Impact of Foliar Fungicides on Frogeye Leaf Spot Severity, Radiation Use Efficiency and Yield of Soybean in Iowa

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Abstract: Frogeye leaf spot, caused by Cercospora sojina K. Hara, is a major soybean (Glycine max L. Merr.) disease that has become more prevalent in the upper Midwest and can be managed with foliar fungicides. Incorporating disease severity into a parameter directly related to yield may better relay the impact of disease on yield and yield components than severity alone. Experiments during the 2018 and 2019 growing seasons in fields located in north central and southwestern Iowa were completed to (i) determine how foliar fungicides affected frogeye leaf spot, remotely sensed plant health indicators, and soybean yield, and (ii) compare the relationship and impact of foliar fungicides and frogeye leaf spot on radiation-use efficiency (RUE) estimated using unmanned aerial vehicle reflectance data. Fungicides affected frogeye severity and yield in one of the three locations; in Lewis 2018, the flutriafol + fluoxastrobin treatment reduced frogeye leaf spot severity by over 50% and increased yield by 19% compared to non-treated controls. Applications of foliar fungicides increased canopy coverage compared to non-treated controls (p = 0.012), but NDVI, SPAD values, and RUE values did not differ between fungicide treatments at all three locations. Estimated soybean RUE values (1.05 to 1.66 g MJ⁻¹) were within the range of known values. Overall, this study indicates that RUE can be a valuable resource to estimate the impact of the disease on yield, however, additional research will be needed to use RUE within certain pathosystems.

Keywords: RUE; frogeye leaf spot severity; soybean yield; foliar fungicides

1. Introduction

Frogeye leaf spot of soybean, caused by the pathogen Cercospora sojina K. Hara, is found worldwide with the potential to cause yield loss of up to 60% [1–5]. Frogeye leaf spot is common in the southern United States due to the favorable environmental conditions and mild winters, but northern states such as Iowa, Ohio, and Wisconsin have seen a rise in prevalence [6–8]. Across the United States from 1996 to 2019, frogeye leaf spot reduced soybean yields by approximately 288,000 metric tons, which equated to an annual loss of $98 million [9]. In Iowa, frogeye leaf spot caused losses of over 43,500 metric tons and $14.5 million annually from 1996 to 2019. In Iowa during 2018, frogeye leaf spot caused an estimated loss of over 360,000 metric tons and over 140,000 metric tons in 2019, which equates to over $119.4 million in 2018 and $46.5 million in 2019 [9].

Frogeye leaf spot symptoms include characteristic foliar lesions that, when mature, are light brown or tan spots surrounded by a dark reddish-brown margin [10] (Figure 1A). Infection can occur at any growth stage of the soybean plant. Lesions can also occur on the stems and pods, but appear different from the distinctive foliar lesions [11] (Figure 1B). C. sojina is an ascomycete that favors warm and humid conditions [10]. The primary inoculum arises from diseased crop residue and infected seed [12]. It has been postulated that this increase in frogeye leaf spot may be attributed to a combination of reduced-till farming practices and warming global temperatures [6,8,13–15]. Management strategies...
for frogeye leaf spot include planting resistant cultivars and applying foliar fungicides [6,8,16]. However, fungicide resistant strains of C. sojina have been identified, first in Tennessee in 2010 [17]. Since then, it has been reported in isolates collected from Alabama, Arkansas, Delaware, Illinois, Indiana, Iowa, Kentucky, Louisiana, Mississippi, Missouri, North Carolina, Ohio, Tennessee, Virginia, and South Dakota [18,19].

![Image of frogeye leaf spot symptoms](image)

**Figure 1.** (A) Symptoms of frogeye leaf spot on soybean leaves. Image: X. Phillips. Taken 09-06-2018 in Lewis, IA, USA. (B) Symptoms of frogeye leaf spot on a soybean pod. Image: X. Phillips. Taken 09-13-2018 in Lewis, IA.

Certain fungicides, specially the Qols, have also been promoted to increase yield even in the absence of disease, and improvements in yield have been shown, although inconsistently [20–23]. Increase in the yield may be attributed to a phenomenon called the “greening effect”, which is described to extend the duration of green leaf area, maintaining photosynthetic efficiency and allowing continuation of dry matter accumulation [24–27]. Joshi et al. [28] reported that application of pyraclostrobin benefited the formation of root nodules; nitrogen fixation was enhanced, growth improved, and yields were greater. Amaro et al. [29] concluded that the physiological effects associated with an application of a quinone outside inhibiting (QoI) fungicide are due to an increase in net photosynthesis. In addition, applications of pyraclostrobin delay senescence by impacting hormonal levels [29].

Yield loss caused by frogeye leaf spot is primarily attributed to a reduction in green leaf area and premature defoliation that occurs when severity is greater than 30% [4]. Reducing the photosynthetic leaf area can result in a reduced seed weight and consequently less yield [4]. Historically, disease severity and the area under the disease progress curve (AUDPC) have been used to measure the impact of disease severity on yield [26,30,31]. However, disease severity is not directly related to yield and the resulting relationship between AUDPC and yield can be inconsistent [32–34]. Instead, yield has more consistently been predicted by healthy leaf area duration and the absorption of solar radiation [34–36].

Yield and green biomass have been correlated with various indices using remote sensing [37]. Handheld active sensors such as a GreenSeeker (Trimble, Sunnyvale, CA, USA) and Soil Plant Analysis Development (SPAD) meters (Konica-Minolta, Chiyoda, Tokyo, Japan) are commonly used to measure plant health and crop nitrogen status [38]. These sensors measure transmittance and reflectance of specific regions of the magnetic spectrum. Raw SPAD values are proportional to the chlorophyll in the leaf and have been used to determine the efficacy of nitrogen treatments [39–44]. The normalized difference
vegetation index (NDVI) is the ratio between the difference and sum of red and near-infrared frequencies. It has been used to monitor health and estimate yield [45,46].

