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Modeling the relationship between estimated fungicide use and disease-associated yield losses of soybean in the United States I: Foliar fungicides vs foliar diseases

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Data Availability Statement: The fungicide data can be obtained directly from the Pesticide National Synthesis Project website: https://water.usgs.gov/nawqa/pnsp/usage/maps/index.php. Summarized yield loss data is available on our GitHub repository at: https://github.com/PSUPlantEpidemiology/FungicideLossData/tree/v1. Any questions can be directed to the lead or corresponding author, as necessary.

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Abstract

Fungicide use in the United States to manage soybean diseases has increased in recent years. The ability of fungicides to reduce disease-associated yield losses varies greatly depending on multiple factors. Nonetheless, historical data are useful to understand the broad sense and long-term trends related to fungicide use practices. In the current study, the relationship between estimated soybean yield losses due to selected foliar diseases and foliar fungicide use was investigated using annual data from 28 soybean growing states over the period of 2005 to 2015. For national and regional (southern and northern United States) scale data, mixed effects modeling was performed considering fungicide use as a fixed and state and year as random factors to generate generalized R^2 values for marginal ($R^2_{GLMM(m)}$; contains only fixed effects) and conditional ($R^2_{GLMM(c)}$; contains fixed and random effects) models. Similar analyses were performed considering soybean production data to see how fungicide use affected production. Analyses at both national and regional scales showed that $R^2_{GLMM(m)}$ values were significantly smaller compared to $R^2_{GLMM(c)}$ values. The large difference between R^2 values for conditional and marginal models indicated that the variation of yield loss as well as production were predominantly explained by the state and year rather than the fungicide use, revealing the general lack of fit between fungicide use and yield loss/production at national and regional scales. Therefore, regression models were fitted across states and years to examine their importance in combination with fungicide use on yield loss or yield. In the majority of cases, the relationship was nonsignificant. However, the relationship between soybean yield and fungicide use was significant and positive for majority of the years in the study. Results suggest that foliar fungicides conferred yield benefits in most of the years in the study. Furthermore, the year-dependent usefulness of foliar fungicides in mitigating soybean yield losses suggested the possible influence of temporally fluctuating abiotic factors on

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the effectiveness of foliar fungicides and/or target disease occurrence and associated loss magnitudes.

Introduction

Soybean [*Glycine max* (L.) Merrill] is a key agricultural commodity in the United States and has been cultivated on 34.7 million hectares on average annually between 2015 and 2019 (USDA-NASS). Similar to the production of other economically important crops, numerous abiotic and biotic stressors like adverse weather, variation in soil characteristics, diseases, insects, and weeds present enormous challenges to soybean production [1, 2]. Soybean diseases are detrimental to production due to their deleterious effects on yield. In the U.S., the average annual disease-associated soybean yield losses are approximately 11% [3]. However, the relative importance of diseases and concomitant yield losses vary both temporally and spatially. For example, total yield losses due to diseases in 2012 was estimated to be 10.07 million metric tons while in 2014 it was 13.94 million metric tons [4]. Among various soybean foliar diseases, Septoria brown spot, caused by *Septoria glycines* Hemmi, and frogeye leaf spot, caused by *Cercospora sojina* Hara, are the most common [1, 5–8] and are also considered to be important yield limiting diseases in soybean [9]. The losses caused by Septoria brown spot range from 196 to 293 kg ha^{-1} [6]. Septoria brown spot can cause up to 2,000 kg ha^{-1} loss in high-yield soybean production systems (>5,000 kg ha^{-1}) [10]. Frogeye leaf spot can result in yield losses from 10 to 60% [11] and seed weight reductions up to 29% [12].

Different management strategies are deployed either individually or in an integrated manner to reduce the losses caused by foliar fungal diseases in soybean production systems. Among these, the use of foliar-applied fungicides has been an important tactic. Fungicide use in soybean has risen dramatically since 2005 [13]. Several reasons were given to explain this increase including: increased availability of fungicides for use on soybean, improved awareness of soybean diseases, the initial observation of soybean rust in North America and the resultant production of specific chemistries to manage this disease that were not widely used, increased soybean commodity price, and promotion of certain fungicides by the manufacturers for their potential physiological benefits that may increase soybean yield even in the absence of disease, a phenomenon in which the term "plant health" has been coined [14, 15].

The quinone-outside inhibitor (QoI; strobilurin) class of fungicides (Fungicide Resistance Action Committee [FRAC] group 11) are commonly used to manage foliar diseases of soybean and these act by binding with complex III of the mitochondrial respiration pathway [16]. Additionally, the demethylation inhibitor (DMI; triazole) class of fungicides (FRAC group 3) are also used in soybean and this class of fungicides inhibit ergosterol biosynthesis by fungi [17]. Recently, active ingredients from the succinate dehydrogenase inhibiting (SDHI; FRAC group 7) class of fungicides were introduced for management of foliar soybean diseases. Similar to QoI fungicides, SDHI fungicides are classified as respiration inhibitors. However, instead of complex III, SDHI fungicides bind at complex II in the mitochondrial respiration pathway [17]. In general, these fungicide groups possess broad-spectrum activity on foliar fungal soybean diseases including Septoria brown spot and frogeye leaf spot [18]. The fungicides within these specific chemical classes can generally be purchased as stand-alone fungicides, especially those products designated as either DMI or QoI. However, stand-alone fungicide products consisting of SDHIs are currently not available and are included as a pre-mix fungicide that contains either one of the other classes (either DMI or QoI) or both of the classes as a three-way fungicide product. The current fungicide production trend from chemical manufacturers is to provide products that contain multiple modes of action to help reduce the development of fungicide resistance. In general, and to more broadly classify the chemical classes as outlined above, following the initial observation of soybean rust in the contiguous U.S., fungicide products were broadly categorized as either curative (DMI) and preventive (QoI and also SDHI).

Although foliar fungicides have extensively been used for soybean production, the extent to which yield losses can actually be mitigated with fungicide application and the subsequent economic return is often questioned. While fungicides are reported to reduce the yield losses when diseases are present [19, 20], the impact of fungicide application on yield in the absence of disease, i.e., the plant health scenario, are inconsistent. Several studies have demonstrated no significant increase in soybean yield with fungicide applications in the absence of disease [20–23], while other studies suggested that yield increases can occur with foliar fungicide application even in the absence of disease [7, 23–25]. Therefore, the economic return following a fungicide application does not intuitively follow a linear trend due to its apparent dependency on multiple factors such as disease pressure, class of fungicide being used (i.e., active ingredient), time of application (growth stage of the plant), and environmental conditions [19, 26, 27].

Widespread fungicide use can ultimately lead to an increased risk of selecting fungicide-resistant strains out of the targeted pathogen population. Fungicide resistance is an issue increasing in importance across soybean production areas in the U.S. as a result of automatic fungicide applications at specific growth stages, as well as fungicide applications with specific fungicide classes where the goal is a curative response [28–31]. Currently, QoI fungicide resistance has been reported for several soybean pathogens in the U.S., including *C. sojina*, in Illinois, Tennessee [32], South Dakota [33], and Mississippi [29]. Zhang et al [31] recently reported QoI resistant *C. sojina* isolates from 14 states including Alabama, Arkansas, Delaware, Illinois, Indiana, Iowa, Kentucky, Louisiana, Mississippi, Missouri, North Carolina, Ohio, Tennessee, and Virginia. Additionally, the fungi responsible for causing Cercospora leaf blight (*C.* cf. *flagellaris*, *C. kikuchii* (Tak. Matsumoto & Tomoy.) M.W. Gardner and *C.* cf. *sigesbeckiae*) have been reported to exhibit resistance to QoI fungicides throughout Louisiana [28]. Moreover, additional anecdotal, unpublished reports of resistance within populations of *S. glyinces* and *Corynespora cassiicola* (Berk. & M.A. Curtis) C.T. Wei, the causal organism of target spot of soybean have recently been made.

