Non-destructive diagnostics for estimating fungal infection in resin-tapped and non-tapped pine trees

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Abstract. In pine stands systematic harvesting of forest products, such as resin extraction, are known to affect trees' vitality and consequently their response to fungal diseases. The latter constitutes a serious threat for standing vigorous trees, thus early warning signals and short diagnosis time of fungal pathogens, are crucial for designing effective forest management practices. In this study, the potential detection of fungal infection in managed pine stands was evaluated, using non-destructive diagnostics. Specifically, indices related to canopy structure and trunks' temperature distribution in 334 resin-tapped and 185 non-tapped pine trees, in 21 stands, on Lesvos Island, Greece, were developed by analysing hemispherical and infrared photographs. In each stand, tree cores were extracted from the 34% of the total trees, while the fungal presence was confirmed (a) by the sudden change in boring resistance and sound during the core extraction and (b) by the discoloration of the tree cores. Logit models were developed to estimate the fungal presence explained by non-destructive diagnostics, both in resin-tapped and non-tapped pine trees. Results showed that fungal presence was successfully classified in (a) 92.6% of the cases as predicted by the canopy structure indices and in (b) 89.8% of the cases explained by the thermal indices. The effectiveness of these non-destructive diagnostics has demonstrated the accurate assessment of pines' fungal infection, in order to prevent or mitigate forest stands' degradation.

Keywords: *Pinus brutia*, fungal detection, thermal imaging, leaf area index, tree coring

1. Introduction

In forest ecosystems, anthropogenic pressures, such as overgrazing, increasing demand for timber, and chronic harvesting of forest products, are degradation factors that can potentially lead to changes in the availability of natural resources, diversification of forest areas, and reduction of their productivity and regeneration. At the same time, biotic (e.g. fungi, bacteria, insects, parasitic plants) or abiotic (e.g. pesticides/herbicides, soil conditions, climatic conditions, water availability) disturbances (Boa 2003), can reinforce the already existing anthropogenic pressures.

One of the most important biotic factors affecting forest systems is fungi. Over the last four decades, the number of invasive fungal pathogens in Europe has increased exponentially (Santini et al. 2013) with fungal infections being considered as the leading causes of infectious diseases in forest trees. The presence of fungal pathogens is difficult to control as their populations show spatio-temporal and genetic variability, depending to a large extent on humidity and ambient temperature, while climate change increases the risk of infection in forest trees (Garrett et al. 2016). In order for fungi to grow, a food source (wood), appropriate temperatures (15 - 30 ^{0}C), humidity in the substrate, and oxygen are required. If one of the above conditions is not met, then the fungus will die or fall into a dormant state. In the case of standing trees, it seems that the most important factor influencing their infestation by the fungal pathogens is the existence of any type of wound. Immediately after the injury is inflicted, the cambium reacts by producing healing tissue. The speed of healing depends on the size of the wound and the vitality of the particular tree. Some coniferous species (mainly pine trees) when injured, secrete resin, placing a barrier at the injury area against drying tissue and attack by insects and pathogens (Reid et al. 1967). If the healing occurs quickly then it is possible to cause only a simple discoloration on the wood. However, if the wound remains open for a longer time period and the temperature and humidity conditions are favorable, then the exposed wood is colonised by bacteria as well as the fungal pathogens.

In pine forest stands, resin extraction disturbs trees as it causes structural damage to their trunks while affecting their growth rate (Papadopoulos 2013), their vitality in general, and consequently their response to fungal diseases. Resin production is the trees' defensive response to wounds (van der Maaten et al. 2017) preventing water loss as well as penetration of insects or other pathogens. In addition, it alters trees' sensitivity to climatic stress, making them less resilient to extreme weather events. In further detail, the wound caused by the resin extraction process, results in creating conditions that lead to the colonisation of internal functional areas or non-functional tissues by fungal communities. The latter constitutes a serious threat regarding standing vigorous trees, it is among the leading causes of their mortality, and contributes greatly to forest stands degradation. As a result, resin collection leaves trees' wounds exposed to pathogens. For these reasons, early warning signals and short diagnosis time of fungal pathogen presence is a crucial element to prevent or mitigate their impact in forest stands. In this respect, the fungal infection presence in pine forests after resin extraction was evaluated, using non-destructive diagnostics; thermal imaging of pines trunks' resinous surfaces, and hemispherical photography of their canopy.

2. Materials & Methods

2.1. Study area

Measurements were carried out in *Pinus brutia* forest stands, on the island of Lesvos which is located at the north-eastern Aegean Sea, and occupies an area of 1,632.8 km^2 (**Fig. 1**). Trees in the study area were tapped during the period of 1930-1970 and data collection took place in the spring of 2018 and 2019, during morning hours (05:00 - 08:00), in order to avoid data alteration due to external factors (e.g. solar radiation).

