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Short Communication

Pyroligneous Acid for Management of *Septoria tritici* Blotch and Productivity of Wheat

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Abstract

Pyroligneous acid (PA), a by-product of biomass pyrolysis, has been proposed as an organic alternative to chemical fungicides. Two experimental phases were carried out in Itapúa, Paraguay, to evaluate the effect of PA on the severity of *Septoria tritici* blotch (STB) and on the productivity of wheat (*Triticum aestivum* L.). The pathogen was identified through morphological characterization of pycnidiospores and lesions, consistent with recent regional reports of *Zymoseptoria tritici*. In Phase I (2019 and 2023 growing seasons), a randomized complete block design (RCBD) with eight treatments was used: a chemical fungicide check (Pyraclostrobin + Epoxiconazole + Fluxapyroxad, 800 mL ha^{-1}), an untreated check (0 mL ha^{-1}), and six increasing doses of PA (1,000, 2,000, 3,000, 4,000, 5,000, and 6,000 mL ha^{-1}). In Phase II (2024 growing season), a factorial RCBD (2×6) compared two wheat varieties (Itapúa 85 and Itapúa 90) and five fungicide treatments (zero, one, or two applications of chemical fungicide or PA at 3,325 mL ha^{-1}, the dose corresponding to the quadratic optimum estimated from Phase I data and falling within the Phase I tested range of 1,000-6,000 mL ha^{-1}. In Phase I, PA doses ≥ 3,000 mL ha^{-1} significantly reduced STB severity and increased spike number per square meter and grain yield compared with the untreated check, although the chemical fungicide produced the highest thousand-grain weight. In Phase II, one application of PA (8.68%) was statistically equivalent to two applications of chemical fungicide (7.98%) in reducing STB severity, while two applications of PA did not improve over the untreated check, possibly due to cumulative phytotoxicity associated with the low pH of the product. No significant treatment effects on yield were observed in Phase II. Collectively, the results suggest that PA at doses of 3,000-6,000 mL ha^{-1} can be a viable, lower-cost alternative for STB management in wheat grown under subtropical conditions of Paraguay.

Categories: Organic agriculture, Use of biostimulants, biofertilizers, bioinoculants in sustainable agriculture

Keywords: septoria tritici blotch, triticum aestivum, wood vinegar, fungicide applications, subtropical wheat

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Introduction

Wheat (*Triticum aestivum* L.) is the most extensively cultivated cereal crop globally and a fundamental pillar of food security [1]. In Paraguay, wheat is the chief winter-season cash crop, accounting for the bulk of farm income during the June-September period in the eastern region of the country [2]. The subtropical conditions of the Itapúa department, characterized by warm temperatures, high relative humidity, and episodic rainfall during the tillering-to-grain-filling period, create a highly conducive environment for multiple fungal diseases. Among these, *Septoria tritici* blotch (STB), caused by *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous, is recognized as one of the most economically important foliar diseases of wheat globally, capable of causing yield losses of 20-50% on susceptible cultivars [3]. In Paraguay, STB was first formally confirmed by morphological and molecular characterization only in 2023, from affected fields in Capitán Miranda and Natalio, Itapúa [4], although epidemics associated with this pathogen had been recorded in the country since 1952 [5]. Other foliar diseases including leaf rust (*Puccinia triticina*), tan spot (*Pyrenophora tritici-repentis*), and wheat blast (*Magnaporthe oryzae* pathotype *Triticum*, MoT) also occur in the region [6], underscoring the complexity of disease management in this environment. Together, these conditions make Itapúa one of the most disease-conducive wheat-producing environments in Paraguay, so that disease-management strategies tested there are evaluated under particularly stringent, high-pressure conditions.

The conventional approach to foliar disease management in wheat has relied predominantly on synthetic fungicides, particularly azole-strobilurin-carboxamide mixtures applied one to three times per season. While these products remain effective when deployed according to integrated pest management (IPM) principles, their continued exclusive use raises serious concerns: the emergence of demethylation inhibitor (DMI)- and quinone outside inhibitor (QoI)-resistant populations of key pathogens has been documented in Brazil and neighboring countries [7,8]; repeated chemical inputs increase production costs for smallholder farmers; and the environmental load of synthetic fungicides in subtropical agricultural systems remains an active area of policy concern. These pressures have stimulated growing interest in organic or biologically derived alternatives that can complement, or partially substitute for, synthetic fungicides within IPM frameworks.

Pyroligneous acid (PA), also known as wood vinegar, is an aqueous by-product of biomass pyrolysis obtained by condensing the smoke generated during the carbonization of lignocellulosic material at 450-600°C [9,10]. PA is a complex liquid composed of water (80-90%) and over 200 organic compounds, of which acetic acid is the most abundant (approximately 50% of the organic fraction), alongside phenols, furans, ketones, methanol, and guaiacols [11,12]. The characteristic pH below 3, conferred by acetic acid, combined with the antimicrobial properties of its phenolic and furfural components, underpins the antifungal activity of PA [13,14]. Recent mechanistic studies confirm that PA inhibits fungal mycelial growth by disrupting membrane integrity and suppressing spore germination; wheat straw vinegar, a PA analog derived from wheat straw pyrolysis, inhibited *Fusarium graminearum* growth and deoxynivalenol (DON) production in wheat with a median effective concentration (EC_{50}) of only 3.1 µL mL^{-1}, making it substantially more cost-effective than commercial fungicides [15]. At the plant level, PA has been shown to upregulate abscisic acid (ABA) signaling and antioxidant enzyme activity in wheat roots [16], increase proline accumulation, and reduce membrane injury under stress conditions [17], suggesting that protection against fungal diseases may partly involve induced systemic resistance (ISR) rather than direct toxicity alone. Additionally, low concentrations of PA promote growth of plant-growth-promoting bacterial genera (*Bradyrhizobium*, *Azospirillum*, *Pseudomonas*) in soil [18], which may further contribute to disease suppression through competition and antagonism. Unlike the curative, systemic activity of triazole-strobilurin-carboxamide fungicides, PA is therefore unlikely to arrest established infections, which makes the timing and frequency of application critical determinants of its field performance, a consideration that motivates evaluating its application frequency under field conditions.

Field-level evidence for PA efficacy against fungal diseases in Paraguay is still limited. The only published field evaluation of PA as a fungicide in the country was conducted on stevia (*Stevia rebaudiana*) at the Center for Technological Development and Innovation (CEDIT), Hohenau, Itapúa, where PA was applied at 30 mL L^{-1} against *Septoria* leaf spot (*Septoria steviae*) and stem rot (*Sclerotium rolfsii*). Results showed variable efficacy across seasons and years, with PA not significantly different from the chemical fungicide (azoxystrobin) or the untreated control in most trials, attributed to

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high plot-to-plot variability and possible environmental degradation of the active compounds [19]. No field-level evaluation of PA for foliar disease management in wheat had been published for Paraguay prior to the present study, highlighting the need for the systematic dose-response and frequency-of-application experiments described here.

Based on the antimicrobial and resistance-inducing properties of PA documented above, we tested three hypotheses: (i) that PA reduces STB severity in a dose-dependent manner across the range tested; (ii) that this efficacy is governed by a minimum threshold dose below which field-level protection is not achieved; and (iii) that application frequency modulates the level of protection conferred by PA relative to synthetic fungicide, given its predominantly contact and induced-resistance mode of action. These hypotheses were addressed sequentially through the two experimental phases described below.

The present study was designed to address these gaps through two experimental phases conducted in Itapúa, Paraguay: Phase I systematically evaluated a range of PA doses across two growing seasons to identify effective dose thresholds, while Phase II assessed the effect of application frequency (one vs. two applications) at a dose derived from Phase I, across two commercial wheat varieties. The specific objectives were: (1) to determine the effect of increasing PA doses (1,000-6,000 mL ha^{-1}) on foliar disease severity, spike number per m^2, thousand-grain weight (TGW), and grain yield in wheat; and (2) to evaluate the effect of application frequency of PA compared with chemical fungicide, and their interaction with wheat genotype, on these same agronomic variables.