In the current paper, we investigate long-term fungicide use patterns and the relationship with soybean yield and the resulting foliar diseases that cause losses. Our primary spatial grain was at the state level, although regional and national level trends were also explored. While numerous individual experiments have been conducted to address the aforementioned issues, a more comprehensive analysis with long term historical data (estimated fungicide use and soybean yield losses as a result of diseases) is currently lacking. Thus, our objectives for this study were to (i) investigate the relationship between foliar fungicide use in the U.S. and estimated yield losses due to foliar diseases, and (ii) investigate the relationship between foliar fungicide use in the U.S. and soybean production/yield at national, regional, and state levels. Findings of this study will aid in informed decision making on spatiotemporally sensitive, economically viable, and environmentally sound use of fungicides to manage soybean fungal diseases in the U.S. Furthermore, results will also provide useful insights into how research, policy, and educational efforts should be prioritized in soybean disease management using fungicides.

Materials and methods

Fungicide use data

Annual state-level foliar fungicide use estimates (in Kg of active ingredient) for soybean were obtained from the Pesticide National Synthesis Project webpage (https://water.usgs.gov/nawqa/pnsp/usage/maps/county-level/StateLevel/HighEstimate_AgPestU sebyCropGroup92to16.txt).

Note that these are all actual use estimates but not amounts that were sold. Please see the webpage earlier cited for detailed information about the methodology used to compute the fungicide use estimates. Foliar fungicides applied to soybean during the period between 2005 and 2015 were considered for this study. The time period was based upon the availability of fungicide use data spanning 28 soybean growing states (AL, AR, DE, FL, GA, IA, IL, IN, KS, KY, LA, MD, MI, MN, MO, MS, NC, ND, NE, OH, OK, PA, SC, SD, TN, TX, VA, WI). Fungicide use data were also classified based on each region where northern states considered for this study included IL, IN, IA, KS, MI, MN, NE, ND, OH, PA, SD, and WI while southern states included AL, AR, DE, FL, GA, KY, LA, MD, MS, MO, NC, OK, SC, TN, TX, and VA. The classification of states into regions was based on the two groups of soybean pathologists collecting disease loss estimate data, NCERA-137 (North Central Extension and Research Activity for Soybean Diseases) and the Southern Soybean Disease Workers.

To compute the fungicide use per unit area within each state (in grams per hectare), the amount provided in the database (in kg) was first converted to grams (g). The soybean planting and harvesting area was retrieved from USDA-NASS database (https://quickstats.nass.usda.gov) for individual states from 2005 to 2015. Fungicide use values (in g) were divided by respective state-wide total soybean (i) planted number of hectares and (ii) harvested number of hectares separately to decide the most appropriate type of explanatory variable (g of fungicide per unit hectarage planted versus g of fungicide per unit hectarage harvested) for use in the study. A simple linear regression analysis showed that two variables were linearly and positively related to each other ($R^2 = 0.9987$, $P < 0.0001$, $y = 0.965x + 0.211$), indicating a high similarity between the two variables. As such, for this study, we report the fungicide concentration in grams of fungicide per harvested hectare (here after mentioned as g/ha).

Yield loss data

Historical soybean yield loss estimates were gathered from soybean Extension specialists and researchers. We considered the soybean losses for the same periods where foliar fungicide data were also available. Soybean losses spanned the same 28 soybean growing states as indicated above. The methodology used to collect and report soybean disease losses have been previously described [4]. Briefly, a spreadsheet was circulated annually to plant pathologists with soybean responsibilities and they provided estimates of the losses associated with a defined set of diseases (n = 23). However, for the purposes of this study we focused on the results related to foliar diseases caused by fungi that could be effectively managed by foliar fungicide application. The methods employed within each state differed with regards to the specific method for estimating losses; however, in general, some of the methods employed were based on each individual's evaluation of cultivar trials, fungicide efficacy plots, specific troubleshooting or field calls, queries of Extension personnel within counties/parishes, statewide plant disease surveys, or plant disease diagnostic laboratory databases.

Given that the historical yield loss data were provided in the form of losses in metric tons (MT) of production, to calculate the loss per soybean disease, we first calculated the loss as a percentage based on overall production (in MT) per state and year using USDA-NASS data. We then calculated the overall loss (as a percentage) due to soybean diseases using Padwick's calculation [34], which is:

$$\text{Loss}(\%) = 100 \times \left[1\text{-}\frac{(100\text{-}Y_1)(100\text{-}Y_2)(100\text{-}Y_3)\ldots(100\text{-}Y_n)}{100^n}\right],$$

where

Y_1, Y_2, Y_3, Y_n, represent the percentage loss due to disease 1, 2, 3, through n, respectively. To estimate the loss due to diseases in terms of yield, we used the average soybean yield per state and year, from which we estimated the yield in the absence of diseases (the percentage loss estimated using Padwick's calculation). The difference between the state average yield and the estimated yield in the absence of diseases was considered as the loss.

Fungicides and their targeted diseases considered

Based on data available in the fungicide and yield loss databases combined with soybean fungicide efficacy summarized by Extension plant pathologists on an annual basis, we concentrated on specific diseases for this study. Foliar fungicides (n = 15) included the following active ingredients within several specific chemical classes as defined by the FRAC: QoIs (FRAC code 11) = azoxystrobin, fluoxastrobin, picoxystrobin, pyraclostrobin, trifloxystrobin; DMIs (FRAC code 3) = cyproconazole, difenoconazole, flutriafol, propiconazole, prothioconazole, tebuconazole, tetraconazole; chloronitrile (FRAC code M 05) = chlorothalonil; SDHI (FRAC code 7) = fluxapyroxad; and methyl benzimidazole carbamate (MBC) (FRAC code 1) = thiophanate-methyl. Although azoxystrobin, pyraclostrobin, and trifloxystrobin have uses as seed-applied fungicides, they were considered as foliar fungicides for this study as they are predominantly used to manage foliar diseases of soybean. The targeted diseases for the foliar fungicides listed above included anthracnose (caused by *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore and several related species), Cercospora leaf blight (purple seed stain: *Cercospora flagellaris*, *C. kikuchii*, *C. sigesbeckiae*), frogeye leaf spot (*Cercospora sojina*), Rhizoctonia aerial blight (*Rhizoctonia solani* J.G. Kühn), Sclerotinia stem rot (White mold: *Sclerotinia sclerotiorum* (Lib.) de Bary), Septoria brown spot (*Septoria glycines*), and soybean rust (*Phakopsora pachyrhizi* Syd. & P. Syd.).

Determination of the relationship between fungicide use and yield losses due to diseases at national and regional scales

As the fungicide use data and yield loss data were classified by states and years, a generalized linear mixed model approach was used to model the data at national and regional scales using a gaussian distribution. Both null (intercept/empty) and full models were fitted. All models contained 'state' and 'year' as random factors while full linear model contained 'fungicide use' as a fixed factor. In addition, full quadratic model was also fitted by incorporating the square term of fungicide use as a fixed factor to the model. Following the methods in Nakagawa and Schielzeth [35], marginal R^2 [$R^2_{GLMM(m)}$; fixed effects] and conditional R^2 [$R^2_{GLMM(c)}$; fixed and random effects] values were computed for the full model to compare the relative contribution of fixed and fixed + random factors to the observed variation of yield loss. Information criterions (AIC and BIC) were calculated using maximum likelihood (ML) specification while all other parameters were generated using restricted maximum likelihood (REML) specification. Analyses were conducted to examine total fungicide use (MT) and total production loss (1,000 MT), as well as total fungicide use per unit harvest area (g/ha) and total yield loss per unit area (kg/ha). Similar analyses were performed considering soybean production data to see how fungicide use affect production. The packages *arm* (version 1.10–1) [36], *lme4* (version 1.1–21) [37], and *MuMIn* (version 1.43.15) [38] in R (version 3.5.1) were used for mixed effect modeling.

