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DETECTION OF *LECANOSTICTA ACICOLA* SPORE LOAD USING SPORE TRAPS

by
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ABSTRACT

Brown spot needle blight (BSNB) is a significant foliar disease affecting pine species, with *Lecanosticta acicola* recognized as the primary causal agent. The disease is historically found throughout the southeastern U.S. from Virginia through Alabama, Florida and west to Louisiana where warm, humid summers sustain multiple infection cycles. Knowledge of the epidemiology and climatic variables that affect its spread will be essential to understand and improve management of the disease. Monitoring *L. acicola* spore dispersal using spore traps has revealed that rainfall, temperature, and relative humidity (RH) independently drive inoculum pressure throughout the sporulation season (March to November). This study used 20 spore traps deployed on 5 BSNB-infected study plots from March to November, to assess the spore load in the plots and how climatic variable affect the spread of the spores. Our mixed-effects models showed that each 10 mm of monthly rainfall increases mean spore counts by ~17%, a 5 °C rise in temperature boosts count by ~19%, and a 5% relative humidity increment nearly doubles spore counts (101%). Poisson incidence-rate ratios (IRR= 1.02-1.26) confirm these effects after accounting for modest between plot heterogeneity (variance = 0.016, SD = 0.13). The peak for spore abundance occurred in July and then it fell in the subsequent months, with November having the lowest spore count. Our results will be useful for managers and landowners to find the best strategies for controlling the disease.

Keywords: brown spot needle blight, foliar pathogen, spore trap

INTRODUCTION

Brown spot needle blight (BSNB), caused by *Lecanosticta acicola* (Thüm.) Syd. & P. Syd., has emerged as a serious constraint on pine health and productivity across North America, Europe and parts of Asia and South America. The pathogen now threatens more than 50 *Pinus* species, causing growth loss, premature needle cast, and under epidemic pressure, plantation failure (van der Nest et al., 2019). Recent interceptions in previously unaffected regions and its listing on multiple quarantine schedules underscore the need for robust surveillance strategies. Disease development hinges on the production and aerial dissemination of conidia from acervuli on infected needles. Conidial release is strongly modulated by moisture and temperature: peaks occur during warm, wet periods, with rainfall events acting as the primary trigger for mass discharge and subsequent wind or splash-borne spread (Wyka et al., 2018c).

In the Atlantic coastal pine stands of northern Spain, daily spore loads recorded with volumetric samplers surged from early autumn, and generalized additive models identified maximum temperature and cumulative precipitation as the best predictors of airborne inoculum (Mesanza et al., 2021b). Because foliar symptoms can remain latent for months, measuring the flux of spores in the forest atmosphere is pivotal for early warning and for parameterizing epidemiological models. Spore traps, which draw a constant air flow across an adhesive surface or tape, remain the essential tool of forest aerobiology; recent innovations include automated image recognition, on-trap DNA extraction, and networked samplers that stream near-real-time data to decision dashboards (West & Kimber, 2015).

These advances shorten the feedback loop between inoculum detection and management action, enabling fungicide timing, targeted silvicultural interventions and quarantine enforcement.

Additionally, combining spore trapping data with spatiotemporal modeling methodologies enhances our understanding of temporal spore release patterns and environmental conditions that favor pathogen establishment, thus informing strategies to mitigate detrimental effects on forest health and biodiversity (Wyka et al., 2018c). Such an integrated approach also fosters deeper knowledge of the pathogen's life cycle, especially its adaptive responses to environmental fluctuations, supporting the development of targeted interventions aimed at increasing forest resilience against invasive pathogens. Moreover, utilizing multi-dimensional data analysis and predictive modeling frameworks can uncover intricate host-pathogen dynamics, identifying critical thresholds and feedback mechanisms that influence host susceptibility. This knowledge is pivotal for developing evidence-based conservation strategies and promoting ecosystem stability amidst biotic invasions (van der Nest et al., 2019).

Furthermore, the application of advanced statistical techniques and machine learning algorithms in analyzing spatiotemporal infection patterns enhances predictive accuracy, enabling timely anticipation of outbreaks and initiative-taking management actions. These methodological advancements not only provide nuanced insights into epidemiological trends associated with *L. acicola* but also empower forest managers and researchers to make informed decisions regarding resource allocation and ecological restoration practices, safeguarding biodiversity, and forest health (West & Kimber, 2015; Mesanza et al., 2021b).

