Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Science

Department of Forest Mycology and Pathology

RELIABILITY OF ROTFINDER INSTRUMENT FOR DETECTING DECAY IN STANDING TREES

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Reliability of Rotfinder instrument for detecting decay in standing trees
Utvärdering av Rotfinder för detektion av röta i stående träd

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INDEX

ABSTRACT

A wood decay detecting instrument, Rotfinder® was evaluated for its accuracy, sensitivity and specificity in detecting decay in standing trees. Five hundred trees were measured in three different stands with Rotfinder at three heights in the stems. The trees were felled and sectioned, and presence or absence of decay was observed for each section. Wood samples were taken from every section and their wood density and moisture content was measured. Rotfinder accuracy of detecting both decayed and healthy trees was 82.7%. The highest sensitivity was obtained at stump height (55.9%). Advanced decay was detected in the 71% of cases while incipient decay was detected in the 23% of cases. Rotfinder has a scale from 0 to 10 which indicates an increasing degree of probability of decay. Rotfinder values higher than 4 indicated more than 90% of probability of finding decay. Rotfinder successful detections (true positives and true negatives) and Rotfinder failures (false positives and false negatives) were compared in terms of moisture content, wood density and N, C, Mg, Ca, K, Na and Mn concentration. True positives had higher moisture content in reaction zone and decay, higher density in reaction zone and higher concentration of calcium and sodium of reaction zone than false negatives. Relationship between Rotfinder values and wood characteristics were studied, and the potassium concentration showed the highest correlation with Rotfinder values.

1.- INTRODUCTION

1.-INTRODUCTION

1.1.-WOOD DECAY CAUSED BY FUNGI

Wood is a material formed by cells. Cell walls are formed by micro fibrils of cellulose and hemi cellulose impregnated with lignin. The stem of a tree has two well differentiated parts: heartwood and sapwood. Heartwood has an inner location and it is formed by dead cells though some residual enzymatic activity can be found (SHIGO & HILLS, 1973). It has a mechanical support function. Sapwood has a peripheral position and is made of living tissue. Its principal function is sap conduction and to store up the reserves (SMITH, 1970). Transformation of sapwood to heartwood is produced by necrosis of the xylem parenchyma.

Decay is a deterioration of wood by enzymatic activities of fungi. (MANION & ZABEL, 1979). Principal types of decay are classified into three groups: soft rot, white rot and brown rot. Soft rot fungi belong mainly to the Ascomycetes and Deuteromycetes phyla. They are present in standing trees but they have a little economic impact. They are frequent in wood in use, e.g. when exposed to high-moisture conditions. These organisms attack cellulose and hemicellulose while the lignin remains almost unaffected. The expression “soft-rot” refers to the fact that a softening of the wood surface is produced when wood is degraded by this type of fungi (ERIKSSON, 1981).

Brown rot and white rot fungi are found in standing trees and can produce a high economic and ecological impact. Most of these fungi belong to Basidiomycetes phylum, although some Ascomycetes can also produce this kind of rot. Wood rotting Basidiomycetes cause every year high losses of timber in forest stands (AGRIOS, 1997). Brown rot fungi mainly feed on cellulose and hemi cellulose. They do not usually degrade the entire cell walls. They leave a lignin residue which gives the decayed wood a brown color appearance. In general, brown rot fungi are more frequently found infecting gymnosperms. Based on weight loss measurements at comparable stages of decay, brown rot fungi reduce more the strength of the wood than white rot fungi (HIGHLEY & KIRK, 1979).

White rot fungi can degrade cellulose, hemi-cellulose and lignin. The capability to degrade lignin may be related to the presence of Mn-peroxidase, lignin peroxidase and laccase-type enzymes. Wood decayed by white rot fungi has a white or bleached appearance. These fungi are typically associated with angiosperms. White rot fungi can survive under more basic conditions than brown rot fungi (HIGHLEY & KIRK, 1979).

1.2.-BUTT AND ROOT ROT CAUSED BY *HETEROBASIDION ANNOSUM*

Heterobasidion annosum (Fr.) Bref. is a basidiomycete that causes root and butt rot in conifer and broadleaf trees. It is a white fungus which can degrade wood cellulose, hemi-cellulose and lignin. The fungus colonizes the root system of the trees, producing nutrient deficiencies and periodic increment reduction that can lead to the death of the tree by weakening or wind-blowing. Diagnostic at genus level can be based on fruiting bodies that appear usually hidden in places under roots or in hollow stumps (KORHONEN, 2002). Differentiation of *H. annosum* species can be also performed by molecular methods as well as by mating tests with homokaryotic isolates of known species (GONTHIER *et al*., 2003).

Heterobasidion annosum produces both sexual (basidiospores) and asexual spores (conidia). Fruiting bodies produce a high amount of basidiospores, especially under moist and warm conditions. Spores are airborne and infect trees mainly through wounds and through nearby recently cut stumps. Once a fungus is established in a stand, the infection spreads among trees by root-to-root contacts. Root infection speed depends on soil type and pH level (KORHONEN, 2002). Propagation of *H. annosum* can be increased after thinning and final cutting operations (PIRI & KORHONEN, 2008). Stress caused by drought and environmental pollution facilitate the spread through the root system as well (KORHONEN, 2002).

Heterobasidion annosum geographical distribution extends into Europe, North America, China and Japan. It was considered for years as a complex of various intersterility groups that finally resulted in different species. Three intersterility groups were recognized in Europe (P, S and F) and two in North America (P and S), but nowadays the names of P, S and F groups are *Heterobasidion annosum* (Fr.) Bref., *Heterobasidion parviporum* Niemelä & Korhonen and *Heterobasidion abietinum* Niemelä & Korhonen respectively (ASIEGBU *et al*., 2005).

European *H. annosum* had a host preference for the tree genera *Pinus*, *Picea*, *Juniperus* and *Betula. H. parviporum* infects *Picea* and *Abies sibirica* and the *H. abietinum* type mainly occurs on *Abies* species in Southern Europe. North American *H. annosum*, usually attack the tree genera *Pinus*, *Juniperus* and hardwoods while *H.parviporum* is usually observed infecting *Abies*, *Tsuga* and *Picea* (KORHONEN & STENLID, 1998). Genetic analyses of these populations show that European and North American *H. annosum* populations form different clades.

H. annosum cause severe root and butt rot on coniferous trees throughout the Northern Hemisphere (WOODWARD *et al.*, 1998). Prevention should be the first step for controlling *Heterobasion annosum*. Silvicultural operations should be reduced to the minimum (MANION, 1991), and should be developed with care in order to not damage root system. After thinning and final cutting operations, application of a compound such as urea, borate or *Phlebiopsis gigantea* (Fr.) Jülich, on the stump surface resulted to be an effective barrier for spore germination (PRATT *et al*., 1998). Planting mixed stands and coniferous resistant species could be also useful to reduce propagation (ASIEGBU *et al*., 2005). If a control program is performed, economic losses can be reduced considerably (KORHONEN *et al.*, 1998).

1.3.-EFFECTS OF DECAY IN LIVING TREES

Rot fungi grow inside tree cells, degrading wall components in order to use them as food. This process could produce weight and strength loss, volume reduction, changes in permeability, increases in electrical conductivity and discoloration of wood (MANION, 1991). Degrading enzymes of the fungus metabolized plant wall components and this produces a loss of weight. Wood decay fungi increase the permeability of the wood which produces the loss of electrolytes such as H^+ and K^+ (AGRIOS, 1997). An increase of electrical conductivity of wood is also associated with wood decay. Decay produces an accumulation of ions in the discolored wood around the decaying region that reflect changes in tree metabolism (MANION, 1991).

Trees principal defense processes are the compartmentalization and the creation of a reaction zone. Trees that are able to wall off infected or injured tissues quickly will survive longer (SHIGO, 1980). Compartmentalization is the typical response used for isolating injured tissues but in decay process can also be used. The barrier zone is formed to isolate damaged tissues and thus localize the spread of pathogens (SHIGO, 1984). Reaction zones are necrotic tissues enriched with inhibitory extractives and are produced in advance of the infection. The accumulation of anti-fungal compounds in this part has provided evidence that this tissue constitutes a defense mechanism (SHAIN, 1979). MANION & ZABEL (1979) resumed principal responses of the tree to invasion by decay microorganism as ethylene production, moisture loss, starch degradation and mineral accumulation.

1.4.-IMPORTANCE OF DETECTION OF DECAY IN TREES

Presence of decay has a great impact on timber value and affects tree growth and its presence should be considered in this selection process. A fast, non-destructive method to measure the relative incidence of decay in standing trees could be used to incorporate the rot information when taking decisions regarding the thinning regime or the time of the final cut (SHORTLE & SMITH, 1987; VOLLBRECHT & AGESTAM, 1995). Furthermore, the loss of the mechanical strength in decayed branches or stems caused by rot fungi represents a threat for mechanical failure in urban environments that can produce significant damages (GUGLIELMO *et al.*, 2007).

1.5.-METHODS TO DETECT THE PRESENCE OF DECAY

In the last years, several devices to detect decay in wood have been developed and tested (MÜLLER *et al.*, 2001; NICOLOTTI *et al*., 2003; BUCCUR, 2005; MACCHIONI *et al*., 2007; TOMAZELLO *et al.*, 2008). The instruments for detecting decay can be classified as destructive and non-destructive, although some of them are weakly destructive devices. A good instrument to detect decay should be simple, economical, accurate, safe to perform and sensitive enough to avoid false positives (SCHULTZE *et al*., 1998).

