The Impact of Temperature and Humidity on Disease Development and Severity of *Burkholderia glumae* in Rice (*Oryza sativa* L.)

Hamood U Rehman[1,2], Norida Mazlan,[1,3], Siti Izera Ismail,[2,3], Nur Azura Husin [4]

[1]Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia, Serdang-43400, Selangor, Malaysia.

[2]Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, Serdang-43400, Selangor, Malaysia.

[3]Laboratory of Climate-Smart Food Crop Production, Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, Serdang-43400, Selangor, Malaysia.

[4]Department of Computer Science, Faculty of Computer Science and Information Technology, Universiti Putra Malaysia, Serdang-43400, Selangor, Malaysia.

Corresponding author: noridamz@upm.edu.my

Bacterial panicle blight (BPB), caused by *Burkholderia glumae*, is a devastating disease of rice, with its severity exacerbated by warm and humid conditions, posing a significant threat to global food security. This study aimed to quantify the relationships between environmental factors, disease development, and yield loss, and to define specific thresholds for predictive disease management. A controlled glasshouse experiment was conducted using a susceptible rice variety, with disease progression, panicle sterility, and microclimate data monitored following inoculation. Results demonstrated a significantly higher area under the disease progress curve (AUDPC) in inoculated plants ($p < 0.001$). A strong positive correlation was identified between final disease severity and panicle sterility ($y = 0.58x + 16$, $R^2 = 0.77$), confirming the direct impact of BPB on yield. Furthermore, receiver operating characteristic (ROC) analysis established actionable forecasting thresholds, identifying maximum relative humidity >83.7% (AUC = 0.773) and temperature >29.7°C as robust predictors of economic damage. This study provides a quantitative framework that bridges the gap between disease epidemiology and practical management. The defined thresholds enable the development of a precision forecasting system, empowering farmers to implement timely interventions and advancing the goal of sustainable, climate-resilient rice production.

Key words: Rice pest management, Bacterial panicle blight, Bacterial grain rot

Introduction

Rice *(Oryza sativa L.)* is a staple food for more than half of the world's population, and its production is increasingly threatened by a range of biotic stresses, among which bacterial panicle blight (BPB), caused primarily by *Burkholderia glumae*, has emerged as a major concern in many rice-growing regions (Rezvi *et al.* 2023, Sabri *et al.* 2023). BPB is notorious for its ability to cause severe yield losses, sometimes reaching up to 75%, by inducing panicle sterility, grain rot, and significant reductions in grain weight and quality (Nandakumar *et al.* 2009, Fory *et al.* 2014a, Wahidah *et al.* 2019). The disease is characterized by a complex infection cycle, with *B. glumae* entering rice plants through infected seeds or environmental sources and maintaining itself as an endophyte during both vegetative and reproductive growth stages (Pedraza et al, 2018; Ortega and Rojas 2021; Ham et al, 2011). Studies have demonstrated that the pathogen can colonize seedlings and persist within the plant at low populations throughout the vegetative stage, before rapidly increasing its biomass as the plant transitions to the reproductive phase, particularly at the onset of panicle initiation and flowering (Li *et al.* 2016). This ability to remain dormant and then proliferate under favorable conditions makes BPB a particularly challenging disease to manage, as visible symptoms often do not appear until the critical flowering and grain-filling stages, by which time significant physiological damage may have already occurred (Ura *et al.* 2006). Climatic factors, especially temperature and humidity, play a pivotal role in the progression and severity of BPB caused by *B. glumae*.

The pathogen thrives under warm, humid conditions, with optimal temperatures ranging between 30°C and 35°C, which coincide with the flowering stage of rice in many tropical and subtropical regions (Shew *et al.* 2019). High night temperatures have been strongly correlated with increased disease incidence and severity, as they favor bacterial multiplication and toxin production (toxoflavin), which is the primary virulence factor responsible for tissue necrosis and panicle sterility (Kim et al., 2004; Kumar et al., 2023; Suzuki et al., 1998). Several field studies have documented that years or seasons with prolonged periods of elevated night temperatures and high relative humidity coincide with severe outbreaks of BPB, leading to substantial yield losses (Francis *et al.* 2013, Ramachandran *et al.* 2021). Conversely, cooler, or drier conditions tend to suppress disease development, highlighting the strong environmental dependency of BPB epidemiology (Song *et al.* 2024). This climatic sensitivity has raised concerns about the potential impact of climate change on BPB prevalence, as increasing global temperatures and altered precipitation patterns could expand the geographic range and severity of the disease (Darmawan et al, 2021).

