

# Monitoring air spreading of *Lecanosticta acicola*: From the traps to the apps

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**Abstract:** *Pinus radiata* suffers from a number of highly damaging diseases of which needle blights are the most serious ones affecting the tree health in Spain. The largest impact of needle diseases in the recorded history of *Pinus radiata* in the North of Spain, was from 2018 to 2020. The severity of the disease has led to a significant modification of the landscape derived from a serious reconsideration of silviculture in the forestry sector. Despite the fact that three species were detected in the studied area: Dothistroma needle blight (DNB), caused by *D. septosporum* and *D. pini* and brown spot needle blight (BSNB) caused by *Lecanosticta acicola*, *L. acicola* is by far the most frequent and abundant.

In order to minimize the infection of *L. acicola* through forest activities, it is important to understand the dynamics of spore dispersal and the favorable environmental conditions for the infection so that those activities that may work as measures to reduce the disease impact, may be temporarily displaced at times in which the effect of them could be more efficient against the disease.

A total of 15 spore traps were placed in *Pinus* plantations. We used the observations of captured spores at these traps to fit a statistical model that estimates spore abundance in terms of weather variables. This model allowed us to identify which variables have a significant effect on the spore count and may be used in the near future to create a management app available to forest owners and managers.

**Keywords:** Generalized additive model (GAM), brown spot needle blight (BSNB), *Pinus* species

## 1. Introduction

Dothistroma needle blight (DNB), caused by *D. septosporum* (Dorogin) M. Morelet and brown spot needle blight (BSNB) caused by *Lecanosticta acicola* (Thüm.) Syd. are the main species involved in the severe defoliation of *Pinus radiata* these days. It is considered that both species have similar life cycles and symptoms (EPPO, 2015; Sinclair et al., 1987). *Lecanosticta acicola* is the most frequently detected in the study area (Ortiz de Urbina et al., 2017).

Control of *L. acicola* is difficult because it is capable of surviving in both dead and living needles in forest ecosystems of *Pinus radiata* and its dispersing capacity in the plantation is extraordinarily efficient. Conidia are spread, mainly by rain splash among vicinal trees, causing a fast disease expansion in pine stands especially in the spring and early summer (EPPO, 2015; Sinclair et al., 1987; Tainter and Baker, 1996). In addition, *L. acicola* haplotypes are adapted to local temperature conditions which contribute to increasing the infectious success of this pathogen (Janoušek et al., 2016). Severe infection has a serious impact in host growth reductions and in extreme cases could cause tree death (EPPO, 2015; Sinclair et al., 1987).

Several measures have been suggested to minimize and prevent needle blight during plantation establishment such as the use of healthy and good quality propagation material (Cordell et al., 1990; Skilling and Nicholls, 1974) for establishing new plantations in areas far from infected pines (Tainter and Baker, 1996). Also the application of thinning treatments showed effectiveness in reducing the severity of the disease native stands of *P. strobus* in the USA (McIntire et al., 2018). However, the local silvicultural management performed mainly in *Pinus radiata* plantations in the Atlantic area, pruning and thinning, did not result in the expected improvement (Ortiz de Urbina et al., 2017).

The periods in which pruning activities of infected pines are relevant to prevent the disease, avoiding rainy or wet periods. Spores are discharged during these conditions and can adhere to the pruning saw blades, constituting a disease pad from infected to healthy trees (Skilling and Nicholls, 1974). The use of chemicals is not considered an option for disease control due to negative environmental impact and the European restriction reference.

The objective of this study was to quantify the precise amount, timing of air dispersal of spores of *L. acicola* in *Pinus radiata* ecosystem representative of the Atlantic climate, with the aim of modelling disease pressures and in the end to be able to predict disease risks in decision support systems of forest management.

## 2. Materials and Methods

### 2.1. Spore traps description

The spore traps used in this study were passive traps by impaction based on the previous design of Iturrutxa and Ganley (2007) for the study of *Diplodia sapinea*. Four microscope slides were positioned vertically on an expanded polystyrene disk of approximately 6 cm of thickness and 9 cm of diameter and covered by a 90 mm diameter petri plate. Four gaps were carved in the polystyrene base so the slides formed a cross shape. To support the petri plate a hole was drilled in its center and a 9 cm nail was inserted, the nail point was affixed to the polystyrene disk. Only one side of the slides was covered with a thin layer of Technical grade soft Vaseline (Panreac Applichem, Barcelona, Spain) before being placed in the base. Each trap was attached to a 1.70 m post.

