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Spacing effects and seed-lot contrasts in field-expressed foliar necrosis, survival, and growth of *Eucalyptus nitens* in a Nelder genetic trial under Atlantic sanitary conditions

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Introduction: Genetic trials can provide comparative evidence on host performance under shared field exposure that is relevant to forest health surveillance, yet field evidence linking early foliar damage, spacing, and later plantation performance remains limited for *Eucalyptus nitens* under Atlantic conditions.
Methods: We analysed a partially imbalanced subset of trees from a Nelder spacing trial in northern Spain, focusing on two seed lots evaluated along a continuous spacing gradient. Tree-level foliar necrosis variables from a 2016 health assessment were related to survival and post-2016 growth measured through 2024. The study assessed field-expressed foliar necrosis under Atlantic sanitary conditions. Although the regional sanitary context is consistent with the occurrence of foliar diseases within the Mycosphaerella–Teratosphaeria complex, no tree-level pathogen confirmation was available. Analyses combined regression models with sensitivity analyses tailored to the clustered design and a leaf-level sensitivity analysis of necrosis severity.
Results: Lower planting density was consistently associated with lower foliar necrosis severity, higher survival probability, and greater post-2016 growth. EUNI-2 showed markedly higher observed survival to 2024 than EUNI-1 (92.5% vs. 70.8%; absolute difference 21.7 percentage points), and this survival advantage remained robust across sensitivity analyses. EUNI-2 also showed lower mean foliar necrosis severity than EUNI-1 (18.67% vs. 26.47%), but support for that contrast between seed lots was weaker under cluster-bootstrap inference. Leaf-level sensitivity analyses nevertheless indicated lower symptom intensity in EUNI-2 and towards the less dense end of the spacing gradient. Early foliar necrosis severity was not clearly associated with later growth.
Discussion: The most robust signals in this field subset were the consistent effect of spacing across sanitary, demographic, and growth responses and the survival advantage of EUNI-2. Taken together, the study shows that a structured genetic trial can generate useful comparative evidence on relative host performance for forest health surveillance and the evaluation and deployment of tree genetic resources under Atlantic plantation conditions.

KEYWORDS
establishment mortality, forest pathology, phytosanitary monitoring, sentinel planting, silvicultural design

1 Introduction

Eucalyptus species are among the most extensively planted hardwoods worldwide and underpin major industrial sectors linked to pulp, timber, fibre, and bioenergy production. Their global expansion has also increased exposure to a wide range of pathogens, among which foliar fungi are especially important because they directly affect the photosynthetically active canopy and, consequently, plantation performance. Foliar diseases are therefore regarded as one of the major health constraints on the sustainability of eucalypt forestry, particularly where host continuity, broad-scale deployment, and favourable environmental conditions may promote pathogen establishment and spread (Burgess and Wingfield, 2017; Crous et al., 2019; Solís et al., 2025).

The biological significance of foliar disease extends well beyond the visible expression of leaf spots, necrosis, or blight, because canopy damage may translate into measurable physiological and productivity losses in affected trees (Pinkard and Mohammed, 2006; Eyles et al., 2013). In young plantations, successful establishment depends critically on rapid canopy development and on the maintenance of functional juvenile foliage, as leaf area strongly underpins radiation capture, carbon gain, and early growth performance in eucalypts (Whitehead and Beadle, 2004). This is particularly relevant because young, fast-growing eucalypt plantations are characterized by abundant juvenile foliage, which may favour pest and pathogen outbreaks and increase the impact of foliar damage on tree performance (Bonora et al., 2020). Recurrent damage during this stage may therefore reduce effective leaf area, constrain carbon assimilation, alter physiological performance, and generate persistent effects on vigour, growth, and survival (Pinkard and Mohammed, 2006; Eyles et al., 2013; Balmelli et al., 2013).

This issue is especially relevant for *Eucalyptus nitens* (H. Deane & Maiden) Maiden, a species of increasing silvicultural importance in cool temperate and humid plantation environments. In northern Spain, *E. nitens* has gained relevance as an alternative plantation species in colder areas and in environments where pests and diseases may constrain the performance of more traditionally deployed eucalypts, making field-based evaluation of host–pathogen interactions particularly important for both pathology and deployment decisions (Pérez-Cruzado et al., 2011; Pérez Cruzado et al., 2015).

Among the foliar diseases affecting eucalypts, one of the most intensively studied syndromes is that historically referred to as *Mycosphaerella* leaf disease. This designation reflects an earlier taxonomic framework under which many associated fungi were grouped, whereas subsequent revisions demonstrated that the disease complex includes species currently assigned to several genera, including *Teratosphaeria*. Within this revised framework, *Teratosphaeria nubilosa* (Cooke) Crous & U. Braun, formerly known as *Mycosphaerella nubilosa*, is recognized as one of the most important foliar pathogens of Eucalyptus in both native and introduced environments (Crous et al., 2019; Hunter et al., 2009). Severe infection by *T. nubilosa* is associated with leaf spotting, necrosis, premature defoliation, and reduced tree performance, particularly under favourable environmental conditions and in susceptible planting material (Hunter et al., 2009; Andjic et al., 2019).

Its relevance is not restricted to distant plantation systems. Earlier reports from Spain also documented *Mycosphaerella* species causing leaf spot on *Eucalyptus globulus*, indicating that foliar pathogens within this broader disease complex were already recognized as a sanitary issue in Iberian plantation contexts (de Blas et al., 2009). More recently, surveys in north-western Spain have shown that diseases caused by *Mycosphaerella*- and *Teratosphaeria*-related fungi are widespread and that *Teratosphaeria* diseases are among the most prevalent and severe foliar health problems in the region; within that complex, *T. nubilosa* was identified as the most prevalent species in affected stands, highlighting its direct sanitary relevance under Atlantic Iberian conditions (Aguín et al., 2013).

Management of foliar diseases in commercial tree plantations remains inherently difficult. Curative control is often impractical at operational scale, and chemical protection is rarely a desirable long-term strategy in plantation forestry. Consequently, the deployment of resistant or less susceptible planting material is widely regarded as one of the most promising options for reducing disease impact. Importantly, field resistance should not be interpreted solely as reduced visible infection, but also as reduced canopy loss, improved survival, or greater tolerance of early damage. Recent syntheses on eucalypt foliar pathogens and host defence mechanisms support the importance of resistant genetic material as a central component of sustainable disease mitigation strategies (Burgess and Wingfield, 2017; Solís et al., 2025; Andjic et al., 2019).

These considerations align with the growing use of arboreta, genetic trials, and surveillance-oriented woody plantings as field platforms for documenting differential host responses to pests and pathogens and for informing the deployment and conservation of tree genetic resources. Genetic trials are particularly informative in this context because they enable structured comparisons among contrasting plant materials under shared environmental exposure, thereby linking visible sanitary outcomes to host identity and host performance (Vettraino et al., 2015; Mansfield et al., 2019; Migliorini et al., 2023). Rather than providing definitive etiological attribution at the individual-tree level, such trials can generate surveillance-relevant evidence on relative host vulnerability, host resilience, and the potential operational value of contrasting planting materials under realistic field conditions.

When such trials are established using a Nelder systematic design, they provide an additional methodological advantage by incorporating a compact and continuous spacing gradient within a relatively small area. Nelder designs were originally developed to study the effects of growing space and plant arrangement and remain especially useful when the objective is to characterize directional biological responses across density gradients rather than to compare only a few discrete spacing treatments (Nelder, 1962; Vanclay, 2006). This feature is particularly relevant for foliar disease studies because planting density may influence not only tree growth and competition but also canopy structure, air movement, moisture retention, and leaf wetness within the canopy, thereby affecting the microenvironment in which foliar infection and damage are expressed (Vidal et al., 2017;

Krupinsky et al., 2002). In the present study, these questions were addressed in the Monte Corona trial, our experimental site in northern Spain, which was established as a Nelder-based Eucalyptus plantation to evaluate the role of spacing in early stand development and in the field expression of foliar necrosis under Atlantic sanitary conditions in which *T. nubilosa* and related foliar pathogens are regionally relevant.

