AUBURN UNIVERSITY
Forest Health Cooperative

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ASSESSMENT OF SEEDLING SUSCEPTIBILITY OF DIFFERENT LOBLOLLY PINE FAMILIES TO *LECANOSTICTA ACICOLA* USING OPEN-TOP CHAMBERS

by
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ABSTRACT

Loblolly pine (*Pinus taeda* L.) is a commercially significant tree species in the southeastern U.S., valued for its rapid growth and adaptability to various soil types. However, its susceptibility to the fungal pathogen, *Lecanosticta acicola*, poses a significant threat to its health and productivity. *Lecanosticta acicola* is known to cause needle blight, leading to reduced photosynthetic capacity, growth inhibition, and, in severe cases, tree mortality. This study evaluated the susceptibility of seventeen loblolly pine families to *L. acicola* using open-top chambers with three different treatments. We quantified inter-family differences in disease severity, explored how chamber treatments shaped disease symptom expression, and identified families with high and low disease tolerance. Our findings highlight that genetic and environmental factors contribute to needle blight resistance, and suggest promising genotypes for breeding programs, ultimately supporting the development of more resilient loblolly pine stands.

INTRODUCTION

Lecanosticta acicola is a fungal pathogen that causes brown spot needle blight disease in loblolly pines. This disease also affects loblolly pine seedlings, particularly in nurseries and young plantations across the southeastern U.S. (Barnes et al., 2019; Sinclair & Lyon, 2005). The disease poses a significant threat to pine establishment and productivity (Barnes et al., 2019; Sinclair & Lyon, 2005). The susceptibility of loblolly pine seedlings to various pathogens has had considerable research, with particular focus on intraspecific variation among different families. Several studies have employed controlled environments, such as open-top chambers, to assess how environmental factors influence disease susceptibility. Chieppa et al., (2017) conducted experiments using seedlings from four loblolly pine families grown within capped open-top chambers subjected to three distinct weekly moisture regimes. This approach allowed for the evaluation of how moisture variability impacts seedling health and disease susceptibility, providing insights into the interaction between genetic factors and environmental conditions.

Intraspecific variation among loblolly pine families has been reported in relation to susceptibility to various diseases, such as Dothistroma needle blight (DNB) and other foliar diseases, emphasizing the importance of genetic factors in disease resistance (Bradshaw et al., 2000). Additionally, variation in susceptibility among loblolly pine families has been observed in nursery settings, with some families exhibiting lower disease incidence, possibly due to inherent genetic resistance (Cordell et al., 1989).

The use of open-top chambers has proven to be effective in isolating and studying genetic and environmental factors thereby providing valuable insights into how these factors may influence disease susceptibility in loblolly pine seedlings. Considering the economic importance of loblolly pines to the southern forestry industry, future research should continue to explore these dynamics to inform breeding and management strategies aimed at enhancing disease resistance in pine plantations. This study seeks to employ open-top chambers with different treatments to assess the susceptibility of seedlings of different loblolly pine families to *L. acicola*.

MATERIALS AND METHODS

Study site and Open-top chambers

The study was conducted at the Atmospheric Deposition Site (approximately 0.02 km^2 in area), located approximately 5 km north of Auburn University Campus. The open-top chambers (OTC) were used as a relatively more controlled environment to replicate the experiment. The OTCs were 4.8 m high, 4.5 m in diameter with aluminum framed structures and chamber plastics attached to each OTC permit adequate airflow (Gilliland et al., 2012; Heagle et al., 1989). Before the study, the vegetation growing in each OTC was killed with a 3% solution of glyphosate. Once dead, the vegetation was cleared, and the ground was covered with landscape fabric to prevent further unwanted vegetation growth within the chambers. Eight chambers were used with inoculation treatments which were either bare ground (negative control), inoculation with infected needles, or inoculation with healthy needles (positive control).