A parameter that incorporates yield-related measurements in addition to disease severity may better characterize the impact of the disease. Radiation use efficiency (RUE) is a measure of the relationship between accumulated biomass and intercepted photosynthetically active radiation (PAR) [47–50]. Madden and Nutter [31] suggested that using the healthy leaf area and RUE could quantify the effects of disease on yield. Foliar disease may be accounted for by removing severity from the green leaf area index (GLAI) by converting the severity rating to a 0–1 scale and multiplying it by the GLAI [31]. There is no standard method of estimating RUE. Gitelson and Gamon [51] proposed that the standard method should be the radiation absorbed by photosynthetically active tissue (RUE_{green}). However, by only considering green leaf area in the RUE estimation, it becomes sensitive to inevitable pigment changes during senescence [51]. Tewes and Schellberg [52] used the RUE_{green} method to estimate RUE of corn. This method was first used to estimate the RUE of soybean using reflectance data from unmodified consumer grade imagery captured by an unmanned aerial vehicle (UAV) [53].

UAVs are increasingly being used in agricultural settings to monitor water status, plant vigor, biomass, and diseases [54–58]. UAVs can be used to capture imagery at high spatial and temporal scales across large areas in a single flight [59]. RGB and multispectral imagery are the forms most utilized to collect disease information [60–65].

Soybean RUE determined on a small plot scale with high sampling frequency under low disease conditions by Phillips et al. [53] was within the range of previously published RUE values [66]. Since RUE is directly associated with above-ground biomass [48], we propose RUE may be a better parameter to monitor the impact of diseases and their management. The objectives of this study were to (i) determine how foliar fungicides affect frogeye leaf spot, remotely sensed plant health indicators, and soybean yield, and (ii) compare the relationship and impact of foliar fungicides and frogeye leaf spot on RUE estimated using UAV reflectance data with a reduced sampling approach across foliar fungicide treatments.

2. Materials and Methods

Field experiments were completed near Kanawha and Lewis, IA in 2018 and Kanawha in 2019. Experiments were laid out in a randomized complete block design with three treatments and four replications. Plots were 7.8 m long, and four rows wide spaced 76 cm apart. The three treatments were (1) non-treated controls, (2) fluspyroxad + pyraclostrobin (Priaxor®, FRAC group 7 + 11, 0.05 L ha⁻¹, BASF, Research Triangle Park, NC, USA), and (3) flutriafol + fluoxastrobim (Preemptor®, FRAC group 3 + 11, 0.06 L ha⁻¹, FMC Corporation, Philadelphia, PA, USA). Cultivars and other experimental details are given in Table 1. Fungicides were applied at the R3 growth stage [beginning pod; 67] at a rate of 0.05 and 0.06 L ha⁻¹ active ingredient for the fluspyroxad + pyraclostrobin and flutriafol + fluoxastrobim treatments, respectively. All four rows were sprayed with a self-propelled research sprayer that delivered fungicides using XR 11002 nozzles with 142 to 189 L ha⁻¹ at 241 kPa powered by CO₂.
Field data were collected every 6–16 days beginning at the R3 growth stage, beginning pod development [67]. At both locations in 2018 there were four sampling dates (SDs) and six SDs in Kanawha in 2019. Each SD was paired with a growing degree day (GDD) sum (Table 1). The GDD was calculated with a base of 10 °C (http://mesonet.agron.iastate.edu, assessed on 16 July 2021). GDD values were summed, beginning at the planting date of the specific site year, through the calendar date of the field sampling. Sampling dates across locations were grouped based on the GDD of the respective SD and is referred to as the growing degree day group (GDDG) (Table 1). Six GDDGs (GDDG-1 to GDDG-6) were created. The range of GDDs within GDDG-1 was 1280 to 1298, 1542 to 1560 for GDDG-2, 1808 to 1858 for GDDG-3, 2140 to 2243 for GDDG-4, 2344 to 2711 for GDDG-5, and 2832 for GDDG-6.

During each sampling, a 0.5 m section from one of the non-yield rows was marked for data collection. This section was kept 0.5 m away from row ends and previously sampled areas to avoid edge effects [68]. From this section of row, frogeye leaf spot severity data, NDVI values, SPAD readings, plant count, and destructive plant samples were collected.

Frogeye leaf spot severity (0–100%) was determined by examining all plants in the 0.5 m sampling section. Both the upper and lower canopies were rated and averaged for a single plot estimation because the leaf area was determined from all of the plants’ leaves. Only the frogeye leaf spot severity recorded on the last SD respective to each site year was used in the analysis because the disease in earlier other dates were at low levels.

SPAD readings were collected with a SPAD-502 m (Konica-Minolta, Chiyoda, Tokyo, Japan) in 2018 and a CCM-200 (Opti-Sciences, Hudson, NH, USA) in 2019. The SPAD-502 m uses 940 nm and 650 nm wavelengths while CCM-200 uses 940 nm and 660 nm wavelengths to measure the ratio vegetative index (RVI). The reading frame of the two devices differ; the area of reading is 0.06 and 0.71 cm² for the SPAD-502 and CCM-200, respectively. This was done by taking 10 readings from the center leaf of a mature, fully expanded trifoliate from the upper canopy. We avoided taking readings directly on frogeye leaf spot lesions. Readings were averaged to a single value. A handheld GreenSeeker (Trimble, Sunnyvale, CA, USA) was used to measure the NDVI of the section of row. The 0.5 m section was measured three times and averaged to a single NDVI rating per SD. Five plants were then destructively sampled by cutting every other plant at the soil line. The five plants were bundled in a plastic bag, and stored at 4 °C until further processing 2 to 10 days later.