Despite this potential, relatively few field studies have used genetic trials not only to compare host materials, but also to evaluate their practical sentinel value for forest health surveillance and the protection of tree genetic resources under realistic plantation conditions. This gap is especially relevant for *Eucalyptus nitens* under Atlantic environments, where juvenile foliar disease, stand density, and establishment success may interact strongly. Because genetic trials expose contrasting host materials to shared local disease pressure, they can generate surveillance-relevant evidence on relative host vulnerability before wider deployment.

Against this background, we used the Monte Corona Nelder trial as a structured field platform to assess whether seed-lot identity and spacing jointly shaped early foliar necrosis, survival, and later plantation performance under Atlantic field conditions. Specifically, we asked whether the two seed lots differed in mean foliar necrosis severity in 2016, whether spacing modified foliar damage, survival, and post-2016 growth, and whether early foliar damage was associated with later performance. By addressing these questions in a shared field setting, the study evaluates the extent to which a genetic trial can generate surveillance-relevant evidence for the evaluation and deployment of tree genetic resources.

2 Materials and methods

2.1 Study site and environmental conditions

The study was conducted at the Monte Corona trial, located in the municipality of Valdáliga, Cantabria, northern Spain. The experimental site forms part of the public forest "Canal de Llaín" (Monte de Utilidad Pública No. 345) and occupies an area of 2.007 ha. The site is located at UTM coordinates 394,870 E and 4,798,651 N, within the nemoral phytoclimatic subregion VI(V), at an elevation ranging from 220 to 265 m above sea level.

Monte Corona is representative of humid Atlantic environments in which foliar diseases of Eucalyptus are of particular sanitary relevance. The site was characterized by low-slope topography, a west-facing aspect, a soil pH of 4.65 in the 0–30 cm layer, cambisol soil of dystric-eutric subtype, and siltstone bedrock. Mean annual temperature was 13 °C, mean temperature of the coldest month was 4 °C, mean annual precipitation was approximately 1,300 mm, and the site experienced an average of 5 frost days per year.

2.2 Trial design and sampling strategy

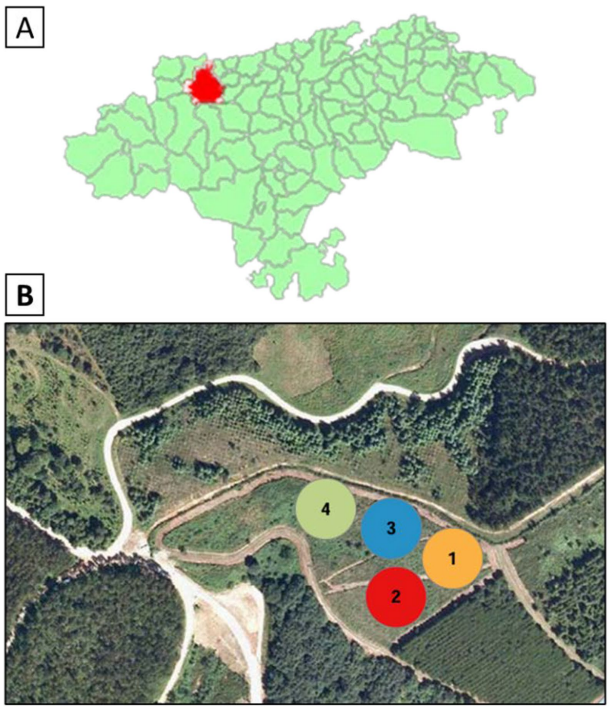
The trial was established as a Nelder type-a systematic spacing design based on concentric arcs, generating a continuous gradient from higher to lower planting density (Nelder, 1962; Vanclay, 2006). The original plantation comprised four Nelder rings, each containing 36 radial lines and 8 trees per radial line, resulting in 288 trees per ring and 1,152 trees across the full experiment. Trees were planted in spring 2012, and missing individuals were replaced in spring 2013. Replacement trees from 2013 could not be retrospectively identified in the final analytical dataset, so their potential influence on later survival and growth estimates could not be evaluated separately.

Two *Eucalyptus nitens* seed lots of contrasting origin were evaluated in the plantation. EUNI-1 corresponded to open-pollinated seed collected from the Chilean Nihuinco seed orchard, whose breeding background is largely based on Central Victoria material. EUNI-2 corresponded to open-pollinated seed collected from a commercial plantation in Guriezo (Cantabria), derived from material established in the former Arcentales arboretum in northern Spain. According to breeder records, these materials differ in their geographic origin and breeding background. In the context of the present study, EUNI-2 was treated *a priori* as the more field-tolerant seed lot and EUNI-1 as the more susceptible seed lot with respect to field-expressed foliar necrosis under Atlantic sanitary conditions. Here, "field-tolerant" refers to comparatively better performance under shared field exposure, based on breeder records and prior operational observations under regional plantation conditions, not to experimentally confirmed resistance to a single pathogen. Because both materials represent open-pollinated seed lots rather than genetically uniform entries, within-treatment heterogeneity was expected.

For the present study, analyses were restricted to trees located on radial lines 1, 10, 19, and 28 within each of the four Nelder rings. All eight tree positions within each selected radial line were considered, resulting in a theoretical sample size of 128 trees. These four radial lines were selected *a priori* because they represented the four quadrants of the Nelder design and allowed spatially distributed sampling to be completed within a narrow time window under a common field protocol. This logistical restriction necessarily limited spatial coverage relative to the complete trial and may not have captured the full extent of fine-scale environmental heterogeneity present across all radial lines. The sampled subset was defined *a priori* and was not selected on the basis of tree health, survival, or growth. Although it captured all four rings and all four quadrants of the design, it did not represent the full spatial and micro-site heterogeneity of the complete trial. Tree position within the spoke was used as a numeric representation of the spacing gradient, with lower values corresponding to higher planting density and higher values to lower planting density. Figure 1 provides a simplified representation of the trial layout, sampled subset, and site location. Within the sampled subset, seed-lot allocation was not fully balanced across sampled radial lines: only four of the 16 sampled ring-by-radial-line units contained both seed lots, whereas the remaining 12 units were single-seed-lot clusters. Because seed-lot allocation in the analysed subset was only partially balanced, contrasts between seed lots from the full-sample models should be interpreted as adjusted associations in a structured field subset rather than as fully randomized within-cluster comparisons. For that reason, we complemented the primary analyses with stress tests restricted to clusters containing both seed lots, cluster bootstrap sensitivity analyses, and leave-one-cluster-out refits. The distribution of seed lots across sampled ring-by-radial-line units is reported in Supplementary Table S5.

2.3 Foliar health assessment

Tree health was assessed on 21 December 2016 during the juvenile phase of plantation development using a standardised field protocol based on ICP Forests crown condition and damage assessment



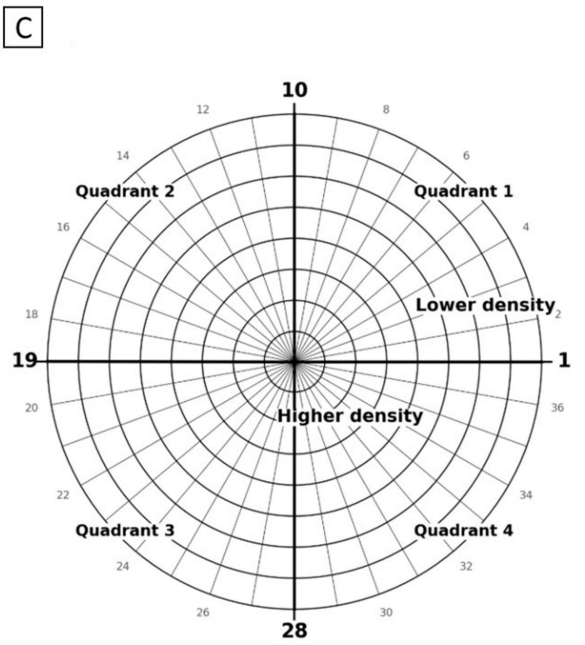


FIGURE 1
Study site and experimental design of the Monte Corona *Eucalyptus nitens* trial in northern Spain. **(A)** Regional location of the study area in Cantabria, Spain, with the municipality of Valdáliga highlighted. **(B)** Orthophoto of the Monte Corona experimental area showing the approximate location of the four Nelder circles established in the trial. **(C)** Schematic representation of the Nelder spacing design, showing the four quadrants, the 36 radial lines, and the density gradient from higher density near the centre to lower density towards the outer part of the design. Radial lines 1, 10, 19, and 28 correspond to the positions sampled in the present study.