### 2.2. Spore traps location and spore measurement

A total of 15 spore traps were placed in the center of *Pinus radiata* plantations severely damaged by needle blight (Fig.1) in 2019. Two traps were located in the province of Alava, two in the province of Gipuzkoa and 11 in the province of Bizkaia. Traps were set on their sites the 7<sup>th</sup> of January (Albina, Oleta, Lezama, Unbe, Pagatza and Elorrio), the 31<sup>st</sup> of January (Mallabia, Muxika, Igorre, Gueñes and Karrantza), and the 4<sup>th</sup> of February (Idiazabal-Larraegi and Azpeitia-Igarate).

Microscope slides were collected approximately every two weeks and spores counted with a microscope using a 40X objective. The measured area was calculated by the following equation: length of measurement on the slide/ field of view (FOV), where FOV is the ratio of the microscope field number and the objective magnification (Meuten et al., 2016). The length of measurement was set to the length of the cover slip. Once the area was determined the number of spores per m<sup>2</sup> was calculated and the spore concentration of the four slides was added.

2.3. Weather variables

We collected data from Euskalmet, the meteorological institute of Basque Country. Euskalmet has a total of 102 weather stations that record several variables on a ten-minute frequency (<https://opendata.euskadi.eus/catalogo/-/estaciones-meteorologicas-lecturas-recogidas-en-2019/>). We aggregated these observations and extracted a total of ten daily measures for each of the traps for the period under study: Average wind speed expressed in kilometers per hour, Air temperature (average, maximum and minimum) expressed in degrees centigrade, Relative air humidity expressed as a percentage (average and maximum), Accumulated precipitation expressed in millimeters, Number of rainy days, Global irradiance on a flat surface expressed in watts per meter<sup>2</sup>

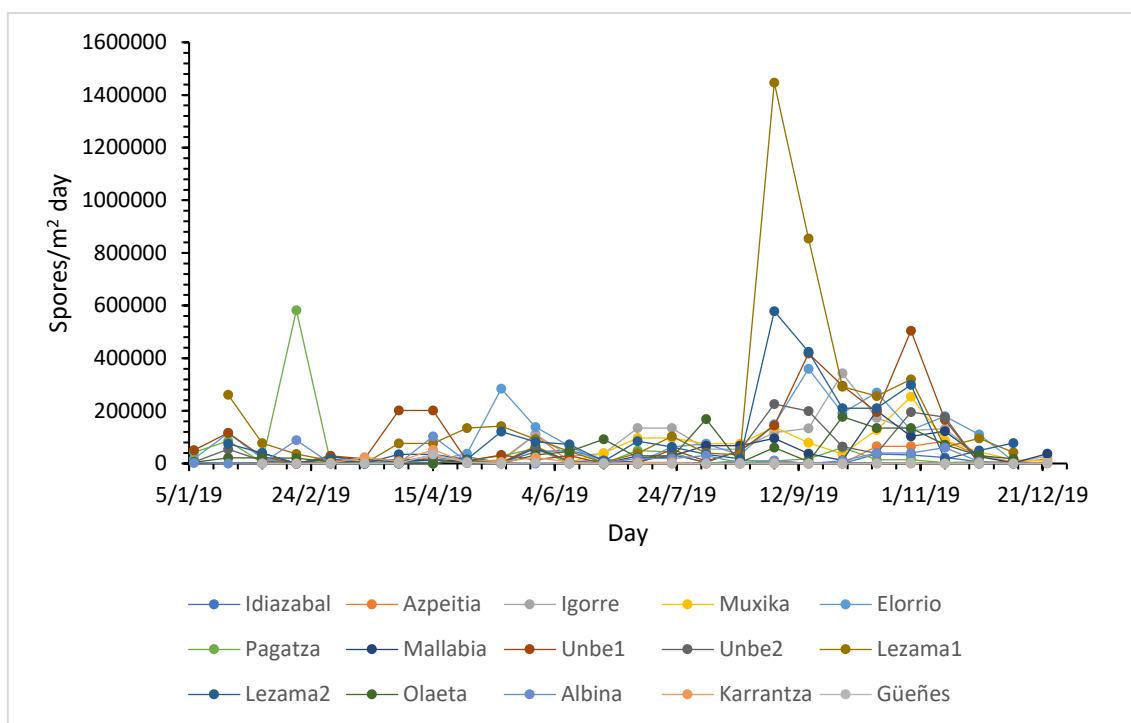
2.4. Statistical analysis

We used Generalized Additive Models (GAM) to analyze the spore abundance dependency to weather data as follows. We first associated a set of observed meteorological data to each of the traps. We started with the closest weather station and completed the unobserved variables with the following nearest station, up to a maximum of the fifth-closest station.