Seedlings

Bareroot seedlings of seventeen commercially grown loblolly pine families were obtained from Aborgen, Westervelt, and the International Forest Company (IFCO) for this study (Table 3.1). In January 2024, 2,550 seedlings (150 per family) were planted in 2.4-liter pots (1 trade gallon) with ProMix BX® peat-based potting mix (Premier Tech, Quebec, Canada). Seedlings were kept in a shade-house and watered weekly for 8 weeks to enable them to acclimate to the environment until they were deployed into the chambers in March 2024. The pine families used for the study are shown in Chapter 3 (Table 3.1).

Inoculation

Eight chambers, and three treatment levels for the chambers: Infected, Positive control and Negative control treatments were used for this experiment. Four chambers were assigned for the infected treatment, and two chambers each for the two control treatments. The inoculum for the infected chambers was obtained by harvesting infected pine needles from diseased trees in Osko Forest. The trees were selected from unburned stands which had trees showing visible crown disease symptoms (> 2/3 disease rating). These trees were felled with a chain saw and the needles were manually ripped off and placed into sterile bags and labelled.

The inoculum for positive control (sterilized needles) treatment was obtained from a controlled-burned stand with relatively healthy/uninfected trees showing no disease symptoms. The trees were felled, and the needles were ripped off manually and placed into separate sterile bags and labelled. The bags were transported to the Forest Health Dynamics Lab, where the needles were sterilized under oven dry heat at 160 ^{0}C for 2 hours, and then allowed to cool under a laminar flow chamber after which it was transported to the OTCs and spread cover the floor of the chambers. The two negative control (no needles) chambers did not have any inoculum at all. Seedlings were set on the bare ground in the chambers. In each chamber, 7 seedlings per family were randomly deployed for a total of 119 seedlings per chamber (Figure 1). The total number of seedlings used in the OTCs was 952. Optimum water content was maintained throughout the exposure period by watering them weekly and then increasing the watering regime to twice weekly during the summer period.

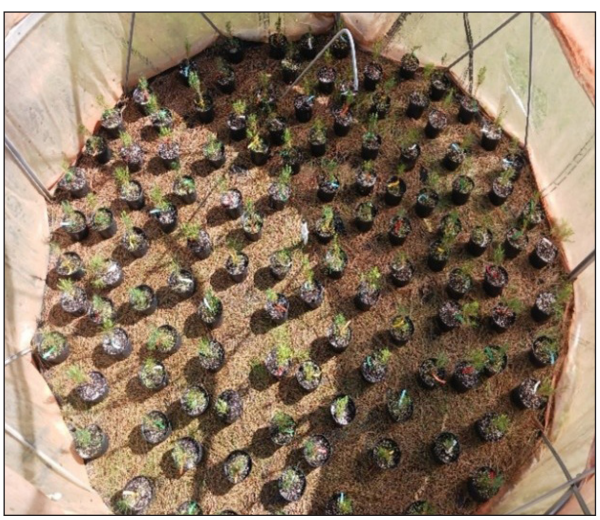

Figure 1. Seedlings deployed in Open-top chambers

Measurements

Root collar diameter (RCD) and height measurements were taken monthly and recorded for all seedlings throughout the study period using a digital caliper and a meter rule. Seedling RCD and height trajectories across families and treatments was used to distinguish lines that tolerate infection maintain near-normal growth from those that simply show low disease and normal growth (Eckhardt et al., 2016; Walkinshaw and Barnett, 1998).

Relative water content was assessed on two randomly selected seedlings per family per chamber. The fascicles were initially weighed to the nearest 0.01g (W_w) and then soaked in distilled water overnight, reweighed in the morning to obtain turgid weights (W_T), dried at 70 ^{0}C to equilibrium, and reweighed (W_D). Fascicle relative water content (RWC_F, %) will be determined by the equation:

$$RWC_F = [(W_W - W_D) / (W_T - W_D)] * 100$$

Disease rating was assessed based on observation of symptoms such as chlorosis, mortality (necrosis or death of needles), infection (tiny black fruiting bodies of the fungus in dead spots or bands), and needle discoloration (presence of brown, tan, or gray lesions on the needles). These symptoms were matched on a five-point scale 0 to 4 i.e.: 0 - No visible symptom, fully healthy needles; 1 - slight infection, very few needles (< 10% chlorotic or bearing small lesions, no chlorosis); 2 - moderate infection, noticeable chlorotic and discrete brown or tan lesions (10-25% of foliage affected); 3 - severe infection, widespread needle discoloration and necrosis (affecting 25-50% of foliage); 4 - Very severe infection (>50% of needles severely necrotic or dead).

