Revista GEAMA – Ciências Ambientais e Biotecnologia
Scientific Journal of Environmental Sciences and Biotechnology
ISNN: 2447-0740

9
2

Wheat photosynthetic parameters influenced by the use of seaweed extract and fungicide

Parâmetros fotossintéticos do trigo influenciados pelo uso de extrato de alga e fungicida

Rafael Dal Bosco Ducatti[1], João Américo Wordell-Filho[2], Siumar Pedro Tironi[3], Sérgio Miguel Mazaro[1]

[1] Federal Technological University of Paraná, Paraná, Brazil
[2] Agricultural Research and Rural Extension Company of Santa Catarina, Santa Catarina, Brazil
[3] Federal University of the Southern Frontier, Santa Catarina, Brazil

Contato: rducatti@alunos.utfpr.edu.br

Keywords
Algomel PUSH
plant elicitation
fluorometer
Solieria chordalis
electron transfer

ABSTRACT
Fungicides and seaweed extracts are commonly employed in agricultural fields, yet their impact on the photosynthetic process in plants remains poorly understood. This study aimed to address this knowledge gap by conducting a controlled experiment, fully isolating the treatments, and investigating the effects of a fungicide containing Trifloxystrobin + Prothioconazole (applied three times), a seaweed extract derived from Solieria chordalis (applied once), and their combination. The study employed four treatments with ten replicates each, using the TBio Audaz® cultivar. Parameters related to chlorophyll excitation were assessed using a portable fluorometer. The findings revealed significant effects on nearly all analyzed parameters. Application of the fungicide resulted in a significant reduction in the flow of electrons to photosystem I (PSI) (φRo) and induced the formation of a larger (ABS/RC) and less efficient (PIABS) antenna system. Moreover, the combined use of the seaweed extract with the fungicide exacerbated the detrimental effects of the pesticide.

Palavras-Chave
Algomel PUSH
eliciação vegetal
fluorômetro
Solieria chordalis
transferência de elétrons

RESUMO
Fungicidas e extratos de algas são amplamente utilizados em campos agrícolas. Entretanto, pouco se sabe sobre o efeito que esses dois componentes exercem sobre o processo fotossintético das plantas. Dessa forma, esse estudo buscou, em ambiente totalmente controlado e com isolamento total dos tratamentos, investigar o efeito de um fungicida a base de Trifloxistrobina + Prothioconazole (três aplicações), de um extrato de algas a base de Solieria chordalis (uma aplicação) e da combinação entre eles. Um total de quatro tratamentos e dez repetições foi utilizado. A cultivar utilizada foi a TBio Audaz®. Avaliou-se parâmetros relacionados a excitação da clorofila com a utilização de um fluorometro portátil. Efeitos significativos foram observados para praticamente todos os parâmetros analisados no trabalho. A aplicação de fungicida reduziu significativamente o fluxo de elétrons até o fotossistema I (PSI) (φRo), da mesma forma que induziu a formação de um maior (ABS/RC) e menos eficiente (PIABS) sistema antena. O uso combinado do extrato de algas junto ao fungicida agravou os efeitos deletérios do defensivo agrícola.

Informações do artigo
Recebido: 21 de junho, 2023
Aceito: 05 de agosto, 2023
Publicado: 23 de agosto, 2023

Introduction

Wheat represents an important staple food for human and animal nutrition (DUCATTI et al., 2022a,b). Nevertheless, this crop is susceptible to a wide range of biotic and abiotic stresses that can hamper its growth and reduce yields (FIGUEROA et al., 2018).

Among the challenges faced by wheat, diseases have a significant impact on crop yield and quality. One such disease is Fusarium Head Blight (FHB), caused by *Fusarium graminearum*, which can lead to yield reductions ranging from 18.6% to 39.9% in Brazilian fields (REIS et al., 2016). Furthermore, the presence of the mycotoxin deoxynivalenol due to FHB can result in complete losses of kernel quality, reaching up to 100% (DUVEILLER et al., 2016).

