.81	0.08	16
	ApronMaxx®	0.82a	0.08	4
	CruiserMaxx®	0.83a	0.06	4
	UTC	0.80a	0.10	8
Iowa	All	0.91	0.08	16
	ApronMaxx®	0.85a	0.12	4
	CruiserMaxx®	0.96a	0.01	4
	UTC	0.92a	0.07	8
Minnesota	All	0.87	0.07	24
	ApronMaxx®	0.86a	0.09	8
	CruiserMaxx®	0.87a	0.06	8
	UTC	0.89a	0.07	8
North Dakota (Leonard)	All	0.83	0.09	24
	ApronMaxx®	0.86a	0.10	8
	CruiserMaxx®	0.82a	0.09	8
	UTC	0.83a	0.10	8
North Dakota (Mapleton)	All	0.92	0.04	24
	ApronMaxx®	0.94a	0.05	8
	CruiserMaxx®	0.91a	0.05	8
	UTC	0.91a	0.02	8
South Dakota	All	0.87	0.05	16
	ApronMaxx®	0.91a	0.06	4
	CruiserMaxx®	0.85a	0.04	4
	UTC	0.86a	0.05	8
Wisconsin	All	0.86	0.09	16
	ApronMaxx®	0.94a	0.03	4
	CruiserMaxx®	0.92a	0.03	4
	UTC	0.96a	0.03	8

Data were normalized by dividing each plot yield by the highest yield result for the site–year to incorporate that source of variability and facilitate economic analysis. Untreated control (UTC) values include plots grown from untreated seed designated for IPM treatment; these were not treated with foliar insecticide in 2012 as soybean aphids did not reach economic threshold levels at any location. Treatment means followed by the same letter within a site are not significantly different (Tukey HSD $\alpha = 0.05$).

Table 4. Summary of normalized yield by state and treatment, 2013

State	Treatment	Mean	SD	*N*
Indiana	All	0.93	0.06	16
	ApronMaxx®	0.91a	0.01	4
	CruiserMaxx®	0.97a	0.05	4
	IPM	0.93a	0.05	4
	UTC	0.91a	0.10	4
Iowa	All	0.82	0.09	16
	ApronMaxx®	0.87a	0.05	4
	CruiserMaxx®	0.73a	0.11	4
	IPM	0.86a	0.11	4
	UTC	0.82a	0.04	4
Kansas	All	0.68	0.12	16
	ApronMaxx®	0.70a	0.06	4
	CruiserMaxx®	0.63a	0.12	4
	UTC	0.75a	0.18	4
	IPM	0.66a	0.11	4
Minnesota	All	0.87	0.04	16
	ApronMaxx®	0.90a	0.05	4
	CruiserMaxx®	0.85a	0.07	4
	UTC	0.84a	0.04	4
	IPM	0.91a	0.08	4
North Dakota (Emerado)	All	0.88	0.03	16
	ApronMaxx®	0.85a	0.06	4
	CruiserMaxx®	0.91a	0.06	4
	UTC	0.88a	0.04	4
	IPM	0.89a	0.05	4
North Dakota (Harwood)	All	0.84	0.01	16
	ApronMaxx®	0.83a	0.13	4
	CruiserMaxx®	0.85a	0.12	4
	UTC	0.86a	0.14	4
	IPM	0.85a	0.14	4
South Dakota	All	0.69	0.14	20
	ApronMaxx®	0.59b	0.12	8
	CruiserMaxx®	0.77ab	0.08	4
	UTC	0.55b	0.11	4
	IPM	0.85a	0.1	4
Wisconsin	All	0.88	0.08	16
	ApronMaxx®	0.89a	0.04	4
	CruiserMaxx®	0.93a	0.06	4
	UTC	0.85a	0.12	4
	IPM	0.86a	0.09	4

Data were normalized by dividing each plot yield by the highest yield result for the site–year to incorporate that source of variability and facilitate economic analysis. Treatment means followed by the same letter within a site are not significantly different (Tukey HSD $\alpha = 0.05$).

for each value of x. Finally, as in Esker and Conley,[38] the cost relative yield (CRY) was calculated as follows:

$$\text{CRY} = \text{Treatment cost}/(P \times \text{Yield})$$

CRY is a unitless value that can be thought of as the minimum percentage in yield gain needed to cover the costs associated with treatment and serves as a ‘break-even’ point within the probability distribution needed to estimate the one-tail probability of breaking even.

