

# Effects of variable root damage caused by *Phytophthora cinnamomi* on water relations of chestnut saplings

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**Abstract** – The effects of *Phytophthora cinnamomi* root damage on water relations of chestnut (*Castanea sativa*) saplings were investigated. The relationship between root damage severity and impact on water relations was studied using a four compartment split-root system. Saplings were submitted to five inoculation levels and two watering conditions: normal (well-watered) or restricted watering. In well-watered saplings, stomatal conductance and whole plant transpiration rate were negatively correlated to the proportion of necrotic roots. Nevertheless, plant hydraulic conductance and leaf water potential were only affected above 90% necrotic roots. Interaction between root damage and water restriction was difficult to assess, since soil moisture decreased only slightly in infested compartments. However, saplings under restricted watering displayed lower stomatal conductance and transpiration values, regardless of root damage severity. Furthermore, the threshold of root damage leading to a decrease in leaf water potential was lower under restricted watering than under normal watering.

*Castanea sativa* / split-root / stomatal conductance / leaf water potential / water stress

**Résumé** – Effets de dégâts racinaires variables causés par *Phytophthora cinnamomi* sur le fonctionnement hydrique de jeunes châtaigniers. Les effets des dégâts racinaires causés par *Phytophthora cinnamomi* sur le fonctionnement hydrique de jeunes châtaigniers (*Castanea sativa*) ont été étudiés. Afin de relier les effets observés à la sévérité des dégâts racinaires, un système de « split-root » à quatre compartiments a été utilisé. Les arbres ont été soumis à cinq niveaux d'inoculation et à deux régimes hydriques (arrosage normal ou arrosage réduit). Chez les arbres normalement arrosés, une diminution linéaire de la conductance stomatique et de la transpiration avec la proportion de racines nécrosées a été observée. Cependant, la conductance hydraulique de l'arbre et le potentiel hydrique foliaire n'ont été affectés qu'à partir de 90 % de racines nécrosées. L'interaction entre les dégâts racinaires et la restriction en eau a été difficile à évaluer car l'humidité du sol a faiblement baissé dans les compartiments inoculés. Cependant, les arbres soumis à une restriction en eau avaient une conductance stomatique et une transpiration faibles quelle que soit la proportion de racines nécrosées. De plus, le seuil de dégâts racinaires entraînant une baisse de potentiel hydrique foliaire était plus faible chez les arbres soumis à une restriction en eau que chez les arbres normalement arrosés.

*Castanea sativa* / split-root / conductance stomatique / potentiel hydrique foliaire / stress hydrique

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## 1. INTRODUCTION

*Phytophthora cinnamomi* Rands is a root rotting pathogen with a wide host range. Its global distribution ranges from tropical and subtropical to temperate regions [30]. One of the most closely studied diseases induced by *P. cinnamomi* is the “jarrah-dieback” (*Eucalyptus marginata* Donn ex Sm.) in Western Australia where it has led to the decline of entire plant communities [21]. In Europe and North America, *P. cinnamomi* is the causal agent of the chestnut ink disease in *Castanea sativa* Mill. and *C. dentata* (Marsh.) Borkh., respectively [5, 14]. The primary symptoms caused by the pathogen are root necroses and a reduction in root growth often inducing a decline eventually leading to tree death [4, 10, 13]. Secondary symptoms observed in susceptible species, such as microphyllly, foliage yellowing, wilting, and ultimately shoot death [27, 30], resemble those of drought. Alteration of plant water relations during disease development, similar to that induced by drought, has been reported in several studies with *Phytophthora* species [12], in particular with *P. cinnamomi* [9]. In *P. cinnamomi* infested forest sites, a decrease in predawn leaf water potential and stomatal conductance has been observed in mature *E. marginata* exhibiting dieback symptoms [6]. A reduction of stomatal conductance was also observed in infected mature trees of *E. macrorrhyncha* F. Muell. before they displayed shoot symptoms [9]. Similar effects on leaf water potential and stomatal conductance were observed in infected avocado trees (*Persea americana* Mill.) together with a decrease in transpiration and soil-to-leaf specific hydraulic conductivity [22]. Under controlled conditions, similar changes in water relations were reproduced in *Isopogon ceratophyllus* R. Br. (a highly susceptible Australian shrub species), displaying symptoms on shoots three months after inoculation with *P. cinnamomi* [9], and in *E. sieberi* L. A. S. Johnson seedlings [10].

However, how *P. cinnamomi* causes tree death is still not clear. Is the damage induced on the plant only related to the reduction in water absorption or is the parasite inducing a generalized dysfunction in water relations? During field experiments, death was found to occur in jarrah when trees had lost an important part of the root system or when they had been girdled at the collar [6, 7, 8], supporting the first hypothesis. However, in inoculated *E. sieberi* seedlings, a 91% reduction in hydraulic conductivity of the root system was followed by a decrease in stomatal conductance, transpiration, leaf water potential and water stress in shoots, despite less than one

sixth of the root system being infected [10]. To explain such a disproportion between the low level of root infection and the induced effects, the authors suggested that a hormonal imbalance or a xylem vessel blockage by tyloses or by macromolecules associated with pathogen enzyme activity might be responsible for the decrease in root hydraulic conductivity.

The aim of our study was to investigate the effects of inoculation by *P. cinnamomi* on water relations of chestnut, for which no data are available. Saplings were submitted to two watering conditions, optimal watering or restricted water supply, in order to study the effects of root damage on tree vulnerability to drought. More precisely, the objective was to address the issue of the relationship between root damage severity and impact on seedling water relations. Because of the difficulty of controlling the level of root infection in potted plants, particularly due to the production of secondary inoculum under moist conditions, chestnuts were grown in a split-root system with four compartments that permitted partial inoculation and thus prevented complete destruction of the root system of inoculated plants. Furthermore, this split-root inoculation approach was used to induce partial infection of the root system which mimics the situation occurring in mature trees in situ [6].

