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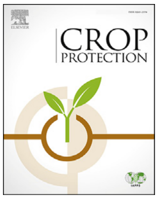

Plot size can influence yield benefits from fungicides on corn

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ABSTRACT

Use of foliar fungicides on corn has increased over the last decade. Part of the reason for this increase is due to physiological benefits on plants from QoI (strobilurin) containing fungicides. However, there remains controversy over how significant yield and economic benefits are from strobilurin fungicides. A potential source of this controversy might be explained by experimental plot size. To better understand grower-relevant yield benefits from fungicides, three hundred and fourteen commercial-strip trials (8.1 ha fungicide treated and 8.1 ha untreated) were conducted on growers' farms across four years, and twenty-five small plot (37.2 m^2 or less) trials were conducted across the corn belt in 2010. Yield benefits from fungicides were much greater in the commercial-strip trials than in the small plot trials. In 2011, twenty-six large plot trials (ranging from 557 to 1394 m^2), were established with efforts made to reduce border and alley effects. Two corn hybrids were evaluated at each of the 26 trial locations, and the results indicated that corn yield benefits from Quadris® fungicide (a solo formulation containing 22.9% azoxystrobin) applied at the V4-V8 growth stage, Quilt Xcel® fungicide (a premix formulation containing 13.5% azoxystrobin and 11.7% propiconazole) applied at the R1 growth stage, or a combination of the two, provided yield benefits similar to those from the commercial-strip trials. The financial gain/loss from the use of fungicides was determined. Using the highest cost estimates for fungicide and applications, Quilt Xcel fungicide applied at the R1 growth stage provided estimated yield benefits of $105, $219, $241, and $278/ha ($19, $65, $74, and $89/A) over the untreated checks in the commercial-strip trials conducted in 2009, 2010, 2012, and 2013, respectively. The average economic benefit to growers over the four year period was $211 ± 37/ha ($62 ± $15/A). Variability in economic benefit not only includes costs associated with fungicides but also includes annual commodity price, disease pressure, and location effects. This study supports the hypothesis that plot size influences assessment of yield effects of fungicides. Yield responses from the small plot, large plot, and commercial-strip trials resulted in increases of 378 kg/Ha (6 Bu/A), 701 kg/Ha (11 Bu/A), and 1132 kg/Ha (18 Bu/A) over the untreated, respectively.

1. Introduction

Due to several driving factors, fungicide use on maize has increased significantly over the past twenty years. First, gray leaf spot, caused by *Cercospora zeae-maydis* Tehon & E. Y. Daniels, became more prevalent in the 1980's and 1990's (Lipps et al., 1996; Lipps, 1987, 1998) concomitant with the adoption of reduced tillage practices in the US (Lipps, 1987, 1998). Gray leaf spot is one of the most yield-limiting fungal diseases of corn (Munkvold et al., 2001) and as a necrotrophic pathogen, the fungus overwinters in corn residues that remain in the field with reduced tillage. Second, with the threat of Asian soybean rust establishing in the US in the early 2000's, the US Environmental Protection Agency (EPA) granted Quarantine Section 18 registrations for several fungicide active ingredients including: cyproconazole, metconazole, myclobutanil, tebuconazole, propiconazole, prothioconazole, tetraconazole, flusilazole, flutriafol, pyraclostrobin, tebuconazole and pyraclostrobin, propiconazole and trifloxistrobin, flusilazole and femoxadone, metconazole and pyraclostrobin, propiconazole and azoxystrobin, and cyproconazole and azoxystrobin (Mueller and Eckermann, 2006). Several of these active ingredients and combinations of active ingredients were not registered in the US prior to this event. Many of the fungicides containing these active ingredients

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eventually received EPA federal registrations (Section 3 registrations) with corn on their labels. A third and more controversial reason for increased use of fungicides on corn has been due to industry promotion of physiological benefits that quinone outside inhibitor (QoI, also known as strobilurin) fungicides provide on some crops, including corn, above and beyond disease control.

Azoxystrobin, the active ingredient in Quadris® fungicide, and one of the two active ingredients in Quilt Xcel® fungicide, increases many of the beneficial antioxidant enzymes in plants and decreases some of the damaging reactive oxygen species (ROX). Azoxystrobin decreases super oxide (O^{-}_{2}) production in wheat while increasing both super oxide dismutase and peroxidase (Wu and von Tiedemann, 2001), which subsequently reduces ozone injury (Wu and von Tiedemann, 2002b). Azoxystrobin also reduces super oxide levels in barley (Wu and von Tiedemann, 2004), which is highly correlated with physiological leaf spot (Wu and von Tiedemann, 2002b; Wu and von Tiedemann, 2004), a malady that causes abiotic necrotic lesions and often decreases yield in parts of Europe (Jabs et al., 2002; Wu and von Tiedemann, 2002a). Azoxystrobin has also been found to increase super oxide dismutase, peroxidase, catalase, ascorbate peroxidase, glutathione reductase, and protein content in spring barley (Wu and von Tiedemann, 2002b). Other strobilurin fungicides have been shown to increase nitrate reductase levels in plants (Glaab and Kaiser, 1999; Wu and von Tiedemann, 2002b; Ruske et al., 2003), resulting in increased protein content (Wu and von Tiedemann, 2002b). Strobilurin fungicides also can reduce transpiration (Grossmann et al., 1999; Nason et al., 2007) and delay senescence in plants (Grossmann and Retzlaff, 1997; Gerhard et al., 1999; Beck et al., 2002). Therefore plants tend to utilize water more efficiently (Giuliani et al., 2011) and the leaves stay green longer (Below and Uribelarrea, 2009; Byamukama et al., 2013). Longer green leaf duration in corn is positively correlated with increased corn grain yields (Tollenaar and Daynard, 1978; Gregersen et al., 2013). The translation of many of these physiological benefits in terms of greener plants, stronger stalks, reduced lodging, and yield benefits have all supported grower adoption of fungicide use on corn.