This study therefore focuses on quantifying *L. acicola* spore load using spore traps deployed across representative pine stands and determining how climatic factors affect spore load and distribution. The resulting framework will contribute to initiative-taking surveillance networks capable of mitigating the expanding footprint of *L. acicola* in commercial and natural pine ecosystems.

MATERIALS AND METHODS

Spore Trap Construction

Twenty spore traps were deployed in March 2024. Each trap was positioned at the center of every subplot. The spore traps were about 1.8m tall and pegged into the ground inclined at an angle of 45°. The free end of the rod is bent downwards, and hole was drilled through it, where a rope was looped through it to enable a motor to be suspended from it. The motors were about 25 inches in length with a 1.3 mm gauge to which two plastic slides per trap were fastened. A plastic disposable cup was looped through the rope to cover the motor and to shield it from excess rain (Figure 1). Microscope slides (plastic) were coated with petroleum jelly (Vaseline®) and labelled with specific numbers for easy identification and matching with plots. A battery-powered rotating motor was tied with a rope to hang on the metal rods of the trap.

Figure 1. Picture of spore trap deployed in the field

Spore trap deployment

The experiment was set up in Cullman county (Osko Forest) (RR25-02, Table 1). Cullman county is in North-Central Alabama. Its average temperature in summer often reaches the upper 80s °F (about 31-32 °C), and winters commonly drop into the lower 30s °F (around 0-2 °C). Annual rainfall typically ranges from about 50 to 55 inches (1270-1400 mm). Rainfall is relatively well-distributed throughout the year, though slightly heavier in late winter and spring months. Five research plots were set up with each plot having four subplots based on Dunn (1999), comprised of one central plot and three sub-plots identical to it. The subplots were located 120 feet from the central plot and at bearings of 120°, 240°, and 360°. Each plot covers an area of approximately 2.3 acres (RR25-02, Figure 1). This privately owned forest consisted of mostly loblolly pines and a few hard woods. This site has moderate presence of brown spot needle blight with around 20-30% of trees showing symptoms of the disease. The slides in the spore traps were replaced weekly and stored in a sterile slide box and transported to the Forest Health Dynamics Laboratory for analysis. The rechargeable batteries in the motors were also replaced weekly to ensure continuous

rotation of the motors for effective spore trapping. The sampling period was from March to November 2024.

Spore slide examination

Spore slides were examined under a compound microscope at 100× magnification. Lactophenol cotton blue dye was used to enhance the contrast. To obtain a more accurate spore count, a grid slide was placed beneath the slide under the microscope. Conidia were identified based on their morphological description as previously described by Jankovský et al., 2009. Other fungi recovered were identified using a manual by Barnett and Hunter (1998).

Weather data

Weather data available online (http://www.weatherspark.com) obtained from the nearest weather stations to the study sites were used to determine the monthly averages for temperature, relative humidity and rainfall (RR25-02, Table 2). To determine the effects of the weather factors on spore loads, the data was incorporated into the model and analyzed (Boateng and Lewis, 2015).

Statistical analysis

The data collected from all twenty traps during the study period was used for statistical analysis using JMP Pro11.2.1. Spore counts from the two slides of each trap were averaged. Mean counts for every bi-weekly period were calculated on all plots. To determine the overall spore released, spore counts were averaged per month and per plot and transformed to fit the assumptions of Analysis of variance (ANOVA) (Boateng and Lewis, 2015). Also, two complementary models (i.e. Poisson regression Generalized Linear Model and Linear Mixed-effects Model) were used to quantify the independent effects of rainfall (mm), temperature (°C) and relative humidity on *L. acicola* spore counts.

RESULTS

Patterns of spore dispersal

The spore-trap system effectively captured *Lecanosticta acicola* conidia across all five plots, and throughout the sampling period. Spores were first recovered during the third week of March (16-29 March) (Table 1), confirming that dispersal had begun soon after the traps were deployed. Counts rose steadily and reached their peak within the period of 7 to 28 July, with plot 11 having an average of 23.9 spores, plots 10 and 14 recorded the lowest overall means 13.0 and 8.7 spores respectively (Table 2). After July, spore numbers declined toward the close of the sampling season. Statistical analysis showed no significant variation in monthly spore totals among the plots ($p = 0.672$). Spores recovered from plots 12 and 13 were 198 and 180 respectively with a mean by month count of 22 and 20 respectively (Table 2). The number of spores released fell sharply after July, and the subsequent months had low counts with the least being in November (spore count = 5) (Figure 2). Negative binomial GLM test showed that there was no significant variation in monthly spore totals among the plots ($p = 0.672$) (Table 3).

