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Benefits and Profitability of Fluopyram-Amended Seed Treatments for Suppressing Sudden Death Syndrome and Protecting Soybean Yield: A Meta-Analysis

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Abstract

A meta-analytic approach was used to summarize data on the effects of fluopyram-amended seed treatment on sudden death syndrome (SDS) and yield of soybean (*Glycine max* L.) in over 200 field trials conducted in 12 U.S. states and Ontario, Canada from 2013 to 2015. In those trials, two treatments—the commercial base (CB), and CB plus fluopyram (CBF)—were tested, and all disease and yield data were combined to conduct a random-effects and mixed-effects meta-analysis (test of moderators) to estimate percent control and yield response relative to CB. Overall, a 35% reduction in foliar disease and 295 kg/ha (7.6%) increase in yield were estimated for CBF relative to CB. Sowing date and geographic region affected both estimates. The variation in yield response was explained partially by disease severity (19%), geographic region (8%), and sowing date (10%) but not by the resistance level of the cultivar. The probability of not offsetting the cost of fluopyram was estimated on a range of grain prices and treatment cost combinations. There was a high probability (>80%) of yield gains when disease level was high in any cost–price combinations tested but very low when the foliar symptoms of the disease were absent.

Since first reported in Arkansas in 1971 (Hirrel 1983), sudden death syndrome (SDS) has become economically important and widespread throughout most regions that produce soybean (*Glycine max* (L.) Merr.) (Hartman et al. 2015). Yield loss estimates from 1996 to 2014 placed SDS as one of the top 10 yield-reducing soybean diseases in the United States, often ranking from second to fifth in importance (Allen et al. 2017; Wrather and Koenning 2009). Yield loss due to SDS may range from 0 to more than 80% depending upon cultivar, stage of the crop at the time when disease symptoms appear, and disease severity (DS) (Roy et al. 1997). From 2010 to 2014, the disease reduced soybean yield by approximately 1.14 million metric tons annually, ranging from 0.60 in 2012 to 1.94 million metric tons in 2010 in the U.S. and Ontario, Canada (Allen et al. 2017).

SDS is caused in the United States and Canada by a soilborne fungus, *Fusarium virguliforme* O'Donnell & T. Aoki (Aoki et al. 2003). The fungus survives as thick-walled chlamydospores in the soil and as mycelium in crop residue and on the cysts of soybean cyst nematodes (SCN) (Roy et al. 1997). The disease cycle begins with the infection of soybean roots soon after the radicles emerge from the seed (Gongora-Canul and Leandro 2011). The fungus causes root rot and produces toxins, including FvTox1, which causes SDS foliar symptoms (Brar et al. 2011; Pudake et al. 2013). Foliar symptoms usually appear around the R3 (beginning of pod) soybean growth stage (Fehr et al. 1971) and are characterized by interveinal chlorosis and necrosis. In a conducive environment, leaves may drop prematurely, leaving petioles intact on the plant. Pod abortion and premature death of the plant without pod set can be observed in severe cases.

Planting resistant cultivars is the primary strategy to manage SDS; however, currently available commercial soybean cultivars are at best partially resistant. In fields with a history of SDS and under favorable environmental conditions for disease, partially resistant cultivars alone have not been enough to control SDS (Kandel et al. 2016b; Leandro et al. 2013). Several other management strategies such as crop rotation, tillage, adjustment of planting date, and SCN management can affect SDS severity (Adee et al. 2016; Hershman et al. 1990; Rupe et al. 1997; Westphal et al. 2014; Wrather et al. 1995; Xing and Westphal 2006) but their effect on SDS is not consistent (Kandel et al. 2016b; Kolander et al. 2012; Xing and Westphal 2006).

Several commercial fungicides from various Fungicide Resistance Action Committee (FRAC) groups, which were tested as seed treatments in multiple combinations by Weems et al. (2015), did not reduce *F. virguliforme* infection or SDS symptom development. More recently, new fungicides have been registered and marketed for SDS management, including fluopyram (ILeVO; Bayer CropScience, Research Triangle Park, NC), a succinate dehydrogenase inhibitor (SDHI, FRAC group 7) fungicide. It was registered in 2014 as a seed treatment fungicide for SDS management and is commercially available for soybean farmers. There has been a growing interest in fluopyram seed treatment for multiple reasons: (i) SDS has been a major yield reducer in many areas; (ii) fluopyram has shown promising results for SDS control (Adee 2015; Gaspar et al. 2017; Kandel et al. 2016a,b; Marburger et al. 2015), even in the absence of aboveground SDS symptoms (Bayer CropScience 2016); and (iii) fluopyram seed treatment is also labeled for activity against the economically important nematodes *Heterodera glycines* Ichinohe, *Meloidogyne*

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incognita (Kofoid & White) Chitwood, and *Rotylenchulus reniformis* Linford & Oliviera (Bayer CropScience 2016). Carbon-14 radiolabeled studies indicate that fluopyram systemically moves into the tap root (J. Riggs, personal communication), which is not common with other seed treatment fungicides (Mueller et al. 2013).

Claims of substantial yield increases, even in the absence of foliar SDS symptoms, may increase fluopyram seed treatment use without considering SDS risk (Bayer CropScience 2016). However, there is limited information available from public field trials about the yield and disease responses to fluopyram in fields with no SDS or varying levels of DS. Fluopyram seed treatment or in-furrow application showed efficacy against SDS symptoms and resulted in increased soybean yields compared with the standard commercial base seed treatments that included fungicide, insecticide, and nematistat active ingredients (prothioconazole + penflufen + metalaxyl and clothianidin + *Bacillus firmus*) (Kandel et al. 2016a,b). However, analysis of variance (ANOVA) for individual field experiments in previous reports also resulted in inconsistent responses ranging from highly significant to only numeric differences between the treatments across the locations (Kandel et al. 2016a,b). In those studies, 91% of the field trials had positive yield response but the difference was statistically significant at $\alpha = 0.05$ only in 32% of the trials. In this meta-analytic study, we obtained additional new data from over 200 field experiments conducted in 12 U.S. states covering almost all states that had SDS reported in previous years and in Ontario, Canada.