To mitigate the incidence of these diseases, the use of fungicides has become a common practice in wheat cultivation. However, over time, fungicides have been found to lose their effectiveness due to various factors, including the excessive use of fungicides without proper rotation of modes of action (CORKLEY et al., 2022).

When fungicides become less effective, their doses are usually raised to maintain an acceptable rate of control of the main diseases affecting the crop (VAN DEN BERG et al., 2016). Unfortunately, this practice can lead to environmental problems (ZUBROD et al., 2019) and may also have negative effects on the crops themselves (LIU et al., 2021). According to some research, fungicide applications can cause adverse effects on plants, such as phytotoxicity (ZUNTINI et al., 2019), decreased chlorophyll content, and photosynthetic issues (SHAHIB & KHAN, 2018; LIU et al., 2021, DUCATTI et al., 2023), as well as reduced pollen grain viability (JUNQUEIRA et al., 2017). On the other hand, seaweed extracts have demonstrated their potential in alleviating stresses caused by both biotic and abiotic conditions (SHUKLA et al., 2021) by enhancing the primary and secondary metabolisms in plants (DUCATTI et al., 2022a).

However, when plants are stimulated, they produce a diverse range of volatile and non-volatile specialized compounds to prime themselves against potential stresses (LORETO & D'AURIA, 2022). Approximately twenty percent of the CO_2 absorbed during photosynthesis is released back into the atmosphere as volatile organic compounds (VOCs) (BALDWIN, 2010). These VOCs have the ability to prime neighboring organisms (MARKOVIC et al., 2019) and can mask the results obtained when working with eliciting compounds (DUCATTI, 2023). Therefore, when testing new eliciting molecules or products as potential solutions to agronomic problems, it is crucial to fully isolate treatments (DUCATTI, 2023).

Keeping these considerations in mind, this study was conducted under a total controlled condition to evaluate the negative impacts of a fungicide based on Trifloxystrobine + Prothioconazole on the photosynthetic parameters (photosynthetic performance) of wheat plants. Additionally, the study explored how a liquid seaweed extract produced from the red seaweed *Solieria chordalis* could mitigate the negative effects caused by the fungicide.

Material and Methods

The research was conducted as a randomized completely design with adaptations. A total of four treatments and fifiteen replicates were used. Wheat plants were grown in 20-L vases filled with a substrate produced with virgin soil and a commercial organic substrate at a ratio of 9:1, respectively. Each vase received twelve plants of the cultivar TBio Audaz®. Table 1 presents the physicochemical characteristics of the substrate used to fill-in the vases.

Table 1. Physicochemical characteristics of the substrate

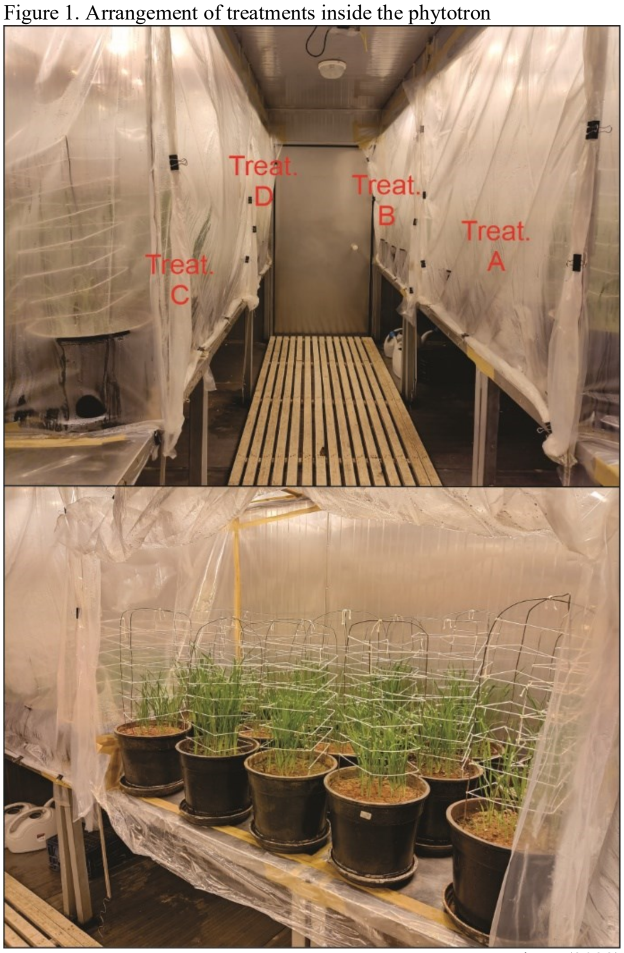
Clay	pH	P	K	OM
(% w/v)	in H_2O	(mg/dm³)	(mg/dm³)	(% w/v)
28,0	6,5	155,9	172,0	4,0
Al	Ca	Mg	Al + H	CEC pH7,0
($cmol_c/dm^3$)	($cmol_c/dm^3$)	($cmol_c/dm^3$)	($cmol_c/dm^3$)	($cmol_c/dm^3$)
0,0	12,4	6,1	1,74	20,66