Table 1. Summary table showing total monthly spore count by plot

Month	Total spores	Mean per plot
March	112	22.4
April	147	29.4
May	159	31.8
June	47	9.4
July	260	52.0
August	23	4.6
September	19	3.8
October	16	3.2
November	5	1.0

Table 2. Summary table showing cumulative spore count by plot from March to November

Plot	Total count	Mean per month	Rank
BS 11	215	23.90	1
BS 12	198	22.00	2
BS 13	180	20.00	3
BS 14	117	13.00	4
BS 10	78	8.70	5

Effects of climatic variables on spore dispersal

The Cullman county weather data (RR25-02, Table 2) from March to November was used to determine the effects of climatic variables on spore abundance and release. There was a positive association found between cumulative rainfall and spore count in the month of July (IRR =1.017, $p = 0.001$) (Table 4). The data further showed a significant relationship ($p < 0.001$) between the expected spore count, temperature and relative humidity. For every

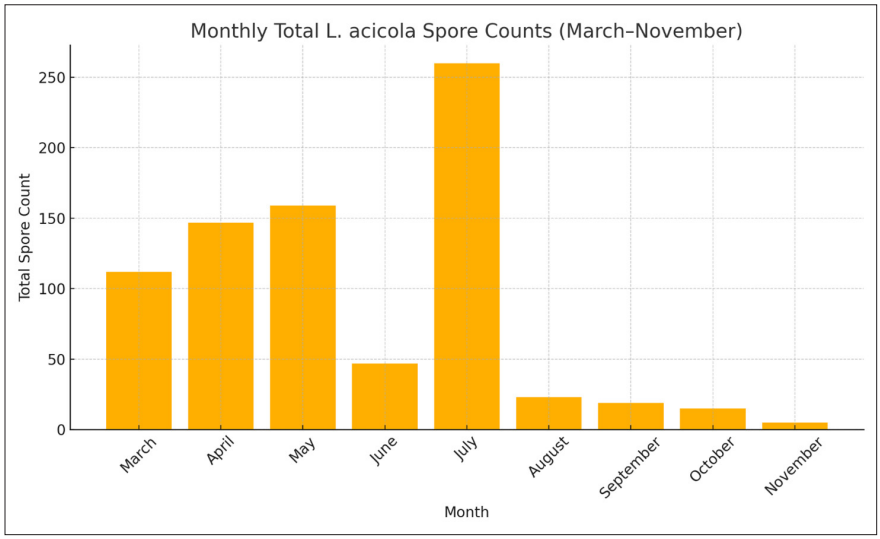

Figure 2. Monthly distribution of *L. acicola* spores across the various plots

Table 3. Statistical test result for across plot variation in spore count

Test	Statistic	Df*	p-value
Negative-binomial GLM	Likelihood ratio = 2.35	4	0.672
Kruskal-Wallis (non-parametric test)	H = 1.92	4	0.750

*Df: Degrees of freedom

Table 4. Summary table for Poisson regression model of climatic variables with spore count

Predictor	Incident-rate ratio†	95% CI*	p-value
Rainfall (mm)	1.017	1.008 – 1.027	0.001
Temperature (^{0}C)	1.037	1.019 – 1.056	< 0.001
Relative humidity (%)	1.225	1.103 – 1.427	< 0.001
Intercept	0.000		

†IRR: Incident-rate ratio = exp(β), values > 1 implies positive association, *CI: Confidence interval.

Table 5. Descriptive statistics for linear mixed-effect model of climatic variable with spore count

Predictor	β (log-scale)*	Std. Error**	Z value	P value
Rainfall (mm)	0.016	0.007	2.32	0.021
Temperature (^{0}C)	0.035	0.012	2.88	0.004
Humidity (%)	0.183	0.064	2.87	0.004
Intercept	-14.85	4.47	-3.33	0.001

*β(log-scale): β- coefficient represents the estimated change in the dependent variable for a unit change in the predictor variable, Std Error: Standard Error.

1°C rise in temperature and every 1% increase in relative humidity, there was a corresponding 3.7% and 5.2% increase in the spore count respectively.

