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SDHI Fungicides. An Important Step in Maintaining Control of Tan Spot and Septoria leaf blotch Diseases of Wheat

Ioannis Vagelas [1*], Achilleas Sourlas [2] & Chris Cavalaris [1]

[1]Department of Agriculture Crop Production and Rural Development, University of Thessaly, Fytokou Str., GR-38446 Volos, Greece
[2]Agricultural Corporation of Dilofo, GR-41500 Larisa, Greece

[*]Corresponding author: Ioannis Vagelas, 1Department of Agriculture Crop Production and Rural Development, University of Thessaly, Fytokou Str., GR-38446 Volos, Greece

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Abstract

In this study, we propose a hypothesis for protecting wheat plants against foliar fungal infections during the early growth stages by treating seeds with fluxapyroxad which is a succinate dehydrogenase inhibitor (SDHI) class fungicide. Fluxapyroxad (Systiva®) is known, from our previous studies, for its systemic action to protect crops from significant wheat pathogens, such as Pyrenophora tritici-repentis and Zymoseptoria tritici. This study investigates the persistence of Pyrenophora tritici-repentis, the causative agent of tan spot disease, as well as other pathogens like Stagonospora nodorum and Mycosphaerella graminicola found in infected crop residues. We emphasize specifically on the potential of using SDHI class fungicides, as seed treatments to reduce the severity of fungal leaf blotch diseases of wheat (Tan spot, Stagonospora nodorum blotch, and Septoria tritici blotch). Through visual assessments of leaf disease and UAV remote sensing data, our findings confirm the effectiveness of Systiva® seed treatments at a dosage of 1.25 cc per kg of seed. This treatment enhances plant growth and reduces disease pressure during the critical early stages of wheat cultivation.

Keywords: Wheat Diseases, SDHI fungicides, Wheat Growth Stages, Wheat Cultural Practices

Introduction

Important Wheat Diseases in Greece – the Field-Crop Status

Wheat crops in central Greece (mainly in the region of Thessaly), can be affected by various fungal pathogens, which can cause significant yield losses and impact grain quality. Based on our research, the most important fungal wheat pathogens are Pyrenophora tritici-repentis, a fungal pathogen that causes tan spot disease in wheat [1]. Zymoseptoria tritici, syn. Mycosphaerella graminicola caused a disease known as Septoria tritici blotch (STB), and Parastagonospora nodorum previously known as Stagonospora nodorum or Phaeosphaeria nodorum caused a disease known as Septoria nodorum blotch (SNB) [1-3]. Those three leaf blotch diseases of wheat occur regularly over large areas in central Greece and affect significantly the wheat yield and grain quality. Our results showed that those three leaf blotch diseases of wheat, especially Stagonospora nodorum blotch, can lead to yield losses in susceptible wheat cultivars, with reported losses of up to 30%, same results were presented by Singh et al provide evidence that about 20% of wheat is lost due to diseases [1, 4, 5].

In detail, our field trails observation in central Greece (2009 up to date), show that:

Pyrenophora tritici-repentis overwinter under infected residues and produced early symptoms on wheat leaf at seedling growth (GS12 or GS13), due to moderate temperatures (10-15°C), and favorable humid conditions.

Pyrenophora tritici-repentis survives the winter in the form of pseudothecia, which are sexual fruiting bodies, and these pseudothecia release ascospores that can infect new wheat plants at seedling growth when two or more leaves have emerged.

Rotating wheat with non-host crops such as legumes can significantly reduce the amount of pathogen inoculum in the field particularly at seedling growth stages but not near tillering stage of wheat.

Those infected residues (wheat stubble) are crucial aspects of forming early STB disease in wheat. Our observations show that Zymoseptoria tritici, the causative agent of Septoria tritici blotch (STB) in wheat, can also persist in infected crop residues, similar to Pyrenophora tritici-repentis.

Such as Pyrenophora tritici-repentis, Septoria spp. survives in the winter on crop residues, and wheat straw left in the field, producing pycnidiospores and ascospores that can infect plants when six or seven leaves emerge.

