ORIGINAl RESEARCH ARTICLE
Agrosystems

Genetic variation for response to mixed triazole and strobilurin application in diverse maize

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Funding information
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Abstract
Strobilurin and triazole classes of fungicides have been reported to have growth-regulating effects on crops in the absence of their target pathogens and to increase maize yield. Because the response of maize (Zea mays L.) plants to a commercially marketed mixture of strobilurin and triazole may not be uniform across genotypes, it may be possible to breed selectively for higher yield responses to application of this chemical treatment. To test this hypothesis, diverse samples of maize inbred lines and hybrids were evaluated for response to a combined mixture of a treatment of strobilurin and triazole. Main effects of treatment and genotype and treatment × genotype interactions were measured on agronomic traits including grain yield and several yield components, lodging, and delayed leaf senescence. Hybrid and inbred genotype main effect variation was significant for all measured traits. Favorable main effects of strobilurin and triazole treatment were observed only for leaf senescence and foliar disease, and genotype × treatment interactions were not significant for yield or yield components. Yield was significantly increased only in two inbred varieties and did not correlate with any known pedigree or genetic relationships. These results suggest that breeding to enhance the response to strobilurin and triazole treatment is not likely to be effective in maize.

1 INTRODUCTION

According to Rademacher (2015), “plant growth regulators can be defined as naturally occurring or synthetic compounds that affect developmental or metabolic processes in higher plants, mostly at low dosages. They do not possess a nutritive value and, typically, are not phytotoxic.” Growth regulators can affect induction of flowering or delay senescence and represent a potential mechanism for yield increases, in addition to genetic improvement through breeding and other agronomic production improvements. Several fungicide classes have growth-regulating effects on plants, and studies have shown measurable yield increases from the application of certain fungicide classes to grain crops in the absence of fungal infection (Blandino, Galeazzi, Savoia, & Reyneri, 2012; Paul et al., 2011; Wu & von Tiedemann, 2001).

Strobilurins are a class of fungicide developed in the 1990s as synthetic mimics of the toxin produced by the fungal genus Strobilurus, among others (Anke, Oberwinkler, Steglich, & Schram, 1977). Strobilurins act as quinone-outside-inhibitors, blocking electron transport at the Cytochrome bc1 complex in the mitochondrial inner membrane (Becker, Von Jagow, Anke, & Steglich, 1981). The first commercial strobilurins were brought to market in 1996 (Bartlett et al., 2002), and numerous studies since then have identified the effects of

Abbreviations: ASI, anthesis–silk interval; GA, gibberellic acid.
strobilurin on wheat, in particular the tendency of strobilurin-treated plant tissue to remain green after untreated tissue has senesced (Grossmann & Retzlaff, 1997; Jones & Bryson, 1998; Wu & von Tiedemann, 2001). This “stay-green” trait was later identified in other small-grain crop species in the field (Bayles & Hilton, 2000) as well as in maize (Byamukama, Abendroth, Elmore, & Robertson, 2013), with several different strobilurin fungicides the subject of these studies.

Venancio, Rodrigues, Begliomini, and de Souza (2003) suggested that the physiological effects of strobilurins might justify their use in maize as growth regulators in the absence of disease. Early studies generally found that there was insufficient evidence to suggest that strobilurin application in small grains was apt to be profitable in the absence of disease (Weisz, Cowger, Ambrose, & Gardner, 2011). More recent studies have attempted to better replicate commercial farming operations, testing for economic yield boost at larger scales, with results suggesting that strobilurin sprays for yield boost are financially justified but that the effects on yield are difficult to measure on small experimental plots (Tedford et al., 2017; Vinelli & Lee, 2015).

The triazoles, another fungicide class, have been shown to reduce production of gibberellic acid (GA) (Rademacher, Fritsch, Graebe, Sauter, & Jung, 1987). Although GA is known to regulate stem length and plant maturation, it is only one element in the regulation of these traits (Evans & Poethig, 1995); however, GA is important in signaling the start of leaf senescence (Fletcher, Sopher, & Vettakkorumakankav, 2000). Wu and von Tiedemann (2001) showed that triazole application, particularly as plants near maturity, aids in retarding leaf senescence and suggested that this stay-green effect may be a result of the triazole’s suppression of GA. Many tests of the use of fungicides as growth regulators in cereal crops have made use of a strobilurin and a triazole in combination (e.g., Tedford et al., 2017) or separately (e.g., Paul et al., 2011). Strobilurin-treated maize has greater stalk strength and less lodging than untreated maize (Kalebich et al., 2017; Paul et al., 2011).