criteria, adapted to young, accessible *Eucalyptus nitens* trees in the Monte Corona trial (ICP Forests, 2016). The assessment focused on foliar necrosis and insect-related damage, both recorded at the leaf level. Because the trees were still small and accessible, eight leaves per tree were sampled directly from accessible foliage under the same field protocol. Foliar necrosis severity was visually estimated for each leaf as the percentage of leaf area affected, predominantly in 5% increments. Insect-related damage was assessed on the same leaves using the same percentage scale. All visual assessments were conducted by a single observer, so inter-observer calibration was not applicable. However, detailed metadata on crown position, height within the crown, exposure, and shoot type were not retained in the available field records and therefore could not be reconstructed retrospectively. These aspects should be considered additional sources of measurement heterogeneity.

These leaf-level observations were used to derive tree-level health variables for the primary statistical analyses, while the original leaf-level data were also retained for an additional sensitivity analysis of symptom intensity. The main health variable used in the present study was tree-level mean foliar necrosis severity, calculated as the arithmetic mean of necrosis severity across the eight assessed leaves. Foliar necrosis incidence was additionally calculated as the proportion of sampled leaves showing non-zero necrosis severity. Insect presence was derived as a complementary binary indicator based on whether insect damage was recorded in at least one of the sampled leaves. Unrecorded variables were treated as missing data in subsequent analyses and were not assumed to represent zero damage.

The foliar variables used here quantify visible necrotic damage recorded in the field under Atlantic sanitary conditions in which *T. nubilosa* and related foliar pathogens are regionally relevant. However, the 2016 assessment did not include pathogen isolation or molecular confirmation at the individual-tree level. Accordingly, the analyses address field-expressed foliar necrosis under Atlantic sanitary conditions rather than symptoms demonstrated to be caused exclusively by *T. nubilosa*.

2.4 Growth measurements and survival assessment

Growth was assessed from total height and basal diameter measurements obtained in 2016 and again on 22 August 2024. These variables were used to quantify post-establishment performance in the present study.

Survival in 2024 was recorded as a binary outcome, indicating whether each sampled tree was alive or dead at the time of reassessment. Because dead trees were not available for remeasurement in 2024, survival analyses included all sampled trees for which status could be established, whereas growth analyses were restricted to surviving trees with complete measurement records.

Post-2016 growth was expressed as the increment between measurement dates. Total height increment was calculated as the difference between height measured in 2024 and height measured in 2016, and basal diameter increment was calculated analogously from the corresponding basal diameter measurements.

2.5 Derived health variables

For analytical purposes, tree-level mean foliar necrosis severity was used as the primary health metric in the present study. This variable was selected because it provided a direct, quantitative, and biologically interpretable measure of field-expressed foliar damage suitable for regression modelling. This choice was also empirically justified because foliar necrosis incidence was high in both seed lots and was therefore less informative for discriminating symptom intensity, whereas mean severity retained broader continuous variation among trees.

Foliar necrosis incidence was analysed as a complementary variable describing symptom occurrence, in order to verify that the main inferences were not dependent on a single representation of early foliar damage. In addition, leaf-level necrosis severity among leaves with non-zero damage was retained for a supplementary sensitivity analysis, in order to evaluate whether the main directional signal was also detectable when symptom intensity was analysed without aggregation to the tree level.

Insect damage was not incorporated into the primary foliar necrosis metric because it represented an additional and non-specific source of foliar injury not directly equivalent to the necrotic damage considered here and was assessed only once. Instead, insect presence was examined separately in complementary descriptive analyses to provide biological context without obscuring the interpretation of the primary health variable.

2.6 Statistical analyses

All statistical analyses were performed in Python 3.13.5 using numpy 2.3.5, scipy 1.17.0, statsmodels 0.14.6, and matplotlib 3.10.8. The analytical dataset was generated by merging the 2016 foliar assessment and the 2024 growth inventory using ring, sampled radial line, tree position within radial line, and seed-lot identity as matching variables. Only trees located on radial lines 1, 10, 19, and 28 within each of the four Nelder rings were included in the analyses.

Variables not recorded during the 2016 health assessment were treated as missing values rather than as true zeros, and no imputation procedure was applied. Accordingly, each model was fitted to the corresponding complete-case subset defined by the availability of the response and explanatory variables.

The analytical framework considered seed-lot identity, ring, and tree position within the Nelder spoke as the main explanatory variables. Ring was retained as a fixed blocking factor to account for broad-scale spatial heterogeneity within the trial. Tree position was treated as a continuous proxy for the spacing gradient, with lower values representing higher planting density and higher values representing lower planting density. The sampled radial lines corresponded to the four quadrants of the Nelder design (NE, SE, NW, SW). Quadrant identity was evaluated as an additional blocking factor in exploratory models; because it did not modify the main inferential conclusions and no quadrant contrast showed clear statistical support, it was not retained in the primary analytical framework.

Because trees were sampled in a structured subset of four radial lines within each of four rings, inferential models were fitted with cluster-robust standard errors, clustering on sampled radial line within ring (16 clusters in total), in order to account for non-independence among trees within the same sampled spoke. Because inference based on conventional cluster-robust standard errors can be optimistic with a limited number of clusters, we also performed non-parametric cluster bootstrap sensitivity analyses for the main regression models, resampling whole sampled radial line-within-ring units with replacement. For each coefficient of interest, bootstrap percentile confidence intervals and sign-based two-sided p-values were used to assess the stability of the main directional conclusions under finite-cluster resampling.

Coefficient stability was further evaluated by sequentially excluding one sampled radial line within ring at a time (leave-one-cluster-out analysis). In addition, because only a subset of sampled clusters contained both seed lots, we fitted a stress-test analysis restricted to the clusters containing both seed lots, using cluster fixed effects to isolate within-cluster contrasts between seed lots. Finally, to assess whether the linear representation of the spacing gradient was adequate, we fitted quadratic-position sensitivity models for the main responses. Results of these quadratic-position sensitivity analyses are reported in Supplementary Table S7.

In this study, full-sample models were used to estimate adjusted associations in the sampled field subset, whereas analyses restricted to clusters containing both seed lots were used as more conservative stress tests for contrasts between seed lots, and bootstrap and leave-one-cluster-out procedures were used to assess robustness.

Tree-level mean foliar necrosis severity in 2016 was analysed as the primary early-health response using a linear regression model with seed-lot identity, ring, and tree position as explanatory variables and cluster-robust standard errors. As a sensitivity check for distributional asymmetry, the same model was additionally fitted on a log-transformed severity scale, log (x + 1). Foliar necrosis incidence was analysed as a complementary response using a binomial generalized linear model based on the number of symptomatic and asymptomatic leaves per tree, again with seed-lot identity, ring, and tree position as explanatory variables and cluster-robust standard errors.

As an additional sensitivity analysis, we re-analysed foliar necrosis severity at the leaf level using generalized estimating equations (GEE) with exchangeable working correlation at the tree level, thereby accounting for within-tree dependence among the sampled leaves. Because foliar necrosis incidence was already high in both seed lots and the grouped-binomial tree-level incidence model did not show clear support for seed-lot or spacing effects, the leaf-level analysis was framed as an analysis of symptom intensity conditional on symptom presence rather than symptom occurrence itself. For that reason, it was restricted to leaves with non-zero necrosis severity and modelled on the log scale. In that analysis, log-transformed leaf-level necrosis severity was modelled as a function of seed-lot identity, ring, and tree position. To evaluate whether the directional signal persisted under the partial seed-lot imbalance of the sampled subset, the same severity model was additionally refitted within the clusters containing both seed lots using cluster fixed effects.