Removing or incorporating infected residues into the soil through plowing can help reduce the pathogen's ability to overwinter and produce spores, but not during the tillering stage.

Further, in our research, the metagenomic data show that Pyrenophora tritici-repentis in central Greece, occurs early in the season at high levels of up to 60% due to crop residues (straw) [4]. Zymoseptoria tritici occurs up to 20% during the tillering stage of wheat, and more than 35% during the booting stage of wheat (flag leaf emergence). Finally, our results show that applying fungicides at critical growth stages, such as late tillering and flag leaf emergence, can handle disease severity caused by Pyrenophora tritici-repentis and Zymoseptoria tritici but does not protect wheat plants in late season e.g. anthesis, and protect plant against other pathogens like Stagonospora nodorum, Fusarium graminearum, and other Fusarium species.

Important Wheat Diseases in Greece - Management with SDHI Fungicides

Based on our long-term wheat crop experience conducted in trails or on a large-field-scale as mentioned above we believe that apart from crop rotation, residue management, plant density, excessive nitrogen, and fungicide application, a seed treatment of wheat with succinate dehydrogenase inhibitor (SDHI) class fungicides such as fluxapyroxad (BASF) or sedaxane (Syngenta) will reduce the severity of both tan spot and STB diseases in wheat crop in Greece.

It is well known that Systiva® (BASF), can control all the major foliar as well as seed-borne and soil-borne diseases with excellent control of Septoria tritici blotch (BASF webpage (https://crop-solutions.basf.com.au/products/systiva).

Besides BASF information we presented field trail results showed that Systiva® seed treatments mainly at dose 150 cc (150 cc per 100 kg of wheat seed) significantly reduce the percentage of infected wheat plants caused by foliar fungal pathogens. Our results showed that can protect plant up to growth stages GS23-25 and GS30-31. We concluded the effectiveness of Systiva® (fluxapyroxas) seed treatments by visual leaf disease assessments and by UAV remote sensing data. Additionally, we showed that metagenomics analyses of microbial communities revealed that Systiva® significantly decreased the infection by P. tritici-repentis and Z. tritici [4].

Based on the information above, we believe that, in central Greece the foliar wheat fungi such as Pyrenophora tritici-repentis and Zymoseptoria tritici present the first risk in the wheat crop for early development of foliar diseases due to straw (stubble) residues. dressing wheat seeds with an SDHI class fungicide will prevent early crop infection from fungus that overwinters on stubble.

In conclusion, the primary focus of this article is to develop a protocol (-?) for preventing early crop infection by key wheat pathogens that overwinter on stubble. This involves treating wheat seeds with an SDHI class fungicide, such as fluxapyroxad.

Key Wheat Pathogens - Fluxapyroxad

The importance of Tan Spot, STB and SNB in wheat: Pyrenophora tritici-repentis is a necrotrophic fungus that causes tan spot disease in wheat, leading to significant crop losses [6]. This pathogen is known for its ability to produce host-selective toxins, such as Ptr ToxA and Ptr ToxB, which play a crucial role in the interaction between the fungus and wheat plants [7, 8]. Pyrenophora tritici-repentis is a homothallic fungus capable of both sexual and asexual reproduction, with sexual reproduction occurring on wheat stubble between crops and asexual reproduction during the growing season on the wheat crop highlighting its ability to persist in the environment [9]. Apart to biological studies, research has also focused on the genetic aspects of the interactions between wheat and Pyrenophora tritici-repentis, emphasizing the importance of understanding the genetics underlying the disease development and host-pathogen interactions [10, 11]. Additionally, studies have highlighted the importance of agronomic practices in controlling wheat leaf diseases, with tan spot caused by Pyrenophora tritici-repentis being a dominant disease in certain years [12].