Destructive instruments have the disadvantage of destroying wood. Among invasive instruments, the Shigometer, the increment borer, the Resistograph® or the fractometer are the most commonly used ("Wood decay detection instruments"). The Shigometer measures electric resistance between tissues by means of applying electricity between two electrodes. A narrow and cylindrical hole is performed in order to introduce these electrodes in the centre of the stem (BUTIN, 1995; SHORTLE, 2000). The increment borer is a tool to extract a core sample from the stem. Afterwards the core sample is examined for presence or absence of decay (STENLID & WÄSTERLUND, 1986). Resistograph is based on measuring resistance of wood. It is an instrument with a 3 mm diameter drill bit and bores into the wood to measure the density or the hardness of the wood. Fractometer is a mechanical device which measures the strength of the wood by measuring its bending angle and the point at which it completely breaks. Results obtained with these instruments may vary depending on spatial position of decay and direction in which measurements are carried out (WANG *et al*. 2004).

Non-destructive devices are preferable since they provide diagnosis without damaging the the trees. One of those, tomography, allows the reconstruction of a cross section and makes the internal state of a tree visible. Tomography is imaging by sections through the use of wave of energy. Many techniques have been develop in tomography as acoustic (Fakopp 2D, Picus®) (WANG *et al.*, 2004, LIANG *et al*., 2007; DEFOLORIO *et al.,* 2008), electric, ultrasonic and georadar (NICOLOTTI *et al*., 2003; BUCCUR, 2005). Other non-destructive methods are based on techniques as X-ray (MACCHIONI *et al*., 2007; TOMAZELLO *et al.*, 2008), thermal imaging (MARTIN *et al.*, 1987) or magnetic resonance imaging (MÜLLER *et al.*, 2001) to detect decay in trees.

1.6.-DESCRIPTION AND CHARACTERISTICS OF ROTFINDER® INSTRUMENT

Rotfinder® was developed by Rotfinder AB in cooperation with Swedish (Skogforsk) and Danish (Skov&Landskab) Forestry Research Institutes. It appeared in summer 2005 and it is a non-destructive instrument for detecting decay in standing trees (Fig. 1 & 2).

Fig. 1 The Rotfinder instrument

The Rotfinder instrument is based on RISE method (Relative Impedance In Situ Examination). By this method, measurements are made by passing a current through the stem with one pair of electrodes, while measuring the induced voltage with another pair of electrodes on the stem surface. The resistivity is defined as a measure of how strongly a material opposes the flow of electric current. Resistivity of the affected wood is lower in decay wood compared to healthy wood (SMITH & SHORTLE, 1988; LARSSON *et al*., 2004).

LARSSON *et al.,* (2004) tested effectiveness of RISE method by examining 300 trees. They concluded that individual tree resistivity depends on time of year (higher resistivity in winter), type of tree (higher in healthy trees), wood temperature (higher in lower temperatures) and wood water content. Another conclusion was that the detection of decay in a single tree based on resistivity measurements was not possible. The method could be used for estimating the amount of rot in the stand when a number of trees of the stand were measured.

The Rotfinder instrument has a scale from 0 to 10 which indicates an increasing degree of probability of decay. Value 0 indicates a healthy tree and the number 10 indicates a high probability of decay. Rotfinder manual claims that the instrument detects decay with a specificity of 95%.

Rotfinder manual warns that the instrument cannot be used to measure trees, whose wood is frozen, or during or immediately after a heavy rain. The presence of compression wood could also produce misjudgments in Rotfinder ("Rotfinder").

Fig. 2 Rotfinder instrument

2.-OBJECTIVES

2.-OBJECTIVES

Decay fungi reduce economic value of wood and reduce branches or stem resistance. Detecting decay in living trees would be useful during a harvesting process, for example to select trees to cut in a thinning process, when a forest land is assessed for sale or for designation as a conservation area and to detect decay trees in gardens or recreational places. A good instrument to detect decay should be simple, economical, accurate, safe to perform and sensitive enough to avoid false negatives (SCHULTZE *et al*., 1998).

Rotfinder® instrument is a non-destructive instrument for detecting decay in standing trees. It was released in the market in summer 2005. It is based on the fact that as the fungus is invading the stem, a movement of water to the area of mycelia growth is produced, mobilizing metal ions released by damaged tree cells. Affected wood has a lower resistivity compared to healthy wood. The resistivity is measured with the RISE four point method where a low frequency alternating current is applied to the stem and the induced voltage is measured between two points along the stem ("Rotfinder").

More than 300 Norway spruce in eleven measurement campaigns were examined in order to validated RISE method (LARSSON *et al.* 2004), nevertheless no independent study about Rotfinder reliability has been carried out. The study of Rotfinder accuracy and to know what is the relation between the responses of the tree and Rotfinder values is necessary. Three heights in each tree were measured in order to know the spread of the fungus and the maintenance of the accuracy of the instrument at different heights.

The main objectives of the study are:

(i) to evaluate Rotfinder accuracy, sensitivity and specificity.

(ii) to evaluate its capacity for quantifying the vertical spread of decay in the stem by comparing results at three heights.

(iii) to correlate Rotfinder detections with tree responses and decay effects: wood water content, wood density and elements concentration.

3.-MATERIALS AND METHODS

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3.1.-STUDY AREA

The field experiments were carried out in three Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) mixed-forests in southern Sweden (Figures 3a & 3b). The chosen forest corresponded to stands at the age of the final cutting and all were placed at Uppland province (Table 1). Plots were placed in 72-88 year old plantations where a total of 500 trees were sampled (Table 1).

Table 1. Characteristics of the plots. Location, nearest village; Municipality; Age of the stand, Sampled trees, number of sampled trees; X-UTM and Y-UTM coordinates of the stand ; Forest name.

Location	Municipality	Age	Sampled trees	XUTM	YUTM	Forest Name
Ingbo	Heby	83	300	6666220	1554311	Näverkärret
Enåker	Heby	88	100	6662064	1553385	Saxenvägskälet
Harsbo	Tierp	72	100	6698003	1586350	Ragfallsvägen

Fig. 3a Position of Uppland in Sweden

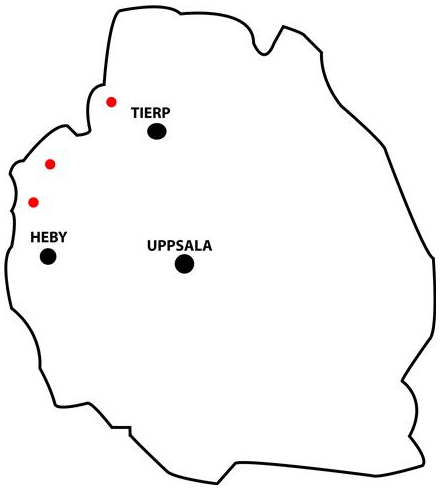

Fig. 3b Location of the plots in Uppland

3.2.-ROTFINDER MEASUREMENTS

All measurements were made between May and July of 2009. Diameter of the tree has to be introduced in Rotfinder instrument before the measurement, so the diameter of the trees was measured by cross callipering. Trees with diameter at breast height smaller than 8 cm or with wounds were discarded. Rotfinder instrument has a scale from 0 to 10: 0 indicates a healthy tree and from 1 to 10 an increasing probability to find decay.

Three measurements were performed at three heights: (i) at stump height 0.30 m from the ground (stump height), (ii) at 0.66 m (middle) and (iii) at 1.30 m (breast height). The place of the measurements was permanently marked.

3.3.-ROT MEASUREMENTS

After the Rotfinder measurements, the trees were felled. Logs were cut approx. 10 cm below the lower measurement to approx. 10 cm above the upper measurement. Every section was observed for the presence of decay and sprayed with a pH indicator: 2,6 Dichlorophenolindophenol (STENLID & WÄSTERLUND, 1986) to determine if a reaction zone was present. This pH indicator shows reaction zone by producing a change in color. Light blue color indicated incipient reaction zone. Also some healthy sections were sprayed to confirm the absence of rot. If a tree showed either decay or reaction zone at one height, it was also examined at the following height until it did not show any symptom.

Presence or absence of decay was noted if any symptom was observed. Furthermore, it was classified into 3 categories: light brown (if the decay has a light color), brown (if it was darker) and soft brown (if the decay was brown and had a soft texture that could be easily broken with the fingers). Figures 4a, 4b and 4c, show different types of decay that were found.