Survival to 2024 was analysed using a binomial generalized linear model with logit link fitted to the full analytical subset with known survival status. Because only 20 death events were observed and small-sample bias or near-separation could affect conventional logistic estimates, Firth's penalized logistic regression was used as the primary robustness-focused inferential model for survival, while the cluster-robust logistic model was retained as a complementary analysis for comparability with the rest of the modelling framework. Because usable early-health data were unavailable for a small number of trees, an additional complete-case survival model including mean foliar

TABLE 1 Analytical sample and variable-specific data availability for the Monte Corona trial subset included in the present study.

Variable/subset	Total	EUNI-2	EUNI-1
Trees targeted	128	80	48
Trees alive in 2024	108	74	34
Trees dead in 2024	20	6	14
Trees with mean necrosis severity data	125	79	46
Trees with necrosis incidence data	125	79	46
Trees included in growth models	107	74	33

The table summarizes the number of trees initially targeted by the sampling design, the number of trees alive and dead in 2024, and the availability of the main early health variables used in the analyses. Mean necrosis severity available refers to trees for which tree-level mean foliar necrosis severity could be calculated from the 2016 foliar assessment. Necrosis incidence available refers to trees for which the proportion of leaves showing necrosis could be derived. Trees included in growth models refers to surviving trees with complete early health and post-2016 growth records.

necrosis severity was evaluated as an exploratory analysis and interpreted cautiously.

Post-2016 growth responses were analysed separately for height increment and basal diameter increment using linear regression models with cluster-robust standard errors. Growth analyses were restricted to surviving trees with complete early-health and growth records. Each growth model included seed-lot identity, ring, tree position, mean foliar necrosis severity, and the corresponding initial tree size measured in 2016. Initial height was included in the model for height increment, and initial basal diameter was included in the model for basal diameter increment.

Residual assumptions for the linear models were assessed by visual inspection of standardised residual-versus-fitted and normal Q–Q plots. Multicollinearity in the growth models was low and did not motivate predictor removal. Variance inflation factors are reported in Supplementary Table S8. Diagnostic plots are shown in Supplementary Figures S1–S3.

Given the moderate sample size and the limited number of death events, interaction terms were treated as exploratory and were not retained in the main inferential models unless they provided clear and stable improvement in fit and interpretability. Results were interpreted on the basis of effect direction, effect magnitude, associated uncertainty, and consistency across related response variables. All tests were two-sided, and statistical significance was assessed at $\alpha = 0.05$.

The statistical reproducibility package supporting the analyses reported in this study has been archived on Zenodo.[1] A minimal dataset sufficient to reproduce the main and supplementary analyses has also been deposited on Zenodo.[2]

3 Results

3.1 Analytical sample and data completeness

A total of 128 trees were included in the analytical dataset, corresponding to the eight tree positions sampled on radial lines 1, 10, 19, and 28 within each of the four Nelder rings. Of these, 80 trees belonged to EUNI-2 and 48 to EUNI-1. By 2024, 108 trees were alive and 20 were dead.

Variable-specific data availability differed slightly among analyses. Mean foliar necrosis severity and foliar necrosis incidence could both be calculated for 125 trees. Post-2016 growth models were fitted on 107 surviving trees with complete records for both growth and early health variables. These differences in sample size were accounted for by fitting each model to the corresponding complete-case subset defined by the availability of the response and explanatory variables (Table 1). The reduction from 34 surviving EUNI-1 trees to 33 trees included in the growth models reflects complete-case filtering due to missing early-health data for one surviving EUNI-1 tree.

Among the 16 sampled ring-by-radial-line clusters, only four contained both seed lots. Because this partial imbalance can widen uncertainty around contrasts between seed lots, additional finite-cluster sensitivity analyses are reported below and in the Supplementary material.

3.2 Early foliar damage in 2016

Mean foliar necrosis severity in 2016 differed between seed lots and across the spacing gradient. Descriptively, EUNI-2 showed lower mean necrosis severity than EUNI-1 (18.67% vs. 26.47%; Table 2 and Figure 2), corresponding to a moderate standardised difference between seed lots (Cohen's d = −0.52).

In the original linear regression model with cluster-robust standard errors, seed-lot identity and tree position both showed statistically supported effects on mean foliar necrosis severity (Table 3). Relative to EUNI-1, EUNI-2 showed lower necrosis severity (beta = −7.89, SE = 3.47, $p = 0.023$), and tree position also had a significant negative effect (beta = −1.68, SE = 0.51, $p = 0.001$), indicating lower necrosis severity towards the less dense portion of the spacing gradient. Ring 4 also showed a comparatively large positive coefficient for necrosis severity (beta = 16.69, $p = 0.055$). However, finite-cluster sensitivity analyses weakened the inferential support for the contrast between seed lots. In the non-parametric cluster bootstrap, the coefficient for EUNI-2 remained negative and similar in magnitude, but the 95% bootstrap interval included zero (95% CI: −14.65 to 1.11; bootstrap $p = 0.086$), whereas the spacing effect remained clearly supported (95% CI: −2.77 to −0.80; bootstrap $p < 0.001$; Supplementary Table S3). In leave-one-cluster-out refits, the coefficient for EUNI-2 and the tree-position coefficient retained the same sign throughout (Supplementary Table S4). When the analysis was restricted to the clusters containing both seed lots only, the coefficient

1 https://doi.org/10.5281/zenodo.19919191

2 https://doi.org/10.5281/zenodo.19919496

TABLE 2 Descriptive statistics by seed lot for mean foliar necrosis severity, foliar necrosis incidence, insect presence, survival to 2024, height increment, and basal diameter increment in the Monte Corona trial subset analysed in the present study.

Variable	Seed lot	*n*	Mean	SD	Median	Min	Max
Mean foliar necrosis severity (%)	EUNI-1	46	26.47	15.52	24.69	0.00	67.14
Mean foliar necrosis severity (%)	EUNI-2	79	18.67	14.55	14.38	3.75	79.38
Foliar necrosis incidence (proportion)	EUNI-1	46	0.84	0.27	1.00	0.00	1.00
Foliar necrosis incidence (proportion)	EUNI-2	79	0.84	0.18	0.88	0.25	1.00
Insect presence (0/1)	EUNI-1	46	0.72	0.46	1.00	0.00	1.00
Insect presence (0/1)	EUNI-2	79	0.85	0.36	1.00	0.00	1.00
Height increment (mm)	EUNI-1	33	1466.82	561.35	1623.00	349.00	2165.00
Height increment (mm)	EUNI-2	74	1545.95	521.87	1673.00	380.00	2367.00
Basal diameter increment (mm)	EUNI-1	33	141.31	93.33	146.38	3.73	328.44
Basal diameter increment (mm)	EUNI-2	74	136.35	101.24	112.34	0.00	374.08
Survival to 2024 (%)	EUNI-1	48	70.83	–	–	–	–
Survival to 2024 (%)	EUNI-2	80	92.50	–	–	–	–

Mean foliar necrosis severity is expressed as the average percentage of leaf area affected per tree. Foliar necrosis incidence is the proportion of sampled leaves showing necrosis among trees with available necrosis observations. Insect presence is expressed as a binary variable indicating whether insect damage was recorded in at least one sampled leaf per tree among trees with available insect observations. Survival to 2024 is expressed as the percentage of trees alive at reassessment. Height increment and basal diameter increment correspond to the difference between the 2024 and 2016 measurements for surviving trees with complete early-health and growth records.