## 2. MATERIALS AND METHODS

### 2.1. Plant material and growth conditions

Chestnut seeds (*C. sativa*) collected in mature stands in Ille-et-Vilaine, Bretagne, France, were surface disinfected by immersing in “Desogerme” (active ingredient: quaternary ammonium salt, 1% v/v, Laboratoires ACI International, Lyon, France) for one hour and placed on wet filter paper to germinate in the laboratory (mid-February 1998). Taproots were cut when 2 cm long to allow the formation of new roots. After one month of growth on perlite in the laboratory, seedlings were removed and excess perlite gently shaken. For each seedling, four roots of equivalent length and diameter were selected (all other roots were removed). Four pots (each with a capacity of 2.5 L) were clipped together to constitute one squared split-root pot with four watertight compartments. One root was placed into each of the four separate compartments and growth substrate added (1/1/1 v/v/v sand, perlite, peat). Seedlings were grown in a glasshouse and fertilized weekly with “Plantprod” (2 g L<sup>-1</sup>; N/P/K 20/20/20 and oligo

elements, Plant Products Co. Ltd, Brampton, Ontario, Canada).

During June 1998, 24 successfully rooted saplings were transferred to an open polytunnel. On July 3, 1998, sapling height and diameter were  $54.5 \pm 22.8$  cm and  $6.13 \pm 1.58$  mm, respectively (mean  $\pm$  S.D.). Saplings were fertilized two more times (July 1998 and April 1999) with  $2 \text{ g L}^{-1}$  and  $4 \text{ g L}^{-1}$  "Osmocote" (N/P/K 10/11/18, Scotts Europe B.V., 6422 PD Heerlen, Netherlands), respectively.

## 2.2. Inoculation

Saplings were inoculated with an aggressive isolate of *P. cinnamomi* (isolate 9 [17]) during mid-July 1998 and at the beginning of the second growth season during mid-May 1999. Five mL of *P. cinnamomi* infected millet seeds were inserted into 8 cm holes, adjacent to the main root in each compartment. Infected millet seeds were prepared as follows: after soaking in water for 24 h, seeds were autoclaved twice at a 24 h interval ( $120^\circ\text{C}$  for 20 min) in glass vials (350 ml). Sterile seeds were inoculated with mycelial discs (diameter, 8 mm, 10 discs per glass vial) taken from the margin of a young colony grown on V8 medium (V8 juice 20%,  $\text{CaCO}_3$  0.2%, agar 1.8%) and incubated in the laboratory at room temperature for three weeks [18]. Five inoculation levels were obtained as follows; 1, 2, 3 or 4 root compartments per sapling were soil infested. No millet seeds were added in control pots. Following inoculation, pots were saturated with water to enhance the production of secondary inoculum in infested compartments.

## 2.3. Watering treatments

Throughout the remainder of 1998, all compartments were maintained at field capacity by drip irrigation (two capillary tubes per compartment, each delivering  $1 \text{ L h}^{-1}$ ). The amount of water given to each sapling was estimated from the mean water loss (measured by weighing the plants once a week) of the non-inoculated saplings. During 1999, half the saplings in each inoculation treatment were kept well-watered ("0W", "1W", "2W", "3W", "4W" saplings, according to the number of infested compartments). The second half of saplings were submitted to a water restriction treatment ("0R", "1R", "2R", "3R", "4R" saplings). This treatment consisted in two periods with reduced watering. During the first period, from June 15 to July 2, "R" saplings were provided

with half of the water delivered to the well-watered saplings. They were re-watered at optimum on July 2 after water relations measurements. On July 13, they were submitted to a second water restriction period by withholding water for seven days. Saplings were then re-watered at the end of the experiment as above.

Soil volumetric water content (VWC,%) was measured one hour after watering in the four compartments of each pot with a ThetaProbe ML2 soil moisture sensor (Delta-T Devices Ltd, Cambridge, UK). Measurements were conducted once a week, before the application of water restriction, at the peak of stress and after the re-watering.

## 2.4. Experimental design

Treatments were arranged in a split-plot design, with two blocks. Watering treatments (2 levels) were assigned to the main plots and the inoculation treatments (5 levels) to the sub-plots. There was one replicate per block for inoculated treatments (1, 2, 3 or 4 infested compartments) and two for the non-inoculated treatment, giving a total of 16 inoculated saplings and 8 non-inoculated saplings.

## 2.5. Root damage assessment and *P. cinnamomi* isolation

All saplings were harvested at the end of the experiment (July 23, 1999). For each compartment, roots were carefully washed. Isolations from root necroses (taproot, lateral roots, fine roots) were made on a sample of 13 saplings (two 0W, one 0S, four inoculated well-watered and six inoculated water-stressed saplings) by plating 5 mm root segments onto PARBHy selective medium (malt 1.5%, agar 1.8%, pimaricin 10 ppm, ampicillin 250 ppm, rifampicin 10 ppm, benomyl 15 ppm, hymexazol 50 ppm) [18]. For each compartment, fine and lateral root damage was assessed visually. The taproot damage was assessed as follows; necrotic taproot length was estimated by adding the lengths of segments presenting necroses and rated to the total length of the taproot. The healthy and necrotic parts of the root system were separated, oven-dried at  $100^\circ\text{C}$  for 48 h and weighed. The necrotic roots ratio was calculated in each compartment as the ratio of the dry weight of necrotic roots to the total root dry weight. The necrotic root ratio for one sapling (NR,%) was calculated by averaging the necrotic root ratios of its four compartments.