In addition to the 2014 soybean price ($371.11 per metric ton), a low ($328.8 per metric ton in 2015) and high ($529.11 per metric ton in 2012) soybean price[5] was selected from the past 5 years (2012–2017) to demonstrate how the net economic return and break-even probability would change under different economic conditions.

2.4 Analysis of foliar insecticide concentrations

The concentration of insecticides in soybean leaves was analyzed using a repeated-measures ANOVA with date, seed genotype and insecticidal treatment as predictors. A Mauchly sphericity test[40] was performed to test for violations of the sphericity assumption. If this assumption was violated, a Greenhouse-Geisser[41] correction was applied. Where significant differences in means were detected with ANOVA, a Tukey (HSD) post hoc analysis was performed.

3 RESULTS

3.1 Efficacy and yield

During 2012 soybean aphid populations did not reach economic thresholds in any treatment at any experimental location, meaning that the IPM treatment did not include a foliar-applied insecticide treatment – in effect, making this treatment a duplicate of the naked seed control plots. In maximum-likelihood models, there were no significant effects on yield for either IPM (estimate = −0.00475 ± 0.017, $t_{27} = -0.289$, $P = 0.775$) or CruiserMaxx® (estimate = −0.0067 ± 0.013, $t_{27} = -0.55$, $P = 0.586$) treatments in 2012 (Fig. 1). However, in 2013 soybean aphid pressure was high at several experimental locations, allowing comparison of treatments in terms of efficacy, cost and yield. In plots where IPM was used, the mean of normalized yield was 12.44 ± 4.77 percentage points higher than in the plots where IPM was not used, and the effect of IPM was significant ($t_{11} = 2.92$, $P = 0.014$, Fig. 1). Plots where CruiserMaxx® seed treatment was used show a similar trend (2.1 ± 2.3 percentage points higher than plots without CruiserMaxx® seed treatment), but are not statistically significant ($t_{31} = 0.96$, $P = 0.341$).

These values were used to calculate the net benefit of each management approach. These calculations used the pooled mean yield across all plots (3133.32 kg ha^{-1} in 2012 and 3285.83 kg ha^{-1} in 2013) as the expected yield. In 2013, the Iowa IPM treatment was mistakenly treated and therefore removed from all subsequent economic calculations. Of the remaining seven IPM sites, three reached the economic threshold of 250 aphids per plant, so treatment frequency for 2013 was set at 0.4286. In 2013, the expected net return for the IPM treatment was $121.07 ha^{-1}, compared to an expected net return of $6.97 ha^{-1} for the CruiserMaxx® seed treatment. Under low and high economic conditions, the expected return ranges from $103.77 to $185.69 ha^{-1} for the IPM treatment and from $4.02 to $18.01 ha^{-1} for the CruiserMaxx® seed treatment.

Based on these parameter values, the breakeven probability can be determined. This breakeven probability is defined as the probability that the net return at a field level will equal or exceed the cost of the treatment (IPM or CruiserMaxx®). Based on the price, cost and yield assumptions outlined above and the parameter estimates in Table 5, the breakeven probabilities are 98.13% for IPM and 59.86% for CruiserMaxx®. Breakeven probabilities of the IPM and CruiserMaxx® treatments ranged from 97.86% to 98.74% and from 56.45% to 67.46%, respectively, under low and high economic conditions.

Despite the lack of aphid pressure in 2012, pest management strategies (i.e., application of seed treatments to soybean

Table 5. Parameter estimates and analysis of the effect of Cruiser and IPM treatment on the mean and standard deviation of normalized yield, 2012 data. IPM treatment was not applied in 2012, as aphids did not exceed treatment thresholds; however, parameter estimates were still made on IPM plots in order to provide input data for the economic analysis

Parameter	Description	Estimate	Error	*t*-statistic	*P*-value
M_{NOIPM}	Mean without IPM	0.891	0.013	69.95	<0.001
M_{IPM}	Mean with IPM	0.887	0.020	46.84	<0.001
IPM	Mean yield effect	−0.005	0.017	−0.29	0.775
M_{NOCRZ}	Mean without Cruiser®	0.891	0.013	69.95	<0.001
M_{CRZ}	Mean with Cruiser®	0.885	0.015	63.85	<0.001
Cruiser	Mean yield effect	−0.003	0.013	−0.55	0.586

Table 6. Parameter estimates and analysis of the effect of each treatment on the mean and standard deviation of normalized yield, 2013 data. IPM and the corresponding no IPM parameter estimates were made based solely upon the three locations at which IPM plots that reached aphid threshold