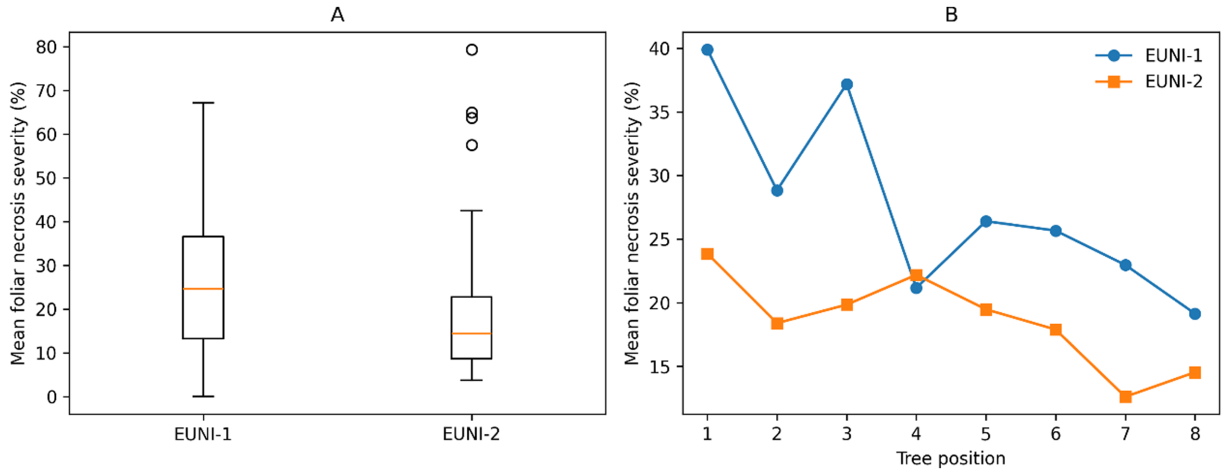


FIGURE 2
Early foliar health in 2016. **(A)** Mean foliar necrosis severity by seed lot. **(B)** Mean foliar necrosis severity along the spacing gradient by seed lot.

for EUNI-2 remained negative but became imprecise (beta = −4.02, $p = 0.213$; Supplementary Table S6). The distribution of seed lots across the sampled clusters is shown in Supplementary Table S5.

A log-transformed sensitivity model preserved the same overall directional pattern, with tree position remaining negatively associated with severity and the coefficient for EUNI-2 also remaining negative, although smaller in magnitude. Because the finite-cluster bootstrap provided a more relevant robustness check for the present clustered design, results are presented on the original percentage scale for biological interpretability.

Because the tree-level contrast between seed lots in mean necrosis severity weakened under finite-cluster bootstrap inference, we additionally evaluated symptom intensity at the leaf level (Table 4). In that sensitivity analysis, restricted to leaves with non-zero necrosis severity, EUNI-2 showed lower symptom intensity than EUNI-1, and severity also declined towards the less dense end of the spacing gradient. In the full leaf-level GEE model, the coefficient for EUNI-2 was −0.450 (SE = 0.082, $p < 0.001$), corresponding to an expected severity approximately 0.64 times that of EUNI-1, whereas tree position was also negative (−0.090, SE = 0.018, $p < 0.001$). Importantly, this directional pattern also persisted in the leaf-level sensitivity analysis restricted to clusters containing both seed lots, in which the coefficient for EUNI-2 remained negative (−0.457, SE = 0.130, $p < 0.001$). Taken together, the tree-level and leaf-level analyses indicate that differences between seed lots and across the spacing gradient were expressed more clearly in symptom intensity than in simple symptom occurrence.

Foliar necrosis incidence showed weaker statistical structure than severity. In the complementary grouped binomial model based on symptomatic versus asymptomatic leaves, neither seedlot identity nor tree position showed clear statistical support (Table 3). Taken together, these results indicate that symptom occurrence was widespread across the analytical subset, whereas symptom intensity provided the more discriminating descriptor

TABLE 3 Cluster-robust models for early foliar health in 2016, including mean foliar necrosis severity and foliar necrosis incidence.

Response variable	Term	Estimate	SE	*z*	*p*	95% CI of estimate
Mean foliar necrosis severity	Intercept	30.36	4.71	6.45	<0.001	21.13 to 39.60
	EUNI-2 vs. EUNI-1	−7.89	3.47	−2.27	0.023	−14.70 to −1.08
	Ring 2 vs. Ring 1	−2.68	4.34	−0.62	0.537	−11.20 to 5.83
	Ring 3 vs. Ring 1	1.26	4.90	0.26	0.797	−8.35 to 10.87
	Ring 4 vs. Ring 1	16.69	8.69	1.92	0.055	−0.34 to 33.73
	Tree position	−1.68	0.51	−3.29	0.001	−2.68 to −0.68
Foliar necrosis incidence	Intercept	1.896	0.829	2.29	0.022	0.271 to 3.520
	EUNI-2 vs. EUNI-1	0.026	0.410	0.06	0.949	−0.778 to 0.831
	Ring 2 vs. Ring 1	−0.572	0.440	−1.30	0.194	−1.433 to 0.290
	Ring 3 vs. Ring 1	−0.108	0.433	−0.25	0.803	−0.957 to 0.741
	Ring 4 vs. Ring 1	0.509	0.841	0.61	0.545	−1.139 to 2.156
	Tree position	−0.030	0.082	−0.36	0.716	−0.190 to 0.130

For categorical predictors, estimates are expressed relative to the reference levels EUNI-1 and ring 1. "EUNI-2 vs. EUNI-1" therefore represents the effect of EUNI-2 compared with EUNI-1, and "Ring 4 vs. Ring 1", for example, represents the contrast between those two rings. Tree position was analysed as a continuous predictor along the spacing gradient, with lower values corresponding to higher planting density and higher values to lower planting density. Mean foliar necrosis severity was analysed with linear regression and cluster-robust standard errors. Foliar necrosis incidence was analysed with a grouped binomial generalized linear model based on the number of symptomatic versus asymptomatic leaves per tree, also with cluster-robust standard errors. SE denotes the standard error of the estimated coefficient; z denotes the Wald statistic; *p* denotes the two-sided *p*-value; and 95% CI denotes the 95% confidence interval of the estimated coefficient. The adjusted R^2 of the mean foliar necrosis severity model was 0.340.

TABLE 4 Leaf-level sensitivity analysis for foliar necrosis severity among leaves with non-zero necrosis.

Model	Term	Estimate (log scale)	SE	*p*	Multiplicative effect on expected severity	95% CI of multiplicative effect
Full leaf-level GEE	EUNI-2 vs. EUNI-1	−0.450	0.082	<0.001	0.638	0.543–0.749
Full leaf-level GEE	Tree position	−0.090	0.018	<0.001	0.914	0.882–0.947
Leaf-level sensitivity restricted to clusters containing both seed lots	EUNI-2 vs. EUNI-1	−0.457	0.130	<0.001	0.633	0.491–0.816
Leaf-level sensitivity restricted to clusters containing both seed lots	Tree position	−0.131	0.031	<0.001	0.877	0.826–0.932

Foliar necrosis severity was re-analysed at the leaf level using generalized estimating equations (GEE) with exchangeable working correlation at the tree level. The full leaf-level model included seed-lot identity, ring, and tree position. To assess whether the directional signal persisted under the partial seed-lot imbalance of the sampled subset, the same severity model was additionally refitted within the clusters containing both seed lots using cluster fixed effects. Estimates are given on the log scale. The multiplicative effect on expected severity was obtained by exponentiating the coefficient estimate. Values below 1 indicate lower expected necrosis severity.

of early sanitary status. This interpretation is also consistent with the similar tree-level mean foliar necrosis incidence observed in both seed lots (0.84 in EUNI-1 and 0.84 in EUNI-2; Table 2), which motivated the leaf-level sensitivity analysis as an assessment of symptom intensity conditional on symptom presence rather than occurrence itself.

3.3 Survival from 2016 to 2024

Observed survival to 2024 was higher in EUNI-2 than in EUNI-1 (92.5% vs. 70.8%; Table 2). This corresponds to an absolute survival difference of 21.7 percentage points between seed lots. In the conventional cluster-robust logistic model fitted to the full analytical subset, both seed-lot identity and tree position showed positive associations with survival. Relative to EUNI-1, EUNI-2 had a higher probability of survival (beta = 1.98, SE = 0.53, $p < 0.001$), corresponding to an odds ratio of 7.26, and tree position also had a significant positive effect (beta = 0.45, SE = 0.12, $p < 0.001$), corresponding to an odds ratio of 1.57 per unit increase along the spacing gradient.