Determination of the relationship between fungicide use and yield losses due to diseases for individual state and year

The objective in this section was to explore the relationships between fungicide use and yield losses due to diseases considering years and states as additional explanatory factors. Regression analysis was conducted in R (version 3.5.1). The model form for this analysis:

$$Y_{ij} = \beta_0 + \beta_1 A_{ij} + \beta_2 B_i + \beta_3 C_j + \beta_{12} A_i B_i + \beta_{13} A_i C_j$$

where, Y_{ij} = soybean yield loss from ith state in jth year; A_{ij} = foliar fungicide use from ith state in jth year; B_i = ith state; C_j = jth year.

Analyses were conducted to examine total fungicide use (MT) and total yield loss (1,000 MT), as well as total fungicide use per unit harvest area (g/ha) and total yield loss per unit area (kg/ha). In addition, similar analyses (as indicated above) were also performed to investigate the relationship between fungicide use and soybean production/yield.

Derivation of soybean yield, harvest, and production zones

One of our objectives in the current study was to explore whether the mean per hectare foliar fungicide use vary by the levels of yield/harvest/production zones. Further, we wanted to perform an exploratory multivariate analysis (see below) by incorporating yield/harvest/production zones and per hectare foliar fungicide use. Therefore, we first derived the said zone types as mention below: (i) Yield zone (1 to 4), based on USDA-NASS estimates at the state level comparing yield (MT/HA) with all state by year combinations, (ii) Harvest zone (1 to 4), based upon USDA-NASS estimates at the state level comparing harvested area (HA) with all state by year combinations, and (iii) Production zone (1 to 4), based upon USDA-NASS estimates at the state level comparing total production (MT) with all state by year combinations. Data points within the minimum to first quartile were classified as Zone 1. Similarly, data points from the first quartile to median, median to third quartile, and > third quartile were classified as zones 2, 3, and 4, respectively. Note that the zones were not solely defined based on geography, in this case state, and are a function of time (temporal scale). As such, the zone of a given data point was relative to the other data points (in terms of yield, harvest area, or total production) within the database. As yield, harvest area, and production within a given state fluctuated over time, the zone classification for a given state varied based on the year. The yield, harvest, and production zones corresponding to foliar fungicide data were therefore derived using soybean yield, harvest, and production data from 2005 to 2015. As these zones do not physically exist, we were not interested in incorporating zones into our mixed model regression analysis.

Factor Analysis of Mixed Data (FAMD)

The objective of this analysis was to explore the clustering patterns of individual data points in the variance maximizing factor map space based upon the levels of qualitative variables (zones in particular, see above). FAMD is a principal component method to analyze a data set containing both quantitative and qualitative variables [39]. FAMD makes it possible to analyze the similarity between individuals (individual data points) by taking into account mixed-variable types. With this analysis, quantitative and qualitative variables are normalized in order to balance the impact of each set of variables. The packages FactoMineR version 1.41 (for the analysis) and factoextra (for data visualization) in R (version 3.5.1) were used for FAMD analysis. Here, total foliar fungicide use in grams of active ingredient (on a per hectare (ha) basis) was used as a quantitative variable while the year, state, region, soybean yield zone, harvest zone, and production zones, were incorporated as qualitative variables.

Analysis of variance (ANOVA)

The objective with this analysis was to see whether the mean foliar fungicide use vary based on the levels of yield, harvest, or production zones. As such, we investigated the main effects of yield, harvest, and production zones on total fungicide use (per ha basis) using the PROC GLIMMIX procedure in SAS (version 9.4, SAS Institute, Cary, NC) at the 5% significance level. Data used to create zones were classified by years and states. We hypothesized that observations (yield/harvest area/production) made in the same year but from different states were correlated, which was a similar hypothesis for observations made in the same state but from different years. Therefore, year and state were considered in the model as random effects. The full linear model that was fitted with yield zone was:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + e_{ijkl}$$

where, Y_{ijkl} is the observed total fungicide use (in grams per hectare) for the l^{th} zone entity (l = 1–77) from the i^{th} yield zone (i = 1–4), j^{th} state (j = 1–28), and k^{th} year (k = 1–11); μ is the overall mean fungicide use common to all yield zones; A_i is the fixed effect of i^{th} yield zone; B_j is the random effect of the j^{th} state; C_k is the random effect of the k^{th} year; eijkl is the residual term for the $ijkl^{th}$ observation. The same model structure was used for harvest and production zones.

Restricted maximum likelihood (REML) was used to compute the variance components. Degrees of freedom for the denominator of F tests were computed using the Kenward-Roger option. Studentized residual plots and Q-Q plots were respectively used to assess the assumptions of identical and independent distribution of residuals, and their normality. Appropriate heterogeneous variance models were fitted whenever heteroskedasticity was observed by specifying a "*random residual/group = x* " statement (where *x* = fixed factor under consideration, ex: harvest zone). The Bayesian information criterion (model with the lowest BIC) was used to select the best fitting model (between homogenous variance vs heterogeneous variance). Mean separation was performed with adjustments for multiple comparisons using the Tukey-Kramer test.

Results

Temporal fluctuation of soybean fungicide use in the United States

Considering total fungicide use (in both MT and g/ha) across 28 soybean growing states, the greatest foliar fungicide use was recorded in 2007 with the lowest recorded use in 2006 (Fig 1A). A 63.5% decrease in foliar fungicide use on a per ha basis was evident from 2007 to 2008. The percentage use increment from 2006 to 2015 was 317% for total fungicide use in MT and 252% for total fungicide use in g/ha, respectively. Despite the annual variation, the total concentration of foliar fungicides used in 28 states showed a general increasing trend from 2005 to 2015.

Spatial fluctuation of soybean fungicide use in the United States

Over an 11-year period, between 2005 and 2015 on a per hectare basis, Louisiana reported the greatest foliar fungicide use (2,309 g) while Kansas reported the lowest (114 g) (Fig 1B). In terms of the total foliar fungicide use (in MT), Florida recorded the lowest (9.7 MT) while Arkansas reported the greatest (1,103.7 MT).

When considered regionally, the total use (MT) of foliar fungicides was 18.7% greater in the southern states (6,451.3 MT) compared to northern states (5,431.2 MT) (Fig 2). Similarly, per hectare total use (g/ha) of foliar fungicides was 521% greater in the southern states (17,437.2 g/ha) compared to the northern states (2,805.7 g/ha) (Fig 2).