The linear mixed effect model showed a modest but non-negligible random-effect by plot variation (variance = 0.016, SD = 0.13), indicating that the little difference in baseline disease pressure was not enough to dominate the overall variability of the experiment (Table 5).

DISCUSSION

Spore trap monitoring has become indispensable for mapping *Lecanosticta acicola* dispersal and forecasting BSNB epidemics. Long-term surveys in Spain's Basque Country, for example, deployed 16 traps over three years, revealing how trap location and sampling duration shape our view of regional spore dynamics (Martínez et al., 2020). Yet passive impact traps have sometimes failed to capture the pathogen's airborne sexual spores, suggesting that conidial splash dispersal and environmental wash-out can limit trap efficiency (González & Pérez, 2018).

Our analyses demonstrate that the key climatic factors of rainfall, temperature, and relative humidity (RH) are each robust, independent drivers of *L. acicola* spore release (Figures 3, 4, 5). In the mixed effects framework, every 10 mm increment in monthly precipitation elevated the geometric mean spore count by 17%, suggesting that rain splash and needle wetness directly facilitate conidial release and dispersal (Sinclair & Lyon, 2005). Likewise, a moderate 5 °C rise in mean air temperature boosted spore abundance by approximately 19 %, consistent with laboratory data showing optimal conidial germination at 20-30 °C (Barnes et al., 2019). The most dramatic effect arose from relative humidity where a mere 5% increase doubled spore numbers (101%), underscoring the pathogen's reliance on sustained humid conditions to form and eject acervuli (Wyka et al., 2018c). Poisson regression corroborated these patterns with incidence rate ratios (IRR = 1.02-1.26 per unit), confirming that each variable exerts an additive influence on inoculum pressure over the March to November

trapping season. The concordance between mixed-effects estimates and IRRs highlights the stability of these relationships even after accounting for plot-level heterogeneity (variance = 0.016, SD = 0.13).

Conidial release in *L. acicola* is facilitated by a mucilaginous matrix; raindrop impact physically ejects spores and creates splash dispersal, so rainfall events are routinely highlighted as the main "switch" for epidemics (Mesanza et al., 2021b). The positive rainfall coefficient here aligns with classic slide-trap studies in the U.S. Lake States, where Skilling & Nicholls (1974) recorded the bulk of conidial showers during wet periods between May and September (Wyka et al., 2018b). Notably, our March rainfall (91 mm) coincided with only moderate counts, while July peaks occurred under less humid but wet conditions (94 mm). This supports field observations that very heavy, persistent rain can wash spores from the air column faster than they are liberated, producing a non-linear response once a saturation threshold is crossed.

Daily maximum temperature emerged as the second-strongest predictor. A recent risk model for Atlantic-coast plantations likewise found higher *L. acicola* spore counts under warmer days, with significant effects above 25 °C (García-García et al., 2025). While laboratory work on central-European isolates places the sporulation optimum at 15-20 °C and > 90% relative humidity (Dvoř ák et al. 2012), southeastern U.S. field data, including our 31-32 °C July maximum (Figure 4) suggests that southern genotypes or local microclimates extend that thermal window. This regional plasticity is important for forecasting disease under climate-warming scenarios.

The results show relative humidity as an amplifying but not merely a permissive factor. Relative humidity above roughly 75% is generally cited as the threshold for conidial development and germination (Agrios, 2005). Our models indicate that once that threshold is met, incremental rises in RH sharply elevate airborne inoculum (IRR ≈ 1.26 per 1% RH). The biology is two-fold: (i) high RH keeps acervular mucilage hydrated, facilitating discharge, and (ii) wet needles prolong germ tube viability, increasing the odds of infection between successive showers. Field guides on needle blights similarly warn that outbreaks can initiate any time there is a coincidence of high RH and spores, even outside the classic spring window. As spore traps evolve from passive collectors to components of integrated surveillance networks, their strategic deployment alongside environmental data will sharpen early-warning systems and improve BSNB management.