On the other hand, Zymoseptoria tritici is another significant pathogen affecting wheat, causing Septoria tritici blotch as mentioned above [1, 4]. This pathogen, in central Greece, along with Pyrenophora tritici-repentis and Parastagonospora nodorum, forms the foliar disease complex of wheat, with each pathogen having specific strategies to cause disease, same results were presented by Kariyawasam et al and by Friesen & Faris [1, 4, 13, 14]. Additionally, the bibliography shows that Zymoseptoria tritici has been reported to dominate in some regions, with its severity exceeding that of tan spot caused by Pyrenophora tritici-repentis [12]. Moreover, research has shown that fungicides have complex effects on the wheat phyllosphere mycobiome, with Zymoseptoria tritici being one of the fungi that can reinfect wheat after early fungicide applications [15].

Septoria nodorum blotch (SNB) primarily affects the lower leaves of the wheat plant, causing dark brown round or lens-shaped spots that coalesce and develop black pycnidia as the lesions mature [16]. The disease can also infect the glumes of wheat, causing dark brown patch-like burn marks that later become purple/brown. SNB can cause significant yield losses in wheat-growing regions, with annual losses of up to 20% possible when susceptible or very susceptible varieties are grown. The disease is favored by warm and humid conditions, and its severity can vary from season to season and between locations. Management of Septoria nodorum blotch involves a combination of cultural practices, such as crop rotation and residue management. Seedborne infection of Stagonospora nodorum can contribute to the initiation of SNB epidemics in winter wheat [16, 17].

Fluxapyroxad - its broad-spectrum antifungal activity: Fluxapyroxad is a pyrazole carboxamide fungicide widely used for its broad-spectrum antifungal activity, high efficiency, and low toxicity registered for uses on a wide range of crops such as cereals, legumes, stone fruits etc [18, 19]. It is structurally related to boscalid but exhibited higher antifungal activity against most important plant pathogenic fungi than boscalid and is showed as a novel highly efficient fungicides pyrazole amide fungicide belongs to 3-(difluoromethyl)-1-methyl-1H-pyrazole-4-acyl group [20]. Fluxapyroxad (BASF), and other recent commercial pyrazole amide derivative of pyrazole-4-carboxylic acid fungicides such as benzovindiflupyr or sedaxane (Syngenta), was used to protect crops from fungal diseases by interfering with key fungal life functions such as spore germination, germ tube growth, appressoria formation, and mycelium growth [21-23]. Fluxapyroxad acts as a succinate dehydrogenase inhibitor (SDHI) and its mechanism of action involves inhibiting the SDH enzyme, disrupting the electron transport in the complex II-oxidation of succinic acid to fumarate in fungi, and disrupting cellular energy metabolism [24-27]. Furthermore, the fungicidal activities of fluxapyroxad, along with other succinate dehydrogenase inhibitors (SDHIs), have been evaluated, and it has been found to inhibit succinate dehydrogenase in complex II of the mitochondrial respiratory chain, leading to the inhibition of mycelial growth within the fungus target species [28].

Apart the mode of action, the toxic effects and potential mechanisms of fluxapyroxad have been studied in zebrafish embryos, indicating its potential impact on aquatic organisms [29, 30]. Additionally, the impact of fluxapyroxad on the microbial community structure and functional diversity in silty-loam soil has been assessed, indicating its potential ecological implications [31]. Research showed that, high fluxapyroxad input (75 mg fluxapyroxad kg^{-1} soil dry weight) increased the microbial stress level [32]. Further, the degradation half-lives of fluxapyroxad in soil were greater than 157 days, demonstrating that it is a persistent pesticide that can potentially contaminate aquatic? and terrestrial ecosystems for long periods of time [33].

Materials and Methods

Study Field

A 0.73 ha field cultivated constantly with winter wheat (variety Simeto) using minimum tillage practices was selected in the region of Larissa, central Greece (22,5838 39,4646, EPSG 4326). The field was divided into two plots, a 0.35 plot that was seeded with untreated seed and served as a control and a 0.38 ha plot where the seed was pre-treated with 1,25 cc of Systiva® (33,3 FS fluxapyroxad) per kg of wheat before sowing. Visual assessments of leaf blotch and tan spot fungicide diseases were carried out during the 2022–2023 growing period focusing on the initial growing stages of the crop, from seedling to tillering and to stem elongation (Zadoks growth scale GS13–GS32 or BBCH growth stage BBCH13-BBCH32). During that period, the infestations come from the inoculum that survives in the previous crop residues (wheat stubble).