Preventive vs curative fungicides

In general, the QoI class of fungicides, commonly referred to as strobilurins are used as preventative fungicides while DMI (or triazoles) are used as curative fungicides. Temporal

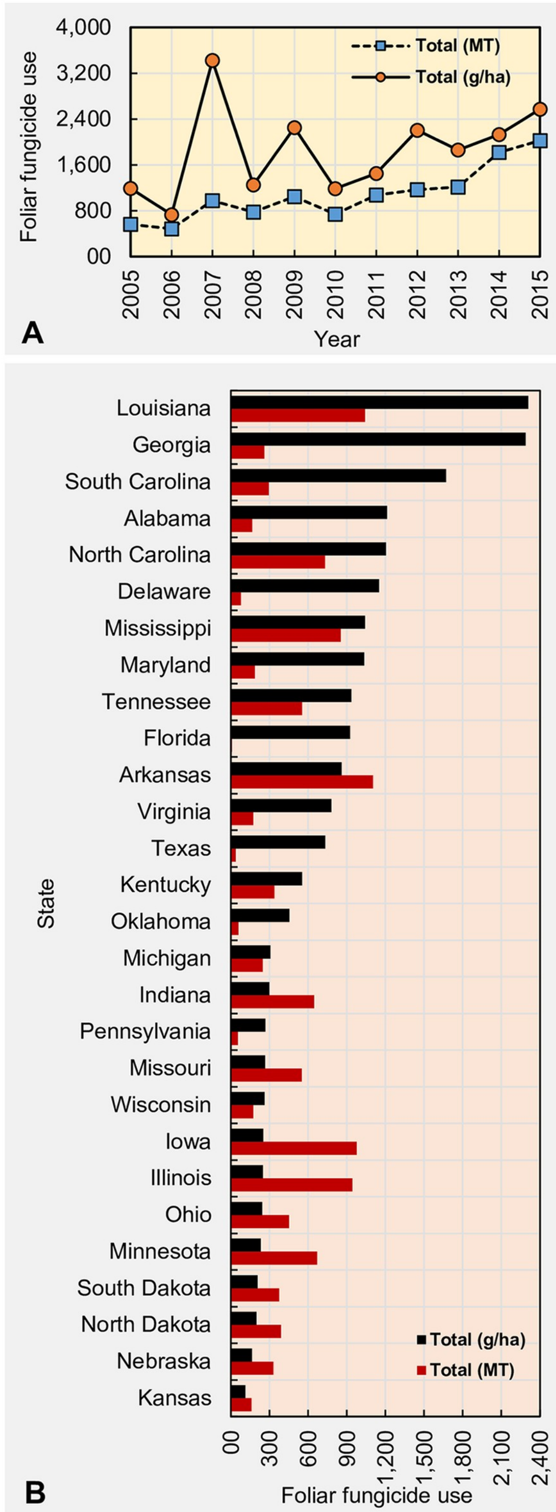

Fig 1. Spatiotemporal foliar fungicide use patterns in the United States. Temporal fluctuation for foliar fungicide use during 2005 to 2015 across all states considered (A) and state-wide use of cumulative foliar fungicides from 2005 to 2015 (B). Fungicides included: quinone outside inhibitors = azoxystrobin, fluoxastrobin, picoxystrobin, pyraclostrobin, trifloxystrobin; demethylation inhibitors = cyproconazole, difenoconazole, flutriafol, propiconazole, prothioconazole, tebuconazole, tetraconazole; methyl benzimidazole carbamates = thiophanate-methyl; multi-site mode of action = chlorothalonil; and succinate dehydrogenase inhibitors = fluxapyroxad.

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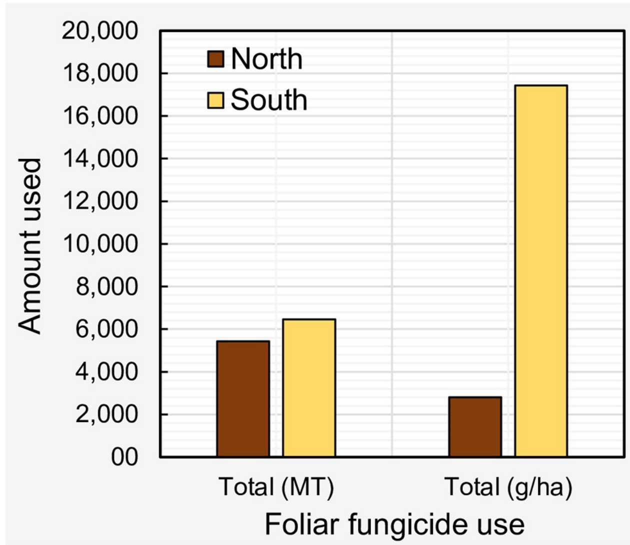

Fig 2. Total foliar fungicide use (from 2005 to 2015) by region. Northern states = IL, IN, IA, KS, MI, MN, NE, ND, OH, PA, SD, and WI; Southern states = AL, AR, DE, FL, GA, KY, LA, MD, MO, MS, NC, OK, SC, TN, TX, and VA. Fungicides included: quinone outside inhibitors = azoxystrobin, fluoxastrobin, picoxystrobin, pyraclostrobin, trifloxystrobin; demethylation inhibitors = cyproconazole, difenoconazole, flutriafol, propiconazole, prothioconazole, tebuconazole, tetraconazole; methyl benzimidazole carbamates = thiophanate-methyl; multi-site mode of action = chlorothalonil; and succinate dehydrogenase inhibitors = fluxapyroxad.

https://doi.org/10.1371/journal.pone.0234390.g002

fluctuations (summed across states) showed that the use of both types of fungicides increased from 2005 to 2015 (Fig 3A). The amount of preventive and curative fungicides used in 2015 were 3.34 and 4.2-fold greater compared to their use in 2005. The use of QoI fungicides, representing = ∑ azoxystrobin, fluoxastrobin, picoxystrobin, pyraclostrobin, and trifloxystrobin, was greater compared to curative fungicides representing = ∑ cyproconazole, difenoconazole, propiconazole, prothioconazole, tebuconazole, and tetraconazole for any given year. Spatially, the greatest and lowest QoI fungicide use, summed across years, was recorded in Iowa and Florida, respectively, while the greatest and lowest DMI fungicide use was recorded in Illinois and Florida, respectively (Fig 3B). In general, QoI fungicide use was greater compared to DMI fungicides except in a few states (Alabama, Delaware, Georgia, South Carolina, and South Dakota).

Mixed effect modeling of annual soybean production/yield losses and annual fungicide use at national and regional levels

At the national scale, where annual total fungicide use and annual total production loss were considered in MT and 1,000 MT, respectively, the marginal model had a very small R^2 (= $\boldsymbol{R^2}_{\mathbf{GLMM(m)}}$) value compared to that of conditional model (= $\boldsymbol{R^2}_{\mathbf{GLMM(c)}}$) (Table 1). Adding a quadratic term for fungicide use (full quadratic model) did not appear to significantly increase the $\boldsymbol{R^2}_{\mathbf{GLMM(m)}}$. The variance component was larger for state compared to year. Results were similar when annual total fungicide use and yield loss were considered in g/ha and kg/ha, respectively (Table 1).

Mixed modeling at regional scales (considering Northern and Southern United States separately) also showed that $\boldsymbol{R^2}_{\mathbf{GLMM(c)}} >> \boldsymbol{R^2}_{\mathbf{GLMM(m)}}$ (S1 and S2 Tables). Adding a quadratic term for fungicide use (full quadratic model) did not result in improved $\boldsymbol{R^2}_{\mathbf{GLMM(m)}}$.

