

Original Article

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***Alternaria* leafspot of cotton in northern Argentina: disease progress, cultivar resistance and fungicide effects under subtropical field conditions**

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Abstract

Alternaria leaf spot of cotton is a widespread foliar disease worldwide; however, information regarding disease progress and cultivar response under field conditions in northern Argentina is limited. This study aimed to evaluate disease progress, commercial cultivar performance, and the effects of fungicide application on plant growth, yield, and fiber quality. Field experiments were conducted during two growing seasons (2021–22 and 2022–23) in Reconquista, Santa Fe, Argentina, using three commercial upland cotton cultivars grown under fungicide-treated and untreated conditions, under high plant density and narrow row system. A commercial mixture of pyraclostrobin and epoxiconazole was applied at 50, 70, and 100 days after sowing. Disease incidence and severity were assessed over time, and plant growth, yield, and fiber quality traits were evaluated. Disease showed clear temporal progression in both growing seasons, reaching 100% incidence at 110 days after sowing, however, mean severity values remained moderate (<10%). The fungicide neither reduced incidence or severity nor improved yield or fiber quality. However, fungicide-treated plots showed significantly higher aboveground biomass, nearly 20% over control, suggesting a physiological effect unrelated to disease suppression, which warrant further research. Despite low disease pressure, cultivars differed in their response to infection, with NuOpal showing the highest susceptibility and Guazuncho 4 the lowest disease accumulation. *Alternaria* leaf spot of cotton progresses in northern Argentina even under unfavorable environmental conditions for fungal epidemics. Significant and consistent differences among cultivars were detected across seasons. Under low disease pressure observed in both seasons evaluated, fungicide applications did not significantly affect disease severity, yield or fiber quality.

Keywords: *Alternaria* leaf spot of cotton, cotton cultivar resistance, pyraclostrobin–epoxiconazole, Argentine cultivars

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Introduction

Cotton (*Gossypium* spp.) is a crop of global importance due to its role as the main source of natural fiber. During 2024–25 cotton season, approximately 30.7 million hectares were cultivated worldwide, with total fiber production exceeding 25 million tons (1). Among cultivated species, upland cotton (*G. hirsutum* L.) accounts for more than 90% of global cotton

fiber production, followed by Pima cotton (*G. barbadense* L.) (2).

In Argentina, cotton production is based exclusively on upland cotton, and during the 2024–25 growing season, more than 600,000 ha were sown in the country, being Santa Fe province the principal producing region, covering 237,000 ha of sown area, with average fiber yield of 510 kg ha⁻¹ respectively (3). Cotton is a strategic crop for northern

Argentina and Santa Fe province, where the entire cotton agro-industrial chain is concentrated, providing employment and economic profit to various families.

However, global cotton crop production is frequently constrained by biotic stresses, among which fungal foliar diseases represent a major limitation (4-10). These diseases have demonstrated reduction of photosynthetically active leaf area, acceleration of leaf senescence and defoliation, and decrease in fiber yield and quality (11, 12).

Among foliar diseases, *Alternaria* leaf spot of cotton (ALS) is a worldwide disease, with reports of yield losses in several cotton producing countries like India (7, 9, 13), Israel (14, 15), Turkey (16) and Zimbabwe (17). In the United States, Faske et al., (18) estimated losses of approximately 2,100 tons due to ALS, and reports of severe defoliation were reported in different cotton growing states like Mississippi (19), Texas (12, 20), Louisiana (21), and New Mexico (22, 23). In Argentina, information regarding yield losses related to ALS is currently unavailable.

ALS symptoms include necrotic lesions on cotyledons, leaves, bracts, petioles and in some cases stems and bolls (16). Initial lesions appear as small pinhead-size spots surrounded by a purple halo, which may expand and coalesce as disease progresses (24), forming irregular shapes and leading to desiccation of cotyledons (25) and premature leaf senescence and defoliation of leaves (11, 21, 26-28).

ALS severity is influenced by cultivar susceptibility, plant nutrition and environmental conditions during crop growth. The disease is frequently associated with stress factors such as drought (29), nutrient imbalance—particularly potassium deficiency—(11, 30-33) and premature leaf senescence (17, 21, 34-39). Under predisposing conditions, ALS may cause substantial economic losses (12, 40).

Management of ALS relies primarily on the use of cultivars with partial resistance and adequate crop nutrition to prevent its incidence (22, 31, 33). When disease symptoms are evident, chemical control using foliar fungicides application is a commonly adopted management decision (6, 28, 41).

In Argentina, ALS is a foliar cotton disease present across cotton growing seasons and regions (42-45). However, despite its widespread occurrence, information regarding disease progress under field conditions, performance of commercial cultivars against ALS, and effectiveness of foliar fungicides remains limited. Particularly, potential impact of fungicide applications on disease development and progress, plant growth, yield and fiber quality under environmental conditions of northern Argentina has not been evaluated.