Materials And Methods

Phase I: dose-response experiment

Site and crop management. Two growing seasons were conducted at Natalio, Itapúa, Paraguay (26°45′57″S, 55°07′06″W). The two seasons differed markedly in weather conditions during the experimental period (Table *1*). The 2019 season was exceptionally dry, with monthly rainfall of only 4.6 mm (June), 3.6 mm (July), 6.8 mm (August), and 8.2 mm (September), totaling 23.2 mm over the June-September period, a mean air temperature of 18.0°C, and a mean relative humidity of 75%. In contrast, the 2023 season was far wetter, with monthly rainfall of 63.0 mm (June), 120.2 mm (July), 144.4 mm (August), and 312.2 mm (September), totaling 639.8 mm, with a mean temperature of 19.0°C and a mean relative humidity of 78%. In both seasons, the wheat variety Itapúa 80 was sown on June 6 using a drill seeder at a density of 350 seeds m-2 with 0.17 m row spacing. Fertilization was applied at 150 kg ha^{-1} of a commercial fertilizer product with a nutrient composition of 18% N, 46% P_2O_5, and 0% K_2O, equivalent to 27 kg N ha^{-1} and 69 kg P_2O_5 ha^{-1}. Insect control in 2019 used Thiamethoxam + Lambda-cyhalothrin (Endigo, 75 mL ha^{-1}), and in 2023 used Dinotefuran + Beta-cyfluthrin (Raptor Duo, 300-500 mL ha^{-1}). Harvest was performed manually per treatment and replication.

Season	Month	Rainfall (mm)	Mean T (°C)	Min T (°C)	Max T (°C)	RH (%)
2019 (Phase I)	June	4.6	18.6	14.4	24.3	83
	July	3.6	14.8	10.7	19.4	81
	August	6.8	17.5	11.6	24.3	72
	September	8.2	21.4	15.8	28.3	65
	Season mean / total	23.2	18.0	13.1	24.1	75
2023 (Phase I)	June	63.0	16.6	10.4	22.2	83
	July	120.2	17.4	11.2	23.4	83
	August	144.4	19.5	11.6	26.3	74
	September	312.2	22.4	16.1	28.7	75
	Season mean / total	639.8	19.0	12.3	25.2	78
2024 (Phase II)	June	200.8	19.2	14.7	24.9	79
	July	85.6	14.9	8.8	19.9	83
	August	99.8	17.4	12.7	22.8	72
	September	56.8	21.8	13.3	28.7	70
	Season mean/total	443.0	18.3	12.4	24.1	76

TABLE 1: Monthly rainfall, mean air temperature, minimum and maximum temperature, and mean relative humidity (RH) during the experimental periods of the three growing seasons (Natalio, Itapúa, for Phase I 2019 and 2023; María Auxiliadora, Itapúa, for Phase II 2024), Paraguay.

Values are derived from daily automatic weather-station records. Rainfall is the monthly total; temperature and RH are monthly means. "Season mean/total" gives the June–September rainfall total and the mean temperature and RH across the period. Mean temperature was 18.0°C (2019), 19.0°C (2023), and 18.3°C (2024); mean RH was 75%, 78%, and 76%, respectively.

Experimental design and treatments. A randomized complete block design (RCBD) with four blocks and eight treatments was used. Plot size was 5.0 × 3.4 m (17 m^2), with a useful harvest area of 3.0 × 2.4 m. Total experimental area was 625.6 m^2 including walkways. Treatments consisted of a chemical fungicide check (T0, Pyraclostrobin 81 g L^{-1} + Epoxiconazole 50 g L^{-1} + Fluxapyroxad 50 g L^{-1}, applied at 700-800 mL ha^{-1}); an untreated check (T1, 0 mL ha^{-1}); and increasing doses of PA

(T2-T7: 1,000, 2,000, 3,000, 4,000, 5,000, and 6,000 mL ha^{-1}). The PA used was the same product described by Sanabria-Velazquez et al. [19]: a blend of 75% *Eucalyptus grandis* (3-5-year-old) and 25% *Azadirachta indica* (5-7-year-old) wood, produced by slow pyrolysis following Campos [20]. All applications were made with a CO_2 backpack sprayer at 150 L ha^{-1}.

Application dates. In the 2019 season, applications were made on: July 13 (37 days after sowing, das = 30 days after emergence, dae), July 29 (53 das = 46 dae), August 14 (69 das = 62 dae), and August 30 (85 das = 78 dae), with regular 16-day intervals between applications. In the 2023 season, applications were made on: July 31 (55 das = 48 dae), August 10 (65 das = 58 dae), August 24 (79 das = 72 dae), and September 11 (97 das = 90 dae). The later start of treatments in 2023 (48 dae vs. 30 dae in 2019) was entirely attributable to the later onset of visible disease symptoms in that season, given that the protocol required reaching approximately 1% field severity before initiating applications. Emergence was estimated at approximately seven days after sowing (June 13) in both seasons.

Variables and evaluations. Disease severity was assessed visually on 10 randomly selected plants per useful plot area, 15 days after each application, using the standard area diagram scale for Septoria leaf blotch of cereals [21] (Key No. 1.6.1). This scale provides reference diagrams at 5, 25, and 50% of leaf lamina area covered by lesions, with interpolation permitted between anchor points; the percentage of leaf area affected by lesions, including any associated chlorotic or necrotic tissue, was estimated on the flag leaf and second leaf of each tiller and averaged across the sample. Plants were not artificially inoculated; disease development in all plots resulted exclusively from natural field infection by the resident pathogen population present in the experimental sites. In the 2019 season, the four evaluations were therefore conducted at 45, 61, 77, and 93 dae, corresponding to the first application at 30 dae plus 15 days for each subsequent interval. In the 2023 season, the four evaluations were conducted at 63, 73, 87, and 105 dae, reflecting the later onset of symptoms and consequently later start of applications (first application at 48 dae). In both seasons, all evaluations were performed after a preceding application, ensuring that severity readings capture treatment effects. Spike number per m^2 and yield were calculated as:

Yield (kg ha^{-1}) = [W × 10,000 × (100 − M)]/(A × 87)

where W is the field weight of grain harvested from each plot (kg), M is the grain moisture content at harvest (%), A is the harvested plot area (m^2), and 87 is a correction factor corresponding to the standard dry matter basis of 87% used for grain commercialization in Paraguay.

Thousand-grain weight (TGW, g) was calculated by counting and weighing 1000 seeds on a precision balance.

Phase II: application frequency experiment

Site and crop management. The experiment was conducted at the Paraguayan Institute of Agricultural Technology (IPTA) experimental station in María Auxiliadora, Itapúa, Paraguay (coordinates 27°09’S, 55°28’W), from June to September 2024. Sowing was on June 15 with a drill seeder at 350 seeds m^{-2}, 0.17 m row spacing, and fertilization at 200 kg ha^{-1} (N 18%, P 47%, K 0%). Seed treatment used fipronil + thiram + carbendazim. Plots were sown directly over soybean stubble. During the Phase II experimental period, monthly rainfall was 200.8 mm (June), 85.6 mm (July), 99.8 mm (August), and 56.8 mm (September), totaling 443.0 mm, with a mean air temperature of 18.3°C and a mean relative humidity of 76% (Table *1*).