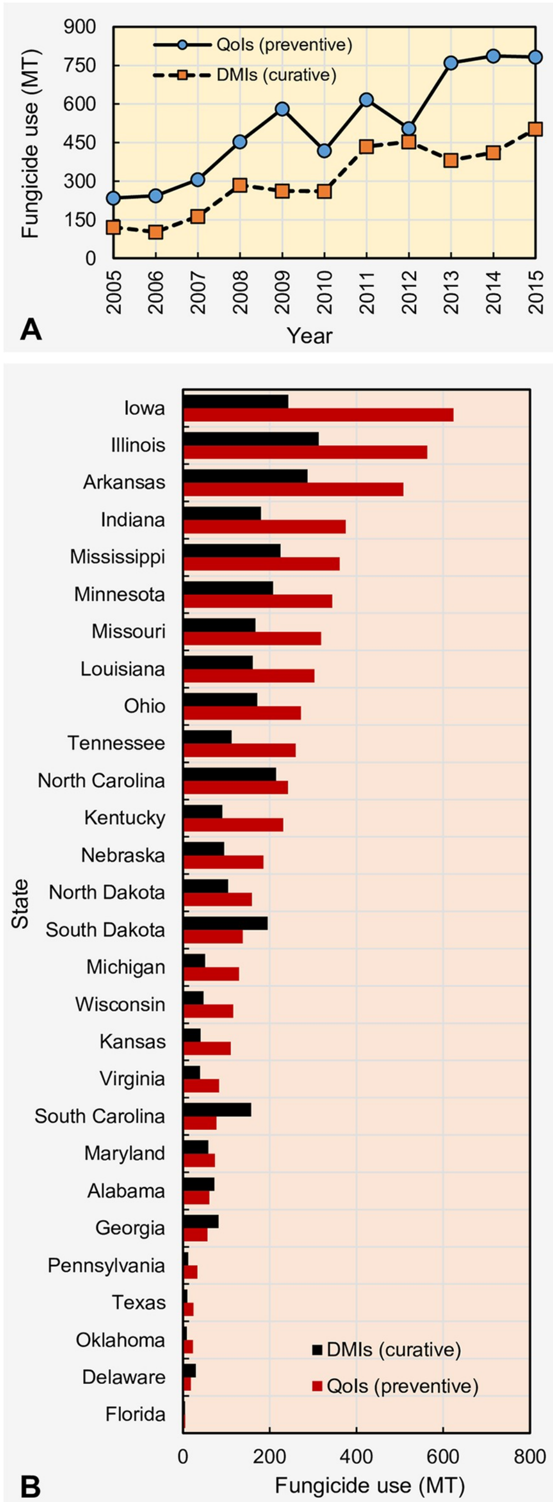

Fig 3. Temporal fluctuation (A) and state-wide variation (B) in the amount of preventive and curative foliar fungicide application use in the United States. Preventive fungicides = quinone outside inhibitors (QoIs) = ∑ azoxystrobin, fluoxastrobin, picoxystrobin, pyraclostrobin, and trifloxystrobin. Curative fungicides = demethylation inhibitors (DMIs) = ∑ cyproconazole, difenoconazole, flutriafol, propiconazole, prothioconazole, tebuconazole, and tetraconazole.

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Table 1. Mixed-effects modelling of the effect of foliar fungicide use on soybean yield losses due to foliar diseases from soybean growing states in the United States during 2005–2015 period at national scale. A = annual total fungicide use in MT and annual total production loss in 1,000 MT. B = annual total fungicide use in g/ha and annual yield loss in kg/ha. States considered for this study included IL, IN, IA, KS, MI, MN, NE, ND, OH, PA, SD, WI,AL, AR, DE, FL, GA, KY, LA, MD, MS, MO, NC, OK, SC, TN, TX, and VA.

	A			B		
Model name	**Null model**	**Full model (L)**	**Full model (Q)**	**Null model**	**Full model (L)**	**Full model (Q)**
Fixed effect	*a* ± SE	*a* ± SE	*a* ± SE	*a* ± SE	*a* ± SE	*a* ± SE
Intercept	68.6 ± 19.2	68.6 ± 18.8	68.6 ± 18.2	72.9 ± 14.5	72.9 ± 14.8	72.9 ± 14.8
Fungicide use	-	96.7 ± 111.3	169.1 ± 119.6	-	131.4 ± 94.3	123.8 ± 97.9
Fungicide use^2	-	-	-153.1 ± 103.1	-	-	25.5 ± 87.2
Random effects	VC	VC	VC	VC	VC	VC
State	7,370	7,104	6,509	4,492	4,711	4,706
Year	938	868	836	344	350	363
Residuals	6,575	6,619	6,651	5,389	5,365	5,379
$\boldsymbol{R^2}_{\mathbf{GLMM(m)}}$	-	0.002	0.012	-	0.005	0.005
$\boldsymbol{R^2}_{\mathbf{GLMM(c)}}$	-	0.547	0.530	-	0.488	0.488
AIC	3,677.5	3,678.8	3,678.6	3,603.8	3,603.8	3,605.8
BIC	3,692.5	3,697.4	3,701.0	3,618.7	3,622.5	3,628.1

L = linear; Q = quadratic; SE = standard error; VC = variance components. $R^2_{\mathrm{GLMM}(m)}$ = generalized R^2 for marginal model; $R^2_{\mathrm{GLMM}(c)}$ = generalized R^2 for conditional model; AIC = Akaike Information Criterion; BIC = Bayesian information criterion.

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Relationship between annual soybean yield losses and annual fungicide use at the state level and across years

Regression analysis indicated that there was no interaction ($\alpha = 0.05$) between total fungicide use (MT) and state, meaning that the relationship between soybean production loss due to diseases (1,000 MT) and total fungicide use (MT) did not vary between states (S3 Table). When losses (kg) and fungicide use (g) were considered on a per hectare basis, a significant relationship was only observed for Pennsylvania (S3 Table). However, the parameter estimate associated with fungicide use (g) for Pennsylvania was positive (S3 Table).

The relationship between soybean production loss due to diseases (1,000 MT) and total fungicide use (MT) was significant in years 2006, 2007, 2009, 2014, and 2015 (S3 Table). Nonetheless, the parameter estimates associated with fungicide use (g) for each of these years were positive (S3 Table). When losses (kg) and fungicide use (g) were considered on a per hectare basis, a significant relationship was only observed for years 2011 and 2015 (S3 Table). For both cases, the parameter estimates associated with fungicide use (g) was negative (S3 Table).

Mixed effect modeling of the relationship between annual soybean production/yield and annual fungicide use at national and regional levels

At the national scale, when annual total fungicide use and annual total production were considered in MT and 1,000 MT, respectively, the R^2 for marginal model (= $\boldsymbol{R^2}_{\mathbf{GLMM(m)}}$) was very small compared to that of conditional model (= $\boldsymbol{R^2}_{\mathbf{GLMM(c)}}$) (Table 2). In fact, the conditional model explained almost entire (98%) variation observed in soybean production at national scale. Incorporation of the quadratic term for fungicide use (full quadratic model) did not improve the $\boldsymbol{R^2}_{\mathbf{GLMM(m)}}$. The state variance component was larger than that of year. Results were similar when annual total fungicide use and yield were considered in g/ha and kg/ha, respectively (Table 2).

Table 2. Mixed-effects modelling of the effect of foliar fungicide use on soybean production/yield from soybean growing states in the United States during 2005–2015 period at national scale. A = annual total fungicide use in MT and annual total production in 1,000 MT. B = annual total fungicide use in g/ha and annual yield in kg/ha. States considered for this study included IL, IN, IA, KS, MI, MN, NE, ND, OH, PA, SD, WI,AL, AR, DE, FL, GA, KY, LA, MD, MS, MO, NC, OK, SC, TN, TX, and VA.

	A			B		
Model name	**Null model**	**Full model (L)**	**Full model (Q)**	**Null model**	**Full model (L)**	**Full model (Q)**
Fixed effect	*a* ± SE	*a* ± SE	*a* ± SE	*a* ± SE	*a* ± SE	*a* ± SE
Intercept	3,153 ± 685	3,153 ± 673	3153 ± 659	2563 ± 110	2563 ± 110	2563 ± 110
Fungicide use	-	2,658 ± 731	3740 ± 801	-	-11.9 ± 414	157 ± 432
Fungicide use^2	-	-	-2068 ± 670	-	-	-474 ± 379
Random effects	VC	VC	VC	VC	VC	VC
State	12,790,225	12,386,051	11,870,689	187,404	187,424	196,392
Year	133,425	105,890	99,484	56,493	56,571	53,200
Residuals	269,348	260,729	254,349	95,081	95,426	95,023
$R^2_{GLMM(m)}$	-	0.002	0.005	-	0.000	0.002
$R^2_{GLMM(c)}$	-	0.980	0.979	-	0.718	0.725
AIC	4,934.5	4,923.7	4,916.2	4,528.1	4,530.1	4,530.6
BIC	4,949.4	4,942.3	4,938.6	4,543.1	4,548.8	4,553.0

L = linear; Q = quadratic; SE = standard error; VC = variance components. $R^2_{GLMM(m)}$ = generalized R^2 for marginal model; $R^2_{GLMM(c)}$ = generalized R^2 for conditional model; AIC = Akaike Information Criterion; BIC = Bayesian information criterion.

