Soil physical properties and the occurrence of chestnut ink disease: a micromorphological study

Propriétés physiques du sol et occurrence de la maladie de l'encre du châtaignier : étude micromorphologique

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ABSTRACT

Ink disease caused by the fungus *Phytophthora cinnamomi* Rands is a major threat to the sustainability of chestnut ecosystems in northern Portugal. Not only soil factors and host resistance but also management practices can influence the occurrence of this disease. Total soil porosity was measured in chestnut groves affected by the ink disease and in healthy ones, at 2 or 3 depth zones. The orchards were located either in mid-slope position or at the foot of the slope. The purpose of the work was to investigate whether or not the occurrence of ink disease would be related with the presence of a plough pan and with the topographical position of the stand. The areas covered by pores with Ø>30 μm (po30) and by pores with Ø>500 μm (po500) were directly quantified on soil thin sections using micromorphological and image analysis techniques. This method was chosen because routine methods fail to detect soil porosity differences in the soils with a clear plough pan. The results show that low absolute total porosity does not directly influence the health status of the orchards. However, the presence of a layer with reduced porosity at 20-35 cm clearly affects the occurrence of ink disease. This plough pan strongly hinders root development and soil aeration and thus makes the trees more sensitive to the attack of *Phytophthora cinnamomi*. The plough pans observed were more pronounced in the foot-slope than in the mid-slope soils. Former studies have shown that lowland groves are also more intensively affected by ink and that there is a close relationship between the number of tillage operations and the incidence of the disease. This study evidences the relationship between the occurrence of *Phytophthora* root disease and the presence of a plough pan, which in turn depends on the location of the stand on the slope. From a methodological point of view it stresses the importance of micromorphological and image analysis techniques to directly determine total soil porosity and to provide complementary and more accurate data than the indirect methods commonly used for quantifying soil porosity.

Key-words: chestnut ink disease, soil porosity, soil micromorphology, image analysis

RESUME

La maladie de l'encre (*Phytophthora cinnamomi* Rands) constitue une des grandes menaces pour le maintien des châtaigneraies cultivées du Nord du Portugal. L'occurence de cette maladie est influencée par des facteurs pédologiques, la résistance de la plante et les pratiques agricoles. L'objectif principal de l'étude est de vérifier si la présence de la maladie est liée à l'existence d'une semelle de labour dans les sols et/ou à la position topographique de la châtaigneraie. Ainsi, la porosité totale du sol a été mesurée à 2 ou 3 profondeurs dans des châtaigneraies attaquées par le champignon et dans des châtaigneraies saines. Les châtaigneraies étudiées sont localisées en mi-pente ou en bas-fonds. Nous avons directement mesuré, en lames minces de sols par des techniques micromorphologiques et d'analyse d'images, les surfaces couvertes par des pores de diamètre supérieur à 30 μm et par des pores de diamètre supérieur a 500 μm. Cette technique a été choisie car les méthodes de routine n'ont pas permis de détecter des variations de porosité à différentes profondeurs, même dans les sols avec une semelle de labour très prononcée. Les résultats indiquent qu'une porosité totale plus basse n'a pas d'influence directe sur l'état de santé des châtaigneraies. D'autre part, la présence dans les sols d'une couche compactée, entre 20-35 cm de profondeur, est clairement associée à l'occurrence de la maladie. Cette semelle de labour restreint le développement des racines et l'aération du sol ; de ce fait, les arbres deviennent plus sensibles à l'attaque du *Phytophthora*. Les semelles de labour sont plus prononcées dans les sols des châtaigneraies en bas-fonds que dans les sols des châtaigneraies en mi-pente. Des études précédentes ont montré que les châtaigneraies en bas-fonds sont aussi plus fortement attaquées par la maladie de l'encre et qu'il existe une relation directe entre le nombre d'opérations de labour et le taux d'incidence de la maladie. Cette étude met en évidence la relation entre la maladie de l'encre sur des châtaigniers et la présence d'une semelle de labour dans les sols, qui, à son tour est reliée à la position topographique de la châtaigneraie. Sur le plan méthodologique, l'étude souligne l'importance de l'utilisation des techniques micromorphologiques et de l'analyse d'images pour mesurer directement la porosité totale du sol et pour fournir des résultats plus précis que ceux obtenus par les méthodes indirectes de quantification de la porosité du sol.