Although physiological effects from strobilurin fungicides on plants are well documented, there remains skepticism that these benefits provide economic value unless disease pressure is high (Munkvold et al., 2001; Paul et al., 2011; Wise and Mueller, 2011; Bradley, 2012). Conflicting results in yield benefits from fungicides between fungicide manufacturers and other researchers have further added to the controversy. However, there are some fundamental differences in trial methodology that potentially could account for some of these differences. Within industry, products are often evaluated in large on-farm strip trials (often referred to as commercial-strip trials). The intent for these trials is to allow growers to see how products perform on their farms versus no treatment and/or other fungicide treatments. Most non-industry research focused on evaluating fungicides for yield benefits have been conducted using small plot trials that are standard for fungicide efficacy testing. Although this plot design is generally sufficient for evaluating fungicide efficacy, small plot trials are often not appropriate for determination of yield benefits for a variety of reasons including border and alley effects (Geater et al., 2004; Wang et al., 2013; Rebetzke et al., 2014). There also is a greater impact on yields from missing plants or inconsistent plant stands in small plots than in large plots. For example, missing 3 plants in a small plot (say stand count of 50 = 6% missing plants) has a much greater impact on yield than does missing 3 plants in a large plot (say stand count of 1000 = 0.3% missing plants).

The objective of the current study was to evaluate the QoI containing fungicides Quadris (Flowable formulation containing 2.08 lb. a.i. of azoxystrobin per gallon) and Quilt Xcel (SC formulation containing 1.02 lb. a.i. propiconazole and 1.18 lb ai azoxystrobin per gallon) for yield effects in commercial-strip trials, small-plot trials, and in large plot trials where border and alley effects were managed, to determine which testing method generates yield results and economic impact reflective of what growers might expect on their farms.

2. Materials and methods

2.1. Commercial-strip trials

From 2009 to 2013 excluding 2011, Syngenta field scientists conducted commercial-strip trials on growers' farms to evaluate Quilt Xcel fungicide for yield effects on corn. Strip trials were not conducted in 2011 due to focused efforts on conducing large plot trials with efforts to minimize border and alley effects (See section 2.3). Each trial consisted of approximately 8.1 ha (20 acres) of corn treated with Quilt Xcel fungicide and 8.1 ha (20 acres) of untreated corn. These trials were non-replicated, but growers were asked to use sections of the field for both the fungicide treated and untreated plots that were as similar to one another as possible, with the same agronomic practices for fertilization and weed control. Growers were also asked to provide yield data from both the fungicide treated and untreated sections of their field at the end of the season. Yield data were collected using growers commercial yield monitors.

A meta-analysis of the data was conducted to determine the overall mean difference between the fungicide-treated and untreated strips. Typically meta-analysis methods are conducted for replicated trials where the within-trial sampling variance is used to weight the results of each trial on the overall mean calculation (Madden and Paul, 2011; Paul et al., 2011). As our trials were non-replicated, each trial was instead assumed to have equal weight. The model was fit using PROC MIXED of SAS, the difference between the treatment and control was set as the response variable, trial was considered a random effect, and year was considered a fixed effect so an overall mean and 95% confidence interval of the mean was found for each year. Using this analysis method, the standard normal test statistic (Z) is used to determine whether the difference in treatments is significantly different from zero.