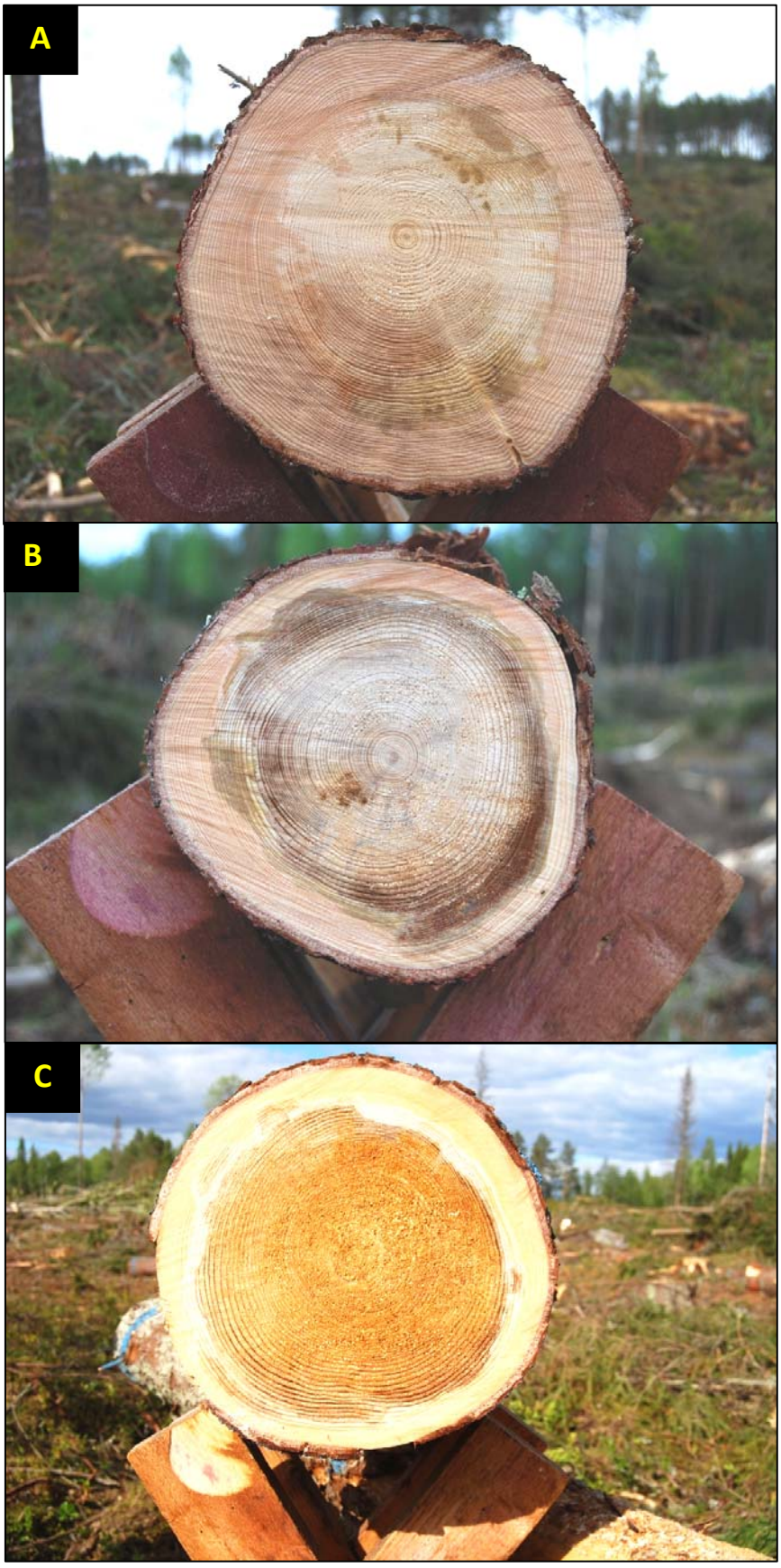

Fig. 4 a Light brown decay, **b** Brown decay, **c** Soft brown decay.

Trees with reaction zone presence were not considered decayed as the reaction zone is a response of the tree to the attack of the fungus. The presence of reaction zone was noted and was classified into two groups: incipient reaction zone if the reaction zone was not visible clear but it appeared after the application of pH indicator 2,6 Dichlorophenolindophenol with a blue color(Fig. 5 a, b); and advanced reaction zone, if it was visible (Fig. 5 c). No blue-colored regions of the stump were observed when reaction zone was absent (Fig. 6 a, b).

Fig. 5 a Incipient reaction zone before application pH indicator **b** Incipient reaction zone after application of pH indicator.

Fig. 5c Visible reaction zone

Fig 6. a Healthy wood, **b** Healthy wood after application of pH indicator.

The measurements were classified into four groups of detection (Table 2): (i) True negatives: Rotfinder value=0 and not rotten; (ii) True positives: Rotfinder value>0 and presence of rot; (iii) False negatives: Rotfinder values of 0 but with decay presence and (iv) False positives: Rotfinder value>0 but not rotten.

Table 2. Rotfinder values and rot presence.

		Rot presence		
		True	**False**	
Rotfinder value	**Positive**[1]	True positive	False positive	Positive predictive value
	Negative[2]	False negative	True negative	Negative predictive value
		Sensitivity	**Specificity**	**Accuracy**

[1] Positive: Rotfinder value >0.
[2] Negative: Rotfinder value =0.

Accuracy was the proportion of true results (both rotten and healthy sections) obtained.

$$Accuracy = \frac{number\ of\ true\ positives + number\ of\ true\ negatives}{number\ of\ true\ positives + false\ positives + false\ negatives + true\ negatives}$$

Sensitivity was the proportion of positives (rotten sections in our study) which were correctly identified. Specificity measures the proportion of negatives (healthy sections) which were correctly identified.

$$Sensitivity = \frac{number\ of\ true\ positives}{number\ of\ true\ positives + false\ negatives}$$

$$Specificity = \frac{number\ of\ true\ negatives}{number\ of\ true\ negatives + false\ positives}$$

In order to know if *Heterobasidion* spp. (Fr.) Bref and *Armillaria* spp. (Fr. ex Fr.) Staude were the fungi that were producing the decay, slices of wood were removed from those trees that presented symptoms of rot. In the field one slice per rot tree was stored in a plastic bag: a total 107 slices of wood were taken to analyze presence of fungi.

Discs were removed and a sub sample of approx. 4.5 cm^3 was kept in hermetic tubes. In all the trees, samples were taken from the three heights. Sapwood samples were taken in all the heights and incipient reaction zone, reaction zone, decay and heartwood when it was possible.

Wood pieces (between 6 and 15 per tree) were taken to analyze moisture content. A total of 771 samples from different wood parts, were analyzed to know moisture content. 228 of sapwood, 97 incipient reaction zone, 132 of reaction zone, 152 of decay and 162 of heartwood. Samples of each group of detection (true negative, true positive, false negative and false positive) were taken (Table 3). Furthermore, in those trees that belong to true positive group of detection, wood samples were taken from each Rotfinder value.

Table 3. Number of samples taken to measure moisture content for each detection group, Rotfinder value and part of the wood.

Detection group[1]	Rotfinder value	Wood part				
		Sapwood	Incipient reaction zone	Reaction zone	Decay	Heartwood
True negatives	0	**54**	**19**	**5**	**0**	**54**
True positives	1	10	7	7	9	6
	2	14	10	10	14	6
	3	10	5	7	10	7
	4	5	2	4	5	3
	5	6	5	6	6	3
	6	8	4	7	8	3
	7	7	5	7	7	2
	8	7	2	7	7	0
	9	8	3	8	8	1
	10	9	2	9	9	1
Total TP		**84**	**45**	**72**	**83**	**32**
False negatives	0	**71**	**28**	**53**	**69**	**57**
False positives	1	11	3	2	0	11
	2	3	0	0	0	3
	3	3	1	0	0	3
	4	2	1	0	0	2
Total FP		**19**	**5**	**2**	**0**	**19**
TOTAL		**228**	**97**	**132**	**152**	**162**

[1] True negatives: Rotfinder value=0 and not rotten; True positives: Rotfinder value>0 and presence of rot; False negatives: Rotfinder values of 0 but with decay presence and False positives: Rotfinder value>0 but not rotten.

Discs were removed and samples were taken following the same manner as in moisture content measurements (see below). We measured the density in all the samples that were taken to measure moisture content but we increased the sample until 919 samples in order to get better estimates on the density. Altogether we collected 273 samples of sapwood, 106 of incipient reaction zone, 165 of reaction zone, 184 samples of decay and 187 of heartwood (Table 4).

Table 4. Number of samples taken for calculates density values for each detection group, Rotfinder value and part of the wood.

Detection group[1]	**Rotfinder value**	**Wood part**				
		Sapwood	**Incipient reaction zone**	**Reaction zone**	**Decay**	**Heartwood**
True negatives	0	**63**	**19**	**9**	**0**	**61**
True positives	1	12	7	8	10	7
	2	16	12	12	16	7
	3	10	5	7	10	7
	4	10	3	9	10	4
	5	6	5	6	6	3
	6	8	4	7	8	3
	7	8	6	8	8	3
	8	8	2	8	8	0
	9	9	3	9	9	1
	10	11	2	11	11	1
Total TP		**98**	**49**	**85**	**96**	**36**
False negatives	0	**93**	**33**	**71**	**88**	**72**
False positives	1	11	3	2	0	11
	2	3	0	3	0	0
	3	3	1	0	0	3
	4	2	1	0	0	2
Total FP		**19**	**5**	**5**	**0**	**16**
TOTAL		**273**	**106**	**165**	**184**	**187**

[1] True negatives: Rotfinder value=0 and not rotten; True positives: Rotfinder value>0 and presence of rot; False negatives: Rotfinder values of 0 but with decay presence and False positives: Rotfinder value>0 but not rotten.

Twenty five trees were chosen in the field to measure element concentration. Trees chosen included healthy trees properly detected (true negatives), rotten trees detected (true positives), rotten trees not detected (false negatives) and healthy trees not detected (false positives) (Table 5). Wood disks were taken in the field following the same procedure as before, and put into bags. In total 60 samples were analyzed: 12 of sapwood, 8 of incipient reaction zone, 13 of reaction zone, 17 of decay and 10 of heartwood.

Table 5. Number of samples taken for element analysis for each Rotfinder detection group and part of wood.

Detection group[1]	**Rotfinder value**	**Wood part**				
		Sapwood	**Incipient reaction zone**	**Reaction zone**	**Decay**	**Heartwood**
True negatives	0	**5**	**0**	**0**	**0**	**5**
True positives	3	2	1	1	2	0
	4	0	1	0	1	0
	6	1	1	1	1	1
	10	0	0	5	5	0
Total T. positives		**4**	**5**	**9**	**11**	**2**
False negatives	0	**0**	**3**	**4**	**6**	**0**
False positives	1	2	0	0	0	2
	5	1	0	0	0	1
Total F. positives		**3**	**0**	**0**	**0**	**3**
TOTAL		**12**	**8**	**13**	**17**	**10**

[1] True negatives: Rotfinder value=0 and not rotten; True positives: Rotfinder value>0 and presence of rot; False negatives: Rotfinder values of 0 but with decay presence and False positives: Rotfinder value>0 but not rotten.