Legend: OM: Organic matter. CEC: Cation exchange capacity

Source: Authors (2023)

Plants were grown in mini chambers placed inside a phytotron (Figure 1). These mini chambers were built using greenhouse plastic film of 150 microns. Each mini chamber had the dimensions of 2.50 x 1.00 meters and accommodate all the vases for each treatment (15 vases). These mini chambers were built to block gas (VOCs) exchange between treatments (Figure 1).

Figure 1. Arrangement of treatments inside the phytotron



Source: Authors (2023)

The growing conditions for all treatments were as follows: Photosynthetic Active Radiation: 700 µmol $s^{-1}.m^{-2}$; Temperature: 32 ± 4 °C during light exposure and 17 ± 2 °C during dark exposure; Photoperiod: 12 hours of light and 12 hours of darkness; Air moisture: 85 ± 5%; Soil moisture maintained at field capacity.

At 25, 41 and 56 days after wheat emergence, each vase received a nutrient supplementation (0.1 $L.vase^{-1}$) of a medium composed of 1.5% w/w KCl, 1.5% w/w $(NH_4)_2SO_4$, 0.05% w/w H_3BO_3, and 0.05% w/w Na_2MoO_4.

The treatments evaluated in the experiment were as follows:

- Treatment A: Untreated control.
- Treatment B: Three applications of the fungicide (Trifloxystrobin 150 $g.L^{-1}$ and Prothioconazol 175 $g.L^{-1}$) – 0.5 $L.ha^{-1}$ at the beginning of the tillering (GS20), booting (GS40), and flowering stages (GS60) (ZADOKS et al., 1974).
- Treatment C: Single application of the Red Seaweed Biostimulant (RSB) (Algomel PUSH® - *Solieria chordalis*) – 1.0 $L.ha^{-1}$ at the beginning of the tillering stage (GS20).
- Treatment D: Combination of Treatment B and Treatment C.

All foliar applications were performed with a portable CO_2 sprayer calibrated to deliver a volume of 200 $L.ha^{-1}$ of the medium prepared.

For the measurements of the physiological parameters involving electron transfer in plants, a portable fluorometer (FluorPen ASCII – Photon Systems Instruments (PSI), Czech Republic) was used. Measurements were performed at 1, 8, 15, 22, 36, 50 and 64 days after the first application of fungicide/seaweed extract. Before each assessment, plants were adapted to dark conditions for 15 minutes. A total of ten measurements were carried out for each treatment in each day of assessment. A period of 5 minutes was allowed between the readings from one treatment to the other. This time was allowed for the dissipation of VOCs and the block of possible interferences from one treatment over the others.