2.2. Small-plot trials

Syngenta field scientists and university plant pathologists conducted replicated small-plot trials in 2010 across many of corn growing states including: Georgia, Illinois, Indiana, Iowa, Kentucky, Minnesota, Missouri, Nebraska, New York, Ohio, Tennessee, and Wisconsin. Two experiments were conducted to evaluate Quadris and Quilt Xcel fungicides for efficacy against corn diseases and measure effects on yields under moderate to high disease pressure (experiment 1) and minimal disease pressure (experiment 2). The objectives of these trials were similar; the main difference was the level of disease pressure present. Eleven trials were established for experiment 1 with hybrids susceptible to gray leaf spot and/or common rust, in locations with a history of disease, and where corn was grown the previous growing season. Fourteen trials were established for experiment 2 growing hybrids with high genetic disease resistance, in locations with a history of low disease pressure, and where corn was not grown the previous growing season. To further maintain disease free plots, Bravo® fungicide (containing the active ingredient chlorothalonil) was used as needed. Bravo fungicide has broad spectrum activity against key fungal pathogens of corn, but imposes no known physiological effects. Trials were established using a randomized complete block design (RCBD) with four to six replications per treatment. The treatments included an untreated check, 0.44 l/ha (6 fl oz./A) of Quadris applied at the V4-V8 growth stage, 0.77 l/ha (10.5 fl. oz./A) of Quilt Xcel applied at the R1 growth stage, and 0.44 l/ha (6 fl oz./A) of Quadris applied at V4-V8 followed by 0.77 l/ha (10.5 fl oz./A) of Quilt Xcel applied at R1. Corn hybrids for most (23/25) of the trials were planted on 76.2 cm (30 inch) wide rows. The two trials with different plant spacing were planted on 97.0 cm (38 inch) and 91.0 cm (36 inch) wide rows. Plot lengths were left to the discretion of the scientists conducting the trials and varied from trial to trial so that the total plot sizes ranged from 14 to 45 m^2 (150–480 ft^2). For each treatment, four rows were treated with fungicide yet only the two center rows were harvested for yield for each trial. Disease severity for gray leaf spot, anthracnose, common rust, and northern corn leaf blight was assessed beginning 28 days after the V4-V8 fungicide application, and at 0, 21–28, and 35–42 days after the R1 fungicide application. The maximum disease severity ratings are reported herein.

The mean difference between each treatment and the control plots within each trial was the response variable used to conduct a separate univariate meta-analysis using PROC MIXED of SAS for each fungicide treatment. As there was replication at each trial location, the within-trial sampling variance was found and its inverse used as the weighting factor in the meta-analysis (Möhring and Piepho, 2009; Madden and Paul, 2011). Trial type (disease-favorable or disease-unfavorable) was included as a moderator variable and trial was included as a random effect as trial locations are intended to be representative of their geographic regions.

2.3. Large-plot trials minimizing border and alley effects

Syngenta agronomists, led by co-author Wayne A. Fithian, created a unique trial design which allowed for large plot sizes and minimal border and alley effects while accommodating the practical aspects of aerial applications (Fig. 1). In 2011, twenty-six trials of their design were established across the US corn-belt. Two hybrids were selected for each trial location based on geographical and commercial relevance (hybrids were selected that are best fit, and sold in each location). They were not consistent across locations, only 9 of the 52 hybrids were planted at two or three locations. For each hybrid and location, placement of the hybrids and Quadris strip treatments were randomized. To facilitate aerial applications of Quilt Xcel perpendicular to the direction of the rows, those treatments could not be randomized between the two hybrids. To reduce border effects a minimum of 12 rows of corn were planted at the edges of the field (Fig. 1). The minimum number of rows for Quadris fungicide test strips was 12 rows and the minimum length of the rows was 305 m (1000 feet) long. To prevent drift effects from areal applications of Quilt Xcel, a buffer strip was included in the middle of the field perpendicular to the corn rows (Fig. 1). Depending on the size of the buffer strip, treated areas ranges from 76.2 to 152.4 m (250–500 ft) long. When plants were at the V4-V8 growth stage, Quadris fungicide was applied at 0.44 l/ha (6 fl oz./A) in 93.5–140.3 L water/ha (10–15 gallons/A) within a strip running the direction of the rows. When corn reached the R1 growth stage, Quilt Xcel fungicide was applied by aircraft at 0.77 l/ha (10.5 fl oz./A) in a minimum of 18.7 L water/Ha (2 gallons water/A) to a strip running perpendicular to the Quadris treated strip. Therefore both of the hybrids in each plot received either: (1) Nothing ‘untreated check’, (2) 0.44 l/Ha (6 fl oz./A) of Quadris applied at the V4-V8 growth stage, (3) 0.77 l/Ha (10.5 oz./A) Quilt Xcel applied at the R1 growth stage, or (4) 0.44 l/Ha (6 fl oz./A) Quadris applied at the V4-V8 growth stage followed by 0.77 l/Ha (10.5 fl oz./A) Quilt Xcel applied at the R1 growth stage (Fig. 1). Yield data were obtained by commercial combine and yield monitors for each of the four treatment sections of each hybrid in all of the twenty-six trials.

Data from these trials were treated similarly as the commercial-strip trial data in that a separate meta-analysis was conducted for each treatment effect versus the untreated control. Due to the lack of consistent hybrids planted across locations, each of the two hybrids per location were considered an individual study in the meta-analysis resulting in 52 hybrid-locations. Each hybrid-location was used as a random effect.