3.4.-SAMPLE HANDLING

3.4.1.-Culture (fungi)

In the laboratory, samples for decay fungi identification were kept at room temperature in the dark. After two-to-three weeks, the slices were observed in the magnifying glass and based on the fungal growth the samples were sorted into four groups; (i) *Heterobasidion* spp. (if typical white conidiophores appeared) (ii) *Armillaria* spp. (if a soft yellowish mycelium was found) (iii) other fungi (white or green mycelium without fruiting bodies) or and (iv) no fungal growth (any fungal structure).

3.4.2.-Moisture content

In the laboratory, all samples were weighed (humid weight Hp) in a scale and dried in the oven at 105º for 24h-48h. Dry samples were weighed again (dry weight Ho). Moisture content was calculated as the proportion of water content (Hp-Ho) contained in the dried samples (Ho).

$$Moisture\,content = \frac{Hp - Ho}{Ho} * 100$$

3.4.3.-Density

The density was obtained in the laboratory by the following steps: samples were placed in the oven at 105º for 24-48h and their dry weight was taken. Later, samples were transferred into the original tubes which had been filled with water. After 24h the samples were soaked with water and the volume was measured following Archimedes principle by introducing the samples in a graduated cylinder with water with a precision of 0.1ml. The density was calculated with the formula:

$$Density = \frac{Dry\,weight\,(gr)}{Volume\,(cm^3)}$$

3.4.4.-Element concentration

Samples were cut into small pieces and ground for 15 minutes and delivered to the Department of Soil and Environment of Swedish University of Agricultural Science (SLU) where the concentration of Carbon (C), Nitrogen (N), Magnesium (Mg), Calcium (Ca), Potassium (K), Sodium (Na) and Manganese (Mn) was measured. Nitrogen and Carbon analysis were performed with CNS 2000 dry combustion instrument and the rest of the elements with ICP Optima 3000DV Optical Emission Spectrometer.

3.5.-STATISTICAL ANALYSIS AND PREDICTIVE MODELS

Data were analyzed with Statistical Analysis System (SAS®) version 9.1 (SAS Procedures Guide, Version 9.1 (2004), Cary, NC: SAS Institute Inc.). First of all, we calculated probability of decay detection between Rotfinder values. Probability of each Rotfinder value was calculated as relation between number of favorable outcomes and total number of possible outcomes. We performed a hypothesis test to see if the probability of decay detection between every decay type (light brown, brown and soft brown decay) was different. The “proc probit” routine of SAS/STAT® was used to perform these analyses.

A one-way ANOVA was used to test the hypothesis that a relationship between decay types and Rotfinder values existed. Tree diameter differences between locations and between groups of detection (true negatives, true positives, false negatives and false positives) were compared also with a one-way ANOVA. We performed the hypothesis test to see if variances in tree diameters were related with groups of detections. The “proc anova” statement was used for these analyses and a level of 5% was used for rejecting the null hypothesis.

We used a hypothesis test to see if means of different wood variables (moisture content, density and element concentration) in different parts of wood (sapwood, incipient reaction zone, reaction zone, decay and heartwood) were different. A one-way analysis of ANOVA was used to test this hypothesis. Each group of detection (true negatives, true positives, false negatives and false positives) was analyzed separately. Once ANOVA showed differences, a Tukey´s HSD test was performed in order to see how means were associated. The same test was used to see if there were differences in the means of moisture content, density and element concentration for each part of wood among groups of detection. Confidence intervals at 95% level and standard errors (SE) were also calculated. The statements “proc means”, “proc anova” and “proc univariate” were used in these analyses.

In order to study the relationship between wood variables and Rotfinder values, a regression analysis was carried out with the Rotfinder values as dependent variables. Values from true positive results were used to calculate the models. Only variables statistically significant at the 5% level were used in the model. The independent variables were: moisture content, density, carbon, nitrogen, magnesium, calcium, potassium, sodium and manganese concentrations from both reaction zone and decay. Some of the variables were transformed with the natural logarithm in order to reduce the variability of the data. In these cases “Ln” in written in front of the variable. Regression coefficients, R-square and “*p*-values” were calculated by means of the “fit” command within the interactive analysis and solutions of SAS/STAT®.

4.-RESULTS

4.-RESULTS

4.1.-RESULTS OF MEASUREMENTS WITH ROTFINDER INSTRUMENT

According to Rotfinder instrument, 103 trees (20.6% of the total) had some probability to be decayed as one of its three measurements was higher than zero. In Ingbo, 16% of trees presented a Rotfinder value different from 0, in Enåker, 29% and in Harsbo, 26%. The Rotfinder values at stump height and number of trees in each location are shown in Table 6.

In all the sections, highest values were recorded at stump height. According to Rotfinder, the 62.1% of rotten trees had rot until 1.30 m; the 8.73% were rotten only until the middle height (0.6 m) and the 29.1% of the rotten trees were rotten only until stump height. Low values (below 5) indicating a low probability to find decay were the most frequent.

Table 6. Number of trees in each location for each Rotfinder value at stump height.

Rotfinder value	Ingbo	Enåker	Harsbo
0	252	71	74
1	12	6	7
2	4	5	7
3	6	2	5
4	5	5	5
5	3	0	2
6	2	2	0
7	5	2	0
8	3	2	0
9	2	1	0
10	6	4	0

The distribution of diameter of the measured trees was not the same in all locations (Table 7). Ingbo presented higher (p<0.0001) mean diameter (22.4 cm) than Enåker (20.8 cm) and Harsbo (20.9 cm). No differences were found between diameters from Enåker and Harsbo.

Table 7. Number of trees in each diametric class. (Diameter at 1.30 m).

Diametric classes	Ingbo	Enåker	Harsbo
<10	0	4	11
10-14	17	37	24
15-19	54	26	26
20-24	107	21	20
25-29	74	8	17
30-34	23	2	1
35-39	20	2	0
40-44	5	0	0

4.2.-ACCURACY, SENSITIVITY AND SPECIFICITY OF ROTFINDER BASED ON THE RESULTS OF VISUAL EXAMINATION.

The visual examination revealed that 108 trees (21.6%) were rotten. In Ingbo, 24.6% of trees were rotten, in Enåker 20%, and in Harsbo 14%. Visual assessment revealed that 63% of the decayed trees were rotten until 1.30 m; 21.3% were rotten until 0.6m, and 17.6% were rotten until stump height. *Heterobasidion* spp. was the most frequently observed fungus found in the decayed trees sampled in this study. It was present in 69.2% of the slices, *Armillaria spp.* was observed in 8% of the samples, 7.48% had unidentified fungi and in 15.9% we did not observe any fungal growth.

The overall accuracy of Rotfinder instrument in our study was 82.7%. The sensitivity was 46.6% and the specificity was 90.4%. True negatives represented 74.4% of the sections, true positives 8.26%, false negatives 9.47% and false positives 7.86%. When measurements are analyzed separately, the highest accuracy and specificity were observed in middle measurements (83.5% and 91.9% respectively) and the highest sensitivity was observed in stump measurements (56.0%) (Table 8).

Table 8. Accuracy, sensitivity and specifity of Rotfinder instrument at the three different heights.

Height	Accuracy	Sensitivity	Specifity
Stump	81.7%	56.0%	88.9%
Middle	83.5%	45.6%	91.9%
Breast	82.9%	35.3%	90.5%

The highest accuracy and specificity were found in Ingbo and highest sensitivity in Enåker. Ingbo had 87.2% accuracy, Enåker measurements showed 75.8% accuracy, and Harsbo had 76% accuracy. Rotfinder estimations, sensitivity and specificity of the locations are shown in Table 9.

Table 9. Rotfinder estimation, accuracy, sensitivity and specifity of every location**.**

Location	Rotfinder Estimation[1]	Accuracy	Sensitivity	Specifity
Ingbo	- 65%	87.2%	42.9%	98.7%
Enåker	+ 53.9%	75.8%	61.1%	79.2%
Harsbo	+ 69%	76%	48.3%	79.1%

[1] Differences between number of rotten trees estimated by Rotfinder and visual examination (% from the total number of measured trees). Signs "+" indicates overestimation and "-"means underestimation.

An increased probability of finding rot with increasing Rotfinder value was confirmed (Fig. 7). Rotfinder values higher than 4 indicated more than 90% probability of finding real rot whereas Rotfinder values higher than 7 indicated 100% success.

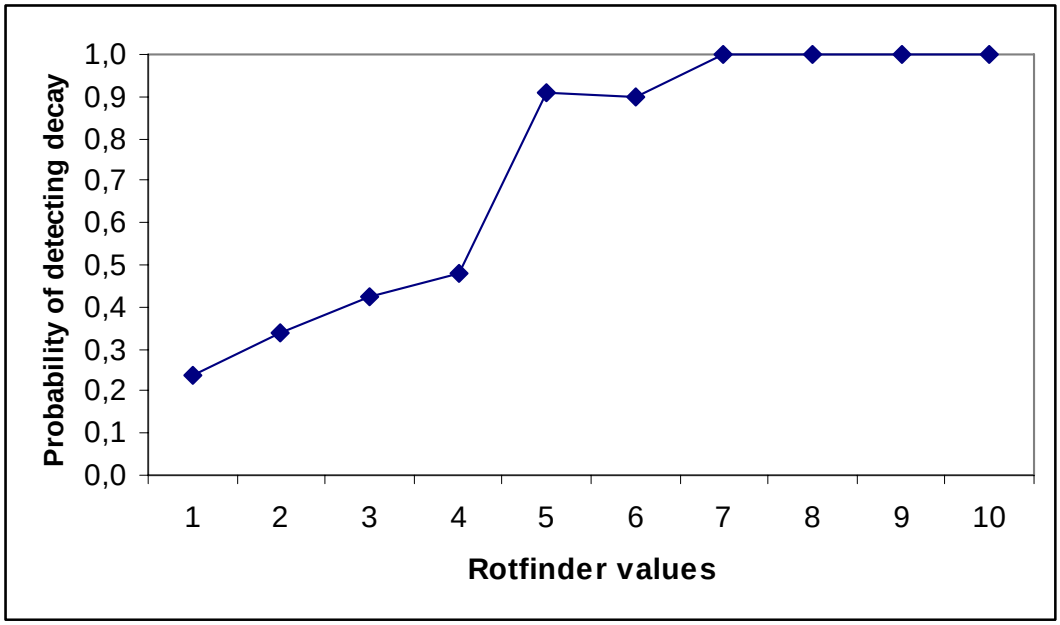

Fig 7. Probability of detecting decay for each Rotfinder value.