Leaves were grouped as “green” and “non-green” to obtain separate green and yellow leaf areas. If a leaf was over 50% yellow for any reason, it was placed into the “non-green” group. All of the leaves from the bundle of five plants were processed to measure

| Site Year | Soil Type/Slope | Cultivar | Planting Population | Planting Date | Fungicide Application * | Sampling Dates (GDD;GDDG) ** | Harvest Date |
|-----------|-----------------|----------|---------------------|---------------|-------------------------|-------------------------------|--------------|
| Kanawha 2018 | Nicollet clay loam/1 to 3 | S28-N6 | 370,000 | 29 May | 30 July | 25 July (1280;1), 9 August (1560;2), 21 August (1808;3), 12 September (2204;4) | 23 October |
| Lewis 2018 | Marshall silty clay loam/2 to 5 | S28-N6 | 370,000 | 9 May | 23 July | 30 July (1858;3), 15 August (2243;4), 6 September (2711;5), 13 September (2832;6) | 24 October |
| Kanawha 2019 | Nicollet clay loam/1 to 3 | S29-K3X | 395,200 | 16 May | 22 July | 25 July (1298;1), 6 August (1542;2), 22 August (1824;3), 2 September (1975), 13 September (2140;4), 23 September (2344;5) | 26 October |

* Fungicides were applied at (R3), beginning pod development [67]. ** Sum of growing degree days (GDD) with a base of 10° Celsius beginning at the planting date.
a “green” and “non-green” leaf area using a LI-3100C (LI-COR, Inc., Lincoln, NE, USA). Leaf area was converted to m² and used to estimate leaf area index (LAI; m² m⁻²) by using the number of plants sampled and the total number of plants in the sampled area [69]. The sum of the green leaf area index (GLAI) and the yellow leaf area index (YLAI) from the “non-green” group, was the total leaf area index (TLAI). The frogeye leaf spot severity rating of each respective date was used to reduce the GLAI and determine the green leaf area index with frogeye leaf spot severity removed (GLAI≤). This was used for all date-specific needs of GLAI.

All leaves, stems, and pods from each plot were placed in brown paper bags and dried for seven days in custom drying bins with fans held at a constant temperature of 60 °C. Sample dry matter (DM) was measured for each bag and adjusted to exclude bag weight (Taylor Precision Products, Oak Brook, IL, USA) Thus, the DM determination at all SDs consisted of the entire plant including seeds.

Images in 2019 were collected with a Phantom 4 Pro (DJI, Shenzhen, China) in 2018 and a Phantom 4 ProV2.0 (DJI, Shenzhen, China), both with an onboard 2.54 cm CMOS 20 MP camera. Image analysis and RUE estimation followed methods performed by Phillips et al. [53]. The inner two rows of each plot were harvested for yield using a 2009 Almaco SPC20 research plot combine (ALMACO, Nevada, IA, USA). Yield calculations were adjusted to 13% seed moisture.

Weather data for site-years was obtained from the Iowa Environmental Mesonet (http://mesonet.agron.iastate.edu, assessed on 16 July 2021) The summer month selection of June–August was compared to the 30-year average for total precipitation and average temperature. In addition, September was included for total precipitation and average temperature and also compared to the 30-year average.

Statistical analyses were performed in SAS 9.4 (SAS Institute, Cary, NC, USA). Analysis of variance was performed for all variables. A mixed model procedure was fitted using Proc Glimmix. Treatment was set as a fixed factor while replication, GDDG (sampling date), and site year were considered random factors. Replication was nested within date-during-analysis within site-years for variables with multiple sampling times. When analysis combined site-years, replication was then nested within a GDDG. Least square means (LS-means) were estimated using the LSmeans statement. Means were separated using “lines” options in the LSmeans statement at a 10% level of significance (α = 0.10). PROC CORR procedure was used to correlate frogeye leaf spot, RUE and yield. The Lewis 2018 site year showed significant errors across all treatments throughout the first replication, and thus the first replication was removed from the analysis.

3. Results

Frogeye leaf spot severity differed across the site-years (Table 2). In Lewis 2018, frogeye leaf spot severity was approximately three and ten times greater than severity at Kanawha 2018 and 2019, respectively (Table 3). Both treatment (p = 0.005) and the treatment × site year interaction (p = 0.010) were significant for frogeye leaf spot severity (Table 2). Non-treated controls had the most frogeye leaf spot, around 9% severity averaged, while the fl✉xapoyroxad + pyraclostrobin and fl✉xafostrobine treatments averaged around 7 and 5%, respectively, over all site-years (Table 3). Overall, the frogeye leaf spot severity of the fl✉xafostrobine treatment was significantly less than the severity of the fl✉xapoyroxad + pyraclostrobin treatment (Table 3). In Lewis 2018, the fl✉xafostrobine treatment reduced frogeye leaf spot severity by over 50% compared to the non-treated controls (Table 4). However, in Kanawha 2018 and 2019, frogeye leaf spot severity across treatments did not differ, although there was numerically less disease with the fungicide treatment (Table 4).
Table 2. Analysis of variance for different variables to test the effects of site-year, treatment, growing degree day group (GDDG), and their combinations in field experiments conducted in Kanawha and Lewis County Iowa in 2018 and 2019.