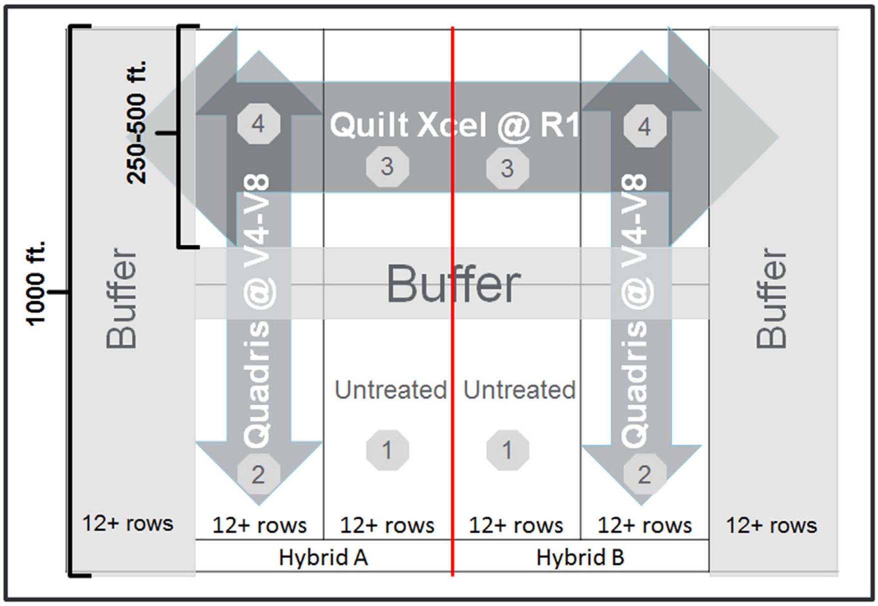

Fig. 1. Plot design for twenty-six large-plot corn trials minimizing border and alley effects, and focused on evaluating yield differences from Quadris® fungicide applied at the V4-V8 growth stage, Quilt Xcel® fungicide applied at the R1 growth stage, or the combination of the two vs. an untreated control. Refer to the text for an explanation of the treatment numbers. To reduce border effects a minimum of 12 rows of buffer corn were planted at the edges of the field as well as across the middle of the field running perpendicular to the direction of corn rows. There was a minimum of 12 rows of corn per treatment and corn was planted on 0.76 m (30 inch) rows. The minimum row length was 305 m (1000 ft). Length of treated rows ranged from 76.2 to 152.4 m (250–500 ft) depending on the width of the center buffer area.

2.4. Probability of recovering the fungicide application costs in the commercial-strip trials

To estimate costs associated with fungicide application and profits associate with yields, we used commodity prices and fungicide costs over the range of years covered in the commercial-strip trials. The average commodity price was \$5.0/Bu ranging from \$3.55/Bu in 2009 to \$6.89/Bu in 2012 (Quick Stats. USDA National Agricultural Statistics Service). Commodity prices are projected to be lower over the next decade with a projected average of \$3.56/Bu from 2015 to 2025 (USDA Agricultural Projections to 2024). Therefore, commodity prices ranging from \$3.00 to \$7.00/Bu in increments of \$1.00/Bu were used. Fungicide and application costs of Quilt Xcel range from \$24 to \$28/A over these years.

For each combination of fungicide (including application cost) and commodity price, the minimum yield (Bu/A) increase required to break even was determined. For example, an 8 Bu/A increase is required when commodity price is \$3/Bu and fungicide application cost is \$24/A. The probability of achieving the minimal yield increase was determined using standardized yield differences from our commercial-strip trials across 4 years, resulting in a single standard normal distribution. Then the probability that the yield difference due to the treatment was at least the yield difference required to break-even (X) was calculated as one minus the probability from the cumulative distribution function of the standard normal distribution ($1-\Phi(X_{std})$). To continue our example, the probability of achieving 8 Bu/A given the resulting trial data is determined by 1-ϕ((8-mean of trial data)/standard deviation of trial data).

3. Results

3.1. Commercial-strip trials

Data from 2009 to 2013, excluding 2011, are presented in piano chart format across the four years (Fig. 2) as well as the meta-analysis results for each year (Table 1). Across those 314 on-farm plots, 4.5% of the time there was a yield decrease, and 95.5% of the time there was a yield increase from Quilt Xcel fungicide over the untreated. From the meta-analysis, the overall mean yield benefit from R1 applications of Quilt Xcel fungicide over the untreated plots was 1132.2 kg/ha (18.0 Bu/A) (Table 1, Fig. 2). Using the highest fungicide and application cost of \$11/ha (\$28/A), the average net revenue benefits from Quilt Xcel fungicide applications at R1 were \$105/ha (\$19/A), \$219/ha (\$65/A), \$241/ha (\$74/A), and \$278/ha (\$89/A) for 2009, 2010, 2012, and 2013, respectively (Table 1). The average net revenue benefit and associated standard errors based on yield and commodity prices across this four year period was \$211/ha ± \$37/ha (\$62/A ± \$15/A) (Table 1).