Sampling and Visual Disease Assessment

Tan spot and Septoria blotch diseases of wheat were visually evaluated in fields based on the plant tissue symptoms affected by the pathogens Pyrenophora tritici-repentis and Septoria spp. Estimates of the percentage of disease (intensity of symptoms) were made by determining the area of blotched, yellowing, and dead leaves on the affected plants by examining samples of at 8–10 plants randomly selected from several spots and estimating the average overall score. The plants were collected from two adjacent rows while walking along the field at a periodic sampling distance of 3m. That way, a total of 24 sampling spots were evaluated from each plot. The sampling spots were also pinpointed with a hand-held GPS (Figure 3d). A standard area diagram (SAD) was used for scoring the severity of the symptoms. The scores 1, 5, 10, 15, 20, 25, 30, 50, and 75 were used to address the percentage of leaf area covered by the disease. SAD diagrams have been broadly used to aid the validation of the severity of crop diseases. According to BBCH-scale or Zadoks growth scale, disease was scored at Seedling growth stages BBCH 13 or GS13 (Zadoks growth scale), and BBCH or GS17 (Zadoks growth scale), (Three, and seven leaves emerged), at Tillering stages BBCH 23 (Main stem and three tillers), BBCH 27 (Main stem and seven tillers) and Stem elongation stage BBCH 32 (Second node detectable).

Remote Sensing Assessment

An aerial survey of the field was performed at the latest GS32 stage of this study using a Parrot Sequoia multispectral sensor on board a Parrot Bluegrass UAV. A flight pattern was designed with the Pix4DCapture mobile app. Multispectral images were captured at a 45 m above-ground level with a forward image overlap of 80% and a side overlap of 70%. A calibration panel provided by Parrot was used to calibrate the multispectral images. Image stitching was accomplished with the Pix4DMapper software. The orthomosaic was imported into QGIS where two common vegetation indices, NDVI and NDRE were estimated [34]. These indices were expected to depict differences in plant vigor and chlorophyll content associated with the disease impacts. A grid of 3x3 m were laid above the two plots (Figure 3d) in the GIS and the two vegetation index layers were sampled at the mean value on each grid cell. The sampled values were used for the statistical comparison of the treatments.

Statistics

An analysis of variance was performed on all datasets using the JASP 0.18.3 open-source software suite (https://jasp-stats.org/). Further, we used Raincloud Plots in JASP to draw meaningful conclusions from a dataset presented in Figures 1, 2 and 4.

Results

Visual Disease Assessment and Wheat Yield

The Analysis of Variance (ANOVA) and the p-values, indicate a significant difference between BBCH growth –scale or Zadoks Growth Stage ($p<0.001$), treatments ($p<0.001$), and BBCH growth –scale vs treatments ($p<0.001$), supporting the hypothesis that Systiva® (fluxapyroxad) seed treatment application protects plants from foliar diseases wheat (Septoria blotch and tan spot diseases). The data with emphasis to interaction BBCH growth –scale vs treatments with $p<0.001$, indicate that blotch diseases of wheat (Septoria diseases and tan spot) were affected by the Systiva® seed treatment application.

The significant results of the Systiva® (fluxapyroxad) seed treatment application compared to the untreated plants (Control) were presented with the post-hoc comparison test. The Tukey post-hoc comparison test showed that Systiva® (fluxapyroxad) protects plants significantly up to the BBCH-27 and BBCH-32 growth stages with a post hoc comparison between treatment ptuckey<0.001. The differences between BBCH Growth Stages and Treatments, such as Systiva® seed treatment application or control, were presented with disease score Figures, Figure 1a for Systiva® seed treatment application, and Figure 1b for Control. From these figures, it is evident that the Systiva® seed treatment application protects plants from foliar diseases up to BBCH-27 and BBCJ-32 growth stages. Using a Raincloud analysis, we presented with a graph (Figure 2) the impact of the Systiva® (fluxapyroxad) application wheat growth stages (BBCH-32 growth stage) compared to untreated Control treatment.