Therefore, the aims of this study were to: (i) characterize ALS progression under field conditions in northern Argentina; (ii) compare Argentine commercial cotton cultivars to ALS; (iii) explore the response of ALS and cultivar performance to a commonly used foliar fungicide under natural disease conditions.

Materials and Methods

Experimental design

Field experiments were conducted during two consecutive cotton growing seasons, 2021-22 (S1) and 2022-23 (S2), at the Reconquista Agricultural Experimental Station of INTA,

Santa Fe province, Argentina (29° 15' S; 59° 44' W, 50 m alt). Sowing dates were Oct-18 2021, for S1, and Dec-16 2022, for S2.

The experimental site is located in a subtropical-subhumid dry transition region, whose rainfall distribution concentrates 70 % of precipitations between October and March (46). The soil type was classified as a silt loam Aquertic Argiudoll (USDA, Soil Taxonomy), Reconquista series (47), presenting the following properties: soil organic matter 1.86 %, available P 23.6 mg kg⁻¹, inorganic N 68.9 mg kg⁻¹ and available K 0.60 cmol kg⁻¹.

In each season, a split-plot design arranged in randomized-complete blocks with four replications was used. Fungicide treatment (fungicide application vs. untreated control) was assigned to the main plots, and cotton cultivars as the sub-plot factor. Three commercial upland cotton cultivars with intermediate cycle to maturity, widely grown in Argentina, were evaluated: Delta Pine 1238 (DP 1238), Guazuncho 4 INTA BGRR (Guazuncho 4), and NuOpal. These genotypes represent more than 65% of cotton grown area in Argentina and Santa Fe province (48, 49). All cultivars contain transgenic traits for resistance to both glyphosate herbicide and lepidopteran insects (50).

Each experimental plot consisted of eight 5-m long rows with 0.52 m of row spacing, commonly known as narrow-row planting system, widely used by farmers in Santa Fe province. Plant density was approximately 192,000 pl ha⁻¹, a high plant density scheme, consistent with local agronomic practices used by farmers in Argentina (50). Fertilization was carried out in two stages: at sowing time with 100 kg ha⁻¹ of diammonium phosphate (18-46-0) and at early vegetative growth with 100 kg ha⁻¹ of urea (N-P-K: 46-0-0). Each experiment was managed according to its individual requirements for insect pest control. ALS developed naturally in the studied plots and no other foliar disease was observed during the study.

To evaluate the effects of foliar fungicide on ALS development, plant growth, yield and fiber quality traits, three applications were performed at 50, 70, and 100 days after sowing (DAS), covering cotton reproductive stage from flowering to boll opening phenological stages (51). A commercial mix (OPERA - BASF) of pyraclostrobin (13.3%) and epoxiconazole (5%) was sprayed at a rate of 0.5 L ha⁻¹. This fungicide is used in Argentina to control foliar diseases of cotton (43).

Meteorological data

Meteorological data were recorded throughout each growing season with the INTA Reconquista weather station located 250 m from the experimental site. Daily records of air temperature, relative humidity (RH), and rainfall were obtained and summarized as monthly mean values for each mentioned condition.

Disease progression measurements

ALS progress was assessed at 60, 80 and 110 DAS, along with flowering, cut out and boll opening crop stages respectively (51). In each plot, 20 monopodial adult fully expanded leaves from the middle part (from node 7 to 13) of different plants were sampled to assess ALS incidence and

severity. Incidence was considered as the percentage of sampled leaves showing at least one spot of ALS of the total leaves evaluated within each plot. Severity was estimated as the mean percentage of leaf area affected with the disease of the 20 leaves sampled, using a diagrammatic scale made from the one published in Fantin et al. (52) but adapted for ALS (Figure 1).