Statistical analysis

The main effects of inoculation treatments on the height and disease rating of the seedlings were analyzed by one-way analysis of variance (Pro GLM, SAS Inc., Cary, NC, USA). Prior to analysis, each dependent variable was checked using Levene statistic test to check for the assumption of equal variances before ANOVA was performed. Differences in disease symptoms of seedlings between chamber treatments were examined. Post Hoc test using Tukey's adjustment for differences in least square means and Bonferroni's correction test for multiple comparisons was used to control Type 1 error due to multiple testing. Differences between means were considered significant at p-values <0.05.

RESULTS

To evaluate the influence of the different chamber treatments on seedling performance, disease rating, height and root collar diameter measurements were assessed. Kruskal-Wallis tests followed by Dunn's pairwise comparisons were used to assess the treatment effects, and family means were ranked within each chamber to examine family-specific responses.

Chamber treatment effects on disease rating of seedlings

Significant differences in disease severity were detected among the chamber treatments ($\chi^2 = 32.54$, $p < 0.001$). Seedlings in the infected chambers displayed the highest mean disease ratings, significantly greater than those in both positive control ($Z = 5.67$, $p < 0.001$) and negative control ($Z = 3.36$, $p = 0.002$) chambers. The contrast between the two control treatments was marginal ($Z = 2.23$, $p = 0.078$), which suggests minimal background disease pressure in the absence of infected inoculum (Figure 2).

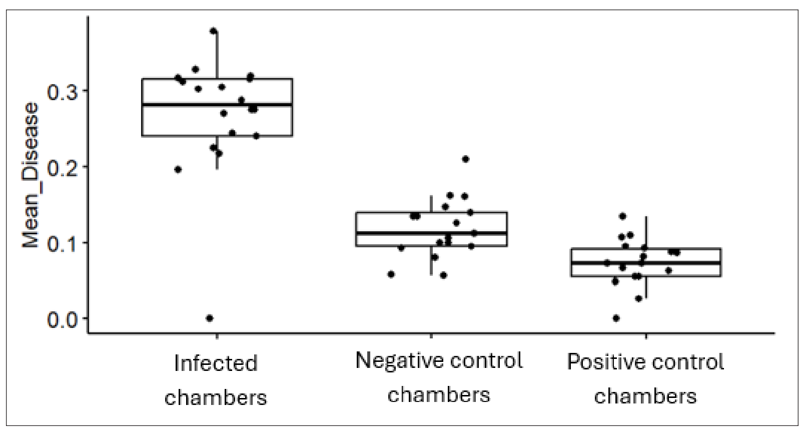

Figure 2. Comparison of mean disease rating across the various chamber treatments

Within the infected chambers, the lowest disease rating was observed in families F13 (Mean = 0.197), followed closely by F14 (0.218) and F17 (0.225), suggesting moderate resistance. Conversely, family F3 had the highest disease severity (0.380), indicating high susceptibility under inoculum pressure. Among control chambers, family F13 again showed the lowest disease in the negative control (0.0579) and maintained low values in the positive control chamber (0.0672), reflecting consistent performance across treatments.

Seedling height response across treatment chambers

Although mean seedling height varied among the various treatments (Figure 3), the Kruskal-Wallis test was not statistically significant ($\chi^2 = 5.08$, $p = 0.079$). A weak trend suggested slightly lower height in the infected group compared to the negative control (Dunn, $p = 0.076$), but no difference was found between the positive and the negative control groups.