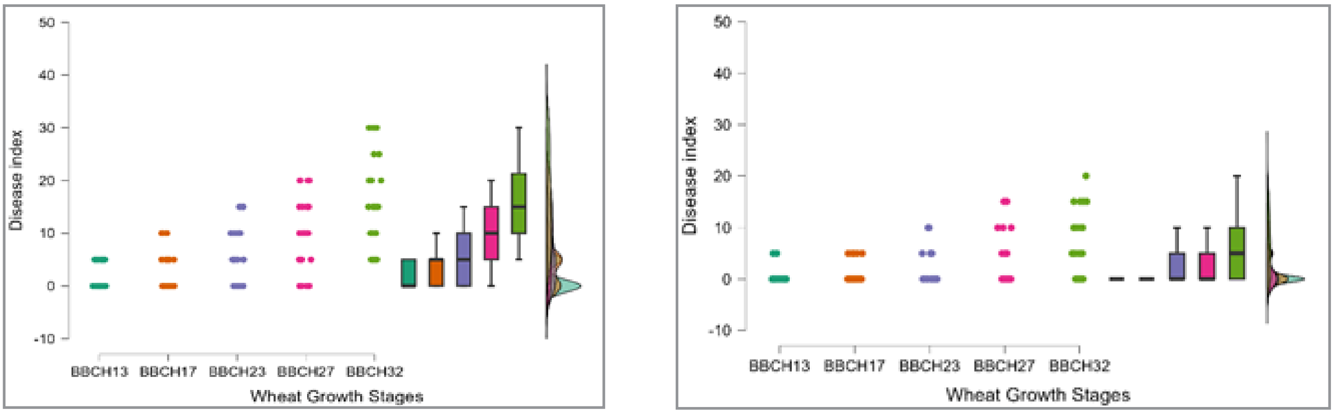

Figure 1: Raincloud results for Disease score and wheat growth stages (BBCH growth stage) on Control treatment (a) and on Systiva® seed treatment (b).

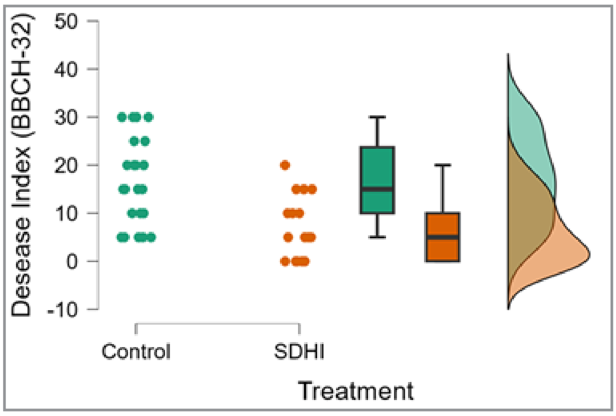

Figure 2: A Raincloud result for Disease score at BBCH-32 growth stage of wheat treated with or without (Control) Systiva® (fluxapyroxad) seed treatment fungicide.

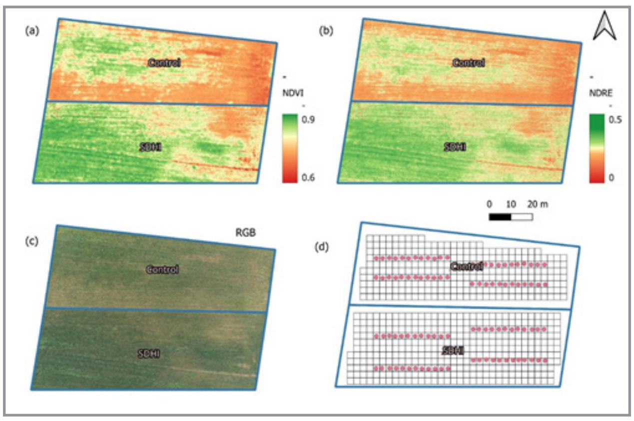

Figure 3: a) NDVI, b) NDRE and c) RGB images captured with a UAV at 11/3/2023. d) 3x3 grid layer for multiple VI sampling used on the statistical analysis. Dots depict the field sampling spots for the visual assessments.