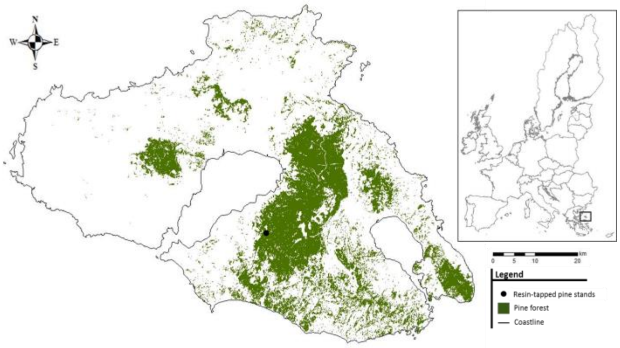

Figure 1. Map of the study area

2.2 Data collection

In 21 forest stands of 900m^2 each, morphometric traits related to 334 resin-tapped and 185 non-tapped pine trees, describing each trees' crown structure and shape, were calculated: (a) Crown Length - CL: the difference resulting by subtracting the height where the first crown branches start from the height of the tree, (b) Crown Ratio - CR: the crown length divided by the tree height, (c) Degree of Spread - DS: the average crown diameter ratio to total tree height, and (d) Crown Projection Ratio - CPR: the ratio of the average crown diameter to the diameter at breast height.

2.3. Hemispherical imaging

The hemispherical images were taken using a Canon EOS 60D camera with a wide-angle lens which was placed on the trunk of each pine tree at a height of 1.3 m from the ground, to photograph the crown area which was located both above the resin scar and on the non-resinous side of the trunk (**Fig. 2**). Hence, the Leaf Area Index (LAI) was calculated, both for the total crown of each pine (LAI_{mean}) and for the crown above the resin (LAI_{resin}) and non-resin ($LAI_{non-resin}$) surface of the trunk. An additional LAI metric, namely LAI_{range}, was also calculated as a representative statistical metric describing tree vitality status.

Figure 2. Sample hemispherical image of a pine tree crown

2.4 Thermal imaging

Pine trees were additionally photographed, using a handheld thermal camera (Testo 875-1i). The thermal images were taken at the part of the trunk where the oldest resin scar was located, from a distance of 3 m (**Fig. 3**). In case where there were more than one scars, the one with the largest dimensions was chosen. Finally, the thermal images were calibrated and analyzed using Testo IRSoft software (**Fig. 3**), while a set of thermal indices were created: a) the minimum temperature (T_{min}), b) the maximum temperature (T_{max}), and c) the temperature range (T_{range}) on the resin scar for each tree, using the ArcGis software.

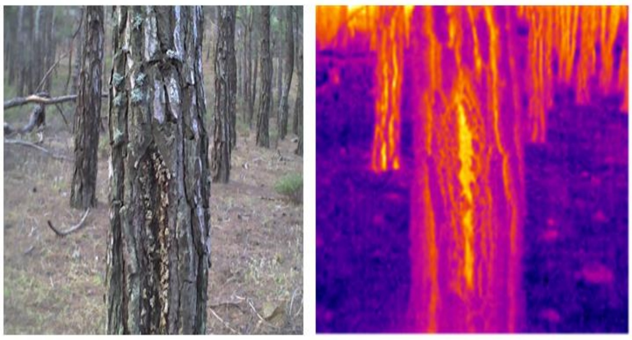

Figure 3. Sample of thermal image of a resin-tapped pine tree

2.5 Assessment of wood decay fungi presence

In each stand, tree cores were obtained from the 34% of the total standing trees, by random sampling, while the fungal presence was confirmed (a) by the sudden change in boring resistance and sound during the coring extraction and (b) by the discoloration of the extracted tree cores.

2.6 Statistical analysis

All statistical analyses were carried out using SPSS software. Data were evaluated for normality and homogeneity with graphical methods and the Kolmogorov-Smirnov test. Binary logistic regression (BLR) models were developed to estimate the fungal presence explained by non-destructive diagnostics, both in resin-tapped and non-tapped pine trees. Predictor variables derived from the LAI (LAI_{mean}, LAI_{resin}, $LAI_{non-resin}$, LAI_{range}) as well as thermal indicators (T_{min},

T_{max}, T_{range}), were evaluated through a backward stepwise procedure, in order to maintain the optimal model.