Parameter	Description	Estimate	Error	*t*-statistic	*P*-value
M_{NOIPM}	Mean without IPM	0.758	0.053	15.96	<0.001
M_{IPM}	Mean with IPM	0.882	0.026	38.70	<0.001
IPM	Mean yield effect	0.124	0.048	2.93	0.014
M_{NOCRZ}	Mean without Cruiser®	0.806	0.026	31.99	<0.001
M_{CRZ}	Mean with Cruiser®	0.828	0.024	35.55	<0.001
Cruiser	Mean yield effect	0.021	0.023	0.96	0.341

seeds and scouting in the IPM treatment) represented a cost of production. Therefore, break-even probabilities were also calculated for 2012. Expected net losses were – \$24.59 ha^{-1} for IPM and – \$27.67 ha^{-1} for CruiserMaxx®, and break-even probabilities were calculated as 13.71% for IPM and 4.77% for CruiserMaxx®. With low and high commodity values, expected net losses and break-even probabilities ranged from – \$23.28 to – \$26.26 ha^{-1} and from 9.44% to 17.85%, respectively, for the IPM treatment, and from – \$25.83 to – \$30.02 ha^{-1} and from 13.29% to 18.65%, respectively, for CruiserMaxx®.

3.2 Cumulative aphid days

In an ANOVA model comparing cumulative aphid-days (CAD) across sites and treatments in 2012, a significant effect of treatment was observed ($F = 2.73$, df = 3, $P = 0.0491$), with no treatment resulting in fewer CAD than any other treatments. There was also a significant effect of study site ($F = 163.807$, df = 6, $P < 0.0001$), but no significant interaction between site and treatment ($F = 0.622$, df = 18, $P = 0.8724$).

In 2013, a significant effect of treatment was observed as well ($F = 11.14$, df = 3, $P < 0.001$), with the CruiserMaxx®, IPM and untreated control treatments displaying significantly fewer CAD than the ApronMaxx® treatments (Fig. 2). There was also a significant effect of study site ($F = 242.24$, df = 7 , $P < 0.0001$), and a significant interaction between site and treatment ($F = 2.81$, df = 21, $p = 0.0003$), indicating that the effects of both treatments in 2013 were dependent upon locality.

3.3 Foliage insecticide concentrations

In samples of soybean leaf tissue, there was a highly significant effect of seed treatment on concentration of thiamethoxam ($F = 20.03$; df = 1120; $P = 0.001$). Analyses were performed on samples collected at each growth stage from emergence (VE) to beginning flowering (R1), at which point analyses were terminated as no statistical differences were observed for the last three samples. As of the V2 growth stage, there was no significant difference in thiamethoxam concentration between treated and untreated plants (Fig. 3). The concentrations of thiamethoxam in leaf tissue from treated plants throughout the collection period ranged from 9075.005 (±4550.564) ng g^{-1} to 0.081 (±0.011) ng g^{-1} leaf tissue. The concentrations of thiamethoxam in untreated seed ranged from 39.320 (±26.365) ng g^{-1} to 0.033 (±0.016) ng g^{-1}. Low levels of thiamethoxam in foliage samples grown from untreated seed are likely due to uptake from field soils containing neonicotinoid residues from previous planting of treated corn and soybean seeds;[3,17,42] both treated seed types had been planted in this field in prior years.

4 DISCUSSION

This work represents the first multi-state comparison of pest management approaches for the key insect pest of soybeans in the upper Midwest: the soybean aphid. During the years of our study, aphid pressure varied within and between sites, allowing us to make some general conclusions for our study area. Our data demonstrate that under both conditions where aphid outbreaks fail to materialize (2012), and conditions where soybean aphids exceed the economic threshold (2013), an IPM approach based on scouting and application of foliar insecticides is the most cost-effective approach for producers. Furthermore, our analyses of soybean foliage throughout the season added to the knowledge base quantifying the window during which pest management benefits from treated soybean seed can be expected. High concentrations of thiamethoxam were observed early in the season (i.e., up to 2 weeks after planting). However, in the Midwestern USA, the phenology of the soybean aphid may not often align with the window of seed treatment efficacy. Although aphids colonize early-season soybeans beginning in late spring, peak rates of immigration into soybean fields occur in early to mid-July, with populations peaking later in the summer.[43] This indicates that, with the notable exception of soybeans planted late in the season (typically as a second crop), most soybeans grown from treated seed