Because only 20 death events were observed, survival inference was further evaluated with Firth-penalized logistic regression and finite-cluster bootstrap sensitivity analyses. In the Firth model, EUNI-2 remained positively associated with survival (beta = 1.82, OR = 6.19, 95% CI: 2.08 to 18.38, $p = 0.001$), and tree position also remained positively associated with survival (beta = 0.41, OR = 1.51, 95% CI: 1.16 to 1.95, $p = 0.002$) (Table 5). The cluster bootstrap gave the same qualitative result: the bootstrap odds-ratio interval for EUNI-2 remained entirely above one (95% CI: 2.27 to 32.45; bootstrap $p = 0.0087$), and the spacing effect also remained robust (95% CI: 1.29 to 2.10; bootstrap $p < 0.001$; Supplementary Table S3). In leave-one-cluster-out refits, the signs of both coefficients remained unchanged (Supplementary Table S4). This pattern also remained directionally consistent in the stress test restricted to clusters containing both seed lots, where the coefficient for EUNI-2 remained positive and statistically supported (cluster-FE logistic estimate = 4.414, $p = 0.042$), while the spacing effect also remained positive (estimate = 1.575, $p = 0.001$; Supplementary Table S6).

TABLE 5 Firth-penalized logistic regression model for survival to 2024.

Term	Estimate (logit)	SE	z	*p*	Odds ratio	95% CI of odds ratio
Intercept	−1.40	0.77	−1.83	0.068	0.25	0.05–1.11
EUNI-2 vs. EUNI-1	1.82	0.56	3.28	0.001	6.19	2.08–18.38
Ring 2 vs. Ring 1	0.77	0.74	1.04	0.299	2.16	0.51–9.18
Ring 3 vs. Ring 1	0.48	0.71	0.68	0.497	1.62	0.40–6.48
Ring 4 vs. Ring 1	0.77	0.74	1.04	0.299	2.16	0.51–9.18
Tree position	0.41	0.13	3.10	0.002	1.51	1.16–1.95

The response variable was survival status in 2024, coded as alive or dead. This model was prioritized for inference because only 20 death events were observed in the analytical subset and Firth penalization reduces small-sample bias and instability under near-separation. Estimates are given on the logit scale. Positive coefficients indicate an increase in survival probability, whereas negative coefficients indicate a decrease. Odds ratios were obtained by exponentiating the penalized logit coefficients. For categorical predictors, estimates are expressed relative to the reference levels EUNI-1 and ring 1. Tree position represents the effect of moving along the spacing gradient from higher to lower density. Supplementary sensitivity analyses based on cluster bootstrap and leave-one-cluster-out refits are provided in the Supplementary material.

Because usable early-health data were unavailable for a small number of trees, the primary survival model was fitted without early-health covariates in order to retain the full analytical subset. In an additional exploratory complete-case survival model including mean foliar necrosis severity, the seed-lot and spacing effects remained positive, but mean necrosis severity did not show clear support. This lack of support also persisted under cluster-bootstrap sensitivity analysis for the complete-case model (OR for necrosis = 1.01; bootstrap 95% CI: 0.94 to 1.08; bootstrap $p = 0.678$; Supplementary Tables S2, S3).

3.4 Post-2016 growth responses

Among trees included in the growth models, mean height increment between 2016 and 2024 was 1545.9 mm in EUNI-2 and 1466.8 mm in EUNI-1, whereas mean basal diameter increment over the same period was 136.3 mm in EUNI-2 and 141.3 mm in EUNI-1 (Table 2).

In the original height increment model estimated with cluster-robust standard errors, tree position had a significant positive effect (beta = 118.58, SE = 15.90, $p < 0.001$), indicating greater height increment towards lower planting density (Figures 3A,C). Initial height in 2016 was also positively associated with subsequent height increment (beta = 1.73, SE = 0.79, $p = 0.028$). EUNI-2 also showed a positive coefficient in this model (beta = 207.02, SE = 84.98, $p = 0.015$), whereas mean foliar necrosis severity did not show clear support. However, finite-cluster sensitivity analyses indicated that support for the coefficient associated with EUNI-2 was weaker than suggested by the asymptotic cluster-robust model alone. In the non-parametric cluster bootstrap, the coefficient for EUNI-2 remained positive but its 95% interval included zero (95% CI: −11.08 to 438.35 mm; bootstrap $p = 0.0665$), whereas the spacing effect remained clearly supported (95% CI: 91.17 to 152.55 mm; bootstrap $p < 0.001$). The sign of the coefficient for EUNI-2 remained unchanged in leave-one-cluster-out refits. Accordingly, although the coefficient for EUNI-2 was positive in the primary model, it should be interpreted as exploratory rather than as a robustly supported seed-lot effect on height increment.

The basal diameter increment model showed a similarly strong effect of tree position (beta = 23.88, SE = 3.86, $p < 0.001$), again indicating greater growth towards lower planting density (Figures 3B,D). Initial basal diameter was strongly and positively associated with subsequent radial growth (beta = 3.55, SE = 0.72, $p < 0.001$). Ring 2 also differed marginally from ring 1 in the original model (beta = 46.06, SE = 23.90, $p = 0.054$). Seed-lot identity and mean foliar necrosis severity did not show clear support in the basal diameter model, and the non-parametric cluster bootstrap likewise did not support a consistent seed-lot effect on radial growth (bootstrap 95% CI for the coefficient associated with EUNI-2: −37.59 to 59.93 mm; bootstrap $p = 0.556$). By contrast, the spacing effect remained robust (bootstrap 95% CI: 16.00 to 30.58 mm; bootstrap $p < 0.001$).

Because these growth models were fitted only among surviving trees, they describe post-2016 growth conditional on survival and do not estimate full-cohort productivity differences between the two seed lots. Taken together, the growth analyses indicate that post-2016 performance among surviving trees was structured more clearly by spacing and initial size than by the single early necrosis assessment. The positive coefficient for EUNI-2 in the height increment model was directionally consistent but weakened under finite-cluster bootstrap inference, whereas no clear seed-lot effect was detected for basal diameter increment. Full coefficient outputs for the growth models are reported in Supplementary Table S1. Finite-cluster bootstrap sensitivity results are summarized in Supplementary Table S3, leave-one-cluster-out coefficient stability is shown in Supplementary Table S4, stress tests restricted to clusters containing both seed lots are reported in Supplementary Table S6, quadratic-position sensitivity analyses are summarized in Supplementary Table S7, and variance inflation factors are reported in Supplementary Table S8.

4 Discussion

The present study shows that a Nelder genetic trial of *Eucalyptus nitens* can generate informative field evidence on spacing effects, differences between seed lots, and later establishment outcomes under Atlantic sanitary conditions. Two findings were particularly robust: the spacing gradient was consistently associated with lower foliar necrosis severity, higher survival, and greater later growth, and EUNI-2 showed a clear survival advantage over EUNI-1. By contrast, the contrast between seed lots in early foliar necrosis was directionally consistent but less secure under finite-cluster bootstrap inference, whereas post-2016 growth contrasts should be interpreted as secondary and conditional on survival. Taken together, these results indicate that the Monte Corona trial generated useful comparative evidence on relative host performance under shared field conditions, while also revealing non-trivial uncertainty around some contrasts between seed lots estimated from a partially imbalanced subset.