| Effect                      | PC    | DM   | CC    | TLAI  | GLAI\(x\) | NDVI  | SPAD  | Frogeye Leaf Spot | RUE (g Mj\(^{-1}\)) | Yield (kg ha\(^{-1}\)) |
|-----------------------------|-------|------|-------|-------|------------|-------|-------|-------------------|---------------------|------------------------|
| Site-year                   | 0.212 | <0.001 | 0.185 | <0.001 | <0.001     | <0.001| <0.001| 0.773             | 0.035               |
| Treatment                   | 0.594 | 0.908 | 0.012 | 0.764  | 0.689      | 0.431 | 0.773 | 0.005             | 0.342               |
| Site-year \(\times\) treatment | 0.824 | 0.552 | 0.987 | 0.378  | 0.477      | 0.952 | 0.860 | 0.010             | 0.462               |
| GDDG                        | 0.039 | <0.001 | <0.001 | <0.001 | <0.001     | <0.001| <0.001| <0.001            | 0.005               |
| Site-year \(\times\) GDDG   | <0.001| 0.429 | <0.001 | <0.001 | <0.001     | <0.001| <0.001| <0.001            | <0.001              |
| Treatment \(\times\) GDDG   | 0.908 | 0.959 | 0.024 | 0.824  | 0.885      | 0.644 | 0.297 |                   |                     |
| Site-year \(\times\) treatment \(\times\) GDDG | 0.968 | 0.991 | 0.984 | 0.982  | 0.945      | 0.999 | 0.878 |                   |                     |

\(z\) RUE = radiation use efficiency; PC = plant count; DM = dry matter; CC = canopy cover; TLAI = total leaf area index; GLAI\(x\) = green leaf area index with disease severity removed; NDVI = normalized difference vegetation index; SPAD = soil plant analysis development.

Table 3. Least square means of yield, frogeye leaf spot severity, and radiation use efficiency (RUE) recorded in field experiments in Kanawha and Lewis county Iowa in 2018 and 2019.

| Site Year       | Treatment \(z\)                  | Frogeye Leaf Spot (%) | RUE (g Mj\(^{-1}\)) | Yield (kg ha\(^{-1}\)) |
|-----------------|----------------------------------|-----------------------|---------------------|-------------------------|
| Kanawha 2018    |                                  | 4.5 b                 | 1.29                | 3174.2 b                |
| Lewis 2018      |                                  | 14.9 a                | 1.38                | 3880.3 a                |
| Kanawha 2019    |                                  | 1.5 c                 | 1.27                | 3840.0 a                |
|                 | NTC                              | 8.9 a                 | 1.19                | 3503.7 b                |
|                 | Fluxapyroxad + pyraclostrobin    | 7.0 b                 | 1.43                | 3571.0 b                |
|                 | Flutriafol + fluoxastrobin       | 4.9 c                 | 1.33                | 3826.5 a                |

\(z\) Means followed by the same letter for site-years and by the same letters for treatments were not statistically different at \(\alpha = 0.1\). Means were separated using the “Lines” option in the LSMEANS statement. \(z\) Treatment = Non-treated controls (NTCs) did not receive a fungicide application; fluxapyroxad + pyraclostrobin (Priaxor, BASF, Research Triangle Park, NC, USA); flutriafol + fluoxastrobin (Preemptor, FMC Corporation, Philadelphia, PA, USA).
Table 4. Least square means \(^*\) of yield, frogeye leaf spot severity, and radiation use efficiency (RUE) for different fungicide treatments in three locations in Iowa during 2018 and 2019.

| Site Year | Treatment \(^*\) | Frogeye Leaf Spot (%) | RUE (g Mj\(^{-1}\)) | Yield \(^*\) (kg ha\(^{-1}\)) |
|-----------|-----------------|---------------------|---------------------|-----------------------------|
| Kanawha 2018 | NTC | 4.8 | 1.37 | 3086.8 |
| | Fluxapyroxad + pyraclostrobin | 3.8 | 1.25 | 3214.6 |
| | Flutriafol + fluoxastrobin | 4.6 | 1.26 | 3221.3 |
| | p value | 0.191 | 0.890 | 0.797 |
| Lewis 2018 | NTC | 19.6 a | 1.05 | 3631.5 b |
| | Fluxapyroxad + pyraclostrobin | 15.8 a | 1.66 | 3725.7 b |
| | Flutriafol + fluoxastrobin | 9.3 b | 1.43 | 4310.7 a |
| | p value | 0.059 | 0.234 | 0.008 |
| Kanawha 2019 | NTC | 2.5 | 1.15 | 3806.4 |
| | Fluxapyroxad + pyraclostrobin | 1.5 | 1.37 | 3779.5 |
| | Flutriafol + fluoxastrobin | 0.5 | 1.29 | 3947.6 |
| | p value | 0.375 | 0.659 | 0.259 |

\(^*\) Means followed by the same letter for site-years and by the same letters for treatments were not statistically different at \(\alpha = 0.1\). Means were separated using the “Lines” option in the LSMEANS statement. \(^*\) Treatments = Non-treated controls (NTCs) did not receive a fungicide application; fluxapyroxad + pyraclostrobin (Priaxor, BASF, Research Triangle Park, NC, USA); flutriafol + fluoxastrobin (Preemptor, FMC Corporation, Philadelphia, PA, USA). \(^*\) Yield was adjusted to 13% seed moisture.