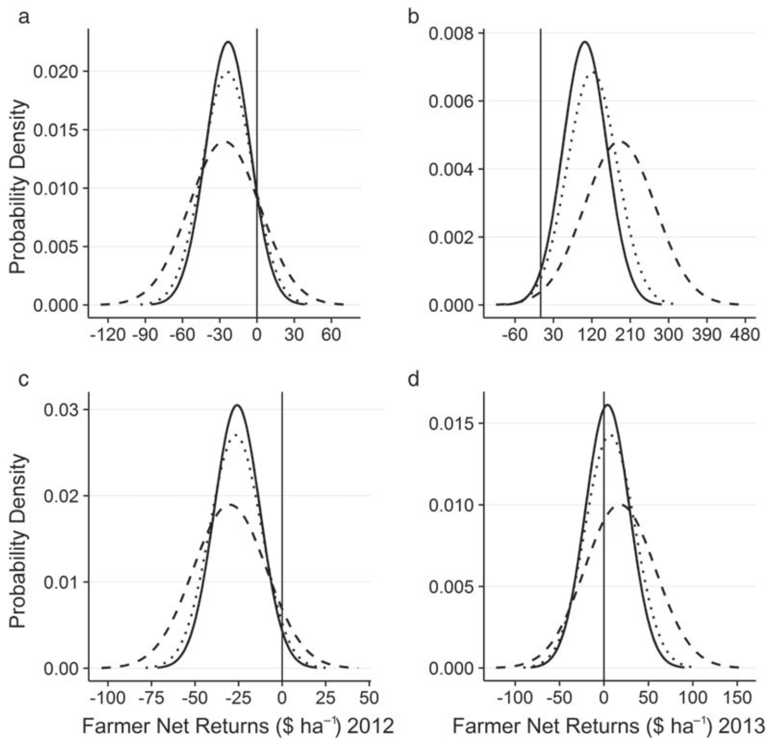

Figure 1. Probability of breaking even (i.e., at least recouping the cost of pest management treatment) under IPM and CruiserMaxx® options. Low ($328.8 ha^{-1}, 2015), medium ($371.11 ha^{-1}, 2014), and high ($529.11 ha^{-1}, 2012) commodity price economic conditions are represented by solid, dotted and dashed lines respectively. For each economic condition, the area under the curve at $x < 0$ represents the probability of a negative economic return and at $x > 0$ of a positive economic return. IPM treatments are denoted by graphs (a) and (b) and CruiserMaxx® treatments are denoted by graphs (c) and (d). As aphids did not reach economic threshold during 2012 the break-even probability of IPM plots includes scouting costs.

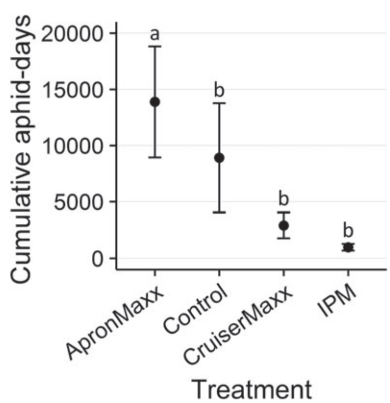

Figure 2. Cumulative aphid–days by treatment in 2013 ($F = 11.14$, df = 3, $P < 0.001$).

will have a window of efficacy that ends well before peak aphid migration into fields. For example, our 2013 foliar treatments of pyrethroid insecticides, applied at the 250 aphids per plant threshold, were applied in mid-August, or approximately 8–10 weeks after planting (Table 2). By this time in the season, concentrations of thiamethoxam in the newest trifoliates (the leaf tissue preferred by aphids and other herbivorous insects) are statistically indistinguishable from untreated plants. Mid- to late-July sampling dates reveal thiamethoxam concentrations of 1.98 and 0.695 ppb for untreated and treated plants, respectively (Table 5), demonstrating uptake from soils even for plants grown from untreated seed. Although additional sites/years with variable patterns of rainfall would likely exhibit some variability in foliage concentrations, our data showing a relatively limited efficacy window are supported by our 2013 yield analyses and suggest that it is unlikely that neonicotinoid insecticides applied as seed treatments will provide significant protection against soybean aphid populations without additional foliar insecticide applications later in the season. A similarly rapid decrease of in-plant concentrations of the neonicotinoid clothianidin was recently reported in field-grown corn (maize) plants, providing further evidence that the high water solubility of neonicotinoids is likely to limit their longevity on or near plants in the field.[44] The pest management window offered by seed treatments has been investigated in soybeans in the past: McCornack and Ragsdale[14] demonstrated that in a field bioassay aphid mortality persisted for up to 49 days after planting, although this study noted that older leaves exhibited higher thiamethoxam concentrations based on aphid mortality. Similarly, Seagraves and Lundgren[11] found that all activity against soybean aphids was gone by 46 days after planting, using observations of aphids placed on the newest trifoliates. Our analyses of insecticide levels in leaf tissue were restricted to the newest trifoliates only, with the rationale that these tissues are preferred by aphids initially colonizing the plants.[45] McCornack and Ragsdale[14] also documented no yield increases attributable to the insecticidal seed treatment in three of four location–years, coincident with low aphid densities, an outcome paralleled by our 2012 results. The 2012 season, where no locations required application of insecticide in the 'IPM' treatment, is representative of a common trend in our study area where populations of soybean aphids can be found in most fields during the season, but a suite of biotic and abiotic factors prevent their densities from reaching the economic