Brown decay was the most frequently observed (44.7%), followed by soft brown (29.2%) and light brown decay (26.1%).

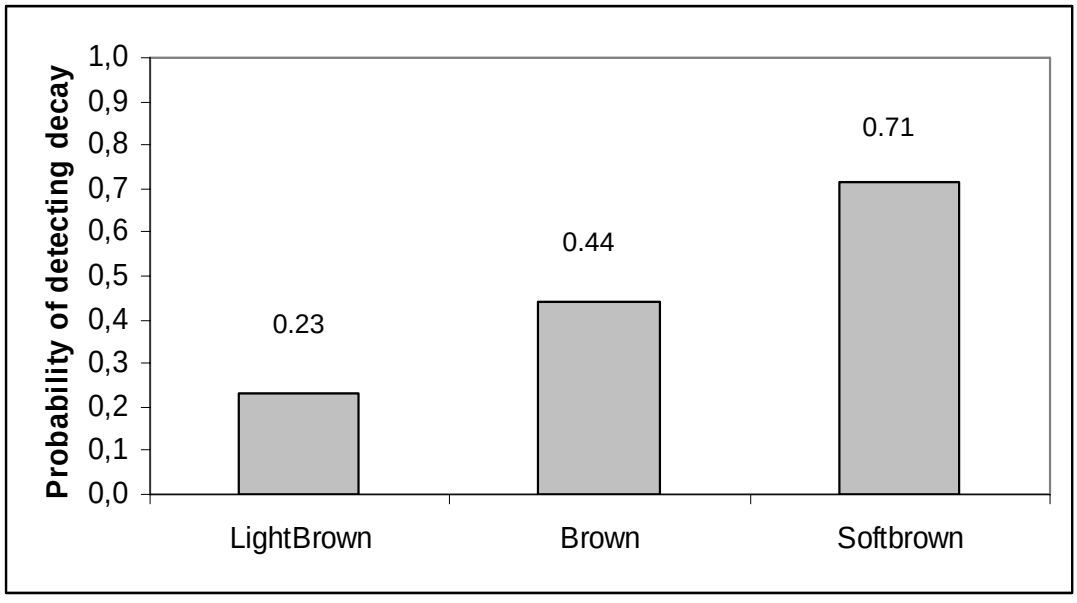

Fig 8. Probability of detecting decay for each decay type

The trees with decay classified as soft brown, representing a more advanced phase of decay, had a higher probability of being detected whereas light brown (incipient decay in most of the cases) had less probability (Fig. 8). Significant differences (p <0.0001) were observed in terms of Rotfinder value between trees with decay classified as soft brown, light brown and brown. Decayed sections with soft brown rot revealed higher Rotfinder values than decayed sections with brown and light brown type of rot. No differences were found between sections with brown and light brown rot (Table 10).

Table 10. Rotfinder mean values for each decay type.

Decay Type	**Rotfinder mean value**	**N[1]**
Light Brown	1.00 b[2]	69
Brown	1.67 b	118
Soft Brown	4.74 a	78

[1] N stands for the number of samples.
[2] Different letters between decay type show values significantly different from p<0.05 (ANOVA Tukey´s HSD test).

Surrounding the decay columns a reaction zone was detected in 91.3% of the samples. Advanced reaction zone was the most common, present in 84.2% of the observed sections. The incipient reaction zone was present in 67.3% of the sections. 25.3% of the sections had only the incipient reaction zone. Both types of reaction zones were present in 42.4% of the sections.

We observed significant diameter differences between true and false sections (p<0.0001) (Table 11). False negatives had higher diameters (p<0.0001) than section from false positives and both true negatives and positives. False positives were smaller than false negatives and both true negatives and positives. No differences were found between diameters from true negative and true positive sections.

Table 11. Diameters (m) of different groups of detections. Mean value ± standard error.

Group of detection[1]	**Diameter measurements**	
	Mean ± SE	**N**[2]
True negatives	23.1 ± 0.239 b[3]	1107
True positives	22.9 ± 0.613 b	123
False negatives	26.1 ± 0.648 a	141
False positives	19.7 ± 0.490 c	116

[1] True negatives: Rotfinder value=0 and not rotten; True positives: Rotfinder value>0 and presence of rot; False negatives: Rotfinder values of 0 but with decay presence and False positives: Rotfinder value>0 but not rotten.
[2] N means number of samples.
[3] Different letters between groups show values significantly different from p<0.05 (ANOVA Tukey´s HSD test).

4.3.-MOISTURE CONTENT, WOOD DENSITY AND ELEMENT CONCENTRATION OF WOOD SAMPLES

4.3.1.-Moisture content

Moisture content in sapwood samples was significantly higher (p<0.0001) than moisture from incipient reaction zone and heartwood in all the groups of detection (Fig.9).

In the group of detection of the true negatives (Rotfinder value=0 and not rotten), samples presented differences (p<0.0001) in moisture content between different parts of the wood (sapwood, incipient reaction zone, reaction zone and heartwood). Moisture content of sapwood samples was higher than moisture content from incipient reaction zone, reaction zone and heartwood (Fig 9a).

In the group of the true positives (Rotfinder value>0 and presence of rot), samples from different parts of the wood also presented differences (p<0.0001) in moisture content. Sapwood moisture content was significantly higher than incipient reaction zone, reaction zone, decay and heartwood moisture content. Furthermore, reaction zone moisture content was higher (p<0.0001) than moisture content from incipient reaction zone, decay and heartwood samples (Fig. 9b)

Samples taken false from negatives (Rotfinder values=0 but with decay presence) showed differences (p<0.0001) among the different parts of the wood. Moisture content was significantly higher in sapwood samples than in incipient reaction zone, reaction zone, decay and heartwood samples. Moisture content of reaction zone parts was also higher than moisture content from incipient reaction zone, decay and heartwood parts (Fig. 9c).

In the group of detection of the false positives (Rotfinder value >0 but not rotten) samples showed significant differences (p<0.0001) of moisture content among wood parts. Moisture content of sapwood was significantly higher than moisture content from incipient reaction zone and from heartwood (Fig. 9d).

Analyses made between groups of detections in each wood part showed that in sapwood samples true positives, false negatives and false positives (p=0.0002) had a higher moisture content than samples from true negative (Fig. 9). In reaction zone samples, moisture content from true positive samples was higher (p<0.0001) than moisture content from true negatives and false negatives. Finally, decay samples from true positives had a higher (p<0.0001) moisture content than samples from false negatives.

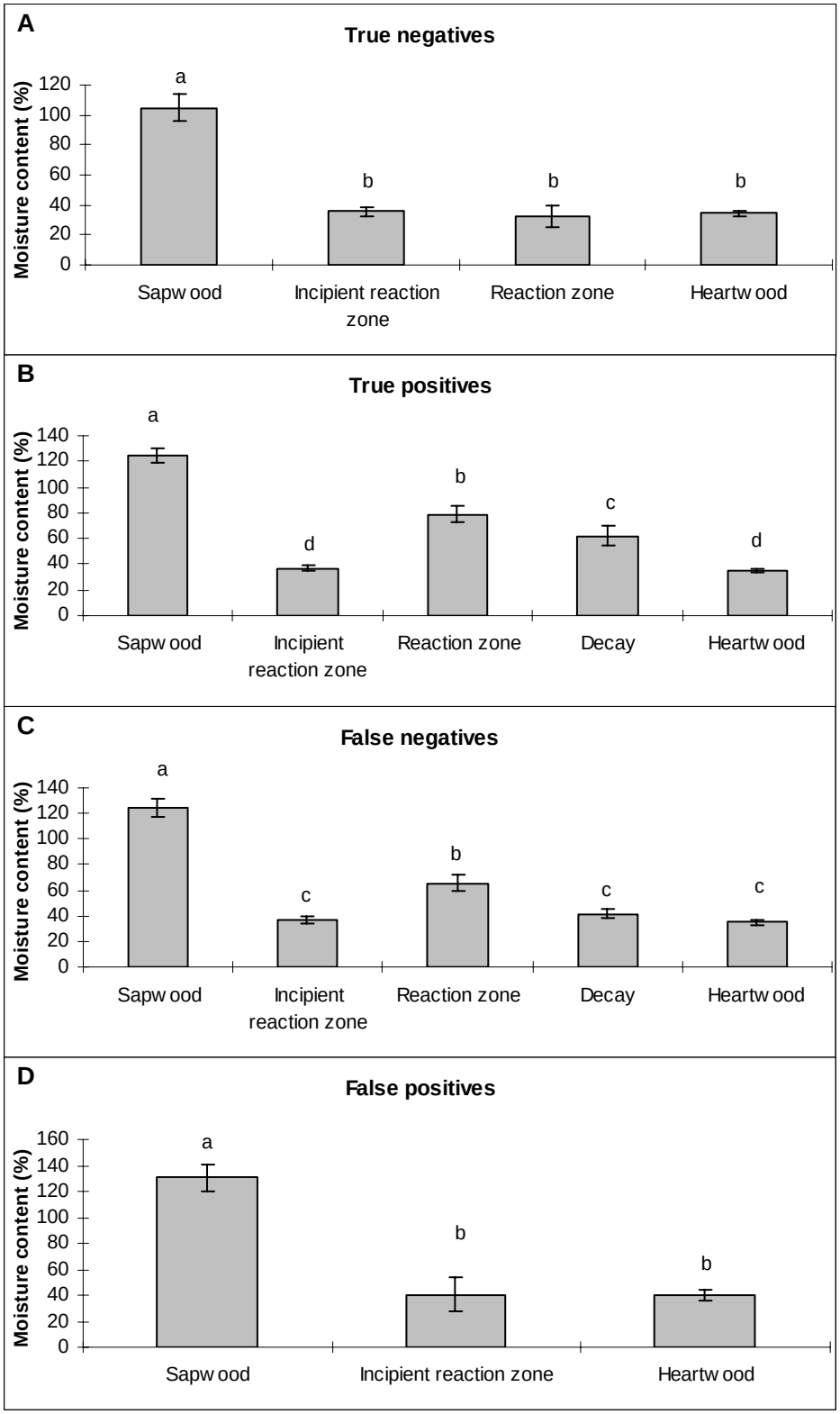

Fig 9a Moisture content in true negative measurements **b** Moisture content in the wood parts in true positives. **c** Moisture content in the wood parts in false negatives. **d** Moisture content in the wood parts in false positive measurements. Means with the same letter were not significantly different at $p<0.05$ (ANOVA Tukey´s HSD test). Bars in all graphics represent confidence intervals at 95%.