A meta-analysis, which provides a quantitative synthesis of results from multiple independent studies, was performed. Meta-analysis has been recognized as a more powerful method to quantitatively synthesize the results from different studies than individual analyses and vote counting (i.e., counting studies with significant positive results) (Madden and Paul 2011). Meta-analysis was first used in plant pathology by Shaw and Larson (1999), and has since become commonly applied to combined data from multiple sources, published or not, to obtain estimates of the size of an effect of one or more treatments of interest relative to another, usually a control (Madden and Paul 2011; Ngugi et al. 2011).

Our objective was to (i) quantify the overall effect of fluopyram-amended seed treatment on foliar SDS and soybean yield; (ii) test whether the variation in the effects could be explained, at least in part, by trial-specific moderator variables; and (iii) calculate the probability of economic benefit in a randomly selected study under various grain price and fungicide cost combinations.

Materials and Methods

Field experiments. Field experiments were conducted in 12 U.S. states (Arkansas, Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, South Dakota, Tennessee, and Wisconsin) and in Ontario, Canada between 2013 and 2015. The number and the name of specific locations (counties) within each state are provided in Supplementary Table S1. These locations were chosen based on SDS severity in previous years ranging from very low to high in order to determine the yield benefits under varying disease levels. Two seed treatments—a standard commercial base treatment (CB) and the commercial base plus fluopyram (CBF)—were tested on multiple cultivars with differing levels of SDS resistance. Fluopyram was applied at 0.15 mg of active ingredient (a.i.) per seed. The same two seed treatments were used across all locations. An untreated control treatment was not included in this study but the fungicides used in the CB were targeted to seedling diseases and are known to have no effect on *F. virguliforme* (Weems et al. 2015). Therefore, the CB served as surrogate for check treatment. The CB included a combination of fungicides (prothioconazole + penflufen + metalaxyl [EverGol Energy, 0.019 mg a.i./seed] and metalaxyl [Allegiance, 0.02 mg a.i./seed]) and an insecticide and a nematistat (clothianidin + *B. firmus* [Poncho/VOTiVO, 0.13 mg a.i./seed]) (Bayer CropScience). All seed treatments were applied by Bayer CropScience using a Hege bowl seed treater (Wintersteiger, Salt Lake City, UT). Red seed colorant (Pro-Ized; Gustafson, LLC, Plano, TX) was applied at the rate of 32.6 µl per 100 g and finisher (Peridiam Precise 1010; Bayer CropScience) was applied at the rate of 65 µl per 100 g on treated seed.

The choice of cultivars across locations was based on adaptability to the locations and SDS resistance. The trial at each location included between 2 and 18 soybean cultivars with different levels of resistance to SDS, and their identity was often unknown by the researchers. The experiments were set in a randomized complete block design with four to six replicates. Plot size differed across the field experiments but individual plots were at least two 5.3-m-long rows spaced at 38.1 to 76.2 cm. Locally adapted soybean cultivation practices were followed for general crop management at each location.

Although the experiments were conducted in fields with a history of SDS, in some locations, including Illinois, Indiana, Minnesota, and Iowa, the plots were artificially infested with locally originated isolates of *F. virguliforme*. The single-spore *F. virguliforme* isolates were used to infest sterile sorghum or oat grains. The infested grains were applied in soil mixing with soybean seed during planting. Irrigation was also applied at some locations to create a more conducive environment for disease development.

Disease assessments were made between the R5 and late R6 growth stages (Fehr et al. 1971) on each plot using the same standard previously published SDS rating scale (Gibson et al. 1994; Kandel et al. 2015a). Disease incidence (DI) was estimated as the percentage of plants with foliar symptoms per plot; usually, the two middle rows of each plot were scored. DS was recorded on a 1-to-9 scale based on area of chlorotic or necrotic lesions on the leaf and premature defoliation, where 1 = 1 to 10% leaf surface chlorotic or 1 to 5% necrotic and 9 = premature plant death. Foliar disease index (FDX) was obtained using the formula FDX = DI × DS scale/9. Yield was obtained from the middle two rows for the plots that had 76.2-cm inter-row spacing and four rows for the plots that had 38.1-cm interrow spacing at harvest maturity (R8) (Fehr et al. 1971). Grain moisture was recorded during harvest and seed weight per plot was adjusted to 13% moisture.

FDX and yield distribution across the studies. ANOVA for the two variables on each trial was performed using PROC GLIMMIX in SAS (version 9.4; SAS Institute Inc., Cary, NC) to obtain treatment *LSmeans* and residual variances (mean sum of squares). In each location, more than one cultivar (with varying resistance to SDS) was tested; therefore, each location–cultivar combination was considered an independent trial (separate study in the meta-analysis). Both seed treatments were randomized and replicated within each cultivar at least four times. Separate ANOVA were performed for each study, considering fungicide treatment as a fixed factor and replication as random factor in the model. The *LSmeans* statement was used to obtain treatment means.

Effect sizes and meta-analyses for the efficacy of the fungicide on FDX and yield. We used the log of the response ratio, $[L = \ln(R)]$, of the two means of treatments ($R = \bar{X}_{CBF} / \bar{X}_{CB}$) as effect size for FDX, given that L has better statistical properties than R (Hedges et al. 1999; Paul et al. 2007). The R is more appropriate than the difference in the means (D) when the variation in the response of interest in the reference treatment is too large (Madden and Paul 2011). The sampling variance (Si^2) of L is given by equation 1 (Hedges et al. 1999; Madden and Paul 2011; Paul et al. 2007)

$$Si^2 = \frac{V}{n}\left(\frac{1}{\bar{X}^2 CBF} + \frac{1}{\bar{X}^2 CB}\right) \quad (1)$$

where i denotes the ith study; V is the residual variance, which was obtained from the primary ANOVA for each study; and n is the replication within the study. The variable $\bar{X}_{CBF}$ is the mean severity index of the CBF treatment and $\bar{X}_{CB}$ is the mean severity index of the base treatment from each study. Overall mean percentage control and its confidence interval (CI) was calculated by back-transforming the mean estimate and respective upper and lower 95% CI of $\bar{L}$ using the formula $\bar{C} = \{1 - [\exp(\bar{L})] \times 100\}$.