## 2.6. Final leaf area estimation

Leaves were harvested at the end of the experiment, oven-dried at 100 °C for 48 h and weighed. Final leaf area (LA, m<sup>2</sup>) was estimated by a linear weight-surface relationship established on a sample of nine saplings (leaf area measured after digitising leaf with “DeltaT Scan” software, A T Delta-T Device, Cambridge, England) where  $LA = 98.976 \times DW$  ( $R^2 = 0.92$ ) with LA defined as final leaf area (cm<sup>2</sup>) and DW as dry weight (g).

## 2.7. Water relations

During 1998, predawn leaf water potential ( $\Psi_{Lpredawn}$ , MPa) was measured every second week with a pressure chamber (Druck Ltd, England) on one leaf per sapling. The stomatal conductance to water vapor ( $g_s$ , mmol m<sup>-2</sup> s<sup>-1</sup>) was measured twice a week (in the morning (07.00 to 09.00 UT) on the lower side of one leaf per sapling) with a LI-1600 steady-state porometer (Li-Cor Inc., Lincoln, NE, USA) equipped with a broad-leaf aperture cap of 2 cm<sup>2</sup>. During 1999, predawn and midday leaf water potential ( $\Psi_{Lmidday}$ , MPa) and  $g_s$  were measured twice a week during the same days. Sapling water loss was estimated once a week during 1998 and 1999 from the loss of weight of pots over three days. To avoid soil evaporation, pots were covered with polystyrene sheets. Transpiration was expressed on a whole sapling basis in mmol s<sup>-1</sup> ( $E_{sapling}$ ), except on July 19, 1999 (last date of measurement) when actual leaf area could be estimated and E was expressed on leaf area basis in mmol m<sup>-2</sup> s<sup>-1</sup>. Soil-to-leaf specific hydraulic conductance ( $L_p$ , mmol m<sup>-2</sup> s<sup>-1</sup> MPa<sup>-1</sup>) was estimated on July 20, 1999, as:  $L_p = E / (\Psi_{Lpredawn} - \Psi_{Lmidday})$ .

## 2.8. Statistical analysis

SAS General Linear Models Procedure was used [19]. The effect of water restriction was studied by a split-plot analysis. For each watering treatment, the effect of inoculation treatments on variables monitored throughout the experiment was studied by repeated measures analysis of variance.

In a second step, the inoculation effect was expressed with the necrotic root ratio (NR) and analysed as a quantitative factor. The relationship between NR and other variables was studied by correlation analysis and a simple linear regression model. For  $g_s$ , a multiple regression model was tested on all the data collected in 1999 for

$\Psi_{Lpredawn} \geq -0.5$  MPa. In this interval, there was a linear relationship between  $g_s$  and the regressors included in the model:  $\Psi_{Lpredawn}$  and NR. The date of measurement was introduced in the model as a classification variable to take climatic conditions into account.

Wilcoxon scores non-parametric test was performed using SAS NPAR1WAY Procedure [19].

## 3. RESULTS

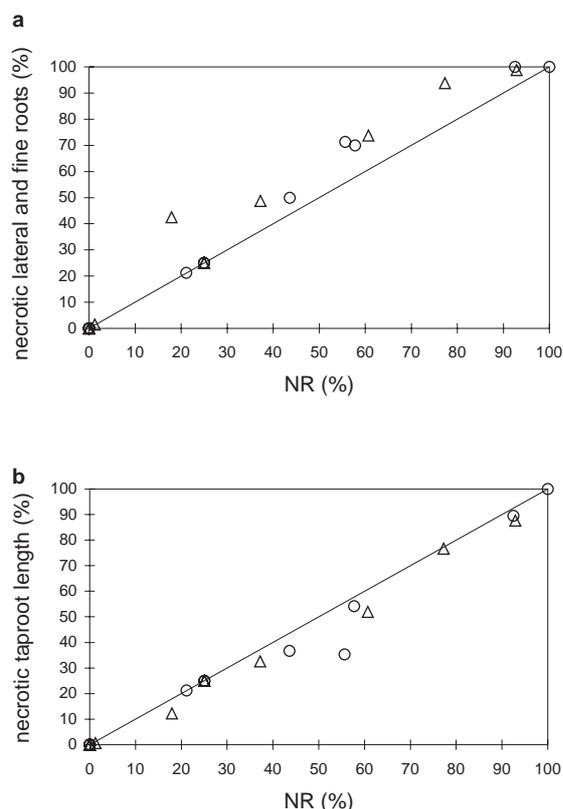
### 3.1. Shoot and root symptoms caused by *P. cinnamomi*

No symptoms were observed during the 1998 growth season. During January 1999, the proximal parts of the roots, which were exposed near the collar, were examined. Root necroses were detected in 14 out of 40 infested compartments. The necroses girdled all proximal roots in six compartments (in one sapling with one infested compartment and in three saplings with four infested compartments). During spring 1999, saplings with four infested compartments displayed a delay in bud break. These saplings continued to develop a smaller number of leaves with a reduced surface area. Only one of these saplings (4W), and three saplings with restricted water supply (one 0R and two 1R), died during summer 1999. Yellowing of leaves and wilting occurred just prior to death. No symptoms occurred on shoots of all other saplings during the experiment.

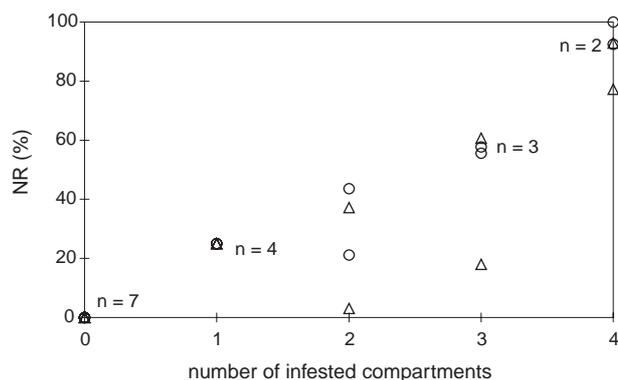
At the end of the experiment, root necroses were visible in all inoculated root compartments, and *P. cinnamomi* was reisolated from necroses of eight inoculated saplings out of the 10 sampled. Only one 0R sapling showed localised necroses in one compartment. This sapling was not taken into account in data analysis. *P. cinnamomi* was not reisolated from this sapling, neither from the other controls (0W and 0R).