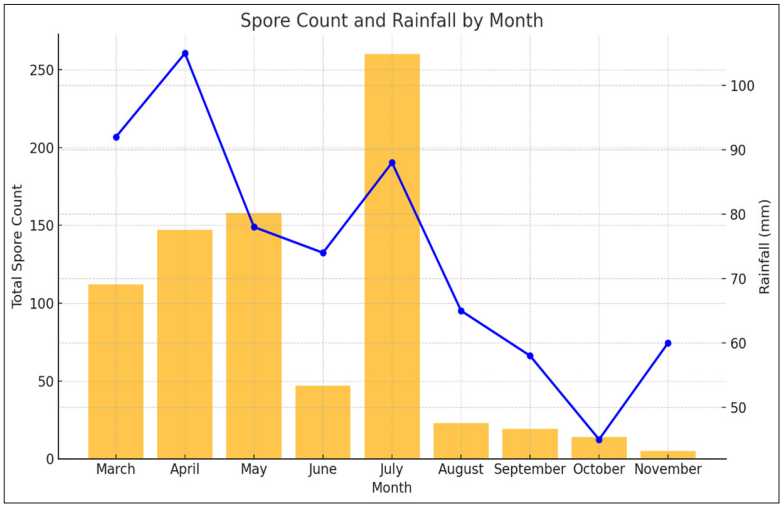

Figure 3. Relationship between Spore count vs Rainfall

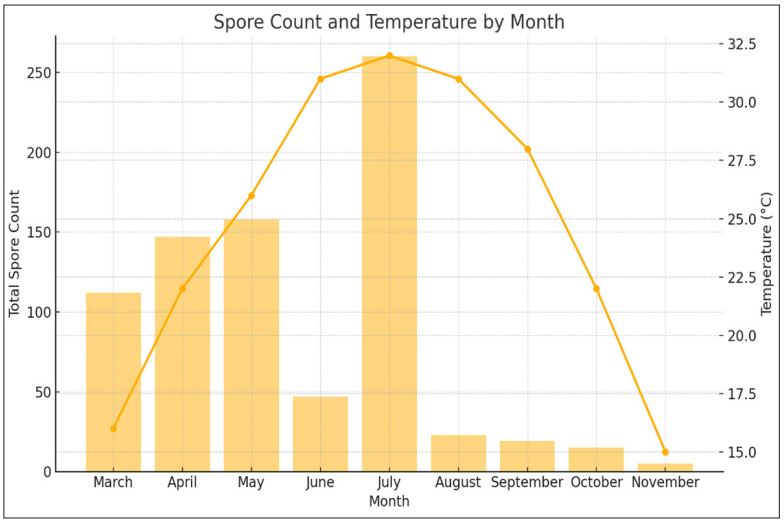

Figure 4. Relationship between Spore count vs Temperature

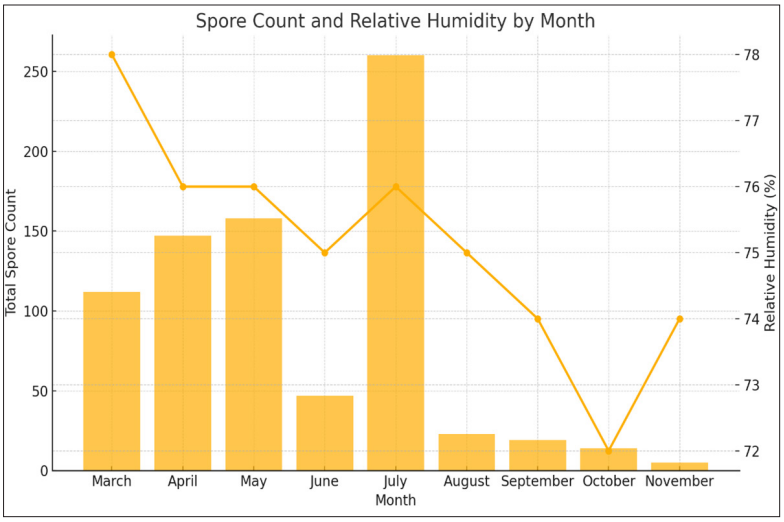

Figure 5. Relationship between Spore count vs Relative humidity

CONCLUSION

In conclusion, spore traps remain an indispensable tool for understanding the dispersal dynamics of *L. acicola*. The choice of trap type, placement, and environmental considerations critically influence the quality and reliability of spore detection data. While active traps like rotating-arm samplers have demonstrated high efficacy, passive traps may require optimization to improve their effectiveness. Integrating spore trapping with molecular detection techniques and environmental data can significantly advance disease prediction and management strategies. Future research should focus on refining trap designs and deployment strategies to maximize detection sensitivity and accuracy in diverse ecological contexts.