The effectiveness of growth regulator application on yield response may depend on cultivar. It is possible that some cultivars would respond more than others, and if such differences were sufficiently strong, this could justify breeding for higher response to growth regulators. To date, studies of growth regulator response have involved only small samples of available cultivars adapted to target production environments, and at least one study indicated variability in cultivar response, although it was not consistent across environments and may have been related to disease resistance rather than growth regulator response per se (da Costa, Cota, da Silva, Meirelles, & Lanza, 2012). Relative to the genetic variability available worldwide in maize, the genetic variation within elite commercial Corn Belt Dent hybrids is limited (Goodman, 2005). Studies of the response to a commercially marketed mixture of strobilurin and triazole in a broader array of maize germplasm may lead to the discovery of a greater range of potential maize response to the application of this mixture, and the most highly responsive germplasm might be used in targeted breeding programs to develop commercial-quality cultivars with significant response to strobilurin and triazole mixture, permitting greater overall maize yields.

The objectives of this study were to test a very diverse sample of maize inbred lines and hybrids for response in yield, yield components, and other agronomic traits to strobilurin and triazole treatment and to test for treatment main effects and treatment × variety interaction. Treatment × variety interactions occur when some varieties are more strongly affected (or affected in a different direction) by the treatment than others, indicating that genotypes vary in their response to treatment. The relative importance of these interactions is an indicator of the potential genetic variability for response to strobilurin and triazole mixture that could be exploited by breeding.

### Core Ideas

- A commercial fungicide mixture has been reported to increase yield in maize.
- We found no variation among diverse maize varieties for response to this mixture.
- Breeding maize for higher response will be difficult.

### 2 MATERIALS AND METHODS

To investigate the potential variability and heritability of maize response to strobilurin and triazole application, we evaluated 21 maize hybrids (Table 1) in replicated trials at three locations in North Carolina in 2016 and 2017. Planting dates, soil types, and monthly weather summaries for the growing seasons at these six environments are provided in File S1. These hybrids were drawn from diverse genetic backgrounds, including high-yielding commercial cultivars, crosses between important public inbreds and commercially developed inbreds with expired plant variety protection certificates, and crosses involving temperate and tropical inbreds and popcorn. A sweet corn hybrid (IL14H × P39) was also planted as part of the evaluation experiments, but every plot was damaged by wildlife feeding, preventing accurate measurement of foliar disease or yield; therefore, no data from this hybrid were included in the analysis. The popcorn hybrid Hp301 × SA24 had very low yields, so total grain yield and grain moisture for this hybrid were not included in analyses.
but they were included in analysis for all other traits, including yield components.

At each location in both 2016 and 2017, the experiment was planted in a split-plot design with three complete replications, where treatment with a mixture of strobilurin and triazole comprised the whole-plot factor and hybrid was the sub-plot factor. Experimental units consisted of four 3.6-m rows planted with the same hybrid; row spacing varied from 76 to 96.5 cm depending on location. Seed was sown at a density of 6.8 seeds m$^{-1}$. The treatments were no treatment (control) or application of fungicide (Quilt XCel, Syngenta) at growth stage V5/V6 and again at growth stage VT. Quilt XCel is a blend of 13.5% azoxystrobine and 11.7% propiconazole. The chemical mixture was applied only to the center two rows of the four-row plots using a backpack sprayer delivering 241.3 kPa through a flat-fan nozzle at the labeled application rate of 0.77 L ha$^{-1}$. All plots within the treated block at an interval (ASI) was the difference between days to silking and days to anthesis.