Inoculation resulted in variable infection damage between compartments. In only four compartments out of 40 (two of them belonging to the same plant), very low root damage was observed, i.e. with a small proportion of necrotic lateral and fine roots (1–10%), and no, or low, taproot damage (c. 5% necrotic taproot length). In 17 compartments, the proportion of necrotic lateral and fine roots was high, between 75% and 100%, and the necrotic taproot length varied between 30% and 80%. The remaining 19 inoculated root compartments were totally destroyed by infection, i.e. with no living roots. In

particular, roots in infested compartments were completely destroyed in all saplings inoculated in one compartment and in the 4W sapling which had died. At the sapling level, the percentage of necrotic lateral and fine roots assessed visually, and the percentage of necrotic taproot length, were highly correlated ( $r^2 = 0.92$ ,  $n = 23$ ,  $P = 0.0001$ ). Both parameters were strongly related to the necrotic root ratio (NR) (figures 1a and 1b), which appeared as a synthetic descriptor of the root damage status of chestnut saplings. NR was therefore used in all further analyses since its estimation was more accurate than for the two others. NR varied between 1% and 100% (figure 2). The effect of inoculation treatment was significant on NR ( $F_{4,8} = 22.65$ ,  $P = 0.0002$ ), which increased, as expected, with the number of infested compartments. The effect of water restriction was also significant: saplings with restricted water supply had significantly lower NR than well-watered saplings ( $F_{1,1} = 289.00$ ,  $P = 0.0374$ ).



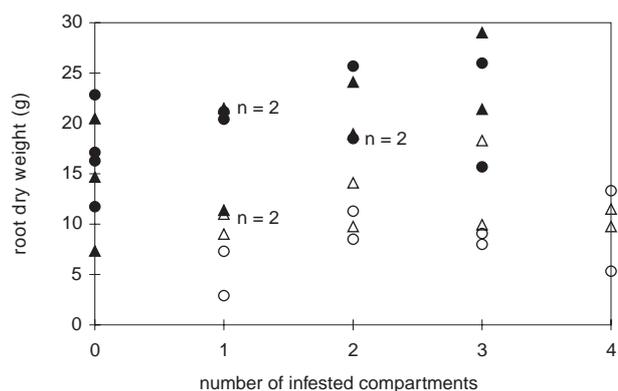
**Figure 1.** Percentage of necrotic lateral and fine roots in relation to the necrotic roots ratio (NR) (a), percentage of necrotic taproot length in relation to the necrotic roots ratio (NR) (b), in saplings of *Castanea sativa* inoculated with *Phytophthora cinnamomi*. Normal watering: circles; Water restriction: triangles. Each point represents an individual sapling.



**Figure 2.** Necrotic roots ratio (NR) in saplings of *Castanea sativa* grown in a four compartment split-root system and submitted to the following treatments: 0, 1, 2, 3 or 4 root compartments infested with *Phytophthora cinnamomi*. Normal watering: circles; Water restriction: triangles. Each point represents an individual sapling.

There was no interaction between inoculation and water restriction on NR.

In each inoculated plant (except in plants with four infested compartments), the mean root weight in infested compartment was lower than in non-infested ones (figure 3). According to Wilcoxon scores, the mean root weight in non-infested compartment of inoculated saplings was higher than the mean root weight in compartment of non-inoculated plants at a 6% significance threshold (figure 3). The reverse was observed for infested compartments ( $P = 0.0147$ ).