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Mixed modeling at regional scale (considering Northern and Southern United States separately) also showed that $R^2_{GLMM(c)} >> R^2_{GLMM(m)}$ (S4 and S5 Tables). Inclusion of the quadratic term for fungicide use (full quadratic model) did not result in improved $R^2_{GLMM(m)}$.

Relationship between annual soybean production/yield and annual fungicide use at state level and across years

Regression analysis showed that there was no interaction (α = 0.05) between total fungicide use (MT) and state. Therefore, the relationship between soybean production (1,000 MT) and total fungicide use (MT) did not vary between states (S6 Table). When soybean yield (kg) and fungicide use (g) were considered on a per hectare basis, a significant relationship was only observed for Texas and Wisconsin (S6 Table). For both states, the parameter estimate associated with fungicide use (g) was negative (S6 Table).

The relationship between soybean production (1,000 MT) and total fungicide use (MT) was significant in years 2007, 2008, 2011, 2012, and 2013 (S6 Table). The parameter estimates associated with fungicide use (g) for each of these years were negative (S6 Table). When yield (kg) and fungicide use (g) were considered on a per hectare basis, a significant relationship was observed for years 2008, 2009, 2012, 2013, and 2014 (S6 Table). For all of these years, the parameter estimates associated with fungicide use (g) were positive (S6 Table).

Factor Analysis of Mixed Data (FAMD)

When FAMD was performed for foliar fungicide use, the variance maximizing data point distribution in the factor map did not show a clear clustering pattern based upon state, year, and yield zone. However, a clear clustering was observed based upon region, harvest zone, and production zone (Fig 4). Factor maps for both harvest and production zones showed that harvest/production zone 1 distantly clusters from harvest/production zone 4 while harvest/production zones 1 and 2 clustered in close proximity in the factor map.

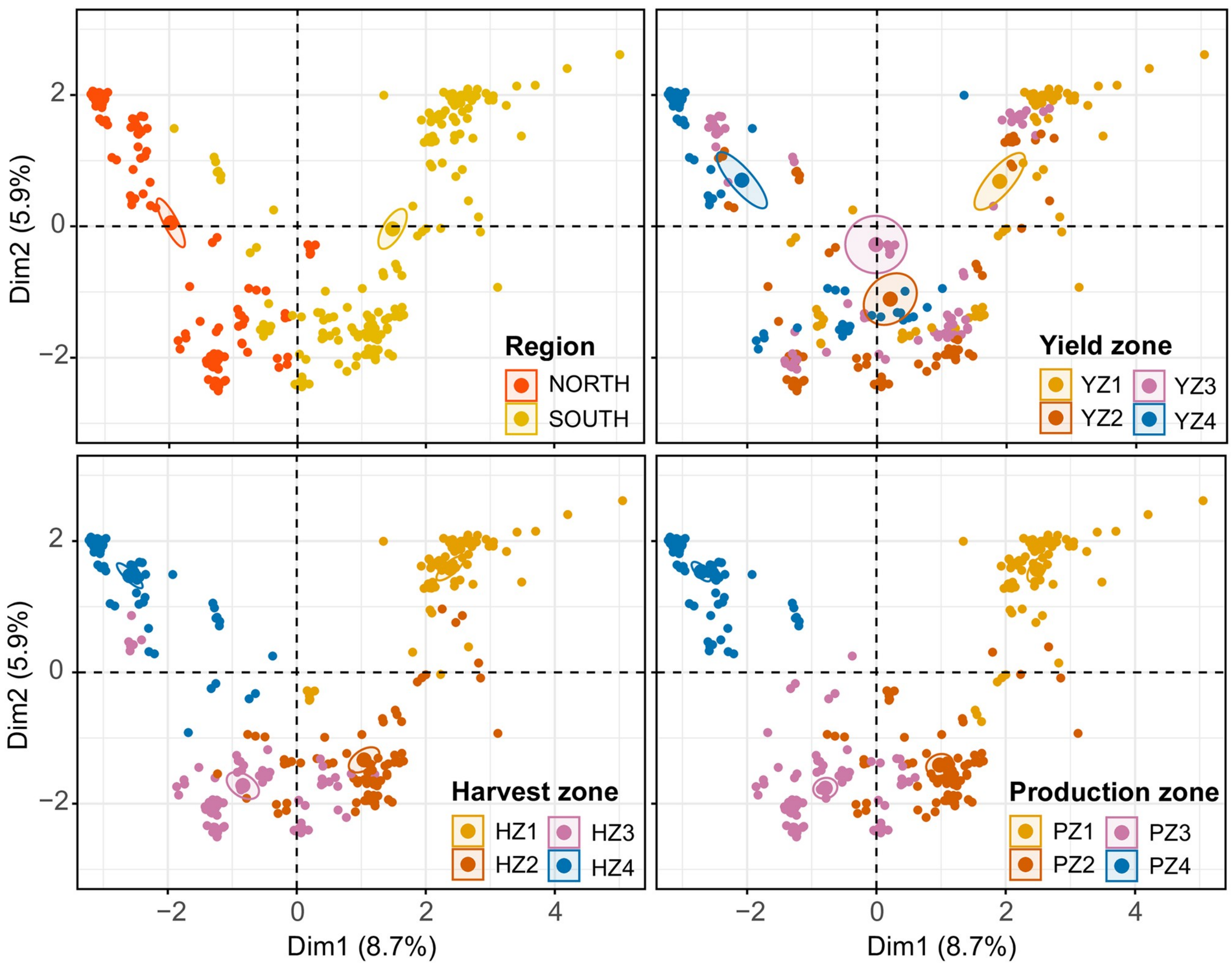

Fig 4. FAMD factor maps obtained from the factor analysis with mixed data approach (FAMD analysis), showing the variance maximizing distribution pattern of data points (n = 308, each data point represent foliar fungicide use in g/ha) in the map space with their clustering patterns based upon state (n = 28), year (n = 11), region (n = 2), and yield/harvest/production zones (n = 4 in each case). Yield/Harvest/Production zones = represent four levels (zone 1 to 4) based on the quartiles within a database containing 308 yield (kg/ha)/harvest area (ha)/production (MT) data points (308 = 11 years × 28 states). Within this database, data points from the minimum to the first quartile were classified as zone 1. Similarly, data points from the first quartile to median, median to the third quartile, and > third quartile were respectively classified as zones 2, 3, and 4. Foliar fungicides included: quinone outside inhibitors = azoxystrobin, fluoxastrobin, picoxystrobin, pyraclostrobin, trifloxystrobin; demethylation inhibitors = cyproconazole, difenoconazole, flutriafol, propiconazole, prothioconazole, tebuconazole, tetraconazole; methyl benzimidazole carbamates = thiophanate-methyl; multi-site mode of action = chlorothalonil; and succinate dehydrogenase inhibitors = fluxapyroxad (effective against anthracnose, Cercospora leaf blight (purple seed stain), frogeye leaf spot, Rhizoctonia aerial blight, Sclerotinia stem rot (White mold), Septoria brown spot, and soybean rust).