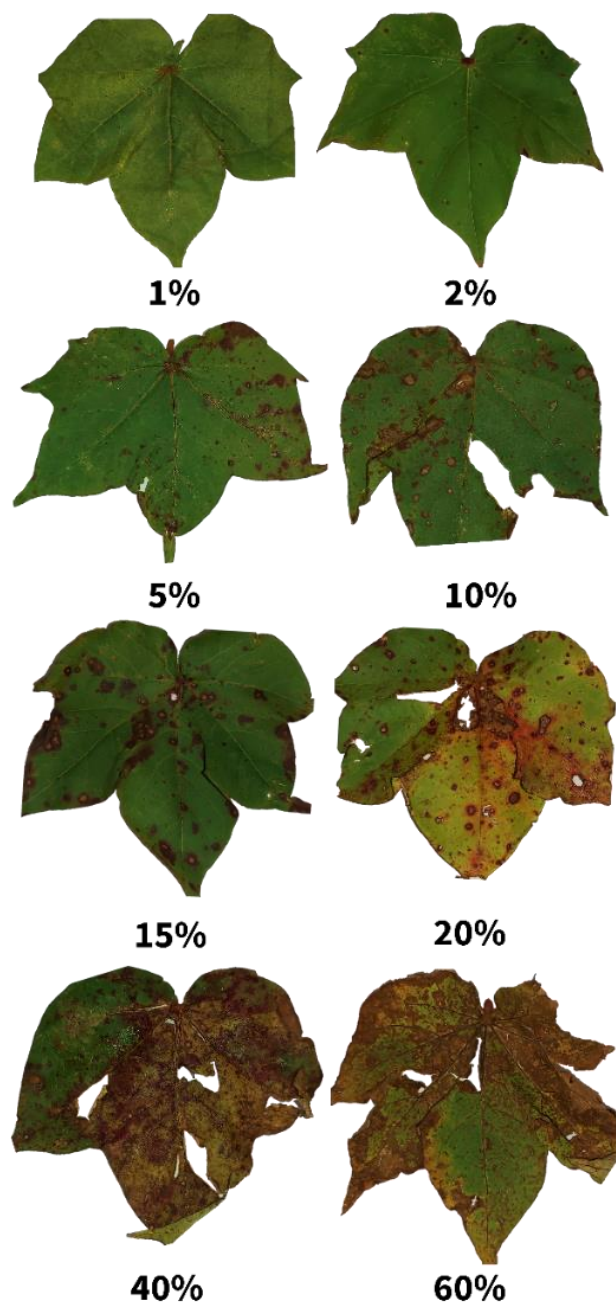


Figure 1. Diagrammatic scale for quantification of severity (%) of ALS in cotton leaves, developed for the present work.

To describe disease progress over time, both incidence and severity were plotted against DAS. In addition, the area under the disease progress curve (AUDPC) was calculated

for disease severity according to the trapezoidal integration method described by Shaner et al. (53):

$$AUDPC = \sum_{i=1}^n \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Where y_i is the disease severity at time t_i , and n is the total number of assessments.

Plant growth measurements

We measured aboveground plant biomass as an indicator of growth and physiological status. Samples were obtained from each plot at three different moments after sowing, at 60, 100 and 130 DAS, according to flowering, cut out and physiological maturity stages of the crop, respectively (51). In each plot, 0.5 m² of plants from the central rows (to avoid border effects) were cut at soil level and separated into leaves, stems, and reproductive structures. All biomass samples were oven-dried at 65 °C until constant weight. Dry weights of individual plant components were recorded, and total dry biomass (TDB) was calculated as the sum of all fractions. Values of weight obtained were transformed into gm⁻² values. Also, plant height was measured, before partitioning for 130 DAS samples, from the base of stem to the top of the apex of sampled plants and expressed in cm values.

Plant mapping, cotton yield and yield component measurements

At crop maturity, 0.5 m² of plants from the central rows were cut and Global Retention (GR) was determined as the ratio of the total number of bolls by the total number of reproductive structures generated per plant (54, 55). For yield determination, bolls from the central rows were manually harvested and seed cotton fiber was collected. Seed cotton fiber was ginned using a laboratory gin machine, and Fiber yield (FY) was determined by weight and subsequently converted into kg ha⁻¹ values. Yield components including boll number (BN) and boll weight (BW) were determined by collecting and weighing 25 bolls from the middle part of plants. BW was determined as the total weight of collected bolls divided by 25, and BN was determined using seed cotton yield and BW values and expressed in bolls m⁻².

Fiber quality measurements

Fiber quality was determined from FY samples obtained after ginning. Analyses were performed at the testing laboratory of the Association for the Promotion of Cotton Production in Santa Fe (APPA, Reconquista, Santa Fe), using a High-Volume Instrument (HVI) (USTER 1000). The following fiber quality parameters were measured: upper half mean length (UHML, in mm), fiber micronaire (Mic), length uniformity index (UI, in %) and fiber strength (Str, in g TEX⁻¹). All measurements were conducted following standard testing procedures recommended for cotton fiber evaluation.

Statistical Analysis

Statistical analyses were performed using Navure software (56), with a significance level across all analyses of $\alpha = 0.05$.

Linear mixed models were used to analyze severity of ALS, AUDPC, plant growth parameters, yield and fiber quality. Model assumptions of normality and homoscedasticity were verified graphically. Particularly, ALS severity data was log-transformed to satisfy the assumption of normality and re-transformed values were informed in results.

For variables measured at different times (Biomass and ALS Severity) a repeated measures analysis was conducted. Sampling moment (Mom), Varieties (Var), Fungicide treatment (Tr), and all interactions between them were considered as fixed effects of the models. Cotton growing Season, Block nested within each Season, and Main Plot within each Block were considered as random effects (structure: 1|Season/Block/MainPlot). For repeated measures analysis, the individual plot was considered a random effect nested within each Main Plot to model temporal correlation (structure: 1|Season/Block/MainPlot/Plot).