Mots-clés : maladie de l'encre sur des châtaigniers, porosité du sol, micromorphologie du sol, analyse d'images

INTRODUCTION

The sustainability of chestnut ecosystems strongly depends on the health conditions of the trees. A major problem of widespread occurrence in chestnut stands in northern Portugal is the ink disease (Abreu *et al.*, 1993; Martins et *al.*, 1997), which is caused by the fungus *Phytophthora cinnamomi* Rands. Because the pathogen is a soilborn fungus, the occurrence of the disease is affected not only by host resistance but also by soil factors, which in turn are influenced by agricultural and management practices. The increase in the incidence of the disease has indeed been associated, already in the years 50, to alterations of these practices (Fernandes, 1955; Guerreiro, 1957).

More recently, Portela *et al.* (1997, 1998) have been studying the relationship between the degree of incidence of ink disease and the topographical position and associated management practices in chestnut orchards in the Trás-os-Montes region. They observed that in stands located in south facing slopes the presence of ink was related to deficient rooting and water stress while in lowland groves the occurrence of the disease was associated to soil compaction resulting from the combined effect of excessive tillage and poor soil structure.

The selection of chestnut orchards to be studied was based on these findings of Portela *et al.* (1997, 1998). Macroscopic observation of the selected soil profiles pointed to the existence, in some of them, of a compacted subsurface layer, which was not reflected by porosity data previously obtained with routine methods of soil physical analysis.

The general purpose of this study was to compare the soil porosity of chestnut groves affected by the ink disease with the soil porosity of healthy ones.

Specific objectives were, on one hand, to investigate whether or not the occurrence of ink disease would be related with the presence of a plough pan and with the topographical position of the stand and, on the other hand, to test the use of micromorphological and image analysis techniques to directly quantify soil porosity in thin sections.

MATERIALS AND METHODS

Sampling and sample preparation

Six profiles, four in chestnut groves affected by ink (P20, P22, P29 and P30) and two (P2 and P32) in healthy ones, were selected for this study. Profiles P2, P32, P20 and P29 were located in mid-slope position and profiles P22 and P30 at the foot of the slope. Depending on the stoniness of the soil undisturbed soil samples in Kubiena boxes or undisturbed soil blocks (2-4/depth zone) were taken at 2 or 3 depth zones (d1<20 cm, d2 = 20-35 cm, d3>35 cm). Depth zone d2 corresponds to the depth at which a plough pan usually occurs.

The samples were impregnated with a polyester resin mixed with a fluorescent dye (Uvitex). After complete drying, two parallel surfaces of each impregnated block were finely polished and photographed under UV light so that the area covered by pores with Ø> 500 µm could be quantified.

The same surfaces were viewed with a fluorescence microscope to produce images (6/surface) suited for the measurement of the area occupied by pores with Ø>30 µm. The photographs were then digitised using a flat bed scanner.

Image analysis

The quantification of total porosity (area % occupied by pores) in soil thin sections was formerly done by point counting or visual estimation but this has been successfully replaced by more accurate and less time consuming techniques such as the use of imaging software (White *et al.*, 1998).

For this study the areas covered by pores with Ø>30 µm (po30) and by pores with Ø>500 µm (po500) were measured on the digital images using the public domain UTHSCSA Image Tool software. This programme permits to determine the number of pixels in the pore space (fluorescent zones) and the total number of pixels in the image.

Data analysis

The hypotheses to be tested were: (i) there are no differences among total porosity at the different levels of the factors - health status, depth zone and topographical position -, and (ii) there is no interaction between the factors.

Total porosity data (po30 and po500) were analysed using a mixed model Analysis of Variance (General Linear Model) with soil profile (P20, P22, P29, P30, P2 and P32) as random factor, depth zone (d1, d2, d3) and topographical position (mid-slope, foot-slope) as within fixed factors and health status (ill, healthy) as between fixed factor.