3.2. Small-plot trials

Yield effects from Quadris fungicide applied at the V4-V8 growth stage, Quilt Xcel fungicide applied at the R1 growth stage, or the combination of the two varied considerably and provided little to no yield benefits in small plot trials (Tables 2 and 3). There were minimal differences in yields for the fungicide treatments between the trials where disease pressure was low (<5%) and those where disease pressure was moderate to high (>5%) (Table 3).

3.3. Large-plot trials minimizing border and alley effects

The average yield benefit from fungicide applications on corn were was lowest 328.3 kg/ha (5.2 Bu/A) with the early application of Quadris fungicide at the V4-V8 growth stage (Table 4). Quilt Xcel applied at the R1 growth stage provided a 6.0 Bu/A yield benefit over Quadris at V4-V8. Application of Quadris at the V4-V8 growth stage followed by Quilt Xcel applied at the R1 growth stage provided the greatest yield benefit of 10.1 Bu/A greater than Quadris

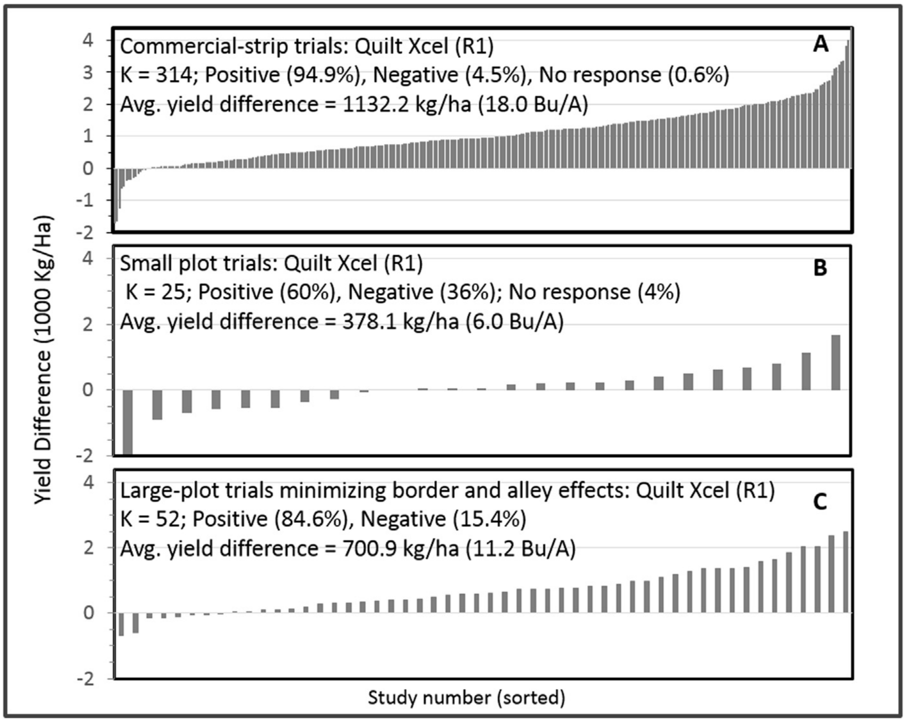

Fig. 2. Meta-analysis results for yield difference between fungicide treated and untreated corn, sorted from lowest to highest for **A**, Quilt Xcel® fungicide applied at the R1 growth stage in commercial-strip trials. **B**, Quilt Xcel fungicide applied at the R1 growth stage in small plot trials; **C**, Quilt Xcel fungicide applied at the R1 growth stage in large-plot trials minimizing border and alley effects.

Table 1
Yield and revenue benefits from one application of Quilt Xcel® fungicide applied at the R1 growth stage of corn in commercial-strip trials across four years (2009, 2010, 2012, and 2013).

Year	Average yield benefit kg/Ha (Bu/A)[a]	SE[b]	No. Obs[c]	Percent of trials with a yield benefit[d]	Commodity price ($/Bu)[e]	Average gross revenue benefit $/Ha ($/A)[f]	Average net revenue benefit from fungicide $/Ha ($/A)[g]
2009	830.33 (13.23)	84.86	92	94.6	3.55	116 (47)	105 (19)
2010	1130.52 (18.01)	64.15	161	93.2	5.18	230 (93)	219 (65)
2012	924.99 (14.74)	132.05	38	100	6.89	252 (102)	241 (74)
2013	1642.74 (26.17)	169.73	23	100	4.46	289 (117)	278 (89)
Avg.	1132.2 (18.0)	112.69 (5.8)	314	96.9	5.02	223 (91)	211 (62)
SE	5.9 (5.8)			6.3	0.71	35.4 (14.4)	37.3 (15.1)