A composite foliar disease rating was taken on each plot using a 0–9 scale. Because each hybrid used in the experiment was not equally susceptible to each disease, the goal of the disease scoring was to generate a rating of overall disease pressure rather than to note individual diseases in each plot. Thus, a plant that had limited gray leaf spot lesions on leaves below the ear leaf would rate an 8, whereas another plant with limited rust on lower leaves would also rate an 8. Overall loss of green tissue was the primary rating objective. Rated diseases included northern corn leaf blight (Elsinoe turcicum Leonard and Suggs), southern corn leaf blight (Bipolaris maydis Nisikado and Miyake), gray leaf spot (Cercospora zeae-maydis Tehon and Daniels), eye spot (Aureobasidium zeae Narita and Hiratsuka), and rust [Puccinia sp. (Pers.)]. A rating of 0 indicated that every plant in the plot was dead from disease; a rating of 9 indicated that no plants showed any signs of disease. All plants with each plot were inspected, and the plot score was assigned based on the mean plant values. Disease rating was accomplished within a week of plot flowering time at each location.

Plant and ear heights were measured approximately 2 wk after flowering, with plant height measured from base to flag leaf node and ear height measured to the insertion point of the primary (highest) ear.

Green leaf counts were made roughly 2 wk after maturity and weekly thereafter until the majority of plants had senesced. Scores are the average number of green leaves (defined as leaves having >50% green tissue) per plant in a plot, with six plants per plot being counted.

Lodging was measured at maturity prior to harvest by counting plants leaning more than 30 degrees from vertical, with broken stalks, or with dropped primary ears. Percent lodging was computed as the number of lodged plants divided by the stand count for the plot. For grain yield and moisture content, the center two rows of each plot were mechanically harvested. Machine-harvestable grain yield and moisture of the two center rows of each plot were measured, and yield was adjusted to 15.5% grain moisture and converted to Mg ha$^{-1}$ based on the area of the plots at each location. Plot-level data for the hybrid experiments are available in File S2.

In 2017, a separate experiment was conducted to measure response to strobilurin and triazole mixture for 40 diverse maize inbred lines (Table 2) using a similar split-plot design with three replications at the same three locations but with a single 3.6-m row as the experimental unit. Treatment application was performed in the same manner as in the hybrid experiment. Plots were scored for the same traits as the hybrids except for grain moisture content because the single-row plots were harvested by hand rather than mechanically. Total ear count per plot was recorded for the inbreds.
TABLE 2  Inbred germplasm used in the study

| Inbred  | Origin     | Germplasm group |
|---------|------------|-----------------|
| 207     | Pioneer Hi-Bred | Ident           |
| 6M502   | DeKalb-Pfizer  | Non-Stiff stalk  |
| A632    | Minnesota    | Stiff stalk      |
| B73     | Iowa         | Stiff stalk      |
| B97     | Iowa         | Non-Stiff stalk  |
| CML103  | CIMMYT      | Tropical         |
| CML228  | CIMMYT      | Tropical         |
| CML247  | CIMMYT      | Tropical         |
| CML277  | CIMMYT      | Tropical         |
| CML322  | CIMMYT      | Tropical         |
| CML333  | CIMMYT      | Tropical         |
| CML52   | CIMMYT      | Tropical         |
| CML69   | CIMMYT      | Tropical         |
| F118    | DeKalb      | Stiff stalk      |
| Hp301   | Indiana      | Supergold popcorn |
| IL14H   | Illinois     | Sweet corn       |
| Ki11    | Thailand     | Tropical         |
| Ki3     | Thailand     | Tropical         |
| Ky21    | Kentucky     | Non-Stiff stalk  |
| LH211   | Holden’s     | Non-Stiff stalk  |
| LH252   | Holden’s     | Non-Stiff stalk  |
| M162W   | South Africa | Non-Stiff stalk  |
| M37W    | South Africa | Mixed           |
| Mo17    | Missouri     | Non-Stiff stalk  |
| Mo18W   | Missouri     | Non-Stiff stalk  |
| Ms71    | Michigan     | Non-Stiff stalk  |
| NC350   | North Carolina | Tropical     |
| NC358   | North Carolina | Tropical     |
| Ob43    | Ohio        | Non-Stiff stalk  |
| Oh7B    | Ohio        | Non-Stiff stalk  |
| P39     | Indiana      | Sweet corn       |
| PHB47   | Pioneer Hi-Bred | Stiff stalk   |
| PHG35   | Pioneer Hi-Bred | Non-Stiff stalk |
| PHHB4   | Pioneer Hi-Bred | Stiff stalk   |
| PHR58   | Pioneer Hi-Bred | Non-Stiff stalk |
| Tx303   | Texas        | Mixed           |
| Va35    | Virginia     | Non-Stiff stalk  |
| W64A    | Wisconsin    | Non-Stiff stalk  |

