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Evaluating potassium phosphonate injections for the control of *Quercus ilex* decline in SW Spain: implications of low soil contamination by *Phytophthora cinnamomi* and low soil water content on the effectiveness of treatments

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Abstract The Iberian forests are suffering severe disease and mortality as a result of decline, with *Quercus ilex* the major species at risk. Trunk injections with potassium phosphonate, which have been used successfully to control *Phytophthora cinnamomi*, were tested against decline. In an area in which *P. cinnamomi* was isolated, *Q. ilex* trees showing different degrees of decline were trunk-injected. Soil properties, and measurements of soil water content (θ) and depth to soil water table were assessed at three sites with markedly different decline incidences. Over the 5 years following the initiation of the experiment, mean symptoms among spring-treated trees and autumn-treated trees, or among trees injected twice a year (spring and autumn), once a year, and non-injected, were not significantly different. No effects of the treatments on shoot growth and acorn production were observed. However, θ values under trees which recovered from decline were higher than θ values under trees which did not recover from decline. At the site with the highest incidence of decline and tree mortality, *P. cinnamomi* was rarely isolated, and the presence of gravel, soil infiltration capacities and water table depth values were significantly higher than at the other sites, water stress being more likely to contribute to decline than *P. cinnamomi*. In areas in which θ is low, the distribution of phosphonate on the tree would be limited. Since the thresholds for phytotoxicity of potassium phosphonate in *Q. ilex* trees at the site studied would be higher than the amounts used, rates of the chemical slightly less than those that cause phytotoxicity should be tested.

Keywords Oak decline · Phosphonic acid · *Phytophthora cinnamomi* · Potassium phosphonate · *Quercus ilex* · Soil water content

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Introduction

Holm oak (*Quercus ilex*) is the main forest tree species in Spain, covering an area of more than 40,000 km^2. This tree species has a critical function as a retardant to soil erosion and desertification, which is considered a primary environmental concern in the Mediterranean basin. Rangelands of scattered *Q. ilex* trees, locally known as 'dehesas', support an astounding diversity of plant and animal life, partly because isolated trees produce acorn crops (400–600 kg ha^{-1} yr^{-1}) about ten times larger than their forest conspecifics (Pulido and Díaz 2005).

A severe decline of *Q. ilex* has been observed since the 1990s in the southern Iberian Peninsula (Brasier

1992; Moreira et al. 2000; Moreira and Martins 2005) and more recently in France (Robin et al. 1998), Italy (Jung et al. 1996; Vettraino et al. 2002) and Morocco (F. Assali and K. Falca, unpublished). The typical symptoms of this phenomenon are (i) a slow decline of the trees showing necrotic leaves, defoliation, dead branches and brown exudation of the trunk, or (ii) a rapid decline followed by the death of the trees in a few weeks (Gallego et al. 1999). Under Iberian conditions, several factors possibly acting in synergy have been proposed to explain the decline: water stress due to severe droughts, soil degradation due to overgrazing, root damage due to deep plowing and fire, bad pruning practices, insect pests (mainly *Cerambyx* spp.), and pathogens such as *Biscogniauxia mediterranea*, *Brenneria quercina* (Biosca et al. 2003), *Pythium spiculum* (Jiménez et al. 2008) and *Phytophthora cinnamomi* (Brasier 1996; Cubera et al. 2009; Gallego et al. 1999; Rodríguez-Molina et al. 2005; Romero et al. 2007; Sánchez et al. 2002). Among the causes involved, *P. cinnamomi* has been proposed as the major factor in *Q. ilex* decline and mortality, based on the facts that the oomycete has been isolated from the soils and roots of a large number of declined trees (Brasier 1992; Brasier et al. 1993; Sánchez et al. 2003, 2006; Tuset et al. 1996) and that pathogenicity tests on *Q. ilex* plants with *P. cinnamomi* satisfied Koch's postulates (Gallego et al. 1999; Robin et al. 1998; Romero et al. 2007). In Central and Eastern European countries, strong associations between the presence of several *Phytophthora* species and mortality and decline of other oak species were found (Balci and Halmschlager 2003; Jung et al. 1996, 2000).