[a] Effect size as mean yield difference between Quilt Xcel fungicide applied at the R1 growth stage and the untreated check over four years. All estimates were significantly greater than zero ($p < 0.001$).
[b] SE = standard error of the mean difference.
[c] Number of field trials conducted each year.
[d] Percentage of observations where yield was higher in the Quilt Xcel treated plots than in the untreated plots.
[e] Commodity price data from USDA Quick Stats (https://quickstats.nass.usda.gov/). To find these data do the following: Open "Data + statistics"; "Access Quick Stats"; "Quick Stats 2.0"; Program: survey; Sector: crops; Groups: Field crops; Commodity: corn; Category: price received; Data item: $/Bu; Geographical level: National; Years: 2009–2013; Period type: Annual; Period: Marketing year.
[f] Average gross revenue benefit calculated by multiplying the average yield benefit by the commodity price.
[g] Average net revenue benefit from fungicide application as calculated by subtracting fungicide and application costs from average gross revenue benefits. Assuming an average fungicide cost of $12/A and average application cost of $16/A for a total of = 28$/A ($11/Ha).

applied at V4-V8.

3.4. Yield comparisons with Quilt Xcel fungicide applications across the three plot sizes

Mean yield differences varied among the three experimental testing systems. As plot sizes increased so did the percentage of trials with a positive yield response. There was a greater percentage of trials with a positive yield response due to Quilt Xcel application in the commercial-strip trials (94.9%) than in either the small plot trials (60.0%) or the large-plot trials (84.6%) (Fig. 2). The average magnitude of yield responses to Quilt Xcel fungicide also increased with plot size. Yield responses from the small plot, large plot, and commercial-strip trials resulted in increases of 378 kg/Ha (6 Bu/A) (Table 3), 701 kg/Ha (11 Bu/A) (Table 4), and 1132 kg/Ha (18 Bu/A) (Table 1) over the untreated, respectively.

3.5. Probability of recovering the fungicide application costs in the commercial-strip trials

Considering the highest application cost of $28/A, and the lowest commodity price of $3/Bu there was a probability of 0.712 that a grower would at least break even (Table 5). Probabilities increase with increasing commodity price and decreasing

Table 2
Small-plot trial dimensions, disease severity, and yield differences for either Quadris® fungicide applied at the V4-V8 growth stage, Quilt Xcel® fungicide applied at the R1 growth stage, or the combination of Quadris applied at V4-V8 followed by Quilt Xcel applied at R1 for trials with moderate to high (≥5%) or low (<5%) disease pressure in 2010.

State	Row Spacing (m)	Plot length(m)	Plot width(m)	Plot area (m^2)	Harvested plot area (m^2)	Reps	Disease severity (%)[a]				Quadris 0.44 l/ha (V4-V8)	Quilt Xcel 0.77 l/ha (R1)	Quadris 0.44 l/ha (V4-V8) fb Quilt Xcel 0.77 l/ha (R1)
							GLS	Anth	Rust	NCLB			
≥**5%** Disease											Yield difference from untreated (1000 kg/ha)		
NE	0.76	9.15	3.0	27.9	14.0	4	23.8	10	5	0	1.13	0.94	1.68
IL-1	0.76	9.15	3.0	27.9	14.0	4	21.2	1.5	10	0	−0.55	−0.68	−0.03
IL-2	0.76	9.15	3.0	27.9	12.6	4	23	0	0	0	0.52	0.21	0.26
IA	0.76	9.15	3.0	27.9	14.0	4	0	0	0	21.2	−0.28	−0.05	0.02
KY	0.76	9.15	3.0	27.9	11.2	4	16.2	0	0	0	−0.68	−0.30	0.16
OH	0.76	12.20	3.0	37.2	18.6	4	2.8	11.5	0	1.8	−0.57	0.12	−0.49
MO	0.76	9.15	3.0	27.9	12.6	4	15.5	0	0	0	0.00	0.44	−0.13
IL-3	0.76	7.62	3.0	23.2	11.6	4	12.8	0	0	0	0.43	−0.24	−0.48
IN	0.76	12.20	1.5	18.6	7.0	4	8.8	0	0	0	−0.53	−0.42	1.15
TN	0.97	9.15	3.8	35.1	14.0	4	8	0	0	0	0.06	1.10	−0.21
KY	0.76	9.15	3.0	27.9	11.2	4	7.5	0	0	0	0.68	1.51	1.24
<**5%** Disease											Yield difference from untreated (1000 kg/ha)		
WI	0.76	9.15	3.0	27.9	14.0	4	1.2	1.6	0.4	0.9	0.17	0.37	−0.50
IN-1	0.76	9.15	3.0	27.9	14.0	6	1.7	0	0	0	0.24	−0.15	0.30
OH	0.76	9.15	3.0	27.9	14.0	4	0.6	0	0	0	0.06	−0.21	−0.07
GA	0.91	7.62	3.7	27.9	14.0	4	0	0	0	0	0.81	0.50	−0.33
IA	0.76	14.63	3.0	44.6	11.6	4	0	0	0	0	−0.07	0.12	0.80
IL-1	0.76	9.15	3.0	27.9	12.6	4	0	0	0	0	0.28	1.06	1.34
IL-2	0.76	6.55	3.0	20.0	10.0	4	0	0	0	0	−1.94	−1.47	−0.98
IN-2	0.76	9.15	3.0	27.9	27.9	4	0	0	0	0	0.05	0.19	−0.49
MN-1	0.76	9.15	1.5	13.9	7.0	4	0	0	0	0	−0.90	1.02	0.58
MN-2	0.76	9.15	3.0	27.9	14.0	4	0	0	0	0	0.25	−0.07	0.50
MO	0.76	12.20	3.0	37.2	37.2	4	0	0	0	0	−0.35	−0.06	0.40
NE	0.76	12.20	3.0	37.2	18.6	6	0	0	0	0	0.61	0.67	0.60
NY	0.76	9.15	3.0	27.9	4.1	4	0	0	0	0	1.66	−0.23	1.36
TN	0.76	9.15	3.0	27.9	14.0	4	0	0	0	0	0.20	0.74	0.83