Mean RUE values were 1.27 to 1.38 g Mj\(^{-1}\) across site-years (Table 3) and did not differ across site-year or treatment (Table 2). RUE values for treatments were 1.19, 1.43, and 1.33 g Mj\(^{-1}\) for non-treated controls, fluxapyroxad + pyraclostrobin treatment, and flutriafol + fluoxastrobin treatment, respectively (Table 3). Although not significantly different, RUE values were numerically greater for fungicide treatments.

The effect of GDDG and the GDDG \(\times\) site-year interaction was significant for plant count (Table 2). The plant count of the measured section of row was greatest at GDDG-1 (GDD ranged from 1280 to 1298) compared to all subsequent groups (Table 2). The plant count across all groups ranged from an average of 11.0 to 13.2 plants (Table 5). Treatment did not affect the plant count in any GDDGs.

Table 5. Least square means \(^*\) of the plant count (PC), dry matter (DM), canopy cover (CC), total leaf area index (TLAI), green leaf area index with disease severity removed, normalized difference vegetation index (NDVI), and soil plant analysis development (SPAD) readings recorded in field experiments conducted in in Kanawha and Lewis County Iowa in 2018 and 2019.

| GDDG \(^\text{y}\) | Treatment \(^z\) | PC | DM (g) | CC (%) | TLAI | GLAI\(_0\) | NDVI | SPAD |
|-----------------|-----------------|----|--------|--------|-------|---------|-------|-------|
| GDDG-1          | NTC             | 13.2 a | 98.6 e | 84.3 c | 2.5 c | 2.0 c | 0.87 a | 40.0 a |
| GDDG-2          | NTC             | 11.9 b | 154.0 d | 92.9 ab | 3.9 ab | 3.7 ab | 0.89 a | 40.8 a |
| GDDG-3          | NTC             | 11.0 b | 201.8 c | 96.0 a | 4.0 a | 3.8 a | 0.88 a | 37.7 a |
| GDDG-4          | NTC             | 11.8 b | 317.8 b | 96.6 a | 3.3 b | 3.1 b | 0.82 b | 34.6 a |
| GDDG-5          | NTC             | 11.2 b | 335.3 ab | 90.7 b | 2.0 c | 1.2 d | 0.63 c | 23.7 b |
| GDDG-6          | NTC             | 11.9 b | 365.0 a | 58.3 d | 0.9 d | 0.5 d | 0.50 d | 17.3 b |
| | Fluxapyroxad + pyraclostrobin | 11.7 | 227.1 | 87.0 a | 2.9 | 2.5 | 0.78 | 33.5 |
| | Flutriafol + fluoxastrobin | 12.0 | 233.6 | 89.4 a | 2.8 | 2.5 | 0.77 | 32.6 |

\(^*\) Means followed by the same letter for site-years and by the same letters for treatments were not Scheme 0. Means were separated using the “Lines” option in the LSMEANS statement. \(^\text{y}\) GDDG = Growing degree day group. \(^z\) Treatments = Non-treated controls (NTCs) did not receive a fungicide application; fluxapyroxad + pyraclostrobin (Priaxor, BASF, Research Triangle Park, NC, USA); flutriafol + fluoxastrobin (Preemptor, FMC Corporation, Philadelphia, PA, USA).
The effect of the GDDG was significant for dry matter, but treatment had no effect (Table 2). The dry matter in the 0.5 m section of row was 98.6 g in GDDG-1, increased with growing degree days (Table 5), and peaked on GDDG-6 (GDD 2832) at 365.0 g (Table 5). Lewis 2018 was the only site year to have significantly different yield and frogeye leaf spot severity across treatments. In Lewis 2018, dry matter was significantly less on the initial date of data collection, GDDG-3 ($p = 0.016$; Table 6). However, the following three sampling periods were not significantly different with regard to dry matter, and decreased numerically after GDDG-4 (Table 6).

Table 6. Least square means of the plant count (PC), dry matter (DM), canopy cover (CC), total leaf area index (TLAI), green leaf area index with disease severity removed, normalized difference vegetation index (NDVI), and soil plant analysis development (SPAD) readings for different growing degree date groups (GDDG) and treatments recorded in a field experiment conducted in Lewis 2018.

| GDDG    | Treatment | PC  | DM (g) | CC (%) | TLAI | GLAID | NDVI | SPAD |
|---------|-----------|-----|--------|--------|------|-------|------|------|
| GDDG-3  |           | 11.3| 254.9 a| 90.7 a | 4.4 a| 4.3 a | 0.88 a| 45.9 a|
| GDDG-4  |           | 11.9| 403.8 a| 99.1 a | 4.3 a| 4.2 a | 0.89 a| 48.0 a|
| GDDG-5  |           | 11.4| 396.2 a| 97.8 a | 2.2 b| 1.9 b | 0.76 b| 45.0 a|
| GDDG-6  |           | 11.9| 371.1 a| 58.3 b | 0.9 c| 0.5 c | 0.50 c| 17.3 b|
| NTC     |           | 11.3| 354.6 | 81.5   | 3.1  | 2.9   | 0.76  | 37.6 |
| Fluxapyroxad + pyraclostrobin |     | 11.5| 346.1 | 87.3   | 3.0  | 2.5   | 0.76  | 40.6 |
| Flutriafol + fluoxastrobin    |     | 12.1| 368.8 | 90.5   | 2.8  | 2.7   | 0.75  | 39.0 |

Means followed by the same letter for site-years and by the same letters for treatments were not statistically different at \( \alpha = 0.1 \). Means were separated using the "Lines" option in the LSMEANS statement. Treatment = Non-treated controls (NTCs) did not receive a fungicide application; fluxapyroxad + pyraclostrobin (Priaxor, BASF, Research Triangle Park, NC, USA); flutriafol + fluoxastrobin (Preemptor, FMC Corporation, Philadelphia, PA, USA).