Within each chamber, families F4 (62.2 cm), F8 (62.0 cm), and F2 (60.7 cm) ranked highest in height under infected needle conditions, suggesting robust growth in height despite pathogen presence. In the negative control, family F8 (58.4 cm) and F2 (57.2 cm) again ranked high, supporting their consistent growth performance. In the positive control chambers, the top four families by height were F18 (64.2 cm), F3 (60.3 cm), and F4, F6 (57.6 cm), highlighting variability in growth performance depending on exposure environment.

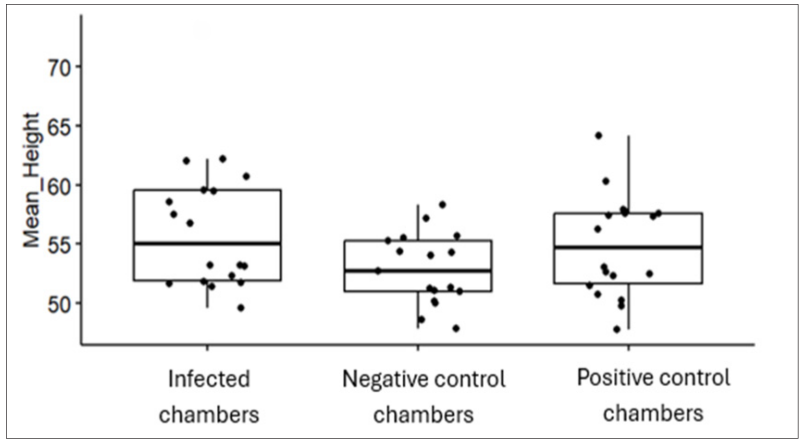

Figure 3. Comparison of mean height across treatment chambers

<u>Root collar diameter (RCD) variation across treatment chambers</u>

Root collar diameter did not vary significantly across chambers ($\chi^2 = 0.998$, $p = 0.61$), and all pairwise comparisons were non-significant ($p > 0.36$). However, within chambers, distinct patterns emerged (Figure 4.4). Under the infected chambers, families F4 (9.46 mm), F5 (9.24 mm), and F8 (8.97 mm) showed the highest average RCD values, indicating potentially strong structural development despite foliar stress. In the negative control chambers, families F8 (8.61 mm), F5 (8.48 mm), and F2 (8.21 mm) ranked highest in RCD, paralleling their height performance. Notably, family F2 performed consistently well across all chambers, maintaining a high height and RCD while demonstrating moderate disease resistance. In contrast, family F3, which had the weakest disease performance under the infected chambers (0.380), also ranked low in growth metrics, suggesting an increased susceptibility. Family F13 demonstrated low disease across all chambers and moderate to low growth, suggesting stable resistance with conservative growth strategy.

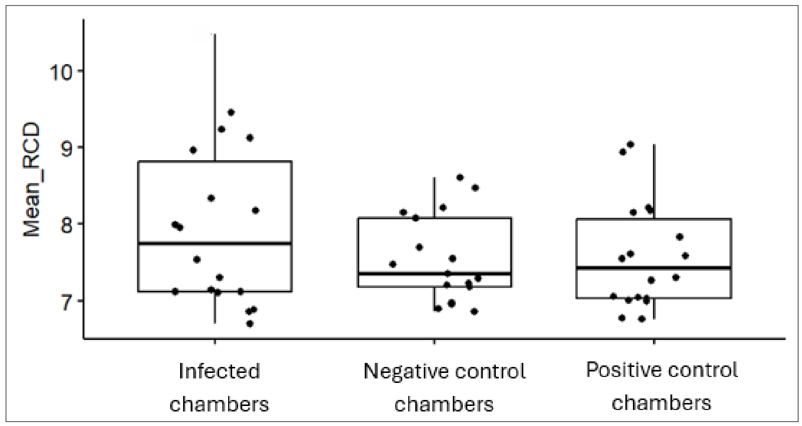

Figure 4. Comparison of mean root collar diameter across treatment chambers

<u>Comparison of family performance across infected chambers based on disease severity, height, and RCD</u>