Experimental design and treatments. The two phases addressed conceptually distinct questions, which dictated different designs: Phase I was a dose-finding experiment in which PA concentration was the sole factor, whereas Phase II shifted the question from how much to how often and on which genotype. A factorial RCBD with a 2×5 arrangement and four blocks was therefore used to resolve both main effects and their interaction. Factor A comprised two wheat varieties (Itapúa 85 and Itapúa 90), and Factor B comprised five application treatments: zero, one, or two applications of chemical fungicide (Azoxystrobin + Ciproconazole, 300 mL ha^{-1}) or PA (3325 mL ha^{-1}). The PA dose was derived from the quadratic dose-response model fitted to final disease severity in the high-pressure 2023 season of Phase I (Figure *3A*; $y = 1.187 \times 10^{-6}x^2 - 7.894 \times 10^{-3}x + 50.51$; $R^2 = 0.50$; $P = 5.5 \times 10^{-4}$): its vertex (−b/2a), corresponding to the dose minimizing late-season severity under epidemic conditions, fell at 3325 mL ha^{-1}. This severity optimum was prioritized over the yield optimum from the same season (≈3,929 mL ha^{-1}; Figure 3B) because the objective of Phase II was to test the biological efficacy of PA within a frequency-of-application framework rather than to maximize agronomic return. Total experimental area was 744 m^2. Applications began at 21 dae and were repeated weekly, using a CO_2 backpack sprayer at 150 L ha^{-1}.

Variables and evaluations. As in Phase I, disease development resulted from natural field infection; no artificial inoculation was performed. Disease severity, spike number per m^2, TGW, and grain yield were measured using the same protocols as Phase I.

Pathogen identification

The causal agent of the foliar disease complex evaluated in both experimental phases was identified as *Zymoseptoria* sp. Identification was performed by light microscopy from symptomatic leaf samples collected in the experimental fields. Pycnidia and conidia were examined using a compound microscope (400× and 1,000×) and the genus and species were assigned based on the morphological descriptions provided by Maidana-Ojeda et al. [4] for the Paraguayan population for the Paraguayan population and by [22]. Characteristic structures observed included spherical to subglobose, partially buried, light brown pycnidia with dark edges embedded in chlorotic-to-necrotic lesions, and hyaline, filiform, multi-guttulate pycnidiospores consistent with *Z. tritici*. This identification is consistent with the first formal report of STB in Paraguay by Maidana-Ojeda et al. [4], made from wheat fields in the same department (Itapúa). Pycnidium formation was confirmed by incubating symptomatic leaf fragments in a humid chamber (lined Petri dish with moistened filter paper, 22 ± 2°C, 12 h light/dark) for 48-72 h, after which mature pycnidia extruding cirrhi of pycnidiospores were observed under a stereomicroscope. Disease symptoms in the field and the pycnidia and conidia observed under stereoscope and microscope (40×) are shown in Figure 1. It should be noted that identification in the present study relied on morphological criteria alone; molecular confirmation was not performed. While the structures observed are fully consistent with the morphological description of the Paraguayan *Z. tritici* population reported by Maidana-Ojeda et al. [4], we acknowledge that morphological identification cannot exclude the co-occurrence of other pycnidial fungi within the foliar disease complex, and the pathogen is therefore referred to at genus level (*Zymoseptoria* sp.) where species-level confirmation was not possible. Disease severity was assessed using the standard area diagram scale for Septoria leaf blotch of cereals [21] (Key No. 1.6.1), estimating the percentage of leaf lamina area covered by lesions on individual flag and second leaves of randomly selected fertile tillers.

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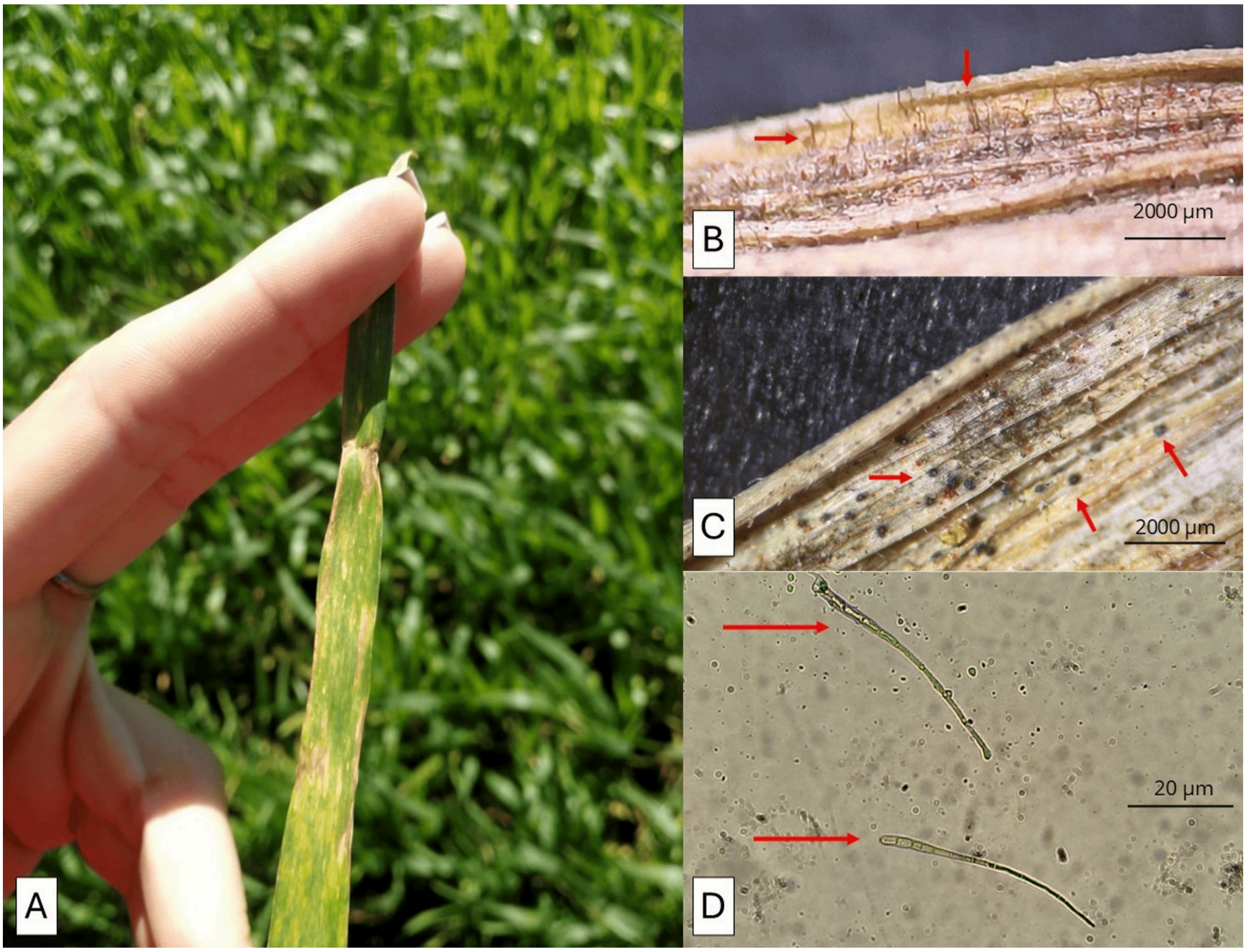

FIGURE 1: Field symptoms and morphological structures of Zymoseptoria sp. associated with Septoria tritici blotch on wheat in Itapúa, Paraguay. (A) Chlorotic-to-necrotic foliar lesions on a naturally infected leaf under field conditions. (B) Lesion surface bearing emerging pycnidiospores, observed under a stereomicroscope (scale bar = 2,000 μm). (C) Dark, partially immersed pycnidia embedded in lesioned leaf tissue, observed under a stereomicroscope (scale bar = 2,000 μm). (D) Hyaline, filiform, multi-septate conidia (pycnidiospores) observed by light microscopy (scale bar = 20 μm). Red arrows indicate the diagnostic structures: emerging pycnidiospores (B), pycnidia (C), and conidia (D).