Canopy coverage varied across treatments and GDDGs (Table 2). Both fungicide treatments resulted in significantly greater ($p = 0.012$) canopy cover compared to non-treated controls, which had 83% CC, by approximately 5 and 8% for the fluxapyroxad + pyraclostrobin and flutriafol + fluoxastrobin treatments, respectively (Table 5). In general, the canopy cover peaked on GDDG-4 at 96.7% and decreased in following GDDGs (Table 5). The canopy cover for Kanawha 2018 was significantly less than that of Kanawha 2019 in GDDGs-1 and 2 (Table 7).
Table 7. Least square means \(^a\) of the canopy cover (CC) measured from experiments in Iowa during 2018 and 2019.

| Site Year | Treatment \(^z\) | GDDG-1 | GDDG-2 | GDDG-3 | GDDG-4 | GDDG-5 | GDDG-6 | \(p\) Value |
|-----------|-----------------|--------|--------|--------|--------|--------|--------|-------------|
| Kanawha 2018 | 81.8 b, C | 90.2 b, B | 98.7 a, A | 93.2 b, AB | 0.012 |
| Lewis 2018 | 90.7 c, A | 99.1 a, A | 97.8 a, A | 58.3 B | \(<0.001\) |
| Kanawha 2019 | 87.0 a, B | 95.6 a, A | 97.4 b, A | 98.4 ab, A | 85.3 b, B | \(<0.001\) |
| \(p\) value | 0.046 | 0.003 | \(<0.001\) | 0.136 | \(<0.001\) |
| NTC | 86.5 a, A | 92.5 a, A | 95.4 a, A | 94.2 a, A | 88.6 a, A | 40.7 a, B | \(<0.001\) |
| Fluxapyroxad + pyraclostrobin | 82.4 a, C | 93.6 a, AB | 96.3 a, A | 97.6 a, A | 91.6 a, A | 60.6 a, D | \(<0.001\) |
| Flutriatol + fluoxastrobin | 84.2 a, C | 92.5 a, B | 96.3 a, AB | 98.1 a, A | 91.8 a, B | 73.4 a, D | \(<0.001\) |
| \(p\) value | 0.481 | 0.863 | 0.814 | 0.512 | 0.776 | 0.246 |

\(^a\) Means within a column followed by the same lower-case letter and means within a row followed by the same upper-case letter do not differ significantly at \(\alpha = 0.1\). Means were separated using the “Lines” option in LSMEANS statement. \(^z\) GDDG = Growing degree day group. The range between GDDs within GDDG-1 was 1280 to 1298, 1542 to 1560 for GDDG-2, 1808 to 1858 for GDDG-3, 1940 to 2243 for GDDG-4, 2344 to 2711 for GDDG-5, and 2832 for GDDG-6. \(^z\) Treatments = Non-treated controls (NTCs) did not receive a fungicide application; fluxapyroxad + pyraclostrobin (Prixor, BASF, Research Triangle Park, NC, USA); flutriatol + fluoxastrobin (Preemptor, FMC Corporation, Philadelphia, PA, USA).

Treatment did not have a significant effect on either TLAI or GLAI\(_{a0}\) (Table 2). Among the treatments, TLAI ranged from 2.8 to 3.0 while GLAI\(_{a0}\) ranged from 2.5 to 2.6. The effect of GDDG was significant for both TLAI and GLAI\(_{a0}\). TLAI and GLAI\(_{a0}\) both plateaued on GDDG-3 at 4.0 and 3.8, respectively (Table 5). Leaf area begins to decline following GDDG-3. The overall values of TLAI and GLAI\(_{a0}\) for Kanawha 2018 did not surpass 2.5 and 2.2, respectively (Table 8). The Kanawha 2019 location had the highest TLAI value on GDDG-2 and GDDG-3 (around 5.2), approximately 16% more than the next-highest value from Lewis 2018 on GDDG-3 (Table 8).

Table 8. Least square means \(^y\) of the total leaf area index (TLAI) and the green leaf area index with frogeye leaf spot severity removed (GLAI\(_{a0}\)) measured from experiments in Iowa during 2018 and 2019 \(^z\).

| Site Year | GDDG-1 | GDDG-2 | GDDG-3 | GDDG-4 | GDDG-5 | GDDG-6 | \(p\) Value |
|-----------|--------|--------|--------|--------|--------|--------|-------------|
| Kanawha 2018 | 2.4 a, A | 2.5 b, A | 2.4 c, A | 1.7 b, B | 0.083 |
| Lewis 2018 | 4.4 b, A | 4.3 a, A | 2.2 a, B | 0.9 C | \(<0.001\) |
| Kanawha 2019 | 2.5 a, C | 5.2 a, A | 5.2 a, A | 4.2 a, B | 1.8 a, D | \(<0.001\) |
| \(p\) value | 0.746 | \(<0.001\) | \(<0.001\) | \(<0.001\) | 0.287 |
| GLAI\(_{a0}\) |
| Kanawha 2018 | 1.6 b, B | 2.2 a, B | 2.2 c, A | 1.4 b, B | 0.068 |
| Lewis 2018 | 4.3 b, A | 4.2 a, A | 1.9 a, B | 0.5 C | \(<0.001\) |
| Kanawha 2019 | 2.5 a, C | 5.2 a, A | 5.0 a, A | 4.0 a, B | 0.8 b, D | \(<0.001\) |
| \(p\) value | 0.003 | \(<0.001\) | \(<0.001\) | \(<0.001\) | 0.004 |

\(^y\) Means within a column followed by the same lower-case letter and means within a row followed by the same upper-case letter do not differ significantly at \(\alpha = 0.1\). Means were separated using “Lines” option in LSMEANS statement. \(^z\) GDDG = Growing degree day group. The range between GDDs within GDDG-1 was 1280 to 1298, 1542 to 1560 for GDDG-2, 1808 to 1858 for GDDG-3, 1940 to 2243 for GDDG-4, 2344 to 2711 for GDDG-5, and 2832 for GDDG-6.