When heterogeneity of variance was detected, it was modeled using VarPower function, and variance structures were compared using AIC to select the best fitting model. For post-hoc comparisons, DGC test was used (57). Non-parametric tests were used for ALS incidence as it did not satisfy the statistical assumptions of normality and homoscedasticity. The Kruskal-Wallis ANOVA for ranks test (58) was used to evaluate incidence over time, and differences between varieties at each time. Post-hoc pairwise comparisons were made using the Wilcoxon test with Bonferroni correction. Additionally, the Mann-Whitney U test was employed to evaluate differences between fungicide treatments over each time.

All figures showed in this study were created using RStudio (59) with the package ggplot2 (60).

Results

Climatic data

Mean temperature records

Both S1 and S2 evaluated cotton seasons recorded mean and maximum temperature values that exceeded respective historical records for December, January and February (Figure 2). As leaf sampling was conducted during reproductive period in both seasons, the sampling moments corresponded with higher temperature periods. Particularly, for S2, although average temperatures were above historical records, they were lower than the corresponding mean values for S1 when plants were at the reproductive period. The studied seasons were considered the warmest cotton seasons recorded from 1970 to that date of time (61, 62).

Mean Relative Humidity

Both seasons recorded RH monthly mean values lower than the corresponding historical mean records during plants reproductive periods. Particularly for S1, RH was close to 55% by the time the crop was beginning to open the capsules, while in S2 during the mentioned stage mean RH was over 70%. These low RH monthly mean values could have influenced, in both evaluated seasons, the dynamics of fungal infections in cotton leaves in the field.

Mean Rainfall

Mean rainfall was lower than the historical records when cotton plants were at the reproductive period. Particularly, for S2 in March, when cotton plants were at the end of flowering stage, rainfall recorded was greater than 100 mm. In both seasons, extended dry intervals and high temperatures were recorded during flowering and boll development, with mean air temperatures exceeding 28 °C and RH below 80%. The mentioned conditions of the seasons studied were considered unfavorable for the development of severe fungal epidemics.

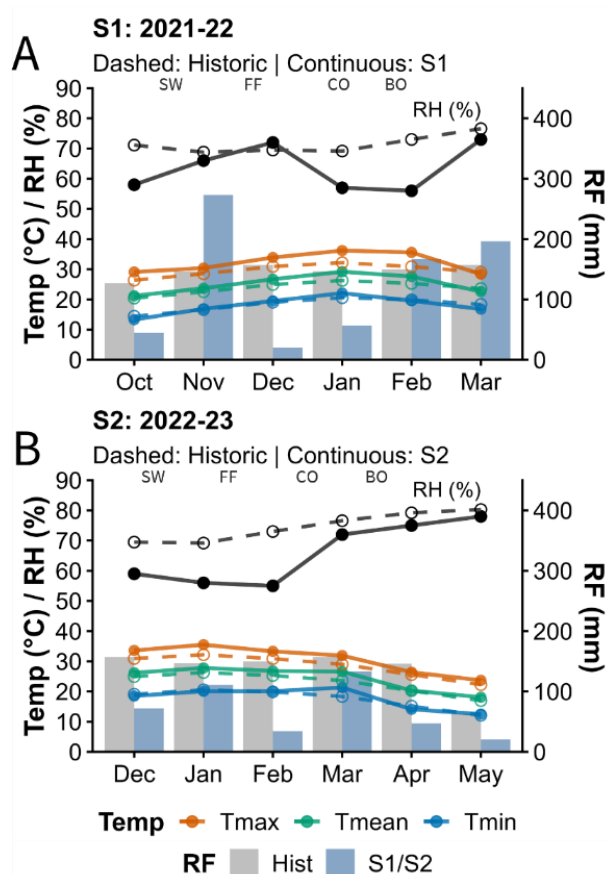


Figure 2. Weather conditions during S1: 2021-22 (A) and S2: 2022-23 (B) studied cotton growing seasons at Reconquista, Santa Fe, Argentina. Monthly mean air temperature (Temp, °C), relative humidity (RH, %) and monthly accumulated rainfall (RF, mm) from S1, S2 and historical data (Hist) obtained from INTA Reconquista Agrometeorological Station (1970-2023). Tmax: maximum mean temperature. Tmean: mean temperature. Tmin: minimum mean temperature. SW: sowing; FF: first open flower; CO: cut out (flowering end); BO: boll opening.

Disease measurements

ALS Incidence and Severity progress

The analysis revealed a significant increase of Incidence over time ($p < 0.05$). At 60 DAS all plants presented at least 55% of Incidence of ALS, increasing steadily and reaching 100% of leaves affected by 110 DAS in all treatments and cultivars (Figure 3). For cultivars, incidence was different only at 80 DAS ($p < 0.05$), being NuOpal the genotype that reached an

ALS of 100%, and the other cultivars reached values of over 90%. However, fungicide application did not significantly affect ALS incidence as no differences were detected between fungicide-treated and untreated plots at any evaluation time ($p>0.05$).