In addition to temperature and humidity, other environmental factors such as rainfall patterns have also been implicated in influenced disease progression. Rainfall and high humidity facilitate bacterial dispersal and infection by providing the moisture necessary for bacterial survival and entry through floral tissues (Chompa *et al.* 2022). Moreover, the timing of these climatic events relative to the rice flowering stage is critical, as infection during early flowering tends to result in more severe grain rot and panicle blight symptoms (Tsushima et al, 1994). Understanding these complex interactions between climatic factors and pathogen biology is essential for developing predictive models and effective management strategies to mitigate BPB impact under varying environmental conditions (Lee et al. 2015; Shew et al. 2019; Perez-Suarez et al, 2022).

Effective management of BPB hinges on a comprehensive understanding of the disease's progression in relation to climatic factors, especially during the early flowering stage, when rice is most susceptible (Faizal Azizi and Lau 2022). Research has shown that the early flowering stage represents a critical window for infection and disease development, as the panicle tissues are more susceptible to bacterial colonization and toxoflavin-induced damage (Sayler et al, 2006). Experimental studies have demonstrated that inoculation during this stage leads to higher disease severity compared to inoculations at other growth stages, underscoring the importance of timing in disease progression (Mizobuchi *et al.* 2018, Riera-Ruiz *et al.* 2018). Moreover, the interaction between host phenology and environmental conditions modulates the severity and spread of BPB, making it necessary to integrate climatic data with phenological observations for accurate disease forecasting (Ramachandran *et al.* 2021).

Recent advances in molecular and epidemiological research have improved the detection and monitoring of *B. glumae* populations, enabling better prediction of disease outbreaks (Maeda *et al.* 2006). For instance, quantitative PCR and other molecular tools have been employed to track bacterial population dynamics on flag leaf sheaths and panicles, revealing that bacterial loads increase sharply at early flowering under favorable climatic conditions (Fang et al. 2009; Zhang et al. 2022; Kim et al. 2012). In Peninsular Malaysia and other tropical rice-growing regions, where warm and humid conditions prevail during the rice flowering period, BPB poses a significant threat to food security and farmer livelihoods (Ramachandran *et al.* 2021). Despite this, there remains a paucity of region-specific studies that comprehensively link climatic variables with disease progression and severity at critical growth stages. This gap underscores the need for localized research to develop predictive models tailored to the unique agro-climatic conditions of Peninsular Malaysia. Such models would facilitate timely interventions and integrated disease management strategies, thereby mitigating yield losses and improving rice production sustainability in changing climate patterns (Rezvi *et al.* 2023).

Materials and Methods

Experimental Site and Plant Material

The study was conducted within a glasshouse structure at Universiti Putra Malaysia (2.984269°N, 101.738036°E). The primary function of this facility was to provide shelter from direct rainfall and exclude large pests, while allowing ambient environmental conditions to prevail. Consequently, abiotic variables such as temperature and relative humidity were not artificially regulated and fluctuated naturally according to external weather patterns. This design was intentional to facilitate the investigation of correlations between these natural diurnal fluctuations and the development of BPB.

The susceptible rice variety MR 297 was utilized in this experiment. Seeds were pre-treated to break dormancy by heating at 40°C for 8 hours, followed by soaking for 18 hours. The pre-germinated seeds were subsequently transplanted into containers (30 × 34.5 × 20 cm), each filled with 8 kg of paddy soil. Each container, representing an individual experimental unit, sustained three plants. All units were spaced 50 cm apart to mitigate risks of cross-contamination and to ensure uniform distribution of sunlight.

Bacterial Inoculum Preparation

A pathogenic strain of *B. glumae* strain GH1 (GenBank accession: PX472914.1) was cultivated, and the inoculum was prepared according to the method of (Fory *et al.* 2014b). The bacterium was cultured on King's B medium and incubated at 30°C for 48 hours. Bacterial cells were then harvested, and a bacterial suspension was adjusted to 1 × 10^8 CFU/mL using sterile distilled water. At the early panicle initiation stage, plants were inoculated using a standardized spray method (Ramachandran *et al.* 2021) to ensure complete panicle coverage. Control plants were treated with an equivalent amount of sterile distilled water. Immediately post-inoculation, all plants were covered with transparent plastic bags for 72 hours to maintain high humidity (>90% RH), creating optimal conditions for infection.

Experimental Design and Disease Assessment

The experiment was established as a Completely Randomized Design (CRD) with two treatments: T1 (Control) and T2 (Inoculated with *B. glumae*), with 12 replications each (N=24). Disease incidence (DI), recorded as the percentage of infected panicles per plant, was assessed every 6 days from panicle initiation to physiological maturity. Data from six key developmental stages, panicle initiation (7 days after inoculation, DAI), early booting, late booting, flowering, milky stage, and maturity are presented herein; the complete longitudinal dataset is available in Supplementary Materials. Disease severity (DS) was assessed at flowering, milky, and maturity stages using a validated 0-5% ordinal scale (Wahidah *et al.* 2019), where 0% indicated no symptoms, 1-2% represented mild discoloration, 3-4% showed necrotic lesions, and 5% reflected severe panicle blight or grain rot. Panicle sterility, calculated as the percentage of unfilled grains relative to the total grains per panicle, was assessed at harvest. Climatic variables (temperature and relative humidity) were monitored daily to ensure environmental consistency.