Statistical analysis

Data from both phases were subjected to analysis of variance (ANOVA) using InfoStat [23]. For Phase I, a randomized complete block ANOVA with blocks as the error term was performed separately for each season. For Phase II, a two-way factorial ANOVA evaluated treatment, genotype, and their interaction effects. Prior to ANOVA, the assumptions of normality of residuals and homogeneity of variances were assessed using the Shapiro-Wilk test and Levene's test, respectively, and were re-checked after transformation. Percentage variables (disease severity) were subjected to the arcsine square-root transformation [$y = \arcsin(\sqrt{(x/100)})$] prior to analysis, as recommended for proportion data. The transformation did not change the significance of any ANOVA result, so means reported in tables and figures correspond to back-transformed values for ease of interpretation, while the Tukey HSD groupings and standard errors were derived from the analysis on the transformed scale. For evaluation 4 (Eval. 4, 105 dae) in the 2023 season, Levene's test indicated significant heterogeneity of variance ($P < 0.01$) that persisted after transformation; in this case the Welch-Satterthwaite correction for unequal variances was applied as a robustness check and confirmed the same statistical conclusions. Where ANOVA indicated significant differences, treatment means were compared using Tukey's honest significant difference (HSD) test (α = 0.05). The coefficient of variation (CV) was calculated on untransformed data and reported for each variable. Additionally, quadratic regression models were fitted to the treatment means of PA-treated plots (0-6000 mL ha^{-1}) for severity and yield variables, and the dose optimizing each response was estimated as the vertex (−b/2a) of the fitted function.

Results And Discussion

Phase I - disease severity

ANOVA indicated significant treatment effects ($P < 0.01$) on foliar disease severity at all four evaluation timings in both the 2019 and 2023 seasons (Table *2*).

In the 2019 season, disease pressure was relatively low (<10% severity in all treatments) (Table *2*, Figure *2*). The untreated check showed the highest severity at the first reading (Eval. 1, 45 dae: 3.96%), while the fungicide check displayed the lowest initial severity (2.37%). From Eval. 3 (77 dae) onwards, the PA) doses of 3,000-6,000 mL ha^{-1} were superior to both the untreated check and the fungicide in reducing severity. By Eval. 4 (93 dae), the 6,000 mL ha^{-1} dose achieved the lowest mean severity (5.43%), followed by the 5,000 mL ha^{-1} dose (5.52%), both significantly below the fungicide check (7.99%) and the untreated check (8.08%).

In the 2023 season, disease pressure was markedly higher than in 2019. All evaluations in 2023 were conducted after applications, at 63, 73, 87, and 105 dae. The pattern at Eval. 1 (63 dae) already reflects 15 days of treatment response: the fungicide check showed the lowest severity (5.3%), while the untreated check (23.5%) and the 1,000 mL ha^{-1} PA treatment (25.1%) showed the highest values. Higher PA doses (3,000-6,000 mL ha^{-1}) ranged from 8.3 to 12.1% at 63 dae, showing intermediate but meaningful early control. By Eval. 4 (105 dae), the fungicide check achieved the lowest severity (23.3%), while the untreated check reached 45.3%. Among PA treatments, the 3,000 mL ha^{-1} dose showed the lowest final severity (34.8%), followed by 4,000 mL ha^{-1} (36.2%). The 1,000 mL ha^{-1} dose was the worst PA performer at Eval. 4 (105 dae: 53.4%), exceeding even the untreated check, suggesting that sub-optimal doses failed to contain epidemic development under high disease pressure (Table *2*, Figure *2*).

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2019 Season				
Treatment (mL ha^{-1})	Eval. 1 (45 dae)	Eval. 2 (61 dae)	Eval. 3 (77 dae)	Eval. 4 (93 dae)
Fungicide check	2.37 ± 0.02 E	6.02 ± 0.09 A	7.12 ± 0.04 A	7.99 ± 0.08 A
0 (Untreated)	3.96 ± 0.09 A	5.96 ± 0.11 A	6.97 ± 0.08 A	8.08 ± 0.14 A
1,000	3.32 ± 0.03 B	5.90 ± 0.07 A	5.95 ± 0.05 B	7.03 ± 0.04 B
2,000	3.12 ± 0.03 BC	5.61 ± 0.15 AB	5.26 ± 0.03 CD	6.91 ± 0.04 B
3,000	3.05 ± 0.08 CD	5.46 ± 0.03 ABC	5.12 ± 0.09 D	6.06 ± 0.07 C
4,000	2.94 ± 0.09 CD	5.18 ± 0.13 BCD	5.55 ± 0.10 C	6.00 ± 0.04 CD
5,000	2.95 ± 0.14 CD	4.90 ± 0.09 CD	5.62 ± 0.12 BC	5.53 ± 0.15 DE
6,000	2.80 ± 0.06 D	4.80 ± 0.21 D	5.56 ± 0.08 C	5.43 ± 0.17 E
F-value	34.62**	15.60**	92.37**	98.79**
CV (%)	4.4	4.0	2.5	2.8
2023 Season				
Treatment (mL ha^{-1})	Eval. 1 (63 dae)	Eval. 2 (73 dae)	Eval. 3 (87 dae)	Eval. 4 (105 dae)
Fungicide check	5.32 ± 0.40 C	4.11 ± 0.67 D	16.12 ± 1.54 C	23.31 ± 0.96 F
0 (Untreated)	23.45 ± 1.50 A	18.87 ± 1.93 A	24.77 ± 1.27 A	45.32 ± 1.33 BC
1,000	25.13 ± 0.77 A	15.30 ± 2.98 AB	21.46 ± 0.59 AB	53.42 ± 1.92 A
2,000	12.28 ± 1.19 B	8.32 ± 0.63 CD	17.65 ± 0.32 BC	38.08 ± 0.54 DE
3,000	12.06 ± 1.63 B	7.68 ± 1.35 CD	19.04 ± 1.30 BC	34.80 ± 0.41 E
4,000	10.19 ± 0.71 B	9.72 ± 1.14 BC	15.78 ± 1.44 C	36.20 ± 0.87 DE
5,000	8.49 ± 0.50 BC	7.82 ± 0.95 CD	15.24 ± 0.45 C	41.00 ± 0.74 CD
6,000	8.29 ± 0.74 BC	3.59 ± 0.34 D	15.06 ± 0.96 C	47.02 ± 1.80 B
F-value	49.98**	12.55**	10.23**	58.45**
CV (%)	14.2	26.5	10.9	5.4

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TABLE 2: Mean foliar disease severity (%) at four evaluation timings in wheat treated with pyroligneous acid (PA) or chemical fungicide across two growing seasons (Phase I, Natalio, Itapúa, Paraguay).

Values are means ± standard error (n = 4 blocks). Within a column, means followed by the same letter do not differ significantly (Tukey HSD, α = 0.05). Severity data were analyzed after arcsine square-root transformation; means shown are back-transformed. ** Significant at p ≤ 0.01; dae = days after emergence.