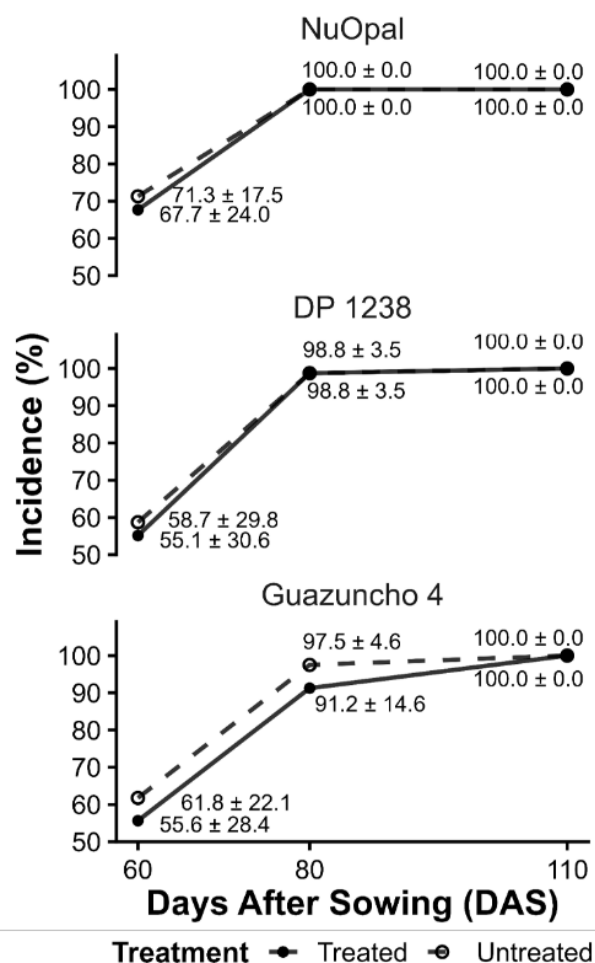


Figure 3. Progress of *Alternaria* leaf spot incidence (%) in cotton cultivars under fungicide-treated and untreated conditions during the experiments. Mean $\pm$ standard deviation values of 20 independent leaves from each plot and sample time are shown. Four replicates per treatment and cultivar were assessed.

Disease severity also increased significantly over time ($p<0.05$), following a pattern consistent with that observed for incidence (Figure 4). Severity levels were generally higher in S2 than in S1 throughout DAS (data not shown). Cultivars significantly differed in their severity response to ALS ($p<0.05$). NuOpal consistently exhibited higher severity values throughout the evaluation period, whereas Guazuncho 4 showed lower disease severity in both growing seasons. DP 1238 displayed an intermediate response. Similar to Incidence, fungicide application did not significantly reduce disease severity ($p>0.05$), and severity progression curves were similar between treated and untreated plots.

ALS AUDPC of Argentinian Cotton Cultivars

The ANOVA performed on the AUDPC values showed significant differences for cotton cultivars in the period evaluated ($p < 0.0001$; Figure 5). Fungicide treated and untreated plots achieved similar levels of AUDPC ($p>0.05$), and no significant interaction Treatment*Cultivar was found ($p>0.05$). Overall, AUDPC values were higher in S2 than in S1.

NuOpal exhibited the highest AUDPC values, Guazuncho 4 the lowest, and DP1238 showed an intermediate response. It is notable that in global terms, for all cultivars, in S2 the highest values for AUDPC were achieved.

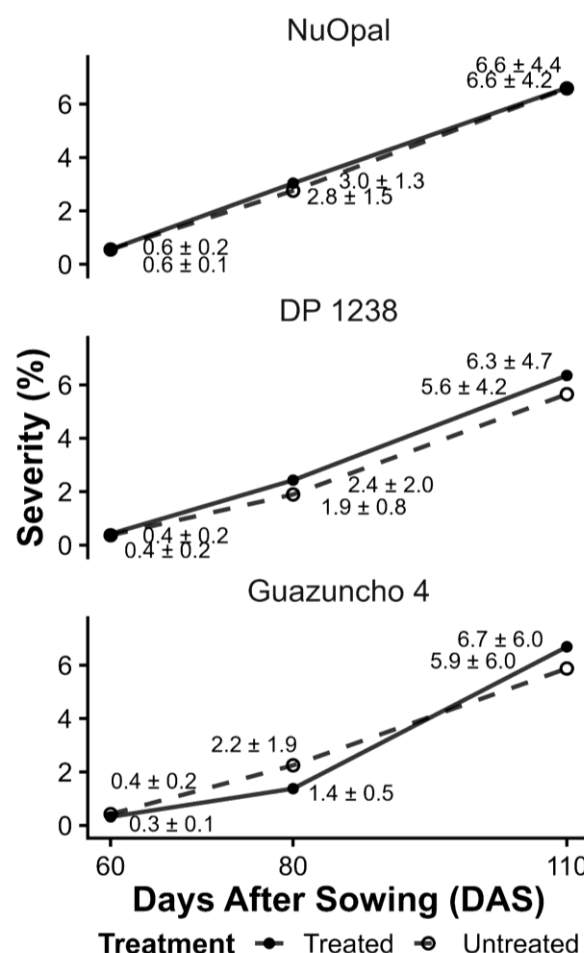


Figure 4. Progress of *Alternaria* leaf spot severity (%) in cotton cultivars under fungicide-treated and untreated conditions during the growing seasons evaluated. Disease severity was visually estimated on fully expanded leaves located in the middle canopy. Mean $\pm$ standard deviation values of 20 independent leaves from each plot and sample time are shown. Four replicates per treatment and cultivar were assessed.