Statistical Analysis

Statistical analyses were conducted using R v4.4.1. Longitudinal disease incidence data were analyzed using a linear mixed-effects model, with Treatment, Time (stages), and their interaction as fixed effects, and a random intercept for Plant ID to account for repeated measures. Post-hoc comparisons at key developmental stages were performed using estimated marginal means with Tukey adjustment. Model assumptions were validated graphically. Disease severity data were analyzed using a cumulative link model for ordinal data, and sterility data were analyzed using an independent-samples t-test. To comprehensively quantify disease progression over time, the Area Under the Disease Progress Curve (AUDPC) was calculated for both disease incidence and severity for each plant using the Agricolae package. Treatment differences in AUDPC values were evaluated using independent samples t-tests. Significance was evaluated at $\alpha = 0.05$.

Results and Discussion

Disease incidence was significantly higher in rice plants inoculated with *B. glumae* (T2) compared to control plants (T1) at all developmental stages. The disease was assessed from panicle initiation to maturity. At panicle initiation (7 days after inoculation, DAI), disease levels in control plants were negligible (0.15%), while inoculated plants showed clear infection (7.54%). This significant difference ($p < 0.0001$) continued through early booting, where T1 showed 0.30% and T2 showed 13.43%, and late booting, where T1 had 0.86% and T2 had 36.99%. The disparity between treatments widened further at later stages (Table 1). By flowering, disease incidence in inoculated plants rose sharply to 48.76%, compared to only 1.15% in controls. This trend continued at the milky stage, with T2 reaching 60.54% and T1 at 1.43%, and at maturity, where T2 had 66.43% and T1 had 1.57%. The difference between treatments remained highly significant ($p < 0.0001$) at every stage.

Table 1: Disease incidence (%) in control (T1) and inoculated (T2) plants across key developmental stages. Values are estimated marginal means ± SE.

Stage	Treatment	Mean ± SE	Lower CL	Upper CL
Panicle Initiation	T1	0.15 ± 0.59	-1.03	1.34
	T2	7.54 ± 0.59	6.36	8.73
Early booting	T1	0.29 ± 0.54	-0.80	1.39
	T2	13.43 ± 0.54	12.33	14.53
Late booting	T1	0.86 ± 0.44	-0.06	1.79
	T2	36.99 ± 0.44	36.06	37.91
Flowering	T1	1.15 ± 0.46	0.18	2.11
	T2	48.76 ± 0.46	47.79	49.73
Milky	T1	1.43 ± 0.54	0.33	2.52
	T2	60.54 ± 0.54	59.44	61.64
Maturity	T1	1.57 ± 0.59	0.38	2.76
	T2	66.43 ± 0.59	65.24	67.62

Note: Values are Estimated Marginal Means ± Standard Error (SE) derived from a linear mixed-effects model. CL = Confidence Level.

The progression of disease is shown in Figure 1. The graph illustrates the stark contrast between the two treatments, with inoculated plants showing a steep, linear increase in disease over time, while disease levels in control plants remained consistently low.