For yield data, the absolute mean difference (D) between CBF and CB plots was used as the effect size, which is appropriate when the yield in the check does not vary much across trials (Machado et al. 2017; Paul et al. 2011). The D was computed as follows: $D = \bar{X}_{CBF} - \bar{X}_{CB}$, where $\bar{X}_{CBF}$ is the mean yield of the CBF and $\bar{X}_{CB}$ is the mean yield of the CB treatments (Madden and Paul 2011; Paul

et al. 2011). Sampling variance of the difference for each study was given by $Si^2 = (2 \times V)/n$, where i denotes the ith study, V is the residual variance, and n is the replication within the study (Paul et al. 2011). Standard error (SE) of $\bar{D}$ was estimated as the square root of sampling variance [$SE(D) = \sqrt{Si^2}$]. The lower and upper limits of the 95% CI of the mean difference were estimated with the *cl* option in the models. Percent yield response to fungicide was calculated as $(\bar{D}/\bar{X}_{CB}) \times 100$.

In the meta-analysis, each study was weighed by the inverse of the variance (weight = $1/Si^2$). Random-effects meta-analyses were performed separately for L and D using PROC GLIMMIX to obtain estimates of ($\bar{L}$) and ($\bar{D}$) and their respective heterogeneity ($\hat{\sigma}^2$) (Madden and Paul 2011; Ngugi et al. 2011; Paul et al. 2011). A standard normal test (Z) was used to determine whether the effect size was significantly different from zero (Madden and Paul 2011; Paul et al. 2007; Paul et al. 2011). Studies was considered as random effects. Contrast estimates and the associated statistics were obtained using the *estimate* statement in PROC GLIMMIX.

Study heterogeneity. To test whether the among-study variance is significantly different from 0, a likelihood-ratio test static was used, as described elsewhere (Madden and Paul 2011; Paul et al. 2011). In addition, a Higgins and Thompson (2002) R^2 statistic was also calculated to determine the impact of among-study variability in the effect sizes. R^2 greater than 1.5 indicated that the impact of among-study variation is large in the meta-analytic result and a need to account the among-study variability in the analysis (Madden and Paul 2011; Paul et al. 2011).

Efficacy of fungicides as influenced by moderator variables. Location- and trial-specific categorical moderators were tested in a mixed-effects model (moderator as fixed effect) to check whether and how much they could explain, at least in part, the heterogeneity in the estimates by reducing the among-study variability (Madden and Paul 2011).

Trials were grouped into three categories based on baseline disease (basedisease) and FDX in CB plots: (i) no disease, FDX = 0; (ii) low disease, FDX > 0 but < 10; and (iii) high disease, FDX ≥ 10. Baseline disease was used as a moderator variable to estimate the treatment effect under varying disease levels. Treatment effect on FDX was only estimated in basedisease categories ii and iii (i.e., categories that had disease symptoms). An FDX of 10 was used as the cutoff to separate the low and high disease categories, because this is when foliar symptoms are clearly observed and this threshold has been used successfully in previous analyses (Kandel et al. 2016a). Total studies analyzed for each group are listed in Tables 1 and 2.

Based on the cultivar resistance rankings provided by seed suppliers, cultivars were grouped in three categories: (i) susceptible, (ii) moderately resistant, and (iii) resistant. Because planting date was reported to have influence on SDS (Hershman et al. 1990; Marburger et al. 2016), it was also tested. Southern U.S. states (Kansas, Missouri, Tennessee, and Arkansas) were excluded in this analysis because of the difference in range of planting dates and the limited number of trials conducted in the southern states. Based on the range of planting dates reported in the data, we grouped studies in three categories: (i) early, for those planted before 1 May; (ii) optimum, for those planted between 1 and 21 May; and (iii) late, for those planted after 21 May.

Trial locations were grouped into three geographical regions: (i) north included South Dakota, Minnesota, Wisconsin, Michigan, and Ontario; (ii) mid included Iowa, Illinois, Indiana, and Nebraska; and (iii) south included Kansas, Missouri, Tennessee, and Arkansas. A separate mixed-effects meta-analysis was performed to obtain $\bar{L}$ and $\bar{D}$ for each level of the moderator variable, whenever significant (Paul et al. 2007). The percentage of variability explained by each moderator variable was calculated as $100 \times (v - r)/v$, where v is the among-study variance in the absence of moderator and r is the residual among study variance when the moderator variable specified.

Prediction and risk analysis. The mean effect size ($\bar{D}$) and the between-study variance ($\hat{\sigma}^2$) from meta-analyses of fluopyram seed treatment effects on yield were used to estimate the effect size of a randomly selected new study. Given the significance of the basedisease, we also estimated the risk of not offsetting the investment on fluopyram for no, low, and high disease conditions based on the new study effect size for a range of soybean prices and treatment costs if using the same practice in this study. The probability estimates were generated separately for each baseline disease category as follows: $p = \phi[(C - \bar{D})/\hat{\sigma}]$, where ϕ denotes the cumulative standard normal function, C (constant) represents an estimated break-even grain yield for a range of seed treatment costs and soybean grain prices, $\bar{D}$ denotes the effect size, and $\hat{\sigma}$ denotes the standard deviation between studies (Paul et al. 2011). $\bar{D}$ and $\hat{\sigma}$ were obtained from the meta-analyses. Soybean price ranges for risk analysis ($0.26 to $0.59/kg) were chosen based on the market price fluctuations in the last 10 years from 2007 to 2016 (($0.28 to $0.52/kg) (USDA-NASS 2017), and cost of seed treatment was assumed ($18 to $48/ha) on the basis of current price for fluopyram seed treatment and seeding rate recommendations. The minimum yield required to offset the fluopyram seed treatment cost was estimated for each grain price and seed treatment cost combination. For example, if grain price is $0.26/kg and seed treatment cost is $18/ha, the break-even grain yield benefit is 70 kg/ha. The probability is the risk of failing to recover the fluopyram seed treatment cost.