The comparative summary of seedling performance under the infected chamber treatment, ranked according to mean disease severity, height, and root collar diameter (RCD) showed that families F13, F14, and F17 exhibited the lowest mean disease severity, suggesting higher tolerance to *Lecanosticta acicola*. Among them, F13 had the best disease ranking (1st), although it ranked low in height (15th) and moderately in RCD. Conversely, F4, F2, and F3 showed superior growth traits; F4 ranked 1st in height and had the highest mean RCD (9.46) but was ranked 5th in disease resistance. F3 had the poorest disease ranking (17th) despite favorable height and RCD. This highlights a trade-off between disease resistance and growth traits in some families, with few families, such as F2 and F4, demonstrating a desirable balance of moderate disease resistance and superior growth performance (Table 1).

<u>Comparison of family performance across negative control chambers based on disease severity, height, and RCD</u>

The comparative performance of seedlings in the negative control chambers with rankings based on mean disease severity, height, and root collar diameter (RCD), revealed that families F13 and F9 exhibited the lowest disease severity (both ranked 1st and 2nd, respectively), suggesting a strong baseline resistance to disease even in the absence of pathogen pressure. Family, F8 attained the greatest mean height (58.4 cm) and highest RCD (8.61 mm), though it ranked 10th in disease resistance. Families such as F2 and F5 combined relatively strong disease performance (ranked 7th and 5th) with robust growth traits, placing them among the most balanced performers. In contrast, F10 and F17 were among the lowest in height and RCD, with F17 maintaining a high disease resistance rank. Overall, the table reveals variation in baseline growth and health traits among families, useful for identifying genotypes with both inherent vigor and disease resilience under non-inoculated conditions (Table 2).

<u>Comparison of family performance across positive control chambers based on disease severity, height, and RCD</u>

The comparison of seedling performance across the positive control chambers showed that Family F16 had the lowest disease severity (ranked 1st), though it ranked low in height (16th), suggesting strong disease resistance but limited growth. Similarly, families F14 and F15 ranked highly in disease resistance but were also among the shortest. In contrast, family F3 ranked last in disease resistance but achieved the highest mean height (60.3 cm) and largest RCD (9.04 mm), indicating a potential trade-off between growth and disease tolerance. Several families such as F2, F4, and F8 demonstrated a favorable balance, combining moderate to high disease resistance with strong growth performance. This variation highlights key genetic differences in growth and health traits under non-infected, but controlled, environmental exposure (Table 3).

Variation in relative water content across chambers

Two-way ANOVA test was conducted to determine the effects of chamber treatments and seedling families on the relative water content. Chamber treatments and family variation did not have any significant effect on the change in the relative water content of the seedlings across all families ($p = 0.315$; $p = 0.975$ respectively) (Table 4).

DISCUSSION

The susceptibility of loblolly pine seedlings to *Lecanosticta acicola* is strongly modulated by ambient environmental stressors. Open top chamber experiments have long been used in studies such as in examining how atmospheric pollutants influence pine physiology and disease susceptibility. By employing open-top chambers to maintain uniform environmental conditions, we minimized external variability and sharpened family level effects (Olszyk, Tingey, & McMichael, 1980). Unlike many prior surveys that focused on testing the susceptibilities of multiple species (Aldrich & McCarty, 2019; Davis & Thompson, 2018), this experiment uniquely targets only loblolly pine families under open-top chambers (with three different treatments). Our findings pointed out that regardless of the treatment used, seedling height increased by approximately 2.00 cm, month by month. This could be explained by the fact that the seedlings were watered consistently throughout the period and increasing the watering regime during the summer when the environment was predominantly dry. Also, relative water content change across the different treatment chambers did not vary significantly ($p = 0.315$) suggesting that the seedlings were well hydrated during the exposure period.