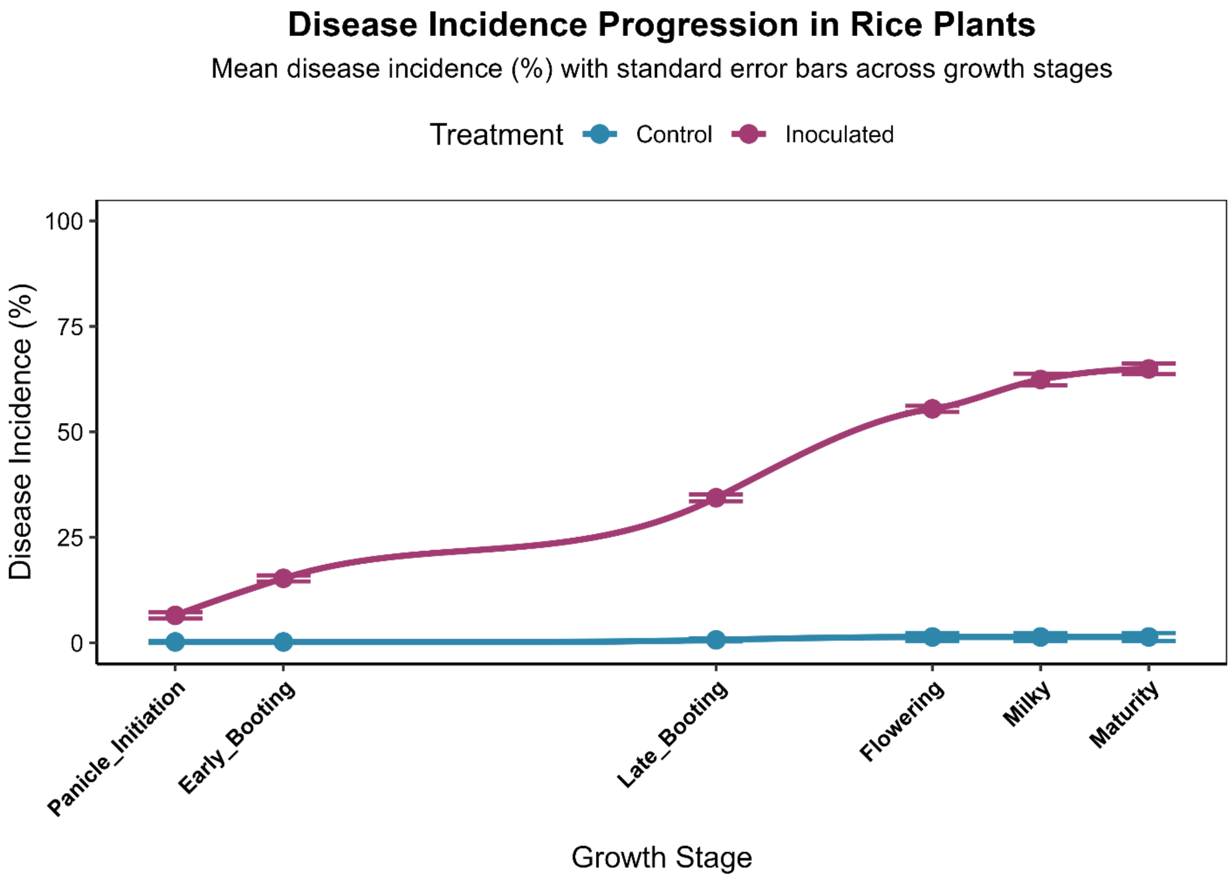

Figure 1: Disease incidence of bacterial panicle blight in control (T1) and inoculated (T2) rice plants across developmental stages. Error bars represent standard error (SE).

Statistical analysis confirmed these observations. A linear mixed-effects model showed that the rate of disease increase was dramatically faster in inoculated plants (1.96% per day) than in controls (0.05% per day). The interaction between treatment and time was highly significant ($p < 0.001$), confirming that the disease progressed differently in each group. The artificial inoculation successfully induced bacterial panicle blight, which developed rapidly and severely, while control plants remained largely healthy**.** This pattern of disease escalation, particularly post-heading, aligns with the typical epidemiology of BPB, where the pathogen rapidly multiplies during the reproductive stage when host defenses may be altered, and nutrient-rich panicles become available (Ortega and Rojas 2021).

Disease Severity Analysis

The results from the Friedman rank sum test revealed significant differences in disease severity across the growth stages in the inoculated plants (T2), with the highest severity observed at the maturity stage. The disease severity at flowering, milky, and maturity stages increased significantly (Table 2), particularly between flowering and maturity. In contrast, the control plants (T1) did not show any significant changes in disease severity across the growth stages, suggesting that the presence of *B. glumae* caused a marked increase in disease as the plants matured. These findings are supported by the post-hoc pairwise comparisons, which confirmed the substantial differences in disease severity between the stages for the inoculated plants. The table displaying the mean values of disease severity at each stage and the figure showing the distribution of disease severity across different growth stages can be placed alongside this section to provide visual clarity and further support the data presented.

Table 2: Disease Severity (%) of rice plants at different growth stages for control (T1) and inoculated (T2) treatments

Treatment	Growth Stage	Mean Severity (%)	Standard Deviation (SD)	Standard Error (SE)
T1	Flowering	0.11	0.38	0.11
	Milky	0.56	1.33	0.38
	Maturity	1.67	3.07	0.89
T2	Flowering	10.00	1.33	0.39
	Milky	30.00	4.31	1.24
	Maturity	76.67	2.70	0.77

The severity percentages represent the mean values of disease severity for each treatment at the Flowering, Milky, and Maturity stages. Standard deviation (SD) and standard error (SE) are provided for each measurement. Disease severity was assessed based on visible symptoms of Burkholderia glumae infection.

Integrated Disease Assessment Using Area Under the Disease Progress Curve (AUDPC)

To comprehensively quantify the total disease pressure over the entire epidemic period and address the temporal nature of disease progression, we calculated the AUDPC (Table 3)for both disease incidence and severity. The AUDPC is a robust, integrative metric that condenses multiple observations into a single value, providing a more complete picture of the epidemic than assessments at single time points, and is a standard for evaluating disease progression in plant pathology (Sharma *et al.* 2022).

Table 3: Area Under the Disease Progress Curve (AUDPC) for disease incidence and severity in rice plants inoculated with *B. glumae*.