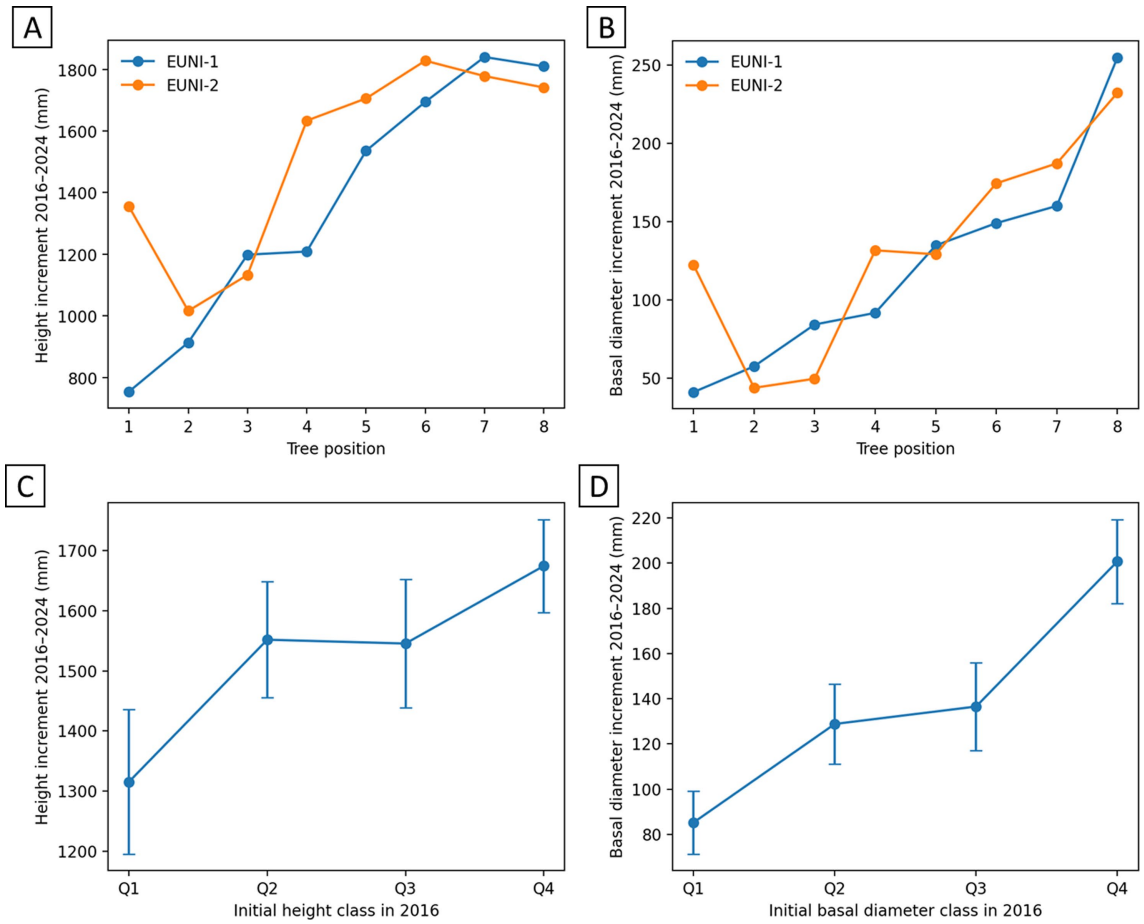


FIGURE 3
Post-2016 growth responses in the Monte Corona trial subset included in the growth models. **(A)** Mean height increment between 2016 and 2024 along the spacing gradient, shown by tree position and seed lot. **(B)** Mean basal diameter increment between 2016 and 2024 along the spacing gradient, shown by tree position and seed lot. **(C)** Mean height increment between 2016 and 2024 across quartiles of initial height measured in 2016. **(D)** Mean basal diameter increment between 2016 and 2024 across quartiles of initial basal diameter measured in 2016. Error bars represent standard errors.

The density-related signal detected in the present study was coherent, biologically plausible, and supported across multiple response variables. Trees located towards the lower-density portion of the Nelder design showed lower mean foliar necrosis severity, while the same gradient was associated with higher survival probability and greater basal diameter increment. Although the available dataset does not permit direct testing of the underlying mechanisms, the direction of the response is consistent with the expectation that planting density can influence both disease expression and later performance by modifying canopy structure, local humidity, leaf wetness duration, competition intensity, and access to resources (Vidal et al., 2017; Krupinsky et al., 2002). In this respect, the Nelder design offered a distinct methodological advantage. Because it incorporates a compact and continuous spacing gradient within a relatively small area, it allows directional biological responses to density to be evaluated more efficiently than experiments restricted to a few discrete spacing treatments (Nelder, 1962; Vanclay, 2006). Under the conditions of the Monte Corona trial, this feature made it possible to detect a consistent spacing-related signal across several response variables and thereby strengthen inference on the joint effects of seed-lot identity and stand structure.

From an applied perspective, the demographic signal remains the most robust difference between seed lots detected in the study. Observed survival to 2024 was clearly higher in EUNI-2 than in EUNI-1, and this contrast remained supported in the primary survival model fitted to the full analytical subset. In operational terms, the key point is that EUNI-2 showed lower early foliar necrosis severity and markedly higher later survival under the shared field conditions of this trial. However, the present dataset does not allow that survival difference to be attributed specifically to lower susceptibility to a particular pathogen or to the 2016 foliar necrosis assessment alone. The higher survival of EUNI-2 should therefore be interpreted as a robust field-performance difference under the shared conditions of the trial, rather than as direct evidence of pathogen-specific resistance. This applied relevance is particularly clear in northern Spain, where *Mycosphaerella–Teratosphaeria*-related foliar disease has already motivated explicit discussion of operational management strategies in eucalypt plantations (Pérez et al., 2016). For deployment decisions, that pattern is likely to be more consequential than modest differences in growth among surviving individuals. In this sense, the results support the broader view that useful field resistance in plantation forestry should be understood not only as reduced visible infection, but also as the capacity to maintain functional performance, especially survival and establishment, under field disease pressure (Burgess and Wingfield, 2017; Solís et al., 2025; Andjic et al., 2019).

The lower early foliar necrosis severity observed in EUNI-2 is consistent with the broader importance of host genetic background

in shaping eucalypt responses to foliar pathogens. *T. nubilosa* is widely regarded as one of the most important foliar pathogens of Eucalyptus, particularly in susceptible planting material and under environmental conditions favourable to repeated infection, where it can induce necrosis, canopy loss, and reductions in tree performance (Hunter et al., 2009; Andjic et al., 2019). Similar patterns of genetic variation in susceptibility have been reported previously in eucalypts, including provenance-level variation in *Eucalyptus nitens* and resistance-related variation in *Eucalyptus globulus* under field disease pressure (Lundquist, 1987; Balmelli et al., 2014). In this context, the lower foliar necrosis severity recorded in EUNI-2 is consistent with the possibility that this material may offer operational advantages under Atlantic plantation conditions. This is relevant not only as a contrast in visible symptoms, but also because lower foliar damage during stand establishment may help maintain photosynthetically active leaf area, reduce cumulative physiological stress, and improve the probability of successful early plantation development (Pinkard and Mohammed, 2006; Eyles et al., 2013).

The contrast between seed lots in early necrosis should nevertheless be interpreted cautiously. Because EUNI-1 and EUNI-2 represent open-pollinated seed lots rather than genetically uniform entries, some within-treatment heterogeneity was expected, which may have attenuated phenotypic contrasts relative to a more genetically homogeneous comparison. In addition, support for that contrast weakened under finite-cluster sensitivity analyses, indicating greater uncertainty than suggested by asymptotic cluster-robust inference alone. The weaker inferential structure of foliar necrosis incidence relative to severity further suggests that symptom intensity, rather than simple symptom occurrence, was the more informative descriptor of early sanitary differentiation in this trial. This interpretation was reinforced by the leaf-level sensitivity analysis, which showed clearer differentiation between seed lots and along the spacing gradient in necrosis severity among affected leaves than in the mere occurrence of necrotic symptoms.

However, only four of the 16 sampled clusters contained both seed lots, so residual confounding between seed-lot identity and fine-scale micro-site heterogeneity cannot be excluded, even after adjustment for ring and additional sensitivity analyses. Accordingly, contrasts between seed lots should be interpreted as adjusted differences within a structured field subset rather than as fully randomized comparisons. The comparatively large coefficient for Ring 4 is also consistent with some residual spatial heterogeneity within the trial, although ring effects were not a primary focus of inference and cannot be interpreted mechanistically from the present dataset. Because the present dataset does not include independent micro-site measurements at that scale, it was not possible to determine whether this signal reflected local environmental heterogeneity, establishment-related differences, or other unmeasured spatial factors.