Families with reduced symptom expression may harbor structural or physiological defenses such as thicker cuticular layers or elevated phenolic compound production that merit targeted biochemical and anatomical analyses (Barnes et al., 2019). Our findings suggest that selecting seedlings from these three families (F13, F14, F8) could promise an approximately 40-50% reduction in disease severity compared to the bottom three low performing families (F3, F8, F15). Moreover, our findings show a significant genotype and environmental interaction: the expression of resistance traits depended on both genetic background and microclimatic factors within the chambers (Zhang et al., 1994). In conclusion, exploring these dynamics is critical for predicting loblolly pine resilience to future BSNB outbreaks and for guiding selection in breeding programs aimed at enhancing durable resistance. Also, seedlings from less tolerant families can be incorporated into early detection-based studies and operations in the future. Susceptible families develop visible symptoms more rapidly and at lower inoculum levels than tolerant genotypes. By deploying these families in sentinel plots or alongside spore-trap networks, managers can detect initial pathogen incursions or emerging more aggressive strains sooner than would be possible using only tolerant stock.

Table 1. Summary of family rankings across infected chambers based on disease severity, height, and RCD

Family	Mean Disease	Disease Rank	Mean Height (cm)	Height Rank	Mean RCD (mm)
F13	0.197	1	51.7	15	7.12
F14	0.218	2	53.2	10	7.11
F17	0.225	3	51.8	13	7.14
F2	0.241	4	60.7	3	8.18
F4	0.245	5	62.2	1	9.46
F5	0.271	6	59.5	5	9.24
F7	0.275	7	51.7	14	9.13
F12	0.276	8	53.1	11	6.89
F10	0.288	9	52.3	12	7.12
F1	0.303	10	56.8	8	7.54
F16	0.305	11	51.4	16	7.31
F6	0.313	12	57.5	7	7.96
F11	0.317	13	53.2	9	6.86
F9	0.317	14	58.5	6	7.99
F15	0.32	15	49.5	17	6.7
F8	0.329	16	62	2	8.97
F3	0.38	17	59.5	4	8.34

Table 2. Summary family rankings across negative control chambers based on disease severity, height, and RCD

Family	Mean Disease	Disease Rank	Mean Height (cm)	Height Rank	Mean RCD (mm)
F13	0.0579	1	51.2	11	7.21
F9	0.0579	2	52.7	9	7.47
F17	0.0813	3	48.6	16	6.9
F4	0.0932	4	55.3	5	8.15
F5	0.0952	5	55.7	3	8.48
F12	0.1	6	50.1	14	6.97
F2	0.101	7	57.2	2	8.21
F7	0.107	8	51	12	6.96
F14	0.113	9	50.9	13	7.29
F8	0.126	10	58.4	1	8.61
F1	0.134	11	55.5	4	7.7
F3	0.134	12	54.3	6	8.08
F10	0.14	13	47.9	17	6.86
F15	0.147	14	54	8	7.36
F11	0.161	15	51.3	10	7.23
F16	0.162	16	50	15	7.54
F6	0.21	17	54.3	7	7.18

Table 3. Summary of family rankings across the positive control chambers based on disease severity, height, and RCD

Family	Mean Disease	Disease Rank	Mean Height (cm)	Height Rank	Mean RCD (mm)
F16	0.0259	1	49.7	16	7.03
F14	0.048	2	50.7	14	6.77
F2	0.05	3	56.3	8	7.55
F15	0.056	4	50.2	15	7
F17	0.056	5	47.7	17	6.76
F10	0.064	6	52.4	11	7.31
F13	0.0672	7	52.6	10	7.61
F7	0.0738	8	51.5	13	7.06
F8	0.0738	9	57.9	2	8.21
F4	0.0813	10	57.6	5	8.18
F6	0.0873	11	57.6	4	7.58
F9	0.088	12	57.3	7	7.84
F1	0.0932	13	57.7	3	7.27
F5	0.0957	14	57.4	6	8.15
F11	0.108	15	53.1	9	7.01
F12	0.11	16	52.3	12	7.04
F3	0.134	17	60.3	1	9.04

Table 4. Two-way ANOVA relationship between treatments and family effects on RWC change

Source	Sum of squares	df	F-statistic	p-value
Treatment	1494.16	2	1.17	0.315
Family	4226.22	16	0.41	0.975
Residual	52861.18	83		

Df: Degree of freedom