Treatment	n	AUDPC Incidence[1]	AUDPC Severity[2]
T1	12	21.3 ± 13.1	4.3 ± 2.3
T2	12	968.4 ± 11.5	220.0 ± 3.4

[1] t-test for Incidence: t(21.6) = -54.384, p = <0.001

[2] t-test for Severity: t(19.5) = -52.276, p = <0.001

This analysis revealed profound and highly significant differences between the treatments (Figure 2). For disease incidence, the mean AUDPC for inoculated plants (T2) was 968.4 ± 11.5, compared to only 21.3 ± 13.1 for control plants (T1) ($t(21.6) = -54.384$, $p < 0.001$). A similarly dramatic difference was observed for disease severity, with T2 plants showing a mean AUDPC of 220.0 ± 3.4, against 4.3 ± 2.3 for T1 plants ($t(19.5) = -52.276$, $p < 0.001$). The stark contrast in AUDPC values, with minimal overlap as visualized in the boxplots, underscores the aggressive nature of the *B. glumae* infection and its successful establishment. This use of AUDPC aligns with established methodologies for bacterial rice diseases, where it serves as a primary metric to discriminate between treatments and genotypes (Adamu *et al.* 2021).

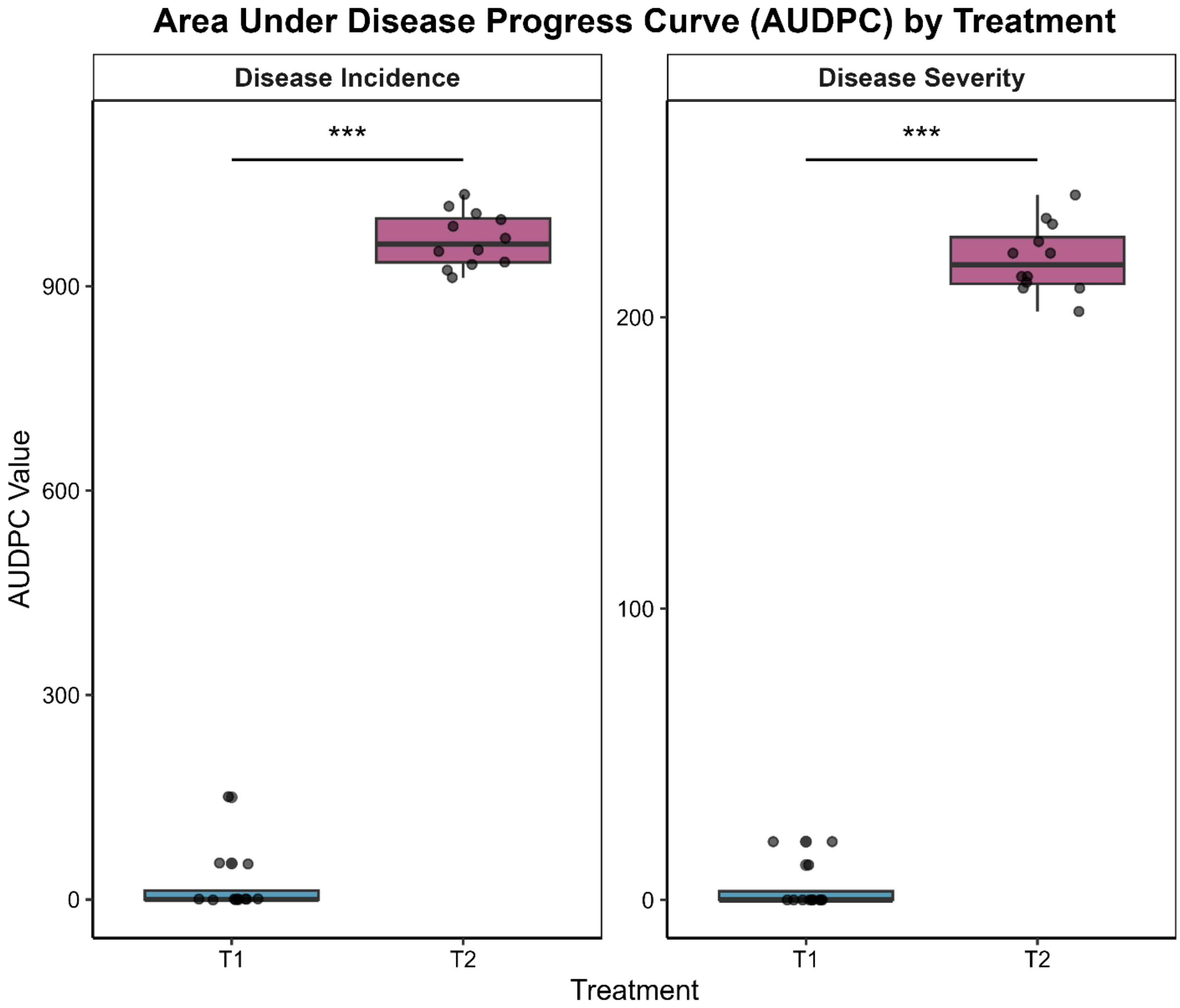

Figure 2. Area Under the Disease Progress Curve (AUDPC) for (A) disease incidence and (B) disease severity in control (T1) and inoculated (T2) rice plants. Boxplots represent the median and interquartile range; whiskers show the data range. *** denotes a significant difference at $p < 0.001$.