Remote Sensing Results

The analysis of variance of the grid sampled data from the two vegetation indices (Table 4) indicated that there was a significant impact of the seed treatment on the two VIs. The post-hoc tests in Table 5 and the rainclouds in Figure 4 reveal that the SDHI seed treated plots presented a higher NDVI and remarkable higher NDRE. These are also visually verified in Figure 3. In Figures 3a and 3b it is worth mentioning the abrupt change of the two VIs at the border line separating the two plots. This is another evidence of the effective protection provided by the SDHI component. The differences were also eye conceivable as revealed by the RGB image in Figure 3c.

Table 1: Analysis of variance results.

Cases	Sum of Squares	df	Mean Square	F	p
NDVI					
treat	0.742	1	0.742	227.357	< .001
Residuals	2.048	628	0.003	0	0
NDRE					
treat	0.654	1	0.654	453.625	< .001
Residuals	0.905	628	0.001	0	0

Note: Type III Sum of Squares

Table 2: Descriptive statistics

treat	N	Mean	SD	SE	Coefficient of variation
NDVI					
Control	276	0.756	0.068	0.004	0.089
SDHI	354	0.825	0.047	0.003	0.057
NDRE					
Control	276	0.226	0.036	0.002	0.161
SDHI	354	0.291	0.039	0.002	0.134

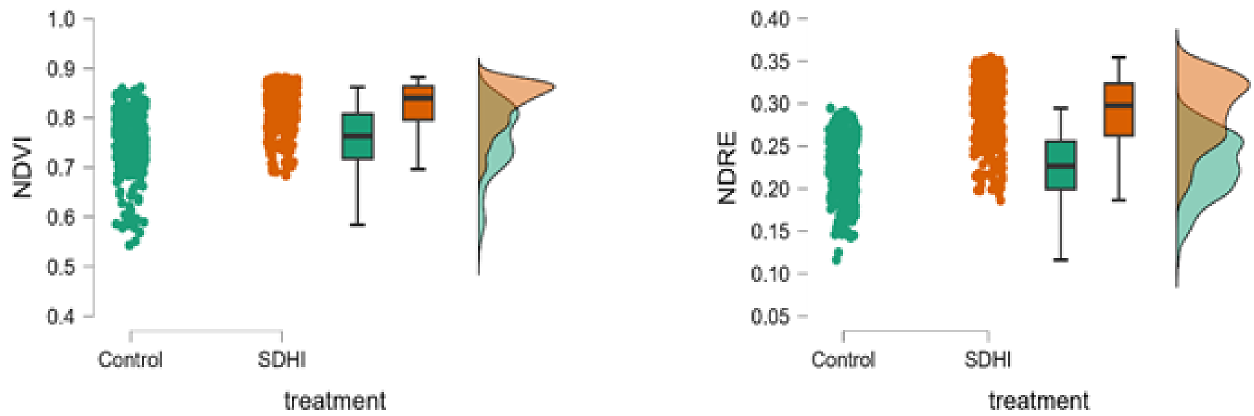

Figure 4: Raincloud results for NDVI (left) and NDRE (right).

Discussion

In our previous report, we presented data that fluxapyroxad, the active ingredient in the Systiva® BASF seed treatment fungicide has a significant impact on diseases such as Septoria tritici blotch and tan spot in wheat crops [4]. Here we provide evidence that even a dose (1,25 cc per kg of seed) of Systiva® seed treatment fungicide reduced significantly the infection rate of Septoria tritici blotch and tan spot diseases. It is well known that Septoria tritici blotch, Stagonospora nodorum blotch, and tan spot have the potential to cause significant grain yield and quality losses in wheat, especially under favorable environmental conditions during flag flag-emergent wheat stage. We believe that those main foliar diseases pose a significant threat to wheat crops in Greece due to climate change (warmer February), and cultural practices (no-till, minimum till), associated with those pathogens' overwinter inoculum.