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Analysis of variance (ANOVA)

ANOVA indicated a significant main effect of harvest zone ($P = 0.0219$), while no differences were observed for yield zone ($P = 0.1904$) and production zone ($P = 0.1127$) on foliar fungicide use. With respect to harvest zone, the foliar fungicide use (g/ha) in harvest zone 1 was significantly greater than that of harvest zone 4 (Fig 5).

Discussion

Use of foliar fungicides has been a major strategy to manage fungal pathogens in agricultural cropping systems following the green revolution. Fungicide usage has increased over the past

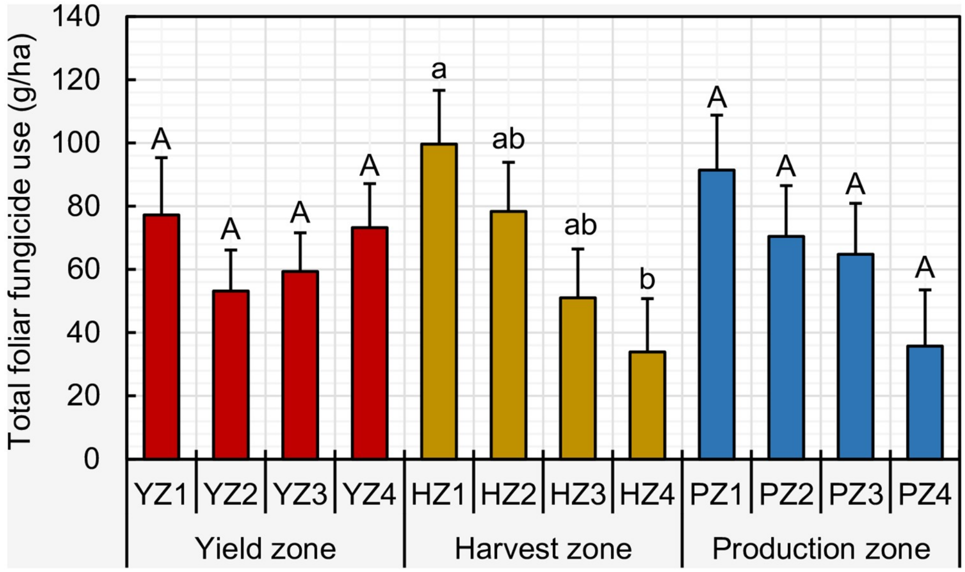

Fig 5. Comparison of the mean per hectare foliar fungicide use (in g) among yield/harvest/production zones. Within each zone type, means followed by a common letter are not significantly different after adjustment for multiple comparisons using Tukey-Kramer test at the 5% level of significance. Error bars represent standard errors. Foliar fungicides included: quinone outside inhibitors = azoxystrobin, fluoxastrobin, picoxystrobin, pyraclostrobin, trifloxystrobin; demethylation inhibitors = cyproconazole, difenoconazole, flutriafol, propiconazole, prothioconazole, tebuconazole, tetraconazole; methyl benzimidazole carbamates = thiophanate-methyl; multi-site mode of action = chlorothalonil; and succinate dehydrogenase inhibitors = fluxapyroxad (effective against anthracnose, Cercospora leaf blight (purple seed stain), frogeye leaf spot, Rhizoctonia aerial blight, Sclerotinia stem rot (White mold), Septoria brown spot, and soybean rust).

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decade especially in soybean production systems. Findings of the current study revealed that the foliar fungicide usage in the U.S. increased by 116% (on a per unit area basis: g/ha) and 260% (on a total usage basis: MT) from 2005 to 2015. Fungicide use was greatest in 2007, which was a year with more widespread soybean rust outbreaks on a national level and the first year that soybean rust moved into the upper Midwest through Texas to Iowa [40]. Furthermore, 2007 was the only year to date that Iowa reported observing the disease [40]. A similar situation occurred in 2009, where an increased incidence of soybean rust was reported. For example, Alabama, Georgia, Mississippi, and Tennessee reported the greatest number of counties with soybean rust [40]. Moreover, on a national basis, more counties/parishes were observed to contain soybean rust during 2009 than any other year [40]. Additionally, 2009 was an exceptionally wet year particularly in the southern U.S., leading to more foliar diseases [40]. All these factors could have specifically contributed to the greater foliar fungicide use in 2009.

The regional level data revealed that foliar fungicide use (total in MT as well and per hectare basis in g) was greater in the southern states compared to the northern states despite the greater land use for soybean production in the northern states. The greater per hectare fungicide use in the south may be due to several reasons. In general, this region has an extended period of soybean planting (March to June) and a prolonged period of disease conducive conditions (warmer and wetter for a longer period of time) compared to the northern U.S. Along with that, soybean rust was first detected in the contiguous U.S. in November 2004 [41] and fungicides were the main method of managing the disease. Even though soybean rust has not posed a major yield loss threat since the initial observation [42], fungicide applications in specific years have likely been driven by the presence of the disease. Lastly, based on observations by Extension specialists, a greater percentage (60–65%) of southern U.S. acres likely receives at least one fungicide application at a specific growth stage as an automatic application in the absence of diseases.

Prophylactic application of foliar fungicides can significantly increase production costs, and subsequently suppress profitability particularly when diseases are absent or are present at low levels [43]. In the current study, we observed that the vast majority of the states have used a greater amount of preventive fungicides as compared to curative fungicide over time. If the application of a preventive fungicide was not made at the suggested growth stage based on plant phenology, such applications may not provide a scenario whereby a reduction in the potential yield losses associated with a given disease were met. Poor fungicide application practices may contribute to a positive relationship between fungicide use and yield losses. For example, the fungicide application timing greatly affects the effectiveness of a fungicide in terms of its ability to suppress the severity of a disease and associated yield losses [19, 26, 44, 45]. Application of labeled fungicides after the establishment frogeye leaf spot [44] and soybean rust [46] could still result in significant yield losses.

Additionally, reduced fungicide efficiency due to a variety of factors such as unfavorable environmental conditions and automatic fungicide application on disease-resistant soybean cultivars can result in a positive relationship between fungicide use and yield losses. For example, compared to the control, application of benomyl at different application timings based on growth stage did not significantly reduce frogeye leaf spot severity or associated grain yield loss on resistant soybean genotypes, although significant disease severity and yield loss reductions were observed with susceptible soybean genotypes [44]. Resistance within the targeted pathogen population to the active ingredient contained in the applied fungicide/s could also contribute to a positive relationship between fungicide use and yield losses [28, 29, 31–33]. Furthermore, fungicides are applied with self-propelled, pull type, or aerial spray applicators in the U.S. Ground applicators create wheel-tracks in the soybean crop, which reduce yield particularly when made during the reproductive growth stages [47]. This also can contribute to positive relationship between fungicide use and soybean yield losses.

One of the major objectives of this study was to investigate the relationship between fungicide use and soybean production/yield loss due to selected foliar diseases using data from different soybean growing states years. Given that fungicide use data and soybean production/yield loss data were classified by state and year, we employed generalized linear mixed model approach to model the effect of foliar fungicide use on soybean yield losses due to selected foliar diseases at national and regional scale by specifying state and year as random effects. The difference of generalized R^2 between marginal (only fixed effects; fungicide use = $\boldsymbol{R^2}_{\mathbf{GLMM(m)}}$) and conditional (fixed and random effects; fungicide use + state + year = $\boldsymbol{R^2}_{\mathbf{GLMM(c)}}$) models were large, with $\boldsymbol{R^2}_{\mathbf{GLMM(m)}} \lll \boldsymbol{R^2}_{\mathbf{GLMM(c)}}$. Given the relatively strong effects of state and year in terms of the overall observed variation, there was not a strong relationship between foliar fungicide use and soybean production/yield loss due to foliar diseases at national and regional scales. As such, we focused modeling efforts to look at the state and year trends.