Also in 2017, four additional traits were measured for all hybrid and inbred plots: ear length, ear tip fill, 50-kernel weight, and kernel row number. Four random ears were sampled from each plot at maturity to measure ear and kernel traits. Ear length was measured using a Fowler 12” Ultra-Cal IV electronic caliper from base to tip of ear. The same caliper was then used to measure the unfilled portion of the ear, and ear fill percentage was calculated from this measurement. Kernel row number was counted on the same four ears. These four ears were then shelled, the kernels were bulked, and 50 kernels were selected at random and weighed to generate a 50-kernel weight for each plot. Plot-level data for the inbred experiments are available in File S3.

Data were analyzed using Proc MIXED in version 9.4 of the SAS System for Windows (SAS Institute, 2013). The following statistical model was used for the hybrid experiment:

\[
Y_{ijkl} = \mu + G_i + T_j + GT_{ij} + D + S + E_k + BE_{l(k)} + TBE_{jl(k)} + GTE_{ijk} + e_{ijkl}
\]

where \(Y_{ijkl}\) represents the measured trait value of an experimental unit, \(\mu\) represents the overall mean, \(G_i\) represents the fixed effect of hybrid, \(T_j\) represents the fixed effect of treatment, \(GT_{ij}\) represents the interaction between hybrid and treatment, \(D\) is a covariate accounting for the effect of disease pressure in each plot, \(S\) is a covariate accounting for the effect of differences in stand count (for dependent variable yield only), \(E_k\) represents the random effect of environment (year-location combination), \(BE_{l(k)}\) represents the random effect of block within environment, \(TBE_{jl(k)}\) represents the interaction of treatment and block within environment (the whole-plot error effect), additional terms represent interactions among variables, and \(e_{ijkl}\) is the residual error.

For the inbred study, the following statistical model was used:

\[
Y_{ijkl} = \mu + G_i + T_j + GT_{ij} + D + S + L_k + BL_{l(k)} + TBL_{jl(k)} + GL_{ik} + TL_{jk} + GTL_{ijk} + e_{ijkl}
\]

where \(Y_{ijkl}\) represents the measured trait value of an experimental unit, \(\mu\) represents the overall mean, \(G_i\) represents the fixed effect of inbred line, \(T_j\) represents the fixed effect of treatment, \(GT_{ij}\) represents the interaction between treatment and inbred line, \(D\) is a covariate accounting for the effect of disease pressure in each plot, \(S\) and \(C\) are covariates accounting for the effect of differences in stand count and harvested ear count (for dependent variable yield only), \(L_k\) represents the random effect of location, \(BL_{l(k)}\) represents the random effect of block within location, \(TBL_{jl(k)}\) represents the interaction of treatment and block within location (the whole-plot error effect), additional terms represent meaningful interactions among variables, and \(e_{ijkl}\) is the residual error. The SAS code used for hybrid experiment analysis is provided in File S4; the SAS code used for inbred experiment analysis is provided in File S5.

Models with and without the disease rating as a covariate were fit to the data from both experiments, and results the results were compared to determine if treatment effects or treatment \(\times\) variety interactions depended on disease pressure.
3 | RESULTS

3.1 | Hybrid experiment

Hybrid main effect was significant \( (p \leq 0.05) \) for all measured traits when foliar disease was adjusted for and for almost all traits without the disease covariate (Table 3). In contrast, the main effect of strobilurin plus triazole treatment in the hybrid study was significant for only three traits: grain moisture, foliar disease score, and late-season green leaf count (Table 3). Although disease pressure was relatively low in hybrids (mean rating 7.7 on 9-point scale, with 9 reflecting no disease present), application of the strobilurin and triazole mixture resulted in an average reduction in observable disease level of 1.7 points on the 9-point scale \( (p < 0.0001) \). Treatment was associated with an increase of 0.5% grain moisture on average (increasing from 17.6 to 18.1%) and a mean increase of 1.4 more green leaves at late-season rating time, reflecting significantly delayed senescence.