Plant growth measures

Total aboveground dry biomass exhibited significantly higher values in fungicide treated than untreated plots, over all DAS evaluated ($p<0.05$; Figure 6). For treated plots, at

130 DAS, TDB was almost 20% higher than untreated plots. No other effect or interaction was found significant for TDB ($p>0.05$). Seasonal differences were also observed, with overall biomass values being higher in S2 than in S1 (data not shown). Fungicide treatment did not produce significant effects for plant height nor the other biomass components evaluated (data not shown). Even TDB was similar between cultivars, other partition parts showed significant differences among cultivars indicating that the commercial genotypes studied in this work were different in terms of their growth and plant assimilate partition.

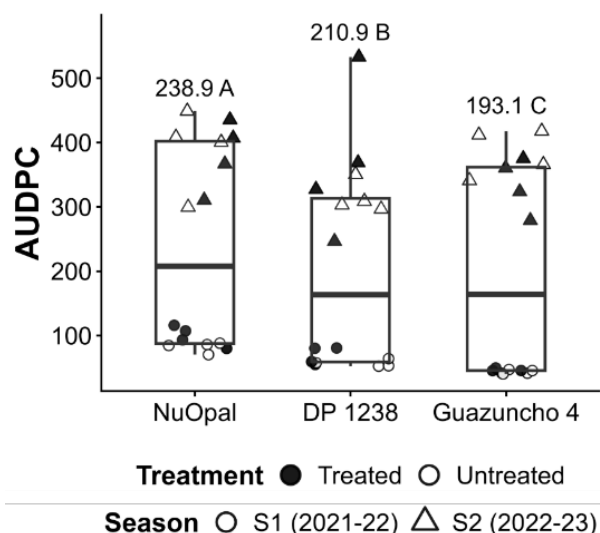


Figure 5. Area Under the Disease Progression Curve (AUDPC) values for ALS severity for cotton cultivars evaluated in the seasons considered (S1 and S2) for fungicide-treated and untreated plots. Above the box plots are shown the model-adjusted means for each genotype. Letters indicate significant differences (DGC test, $p<0.05$).

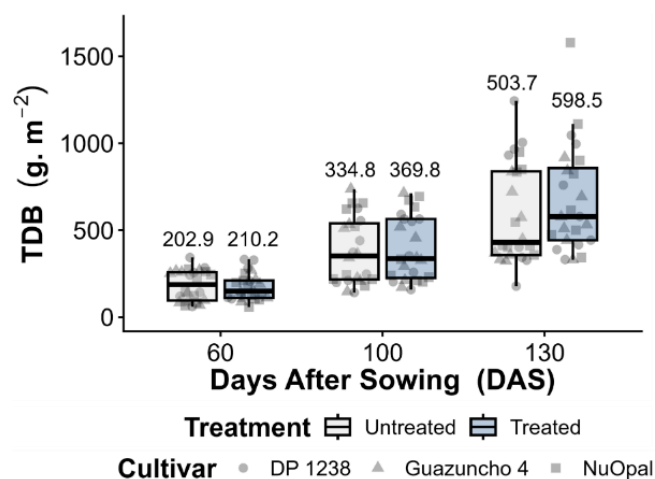


Figure 6. Total aboveground dry biomass of the three cultivars grown under fungicide treated and untreated

conditions during evaluated seasons S1 and S2. Values above boxplots indicate model adjusted means from ANOVA.

Crop yield, yield components and fiber quality traits

ANOVA indicates that fungicide treatment did not significantly affect GR, yield (FY), yield components (LY, BN, BW) or fiber quality traits (UHML, UI, Mic, Str) ($p>0.05$; Table 1). Additionally, no significant interaction between fungicide treated plots and cotton cultivars was found at any of the mentioned variables. Cultivar effects were observed for GR, FY, LP and all fiber quality traits ($p<0.05$).

Discussion

ALS progress over time

Our results indicate that ALS progresses in cotton crop in northern Argentina, in terms of incidence and severity, even under unfavorable conditions for fungal epidemics. In seasons S1 and S2, ALS reached 100% incidence by late reproductive stage, and disease severity increased gradually and remained at low levels by the end of crop cycle. These low values are closely related to constraining weather conditions registered during evaluated seasons, with average maximum temperatures that exceeded 30 °C, and relative humidity values below 80%, limiting ALS development and resulting in low severity levels. These results are consistent with reports which show that ALS is influenced by humidity and temperature (9, 11, 12, 21, 28), favorable with temperatures between 20 and 30 °C and relative humidity over 80% and inhibited when temperatures are high and relative humidity is low (29).