Mean total porosities at the different levels of the health status, depth zone and slope position combinations were estimated from the best fitting model (i.e. the model with only the significant main effects and significant interaction terms, plus possibly the non significant lower order terms included in the latter). Pairwise comparisons between the estimated marginal means were carried out with Bonferroni adjustment.

RESULTS

The descriptive statistics for the total porosity measurements (po30 and po500) are compiled in Table 1.

The results of the statistical analysis are given in Table 2, Table 3 and Figure 1.

As can be seen from Table 2 total porosity is affected by health status, topographical position of the orchard and depth zone and/or their interactions. The effect of depth zone is significant and, more importantly, it is modified by the health status and, for po30, also by the topographical position of the orchard.

Pairwise comparisons in Table 3 show that in healthy chestnut groves the differences between porosities in d1, d2 and d3 are not significant while in affected groves total porosity in d2 is significantly smaller than in d1 and d3. This is well illustrated by the images in Figure 2.

The graphs in Figure 1 again suggest that the effect of depth zone is modified not only by the health status but also by the topographical position.

DISCUSSION

From the data in Table 1 and the graphs in Figure 1 it can be deduced that low absolute total porosity values do not directly influence the health status of the orchards. On the contrary, the presence of a layer with reduced porosity (plough pan) at d2 clearly affects the occurrence of ink disease in chestnut groves (Table 2, Table 3, Figure 1 and Figure 2).

Mean soil porosity values for po30 (Table 1) have in the ink affected orchards larger standard deviations than in the healthy ones. This indicates a high variation of total porosity within the same depth zone and suggests the presence of micro-sites with very low porosities in the soil of ink affected groves.

The plough pan is more pronounced in the foot-slope than in the mid-slope soils as illustrated by the graphs in Figure 1. This is in line with the studies by Portela *et al.* (1998) that show that lowland groves are also more intensively affected by ink and that there is a close relationship between the number of tillage operations and the incidence of the disease. According to these authors the lowland soils have high amounts of fine sand and silt and consequently are submitted to frequent tillage to break up soil crusts.

The plough pan strongly hinders root development and soil aeration and thus makes the trees more sensitive to the attack of *Phytophthora cinnamomi*. Low oxygen availability, even for short periods, may favour *Phytophthora* root disease (Shea & Broadbent, 1983).

This study evidences the relationship between the occurrence of *Phytophthora* root disease and the presence of a plough pan, which in turn depends on the location of the stand on the slope. Attention should thus be focussed on minimising tillage operations in chestnut orchards.

From a methodological point of view it stress the importance of micromorphological and image analysis techniques to directly determine total soil porosity and to provide complementary and more accurate data than the indirect methods commonly used for quantification of soil porosity.

health status	soil profile	depth	area % occupied by pores with ∅>30 μm (po30)			area % occupied by pores with ∅>500 μm (po500)		
			mean	std. dev.	N	mean	std. dev.	N
ink affected	P20 (mid-slope)	d1	14.6	3.7	36	6.1	1.2	4
		d2	14.3	3.6	48	3.4	1.9	4
	P22 (foot-slope)	d1	10.7	3.5	36	3.5	0.8	3
		d2	5.5	2.4	36	1.5	0.7	3
		d3	10.0	2.8	36	4.3	1.0	4
	P29 (mid-slope)	d2	9.1	4.2	24	4.1	1.6	2
		d3	14.5	2.1	12	5.4	-	1
	P30 (foot-slope)	d1	9.7	3.1	36	3.0	0.5	3
		d2	5.7	2.3	36	1.3	1.0	3
healthy	P2 (mid-slope)	d1	7.7	1.8	12	2.3	-	1
		d2	8.0	1.6	12	2.3	-	1
		d3	8.0	2.0	24	1.6	0.1	3
	P32 (mid-slope)	d1	10.5	1.9	24	4.6	0.4	2
		d2	7.0	2.2	12	3.6	-	1
		d3	7.8	1.7	36	3.6	1.4	3