The interaction of treatment and hybrid, a measure of variation among hybrids for response to treatment, was significant for only two traits: disease rating \( (\text{as also reported previously by da Costa et al. [2012]} \) and ASI (Table 3; Files S6–S9). Disease score response to treatment ranged from 1.2 points for PHB47 × LH51 to 2.3 points for B73 × LH211; the reduction was also significant for each hybrid individually (Files S6 and S7). Only two hybrids had significant individual responses to treatment for grain moisture, and moisture was increased by treatment in both two cases (Figure 1). Treatment × environment interaction variance components were relatively small for all traits and were always smaller than hybrid × environment interaction variances (File S10). The variance component for the three-way interaction of treatment, hybrid, and environment was also smaller than the hybrid × environment variance component for all traits except ASI.

Because grain yield differences may be difficult to identify in small research plots, we measured yield components, including ear length, percent cob fill, kernel row number, and kernel weight, in the second year of the experiment. If there were any patterns of increased ear length, fill rate, row number, or kernel size, these might be extrapolated to suggest potential yield effects at larger scales. However, none of these yield components was significantly altered by treatment (Table 3).

Anthesis–silking interval is frequently used as a gauge of plant stress during the critical flowering period (Hall, Vilella, Trapani, & Chimenti, 1982). If treatment withfungicide reduces plant stress, this might be reflected in reduced ASI for treated plants, which may suggest potential yield benefits if replicated at large scale. Anthesis–silking interval varied significantly because of the interaction of material and treatment (Table 3) but not in a consistent direction. Anthesis–silking interval increased with strobilurin plus triazole application for some hybrids but decreased for others (Figure 1). Anthesis–silking interval was not correlated with yield or with any yield component.

Aside from disease score and late-season green leaf count (which had consistent increases due to treatment), 12 agronomic and yield component traits were measured on hybrids, resulting in a total of 252 trait–hybrid combinations (although yield and moisture were not measured for the popcorn hybrid). For 12 of the 250 measured trait–hybrid combinations (4.8%), the \( p \) value associated with the simple treatment effect specific to that hybrid was significant at \( \leq 0.05 \) (Figure 1). This is about the proportion of significant tests expected under the null hypothesis of no responses to treatment. Multiple testing correction \( (\text{e.g., Tukey or Bonferroni}) \) would result in no comparisons being significant. Even without correcting for multiple testing, no clear pattern of treatment response was observed: responsive hybrids were not from common germplasm groups, nor were hybrids that showed response to treatment in one trait more likely to show response in another (Figure 1).

Although disease rating was itself significantly reduced by treatment, when disease pressure was included as a covariate in the analysis of other traits, it had little effect. The inclusion of the disease pressure covariate had relatively little effect on the statistical significance of the treatment or treatment × environment effects. Table 3 shows the few exceptions to this rule; however, again no clear patterns emerge regarding value of disease covariate in significance of measured effect.

3.2 | Inbred experiment

The inbreds used in this study represent an even wider array of maize diversity than the hybrids. However, our findings are consistent with the hybrid experiment in that no pattern of significance emerged among related germplasm. The inbred main effect was strongly significant for every trait, whereas the treatment main effect was significant only for disease rating \( (p = 0.04) \), days to anthesis, and ASI (Table 3). The interaction of inbred with treatment was significant only for lodging and stay-green. The traits for which the treatment × genotype interaction was significant in the inbred experiment are entirely distinct from those for which the interaction was significant in the hybrid experiment.