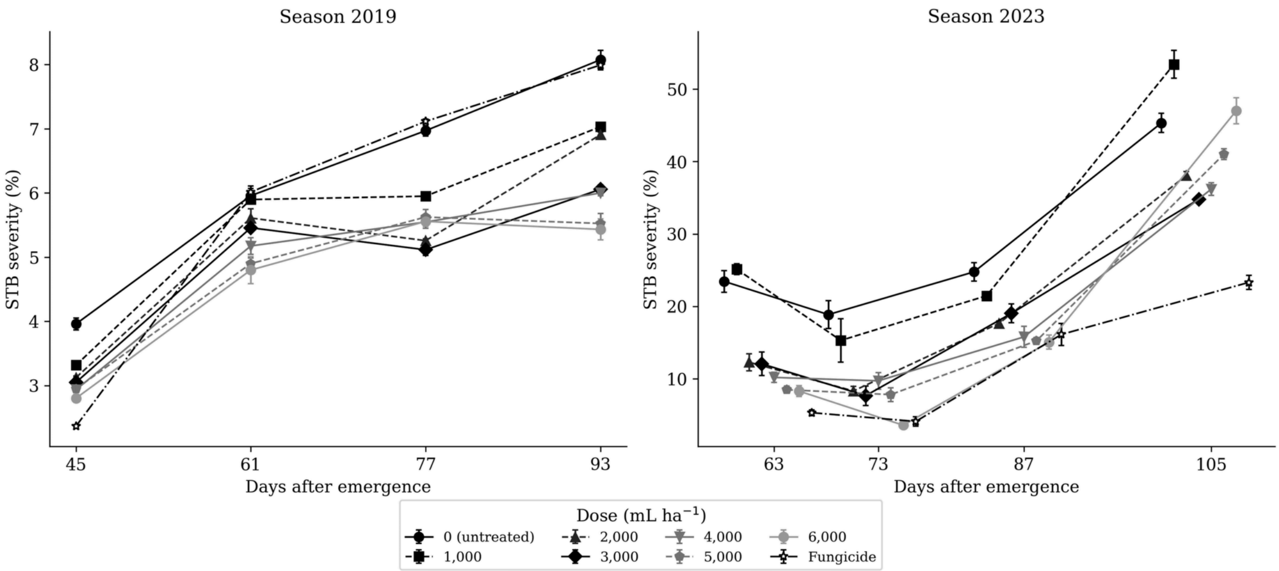

FIGURE 2: Temporal progression of Septoria tritici blotch (STB) in wheat treated with increasing doses of pyroligneous acid (PA) or chemical fungicide, evaluated 15 days after each application. Season 2019: evaluations at 45, 61, 77, and 93 days after emergence (dae); Season 2023: evaluations at 63, 73, 87, and 105 dae. Left: 2019 season (Natalio, Itapúa, Paraguay, low disease pressure). Right: 2023 season (high disease pressure). Points are treatment means (n = 4).

The Phase I results strongly support a dose-responsive relationship between PA and foliar disease suppression up to approximately 5,000-6,000 mL ha^{-1}. In the 2019 season, the 3,000-6,000 mL ha^{-1} range consistently produced lower late-season severity than the chemical fungicide check. Under the low disease pressure of 2019, the frequent (16-day interval) applications of a contact/protective product may have maintained a more continuous protective layer on expanding foliage than the finite residual activity of a single-mode-of-action systemic mixture, which would help explain why the higher PA doses outperformed the systemic chemical check in reducing late-season severity in that season. The antifungal mechanism of PA is now better understood: the combination of low pH (driven by acetic acid, which constitutes approximately 50% of the organic fraction) and phenolic compounds such as guaiacol and syringol disrupts fungal membrane integrity and inhibits mycelial elongation [13,14]. A direct comparison between wheat straw vinegar and synthetic fungicides against *Fusarium graminearum* confirmed that pyroligneous-type liquids can achieve fungicidal thresholds sufficient to protect wheat under field conditions, provided concentration is adequate [15]. The dose threshold of 3,000 mL ha^{-1} identified in Phase I is broadly consistent with this mechanistic framework, as sub-threshold doses would likely be insufficient to maintain inhibitory acetic acid concentrations on the leaf surface after dilution with the spray carrier volume.

The marked difference in disease dynamics between the 2019 and 2023 seasons reflects both contrasting disease pressure levels and a difference in evaluation timing. In 2019, all four evaluations (45, 61, 77, and 93 dae) were performed after treatments had been applied, under low disease pressure (maximum severity <10%), producing an orderly and progressive dose-response. In 2023, the first evaluation at 63 dae already captured 15 days of treatment response after

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the first application at 48 dae. At that point (Eval. 1, 63 dae), the fungicide check showed only 5.3% severity while the untreated check reached 23.5%, a clear and immediate treatment differentiation absent in 2019 at the equivalent evaluation, reflecting the much higher inoculum pressure in 2023. This contrast is mechanistically consistent with the biology of *Z. tritici*, whose pycnidiospores are dispersed by rain-splash from gelatinous cirrhi and require prolonged leaf wetness for germination and infection. The near-absence of rainfall during the 2019 season (23.2 mm total over June-September) would have severely limited both spore dispersal and infection, whereas the abundant and progressively increasing rainfall of 2023 (639.8 mm total, rising from 63 mm in June to 312 mm in September) provided highly conducive conditions for epidemic development. Notably, the September peak in rainfall coincided with the highest recorded disease severities at the final evaluation, while mean air temperature (18.0 vs. 19.0 °C) and relative humidity (75 vs. 78%) were broadly similar between the two seasons. This indicates that moisture availability, rather than temperature, was the principal environmental driver of the contrasting disease pressure between 2019 and 2023.

The later start of applications in 2023 (48 dae vs. 30 dae in 2019) was entirely determined by the 1% symptom threshold protocol: symptoms appeared later in absolute terms but escalated more rapidly once established, resulting in substantially higher final severity values. Among PA doses, the 1,000 mL ha^{-1} treatment showed the highest severity at Eval. 4 (105 dae: 53.4%), exceeding even the untreated check (45.3%). This finding suggests that sub-optimal PA concentrations may fail to contain epidemic development once disease pressure exceeds a critical level, and doses ≥ 3,000 mL ha^{-1} should be considered the minimum effective dose under conditions of high epidemic potential. The weaker regression fit for the 2023 final evaluation (R^2 = 0.50 vs. 0.97 in 2019) reflects the non-monotonic response at 1,000 mL ha^{-1} and the generally higher variability under epidemic conditions. An alternative, non-exclusive interpretation is that the sub-threshold 1000 mL ha^{-1} dose exerted a hormetic or phyllosphere-altering effect: low concentrations of the bioactive compounds in PA may have been insufficient to inhibit the pathogen while still perturbing the resident leaf microbiota or inducing mild tissue stress that predisposed the lamina to faster colonization, consistent with the documented capacity of low PA concentrations to modify microbial community composition [18]. As with the Phase II result, no leaf-stress symptoms attributable to the 1,000 mL ha^{-1} treatment were systematically recorded, so this interpretation remains speculative and warrants targeted investigation, including phyllosphere microbiome characterization across the dose gradient.

Phase I - spike number per m²

Significant treatment effects ($P < 0.01$) on spike number per m^2 were found in the 2019 season (F = 27.20) but not in 2023 (F = 1.49, $P = 0.22$) (Table *3*). In 2019, the untreated check produced markedly fewer spikes (209.3 m^{-2}) than all treated plots. The highest spike counts were observed at 6,000 mL ha^{-1} PA (395.5 m^{-2}) and the 5,000 mL ha^{-1} dose (346.0 m^{-2}), both exceeding the chemical fungicide (335.3 m^{-2}).

Phase I - thousand-grain weight

Thousand-grain weight (TGW) was significantly affected by treatments in both seasons ($P < 0.01$; Table *3*). In both 2019 and 2023, the chemical fungicide produced the highest TGW (33.4 and 35.0 g, respectively), while the untreated check had the lowest (27.8 and 32.4 g). Among PA treatments, TGW values increased up to the 4,000 mL ha^{-1} dose in 2019 (31.3 g) before stabilizing. The chemical fungicide consistently produced the highest TGW in Phase I (33.4 g in 2019; 35.0 g in 2023), suggesting that its systemic, curative-and-preventive mode of action provides more complete protection during the critical grain-filling window than the more contact- and induced systemic resistance (ISR)-based activity of PA.

Phase I - grain yield

Significant yield differences were detected between treatments in both 2019 (F = 4.89, $P < 0.01$) and 2023 (F = 5.22, $P < 0.01$) (Table *3*). In 2019, yields ranged from 1653 kg ha^{-1} (untreated check) to 2,068 kg ha^{-1} (chemical fungicide). PA treatments improved yield compared with the untreated check in a dose-responsive manner; the 3,000-6,000 mL ha^{-1} range produced 1,907-1,978 kg ha^{-1}, not significantly different from the chemical fungicide. In 2023, the 3,000 mL ha^{-1} treatment recorded the highest PA yield (2,376 kg ha^{-1}), equivalent to the fungicide check (2,330 kg ha^{-1}). The untreated check yielded 2,053 kg ha^{-1} in 2023, reflecting the generally higher productivity of that season.