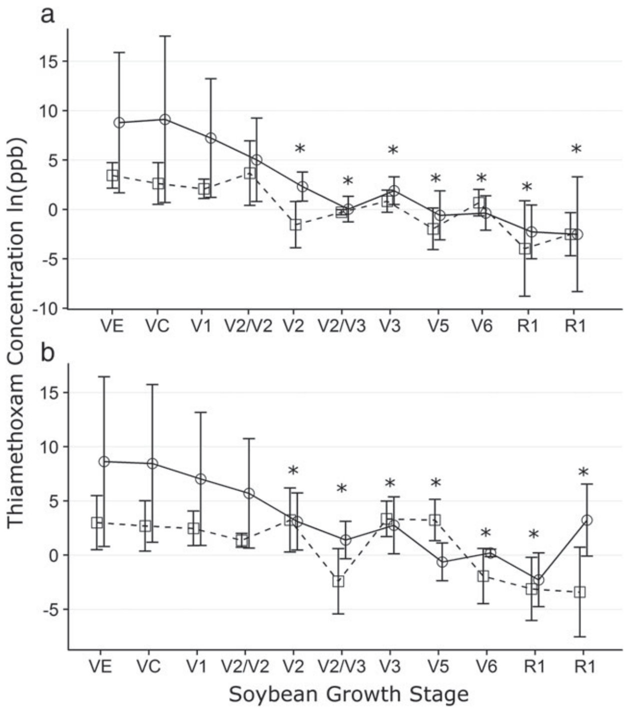

Figure 3. Concentrations of natural log-transformed thiamethoxam ± standard error (ppb) determined for newest trifoliate of soybean at each growth stage. Soybean variety Rag1 is represented by graph (a) and variety 76R by graph (b). CruiserMaxx-treated seed is represented by empty circles with solid lines whereas the untreated control is represented by empty squares with dashed lines. Stages with no significant differences between treatments are marked with an asterisk ($P > 0.05$).

threshold and intervention is often not required to protect the yield potential of the crop.

Our analyses of 2013 aphid pressure throughout the season at each location (expressed as CAD) revealed that thiamethoxam seed treatment can suppress aphid populations relative to untreated seed (Fig. 1), although this suppression was ultimately not sufficient to have a significant impact upon yield. The IPM treatment at the same sites provided significant benefit in terms of soybean yield. This demonstrates a fundamental difference between the IPM approach and neonicotinoid seed treatments: the former has an inherent advantage because it results in no action being taken unless the pest reaches the economic threshold – a threshold based upon years of information regarding soybean aphid population dynamics in the field. Our data show that, while insecticidal seed treatments can kill soybean aphids, this mortality may not provide value to producers if it is not timed to match economic infestations.

Scouting costs are a key variable in calculation of the relative benefits of IPM, and few current and comprehensive data sources exist for assessing this cost. In developing our estimates of \$18.37 ha^{-1}, we included nine states for which scouting cost data were available (summarized in Mitchell[39]); only three of these (IA, MI, MO) are located in areas where soybeans are widely grown. Use of those three values alone would have resulted in a scouting cost estimate of \$14.77 ha^{-1}; this ∼20% reduction in potential costs would render the scout and treat option even more attractive economically.