At present, no direct control measures have been reported to assist in the recovery of *Q. ilex* trees from decline, with the exception of trunk injection of potassium phosphonate (Fernández-Escobar et al. 1999). Phosphonates are fully systemic fungicides, i.e., being xylem- and phloem-translocated, with both downward and upward movement in the host. The precise mode of action of phosphonates is unknown (Guest and Grant 1991; Guest et al. 1995), but it is believed that they cause fungistasis and a stimulation of the defense mechanisms of the fine roots, leading to an increase of the fine root system vigor, followed by the recovery of the crown condition. Trunk injections do not require special equipment or high labor costs, and for these reasons injections of potassium phosphonate are being extensively commercialized and used in south–central Spain against oak decline. However, recent observations on treated trees have reported a lack of effectiveness of the potassium phosphonate injections (Porras et al. 2007; Tuset and Sánchez 2004). In south–west Spain, in 10 out of 11 treated and declined *Q. ilex* stands, trees did not recover significantly in comparison with the controls (Porras et al. 2007). Scientific support of the efficiency of injections under natural conditions is lacking. The main purpose of the present work was to evaluate under field conditions, over a 5-year period, the effectiveness of potassium phosphonate treatments for the control of *Q. ilex* decline. The following questions were addressed: (i) at which stage of tree decline are treatments more effective?, (ii) during which phenological period (spring, autumn, or both spring and autumn) is it better to treat trees?, and (iii) how do the soil properties, soil water content, and soil water table depth affect the effectiveness of the treatments? Finally, we examined the effect of potassium phosphonate treatments on tree growth and acorn production.

Materials and methods

Study area

The experiment was carried out at Dehesa de Santa Amalia (39°44′N, 5°59′W), 5 km south–east of Torrejón el Rubio (Extremadura, W Spain). This *Q. ilex* open woodland is approximately 360 m a.s.l., and occupies ~800 ha. The climate is Mediterranean with a mean annual rainfall of 597 mm which falls mainly from October to May (climatic data from the nearby meteorological station, Cáceres, 39°28′N, 6°20′W; 405 m a.s.l.). Mean minimum and maximum temperatures occur during January (3.4°C) and July (35.5°C), respectively. Soils are mainly chromic Luvisols (FAO) developed over tertiary sediments with abundant quartzite gravels. The dominant tree species is *Q. ilex* (15–20 trees per ha), although *Q. suber* and *Pyrus bourgaeana* are also present (<1 tree per ha), and the main understorey plant species are *Cistus ladanifer* and *Lavandula stoechas*. Current land-uses are extensive grazing by cows and sheep, and hunting.

A severe mortality of *Q. ilex* trees has been observed in Dehesa de Santa Amalia since 1997, and due to positive isolations of *P. cinnamomi* by the

regional sanitary services (Junta de Extremadura) and the absence of other pests or pathogens involved in the decline, the owners were told to inject their trees with potassium phosphonate as the only solution available. Therefore, the study area appeared suitable to test the efficacy of potassium phosphonate to control *Q. ilex* decline. The efficiency of potassium phosphonate injections was studied at two levels: (i) within an extensive area, in which disease symptoms, shoot growth and acorn production of treated trees were evaluated, and (ii) within a reduced area (sites 1, 2 and 3, Fig. 1), where under treated trees, pathogen isolation and quantification, and soil physical properties were recorded; and soil water table depth, soil infiltration, and soil water content measurements were performed.