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A consistent pattern across both experimental phases was the limited or absent translation of disease severity differences into statistically significant yield differences. In Phase II, no treatment effect on yield was detected whatsoever ($F = 0.35$, $P = 0.84$). This decoupling between severity and yield is a well-recognized phenomenon in subtropical wheat pathosystems, where moderate foliar disease can be compensated by plasticity in spike number, tiller survival, and grain filling, particularly when the disease complex does not include spike blast, which directly destroys grain [24]. A similar yield-severity decoupling was observed by Sanabria-Velazquez et al. [19] in stevia, where PA failed to produce yield benefits despite showing some disease reduction. In the 2023 season specifically, the late onset of the epidemic is the most plausible explanation for the limited yield penalty despite final severities of 45-53%. Because applications did not begin until 48 dae (when the 1% threshold was reached) and peak severities were recorded at the final evaluation (105 dae), the most damaging disease levels coincided with late grain filling, after the crop had already passed the critical stages of spike formation and early grain set. This phenological escape is consistent with the observation that, in 2023, treatment-related yield differences were driven almost entirely by thousand-grain weight rather than by spike number per m^2 (which did not differ significantly among treatments; $F = 1.49$, ns), indicating that whatever protective benefit PA conferred operated principally during grain filling rather than during the earlier reproductive phases. The very high coefficient of variation (CV) (25.86%) observed in the Phase II yield data further underscores the confounding role of spatial variability in small-plot yield estimates. This high CV likely reflects a combination of small plot size, environmental heterogeneity across the experimental area, and the manual harvest method, all of which reduce the statistical power to detect treatment effects on yield and may mask real biological trends; this limitation should be borne in mind when interpreting the Phase II yield results. Future studies should incorporate quantitative area under the disease progress curve (AUDPC) analysis and economic threshold modeling to better delineate the conditions under which PA provides not only biological but economic returns above the cost of application.

Phase I - dose-response regression analysis

Quadratic regression models were fitted to the means of PA-treated plots (0-6,000 mL ha^{-1}, excluding the fungicide check) to estimate the dose that optimizes each variable (Figure *3*). In 2019, regression models explained a high proportion of variance for all variables (R^2 = 0.92-0.97), indicating a strong and consistent dose-response under low disease pressure. The model for Eval. 4 (93 dae) predicted that severity continues to decline beyond 6,000 mL ha^{-1}, with no clear minimum within the tested range (optimal dose estimated at 6,877 mL ha^{-1}), suggesting that the protective effect of PA had not reached a plateau. For grain yield, the quadratic model identified an optimal dose of approximately 5,437 mL ha^{-1} (R^2 = 0.94), with an estimated maximum yield of 1,956 kg ha^{-1}. Similarly, early-season severity (Eval. 1; 45 dae) was minimized at ~4,922 mL ha^{-1} (R^2 = 0.92). Together, these models suggest that a dose in the range of 4,500-6,000 mL ha^{-1} maximizes the agronomic benefit of PA under low-to-moderate disease pressure conditions similar to 2019.

In 2023, under higher disease pressure, the dose-response models were less precise. For Eval. 4 (105 dae), the R^2 was only 0.50, reflecting the non-monotonic response driven by the anomalously high severity at 1,000 mL ha^{-1}. Despite this, the quadratic minimum was estimated at 3,325 mL ha^{-1}, suggesting that doses around 3,000-4,000 mL ha^{-1} provide the best disease suppression under epidemic conditions. For grain yield, the 2023 model was more robust (R^2 = 0.73) and identified an optimal dose of approximately 3,929 mL ha^{-1}, with an estimated peak yield of 2,305 kg ha^{-1}. Combining both seasons, the dose range of 3,000-5,000 mL ha^{-1} emerges as the practical optimum zone for PA in subtropical wheat production in Itapúa, balancing disease suppression and yield gain across contrasting epidemic conditions.

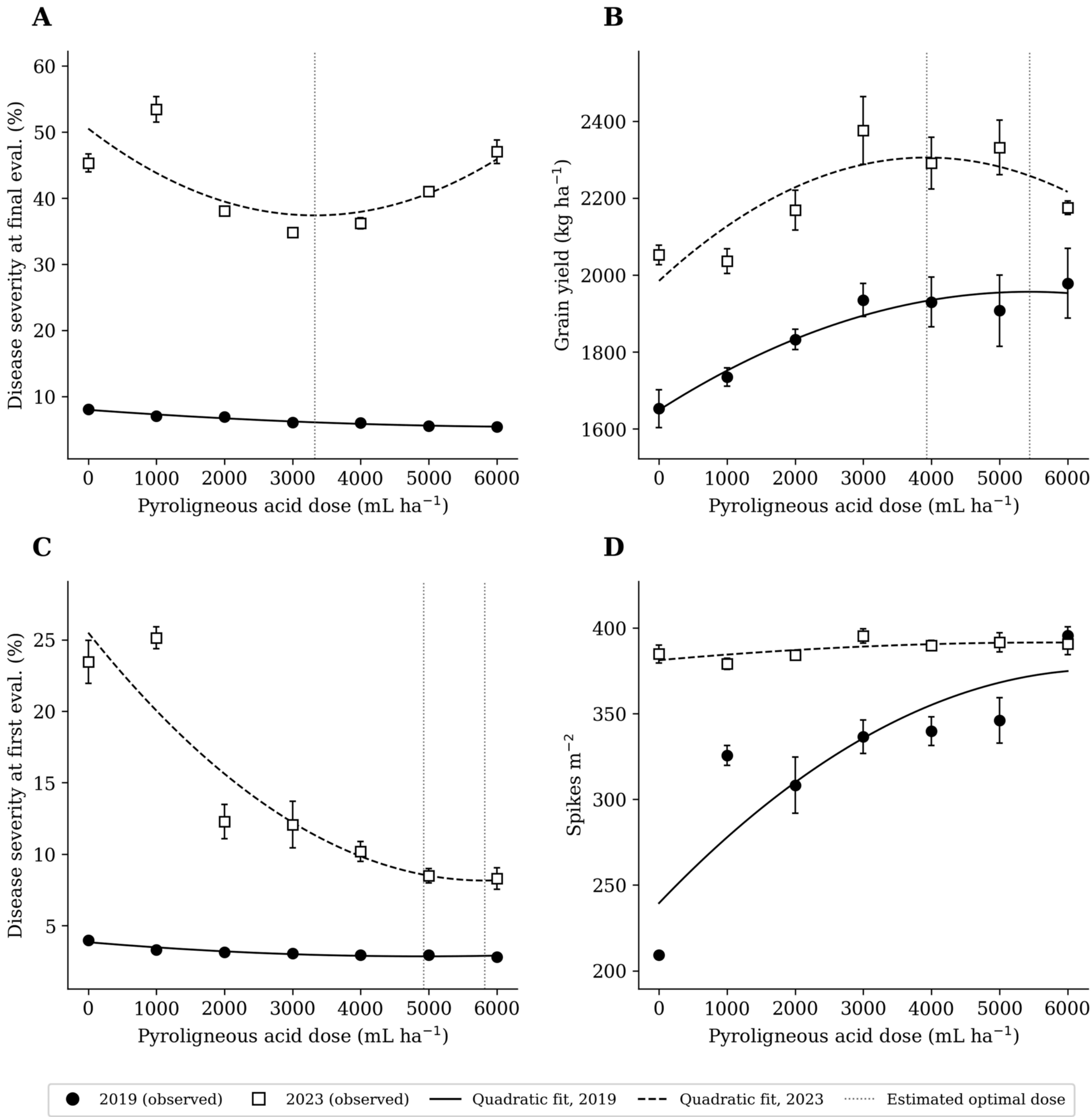

FIGURE 3: Dose-response relationships between pyroligneous acid (PA) dose and (A) final disease severity (Eval. four: 93 dae in 2019, 105 dae in 2023), (B) grain yield, (C) initial disease severity (Eval. one: 45 dae in 2019, 63 dae in 2023), and (D) spike number per m^2 for two growing seasons in Natalio, Itapúa, Paraguay.