Results

FDX and yield data. FDX and yield data varied among studies and between treatments within the studies. Box plots showing the distribution of raw data from experimental plots for foliar disease and yield are given in Figure 1. In general, mean FDX was lower in CBF-treated plots than in CB plots (Fig. 2A). Mean FDX across the studies was 0.1 to 94.5 with overall mean 19.5 ± 1.6 in CB, and 0 to 78.9 with overall mean 13.2 ± 1.4 in CBF treatments (Fig. 2A). Slightly over 50% of the studies had FDX greater than 10. Other foliar diseases that can be confused with SDS such as brown stem rot caused by *Cadophora gregata* and stem canker caused by *Diaporthe phaseolorum* var. *caulivora* were not observed in significant proportions in any locations. We confirmed presence of *F. virguliforme* in roots collected from border rows by running a specific quantitative polymerase chain reaction protocol (Kandel et al. 2015b; Wang et al. 2015).

Mean soybean yield was different among studies and between treatments within the studies. In general, mean yield was greater in CBF-treated plots than in CB plots. Mean yield across the studies ranged from 764 to 6,215 kg/ha with an overall mean of 3,866 ± 67 kg/ha in the CB treatment, and from 924 to 6,768 kg/ha with overall mean of 4,168 ± 71 kg/ha in the CBF treatment (Fig. 2B). Yield response to fluopyram treatment was mostly positive, ranging from 542 to 1,848 kg/ha. There were 51 studies with no foliar symptoms and 209 studies with foliar symptoms. Of the 209 studies with foliar symptoms, 85% of the studies had a positive yield response. In 51 studies with no SDS foliar symptoms, yield response to the fluopyram treatment was positive in 53% of the trials.

Meta-analysis. Standard test statistics from the meta-analysis showed that the overall log-transformed response ratio ($\bar{L}$) was negative (−0.44 ± 0.04) and significantly different from zero ($P < 0.001$). The corresponding back-transformation of the estimate normalized to percent control was 35.4%, suggesting that fluopyram contributed to suppress the disease compared with the CB plot. The lower (CI_L) and upper (CI_U) limits of 95% CI around ($\bar{L}$) were –0.52 to –0.35, respectively, corresponding to 29.6 to 40.7% control after back-transformation.

The overall yield response (all 260 studies) to fluopyram was positive. The $\bar{D}$ was 295 ± 23.5 kg/ha (95%CI = 249 – 342 kg/ha) and significantly different from zero ($P < 0.01$). CBF-treated plots resulted in 7.6% more grain yield than the CB plots, based on the estimates of both treatments.

Heterogeneity and moderator effects. Based on the likelihood ratio test, the estimated among-study variances for FDX ($\hat{\sigma}^2_{FDX} = 0.1551$) and yield ($\hat{\sigma}^2_{yield} = 106627$) were significantly different from zero ($P < 0.001$) for models fitted with and without the random effect of trial. The corresponding R^2 was greater than 1.5 for FDX ($R^2_{FDX} = 10.4$) and for yield ($R^2_{yield} = 11.0$), indicating that there was a considerable impact of among-study variance, which was explained, in part, by some moderator variables (Tables 1 and 2).

All moderators tested in this study, with the exceptions of baseline disease ($P = 0.0679$) and cultivar resistance ($P = 0.8349$), significantly affected $\bar{L}$ (Table 1). Date of planting influenced the disease control, which was greater in early plantings (53%) than the later ones: 26 and 40% for optimum and late plantings, respectively. Percent control was greater in the mid geographic region (40%; Iowa, Illinois, Indiana, and Nebraska) than the north and south regions (Table 1).

Except cultivar resistance ($P = 0.2076$), all other moderator variables also significantly affected $\bar{D}$ (Table 2). Baseline disease also influenced the effect size, with the greatest $\bar{D}$ in high disease and lowest $\bar{D}$ when disease was absent (Table 2). The $\bar{D}$ (38 kg/ha) when disease was absent did not differ from zero ($P = 0.4389$). The $\bar{D}$ was positive and significantly different from zero in studies with both low and high disease categories. The $\bar{D}$ was 268 kg/ha (or 6.2% increase) in studies with low disease. The $\bar{D}$ in high disease category was 449 kg/ha (13.2% increase). The difference between the upper and lower limit (the width) of 95% CI around the $\bar{D}$ was similar in both low and high disease categories, with 0.6 kg/ha narrower width in studies with high disease. The influence of planting date on $\bar{D}$ was significant, with the greatest response observed in optimum planning. The $\bar{D}$ was not different from zero in late planting. Study location also influenced the $\bar{D}$, where the south locations had the lowest $\bar{D}$ (Table 2).