Plant material and treatments

Four hundred *Q. ilex* trees were preselected within an extensive area of 67 ha affected by decline. Trees were 120–140 years old, 8 m high, 35–55 cm in trunk diameter at breast height, and with crown diameters of 9–16 m. In April 2002, trees were first rated according to the following decline categories (Fernández-Escobar et al. 1999): 0 = healthy trees (crown transparency ≤5%); 1 = trees showing few decline symptoms, i.e., necrotic leaves, defoliation and dead branches (crown transparency 6–20%); 2 = trees showing moderate symptoms (21–40%); 3 = trees showing pronounced symptoms, indicating an advanced stage of decline (41–60%); and 4 = very advanced decline (>60%). After this first rating, 48 trees per decline category were labeled and selected for the experiment (N=240), rejecting those which clearly differed in trunk diameter or crown size from the average.

Each group of 48 trees was divided into four subgroups (n=12): (i) untreated control; (ii) trees treated in spring (April); (iii) trees treated in autumn (October); and (iv) trees treated in both spring and autumn (April and October). Treatment timing was designed according to the assumption that during autumn and spring maximum infections of *Q. ilex* roots by *P. cinnamomi* occur (Rodríguez-Molina et al. 2005). Treatments consisted of trunk injections of 28 $g l^{-1}$ of potassium phosphonate, i.e., phosphonic acid neutralized with potassium hydroxide to pH 5.5 (2.8% H_3PO_3 and 2.5 KOH, w/v). The injection treatments were carried out with Fertinyect® capsules (Fertinyect SL, Córdoba, Spain) following Fernández-Escobar et al. (1999). The method consisted of a pressurized capsule containing 225 ml of the solution to be injected, which was connected to a plastic injector that was inserted into a drilled hole, 4.5 cm deep and 6 mm in diameter. Injections were done at the base of the trunk, 20–25 cm above the soil surface, and the capsules were placed ~30 cm from each other. Each treated tree received three to five

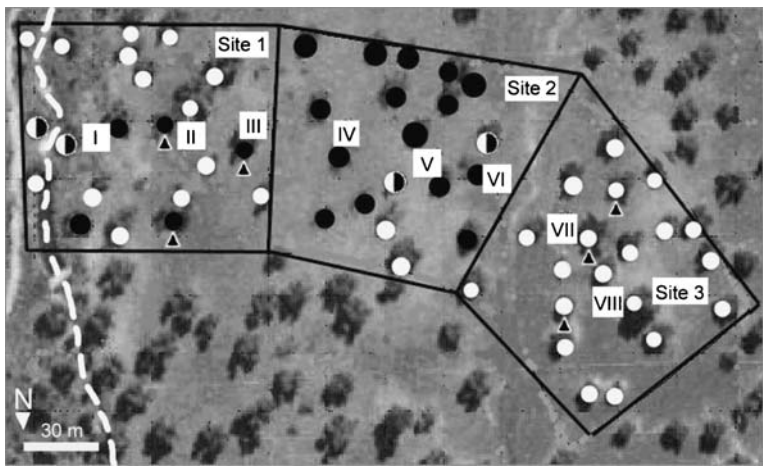

Fig. 1 Orthoimage of the Dehesa de Santa Amalia, Cáceres, Spain. Within the three sites studied intensively, white circles indicate healthy *Quercus ilex* trees or trees showing few decline symptoms (score 1); half-filled circles indicate trees showing moderate symptoms of decline (score 2); and black circles indicate trees showing pronounced symptoms of decline or dead trees (scores 3–5). White squares indicate location and numeration of piezometers used for water table measurements, black triangles indicate location of TDR-probes, and the discontinuous line indicates the course of a stream

simultaneous injections, depending on the size of the tree. The capsules were left on the tree until complete uptake of the solution, which usually occurred within the first 2 h. Treatments were carried out during 2002 and 2003. Trees belonging to groups (ii) and (iii) were injected once a year, in April and October, respectively, and trees belonging to group (iv) were injected twice a year, in April (spring) and October (autumn). Treatments were arranged in a randomized design within the five decline categories described.