Points are treatment means ($n = 4$); error bars represent ± standard error of the mean. Curves represent fitted quadratic regression models; vertical dotted lines indicate estimated optimal doses. Fitted quadratic models (y = dose in mL ha^{-1}): (A) final disease severity - 2019: $y = 5.458 \times 10^{-8}x^2 - 7.507 \times 10^{-4}x + 7.98$ ($R^2 = 0.97$; optimal dose 6,877 mL ha^{-1}); 2023: $y = 1.187 \times 10^{-6}x^2 - 7.894 \times 10^{-3}x + 50.51$ ($R^2 = 0.50$; 3,325 mL ha^{-1}). (B) grain yield - 2019: $y = -1.040 \times 10^{-5}x^2 + 1.131 \times 10^{-1}x + 1648.84$ ($R^2 = 0.94$; 5,437 mL ha^{-1}); 2023: $y = -2.077 \times 10^{-5}x^2 + 1.632 \times 10^{-1}x + 1984.74$ ($R^2 = 0.73$; 3929 mL ha^{-1}). (C) Initial disease severity - 2019: $y = 4.086 \times 10^{-8}x^2 - 4.022 \times 10^{-4}x + 3.84$ ($R^2 = 0.92$; 4,922 mL ha^{-1}); 2023: $y = 5.124 \times 10^{-7}x^2 - 5.962 \times 10^{-3}x + 25.49$ ($R^2 = 0.86$; 5,818 mL ha^{-1}). (D) Spike number per m^2 - 2019: $y = -3.170 \times 10^{-6}x^2 + 4.156 \times 10^{-2}x + 239.48$ ($R^2 = 0.78$; 6,556 mL ha^{-1}); 2023: $y = -3.095 \times 10^{-7}x^2 + 3.571 \times 10^{-3}x + 381.13$ ($R^2 = 0.50$; 5,769 mL ha^{-1}).

Delta-method 95% confidence intervals for the estimated optimal doses: final disease severity - 2019: 6,877 mL ha−1 [95% CI: 2,341–11,413]; 2023: 3,325 mL ha−1 [95% CI: 1,964–4,686]. Grain yield - 2019: 5,437 mL ha−1 [95% CI: 2,834–8,041]; 2023: 3,929 mL ha−1 [95% CI: 2,362–5,496]. CI for spike number per m^2 extended outside the tested range and is not reported.

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2019 Season			
Treatment (mL ha^{-1})	Spikes m^{-2}	TGW (g)	Yield (kg ha^{-1})
Fungicide check	335.3 ± 12.5 B	33.40 ± 0.69 A	2,068 ± 54 A
0 (Untreated)	209.3 ± 1.7 C	27.83 ± 0.34 C	1,653 ± 50 C
1,000	325.5 ± 5.8 B	31.05 ± 0.38 AB	1,735 ± 24 BC
2,000	308.3 ± 16.3 B	30.32 ± 0.50 B	1,833 ± 27 ABC
3,000	336.5 ± 9.7 B	30.59 ± 0.72 B	1,935 ± 43 ABC
4,000	339.8 ± 8.3 B	31.31 ± 0.60 AB	1,930 ± 65 ABC
5,000	346.0 ± 13.2 B	31.09 ± 0.52 AB	1,908 ± 93 ABC
6,000	395.5 ± 5.0 A	29.60 ± 0.21 BC	1,978 ± 91 AB
F-value	27.20**	9.15**	4.89**
CV (%)	5.6	3.2	5.9
2023 Season			
Treatment (mL ha^{-1})	Spikes m^{-2}	TGW (g)	Yield (kg ha^{-1})
Fungicide check	393.5 ± 4.7 ns	34.95 ± 0.31 A	2,330 ± 66 AB
0 (Untreated)	384.8 ± 5.2 ns	32.38 ± 0.11 B	2,053 ± 25 BC
1,000	379.0 ± 3.2 ns	33.08 ± 0.25 B	2,036 ± 32 C
2,000	384.0 ± 2.5 ns	33.51 ± 0.39 B	2,169 ± 52 ABC
3,000	395.3 ± 4.2 ns	32.32 ± 0.35 B	2,376 ± 88 A
4,000	389.8 ± 2.8 ns	32.66 ± 0.32 B	2,291 ± 67 ABC
5,000	391.5 ± 5.5 ns	32.64 ± 0.21 B	2,332 ± 71 AB
6,000	390.5 ± 6.2 ns	32.55 ± 0.22 B	2,175 ± 18 ABC
F-value	1.49 ns	9.60**	5.22**
CV (%)	2.2	1.6	4.7

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TABLE 3: Mean spike number per m², thousand-grain weight (TGW), and grain yield of wheat treated with pyroligneous acid (PA) or chemical fungicide (Phase I, Natalio, Itapúa, Paraguay).

Values are means ± standard error (n = 4 blocks). Within a column, means followed by the same letter do not differ significantly (Tukey HSD, α = 0.05). ** Significant at p ≤ 0.01; ns = not significant.

Phase II - disease severity

Treatment significantly affected foliar disease severity (F = 48.67, $P < 0.01$; CV = 6.19%), while genotype and the treatment × genotype interaction were non-significant (Table 4). The untreated check showed the highest severity (11.22%). Two applications of chemical fungicide produced the lowest severity (7.98%), statistically equal to one application of PA (8.68%, group AB). Two applications of PA (10.33%) significantly reduced severity relative to the untreated check but were significantly less effective than a single PA application, indicating that the second application did not enhance, and may have partially offset, the protection conferred by the first. One application of chemical fungicide (9.33%) occupied an intermediate position.

Treatment	Severity (%)	Tukey 5%	Spikes m^{-2}	Tukey 5%
0 Applications (Untreated)	11.22	D	288.88	A
1 Application — fungicide	9.33	BC	293.13	A
2 Applications — fungicide	7.98	A	295.50	A
1 Application — PA (3325 mL ha^{-1})	8.68	AB	288.75	A
2 Applications — PA (3325 mL ha^{-1})	10.33	C	288.75	A
Mean	9.51		291.00	
F-value (treatment)	48.67**		0.68 ns	
F-value (genotype)	ns		22.05**	
CV (%)	6.19		3.88	

TABLE 4: Foliar disease severity (%) and spike number per m² of two wheat varieties under five treatment regimes (Phase II, María Auxiliadora, Itapúa, Paraguay, 2024).

Means averaged across both varieties (Itapúa 85 and Itapúa 90). Treatment × genotype interaction was not significant for either variable. ** Significant at $P \leq 0.01$; ns, Not Significant; PA, Pyroligneous Acid.

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The most surprising result of this study was the failure of two PA applications to improve, and in some cases worsen, disease control relative to a single PA application in Phase II (two-application PA: 10.33% severity; one-application PA: 8.68%, statistically equivalent to two chemical fungicide applications: 7.98%). While Phase I suggested that higher doses over time are beneficial, Phase II showed that repeated applications at a fixed dose of 3,325 mL ha^{-1} did not compound the effect. Two non-exclusive explanations may account for this. First, phytotoxicity at elevated cumulative doses cannot be excluded: the acidity and phenolic content of PA can cause foliar damage when exposure is repeated, potentially counteracting disease suppression in two ways: by impairing the photosynthetic capacity of treated leaves, and by inducing micro-injuries or micro-fissures in the leaf cuticle through which *Z. tritici* pycnidiospores could more readily penetrate and establish infection [13,17]. We note, however, that no systematic visual scoring of phytotoxicity (e.g., leaf burning or chlorosis attributable to the spray rather than to disease) was recorded in this trial; the phytotoxicity explanation therefore remains a hypothesis requiring direct measurement in future work. Second, it is possible that the ISR mechanism activated by a single PA application, upregulation of ABA signaling and pathogenesis-related (PR) proteins [16], does not scale linearly with application number. The relationship between PA application frequency and the magnitude of ISR merits dedicated investigation.