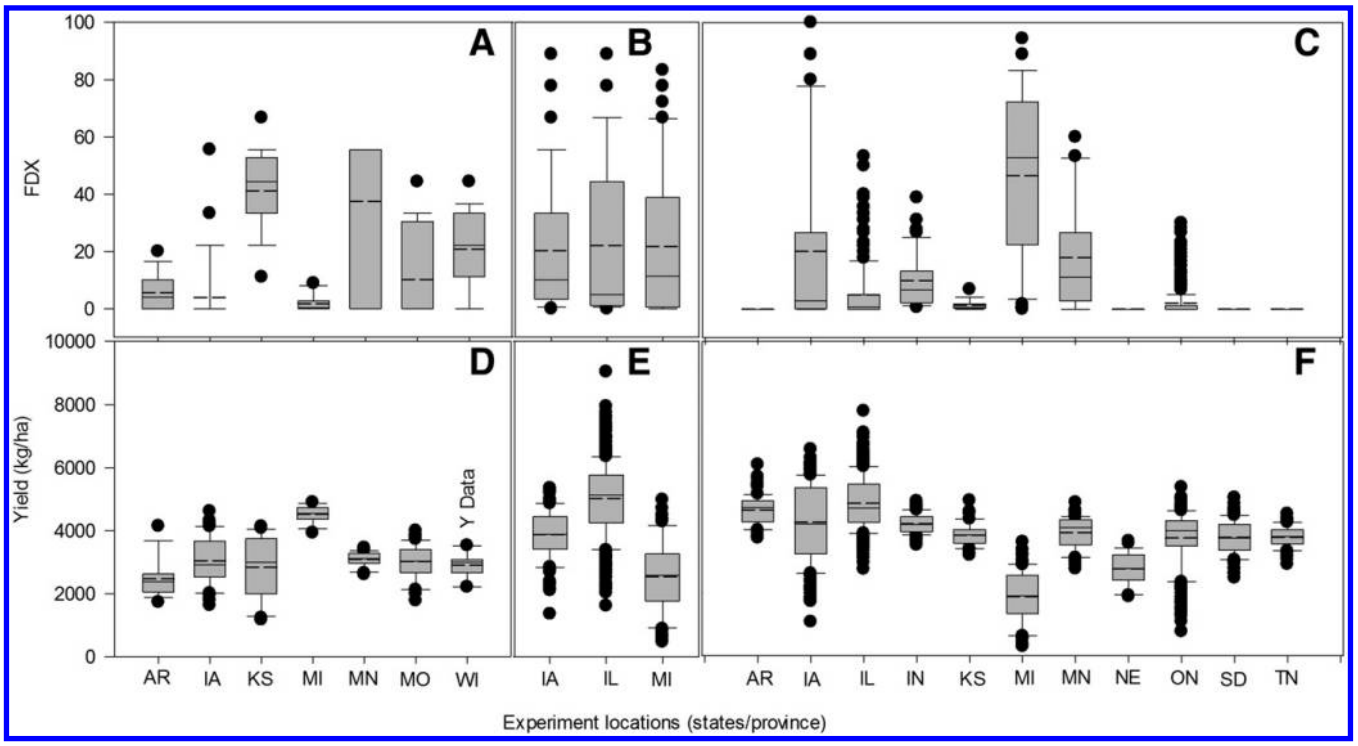

Fig. 1. Box plots showing the distribution of raw data from experimental plots grouped by state and years for foliar disease (sudden death syndrome) index (FDX = disease incidence × disease severity/9) in **A,** 2013; **B,** 2014; and **C,** 2015 and yield in **D,** 2013; **E,** 2014; and **F,** 2015. Solid and broken lines within each box represent the median and mean, whereas the top and bottom lines of the boxes are 75th and 25th percentiles, respectively, and vertical lines extending from the box are 90th and 10th percentiles.

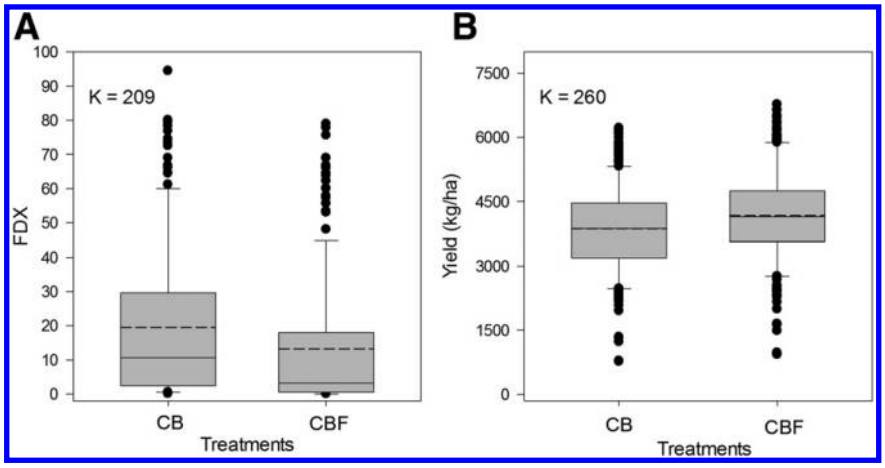

Fig. 2. Box plots summarizing distribution of commercial base (CB) and CB plus fluopyram (CBF) seed treatment *LSmeans* data from all studies for **A,** foliar disease index (FDX = disease incidence × disease severity/9) and **B,** yield. CB included prothioconazole + penflufen + metalaxyl (EverGol Energy, 0.019 mg a.i./seed), metalaxyl (Allegiance, 0.02 mg a.i./seed), and clothianidin + *Bacillus firmus* (Poncho/VOTiVO, 0.13 mg a.i./seed) (Bayer CropScience). Fluopyram (ILeVO, Bayer CropScience) was added at 0.15 mg a.i./seed. Solid and broken lines within each box represent the median and mean, whereas the top and bottom lines of the boxes are 75th and 25th percentiles, respectively, and vertical lines extending from the box are 90th and 10th percentiles. *K* is the number of studies used in the analysis. Studies were conducted in multiple locations in the United States and Ontario, Canada in 2013 through 2015.

Prediction and risk analysis. For all price and cost combinations, the probability of not offsetting the fluopyram cost (P_{loss}) was greater than 49% when no foliar symptoms occurred in CB plots (Fig. 3A). The P_{loss} decreased with increasing FDX. In the low disease category, the P_{loss} ranged from 21 to 41% for all price-cost combinations (Fig. 3B). In the high disease category, the P_{loss} was <20% for all price-cost combinations (Fig. 3C). P_{loss} values increased with increasing cost of seed treatment at any given soybean price and decreased with increasing grain prices. For example, when treatment cost increased from $18 to $48/ha at a grain price of 0.37 kg/ha, the P_{loss} increased from 52 to 63% in the absence of FDX, from 23 to 32% with low FDX, and from 9 to 14% with the high FDX. At a given treatment

Table 1. Influence of moderator variables on the effect sizes, log response ratio (L) of commercial base (CB) to CB plus fluopyram (CBF) seed treatment means for foliar disease index (FDX), and corresponding statistics based on mixed effect meta-analysis model of studies carried in multiple U.S. states and Ontario, Canada in 2013 through 2015