Fungicide treatment did not significantly affect NDVI or SPAD (Table 2). Overall, the non-treated controls combined across all site-years had 0.78 NDVI and 32.7 SPAD. GDDG significantly impacted both variables. NDVI was 0.87 and SPAD was 40.0 in the first sampling. Both numerically plateaued on GDDG-2 with NDVI 0.89 and SPAD 40.8 (Table 5). NDVI values began to significantly decline after GDDG-3 (NDVI value 0.88), whereas SPAD values began to diminish after GDDG-4 (SPAD reading 34.6) (Table 5).
Yield varied across all site-years and treatments at Lewis 2018 (Tables 2 and 3). Across site-years, yield was greatest in Lewis 2018, at approximately 22% more than the yield in Kanawha 2018 (Table 3). Treatment effect was statistically significant for yield (Table 2). Overall, flutriafol + fluoxastrobin treatment had the greatest yield, which was 9% more than non-treated controls. Yields of the flupafoxrad + pyraclostrobin treatment and non-treated controls were statistically similar. Treatment × site-year interaction was significant for yield ($p = 0.08$); thus, we analyzed yield by site-year. Treatment difference was significant ($p = 0.059$) in Lewis 2018 only, although similar trends were observed in other locations. In Lewis 2018, soybeans treated with flutriafol + fluoxastrobin produced 19% more yield than the non-treated controls (Table 4). Correlations among yield, frogeye leaf spot severity, and RUE were not significant (Table 9). Correlation coefficients ranged from $-0.17$ to $0.15$.

**Table 9.** Pearson’s rank correlations between yield, frogeye leaf spot, and radiation use efficiency (RUE) combined across years and locations.

| Variable          | Yield    | Frogeye Leaf Spot | RUE       |
|-------------------|----------|-------------------|-----------|
| Yield             | −        | 0.15 (0.420)      | $-0.17$ (0.357) |
| FLS               | 0.15 (0.420) | −                 | 0.16 (0.404)  |
| RUE               | $-0.17$ (0.357) | 0.16 (0.404)      | −         |

During June to August in Kanawha 2018 and Lewis 2018, total precipitation was approximately 39 and 47% greater than the 30-year average, respectively (http://mesonet.agron.iastate.edu; accessed on 16 July 2021; Table S1). However, precipitation in Kanawha 2019 was approximately 20% less than the 30-year average. During the month of September, total precipitation was 3 and 2 times greater than the 30-year average for Kanawha and Lewis in 2018, respectively. The average temperature of all site-years from June–August did not vary by more than ±2% from the 30-year average. It was slightly warmer in 2018 site-years and roughly 2% warmer in Lewis compared to Kanawha (Table S1).

4. Discussion

The RUE of soybean was estimated on a small plot scale as described previously [53]. This study was done to determine if RUE could be used to monitor the impact of diseases and their management, specifically frogeye leaf spot and foliar fungicide applications on soybean yield. All estimated RUE values in this study fell within the range of previously reported values of 0.60 to 2.53 g MJ$^{-1}$ [53,66]. In our studies, RUE did not differ between field trial locations or among treatments within locations, even though frogeye leaf spot and yield did. Across site-years, Lewis 2018 had the greatest numerical value of RUE, the greatest average yield, and the most frogeye leaf spot severity. However, RUE values did not differ significantly among treatments, although the fungicide treatments tended to have greater RUE than the non-treated controls.

The severity levels of frogeye leaf spot were removed from the GLAI on each date. The level of frogeye leaf spot severity where it starts to affect yield is still not well defined. Previous studies have shown that flupafoxrad + pyraclostrobin provided a yield benefit of 6% at frogeye leaf spot severity levels of approximately 11%, while flutriafol reduced severity by 81% and provided a yield benefit of 10% compared to the non-treated controls, that had approximately 20% severity [70]. By removing the frogeye leaf spot severity, the GLAI, and subsequently the APAR, were reduced during early sampling dates. But, if the level of severity was not impacting DM, this could bolster early sampling dates on the regression plot and potentially wash out effects when severity reaches yield-reducing levels at later dates. Srinivasan et al. [71] showed reducing LAI increased RUE, if DM was maintained. In Lewis 2018, it was expected for there to be a difference in RUE across treatments because of high disease severity and the influence of treatments on disease
severity and yield; however, the reduced number of sampling units to derive RUE may have contributed to the lack of significant differences between treatments. The DM and GLAI, the components needed for RUE derivation, did not increase across sampling dates, perhaps due to the shorter sampling interval and duration compared to the other locations. At this location, the first date of sampling occurred seven days after treatment application, at the R4 growth stage, full pod [67], while at the other locations, sampling began prior to R4.

In this study, there were significant differences across site-years with regards to frogeye leaf spot severity and yield, which may have been related to weather. Both locations in 2018 received substantially more precipitation during the summer months than the 30-year average. In contrast, the 2019 location received less precipitation compared to 2018 locations. Temperatures were slightly warmer in Lewis 2018. The ideal environmental conditions for frogeye leaf spot development are 27 °C with 72 h of leaf wetness [72].