To avoid multicollinearity effects of weather variables, Pearson correlations were calculated, finding several groups of highly correlated variables (for instance, those measuring air temperature). We then fitted several models to subsets of the data. Each of them included as explanatory covariates a single variable from each of the highly correlated groups of variables (for instance, only average air temperature and not maximum or minimum temperature). In addition to this, time and trap location were modelled as a non-linear component and a random effect, respectively. We then chose the best model in AIC score from this family of models.

3. Results

All 16 samplings showed positive detection of *L. acicola* although for each sampling the number of captured spores was very variable across the 15 traps. During the collection period the general pattern of spore dispersal was a maximum peak from September to November (Fig.1), with a small increase in spore concentration occurred in May and July. There are a few exceptions where this second peak was almost as high as the one detected in September (Elorrio, Azpeitia and Idiazabal) or it happens in early August (Olaeta). Three locations did not register maximum spore amounts at those times, but in February (Pagatza) or April (Albina, Güeñes and Karrantza). The maximum spore numbers were recorded in the traps of Lezama1 (1446791 spores/m<sup>2</sup> per day), Pagatza (582592 spores/m<sup>2</sup> per day), Lezama2 (578716 spores/m<sup>2</sup> per day) and Unbe1 (504439 spores/m<sup>2</sup> per day), these were the traps located nearest the coast, in the plantations with the highest level of severity of *L. acicola*.



The model that best fitted the number of trapped spores included the maximum temperature and precipitation recorded in the period when spores were collected, which had an increasing effect on the spore count. The rest of the weather variables were not statistically significant. The model showed a significant improvement compared to a null model that only included time and trap location as covariates (over 15% improvement in deviance explained) and a negligible loss in precision compared to the full model with all the weather variables as covariates (<1% difference in deviance explained). We conclude that this study identifies the weather variables that better explain the dispersal and deposition of *Lecanosticta acicola* spores in the Northeast Spain and it will be used in future works to predict their dispersion.

## References

- Cordell, C. E., Anderson, R. L., & Kais, A. G. (1990). Brown-Spot needle blight In: Southwide Forest Disease Workshop, (Boone, A. J., Anderson, R. L., Fenn, P., Powers, H. R., & Stambaugh W. J., eds.), pp. 18–19. Charleston, South Carolina: South Carolina Forestry Commission, Insect and Disease Section, Columbia, South Carolina.
- EPPO (2015), PM 7/46 (3) *Lecanosticta acicola* (formerly *Mycosphaerella dearnessii*), *Dothistroma septosporum* (formerly *Mycosphaerella pini*) and *Dothistroma pini*. EPPO Bull., 45, 163-182. <https://doi.org/10.1111/epp.12217>
- Iturrutxa, E., & Ganley, R. (2007). Dispersión por vía aérea de *Diplodia pinea* en tres localidades de la cornisa cantábrica. Boletín de Sanidad Vegetal y plagas 33, 383-390.
- Janoušek, J., Wingfield, M. J., Marmolejo Monsivais, J. G., Jankovský, L., Stauffer, C. Konečný, A., & Barnes, I. (2016). Genetic analyses suggest separate introductions of the pine pathogen *Lecanosticta acicola* into Europe. *Phytopathol.* , 106(11), 1413–1425.
- McIntire, C. D., Munck, I. A., Ducey, M. J., & Asbjornsen, H. (2018). Thinning treatments reduce severity of foliar pathogens in eastern white pine. *Forest Ecol. Manag.* 423, 106–113.
- Meuten, D. J, Moore, F. M., & George, J. W. (2016). Mitotic count and the field of view area: Time to Standardize. *Vet. Pathol.* 53(1), 7-9. doi: 10.1177/0300985815593349. PMID: 26712813.
- Ortiz de Urbina, E., Mesanza, N., Aragonés, A., Raposo, R., Elvira-Recuenco, M., Boqué, R., Patten, C., Aitken, J., & Iturrutxa, E. (2017). Emerging needle blight diseases in Atlantic Pinus ecosystems of Spain. *Forests* , 8(1), 18.
- Sinclair, W. A, Lyon, H. H., & Johnson, W. T. (1987). *Diseases of Trees and Shrubs*. Comstock Publishing Associates, London (GB).
- Skilling, D. D. & Nicholls, T.H. (1974). Brown spot needle disease – biology and control in Scotch pine plantations. US Department of Agriculture. Forest Service Research Paper, NC-109, 1–19.
- Tainter, F. H. & Baker, F. A. (1996). Brown spot In: *Principles of Forest Pathology*, pp. 467–492. New York, USA: John Wiley.