The parameters analyzed with the fluorometer were:

- φ_{Po}: maximum quantum yield of PSII
- ψ_o: probability with which a PSII trapped electron is transferred from Q_a to Q_b
- φ_{Eo}: quantum yield of the electron transport flux from Q_a to Q_b
- φ_{Ro}: quantum yield of the electron transport flux until the PSI electron acceptors
- PI_{ABS}: performance index for energy conservation from photons absorbed by PSII antenna, to the reduction of Q_b
- ABS/RC: absorption flux per center of reaction (apparent size of the antenna system)
- T_{Ro}/RC: trapped energy flux per center of reaction
- E_{To}/RC: electron transport flux per center of reaction
- D_{io}/RC: dissipated energy flux per center of reaction

The data of all measurements were combined, and the mean was used to perform the statistical analyses between the different treatments.

All statistical analyses were performed through R software. All data were first submitted to the analysis of outliers with their elimination. Thereafter, data were checked for their normality (Shapiro-Wilk). When data presented a normal distribution, they were analyzed through Analysis of Variance (5%) and their means, when significant, were compared using Tukey's HSD (5%). Data that showed a non-normal distribution were transformed. The transformations are described in each table/figure.

Results and Discussions

The analysis of variance indicated that all parameters, except for the electron transport flux per center of reaction (ETo/RC) (Table 2), exhibited significant differences among the treatments.

After a period of at least 15 minutes of adaptation to darkness, chlorophyll *a* displays fluorescence characteristics known as the Kautsky effect when it is illuminated and excited for specific durations (milliseconds). These fluorescence characteristics can be measured using specialized instruments and are represented by OJIP curves. In the OJIP curve, the "O" represents the original values, which indicate the minimum fluorescence yield. The "J" point signifies the accumulation of reduced Quinone A (Qa-), while the "I" point represents the partial reduction of the Qb- pool accompanied by the accumulation of Qa-. The peak of fluorescence is denoted by the letter "P" (STIRBET & GOVINDJEE, 2011; MARTINAZZO, 2011).

The analysis of the OJIP curve parameters allows for the calculation of the energy fluxes, in the form of electrons generated through water photolysis and chlorophyll excitation, between the photosystems (PSII and PSI) for ATP and NADPH production (STIRBET & GOVINDJEE, 2011; TAIZ et al., 2017).

Based on the fluorometer results, "Treatment B", "Treatment C", and the combination of the fungicide and the seaweed extract (Treatment D) exhibited the highest maximum quantum yield of PSII (φPo) (Figure 2a). This indicates that the seaweed extract alone was effective in enhancing the maximum quantum yield of PSII when compared to the untreated control (Treatment A).

Table 2. Analysis of variance for the physiological parameters analyzed through the fluorometer

Parameter	Factor	DF	SQ	MS	F	*p* value
φ_{Po}	Treat.	3	0,0043	0,00143	4,90	0,0025*
	Error	273	0,0798	0,00029		
	Total	276	0,0841			
ψ_o	Treat.	3	0,0401	0,01338	3,66	0,0129*
	Error	273	0,9976	0,00365		
	Total	276	1,0378			
φ_{Eo}	Treat.	3	0,0330	0,01101	3,84	0,0101*
	Error	273	0,7814	0,00286		
	Total	276	0,8144			
φ_{Ro}	Treat.	3	0,0428	0,01428	13,08	<0,0001*
	Error	273	0,2979	0,00109		
	Total	276	0,3408			
PI_{ABS}	Treat.	3	11,627	3,87587	7,54	<0,0001*
	Error	273	140,16	0,51341		
	Total	276	151,78			
ABS/RC	Treat.	3	1,0508	0,35028	11,74	<0,0001*
	Error	273	8,1418	0,02982		
	Total	276	9,1926			
TR_o/RC	Treat.	3	0,4411	0,14704	11,48	<0,0001*
	Error	273	3,4943	0,01280		
	Total	276	3,9354			
ET_o/RC	Treat.	3	0,0719	0,02397	1,87	0,1343
	Error	273	3,4938	0,01279		
	Total	276	3,5657			
Di_o/RC	Treat.	3	0,1353	0,04510	8,63	<0,0001*
	Error	273	1,4261	0,00522		
	Total	276	1,5614			

Legenda: *significant for the F test (5%); DF: degrees of freedom; SQ: sum of squares; MS: mean square.