Symptom evaluation, tree growth and acorn production

The results were evaluated by measuring the health condition of the 240 trees on the decline scale described previously. Within this scale, the category 5 = dead tree was also added. Tree condition was evaluated nine times (every 3 months) between April 2002 and April 2004, and a tenth assessment was done in April 2007. This last assessment was used to group the trees into (i) those that had improved in health following the treatments, and (ii) those that declined in health following the treatments. Vegetative growth was determined in November 2002 and 2003 by measuring shoot lengths of the current season of all trees of the experiment (N=240). Measurements were carried out on four terminal shoots per tree, located on the north, south, east and west sides of the tree, at approximately 2.5 m height. In a previous experiment, when measuring four or 12 terminal shoots of the same tree, no significant differences in average shoot length were found (P> 0.05). Acorn production was estimated in November 2002 and 2003 by counting the number of acorns on four branches approximately 1 m long and 2 m above ground on the north, south, east and west sides of the tree (N=240). Similarly, when counting the acorns on four or 12 branches of the same tree, no significant differences in acorn production were found (P>0.10).

Pathogen isolation and quantification

In November 2002, soil and root samples were taken from a reduced area comprising three sites with markedly different oak decline incidences (Fig. 1). These sites were selected because they were approximately in the middle of the extensively studied area, and because site 1 was crossed laterally by a perennial stream (Fig. 1). Flooding has been reported to be particularly favorable for infections of *P. cinnamomi* on *Q. ilex* roots (Sánchez et al. 2002, 2005; Tuset and Sánchez 2004). At the time of sample removal, sites 1, 2 and 3 had 2, 5 and 0 dead trees, respectively, and an average tree rating of 2.2, 4.8 and 0.1, respectively. Soil and root samples were taken under three trees per site, rated in November 2002 as 1 or 2. Approximately 300 ml of soil and ~20 necrotic roots per tree were sampled at distances of 2 m from the north side of the trunks, at a depth of 30 cm. The presence of *P. cinnamomi* was assessed in soil suspensions in deionized water, baited by floating 15–20 small pieces (2× 2 mm) of young *Eucalyptus camaldulensis* leaves under laboratory conditions (20°C and diffuse daylight). After 3 days of incubation, baits were removed, washed, surface-sterilized (60 s in 1% aqueous sodium hypochlorite) and blotted dry. Baits were then transferred to nistatin–ampicillin–rifampicin–pentachloronitrobenzene–hymexazol cornmeal agar (NARPH) selective medium. NARPH is a modified medium of PARPH (Jeffers and Martin 1986), in which pimaricin is exchanged for nistatin at the same concentration, and hymexazol is obtained from Tachigaren® LS (Comercial Química Massó SA, Barcelona). Isolation was also attempted from root samples. Roots were cut into 1 cm segments, surface-sterilized (2 min in 1% aqueous sodium hypochlorite), rinsed with sterile water, blotted dry and plated onto the selective medium. All plates were incubated at 24°C in the dark, and after 2–3 days, selected isolates were transferred to carrot agar (CA) medium. Identification of the colonies isolated from roots and soil was carried out by microscopic observations from cultures incubated on CA medium at 24°C in the dark for 4–6 days. Distinctive structures, such as clustered hyphal swellings for *P. cinnamomi* (Erwin and Ribeiro 1996) and oospore ornamentation for *Py. spiculum* (Paul et al. 2006) were observed after transfer to a glass microscope slide and staining with acid fuchsin in lactophenol.