Phase II - spike number per m^2, TGW, and grain yield

Treatments did not significantly affect spike number per m^2, TGW, or grain yield in Phase II ($P > 0.05$ for all variables, Table *5*). Only the genotype effect on spike number per m^2 and TGW was significant. Itapúa 85 produced more spikes per m^2 (298.9 vs. 283.1 for Itapúa 90; F = 22.05, $P < 0.01$), while Itapúa 90 showed a substantially higher TGW (41.5 g vs. 30.0 g for Itapúa 85; F = 319.08, $P < 0.01$), reflecting the distinct grain morphology characteristics of each variety. The absence of treatment effects on yield (F = 0.35, $P = 0.84$; CV = 25.86%) likely reflects the relatively low and uniform disease pressure experienced during Phase II, together with the high yield variability observed (CV = 25.86%). Notably, this low disease pressure cannot be attributed to moisture limitation, since rainfall in 2024 was not limiting (June-September total ≈ 443 mm; Table *1*); the moderate disease levels may instead reflect differences in initial inoculum, the soybean-stubble rotation on which the trial was sown, or the genotypes evaluated, rather than environmental conditions *per se*. Under such conditions, the potential yield benefit of disease control may be insufficient to overcome plot-to-plot variability.

Genotype	TGW (g)	Tukey 5%	Yield (kg ha^{-1})	Tukey 5%
Itapúa 85	30.0	A	1,168	A
Itapúa 90	41.5	B	1,012	A
Mean	35.75		1,090	
F-value (genotype)	319.08**		3.39 ns	
F-value (treatment)	2.38 ns		0.35 ns	
CV (%)	6.02		25.86	

TABLE 5: Thousand-grain weight (TGW) and grain yield of two wheat varieties (Itapúa 85 and Itapúa 90) under five treatment regimes (Phase II, María Auxiliadora, Itapúa, Paraguay, 2024). Treatment effect was not significant for any variable; only genotype effects on TGW and yield are shown.

** Significant at $P \leq 0.01$; ns, Not Significant. Means are averages across all treatments ($n = 24$ per genotype).

The absence of a significant treatment × genotype interaction for disease severity in Phase II, despite the significant main effect of genotype on spike number and TGW, suggests that the relative benefit of PA is largely independent of the varietal background tested. Both Itapúa 85 and Itapúa 90 responded similarly to PA and fungicide treatments in terms of disease suppression, which is a practically useful finding since it implies that dose recommendations developed on one variety can likely be extrapolated to others with similar disease susceptibility profiles. The significant difference between varieties in spike number (298.9 vs. 283.1 m^{-2}) and TGW (41.5 vs. 30.0 g) suggests that the two varieties employ contrasting yield-formation strategies (spike number vs. grain weight), which has implications for the relative sensitivity of each component to foliar disease: varieties relying more heavily on TGW for yield formation (Itapúa 90) may be more sensitive to fungicidal protection during grain filling, while those relying on spike number (Itapúa 85) may benefit more from early-season protection. This hypothesis was not supported in Phase II data but merits investigation in future studies incorporating season × variety × treatment designs.

Practical implications

For wheat farmers in Paraguay, the results collectively suggest that PA applied at 3,000-6,000 mL ha^{-1} can serve as a viable, lower-cost complement to, or partial substitute for, chemical fungicides in seasons of low-to-moderate disease pressure. Given that PA is a by-product of charcoal production, an industry already well established in Itapúa, local sourcing is feasible and could substantially reduce input costs for smallholders. The timing of the first application is critical: in 2019, spraying began at 30 dae when disease had just reached the 1% threshold, and the dose-response was strong and orderly. In 2023, the threshold was not reached until 48 dae; despite the later start, PA doses ≥ 3,000 mL ha^{-1} still provided meaningful suppression. This suggests that strict adherence to the 1% symptom trigger, rather than calendar-based spraying, is the key operational factor for PA efficacy. A rational recommendation emerging from both seasons is a single application of 4,000-5,000 mL ha^{-1} at the 1% field severity threshold as a cost-effective strategy. Although Phase I suggested that additional applications under sustained high pressure may be beneficial, the Phase II result, in which a second application conferred no additional benefit and showed a trend toward reduced efficacy, indicates that the optimal application frequency still requires calibration; repeated sprays should therefore not be recommended as a fixed protocol until the cause of this non-additive response has been clarified. Sub-threshold dosing (<3,000 mL ha^{-1}) should be avoided, particularly in high-pressure years where it may fail to contain epidemic development. Integrating PA applications with reduced rates of chemical fungicides, as has been explored for other bio-based products, may offer synergistic benefits and merits experimental evaluation. The present study is also limited by its focus on a single PA product [19]; given that PA composition varies substantially with feedstock and pyrolysis temperature [10], the dose recommendations presented here should be validated with locally produced PA batches before wide-scale adoption.

A simple cost comparison supports the economic rationale. At prevailing local market prices in Itapúa (2024-2025), pyroligneous acid costs approximately USD 0.82 L^{-1}. At the effective doses identified here, the product cost of a single PA application therefore ranges from about USD 2.45 ha^{-1} (3,000 mL ha^{-1}) to USD 4.91 ha^{-1} (6,000 mL ha^{-1}), and was USD 2.72 ha^{-1} at the 3,325 mL ha^{-1} dose used in Phase II. By comparison, the product cost of a single fungicide application in Paraguay averages approximately USD 25 ha^{-1}. Because both PA and synthetic fungicides are applied with identical equipment and carrier volume (150 L ha^{-1}), operational application costs (labor, fuel, machinery) are equivalent, and the product-cost difference, an approximately 80-90% reduction in input cost per application, translates directly into savings for the grower. This margin is substantial enough that, even allowing for the somewhat lower grain quality (TGW) observed under PA in Phase I, PA remains an attractive option for low-input systems; nevertheless, a full economic analysis incorporating yield response and disease-pressure scenarios remains necessary to confirm net returns.

Conclusions

Across two experimental phases, PA showed potential as a complementary tool for managing foliar disease in subtropical wheat production in Paraguay, although its effects were not consistent across all variables and phases. In Phase I, PA doses of 3,000-6,000 mL ha^{-1} significantly reduced foliar disease severity relative to the untreated check in both the low-pressure (2019) and high-pressure (2023) seasons, and produced spike counts and grain yields statistically comparable to the chemical fungicide. The chemical fungicide consistently produced the highest TGW, indicating that it remained superior for grain filling, plausibly because of its systemic mode of action. The yield benefits observed in Phase I were not replicated in Phase II, where no significant treatment effect on yield was detected. This inconsistency, together with the high coefficient of variation recorded for yield in Phase II (25.86%), indicates that the experimental precision for yield in that phase was limited and that the disease-suppression results should not be interpreted as evidence of a reliable yield benefit under low disease pressure. Claims regarding the agronomic value of PA should therefore be regarded as provisional and contingent on disease pressure.

In Phase II, a single application of PA was statistically equivalent to two applications of chemical fungicide in reducing leaf-spot severity, whereas a second PA application provided no additional benefit and showed a trend toward reduced efficacy. This non-additive response, potentially attributable to cumulative phytotoxicity associated with the low pH of PA or to a non-linear induced-resistance response, indicates that the optimal application frequency of PA still requires calibration before repeated-spray programs can be confidently recommended. Overall, PA is a promising, lower-cost candidate for integration into disease-management programs in low-input wheat systems, best deployed as a protective treatment initiated at the 1% severity threshold rather than on a calendar basis. However, the proposed dose range (3,000-6,000 mL ha^{-1}) and application frequency should be validated before wide-scale adoption, and sub-threshold doses (<3,000 mL ha^{-1}, such as the 1,000 mL ha^{-1} tested here) should be avoided as ineffective and potentially counterproductive. Future research should examine: (i) the combination of PA with reduced doses of chemical fungicides in integrated programs; (ii) multi-location and multi-year validation across the agro-ecological conditions of Paraguay; (iii) the on-farm economic viability of PA-based programs, including a formal cost comparison; and (iv) the physiological basis of the non-additive response to repeated PA applications, including direct assessment of phytotoxicity.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Disclosures

Plant subjects: All authors have confirmed that this study did not involve any herbarium specimen. **Human subjects:** All authors have confirmed that this study did not involve human participants or tissue. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Data Availability Statements

The datasets (and/or code) supporting this study are available from the corresponding author upon reasonable request.

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