Source: Authors (2023)

Additionally, the treatment with the seaweed extract (Treatment C) displayed the highest quantum yield of electron transport flux until the PSI electron acceptors (φRo). This indicates a positive impact on photosynthesis (Figure 2d). Interestingly, the treatments involving fungicides (Treatments B and D) showed the lowest φRo (Figure 2d). These findings support the notion that fungicides can have a detrimental effect on the electron transport flux from PSII to PSI (SHAHIB & KHAN, 2018).

Additionally, it can be observed that the apparent size of the antenna system (ABS/RC - Figure 2f) in Treatment B ("Only fungicide (3x)") and Treatment C ("Seaweed extract (1x)") is relatively smaller compared to the other treatments. This suggests that the plants may be compensating for a weaker quantum yield of PSII (φ_{Po}) by increasing the size of the antenna system. By expanding the antenna system to capture more photons from light, more electrons can reach the photosystems' reaction centers.

Looking at Figure 2e, which indicates the efficiency in conserving the photons captured by the antenna system, it is clear that Treatment B ("Only fungicide (3x)") and Treatment C ("Seaweed extract (1x)") have more efficient antenna systems. Their higher efficiency in conserving captured photons results in smaller energy fluxes per chlorophyll center of reaction, as shown in Figure 2g (T_{Ro}/RC) and 1h (D_{io}/RC).

According to Feng et al. (2020), strobirulins, such as Trifloxystrobin, have a mode of action that blocks the transport of electrons in the cell respiratory chain. Although the respiratory chain primarily occurs in mitochondria, strobirulins might also affect the transport of electrons from PSII to PSI, thereby hindering ATP and NADPH production during photosynthesis. When electron transport is blocked, an overaccumulation of electrons occurs in the plant's antenna system. This leads to the formation of highly charged and excited chlorophylls called chlorophyll triplets, which promote the formation of Reactive Oxygen Species (ROS) by transferring electrons to oxygen. If carotenoids, which usually absorb and consume these electrons as a defense mechanism in plants, are unable to do so, these ROS can cause chlorophyll degradation, oxidative bursts, and cell peroxidation (VINKLÁREK et al., 2018).

This explains why fungicides induce a higher activity of antioxidant enzymes such as catalase, superoxide dismutase, ascorbate peroxidase, among others (LIU et al., 2021). Liu et al. (2021) demonstrated that the use of difenoconazole-based fungicides affected the development of wheat plants due to the oxidative burst caused by the fungicides (ROS production) and subsequently, the reduction of chlorophyll content caused by ROS.