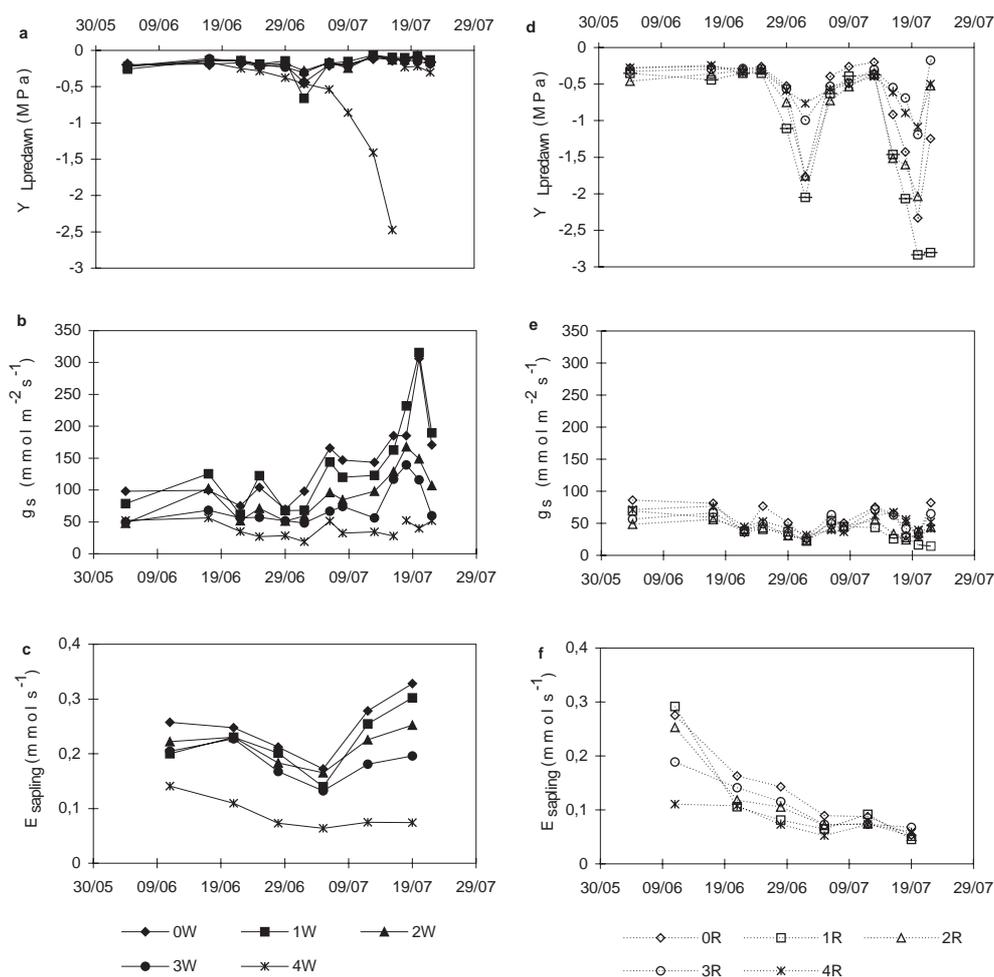


**Figure 3.** Mean root dry weight of non-infested compartments (black symbols) and of infested compartments (open symbols) in saplings of *Castanea sativa* grown in a four compartment split-root system and submitted to the following treatments: 0, 1, 2, 3 or 4 root compartments infested with *Phytophthora cinnamomi*. Normal watering: circles; Water restriction: triangles. Each point represents an individual sapling.

### 3.2. Time course of water relations of well-watered saplings

During the whole experiment, no significant inoculation effect was observed on predawn leaf water potential ( $\Psi_{Lpredawn}$ , figure 4a) and  $\Psi_{Lmidday}$  (not shown) of well-watered saplings, except in the 4W sapling that eventually died as mentioned earlier. During 1998, no effect of inoculation was shown on  $g_s$  and  $E_{sapling}$ . During June 1999,  $g_s$  and  $E_{sapling}$  values ranged from 48 to 126  $\text{mmol m}^{-2} \text{s}^{-1}$  and from 0.14 to 0.26  $\text{mmol s}^{-1}$ ,

respectively (figures 4b and 4c). At the beginning of July 1999,  $g_s$  and  $E_{sapling}$  increased in all the treatments except in the 4W treatment (figures 4b and 4c).  $g_s$  and  $E_{sapling}$  values decreased with the number of infested compartments. According to the repeated measures analyses of variance, the effect of inoculation was significant on  $g_s$  and  $E_{sapling}$  ( $F_{4,5} = 13.15$ ,  $P = 0.0073$  and  $F_{4,5} = 5.50$ ,  $P = 0.0448$ , respectively). The effect of inoculation was significant at the last three measurement dates for  $g_s$  (July 16, 18, 20) and was significant from June 21 onwards for  $E_{sapling}$ .



**Figure 4.** Time course of predawn leaf water potential ( $\Psi_{Lpredawn}$ ) (a, d), stomatal conductance ( $g_s$ ) (b, e) and whole plant transpiration ( $E_{sapling}$ ) (c, f) measured in 1999 on *Castanea sativa* saplings grown in a four compartment split-root system and submitted to the following treatments: 0, 1, 2, 3 or 4 compartments infested during 1998 with *Phytophthora cinnamomi*, combined with two watering conditions: normal watering (W) or water restriction (R). The first water restriction period was imposed between June 15 and July 2 and the second between July 13 and July 20. Stomatal conductance was measured at  $\text{RH} = 56.8 \pm 9.8\%$  and  $T = 23.1 \pm 2.2^\circ\text{C}$  (mean  $\pm$  S.D.). For each treatment,  $n = 2$  except for 0W ( $n = 4$ ) and 0R ( $n = 3$ ). At the last measurement dates,  $n = 1$  for 4W (discontinuous curve).

**Table I.** Soil volumetric water content (VWC, %) measured on July 20, 1999, in each root compartment of *Castanea sativa* saplings grown in a split-root system and submitted to the following treatments: 0, 1, 2, 3 or 4 compartments infested with *Phytophthora cinnamomi* combined with two watering treatments, normal watering or restricted water supply. Values are means  $\pm$  S.D., calculated over non-infested and infested compartments for each inoculation and watering treatment.

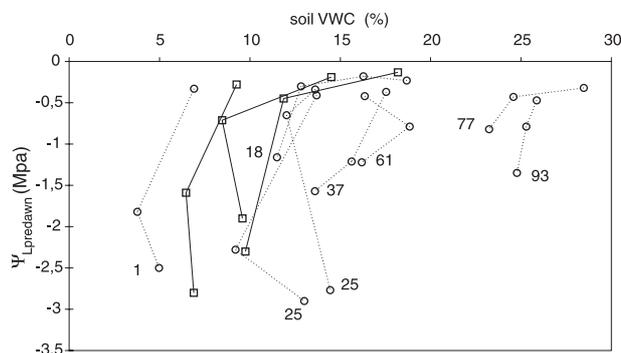
	Inoculation treatment: number of infested compartments				
	0	1	2	3	4
Soil VWC in non-infested compartments					
Well-watered saplings	26.4 $\pm$ 4.3 <i>n</i> = 16	24.1 $\pm$ 3.6 <i>n</i> = 6	22.1 $\pm$ 4.1 <i>n</i> = 4	20 $\pm$ 1.5 <i>n</i> = 2	–
Saplings with restricted water supply	8.7 $\pm$ 2.4 <i>n</i> = 12	5.4 $\pm$ 2 <i>n</i> = 6	5.4 $\pm$ 0.1 <i>n</i> = 4	5.3 $\pm$ 1.8 <i>n</i> = 2	–
Soil VWC in infested compartments					
Well-watered saplings	–	31.5 $\pm$ 1.8 <i>n</i> = 2	29.8 $\pm$ 1.6 <i>n</i> = 4	29.3 $\pm$ 2.7 <i>n</i> = 6	31.2 $\pm$ 5.6 <i>n</i> = 8
Saplings with restricted water supply	–	31.5 $\pm$ 0.9 <i>n</i> = 2	13.1 $\pm$ 10.7 <i>n</i> = 4	16.7 $\pm$ 9 <i>n</i> = 6	24 $\pm$ 10.3 <i>n</i> = 8