				Effect size[a]						Control efficacy (%)[b]		
Moderate variables[c]	Category[d]	K[e]	FDX CB[f]	$\bar{L}$	se($\bar{L}$)	CI_L	CI_U	Z	P	$\bar{C}$	CI_L	CI_U
Base disease	Low	104	3.2	–0.57	0.08	–0.74	–0.40	–6.79	<0.0001	43.3	33.0	52.0
(6%, P = 0.0679)	High	105	35.5	–0.39	0.05	–0.49	–0.29	–7.80	<0.0001	32.1	25.0	38.5
Cultivar resistance	S	36	29.8	–0.46	0.09	–0.64	–0.27	–4.94	<0.0001	36.6	23.8	47.2
(5%, P = 0.8349)	MR	62	20.8	–0.39	0.08	–0.55	–0.23	–4.81	<0.0001	32.3	20.5	42.4
	R	87	15.2	–0.39	0.07	–0.53	–0.26	–5.84	<0.0001	32.5	22.9	40.9
Date of planting	Early	78	5.7	–0.75	0.09	–0.93	–0.57	–8.07	<0.0001	52.8	43.3	60.7
(12%, P = 0.002)	Optimum	104	30.5	–0.30	0.05	–0.41	–0.19	–5.64	<0.0001	26.0	17.7	33.4
	Late	18	21.6	–0.50	0.11	–0.73	–0.28	–4.46	<0.0001	39.6	24.3	51.8
Location	North	66	20.0	–0.23	0.08	–0.39	–0.08	–2.97	0.0039	20.7	7.4	32.1
(11%, P = 0.0128)	Mid	134	20.1	–0.51	0.05	–0.61	–0.41	–9.91	<0.0001	40.1	33.6	45.9
	South	9	7.0	–0.48	0.20	–0.88	–0.09	–2.41	0.017	38.3	8.4	58.4

[a] Effect size: $\bar{L}$ = the log response ratio of CB to fluopyram seed treatment ($\bar{X}_{CB}/\bar{X}_{CBF}$), se($\bar{L}$) = standard error of $\bar{L}$, CI_L and CI_U = lower and upper limits of the 95% confidence interval of $\bar{L}$, and P = significance level of the effect size. P values were testing the null hypothesis that the effect size is not different from zero.

[b] Percent disease controls ($\bar{C}$) and their confidence interval was calculated by back-transforming the log response ratio and their upper and lower limits using the formula $\bar{C} = \{1 - [\exp(\bar{L})] \times 100\}$.

[c] Number in parenthesis in moderator variables column denotes the percent heterogeneity explained by the specified moderator variable and P values were testing the null hypothesis that there is no difference among the categories within the moderator variable. The percentage of variability explained by each moderator variable was calculated as $100 \times (v - r)/v$, where v is the among study variance in the absence of moderator and r is the residual among study variance when the moderator variable specified.

[d] Categories: Baseline disease = no foliar disease (FDX = 0), low disease (FDX > 0 but < 10), and high disease (FDX ≥ 10); cultivar resistance to sudden death syndrome = susceptible (S), moderately resistant (MR), and resistant (R); date of planting (DOP) = early (before 1 May), optimum (1 to 21 May), and late (after 21 May); location = north (South Dakota, Minnesota, Wisconsin, Michigan, and Ontario), mid (Iowa, Illinois, Indiana, and Nebraska), and south (Kansas, Missouri, Tennessee, and Arkansas).

[e] Total number of studies used in the analysis.

[f] FDX in CB plots.

Table 2. Influence of moderator variables on the effect sizes ($\bar{D}$), the yield difference between fluopyram seed treatment and a standard commercial base seed treatment (CB, check), and corresponding statistics and probability values based on mixed effect meta-analysis model of studies carried in multiple U.S. states and Ontario, Canada in 2013 through 2015

				Effect size[a]						
Moderator variables[b]	Category[c]	K[d]	Yield CB (kg/ha)[e]	$\bar{D}$	se($\bar{D}$)	CI_L	CI_U	Z	P	Yield difference (%)[f]
Base disease	No	51	3,796.4	37.5	48.4	–57.8	132.8	0.8	0.4389	1.0
(19%, P = < 0.0001)	Low	104	4,354.8	268.3	34.4	200.5	336.0	7.8	<0.0001	6.2
	High	105	3,414.6	449.3	34.2	381.9	516.8	13.1	<0.0001	13.2
Cultivar resistance	S	45	3,559.5	387.2	54.4	279.9	494.5	7.1	<0.0001	10.9
(7%, P = 0.2076)	MR	77	3,794.4	304.6	42.4	220.9	388.3	7.2	<0.0001	8.0
	R	105	4,081.4	270.9	36.2	199.4	342.3	7.5	<0.0001	6.6
DOP	Early	85	4,405.9	249.5	40.4	169.9	329.1	6.2	<0.0001	5.7
(10%, P = < 0.0001)	Optimum	121	3,521.2	439.8	33.3	374.3	505.4	13.2	<0.0001	12.5
	Late	21	3,682.1	40.0	74.3	–106.7	186.7	0.5	0.5908	1.1
Location	North	33	3,853.1	261.8	39.6	183.8	339.8	6.6	<0.0001	8.2
(8%, P = 0.0001)	Mid	84	3,175.8	370.1	31.2	308.5	431.6	11.8	<0.0001	8.7
	South	33	3,853.1	79.3	62.2	–43.3	201.9	1.3	0.204	2.1

[a] Effect size: $\bar{D}$ = difference in mean for fluopyram seed treatment relative to base treatment mean ($D = \bar{X}_{CBF} - \bar{X}_{CB}$), se($\bar{D}$) = standard error of the difference, CI_L and CI_U = lower and upper limits of the 95% confidence interval of the mean difference, and P = significance level of the effect size. P values were testing the null hypothesis that the effect size is not different from zero.

[b] Number in parenthesis in moderator variables column denotes the percent heterogeneity explained by the variable and P values were testing the null hypothesis that there is no difference among the categories within the moderator variable. The percentage of variability explained by each moderator variable was calculated as $100 \times (v - r)/v$, where v is the among study variance in the absence of moderator and r is the residual among study variance when the moderator variable specified.