For foliar fungicide applications, azoles, such as prothioconazole, tebuconazole, and epoxiconazole, have been widely used to control Septoria tritici blotch (STB) [35, 36]. However, resistance has developed due to mutations in the CYP51 gene, leading to reduced sensitivity so newer azoles like mefentrifluconazole show promise due to their unique structure, which not yet been compromised by resistance [37, 38, 35]. Quinone outside inhibitors (QoIs) such as azoxystrobin have seen widespread resistance, particularly due to the G143A mutation, which remains prevalent even without fungicide pressure [39-41]. Moreover, for foliar fungicide applications, succinate dehydrogenase inhibitors (SDHIs) like benzovindiflupyr and fluxapyroxad are also used, but resistance has emerged, necessitating careful management [42-44]. Further research shows that combining fungicides with different modes of action, such as azoles and SDHIs, can slow resistance development. However, while beneficial for azoles, this strategy may not always be effective for SDHIs [43, 44]. Overall, as foliar fungicides are still commonly relied upon for disease control we believe that mixtures of DMIs and SDHIs have shown improved control and yield benefits compared to DMIs alone [45].

The application of foliar fungicides, specifically demethylation inhibitors (DMIs) such as pyraclostrobin, propiconazole, and a combination of protioconazole and trifloxystrobin, has proven effective in reducing lesion expansion and managing tan spot in wheat [46]. However, resistance to fungicides, particularly strobilurins, has been observed. This resistance is associated with the G143A mutation in the pathogen population, which leads to decreased efficacy of these treatments [47]. Research has shown that the presence of wheat straw on the soil surface, particularly in no-tillage systems, is linked to increased severity of tan spot. This is due to the retention of infected straw, which serves as a source of inoculum [46, 48, 49]. Additionally, this straw can harbor the inoculum for other diseases such as wheat leaf blotches, including Pyrenophora tritici-repentis and Septoria leaf blotch [50]. Our unpublished observations indicate that implementing crop rotation or tilling the straw can effectively manage tan spot by disrupting the disease cycle and reducing disease severity by eliminating primary sources of infection. Based on this, we believe that proper management of wheat straw and crop residues is crucial for minimizing disease spread and maintaining healthy wheat crops.

The bio-stimulant effects of seed-applied sedaxane ® an SDHI fungicide used as a seed treatment, have shown both protective and growth-enhancement effects in crops like maize and wheat [51]. Research showed that sedaxane-treated seeds exhibit improved root development and increased nitrogen metabolism, which can enhance early plant growth and potentially improve resistance to biotic and abiotic stresses [51]. In the same way, in our research, we show that applying fluxapyroxad as a seed treatment fungicide reduces the disease severity and improves wheat growth effects. Field studies have shown that SDHI foliar applications can reduce STB severity and ascospore release, potentially lowering disease pressure but the increasing frequency of SDHI-resistant isolates in Europe highlights the need for ongoing monitoring and implementation of anti-resistance strategies [45, 52].

Based on the information provided, we propose an alternative strategy for managing foliar diseases in wheat by limiting the number of foliar fungicide applications and using SDHIs as a seed treatment. Our evidence shows that seeds treated with Systiva® can enhance plant growth in wheat crops and reduce disease pressure from key foliar pathogens during the early growth stages. Additionally, we propose, for a future experiment, a more sustainable approach to managing these important foliar diseases by combining SDHI seed treatment fungicides with a foliar application of other fungicides, specifically DMIs, applied at the flag leaf stage of wheat.

Conclusion

The present study highlights the potential of using succinate dehydrogenase inhibitor (SDHI) class fungicides such as fluxapyroxad as a seed treatment to control tan spot and septoria leaf blotch diseases of wheat. The study provides evidence that even at a dose of 1.25 cc of Systiva® seed treatment per kg of wheat seed, the fungicide can significantly reduce the infection rate of tan spot and Septoria blotch diseases. Our data indicates that two primary foliar diseases (tan spot and Septoria blotch diseases), affecting wheat crops in Greece are becoming more severe due to climate change and the spread of sustainable farming practices such as no-till and minimum tillage. In this article, we provide evidence that protecting crops from overwintering inoculum is essential to prevent early infections, which can lead to significant yield losses.

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