Throughout the experiment, the glasshouse conditions were highly conducive to BPB development. The mean maximum temperature was 34.5 ± 1.9°C (Table 4), which falls squarely within the well-documented optimal range of 30–35°C for *B. glumae* growth, infection, and toxoflavin production (Rahman *et al.* 2024). Similarly, the mean maximum relative humidity was 85.8 ± 3.4%. High humidity (≥80%) is a consistently identified requirement for BPB onset and spread, facilitating bacterial survival, dispersal, and infection through floral tissues (Shew *et al.* 2019, Safni *et al.* 2020). The combination of these warm and humid conditions created an environment highly favorable for the epidemic, as definitively reflected in the high AUDPC values of the inoculated plants.

Table 4: Environmental Conditions During Experimental Periods, Values represent mean maximum temperature/humidity ± standard deviation

Experimental Period	Max Temperature (°C)	Max Humidity (%)	Days	Notes
Entire Period (DAI 0-34)	34.5 ± 1.9	85.8 ± 3.4	34	Optimal range: 28-35°C for *B. glumae* growth[1]

[1] *Burkholderia glumae* optimal growth temperature range

Disease Incidence and Severity

A strong positive relationship was observed between disease incidence and severity at the plant level (Figure 3). Linear regression analysis revealed that disease severity was a highly significant predictor of disease incidence ($y = 0.8091x$, $R^2 = 0.9852$, $p < 0.001$), indicating that approximately 98.5% of the variation in incidence was explained by changes in severity. This demonstrates that plants exhibiting more severe symptoms on their panicles were also those with a higher proportion of infected panicles. This tight coupling of incidence and severity underscores a uniform disease response across the plant, rather than a patchy infection pattern.

The data points, each representing an individual plant's measurements at maturity, form a consistent upward trend. This relationship is particularly critical during the flowering and milky stages, when rice plants are most susceptible to pathogens like *B. glumae*.

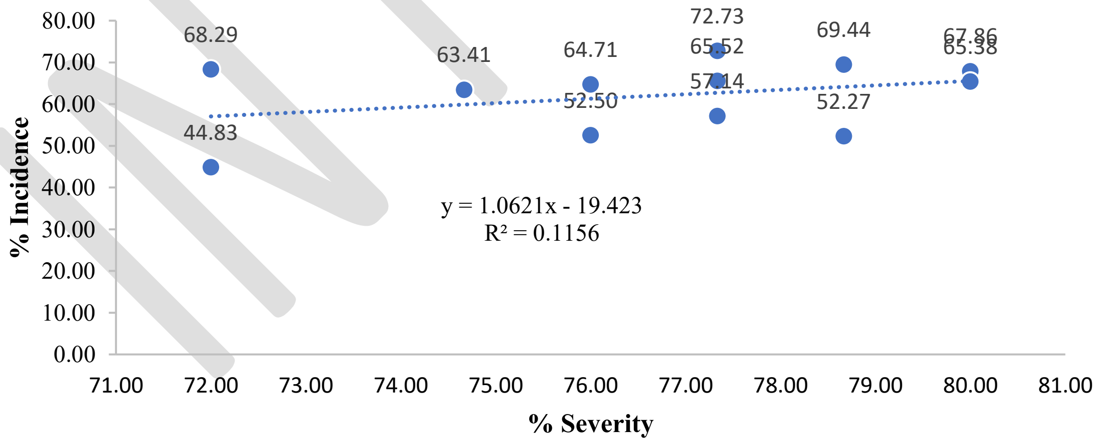

Figure 3: Relationship between disease incidence and severity of bacterial panicle blight in rice at physiological maturity. Each data point represents an individual plant (n=24). The solid regression line ($y = 0.8091x$) and shaded confidence band are shown, with the coefficient of determination (R^2) indicating the proportion of variance in incidence explained by severity.

These results are consistent with the global epidemiology of BPB. For instance, Echeverri-Rico et al. (2021) reported that in Colombia, temperatures above 34°C and relative humidity above 80% favored the spread of *B. glumae*, conditions that are congruent with the conducive environment recorded in our study. Their finding that minimum temperatures above 23°C post-flowering correlate with disease incidence aligns with our results, highlighting the role of warm, humid conditions in driving both disease severity and incidence.