[a] Disease severity in the untreated plots. GLS = gray leaf spot; Anth = anthracnose; Rust = common rust; NCLB = northern corn leaf blight.

Table 3
Yield effects from one application of Quadris® fungicide applied at the V4-V8 growth stage of corn, Quilt Xcel® fungicide applied at the R1 growth stage, or Quadris applied at the V4-V8 growth stage followed by (fb) Quilt Xcel applied at the R1 growth stage in small plot trials under low disease pressure (<5%) or moderate to high disease pressure (≥5%).

Fungicide		Average yield benefit kg/ha (Bu/A)[a]	SE[b]	95% Confidence interval	Z[c]	P[d]
Quadris (V4-V8)		147.05 (2.34)	110.91	(-70.35,364.45)	1.33	0.185
	<5%	181.75 (2.90)	132.06	(-77.12,440.62)	1.38	0.169
	≥5%	112.35 (1.79)	178.22	(-236.99,461.69)	0.63	0.528
Quilt Xcel (R1)		378.14 (6.02)	112.45	(157.72,598.56)	3.36	<0.001
	<5%	409.45 (6.52)	134.18	(146.43,672.48)	3.05	0.002
	≥5%	346.82 (5.53)	180.28	(-6.57,700.21)	1.92	0.054
Quadris (V4-V8) fb Quilt Xcel (R1)		278.02 (4.43)	122.47	(37.95,518.1)	2.27	0.023
	<5%	439.22 (7.00)	147.09	(150.89,727.55)	2.99	0.003
	≥5%	116.83 (1.86)	194.99	(-265.39,499.05)	0.60	0.549

[a] Effect size as mean yield difference between Quilt Xcel fungicide applied at the R1 growth stage and the untreated check over four years. All estimates were significantly greater than zero ($p < 0.001$).
[b] SE = standard error of the mean yield difference.
[c] Z = (standard normal) statistic from the meta-analysis.
[d] P = significance level for the mean yield difference.

Table 4
Yield effects from one application of Quadris® fungicide applied at the V4-V8 growth stage of corn, Quilt Xcel® fungicide applied at the R1 growth stage, or Quadris applied at the V4-V8 growth stage followed by (fb) Quilt Xcel applied at the R1 growth stage in large-plot trials minimizing border and alley effects.

Fungicide	Average yield benefit kg/ha (Bu/A)[a]	SE[b]	95% Confidence interval	Z[c]	P[d]
Quadris (V4-V8)	328.3 (5.2)	89.3	(153.3503.3)	3.68	<0.001
Quilt Xcel (R1)	700.9 (11.2)	97.6	(509.6892.3)	7.18	<0.001
Quadris (V4-V8) fb Quilt Xcel (R1)	893.4 (15.3)	104.6	(688.3,1098.5)	8.54	<0.001

[a] Effect size as mean yield difference between the treatments and the untreated check.
[b] SE = standard error of the mean yield difference.
[c] Z = (standard normal) statistic from the meta-analysis.
[d] P = significance level for the mean yield difference.

Table 5
Probability of at least breaking even following an application of Quilt Xcel® fungicide at the R1 growth stage given ranging application costs and with corn commodity prices ranging from $3.00 to $7.00/Bu.

Treatment[a]	Commodity price[b]	Probabilities of breaking even on fungicide treatment								
		Fungicide and application cost[c]								
Quilt Xcel (R1)	($/Bu)	$24.00	$24.50	$25.00	$25.50	$26.00	$26.50	$27.00	$27.50	$28.00
	$3.00	0.745	0.741	0.737	0.733	0.728	0.724	0.720	0.716	0.712
	$4.00	0.790	0.788	0.785	0.782	0.779	0.777	0.774	0.771	0.768
	$5.00	0.815	0.813	0.811	0.809	0.807	0.805	0.803	0.801	0.799
	$6.00	0.831	0.829	0.828	0.826	0.824	0.823	0.821	0.819	0.818
	$7.00	0.841	0.840	0.839	0.837	0.836	0.835	0.833	0.832	0.831

[a] Quilt Xcel fungicide applied at 0.77 l/ha (10.5 fl oz./A) at the R1 growth stage of corn.
[b] Commodity prices used in the rewards analysis ranged from $3-$7/Bu in increments of $1.
[c] Fungicide and application costs reflective of 2015 market prices.

application costs; with the maximum estimated probability of 0.841.