Over the world, ALS is caused by two mainly cited *Alternaria* species: *A. alternata* and *A. macrospora*, being the second one considered more aggressive (11). Although pathogen identification was not formally carried out in the present study, preliminary observations and isolations conducted in the region across cotton seasons have frequently recovered *A. alternata* from symptomatic cotton leaves. This observation, together with previous reports from other cotton-growing regions like USA (23), Australia (25) and Turkey (16), could suggest that less aggressive *Alternaria* species may be involved in ALS. The predominance of a less aggressive *Alternaria* in northern Santa Fe could partially explain, along with unfavorable weather conditions, the low severity values observed through seasons. However, this assumption must be taken cautiously, as we have not carried out specific field surveys to identify the pathogen in the present study. Therefore, further studies involving pathogen identification in northern Argentina are needed to establish their possible relationship with low disease severity values observed.

Despite warmer and drier conditions, ALS established and progressed in cotton crop in northern Argentina. However, low RH and scant rainfall records limited ALS lesion expansion and inhibited conidia formation and dissemination, so disease severity remained low despite its establishment. Our results suggest that ALS is a disease present in cotton in Argentina and progressing over time but without influencing high damage to the crop in seasons unfavorable for fungal epidemics.

Table 1: Model-adjusted means from ANOVA on Global Retention (GR), Fiber Yield (FY), yield components: Lint Percentage (LP), Boll Number (BN) and Boll Weight (BW), and Fiber Quality traits: Fiber Length (UHML), Uniformity Index (UI), micronaire (Mic), and Strength (Str); for Fungicide treatment (Tr), Cultivar (Cv) and Interaction (Tr*Cv). Different letters indicate significant differences: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. NS: not significant ($p > 0.05$). SOV (Source of Variation)

SOV	Retention	Yield and components				Fiber Quality traits			
	GR (%)	FY (kg ha ⁻¹)	LP (%)	BN	BW (g)	UHML (mm)	UI (%)	Mic	Str (g TEX ⁻¹)
Tr	NS	NS	NS	NS	NS	NS	NS	NS	NS
Treated	19.69	574.14	38.42	34.60	3.59	24.84	78.78	4.29	25.43
Untreated	19.38	570.79	38.61	34.33	3.73	25.02	79.27	4.32	26.24
Cv	**	*	***	NS	NS	*	***	***	***
Guazuncho 4	20.90A	587.15A	37.28B	35.29	3.58	24.47B	78.09B	3.79C	24.44B
DP 1238	19.14B	579.33A	40.99A	33.96	3.69	25.16A	79.89A	4.72A	26.72A
NuOpal	18.57B	550.90B	37.28B	34.14	3.70	25.16A	79.10A	4.39B	26.35A
Tr*Cv	NS	NS	NS	NS	NS	NS	NS	NS	NS

Cotton commercial varieties in Argentina differ in terms of resistance against ALS

Significant differences were found between cultivars evaluated against ALS in the seasons studied. Although none of them showed complete resistance, our results demonstrate the existence of different quantitative resistances between cultivars, as they showed significant differences in terms of incidence, severity and AUDPC. NuOpal achieved earliest Incidence and higher Severity and AUDPC, followed by DP 1238 and Guazuncho 4 showing the highest level of partial resistance to ALS. Similar cultivar-dependent responses to ALS have been reported in several works around the globe like USA (22), Brazil (38, 40, 63, 64), and India (65), showing high diversity of quantitative and environmentally variable resistance. To our knowledge, this is among the first report of different levels of resistance among commercially available cultivars to fungal leafspot pathogens in Argentina. Even under low ALS disease pressure, cultivar behavior was consistent among two consecutive growing seasons, suggesting stable quantitative resistance responses.

Even in unfavorable seasons for ALS aggressiveness, the existence of differential ALS resistant cotton genotypes provides new prospects for integrated management of the disease, especially for genetic disease control, and potentially in seasons or growing regions with predisposing climates for ALS development during cotton crop cycle. In the absence of yield loss, ALS susceptible genotype use could increase fungal inoculum and establish possible future disease problems (38). In addition, in seasons and regions with favorable conditions for ALS development during crop cycle, the use of highly susceptible germplasm could lead to premature severe defoliation.

Future research should involve multiple cotton cultivar screening in contrasting environmental seasons, to determine a wider stability of the behavior against ALS found among Argentine commercial cultivars. In addition, the findings presented establish that ALS field resistance is shown even in unfavorable disease seasons. These results could be useful tools to determine quantitative disease responses for cotton breeding programs.