Eleven agronomic traits (excluding disease and green leaf scores) measured on 39 inbreds resulted in a total of 429 comparisons of specific genotype–treatment effects (Files S11–S14). In 24 of these trait-inbred comparisons...
TABLE 3  Adjusted means of treated and control plots, response to application of a mixture of strobilurin and triazole measured as the difference between treated and control groups, and significance of treatment main effects, genotype main effects, and treatment × genotype interactions for both hybrids and inbreds

|                           | Yield | 50-kernel wt. | Kernel row no. | Cob fill | Ear length | Grain moisture | Days to anthesis | ASI | Ear height | Plant height | Lodging | Disease | Late green leaves |
|---------------------------|-------|---------------|----------------|----------|------------|----------------|------------------|-----|------------|-------------|---------|---------|-------------------|
| Hybrid experiment group means |       |               |                |          |            |                |                  |     |            |              |         |         |                   |
| Treated                   | 9.0   | 13.6          | 15.3           | 86.1     | 17.9       | 18.1           | 66.0             | 0.7 | 110.5      | 195.5       | 6.9     | 8.6     | 4.3               |
| Control                   | 9.0   | 13.4          | 15.0           | 86.6     | 17.4       | 17.6           | 66.6             | 0.6 | 111.9      | 193.0       | 10.2    | 6.9     | 3.4               |
| Treated – control         | 0.0   | 0.2           | 0.3            | –0.05    | 0.4        | 0.5            | –0.6             | 0.1 | –1.4       | 2.5         | –3.4    | 1.7     | 1.0               |

Hybrid experiment significance of F tests

|                           | Treatment without disease covariate | Treatment with disease covariate | Hybrid without disease covariate | Hybrid with disease covariate | Treatment × hybrid without disease covariate | Treatment × hybrid with disease covariate |
|---------------------------|-------------------------------------|----------------------------------|----------------------------------|----------------------------------|---------------------------------------------|-------------------------------------------|
|                           | ***                                 | **                               | ***                              | ***                              | ***                                         | **                                         |
|                           | ***                                 | NA                               | ***                              | ***                              | ***                                         | ***                                       |
|                           | ***                                 | ***                              | ***                              | ***                              | ***                                         | ***                                       |
|                           | ***                                 | ***                              | ***                              | ***                              | ***                                         | ***                                       |
|                           | ***                                 | ***                              | ***                              | ***                              | ***                                         | NA                                        |

Inbred experiment group means

|                           | Treated | 497 | 11.9 | 13.3 | 85.6 | 14.1 | 10.6 | 72.0 | 2.0  | 75.2 | 154.0 | 5.8  | 8.0  | 7.7  |
|---------------------------|---------|-----|------|------|------|------|------|------|------|------|-------|------|------|------|
| Control                   | 495     | 11.8| 13.3 | 85.2 | 14.1 | 10.7 | 72.6 | 2.7  | 77.4 | 157.7 | 7.0  | 6.9  | 7.2  |
| Treated – control         | 2       | 0.0 | 0.0  | 0.4  | 0.0  | (0.1)| –0.6| –0.6 | –2.2 | –3.7 | –1.2  | 1.1  | 0.5  | (Continues) |

(Continues)
| Yield | 50-kernel wt. | Kernel row no. | Cob fill | Ear length | Grain moisture | Days to anthesis | ASI | Ear height | Plant height | Lodging | Disease | Late green leaves |
|-------|---------------|----------------|----------|------------|----------------|------------------|-----|------------|--------------|---------|---------|------------------|
| Mg ha$^{-1}$ g | count | % | cm | % | ——— d ——— | ——— cm ——— | % | 9-point scale | count |

**Inbred experiment significance of F tests**

| Treatment without disease covariate | * | ** | NA |
| Treatment with disease covariate | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| Inbred without disease covariate | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** |
| Inbred with disease covariate | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | *** | NA | *** |
| Treatment × Hybrid without disease covariate | * | ** | * |
| Treatment × Hybrid with disease covariate | * | NA |

*Note.* ASI, anthesis–silk interval. NA, not available.

*Significant at the .05 probability level.