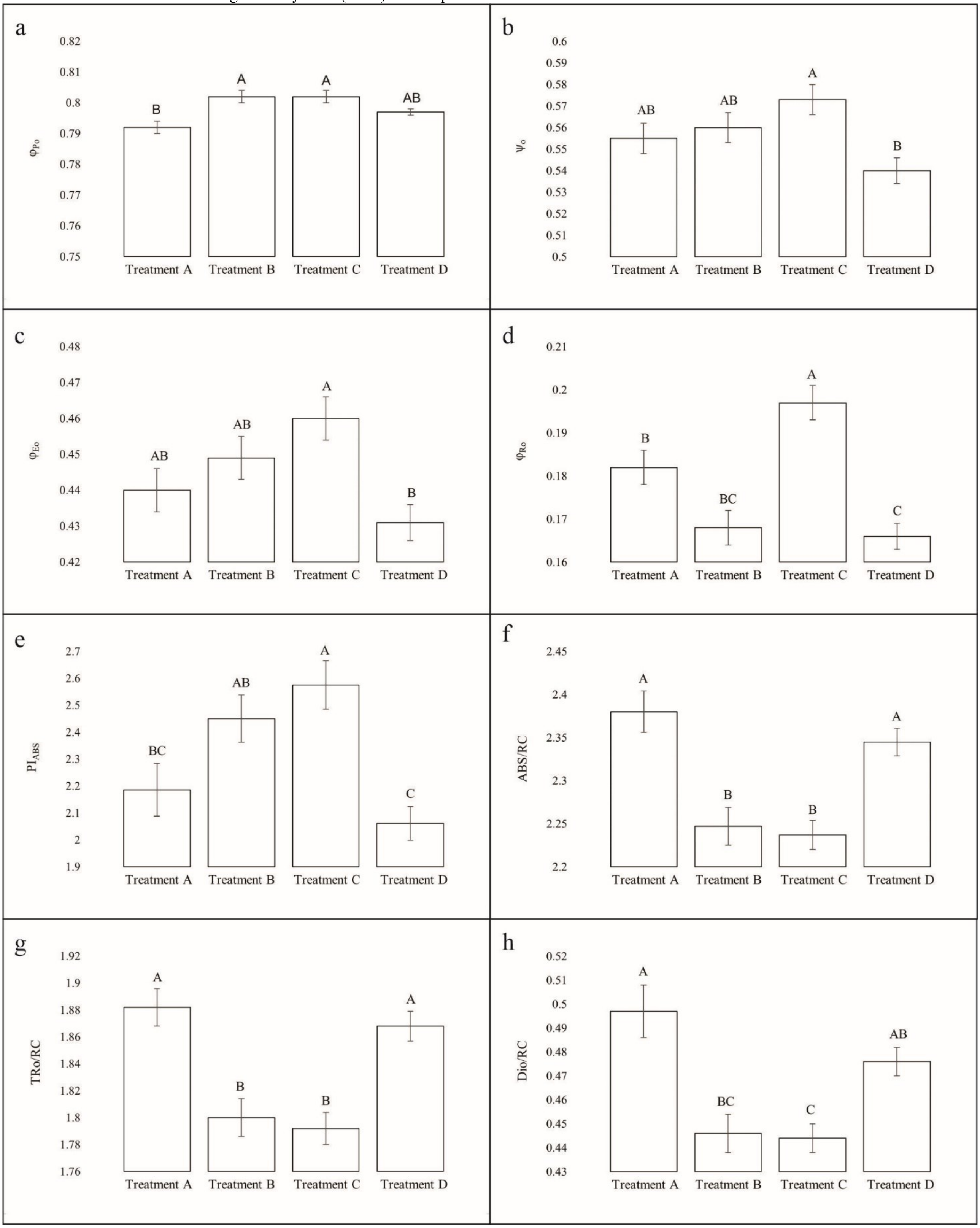
In the present study, the fungicide used is based on Trifloxystrobin + Prothioconazole, which may have also affected chlorophyll synthesis and photosynthetic rates in wheat plants.

The combined application of the seaweed extract and the fungicide resulted in a further decrease in the electron transport flux. This indicates that the combination treatment (Treatment D) had a more pronounced negative impact on the electron transport flux when compared to the sole use of the fungicide or the seaweed extract.

It is important to note that fungicides, in general, can have detrimental effects on various aspects of plant physiology, including photosynthesis and overall growth parameters (JUNQUEIRA et al., 2017; SHAHIB & KHAN, 2018; ZUNTINI et al., 2019; LIU et al., 2021). Despite these potential drawbacks, the use of fungicides is still recommended due to their significant benefits in protecting plants against pathogen attacks. The advantages of disease control usually outweigh the potential negative impacts on plant development.

Therefore, it is crucial to carefully consider the dosage, timing, and application methods of fungicides to minimize their negative effects while maximizing their effectiveness in managing plant diseases.

Figure 2. Statistical differences between the treatments for the physiological variables analyzed. Columns with the same letters within each figure do not differ from each other according to Tukey HSD (< 5%). Bars represent the standard error.



Legend: Treatment A: Untreated control; Treatment B: Only fungicide (3x); Treatment C: Only the Red Seaweed Biostimulant (1x); Treatment D: Combination of treatments B and C.

Source: Authors (2023)

Conclusions

Fungicides have been found to have a detrimental effect on photosynthesis by impeding the flow of electrons from PSII to PSI, thus impairing the production of ATP and NADPH. This disruption in electron transport hampers the overall efficiency of photosynthesis.