### 3.3. Time course of water relations of saplings with restricted water supply

As expected,  $\Psi_{Lpredawn}$  (figure 4d) and  $\Psi_{Lmidday}$  (not shown) decreased with decreasing water supply in all inoculation treatments. However,  $\Psi_{Lpredawn}$  of 3R and 4R saplings remained higher than that of 0R, 1R and 2R saplings. The effect of inoculation was significant on July 20 ( $F_{4,5} = 7.91, P = 0.0217$ ). All saplings recovered high water potentials after the second re-watering, except one 0R sapling and the two 1R saplings which eventually died as mentioned earlier.

It has to be noticed that the water restriction treatment resulted in a variable desiccation of the substrate in infested or non-infested compartments. On July 20, at the end of the second water restriction period, soil VWC had dropped to 5–9% in non-infested root compartments, regardless of inoculation treatment (table I). However, in infested compartments, restricted watering resulted in a noticeable decrease in soil VWC only for 2R and 3R plants. Even for these plants, mean soil VWC remained above 13%. As expected, no decrease in soil VWC occurred in compartments with 100% necrotic roots, such as in the two 1R saplings.

The decrease in  $\Psi_{Lpredawn}$  in relation to soil VWC during the second water restriction period is presented on figure 5. Saplings reached the same  $\Psi_{Lpredawn}$  value (i.e. –0.8 MPa) at mean soil VWC values per plant increasing with root damage: less than 13% for NR  $\leq$  25%, 17–19%

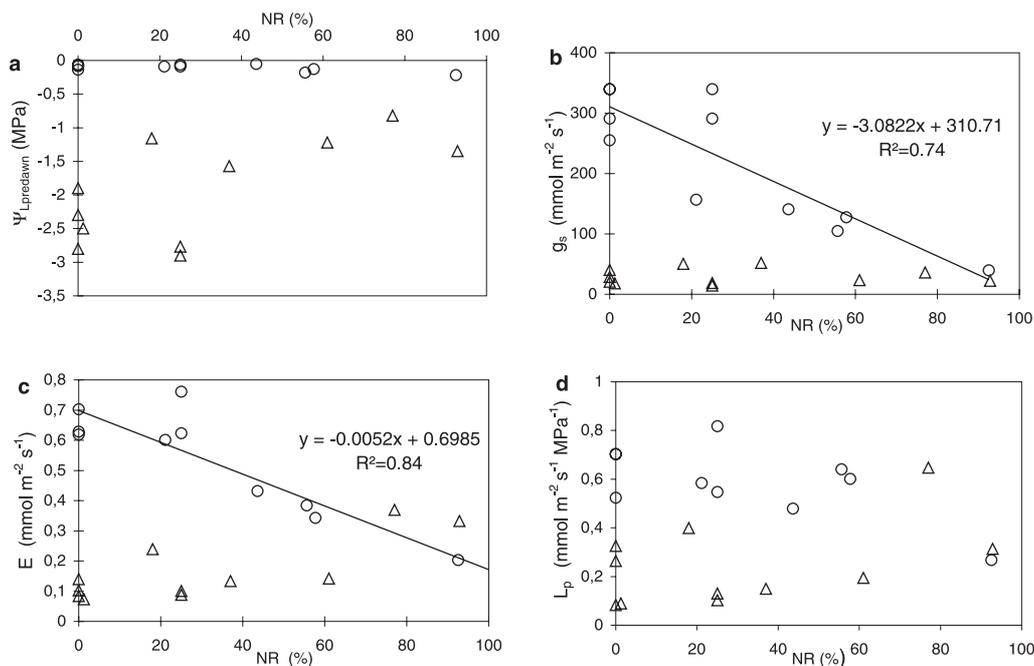


**Figure 5.** Predawn leaf water potential ( $\Psi_{Lpredawn}$ ) of *Castanea sativa* saplings with restricted water supply in relation to the mean soil volumetric water content (VWC) over the four compartments, during the second water restriction period. Each curve represents one individual with measures performed on July 13, 16 and 20, 1999. Non-infested saplings: normal line with squares; Infested saplings: dotted line with circles. The number indicates the necrotic roots ratio of the sapling.