[c] Categories: Baseline disease based on foliar disease index (FDX) on CB plots = no disease (FDX = 0), low disease (FDX > 0 but < 10), and high disease (FDX ≥ 10); cultivar resistance to sudden death syndrome = susceptible (S), moderately resistant (MR), and resistant (R); date of planting (DOP) = early (before 1 May), optimum (1 to 21 May), and late (after 21 May); location = north (South Dakota, Minnesota, Wisconsin, Michigan, and Ontario), mid (Iowa, Illinois, Indiana, and Nebraska), and south (Kansas, Missouri, Tennessee, and Arkansas).

[d] Total number of studies used in the analysis.

[e] Yield in CB plots.

[f] Percent difference was calculated using the formula Percent difference = $(\bar{D}/checkmean) \times 100$.

cost of $48/ha, when the soybean grain price increased from $0.26 to 0.59/ha, the P_{loss} decreased from 70 to 56% with no disease, from 39 to 26% with low disease, and from 19 to 11% with high disease.

Discussion

This is the first meta-analysis that combined data from over 200 fluopyram efficacy trial conducted under various field and management conditions across 13 states or provinces of the leading soybean-producing region of the world. An overall mean increase of 295 kg/ha in yield, relative to base seed treatment, was estimated when the seed treatment incorporated fluopyram. This result agrees with previous reports from experiments conducted in the presence of SDS (Adee 2015; Kandel et al. 2016a,b; Marburger et al. 2015) but the yield response in the present study (7.6%) was greater compared with a previous report (5.5%), which analyzed fewer (n = 22) studies (Kandel et al. 2016a). This shows that the estimated effect size is more precise with greater sample size. Although meta-analysis is generally perceived as an approach to analyze data from published studies, it can be used when original observations are available to obtain the means and sampling variances measures (Madden and Paul 2011). Here, we used data collected before and at the time of the fluopyram commercialization for soybean; therefore, most have not been published.

Previous conclusions from public research trials examining the yield response to fluopyram seed treatment were mainly based on simple arithmetic means across trials (meaning that the same weight was given to studies with low or high sampling variance) or counting significant P values (Adee 2015; Kandel et al. 2016b; Marburger et al. 2015). Moreover, the trials were not treated as random effects, and both significant and nonsignificant effects were reported for individual-trial analyses (Kandel et al. 2016a,b). The nonsignificant results in previous studies could have been due to the low power of statistical tests for individual studies associated with a low number of replicates and a high degree of variability in these experiments. Our study provides additional evidence, in the plant pathology field, that meta-analysis is statistically more powerful than individual studies to detect treatment effects (greater probability of rejecting null hypothesis when alternative hypothesis is true) (Madden and Paul 2011).

The magnitude of yield response to fluopyram seed treatment was affected by the baseline SDS level, as measured by FDX, which tended to increase with high disease intensity. Similarly, greater yield response was reported when disease was at higher levels in our previous studies (Kandel et al. 2016a,b). In fact, Marburger et al. (2015) reported positive yield responses to fluopyram in Wisconsin only when disease pressure was moderate to severe. Even though, in the present study, overall response was not significant, some studies reported a positive yield response with fluopyram treatments in the absence of foliar SDS symptoms. This might be related to experimental error, root rot, or nematode management (Beeman and Tylka 2018; Zaworski 2014). It is not yet clear how much the root rot phase of *F. virguliforme* contributes to yield loss, which is worth further investigation. There are reports showing negative correlations between root colonization and soybean yield (Luo et al. 2000) and differences in root rot severity caused by *F. virguliforme* between treatments with and without fluopyram seed treatment (Kandel et al. 2016a).

Our meta-analysis showed that the fluopyram seed treatment reduced, on average, 35% of FDX relative to CB, which agrees with a previous report (Kandel et al. 2016a). Cultivar resistance did not explain the variation in disease control and yield response to fluopyram, suggesting that cultivar response to SDS did not significantly influence the efficacy of fluopyram seed treatment. Yield benefits ranged from 7 to 11% from resistant to susceptible cultivars. This is likely due to similar FDX in check plots in resistant and susceptible cultivars observed in many locations. Previous studies have also reported that many cultivars that are labeled as moderately resistant did produce the same level of SDS as the susceptible cultivars when environmental conditions were highly conducive for this disease (Kandel et al. 2016a,b). The yield response was greatest in the optimum window of planting, which is between 1 and 21 May, but not significant for later plantings. This is consistent with our previous results (Kandel et al. 2016b), where the yield response to fluopyram seed treatment was lower in June than May plantings. This might be due to reduced root rot in late plantings (Kandel et a. 2016b) that may have compromised the efficacy of fluopyram seed treatment for SDS control. In Wisconsin, fluopyram seed treatment resulted in lower FDX scores in May but not in June plantings (Vosberg et al. 2017). Yield response to fluopyram was also lowest in the southern region, perhaps due to a lack of or very low levels of foliar disease symptoms present in these locations.

We found that crop yield responded to fluopyram for a range of disease categories. However, this is not enough information for a farmer to decide on a product and technology. The predictions regarding the estimate of future outcome and the probability of recovering cost under different scenarios are particularly important. Based on the United States Department of Agriculture–National Agricultural Statistics Service data, soybean price in the last 10 years (2007 to 2016) ranged from $0.28 to $0.52/kg. The chances of not recovering fluopyram cost at current soybean prices and at a range of application costs was high when no foliar disease was observed, even when those fields had a history of SDS. There was a high probability of recovering investment on the fluopyram treatment when the