**Significant at the .01 probability level.

***Significant at the .001 probability level.
4 | DISCUSSION

The goal of this experiment was to determine if there is heritable variation in maize breeding germplasm for response to treatment with blended strobilurin and triazole fungicides beyond effects that might be attributable to the effects of protection against disease. The existence of heritable variation for response to the application of the mixture of strobilurin and triazole would suggest that specific hybrids could be selected for optimal response to such treatment and perhaps that new germplasm sources with greater responses could be identified and incorporated into commercial breeding programs. Our findings suggest limited variability in response to treatment even across a very broad range of genetically diverse maize germplasm. The interaction between hybrid or line and treatment was not significant for yield or any yield components. In the few cases where the interaction was significant, we observed no consistency between treatment and direction of response, which suggests limited or no heritable variation for response to this particular mixture of chemicals. The few individual cases of hybrids or inbreds responding for particular

(5.5%), the $p$ value associated with the difference between treatment and control was $\leq 0.05$ (Figure 2). As in the hybrid study, this is about the proportion of significant tests expected under the null hypothesis of no responses to treatment, and multiple-test correction would result in no significant differences. Also, as in the hybrid experiment, no consistent pattern of treatment response with respect to genotypes was observed (Figure 2). Treatment × environment and treatment × environment × hybrid interaction variance components were smaller than the inbred × environment variance component for all traits (File S15).
traits did not exhibit any clear pattern. The responses were not consistent within germplasm groups; nor were they consistent across traits for a particular hybrid (Figures 1 and 2). Commercial hybrids developed by Syngenta were among those with the strongest delayed-senescence effect, suggesting that if this particular response is genetically controlled, it has already been exploited in this commercial breeding program.

This experiment was conducted under natural disease conditions; the observed disease pressure was low in most environments. Despite the low incidence of disease, the one trait for which the treatment response was clearly significant was the disease rating itself. Treated plots were lower in disease pressure than untreated plots, and this was significant for all hybrids individually and, on average, across hybrids. This result demonstrates that the fungicide is effective at reducing disease symptoms even in relatively low-disease environments. Using the disease score as a covariate in the experimental models produced minor and inconsistent changes in results, showing that the reduction in pathogen pressure, although significant, was not sufficient to create a clear agronomic benefit in the environments studied. Our results suggest that treatment effects were too small on most traits to provide a clear or consistent response.

Producers rarely wish to apply pesticides of any kind when the pest pressure is below a certain economic threshold. Other studies have found yield increases in reportedly low-disease conditions with the application of mixed strobilurins and triazoles (da Costa et al., 2012; Tedford et al., 2017). Our results suggest that the effects of application of a strobilurin and triazole mixture on yield are negligible or very small and may be dependent on unknown environmental conditions.

The results of this study do not suggest that there is any compelling reason to spray this mixture of fungicides in the absence of disease purely for their growth-regulating effects.
Such effects, where present, are small and inconsistent. The potential for development of fungal resistance to these chemicals when routinely spraying pesticides in low-disease conditions is well established (Gisi, Sierotzki, Cook, & McCaffery, 2012; Lucas, Hawkins, & Fraaije, 2015). The potential consequences of promoting the evolution of fungal resistance and the loss of effective fungicidal chemistries by using these products is a contraindication to their use in the absence of substantial disease pressure.

Further study may suggest potential economic benefits of fungicide application in low-disease environments, but the results of this study do not support application of strobilurin and triazole mixture in the absence of substantial disease pressure. Additionally, future work may illuminate any connection between delayed senescence and potential yield benefits. The only significant correlation with delayed senescence, however, was with increased grain moisture at harvest, which is unfavorable to the grower. Increased grain moisture may result in maize being left in the field beyond the traditional harvest dates to realize a yield increase connected to delayed senescence, which is unlikely to be desirable. This study examined the effect of a combined strobilurin–triazole mixture, and inferences from these results do not extend to the effects of either chemical alone. We are unaware, however, of any reports in the literature demonstrating antagonistic effects of strobilurins and triazoles when combined. Studies comparing the effects of mixtures to separate treatments of strobilurins and triazoles on agronomic performance in wheat (Triticum aestivum L.) and rapeseed (Brassica napus L.) found no antagonistic effects and for some traits found favorable synergism (Ijaz & Hornermeier, 2012; Jones, 2000). Therefore, there is likely little potential value in additional studies of the growth-regulating effects on maize of either chemical alone.

CONFLICT OF INTEREST
M.S.W. was supported by a grant from Syngenta to NC Agriculture Foundation.

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**How to cite this article:** Woore MS, Holland JB. Genetic variation for response to mixed triazole and strobilurin application in diverse maize. *Agrosyst Geosci Environ*. 2020;3:e20054. https://doi.org/10.1002/agge.20054.