Effect of fungicides over disease progress, plant growth, yield, and fiber quality

The present work showed that three foliar applications of commercial doses of pyraclostrobin and epoxiconazole did not produce significant reduction of ALS incidence, severity or AUDPC under the climatic conditions registered. However, these results must be interpreted with caution because the study presents limitations, as ALS severity remained low during both growing seasons. The low disease pressure could have restricted the ability to fully assess fungicide performance.

In this context, these findings are associated with the unfavorable meteorological conditions observed during evaluated seasons, which resulted in low ALS pressure and limited the opportunity for fungicides to express their disease-control potential. Consequently, no measurable disease-control benefit was detected.

There are multiple studies evaluating the effect of ALS in cotton crop yield and quality loss, and the effect of different fungicide molecules over ALS control and productivity in different countries like India (7, 9, 66-69), USA (12) and Israel (14, 15). In Argentina, the only work that evaluated fungicides on cotton crop is the study of Tarragó et al. (43) in Chaco province. In the study mentioned, the authors report yield and fiber quality gain when using the fungicides of the present study, as it was particularly effective over *Ramularia* leaf spot and ALS severity decrease. Our work is the first study of leaf diseases of cotton in Santa Fe province. However, in the present work the only disease registered was ALS in low levels of severity in both evaluated seasons, and no significant improvement of boll retention, yield or fiber quality was achieved in treated plots compared to control.

In this context, several works across different states of USA (21, 33, 35, 70) are consistent with our findings, as they have reported that during seasons with unfavorable environmental conditions for disease development, resulting in low ALS pressure, fungicide application did not significantly improve yield or fiber quality.

As shown previously, climatic conditions are determining factors for ALS disease pressure and therefore fungicide measurable effects on disease control. As previous studies

showed disease control over ALS using foliar fungicides in disease-favorable conditions (7, 9, 12, 14, 15, 43, 66-69), the findings in our study may not represent higher disease pressure scenarios. Future studies should clarify the fungicide mix used efficacy over ALS in more favorable disease scenarios, in order to establish whether benefits in disease control could be measured. However, despite lack of ALS measurable control, fungicide-treated plots exhibited 19,5% higher total aboveground dry biomass compared with untreated plots at 130 DAS. This significant increase in TDB could be explained by physiological effects provoked by fungicides, but independent of disease control. One effect that could explain the higher biomass is the “stay-green” effect observed when crops are treated with strobilurin based fungicides. Previous studies have associated this effect with senescence delay of leaves, photosynthetic efficiency promotion by ethylene modulation and nitrogen assimilation dynamics (71-74). While the increase in TDB found in this work is consistent with these previously reported physiological effects, the mechanisms involved were not evaluated in the present study and therefore remain speculative. Further studies involving leaf senescence measures, nitrogen enzyme related activities, photosynthesis efficiency or hormone implications should be developed to deepen knowledge of mechanistic approaches involved in biomass accumulation by fungicide application in cotton.

However, the previous finding was not accompanied by increases of boll retention, fiber yield or quality. The proposed stay-green effect achieved by fungicide application could have influenced photosynthetic carbon uptake, increasing plant source activity, but without being reflected in higher reproductive allocation to reproductive structures, therefore not increasing boll retention, or sink-associated variables.

In this aspect, Scarpin et al. (50, 55) showed that even with significant genetic progress of cotton yield in Argentine cotton commercial cultivars (including the ones evaluated in the present study), no significant progress of TDB was achieved, indicating that yield could have a better association with improved reproductive allocation or higher boll retention than increments in TDB. These findings could partially explain our results, as even with increments in above-ground biomass, no significant higher yield and quality values were obtained. Further studies should involve the relationship between productivity and biomass accumulation effects induced by fungicides in cotton.

Limitations of the study

Our work underlines some limitations. At first, pathogen identification was not conducted and only one fungicide mixture was evaluated. Moreover, physiological approaches were not studied to fully explain biomass accumulation by fungicide treatment. Therefore, conclusions regarding fungicide effectiveness should be restricted to the environmental conditions and disease levels observed in this study.

Future research should focus on elucidating physiological bases of biomass effects caused by fungicide applications, involving different molecules, doses and evaluated regions. In addition, pathogen molecular identification should

provide with valuable information about the disease in Argentina and the interaction with fungicide sensitivity and commercial cultivars responses.

Conclusion

Our findings confirm that *Alternaria* leaf spot of cotton is an established and progressive disease in the region of northern Argentina, even under not favorable climatic conditions for fungal epidemics. In addition, Argentine commercial cotton cultivars differed significantly in their resistance to the disease. Guazuncho 4 showed the lowest disease accumulation, followed by DP1238, while NuOpal was the most susceptible genotype. The consistency of these differences across two seasons suggests stable basis for resistance, with practical implications for cultivar selection for cotton breeding in Argentina. Under the low disease pressure observed, three foliar applications of pyraclostrobin + epoxiconazole did not significantly affect disease progress, yield or fiber quality. However, while fungicide applications increased almost 20% total aboveground dry biomass, the proposed physiological responses independent of measurable disease suppression remains hypothetical and warrant further research.

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