3. Results and Discussion

3.1 Crown structure, LAI, and thermal indices related to P. brutia forest stands

Trees (N = 519) had an average height of 13.86 m (SD 4.10) and perimeter of 118.06 cm (SD 36.75). Descriptive statistics of resin-tapped and non-tapped trees are shown in **Table 1**.

Table 1. Descriptive statistics for pine trees' variables

	Resin-tapped		**Non-tapped**	
	Crown indices			
	Mean	**SD**	**Mean**	**SD**
CL (m)	6.01	2.75	3.92	2.01
CR	.39	.11	.31	.11
DS	.44	.17	.28	.13
CPR	14.38	3.73	12.03	3.83
	LAI metrics			
LAI_{mean}	1.07	.44	1.23	.48
LAI_{resin}	.87	.57	1.30	.56
$LAI_{non-resin}$	1.27	.51	1.15	.52
LAI_{range}	.58	.45	.32	.38
	Thermal indices			
T_{min} (°C)	13.53	3.59	12.88	5.34
T_{max} (°C)	16.10	3.43	13.70	5.28
T_{range} (°C)	2.57	1.12	.82	.45

The mean age of the pines from which cores were obtained (N = 175) was 77.1 years (SD 20.33), while they were resin-tapped at the age of 33.92 (SD 18.07). Fungal infestation was identified in 52.5% (92 trees), 46.2% (81 trees) did not appear fungal signs while in 2 trees it was particularly difficult to identify the presence or absence of fungi (**Table 2**).

Table 2. Descriptive statistics of pine trees' fungal infestation in relation to their age

	Fungal infestation	**N**	**Mean**	**SD**
Age	Presence	92	83.39	20.65
	Absence	81	70.44	17.65
	Total	173	77.33	20.31

3.2. Logistic regression models in estimating fungal infestation

In order to examine whether non-destructive diagnostics explain the probability of the presence or the absence of fungal infestation, three BLR models were used. The first one used variables related to pines' crown structure and shape as well as the ones resulting from the LAI, the second one the thermal indices, while the third one a combination of both diagnostics. All the aforementioned variables were entered into the BLR models as the predictive factors while an indication of the amount of variation explained by the models was provided by Nagelkerke's R square, while the overall significance of the models was assessed by the Hosmer and Lemeshow goodness of fit test. To assess the discrimination ability of the models, a classification table of observed and predicted values regarding the probability of fungal presence was computed and evaluated by receiver operating characteristic (ROC) curve analysis. To correctly discriminate fungal infected and non-infected trees, the area under the ROC curve (AUC), as a measure of the average value of sensitivity for all possible values of specificity, with a threshold resulted from Youden's index, was selected.

3.2.1 Crown and Leaf area indices

The BLR identified six significant variables that contributed the most in predicting fungal infestation (**Table 3**). The Nagelkerke R^2 showed that these variables explained 82.8% of the total variance of the data. In addition, the Hosmer & Lemeshow test showed that the model's goodness of fit can be accepted, due to the absence of chi-square significance (.555).

Table 3. Logistic regression model where B = logistic coefficient; S.E. = standard error of estimate; Wald = Wald chi-square; df = degree of freedom; sig. = significance.

Predictor	**B**	**S.E.**	**Wald's χ^2**	**df**	**Sig.**
CL	1.33	.32	16.44	1	≤.001
CR	-23.80	6.74	12.44	1	≤.001
DS	16.90	4.11	16.84	1	≤.001
CPR	-.53	.15	12.30	1	≤.001
LAI_{resin}	-4.92	.88	31.25	1	≤.001
LAI_{range}	5.07	1.08	22.11	1	≤.001
Constant	4.33	1.75	6.08	1	.014

The overall predicted accuracy of the model was 92.6% and in particular, 94.6% for presence and 90.4% for the absence of fungal infestation at the 173 trees in the study area. Finally, the area under the ROC curve for the model was .974 with an estimated standard error of .010, which indicates a very successful and trusted model for estimating the probability of fungal infestation presence at the studied trees (**Fig. 4**).

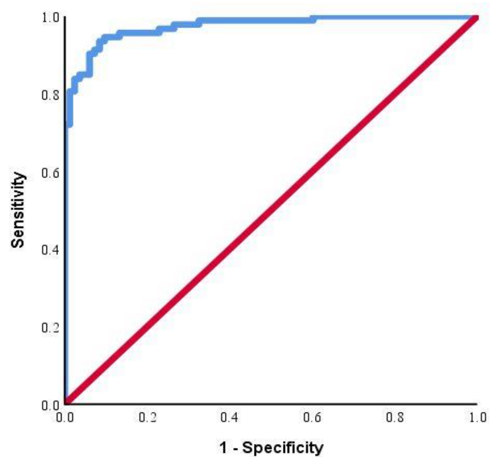

Figure 4. The ROC curve for logistic regression model using the Crown and Leaf Area indices

3.2.2. Thermal indices

The area under the ROC curve (AUC = .946; S.E. = .016; 95% CI .914-.978; p < .0001) correctly classified the fungal infected and non-infected pines in 90.3% of the cases (**Fig. 5**).