4.3.2.-Density

Samples taken from true negatives (Rotfinder value=0 and not rotten) did not show density differences between sapwood, incipient reaction zone, reaction zone and heartwood (Fig. 10a).

In the group of detection called true positives (Rotfinder value>0 and presence of rot) samples showed differences (p<0.0001) in density among wood parts . Reaction zone density was significantly higher than sapwood, incipient reaction zone, decay and heartwood ones. Density from sapwood and incipient reaction zone samples was significantly higher than density from decay samples (Fig. 10b).

In false negatives (Rotfinder values=0 but decay presence), density from reaction zone was significantly (p<0.0001) higher than density from decay and heartwood samples (Fig. 10c). No differences were found among density from sapwood, incipient reaction zone, decay and heartwood.

Samples from group of detection called false positives (Rotfinder value>0 but not rotten) did not show differences of density between sapwood, incipient reaction zone and heartwood samples (Fig. 10d).

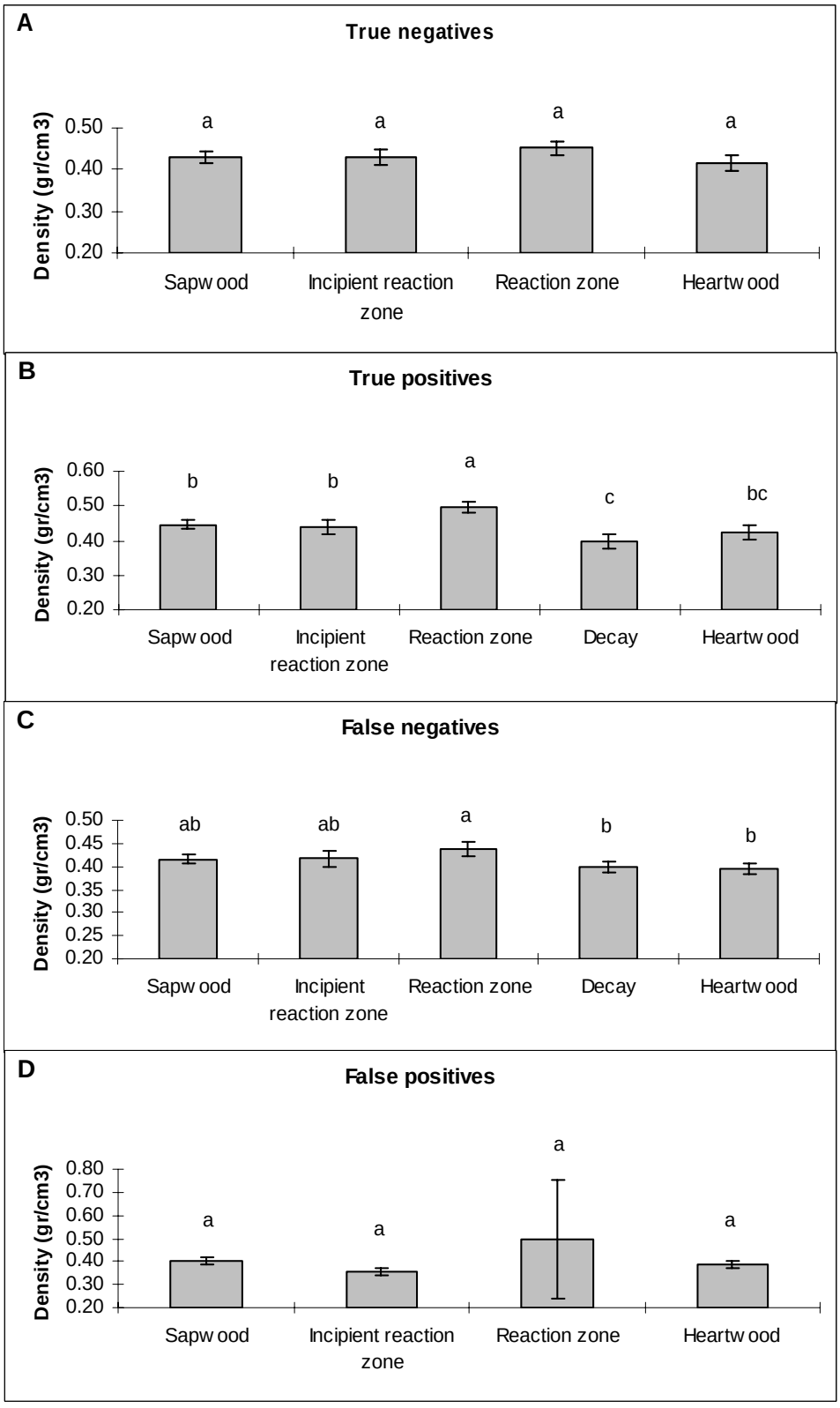

Fig. 10a Density in true negative measurement **b** Density of the wood parts in true positives. **c** Density of the wood parts in false negatives. **d** Density of the wood parts in false positive measurement. Means with the same letter were not significantly different at $p<0.05$ (ANOVA Tukey HSD test). Bars in all graphics represent confidence intervals at 95%.

Neither heartwood nor decay samples showed any significant differences among the four detection groups. Sapwood density was significantly higher (p=0.0007) in true positive detections than in false negatives and false positives. Incipient reaction zone had higher (p=0.014) density in true positive samples than in false positive ones. Reaction zone samples presented higher (p<0.0001) density in true positive detections than in false negatives (Fig. 10).

4.3.3.-Element concentration

In the group of detection called true positives (Rotfinder value>0 and presence of decay), samples showed differences in some element concentrations among the different parts of the wood. In calcium, potassium magnesium and manganese decay and reaction zone concentrations were significantly higher than concentrations from sapwood samples. Neither nitrogen, nor carbon nor sodium presented differences between wood parts (Table 12).

Table 12. Element concentration obtained in wood samples for true positive measurements. Mean value ± standard error.

Element[1]	***P*-value**[2]	**Wood part**				
		Sapwood	**Incipient reaction zone**	**Reaction zone**	**Decay**	**Heartwood**
N (%)	0.130	0.040±0.009a[2]	0.040±0.008a	0.055±0.015a	0.064±0.020a	0.033±0.008a
C (%)	0.114	50.3±0.654a	49.8±0.375a	50.5±1.12a	49.4±0.527a	50.0±5.80a
Ln Ca (mg/kg)	0.0003	6.58±0.285c	6.96±1.80bc	7.77±0.378a	7.46±0.366ab	6.87±1.80bc
Ln K* (mg/kg)	<0.0001	5.70±0.423c	6.74±0.669bc	7.82±0.525ab	8.33±0.617a	5.71±6.37bc
Ln Mg* (mg/kg)	<0.0001	4.25±0.161c	5.09±0.369bc	5.68±0.279ab	6.13±0.413a	4.60±0.928c
Ln Mn* (mg/kg)	<0.0001	3.75±0.509c	4.06±0.438bc	5.08±0.422a	4.89±0.471ab	4.60±0.774abc
Ln Na (mg/kg)	0.630	1.37±0.480a	1.94±1.08a	2.29±0.544a	2.59±0.837a	

[1] Variables tagged with"*" presented significant differences between wood parts.

[2] *P*-value for ANOVA.

[3] Different letters between rows show values significantly different from p<0.05 (ANOVA Tukey´s HSD test).

In the group of detection called true negatives (Rotfinder=0 and not rotten) sapwood manganese concentration was significantly higher (p=0.024) than heartwood manganese concentration (Table 13). No other differences in element concentration were found among sapwood and heartwood samples.

Table 13. Element concentration in true negative samples. Mean value **±** standard error.

Element[1]	***P*-value**[2]	**Wood part**	
		Sapwood	**Heartwood**
N (%)	0.524	0.037 ± 0.014	0.033 ± 0.008
C (%)	0.539	50.3 ± 0.601	50.1 ± 0.768
Ln Ca (mg/kg)	0.175	6.67 ± 0.430	6.92 ± 0.221
Ln K (mg/kg)	0.522	5.69 ± 0.906	5.46 ± 0.427
Ln Mg (mg/kg)	0.159	4.25 ± 0.331	4.59 ± 0.531
Ln Mn * (mg/kg)	0.024	4.94 ± 1.61 b[3]	4.75 ± 0.424 a
Ln Na (mg/kg)	0.535	0.958 ± 1.61	0.471 ± 2.03

[1] Variables tagged with"*" presented significant differences between wood parts.
[2] *P*-value for ANOVA.
[3] Different letters between rows show values significantly different from p<0.05 (ANOVA Tukey´s HSD test).