Despite peer-reviewed publications demonstrating inconsistent benefits of seed treatments for soybean aphid management, adoption rates continue to climb.[6,27] Assuming that both treated and untreated soybean seeds are equally available in the region, climbing adoption rates may indicate that producers perceive a greater benefit to seed treatments than actually exists, and consequently under-invest in scouting and achieve only suboptimal aphid management as a result. Our 2013 data support this notion, showing that the most conservative (i.e., risk-averse) and flexible approach for managing soybean aphids in the Midwest is the use of the established 250 aphids per plant treatment threshold.[29] In 2013 field experiments this approach resulted in the lowest aphid pressures and best economic returns. However, regional differences clearly exist as pest pressures and species complexes vary throughout the soybean planting areas of the USA. In cases where scouting is impractical, prophylactic approaches may provide a welcome option. For example, a recent study in the mid-South USA demonstrated that soybeans grown from seed treated with neonicotinoid insecticides offered superior yields compared with seeds treated with fungicide only – a net benefit overall of US \$31 ha^{-1},[20] although an IPM-based approach for insect pest management was not examined in this study.

Our analysis is focused on the soybean aphid – the key pest in the region where most soybean production occurs in the USA – and our results highlight the economic benefits of a monitoring and threshold-based IPM approach for this pest. However, the results are less clear for a management approach based on neonicotinoid seed treatments: while the current study found no statistically significant effect of seed treatments on yield, we did observe a significant effect on aphid pressure (in terms of CAD); and other work has reported benefits of seed treatment in years when aphid populations are high.[8] A previous study, analyzing data from the initial phase of soybean aphid colonization of North America, found that high-aphid and low-aphid years often alternated,[46] resulting in an approximately even chance that a given year will be a high-aphid year. Since the initial invasion, a combination of increased insecticide applications and predation by the predacious ladybird beetle *Harmonia axyridis* have resulted in a loss of this trend.[47] In fact, correlative data analysis by Bahlai *et al.*[47] demonstrates that widespread seed treatment use may have offered regional benefits by reducing aphid populations early in the season from 2005 to 2011 and offer the hypothesis that toxic soybeans early in season may operate in a manner similar to predation pressure. More recently, however, the pest status and frequency of soybean aphid have diminished. According to 4 years of publically available, aphid-monitoring data from the IPM–Pest Information Platform for Extension and Education (IPM-PIPE) project (project overview provided in VanKirk *et al.*[48]), economically injurious populations of soybean aphids occur infrequently. For example, in the seven states sampled for the present study, the IPM-PIPE website reports that aphid populations exceeded the treatment threshold in only 9.4% of plots sampled over the 4 years of monitoring, and populations exceeding the economic injury level occurred in less than 3.6% of plots. While a more consistent, early-season, annual pest may be well suited to prophylactic approaches such as neonicotinoid seed treatments, the population trends outlined above make the soybean aphid system an ideal fit for a flexible, responsive IPM approach. Substituting 9.4% as the 'probability of application', and using the IPM effect calculated from 2013 data, the expected net returns for IPM management increase to \$130.86 ha^{-1} with a 98.77% break-even probability.

Soybean plants are occasionally attacked by secondary pests, including bean leaf beetle and below-ground pests. The cost–benefit analysis in our study was based on the economic variables we specify above and observed yields from field plots. This incorporates any yield-reducing pressure from such incidental pests that might have been present (although not quantified) during our study. Because we did not document the presence or absence of these insects, they would merit further investigation in regions where they are of concern. Aside from soybean aphids, our study region is characterized by low pest pressure in soybeans. A recent study documenting the benefits of foliar insecticide and fungicide applications across the North-Central USA reported very low insect and fungal pathogen pressure across the region during the study period of 2008–2014, and a corresponding benefit of these pesticide applications less than 50% of the time.[49]

Finally, we reiterate that our study is focused on short-term (i.e., yearly) comparisons of costs and benefits of these pest management approaches as they apply to soybean aphids. Although not part of our study, the risk of insecticide resistance in this species has been demonstrated in its native range,[50] and this is an important consideration in developing sustainable management approaches. One key corollary of an IPM approach to pest management is a reduction in the selection pressure that leads to insecticide resistance.

5 CONCLUSIONS

Although insecticides are useful tools in modern agriculture, their benefits may be offset when prophylactic treatments are widely applied, particularly in the absence of pests. In the case of soybean production, our results support those of other studies,[11,14] demonstrating that thiamethoxam levels in foliage are too low to provide reliable pest management benefits for the key pest in the region – the soybean aphid – during the critical period of population buildup and subsequent potential for yield loss. In the case of soybean aphid in the Midwest, which occurs sporadically at levels that impact crop yield, the likelihood of realizing a net benefit of these prophylactic approaches is even lower. Conversely, our data support the notion that the use of scouting, coupled with appropriate economic thresholds,[29] remains the most flexible and cost-effective approach for this occasionally troublesome pest.

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