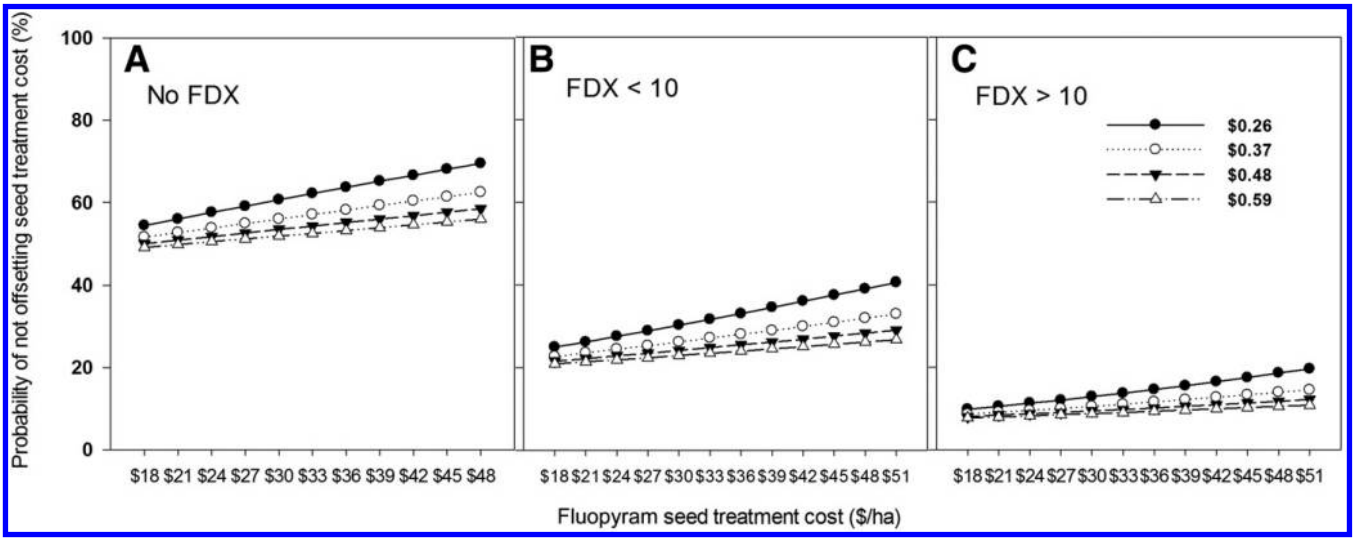

Fig. 3. Probability of not offsetting fluopyram seed treatment cost for a range of treatment costs and soybean market prices based on estimated mean yield difference ($\bar{D}$), and between-study variance $\hat{\sigma}^2$ from meta-analyses. Probability estimates were performed separately under different baseline disease scenarios for studies with **A,** no foliar disease index (FDX) (no disease); **B,** FDX > 0 but < 10 (low disease,); and **C,** FDX ≥ 10 (high disease).

disease level was high, across a range of fungicide cost–soybean price combinations tested.

Although the yield response and, consequently, the probability of profitability was significantly influenced by baseline disease, the inherent genetic yield potential and root rot resistance of a soybean cultivar, other disease and insect pressure, soil fertility and other environmental factors, weed pressure, cropping practices, and so on might also influence the effect size and profitability. Therefore, further investigations of how these factors influence yield response to fluopyram seed treatment may be needed to better explain under which conditions fluopyram seed treatment is consistently profitable. In addition, although an FDX of 10 was used as a threshold in this study based on a previous report (Kandel et al. 2016a) and the overall average FDX of the experiments used in the analysis, it is not clear what this level of disease means regarding the magnitude of yield loss to SDS and a decision threshold for fluopyram seed treatment. Luo et al. (2000) reported 18 to 29 kg/ha yield loss for each FDX unit increase; however, the relationship between FDX and yield loss is not linear because yield loss is also affected by the soybean growth stage at the time of disease onset (Roy et al. 1997).

Yield response to fluopyram seed treatment might be different when there is very high DS (for example, >50 FDX), which we did not investigate in this study because few locations had this disease level. However, this information will be useful for future studies to develop risk-assessment models based on field history and weather forecasts and to refine seed treatment recommendations under different scenarios.

Fluopyram can cause phytotoxicity in emerging seedlings, especially in cool, wet conditions (Wise et al. 2015). This has been a concern for soybean farmers because the phytotoxicity has the potential to lead to stand loss (Kandel et al. 2016a,b). Observations from the present study (data not shown) confirmed the results from previous reports (Gaspar et al. 2017; Kandel et al. 2016a,b) that there can be a slight reduction in plant population. However, there has been no report of yield loss due to the reduction in plant population by fluopyram, likely because soybean has an inherent ability to compensate (Board 2000; Carpenter and Board 1997).

The findings of this study have implications for integrated management of SDS. For example, fluopyram seed treatment is not recommended when SDS risk is low. However, risk is difficult to assess because methods for routine quantification of *F. virguliforme* in soil (Kandel et al. 2015b; Wang et al. 2015) or models for predicting SDS are currently not available. Field history can be used to assess risk, although SDS development will also be influenced by weather conditions (Leandro et al. 2013).

Although farmers have a new tool to manage SDS, the selection of cultivars resistant to foliar SDS should be the first management option, with fluopyram seed treatment suggested in fields at higher risk for SDS development; our data might be used as indicative of these regions. Two scenarios where fluopyram did not consistently provide a yield benefit that matched the cost of treatment were June-planted soybean and fields that had no foliar symptoms. Although farmers cannot predict whether foliar symptoms of SDS will develop in a particular season, using fluopyram in fields with no history of SDS may not be warranted. However, this recommendation does not account for fields that have damaging nematode populations but no history of SDS, which was outside the scope of our research. Our research demonstrates that fluopyram seed treatment can complement the resistant cultivars or other management tactics that are not sufficient to control SDS. However, the additional cost of treatment and the associated risk of not recovering fungicide cost should be considered.

Another advantage in selectively using fluopyram under higher risk of SDS is to preserve the activity of fluopyram against the fungus. This selectivity would be part of a resistance management strategy, which may be needed because several fungal species and lab mutants have been identified with reduced sensitivity to SDHI fungicides (FRAC 2017). Integrating other management practices for SDS such as selection of resistant varieties, SCN management, and crop rotation should be considered as part of a resistance management plan. Monitoring the sensitivity to fluopyram is also warranted when the fungicide is widely adopted by soybean farmers, and the control levels estimated here can be considered as baseline.

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