Samples from the group of detection false negatives (Rotfinder values=0 but decay presence) did not present any significant differences between the parts of the wood (incipient reaction zone, reaction zone and decay) (Table 14).

Table 14. Element concentration in false negatives samples. No significant differences were found in any variable. Mean value ± standard error.

Element	***P*-value**[1]	**Wood part**		
		Incipient reaction zone	**Reaction zone**	**Decay**
N (%)	0.620	0.041 ± 0.007	0.045 ± 0.004	0.053 ± 0.009
C (%)	0.392	50.7 ± 0.182	49.9 ± 0.225	49.9 ± 0.443
Ln Ca (mg/kg)	0.072	6.59 ± 0.236	6.80 ± 0.262	7.52 ± 0.258
Ln K (mg/kg)	0.2945	6.81 ± 0.256	7.38 ± 0.285	7.47 ± 0.253
Ln Mg (mg/kg)	0.224	4.92 ± 0.354	5.17 ± 0.286	5.76 ± 0.322
Ln Mn (mg/kg)	0.665	4.46 ± 0.352	4.06 ± 0.718	4.74 ± 0.456
Ln Na (mg/kg)	0.103	1.77 ± 0.327	1.38 ± 0.238	2.09 ± 0.138

[1] *P* value for ANOVA.

Samples taken from the group of false positives (Rotfinder value>0 but not rotten), presented significant differences in some element concentrations among wood parts (Table 15). Concentrations of calcium (p=0.004) and magnesium (p=0.002) were significantly higher in heartwood parts than in sapwood samples.

Table 15. Element concentration in false positive samples. Mean value ± standard error.

Element[1]	***P*-value**[2]	**Wood part**	
		Sapwood	**Heartwood**
N (%)	0.576	0.042 ± 0.005a[3]	0.039 ± 0.002a
C (%)	0.456	50.0 ± 0.310a	50.4 ± 0.240a
Ln Ca * (mg/kg)	0.0004	6.44 ± 0.016 b	6.95 ± 0.044 a
Ln K (mg/kg)	0.526	5.59 ± 0.208a	5.39 ± 0.210a
Ln Mg* (mg/kg)	0.002	4.10 ± 0.043 b	4.53 ± 0.046 a
Ln Mn (mg/kg)	0.123	3.59 ± 0.128a	4.17 ± 0.273a
Ln Na (mg/kg)	0.130	2.58 ± 0.148a	0.89 ± 0.875a

[1] Variables tagged with"*" presented significant differences between wood parts.

[2] *P* value for ANOVA.

[3] Different letters between rows show values significantly different from $p<0.05$ (ANOVA Tukey´s HSD test).

Analyses for every wood part between groups of detection showed no differences for sapwood and heartwood samples. Incipient reaction zone of false negatives (undetected decay) showed higher (p=0.008) carbon concentration than true positives (correctly detected decay) (Fig. 11a). The reaction zone of undetected decay samples presented lower sodium (p=0.038) and calcium (p=0.008) concentration than in correctly detected decay samples (Fig. 11b & 11c). Also, decay samples from true positive (detected decay) detections tended to be higher (p=0.056) in potassium concentration than the ones from false negative (undetected decay), but the difference was not significant.

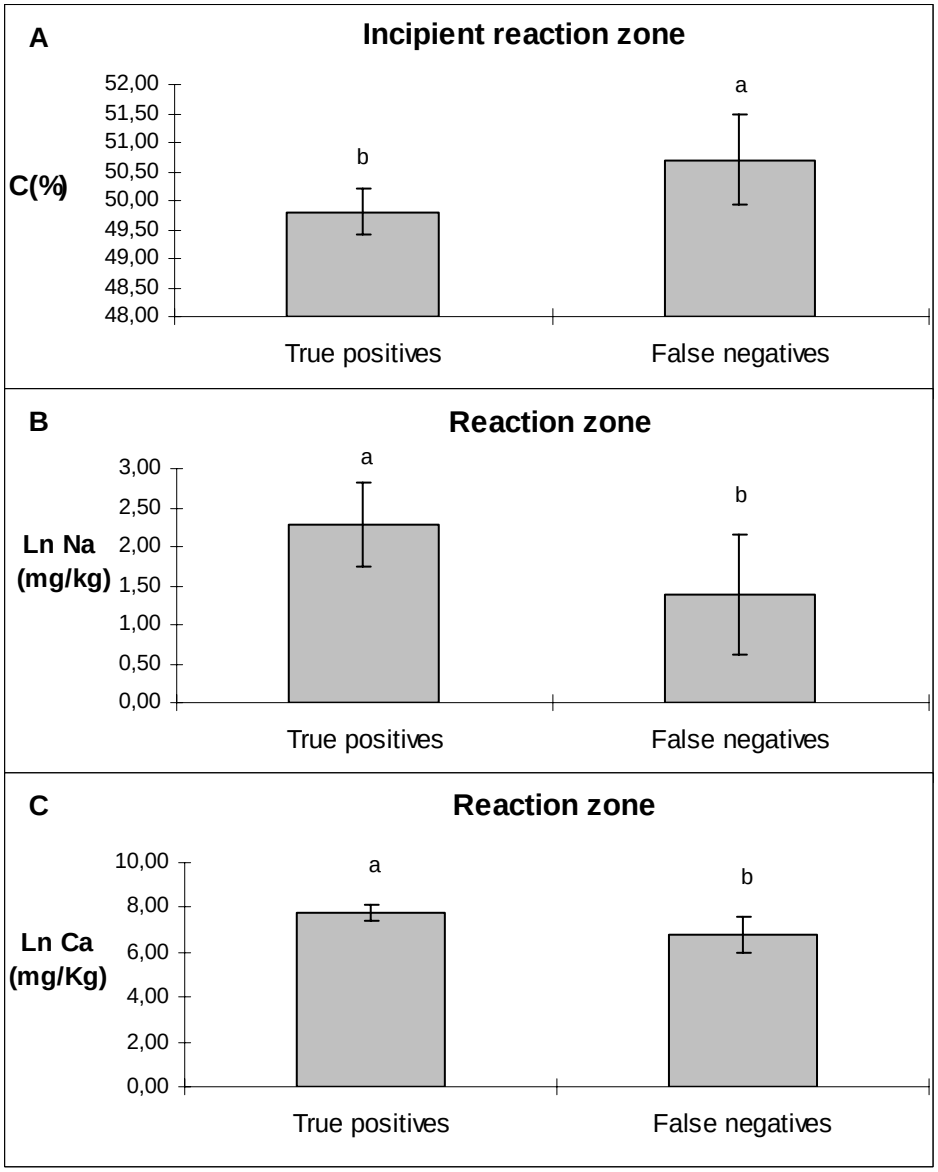

Fig. 11a Means for C concentrations for true positives and false negative in incipient reaction zone samples **b** Means for Ln Na concentrations for true positives and false negatives from reaction zone samples **c** Means for Ln Na concentrations for true positives and false negatives from reaction zone samples. Different letters means significative differences (5% level) in ANOVA Tukey´s HSD test. Bars show confidence interval for 95%.

4.4.-PREDICTIVE MODELS FOR ROTFINDER VALUES

Decay and reaction zone samples showed the highest concentrations of elements (Table 16), thus only parameters of decay and reaction zone were used for studying the correlation between Rotfinder values and the concentration of the elements. Rotfinder values had a significant correlation with decay moisture content, reaction zone density, decay density, nitrogen concentration of reaction zone, nitrogen concentration of decay, carbon concentration of decay, Ln potassium concentration of decay, Ln sodium concentration of reaction zone and Ln sodium concentration of decay (Table 17).

Table 16. Element concentration in every wood part. Mean value.

Element	**Wood part**				
	Sapwood	**Incipient reaction zone**	**Reaction zone**	**Decay**	**Heartwood**
N (%)	0.044	0.045	0.048	0.054	0.036
C (%)	50.1	50.1	49.5	50.0	50.0
Ln Ca (mg/kg)	6.46	6.79	7.49	7.48	6.73
Ln K (mg/kg)	5.7	6.61	7.67	8.08	6.35
Ln Mg (mg/kg)	4.26	4.93	5.55	6.05	4.62
Ln Mn (mg/kg)	3.39	3.91	4.67	4.76	4.21
Ln Na (mg/kg)	1.9	1.88	2.1	2.51	1.99

Rotfinder values showed the highest correlation with potassium concentration of the decay (p<0.0001) (Fig.12). Otherwise, potassium in reaction zone did not correlate with Rotfinder values (p=0.1500). Rotfinder values showed a positive correlation (p=0.0001) with decay moisture content but a low R-square. Our results showed that Rotfinder values presented a positive correlation with reaction zone density (p<0.0001) and a negative correlation with decay density (p=0.017).

Table 17. Correlation between Rotfinder values and moisture content, density and element concentration from reaction zone and decay parts.

Independent variable	Model[1]	R-square	*P*-value
Decay moisture content	R= 0.87+0.0356DMC	0.097	0.0001
Density Reaction zone	R=-7.82+22.76DRZ	0.217	<0.0001
Density Decay	R=5.77-7.64DD	0.031	0.017
N concentration Reaction zone	R=-2.96+150NRZ	0.317	0.045
N concentration Decay	R=-0.971+84.6ND	0.288	0.027
C concentration Decay	R=128-2.50CD	0.280	0.029
Ln K Decay	R=-28.0+4.00LnKD	0.711	<0.0001
Ln Na Reaction zone	R=-2.80+3.814LnNaRZ	0.401	0.020
Ln Na Decay	R=-2.99+3.03LnNaD	0.547	0.001

[1] DMC, decay moisture content (%); DRZ, density of the reaction zone (gr/cm^3); DD, density of the decay (gr/cm^3); NRZ , Nitrogen concentration of the reaction zone (%); ND, Nitrogen concentration of the decay (%); CD, carbon concentration of the decay (%); LnKD, Ln of potasium of the decay; LnNaRZ, Ln of sodium of the reaction zone; LnNaD, Ln of sodium of decay.