The synergistic increase in incidence and severity provides a clear mechanism for the substantial yield losses documented in BPB outbreaks. Previous research has reported yield losses up to 75% in severe epidemics (Echeverri-Rico *et al.* 2021). When a high percentage of panicles are infected (high incidence), and the infections are destructive (high severity), the cumulative damage is amplified, leading to significant yield reduction. The strong, positive correlation observed in our study is therefore consistent with global observations of BPB and confirms that these two disease components act in concert to determine overall crop damage. The consistency of these findings across different geographical regions reaffirms temperature and humidity as fundamental drivers of BPB epidemics.

Disease Severity and Sterility

The relationship between disease severity and panicle sterility revealed a significant positive correlation (Figure 4). The regression ($y = 0.58x + 16$, $R^2 = 0.77$) demonstrates that disease severity is a primary determinant of yield loss, accounting for 77% of the variation in sterility. This finding is consistent with the pathophysiological model established by (Tsushima *et al.* 1994), who identified severely diseased panicles as key predictors of yield loss. The strength of this correlation underscores the value of visual disease assessment as a reliable proxy for predicting economic damage in BPB-affected rice.

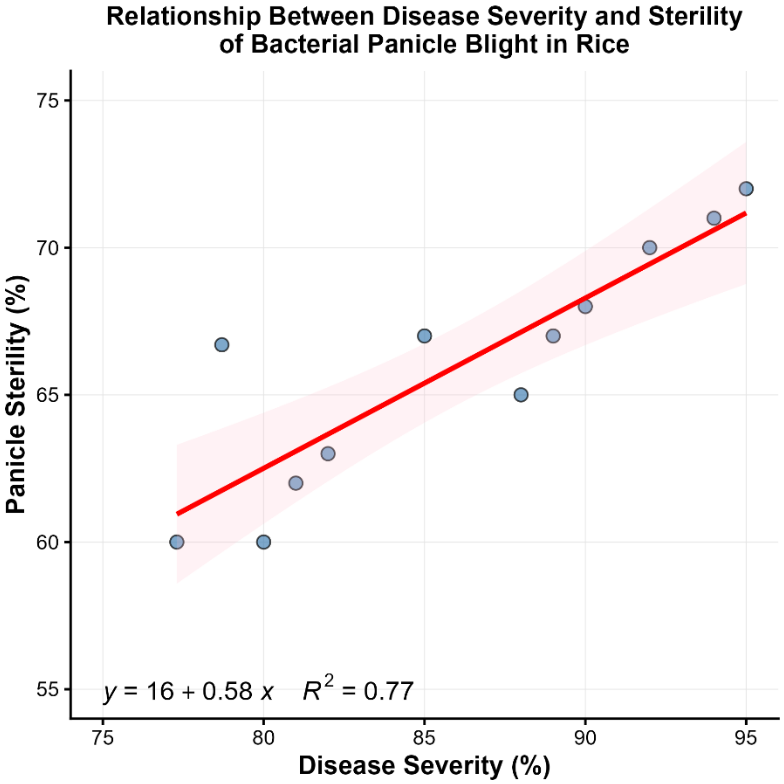

Figure 4: Relationship between disease severity and panicle sterility in rice plants infected with bacterial panicle blight (BPB). Each point represents an individual observation. The solid red line shows the linear regression ($y = 0.58x + 16$), with the shaded area indicating the 95% confidence interval. The coefficient of determination ($R^2 = 0.77$) indicates that 77% of the variation in panicle sterility can be explained by disease severity.

From Correlation to Forecasting: Defining Actionable Thresholds

While disease severity is a robust post-infection indicator, proactive management requires predictive environmental parameters. Our study confirms the role of warm, humid conditions in driving BPB epidemics, as highlighted by (Shew *et al.* 2019, Priyadarsini *et al.* 2023). To move from general association to specific forecasting, we employed ROC analysis to identify precise intervention thresholds (Figure 5). Maximum relative humidity exceeding 83.7% (AUC = 0.773) emerged as a robust predictor of economic damage, while temperatures above 29.7°C provided additional, though more modest, predictive value (AUC = 0.561).

These evidence-based thresholds form the core of a practical forecasting model. We propose that extension services issue "BPB Risk Alerts" when either humidity surpasses 83.7% or temperature exceeds 29.7°C during the critical panicle initiation to flowering stages. This enables targeted