Table 1. Descriptive statistics for the total porosity values

Factor	df	P-values (pore ∅>30 μm)	P-values (pore ∅>500 μm)
health status	1	0.11	0.08
slope position	1	0.13	0.05
depth zone	2	<0.0001	0.05
profile (health status * slope position)	3	<0.0001	0.04
depth zone * slope position	2	<0.0001	-
depth zone * health status	2	<0.0001	0.04

Table 2. Best fitting GLM model and P-values for factors

Health status	Slope position	Comparison	Significance[(1)]	
			(po30)	(po500)
ill	mid-slope	d1 vs d2	**	*
		d1 vs d3	NS	NS
		d2 vs d3	**	NS
ill	foot-slope	d1 vs d2	**	*
		d1 vs d3	NS	NS
		d2 vs d3	**	**
healthy	mid-slope	d1 vs d2	NS	NS
		d1 vs d3	NS	NS
		d2 vs d3	NS	NS

(1) ** significant at the 1% level; * significant at the 5% level; NS not significant

Table 3. Pairwise comparisons between total porosity at the different depth zones

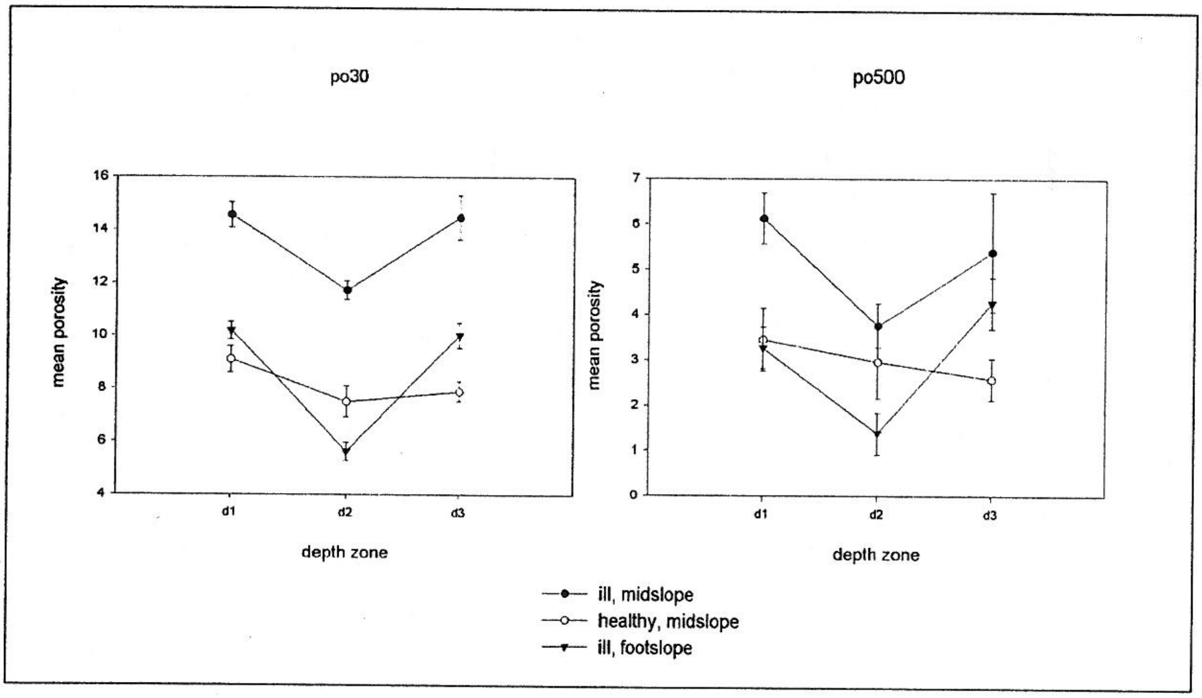

Figure 1. Estimated marginal means for po30 and po500 with error bars (± 1 standard error)