From an operational perspective, the relevance of these contrasts does not depend entirely on attributing symptoms exclusively to *T. nubilosa*. Under Atlantic plantation conditions, foliar damage is often expressed within a broader *Mycosphaerella–Teratosphaeria* disease complex, and for deployment decisions the key question is whether planting material shows lower field-expressed foliar damage and better survival under shared exposure conditions (Aguín et al., 2013). Against that standard, the directional consistency of the EUNI-2 signals across tree-level and leaf-level analyses, together with its robustness in the survival models, provides useful operational evidence even in the absence of individual-tree pathogen confirmation.

Growth responses also showed a consistent association with spacing, although the signal was stronger and more structured for basal diameter than for height. Height increment was positively associated with tree position and initial height, whereas basal diameter increment was positively associated with tree position and initial basal diameter. This indicates that lower density was associated not only with reduced foliar damage and higher survival, but also with improved subsequent growth performance. Such a pattern is not unexpected in spacing experiments, where diameter growth often responds more directly to growing space and competitive release than height growth, particularly after the earliest establishment phase (Vanclay, 2006). The positive effects of initial tree size further indicate that pre-existing differences among surviving trees contributed to subsequent growth trajectories, reinforcing the view that post-2016 performance was shaped not only by early health status but also by later stand differentiation.

Although the original height increment model detected a positive coefficient for EUNI-2 in addition to a strong spacing effect, finite-cluster bootstrap analyses indicated that support for that coefficient was weaker than for the spacing effect, and the bootstrap confidence interval included zero. Accordingly, the positive coefficient for EUNI-2 in the height increment model should be regarded as directionally consistent but exploratory, rather than as one of the most strongly supported results of the study. Its adjusted R^2 was 0.278, indicating moderate explanatory power under heterogeneous field conditions, which is not unexpected given the substantial residual heterogeneity that typically characterizes tree-level growth in field trials.

In contrast, early foliar necrosis severity did not show statistically supported associations with post-2016 growth in the cluster-robust growth models. This result does not imply that early damage was biologically irrelevant; rather, it indicates that, in this dataset, the influence of early foliar damage on later growth was weaker or less detectable than its influence on later sanitary differentiation and survival, even though early foliar injury may still have important physiological and developmental consequences during plantation establishment (Pinkard and Mohammed, 2006; Eyles et al., 2013). This cautious interpretation is also consistent with previous field evidence showing that foliar disease in young eucalypt plantations can affect both growth and survival, although the strength and timing of those effects may vary among host materials and plantation contexts (Balmelli et al., 2014). Several non-exclusive explanations may account for this pattern: growth analyses were restricted to surviving trees; post-2016 growth integrated several years of stand development during which spacing-related competition, local site heterogeneity, and size-dependent growth dynamics may have become stronger determinants of performance than the single 2016 health assessment (Vanclay, 2006); and no repeated sanitary assessments were available to determine whether the 2016 observations reflected an isolated episode or a more persistent juvenile-stage pattern.

Taken together, these considerations suggest that early foliar damage in this trial was expressed more clearly in sanitary differentiation and later survival than in subsequent growth among surviving trees. The 2016 health assessment should therefore be interpreted as an early sanitary snapshot rather than as a direct causal measure of the processes determining survival and growth through 2024; no repeated sanitary assessments were conducted after 2016, so no

intermediate monitoring data suitable for formal analysis were available between the two assessment dates, and unrecorded intermediate sanitary or environmental events may also have contributed to later outcomes. Because mortality differed markedly between seed lots, the surviving EUNI-1 trees are unlikely to represent the original cohort without selection, so the growth contrasts should be interpreted as survivor-conditioned estimates rather than as measures of full productivity or whole-cohort performance. Because the growth analyses were also restricted to a subset of radial lines, the absence of clear seed-lot differences in growth should not be extrapolated directly to the complete trial without further evaluation. Insect-related damage may likewise have contributed additional foliar stress; however, insect presence was descriptively higher in EUNI-2 than in EUNI-1 (0.85 vs. 0.72; Table 2), making it unlikely that lower insect pressure explains the survival advantage observed in EUNI-2, although a contributory role as an additional stressor cannot be entirely excluded. Similarly, because replacement trees from 2013 could not be retrospectively identified, their potential contribution to heterogeneity in the early health, survival, or growth comparisons could not be assessed directly.

Beyond the specific host–pathogen interaction examined here, the study highlights the broader value of genetic trials and surveillance-oriented plantings for forest health research. Public arboreta, genetic trials, and surveillance-oriented plantings are increasingly recognized as useful tools for documenting differential host susceptibility, detecting pest and pathogen associations under shared environmental conditions, and informing risk assessment frameworks (Vettraino et al., 2015; Mansfield et al., 2019; Migliorini et al., 2023). Although the Monte Corona trial was not established as a formal biosecurity sentinel plantation, the present results show that a trial combining contrasting plant materials, structured field assessment, and a systematic spacing gradient can generate useful comparative evidence on relative host performance and on how stand structure may modulate the expression and consequences of early foliar damage. This is also directly relevant to the protection and deployment of tree genetic resources: EUNI-2 combined lower early foliar damage with markedly higher observed survival under shared field conditions, providing evidence relevant to the selection and deployment of resilient planting material in Atlantic Eucalyptus plantations and supporting the broader view that genetic trials can contribute not only to silvicultural comparison, but also to forest health surveillance and evidence-based management of tree genetic resources.

The study also has limitations that should be acknowledged explicitly. First, the analysis was restricted to a subset of radial lines within a single trial, so spatial replication across sites was not available. Second, seed-lot allocation within the sampled subset was only partially balanced across sampled radial lines, and only four of the 16 sampled ring-by-radial-line clusters contained both seed lots. Third, early sanitary status was represented by one field assessment in 2016; repeated juvenile-phase assessments were not available. Fourth, the 2016 foliar assessment quantified visible necrosis but did not include tree-level pathogen isolation or molecular confirmation, so the study cannot attribute symptoms exclusively to *T. nubilosa*. Fifth, cause of death was not established for dead trees. Sixth, the number of death events remained limited for detailed multivariable mortality modelling. Seventh, the adjusted role of necrosis severity in survival could only be evaluated on a reduced complete-case subset. Finally, because replacement trees from 2013 could not be retrospectively identified in the analytical dataset, it was not possible to evaluate whether their distribution across seed lots or sampled sectors may have introduced additional heterogeneity into the early health, survival, or growth comparisons.

Under Atlantic field conditions favourable to foliar disease, the Monte Corona trial shows that structured genetic trials can generate comparative evidence on host performance and on the interaction between seed-lot identity and stand structure. Future work could strengthen causal interpretation by combining repeated juvenile-phase sanitary assessments, tree-level molecular confirmation of the main foliar pathogens involved, and replication across additional Atlantic sites. Such extensions would help determine whether the 2016 field signal captured a transient damage episode or a persistent early-life sanitary trajectory linked to later survival and performance, and would strengthen both the operational use of genetic trials for resilient deployment decisions and the evidence base for tree genetic resource management under Atlantic plantation conditions where foliar disease is already recognized as an operational management challenge.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Author contributions

AB-D: Formal analysis, Methodology, Data curation, Conceptualization, Writing – original draft, Investigation, Visualization. LF: Validation, Data curation, Writing – review & editing, Investigation. CT: Writing – review & editing, Resources, Investigation. AB: Writing – review & editing, Project administration, Supervision, Conceptualization, Investigation. JD: Project administration, Methodology, Supervision, Funding acquisition, Conceptualization, Writing – review & editing.

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Conflict of interest

Author CT was employed by company Ence Energía y Celulosa, S.A.

The remaining author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2026.1869817/full#supplementary-material

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