4. Discussion

There has been a substantial increase in the use of fungicides on corn in the US, even under low disease pressure. Some scientists have challenged whether it is economical for a grower to use a fungicide for benefits beyond disease control (Munkvold et al., 2008; Paul et al., 2011; Wise and Mueller, 2011; Mallowa et al., 2015). These challenges are warranted as yield results from small plot studies typically used to evaluate fungicide efficacy have not reflected positive return on investment to growers (Paul et al., 2011). However, large scale Industry demonstration plots where at least 20 acres of corn are treated and 20 acres are left untreated provide greater yield differences leading to increased economic returns. This discrepancy in yield results lead us to suspect that plot size might play a critical role in accurate assessment of yield benefits from fungicides on corn. After testing small plots, large plots, and strip plots our results illustrate that yield differences from fungicides increased with increasing plot size. The increase was sufficient enough that even with low commodity prices ($3.00/Bu) and fungicide application costs growers have a high probability (about 0.73) of making a profit on their investment.

Other researchers have suggested that assessment of crop yields can be influenced by plot size and other factors that can influence yield. Geater et al. (2004) demonstrated that by increasing the number of rows in a plot they could minimize error due to border effects among corn hybrids, and that by increasing the plot length, they could minimize alley effects among hybrids. Their work was focused on determining the influence of plot size on yields for breeding research and was not directed at evaluating fungicides. However, the same principles hold true for evaluation of fungicide for yield benefits under low disease pressure. These results are consistent with results from other field trials where yields have been demonstrated to be higher near alleys than plants toward the center of the plot (Arny, 1922; Wilcox, 1970; Holman and Bednarz, 2001; Geater et al., 2004; Vincelli and Lee, 2015). This could potentially be due to less competition and/or better light interception with fewer surrounding plants near the edges of the field. Most plants in small plots are not far away from an edge and they are likely near their maximum yield potential. Therefore, a fungicide application may only add a limited benefit. However, within large fields and away from the edges per plant yields are lower, which provides room for yield improvements with products that can mitigate plant stresses, like QoI fungicides. This is only one possible hypothesis that may explain the controversy in yield results from small plot trials and larger plot trials.

Although yield is a critical factor, it is only one component of the benefits that growers can gain from QoI fungicides like Quadris and Quilt Xcel. QoI fungicides can improve corn stalk quality and reduce lodging (Tenuta and Hooker, 2009; Mahoney et al., 2015). Both of these attributes provide additional benefits to growers by improving harvest efficiency, as the combine can harvest the crop at a faster pace. This reduces fuel consumption, equipment wear-and-tear, and labor costs. Reducing lodging also means that growers can leave corn in the field longer and allow the crop to dry down naturally instead of harvesting early in a race against the weather. This also saves growers costs of drying their corn. Reduced lodging also means less seed is returned to the field, reducing volunteer corn the following season. There are significant economic benefits to growers by reducing volunteer corn in soybean fields following corn (Beckett and Stoller, 1988).

Clearly more research is needed to determine the most appropriate plot dimensions, including number of border-rows, fungicide treated rows, and harvested rows in order to obtain yield results reflective of growers' fields. The authors encourage others to conduct trials in larger plots, managing border and alley effects. Recently, some researchers began testing fungicides for yield benefits on corn using larger trial designs (Vincelli et al., 2013a, 2013b, 2013c; Vincelli and Lee, 2015). In 2013, Vincelli et al. (2013b,c) reported that a single QoI fungicide application increased corn yields by 1251.2 kg/ha (19.9 Bu/A), and 4287.9 kg/ha (27.6 Bu/A) over the untreated checks.

Although the studies reported herein illustrate the value of increasing plot size when evaluating fungicides for yield benefits under minimal disease pressure, we must recognize the limitations and challenges of testing in larger plots. Larger plots require more land and resources, both of which limit the number of products that can be tested. With larger plots, one also needs to pay close attention to differences across the field and attempt to provide consistent and uniform field conditions across all plots. Large plot trials with early-stage and/or unregistered compounds pose additional resource constraints as they require crop destruction. Finally, not all corn hybrids respond the same way to QoI fungicides (Bradley et al., 2008). Some hybrids are very responsive where others are less responsive, or not responsive at all. It is important to recognize this and to include at least one or more commercially available responsive hybrid(s) that are appropriate for the testing geography when evaluating QoI fungicides for potential yield benefits on corn.

It remains unclear what the optimal plot size should be to evaluate fungicides for yield benefits under minimal disease pressure. However, it is clear that we need to rethink the way we test fungicides for yield benefits particularly when disease pressure is low. It is important to recognize this and establish appropriate field trials if yield is an important component to be measured, and if the results are to be used to provide meaningful guidance to growers.

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