While both fluxapyroxad + pyraclostrobin and flutriafol + fluoxastrobin have been shown to reduce frogeye leaf spot [70,73], there were differences in their effectiveness in these studies. Severity of frogeye leaf spot when treated with flutriafol + fluoxastrobin averaged 50% less than that of the fluxapyroxad + pyraclostrobin treatment. Similarly, Mengistu et al. [70] reported that the active ingredient flutriafol controlled frogeye leaf spot better than fluxapyroxad + pyraclostrobin and difenconazole + azoxystrobin in some years. QoI fungicide resistance in isolates of C. sojina collected from Iowa has been reported [19]. Mengistu et al. [70] cautioned that additional pressure on the pathogen population to develop DMI resistance exists, when resistance to QoI is likely already present.

At Lewis 2018, yield was protected by application of flutriafol + fluoxastrobin applied at R3. The predominant disease recorded in these soybeans was frogeye leaf spot; however, Septoria brown spot was also present. The yield and frogeye leaf spot severity of the fluxapyroxad + pyraclostrobin treatment did not differ from the non-treated controls. QoI-containing fungicides promoted to have plant health benefits provided varying levels of protection to frogeye leaf spot, while failing to impact yield at low disease levels [20–23,70]. In agreement with Swoboda and Pedersen [74], fungicide application did not benefit yield at low levels of disease. Swoboda and Pedersen [74] did see that total biomass was increased by 10% with a pyraclostrobin treatment, mainly due to the increased stem weight. Phillips et al. [75] also found that pyraclostrobin + fluxapyroxad applied at R3 increased the DM of stems at harvest.

The promoted yield benefit in soybean by prophylactic QoI fungicide application is inconsistent [20–23,53]. There are various explanations for how a QoI fungicide application might improve yield. Amaro et al. [29] concluded that the physiological benefits to healthy plants from QoI fungicide application are due to an increase in net photosynthesis. In our study, canopy cover of both fungicide treatments was greater compared to non-treated controls. However, the increase in net photosynthesis that may have occurred due to the additional canopy cover did not result in yield increases under the low disease conditions in our study. Additionally, in our studies, canopy cover was approximately 85% at R3 in 2018 and 2019 in Kanawha. Since full canopy cover by, or soon after, R1 is needed for maximum yield [76–78], our research suggests that a yield response associated with fungicide application may not occur if full canopy cover is not attained by flowering.

The application of QoI fungicides can increase leaf greenness, chlorophyll content, and delayed senescence [79,80]. No effect of treatment for NDVI values across treatments was detected in any site year under low frogeye leaf spot conditions. Even at the Lewis 2018 location, where frogeye leaf spot severity was significantly different across treatments, no treatment difference was observed for NDVI. Similarly, no differences in SPAD readings among treatments in all site-years were detected. Since the exact reading frame cannot be seen when taking the SPAD value, and SPAD meters used here cover a
very small area (0.06–0.71 cm²) of the leaf, we cannot be certain of the condition of the measured tissue. With no differences between treatments and readings from the active sensors, perhaps the impact of frogeye leaf spot on the leaf is local to the lesion, as opposed to the negative impact the soybean rust fungus, Phakopsora pachyrhizi, has on non-lesion green leaf area [26].

Yield at Kanawha 2018 was 18% less than the yield at Lewis 2018, despite the same cultivar, planting population, and significantly less frogeye leaf spot severity. This was evidenced by the fact that Kanawha 2018 showed nearly half TLAI and GLAI₀ of Lewis 2018 across multiple GDDGs. Differences in LAI values also will affect RUE values. Srinivasan et al. [71] modeled that the optimal LAI of a modern U.S. Midwest soybean cultivar under current CO₂ was 4.2. TLAI values in Lewis 2018 and Kanawha 2019 were above the optimal value identified by Srinivasan et al. [71], but below the optimal value by approximately 43% in Kanawha 2018. It was shown that decreasing their observed LAI of 6.8 to optimum model values would result in an increase of yield [71]. Srinivasan et al. [71] also describes an increase in RUE, attributed to the maintenance of DM, and diminishing APAR caused by reduced LAI. In addition, Srinivasan et al. [71] found that the RUE was 9% greater at the optimal modeled LAI than their observed value, despite the modeled LAI value being approximately 40% less than that observed.

5. Conclusions

RUE is capable of being calculated on a small plot scale with the help of UAV reflectance data. The foliar fungicides used in this study resulted in increased canopy cover compared to non-treated controls. However, this additional canopy cover did not result in a significant yield benefit in two of the three locations. The yield benefit of a fungicide application was seen at one location that had greater levels of frogeye leaf spot severity. No other physiological advantage was detected by application of foliar fungicides at low frogeye leaf spot disease pressure. RUE values, NDVI, and SPAD values, however, failed to capture the protection that fungicides provided to yield against frogeye leaf spot. The level of severity at which frogeye leaf spot begins to impact yield is unknown. Since soybean produces an overabundance of leaves, non-limiting levels of frogeye leaf spot could result in greater RUE estimations if the dry matter produced remains the same. This highlights the issues of understanding how diseases affect soybean yield. Accounting for disease severity when it is not impacting yield may result in an inflated RUE value. Using RUE to estimate impact of disease on yield remotely will be a valuable resource; however, confounding factors (accounting for disease, component collection method) will require additional work to use RUE within certain pathosystems.

Supplementary Materials: The following are available online at www.mdpi.com/article/10.3390/agronomy11091785/s1, Table S1: Precipitation and average temperature during the summer period of June-August and September and the 30-year average for site years in Iowa.

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