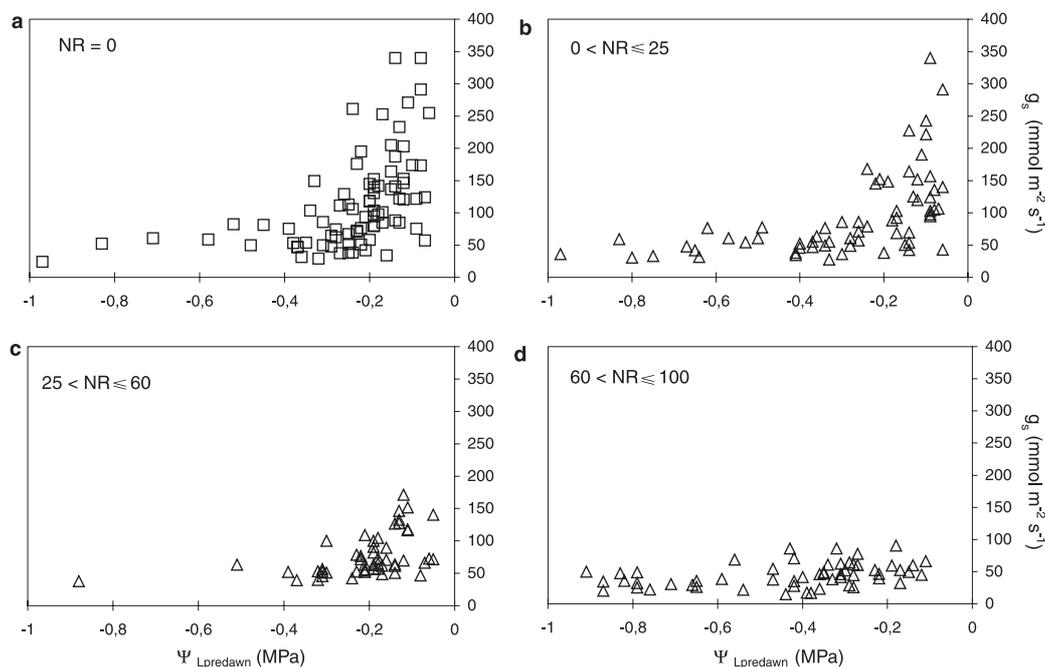
for NR = 37–61% and more than 23% for NR = 77–93%. However, soil VWC was very heterogeneous among the four compartments of a plant. For example, the plant with 37% NR had soil VWC values of 11.6% and 2.8% in the non-infested compartments and 30.9% and 24.8% in the infested compartments.

In all saplings under restricted water supply,  $g_s$  remained at a low level, between 14 and 86  $\text{mmol m}^{-2} \text{s}^{-1}$ ,





**Figure 7.** Predawn leaf water potential ( $\Psi_{Lpredawn}$ ) (a), stomatal conductance ( $g_s$ ) (b), transpiration ( $E$ ) (c), and soil-to-leaf hydraulic conductance ( $L_p$ ) (d), in relation to the necrotic roots ratio (NR) in saplings of *Castanea sativa* inoculated with *Phytophthora cinnamomi*. Normal watering: circles; Water restriction: triangles. Each point represents an individual sapling. Measurements were made on July 20, 1999, except for transpiration (made on July 19, 1999). Stomatal conductance was measured at  $RH = 60 \pm 2.3\%$  and  $T = 23.8 \pm 0.6$  °C (mean  $\pm$  S.D.).



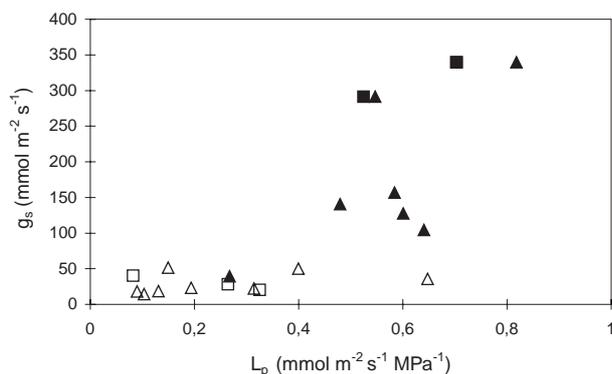
**Figure 8.** General plot of stomatal conductance ( $g_s$ ) as a function of predawn leaf water potential ( $\Psi_{Lpredawn}$ ) of *Castanea sativa* saplings inoculated with *Phytophthora cinnamomi* with different necrotic roots ratios (NR). (a)  $NR = 0\%$  ( $n = 76$ ); (b)  $0 < NR \leq 25\%$  ( $n = 64$ ); (c)  $25 < NR \leq 60\%$  ( $n = 45$ ); (d)  $60 < NR \leq 100\%$  ( $n = 39$ ). Measurements were conducted during 1999.

**Table II.** General linear model relating stomatal conductance to predawn leaf water potential ( $\Psi_{L_{\text{predawn}}}$  limited to the range  $> -0.5$  MPa), necrotic roots ratio (NR) and date of measurement (D), from all data collected in 1999, from *Castanea sativa* saplings inoculated with *Phytophthora cinnamomi*.

Source	DF	type III SS	F	Pr > F	Estimate (min, max)	T for H0 : parameter = 0	Pr >  T
model	26	607150.84	18.43	0.0001			
error	197	249564.76					
NR	1	114019.47	90.00	0.0001	-0.93	-3.13	0.0020
$\Psi_{L_{\text{predawn}}}$	1	48279.58	38.11	0.0001	161.03	6.17	0.0001
D	12	275247.79	18.11	0.0001	(-76.8, 57.0)	(-4.65, 2.89)	(0.0001, 0.0042)
NR $\times$ D	12	73841.60	4.86	0.0001	(-1.97, 0.86)	(4.16, 2.17)	(0.0001, 0.0314)
intercept					167.71	12.81	0.0001

The effect of root damage on the relationship between  $g_s$  and  $\Psi_{L_{\text{predawn}}}$  was therefore studied at each date of measurement. This model was significant at all dates except on June 4 and on July 6. The parameter relating  $g_s$  to NR was always negative, decreasing during the season from  $-0.35$  ( $P = 0.0382$ ) on June 4, to  $-3.21$  ( $P = 0.0063$ ) on July 20.