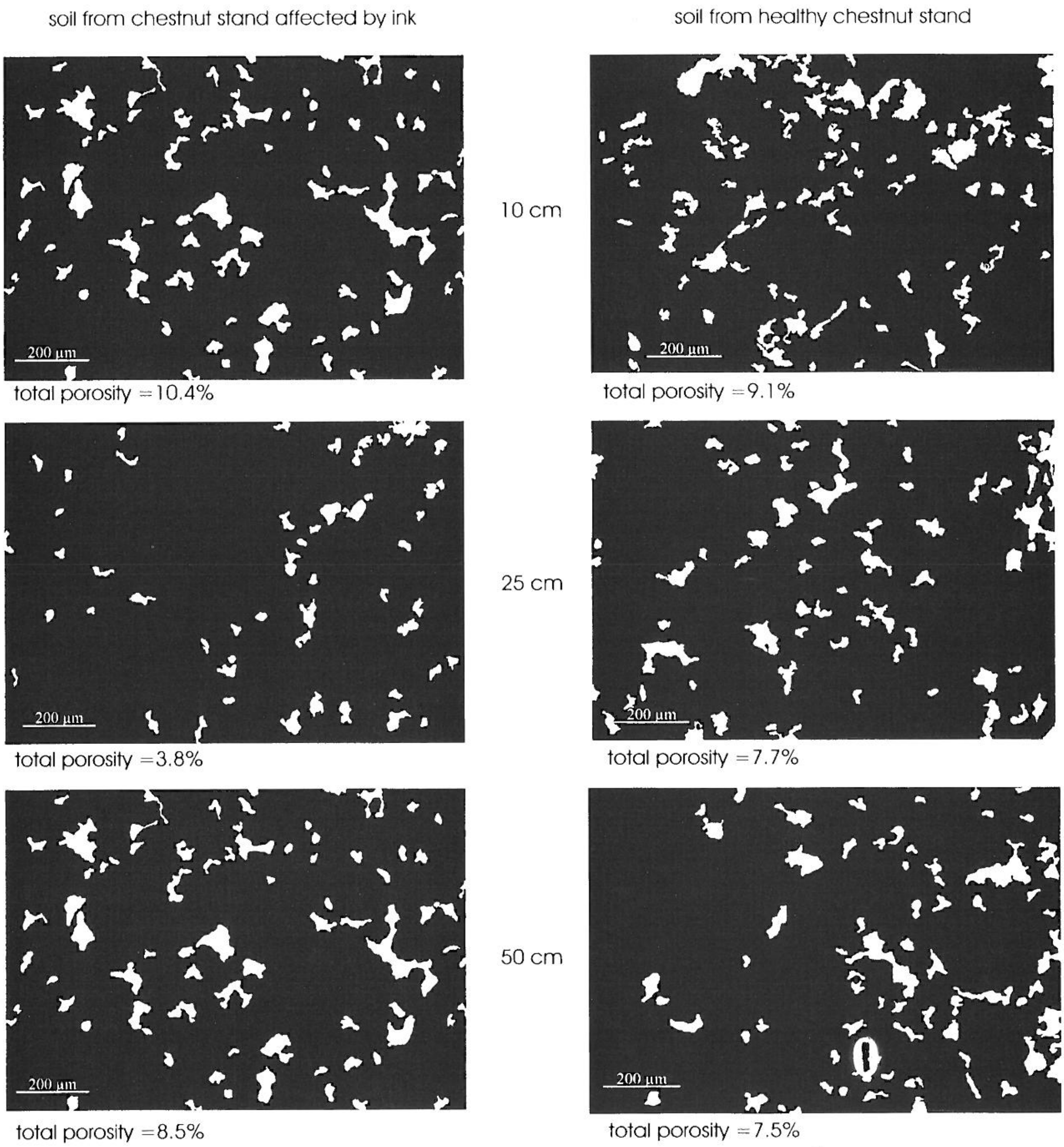

Figure 2. Images of the porosity variation with depth in soils of chestnut groves affected by ink disease and in soils of healthy groves

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