Furthermore, when seaweed extract is combined with fungicides, it exacerbates the negative impacts on photosynthesis. The exact mechanism by which this occurs may vary, but it is likely that the combined application of seaweed extract and fungicides leads to an additive or synergistic effect, intensifying the adverse effects on photosynthetic processes.

References

BALDWIN IT. Plant volatiles. **Current Biology**, v. 20, p. 392-397, 2010. doi: 10.1016/j.cub.2010.02.052

CORKLEY, I.; FRAAIJE, B.; HAWKINS, N. Fungicide resistance management: Maximizing the effective life of plant protection products. **Plant Pathology,** v. 71, p. 150-169, 2022. doi: 10.111/ppa.13467

DUCATTI, R. D. B. Plant elicitation: The generation of misleading and biased information. **Journal of Plant Growth Regulation**, v. 42, p. 3785-3788, 2023. doi: 10.1007/s00344-022-10838-4

DUCATTI, R. D. B.; WORDELL-FILHO, J. A.; TIRONI, S. P.; TRAMONTIN, M. A.; RADUNZ, A. L.; DA SILVA, S. M.; MAZARO, S. M. Wheat elicitation performed in open field with the use of different elicitors. **Contribuciones a las Ciencias Sociales,** v. 7, p.6165-6185, 2023. doi: 10.55905/revconv.16n.7-057

DUCATTI, R. D. B.; TIRONI, S. P.; WORDELL-FILHO, J. A.; MAZARO, S. M. Carrageenan as an elicitor of wheat's mechanisms of defense. **Agronomy Science and Biotechnology**, v. 8, p.1-11, 2022a. doi: 10.33158/ASB.r152.v8.2022

DUCATTI, R. D. B.; WORDELL-FILHO, J. A.; MAZARO, S. M. Deoxynivalenol biosynthesis and accumulation in wheat under application of carrageenan. **Revista de Ciências Agroveterinárias**, v. 21, p.176-181, 2022b. doi: 10.5965/223811712122022176

DUVEILLER, E.; HE, X.; SINGH, P. K. Wheat blast: An Emerging Disease in South America Potentially Threatening Wheat Production. In: BONJEAN, A.; VAN GINKEL, M. (Eds.). **World Wheat Book, Volume 3. A History of Wheat.** Paris: Lavoisier, 2016.

FENG, Y.; HUANG, Y.; ZHAN, H.; BHATT, P.; CHEN, S. An overview of strobilurin fungicide degradation: current status and future perspective. **Frontiers in Microbiology**, v. 11, article 00389, 2020. doi: 10.3389/fmicb.2020.00389

FIGUEROA, M.; HAMMOND-KOSACK, K. E.; SOLOMON, P. S. A review of wheat diseases – a field perspective. **Molecular Plant Pathology**, v. 19, p. 1523-1536, 2018. doi: 10.1111/mpp.12618

JUNQUEIRA, V. B.; COSTA, A. C.; BOFF, T.; MÜLLER, C.; MENDONÇA, M. A. C.; BATISTA, P. F. Pollen viability, physiology, and production of maize plants exposed to pyraclostrobin + epoxiconazole. **Pesticide Biochemistry and Physiology**, v. 137, p. 42-48, 2017. doi: 10.1016/j.pestbp.2016.09.007

LIU, R.; LI, J.; ZHANG, L.; FENG, T.; ZHANG, Z.; ZHANG, B. Fungicide difenoconazole induced biochemical and developmental toxicity in wheat. **Plants**, v. 10, artigo 2304, 2021. doi: 10.3390/plants10112304

LORETO, F.; D'AURIA, S. How do plants sense volatiles sent by other plants? **Trends in Plant Science**, v. 27, p. 29-38, 2022. doi: 10.1016/j.tplants.2021.08.009

MARKOVIC, D.; COLZI, I.; TAITI, C.; RAY, S.; SCALONE, R.; ALI, J. G.; MANCUSO, S.; NINKOVIC, V. Airborne signals synchronize the defenses of neighboring plants in response to touch. **Journal of Experimental Botany**, v. 2, p. 691-700, 2019. doi: 10.1093/jxb/ery375

MARTINAZZO, E. G. Atividade fotossintética em plantas do gênero Prunus. 59f. Tese (Doutorado em Fisiologia Vegetal) – Programa de Pós-Graduação em Fisiologia Vegetal, Universidade Federal do Pelotas. Pelotas, RS, 2011.