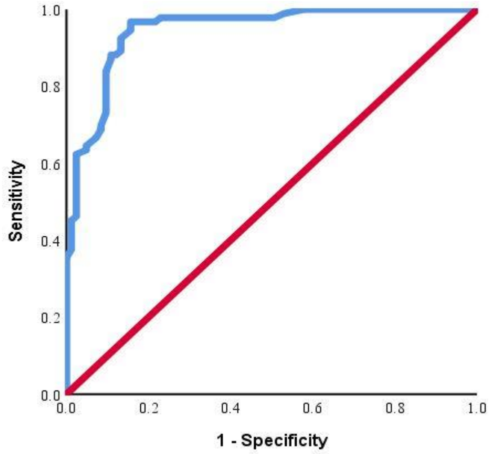

Figure 5. The ROC curve for logistic regression model using the thermal indices

From the three thermal indices, the BLR model identified T_{range} as the most important factor that best separates the examined cases (p <.001; **Table 4**), with a predicted classification accuracy of 89.8%; 86.7% for absence and 92.5% for the presence of fungi. The Nagelkerke R^2 indicates that the model explains 74% of the total variance of the dependent variable and the result of the Hosmer - Lemeshow test shows a chi-square value of 12.953 (p = .113) which estimates that the model fits the data at an acceptable level.

Table 4. Logistic regression model where B = logistic coefficient; S.E. = standard error of estimate; Wald = Wald chi-square; df = degree of freedom; sig. = significance.

Predictor	**B**	**S.E.**	**Wald's χ^2**	**df**	**Sig.**
T_{range}	2.59	.36	51.37	1	≤.001
Constant	-4.88	.72	45.96	1	≤.001

3.2.3 Combining non-destructive diagnostics

A combination of diagnostics from the different non-destructive methods, i.e. the canopy structure indices (CL, CR, DS, CPR, LAI_{mean}, LAI_{resin}, $LAI_{non\text{-}resin}$, LAI_{range}) and the thermal indices (T_{min}, T_{max}, T_{range}) were additionally entered into the model as predictors of fungal infestation. Results showed that the BLR model (**Table 5**) presented a classification accuracy of 91.4% for fungal presence and 96.4% for absence, the AUC was .977 (S.E. =.010; 95% CI .957-.997; p < .0001; **Fig. 6**), the Nagelkerke R^2 = .839, and the Hosmer - Lemeshow test was 10.999 (p = .202).

Our analysis provides evidence of the effectiveness of these non-destructive diagnostics which had demonstrated the rapid and accurate assessment of pines' fungal infection, in order to prevent or mitigate forest stands' degradation.

Table 5. Logistic regression model where B = logistic coefficient; S.E. = standard error of estimate; Wald = Wald chi-square; df = degree of freedom; sig. = significance.

Predictor	**B**	**S.E.**	**Wald's χ^2**	**df**	**Sig.**
CL	.81	.32	6.42	1	.011
CR	-17.23	6.63	6.75	1	.009
DS	13.41	4.22	10.09	1	≤.001
CPR	-.54	.16	10.40	1	≤.001
LAI_{range}	2.75	.80	11.65	1	≤.001
T_{range}	2.98	.55	29.20	1	≤.001
Constant	-2.94	1.74	2.87	1	.09

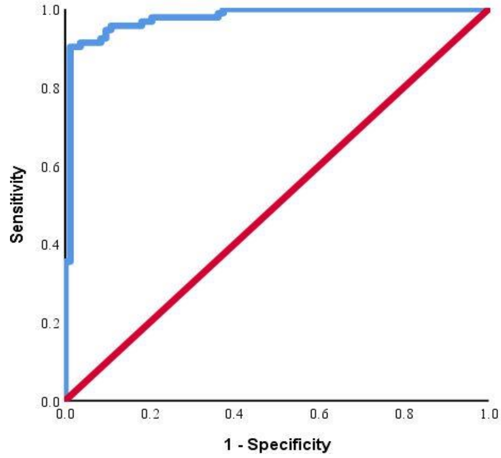

Figure 6. The ROC curve for logistic regression model combining non-destructive diagnostics

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