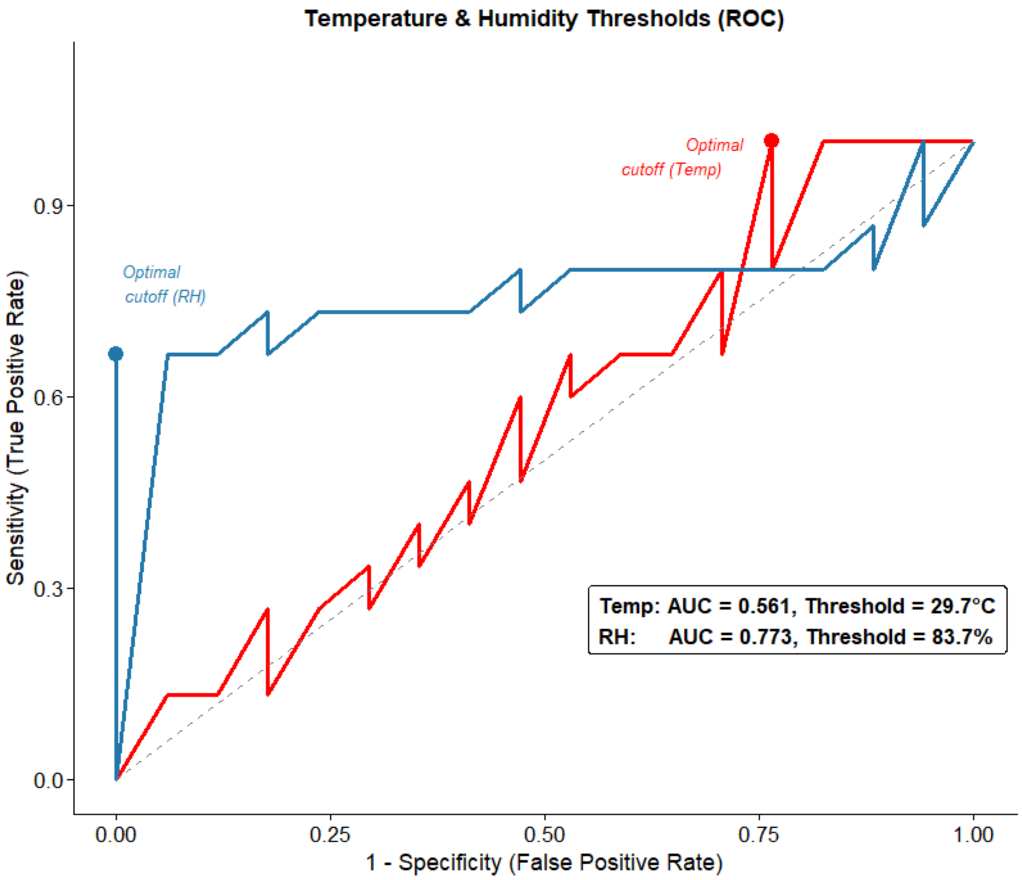

Figure 5. Receiver operating characteristic (ROC) curve analysis for predicting bacterial panicle blight economic damage based on temperature and relative humidity thresholds.

interventions: adjusting irrigation to reduce canopy moisture, employing microclimate management during temperature spikes, and initiating bactericide applications when field scouting confirms disease severity is approaching the 78% economic threshold. This integrated approach synchronizing environmental monitoring with disease assessment optimizes resource allocation and minimizes unnecessary chemical inputs.

The superior predictive power of humidity aligns with the known biology of *B. glumae*, where free moisture is critical for bacterial colonization and disease progression. This threshold-driven model facilitates a necessary shift from calendar-based spraying to precision management, responding to actual risk conditions.

Future Research Directions

Validation of these thresholds across diverse agro-ecological zones and rice cultivars is a critical next step to establish universal applicability. Furthermore, the development of integrated decision-support tools that incorporate real-time meteorological data represents a promising avenue for digital agriculture. Investigating the molecular mechanisms underlying the 83.7% humidity threshold could reveal novel aspects of bacterial pathogenesis, while exploring genotype-by-environment interactions may accelerate the development of BPB-resilient varieties, securing sustainable rice production against this escalating threat.

Conclusion

This study establishes a quantitative framework for predicting bacterial panicle blight (BPB) impact by integrating disease progression, yield loss, and critical environmental thresholds. We demonstrate that under conditions representative of tropical rice-growing regions, *B. glumae* infection causes significant yield loss primarily through panicle sterility, which is strongly predicted by visual disease severity ($R^2 = 0.77$). Crucially, our work advances beyond correlation by identifying specific, actionable environmental parameters for forecasting: maximum relative humidity above 83.7% and temperature exceeding 29.7°C serve as robust triggers for disease risk alerts.

The principal novelty of this research lies in the development of an evidence-based, multi-parameter forecasting model that bridges the gap between pathological understanding and on-farm decision-making. By defining precise humidity and severity thresholds, we provide a foundation for moving from calendar-based sprays to precision management. This contributes directly to sustainable intensification goals by enabling timely interventions such as adjusted irrigation or targeted bactericide application that optimize resource use and minimize environmental impact.

While these findings are validated under controlled conditions, they provide a critical proof-of-concept for developing climate-resilient BPB management. Future research should focus on validating these thresholds across diverse agro-ecosystems and integrating them into digital decision-support tools for extension services. Ultimately, this work provides a scientific basis for safeguarding rice productivity in the face of escalating climate variability and emerging biotic threats, directly supporting the global pursuit of food security.

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Conflict of Interest

The authors do not have any conflicts of interest.

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