PONTS, N. Mycotoxins are a component of *Fusarium graminearum* stress-response system. **Frontiers in Microbiology**, v. 6, Article 1234, 2015. doi: 10.3389/fmicb.2015.01234

REIS, E. M.; BOARETO, C.; DANELLI, A. L. D.; ZOLDAN, S. M. Anthesis, the infectious process and disease progress curves for fusarium head blight in wheat. **Summa Phytopathologica**, v. 42, n. 2, p. 134-139, 2016. doi: 10.1590/0100-5405/2075

SHAHID, M.; KHAN, M. S. Cellular destruction, phytohormones and growth modulating enzymes production by *Bacillus subtilis* stain BC8 impacted by fungicides. **Pesticide Biochemistry and Physiology**, v. 149, p. 8-19, 2018. doi: 10.1016/j.pestbp.2018.05.001

SHUKLA, P. S.; BORZA, T.; CRITCHLEY, A. T.; PRITHIVIRAJ, B. Seaweed-Based Compounds and Products for Sustainable Protection against Plant Pathogens. **Marine Drugs**, v. 19, artigo 59, 2021. doi: 10.3390/md19020059

STIRBET, A; GOVINDJEE. On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and Photosystem II: Basics and applications of the OJIP fluorescence transient. **Journal of Photochemistry and Photobiology B: Biology**, v. 104, p. 236-257, 2011. doi: 10.1016/j.jphotobiol.2010.12.010

TAIZ, L.; ZEIGER, E.; MOLLER, I. M.; MURPHY, A. Plant physiology and development. 6th edition. Artmed: Porto Alegre, 2017.

VAN DEN BERG, F.; PAVELEY, N. D.; VANDEN BOSCH, F. Dose and number of applications that maximize fungicide effective life exemplified by *Zymoseptoria tritici* on wheat –a model analysis. **Plant Pathology,** v. 68, article 12558, 2016. doi: 10.1111/ppa.12558

VINKLÁREK, I. S.; BORNEMANN, T. L. V.; LOKSTEIN, H.; HOFMANN, E.; ALSTER, J.; PSENCIK, J. Temperature Dependence of Chlorophyll Triplet Quenching in Two Photosynthetic Light-Harvesting Complexes from Higher Plants and Dinoflagellates. T**he Journal of Physical Chemistry** B, v. 122, p. 8834-8845, 2018. doi: 10.1021/acs.jpcb.8b06751

ZADOKS, J.C.; CHANG, T.T.; KONZAK, C.F. A decimal code for the growth stages of cereals. **Weed Research**, v. 14, p. 415-421, 1974. doi: 10.1111/j.1365-3180.1974.tb01084.x

ZUBROD, J. P.; BUNDSCHUH, M.; ARTS, G.; BRÜHL, C. A.; IMFELD, G.; KNÄBEL, A.; PAYRAUDEAU, S.; RASMUSSEN, J. J.; ROHR, J.; SCHARMÜLLER, A.; SMALLING, K.; STEHLE, S.; SCHULZ, R.; SCHÄFER R. B. Fungicides: An overlooked pesticide class? **Environmental Science & Technology**, v. 53, p. 3347-3365, 2019. doi: 10.1021/acs.est.8b04392

ZUNTINI, B.; ALVAREZ R. C. F.; THEODORO, G. F. Effect of adding fungicide to mixtures of triazoles and strobilurins in the control of downy mildew and Asian soybean rust. **Pesquisa Agropecuária Brasileira**, v. 49, e53688, 2019. doi: 10.1590/1983-40632019v4953688