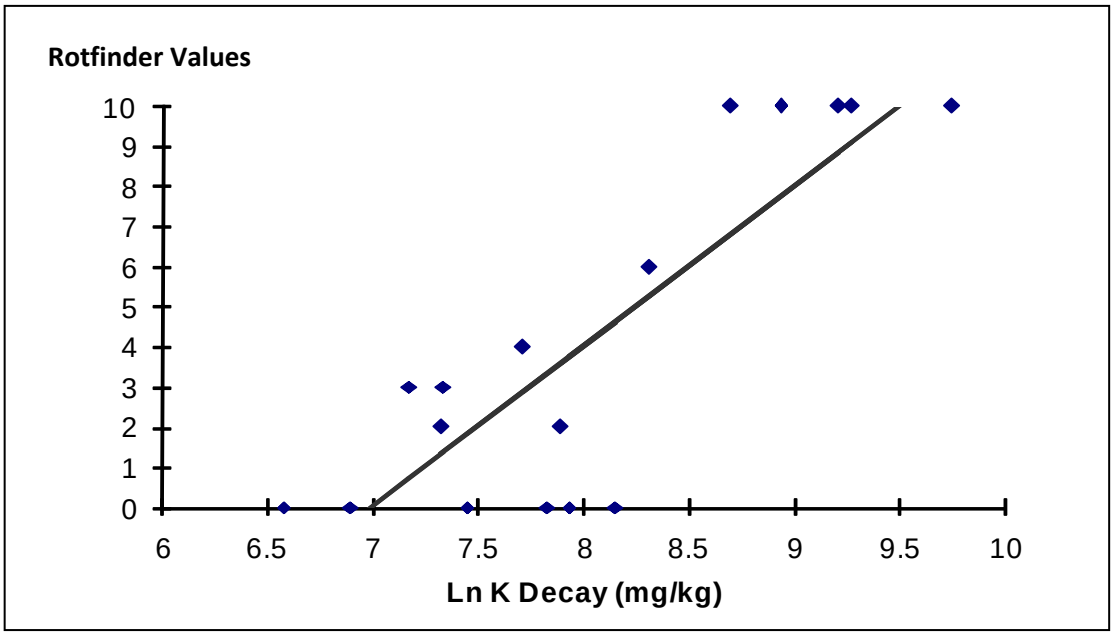

Fig 12. Correlation between Rotfinder values and Ln K concentration in decayed wood

Rotfinder values showed a positive correlation with nitrogen concentration of decay (*p*=0.027) but the model presented a low R-square value (0.288). Model that correlated Rotfinder values and nitrogen concentration of reaction zone presented also a positive correlation (*p*= 0.045) and a low r-square (0.317). Rotfinder values had a negative correlation with carbon (p=0.029) but a low r-square (0.28). Finally, Rotfinder values were positive correlated with sodium concentration in both the reaction zone (*p*=0.020) and in the decay (*p*=0.001).

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5.- DISCUSSION

5.-DISCUSSION

Considering all the measurements in our study, the Rotfinder instrument had an overall accuracy of 82.7% detecting true results and a sensitivity of 46.6% detecting true rotten trees. In one measured stand (Ingbo) Rotfinder underestimate true number of decayed trees by 65% and in the other two stands (Enåker and Harsbo) Rotfinder overestimate the number of decayed trees by 53.9%-69% at stump height.

When considering soft brown decayed trees representing the most advanced phase of decay, the sensitivity of Rotfinder was 71%. In this type of decay, the instrument tended to show high values, associated with a higher probability of rot detection. Rotfinder had a low sensitivity of detecting light brown decay (23%), the incipient phase of decay related with low values of Rotfinder.

Sensitivity of Rotfinder at stump height was 56.0%, which is comparable with the sensitivity of detecting decay with the increment borer method: 61% in STENLID & WÄSTERLUND (1986) study and 60% in SWEDJEMARK & KARLSSON (2004). At breast height sensitivity of Rotfinder decreases to 35% while sensitivity of the increment bore extraction method at this height was 40-70% (STENLID & WÄSTERLUND, 1986).

Rotfinder always gave lower values at breast height (1.3m) and at middle height (0.6m) than at stump height (0.3m). This was because decay colonization starts in the roots and advance along the stem producing a decay column which increases in height while decreases in cross-section area (KORHONEN, 2002; ASIEGBU *et al*., 2005). Probability of detecting vertical presence of the fungus would vary between 45.6% if decay was present at 0.6m and 35.3% if decay reached 1.30m. Accuracy of the instrument was similar in the three heights, but sensitivity was decreasing from stump to breast. It can be related with the presence and the shape of the decay column: it is wider at the bottom and narrower as the height increases. The most advanced phase of decay would be normally placed at stump height and thus the sensitivity of Rotfinder at this height would be higher. In the same way at breast height we would normally find incipient phase of decay, which is also related with lower probability of detection. Rotfinder can be used to detect vertical spread of the decay especially in cases of advance phase of decay along the stem, but if

the colonization of the column of the decay is small, sensitivity of Rotfinder instrument would decrease along the stem.

In order to identify the causal decay fungus in our study, some samples were cultivated. *Heterobasidion* spp. was the most common fungus causing decay in the studied stands. The proportion of decayed trees infected by *Heterobasidion* spp. of 70% what is in line with previous studies developed in Scandinavian countries in which *Heterobasidion* spp. was the fungus which cause the major part of butt rots: 60-80% (STENLID & WÄSTERLUND, 1986; VOLLBRECHT *et al*. 1995).

A decrease in moisture content between reaction zone and sapwood was evident in all the cases either well predicted or not by Rotfinder instrument. DEFOLORIO *et al.,* 2008 also observed a decrease in moisture content in wood parts with presence of incipient decay in Douglas fir, beech, oak and sycamore trees. MANION & ZABEL (1979) predicted a moisture loss as a response of the tree to invasion by decay micro organisms. In reaction zone, moisture contents were significantly higher in true positives (detected decay, 70.0%) than in false negatives (undetected decay, 65.7%). In decay samples these differences were also present: true positives (62.1%) had higher moisture content than false negatives (41.7%) which can suggest that Rotfinder is sensitive to moisture content, and thus these samples were not properly detected.

Rotfinder misjudgments could relate to a low concentration of ions in the decayed parts. In true positive detections calcium, potassium, magnesium and manganese concentrations showed significant differences between wood parts (sapwood, incipient reaction zone, reaction zone, decay and heartwood). The concentrations of all the studied ions in the decayed area and in the reaction zone were significantly higher than in the sapwood. SHAIN (1979) observed an increase of concentration of these four elements in the reaction zone and decayed tissues. On the contrary, false negatives (undetected decays) did not show any difference among the wood parts, which can suggest that the response of the tree in these sections were not as strong as in true positives, and thus concentrations were lower. Reaction zone and decay element concentrations were higher than the ones that presented the other parts. This agrees with other studies results, which confirm that an increase in ion concentration is a consequence of the response of the tree

to infection (SHORTLE & SMITH, 1987). High percent of decay in our study presented either incipient reaction zone, reaction zone or both. Reaction zone is considered as a response of the tree to the fungus infection (SHORTLE & SMITH, 1987; SHAIN, 1979; SHIGO, 1984). Samples of true positives were significantly higher from false negatives, in reaction zone samples in calcium and sodium and almost significant in potassium in decay parts. Perhaps these differences provoked Rotfinder mistakes and misjudgments because a change in electrolytic state is a first response of the tree to the fungus presence, (SHIGO, 1984) and it seems that false negative samples had a lower response.

Rotfinder could be indirectly detecting K concentration. The potassium concentration showed the highest correlation between Rotfinder values in the decay area. It was observed that absolute concentrations of potassium in reaction zone and decay parts were normally higher than concentrations of any other element. According to SHORTLE & SMITH (1987) and NICOLOTTI *et al*., (2003) white rot fungi create an accumulation of cations and specially K-ions that, thanks to their high mobility produce a reduction of the wood resistivity even from the very beginning of the decaying process. Since Rotfinder instrument is based on tree resistivity, potassium concentrations could explain Rotfinder values.

Sensitivity of Rotfinder instrument in advanced stages of decay is relatively high, which makes Rotfinder a proper instrument for detecting advanced decay in standing individual trees. Depending on the aim of the sampling, the sensitivity of Rotfinder to detect incipient decays may be an issue. Rotfinder could be used for detecting trees with a high probability of collapse, since the presence of high decay volumes has associated a high risk of mechanical failure. If Rotfinder is compared with other techniques we found that it has the same difficulties than Picus® acoustic tomography to detect incipient decay stages (DEFLORIO *et al.,* 2007). Magnetic resonance imaging (MÜLLER *et al.*, 2001) and several tomography investigations performed by NICOLOTTI *et al.* (2003) showed positive results in detecting decay at a very early state of infection but they have not been proved yet in standing trees.

In conclusion, the Rotfinder instrument properly detected advanced stages of decay and it seemed to be more efficient when the tree response was stronger. The high values of Rotfinder (5 to 10) gave a very high probability of detecting rotten trees. Furthermore, Rotfinder correctly classified some trees in low Rotfinder values (1 to 4). As the aim of this research was to determine the accuracy of Rotfinder for values 1 to 4, a risk of failure should be assumed when making a decision about the stage of decay. Furthermore, Rotfinder misjudgments may be explained by incipient decay presence, moisture content in reaction zone and decay and element concentrations, especially in lower potassium. It is likely that percentage of the section affected by decay could have also influence the Rotfinder sensitivity. Nevertheless, the use of this non-destructive device might be useful when making decisions in parks, gardens and forest stands, especially if trees have an elevated degree of decay.

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