We did not observe strong, negative relationships between yield losses and fungicide use at the state level. The general lack of model fit between soybean production/yield loss and fungicide use can be contributed by the type of data that we used for this study. For instance, although the fungicide use data available in the Pesticide National Synthesis Project webpage is not sales data but actual use data, they are still estimated values. The methods applied in the Pesticide National Synthesis Project are robust but still may differ from the actual use. Furthermore, the yield losses considered in this study were all estimated values based on data provided by soybean disease experts. While the loss computations incorporated those expert's estimations, along with the use of Padwick's calculation to calculate the overall loss due to diseases, it is still possible that the computed yield losses are different from actual yield losses. Therefore, we recognize that there may be some differences between the observed between soybean yield losses and fungicide use may different from actual trends.

Analyses conducted at the state level showed no significant relationship between soybean production/yield losses and foliar fungicide use for a vast majority of the states. Although a significant relationship between per hectare total yield losses (kg) due to foliar diseases and per hectare total foliar fungicide use (g) was observed for Pennsylvania, the relationship was positive. Therefore, at the state level, our findings do not provide strong statistical evidence to support the usefulness of foliar fungicide application to mitigate foliar disease-associated soybean production/yield losses. The regression analysis considering temporal aspect showed significant relationship between soybean yield loss (kg/ha) and foliar fungicide use (g/ha) for years 2011 and 2015. The negative parameter estimates for fungicide use in these two years indicated that fungicide application was related to yield losses due to foliar disease in a manner suggestive that fungicides reduced the impact of diseases. Furthermore, the observed positive coefficients for soybean yield (kg/ha) and foliar fungicide use (g/ha) for years 2008, 2009, 2012, 2013, and 2014 was suggestive of a positive benefit of foliar fungicides.

Results from the factor analysis with mixed data (FAMD) showed clear distinction between yield/harvest/production zone 1 and 4 based on foliar fungicide use, suggesting contrasting fungicide use differences between these zones. In general, the mean per hectare foliar fungicide use was greater in low yield/harvest/production zones while the use was lower in high yield/harvest/production zones. However, it may be possible that soybean farmers in low yield/harvest/production zones tend to apply foliar fungicides based on a perceived yield benefit as the result of an application made at a specific growth stage, rather than based upon disease observations or soybean cultivar disease tolerance. In fact, previous studies suggested that yield increases can occur following foliar fungicide application irrespective of the presence/absence of diseases [7, 15, 23–25, 48–51]. The yield response in the absence of disease has been partly attributed to the physiological changes that have been reported to occur in the plants following fungicide application with certain chemistries [14]. Increased yield in response to some fungicides such as QoIs have been observed even in the absence of foliar diseases due to their non-fungicidal physiological changes in, for example, soybean [22, 52, 53], wheat, and barley [53–55]. Some of these plant physiological changes include increased leaf greenness, chlorophyll content, photosynthetic rates, and water use efficiency, as well as delayed senescence [48, 50, 53, 54, 56]. Previous studies also reported that foliar application of pyraclostrobin enhance the growth, nitrogen assimilation, and yield of soybean [57] and wheat [58, 59]. Therefore, as revealed by the current study, it appeared that the farmers in the historically low yield/harvest/production zones tend to use foliar fungicide applications with the expectation of a yield increase.

In the current study, it was not possible to determine the relationship between yield losses caused by a single disease and the amount of a labeled fungicide used to control that disease. This was because each fungicide considered in this study may effectively control more than one disease. For instance, QoI fungicides can be used to manage anthracnose (*Colletotrichum truncatum*), Cercospora leaf blight (*Cercospora kikuchii*), frogeye leaf spot; pod and stem blight (*Diaporthe phaseolorum*); Rhizoctonia aerial blight (*Rhizoctonia solani*), and Septoria brown spot [6, 7, 60, 61]. Based on the manner in which the information in the fungicide use database is provided, there is no way to tell what the fungicide specifically targeted. Therefore, relationships between total yield losses caused by all foliar diseases and total concentration of foliar fungicide used were considered for this study.

Although we have previously estimated soybean yield losses due to various diseases for the period between 1996 and 2015 [62], the corresponding annual state-level foliar fungicide use estimates were not available for the entire period in the Pesticide National Synthesis

Project database (https://water.usgs.gov/nawqa/pnsp/usage/maps/county-level/StateLevel/HighEstimate_AgPestU sebyCropGroup92to16.txt). Therefore, the foliar fungicides used between 2005 and 2015 were considered for the current study. With the data used for this study, it was not possible to conduct a realistic economic analysis to determine whether fungicide application was cost effective. Unless there is an appropriate control for comparison, one could not determine the economic yield savings as a result of fungicides applied. Moreover, it is likely that the physical yield losses could have potentially been greater if fungicides were not applied. In addition, the fungicide database only contains information regarding the use of active ingredients and does not include such information as to whether or not a particular active ingredient was applied as a stand-alone fungicide product or in the form of a pre-mixture of more than one chemical. Based on the commercial product and company, the same active ingredient can be marketed under several different trade names and in some cases the products can be priced differently depending on retail outfit. Annual fluctuations as well as locational variations in fungicide application cost (i.e., aerial application versus ground application) and soybean commodity price also are contributing factors as to why a comprehensive economic analysis is less realistic.

In summary, our paper focused on understanding the patterns of foliar fungicide use and its relationship with soybean yield losses due to fungal pathogens (targets of fungicides considered in the study) at broader geographic (national/regional/state) and temporal scales. The trends that we see at such scales may or may not necessarily reflect/represent what each individual soybean farmer would have experienced at a farm scale. In other words, we cannot simply extrapolate the individual farm-level response in relation to his fungicide use and yield losses profiles. Our goal was not to facilitate the fungicide application decision making at the individual farm level, rather we focused on understanding fungicide use patterns and their degree of utility in terms of reducing foliar disease associated yield losses at a broader geographic scale. Nonetheless, our results do provide some guidance in that we suggest that farmers should not rely on fungicides as the sole management strategy to manage foliar diseases in soybean. Instead, location specific best management practices such as optimum maturity group, planting date, seeding rate, row spacing, crop rotation, fertilizer, field history as it relates to disease incidence, and irrigation regime as well as use of genetic resistance should be emphasized to decrease the probability of disease incidence. When necessary, farmers should make informed decisions as to the use of foliar fungicides with special emphasis on application timing (disease susceptible plant growth stage). In conclusion, rather than using fungicides as a routine practice, farmers should treat foliar fungicides as an integral component of a sound integrated pest management system.

Supporting information

S1 Table. Mixed-effects modelling of the effect of foliar fungicide use on soybean yield losses due to foliar diseases from soybean growing states in the northern region of the United States during 2005–2015 period.
(DOCX)

S2 Table. Mixed-effects modelling of the effect of foliar fungicide use on soybean yield losses due to foliar diseases from soybean growing states in the southern region of the United States during 2005–2015 period.
(DOCX)

S3 Table. Parameter estimates/coefficients from the joint regression analysis conducted to test the significance of the relationship between fungicide use and soybean production/

yield losses due to diseases for individual state and year.
(XLSX)

S4 Table. Mixed-effects modelling of the effect of foliar fungicide use on soybean production/ yield from soybean growing states in the northern region of the United States during 2005–2015 period.
(DOCX)

S5 Table. Mixed-effects modelling of the effect of foliar fungicide use on soybean production/ yield from soybean growing states in the southern region of the United States during 2005–2015 period.
(DOCX)

S6 Table. Parameter estimates/coefficients from the joint regression analysis conducted to test the significance of the relationship between fungicide use and soybean production/ yield for individual state and year.
(XLSX)

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