The relationship between estimated soil-to-leaf hydraulic conductance ( $L_p$ ) and stomatal conductance ( $g_s$ ) is shown on figure 9. Low  $L_p$  ( $0.08$ – $0.40$   $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ ) and  $g_s$  ( $14$ – $52$   $\text{mmol m}^{-2} \text{s}^{-1}$ ) values were observed in all saplings with restricted water supply, except in one 4R



**Figure 9.** Stomatal conductance ( $g_s$ ) in relation to soil-to-leaf hydraulic conductance ( $L_p$ ) on July 20, 1999, in saplings of *Castanea sativa* inoculated with *Phytophthora cinnamomi*. Non-infected saplings: squares; Infected saplings: triangles; Normal watering: black symbols; Water restriction: open symbols. Each point represents an individual sapling.

sapling, and in one well-watered sapling which had 92% NR. In the other well-watered saplings,  $L_p$  ranged from  $0.48$  to  $0.82$   $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ . For these higher values of  $L_p$ , saplings displayed different  $g_s$  values. One group with non-inoculated saplings and the two 1W saplings with 25% NR had high  $g_s$  values. The other group, including saplings with 21% to 58% NR had significantly lower  $g_s$  values than the non-infected saplings, according to Wilcoxon scores ( $P = 0.0497$ ).

#### 4. DISCUSSION

The effects of inoculation by *P. cinnamomi* on water relations of chestnut saplings were investigated. In agreement with earlier studies [16, 18], chestnut saplings were very susceptible to *P. cinnamomi* infection. The invasion pattern of the root system by *P. cinnamomi* was quite similar to that occurring in the susceptible tree species, *Banksia grandis* Willd. [21], as the parasite was able not only to infect fine roots but also to colonize lateral roots and finally the taproot. However, only one case of mortality was observed in a sapling that displayed 100% necrotic roots. Indeed, the partitioning between healthy and infected root compartments prevented the total destruction of the root system in most of the saplings. It was therefore possible to monitor the reactions of infected plants over two years whereas in pathogenicity tests with young seedlings, inoculation with *P. cinnamomi* has led to death within a few weeks [18].

The split-root system allowed obtaining a large range of root damage severities (1–100% necrotic roots) by modulating the number of infested compartments. The necrotic roots index was a pertinent descriptor of the visible damage caused by infection on all root system. However, inoculation probably also induced root loss, as seen by the difference in total root biomass between infested and non-infested compartments, which could not be taken into account. Conversely, a compensatory root growth in non-infested compartments of inoculated plants is strongly suggested by the higher root biomass observed in these compartments as compared to controls. This compensatory root growth, together with an efficient compartmentalization of infection at root collar, may explain why plants inoculated in one compartment (which was totally destroyed) responded very similarly to controls.

The proportion of necrotic root biomass assessed at the end of the experiment allowed us to study the relationship between root damage severity and impact on water relations of chestnut. The recorded physiological parameters varied in their response to root damage severity. In well-watered saplings, the most significant effect of inoculation was a decrease in stomatal conductance, as already reported in eucalyptus [6] and avocado trees [22] following *P. cinnamomi* infection. In our study, stomatal conductance, and hence transpiration, decreased linearly with the proportion of necrotic roots. Moreover, this decrease occurred in early stages of infection since it was observed during the first year of the experiment. Conversely, soil-to-leaf hydraulic conductance and leaf water potential were not affected up to a high fraction of necrotic roots (50–60%). Up to this threshold, inoculated saplings exhibited the same healthy root biomass and final leaf area than the non-inoculated saplings which probably allowed them to maintain almost normal water relations. Finally, hydraulic conductance and leaf water potential only dropped in the most infected saplings, which had more than 90% necrotic roots. Crombie and Tippett [6] also found that in natural forest site infections of mature eucalyptus by *P. cinnamomi*, the decrease in stomatal conductance was an earlier indicator of decline than leaf water potential. In *E. sieberi*, a much lower root infection threshold than in our study (*c.* 15%) was associated with a dramatic change in water relations: there was a decrease in stomatal conductance associated with a strong reduction in root hydraulic conductivity, transpiration rate and leaf water potential [10]. The age of plants, and thus, the development of the root system, may be an important factor in the impact of *P. cinnamomi* infection on plant water relations.

In our experiment, the interaction between root damage and water restriction was difficult to assess since soil moisture decreased only slightly in infested compartments. Nevertheless, water restriction reduced root damage induced by *P. cinnamomi* as reported in other studies [23, 24]. Furthermore, the effect of water absorption deficit induced by *P. cinnamomi* was clearly demonstrated in the water restriction experiment. In spite of a high volumetric soil water content in infested compartments, water absorption was too small to maintain an adequate water status when water was limiting in non-infested compartments. Therefore, the thresholds of root damage induced by *P. cinnamomi* leading to an alteration of water relations are likely to be reduced with increasing drought. The high leaf water potential of plants with a high proportion of their root system destroyed, measured at field capacity, is, therefore, a poor indicator of their ability to endure drought. The meaning of predawn leaf water potential was similarly questioned in the case of high soil heterogeneity [1].

In spite of the high susceptibility of chestnuts to *P. cinnamomi*, inoculated saplings showed several responses to infection both in the short term (decrease in stomatal conductance) and in the longer term (reduced leaf area) in order to reduce water stress. Most of these responses are also commonly observed during drought [11, 20, 26] or after root pruning [7]. Moreover, our results suggest that inoculated plants showed a specific decrease in stomatal conductance which was not related to a decrease in soil-to-leaf hydraulic conductance or predawn leaf water potential. This stomatal closure might be triggered by metabolites resulting from infection. Degradation products of the plant cell walls were shown to induce reactions leading to stomatal closure [15]. The involvement of hormonal imbalance [3, 9, 10], toxins produced by the pathogen [2, 25, 29], or phytoalexins produced by the host in response to pathogen attack [28] might also be hypothesized.

In conclusion, the alterations of water status in chestnuts inoculated with *P. cinnamomi* appeared to be mainly a result of root damage severity, interacting with soil water content. The plants reacted very efficiently to reduce infection induced water stress but decline was inevitable when nearly all root capacity was destroyed or when it was insufficient in regards to soil water content. Under natural conditions, the alternance of wet periods that are favourable to *P. cinnamomi* infection, and thus weaken the tree, and of dry periods which exacerbate water stress effects, could lead to tree decline.

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