In March 2005 and in November 2008, soil and root sampling from the same three trees per site was repeated. In addition, quantification of *P. cinnamomi* and *Py. spiculum* colonies per g of soil was assessed following Romero et al. (2007). Erlenmeyer flasks containing 200 ml of distilled water and 0.4 g of agar (Scharlau Chemie SA, Barcelona, Spain) were shaken and sterilized, and 10 g of a sieved (2 mm pore diameter) soil sample were introduced. Soil samples

were obtained from sites 1, 2 and 3, and only one flask per site was used. After shaking, 1 ml aliquots were taken from the soil–water agar mix, and plated on petri dishes containing 20 ml of NARPH medium. This dilution was shown to produce a countable number of *P. cinnamomi* and *Py. spiculum* colonies from soil samples of declining oaks, varying between 4 and 49, and 3 and 110 propagules per g soil, respectively (Romero et al. 2007). Forty plates per flask was used; thus 2 g of soil per site was screened. After 1 day of incubation at 24°C in the dark, the agar surface of each plate was washed with sterile water, removing the soil–water agar mix. The plates were sealed with parafilm, and after 2 days of incubation at 24°C in the dark, the number of *P. cinnamomi* and *Py. spiculum* colonies were identified under a dissecting microscope and counted (Romero et al. 2007). The number of colonies was referred to grams of soil.

Soil water table depth measurements

Variation of the soil water table depths of sites 1–3 was assessed through piezometers installed perpendicularly to the stream (Fig. 1). Eight pierced PVC tubes (5 cm diam, 120 cm length) were used as piezometers, and tubes I and V had the lowest and highest elevation, respectively (Fig. 2a). Piezometers were inserted to a depth of 1.2 m after soil drilling. Soil was drilled with a stainless steel soil column cylinder with a cutting shoe and a removable cover (10 cm diam, 1 m length; Eijkelkamp; http://www.eijkelkamp.com/Portals/2/Eijkelkamp/Files/P1-21e.pdf), plus a 1-m extension rod, which were inserted into the soil with a heavy electric-powered percussion hammer (Makita HM1800). To avoid obstruction, the upper part of each tube was covered. The water table depth was obtained by inserting a 1.2 m bamboo cane inside each tube and, after removal, by measuring the dry length portion. Measurements were taken monthly, from January 2003 to April 2004.

Soil sampling for physical properties

The extracted soil cores taken from where the piezometers were installed, were used to determine some physical properties of soil. Soil cores from tubes I–II (site 1), IV–V (site 2), and VII–VIII (site 3) were used, with approximately 10*l* of soil sampled per piezometer. To assess soil bulk density, soil cores

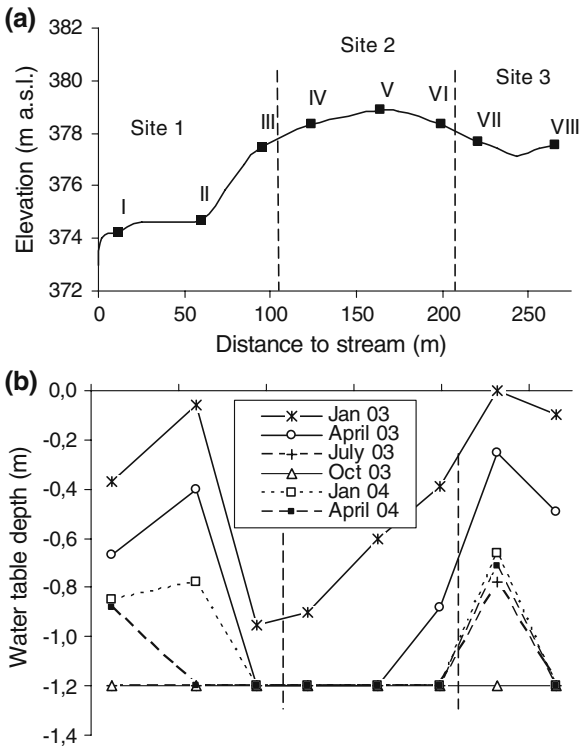

Fig. 2 Elevation of piezometric tubes, and water table depth measurements at sites 1–3 (see Fig. 1). **a** Roman numerals refer to tubes installed at sites 1, 2 and 3, with *Quercus ilex* trees showing intermediate, very advanced and little decline, respectively. **b** Water table depth values obtained from January 2003 to April 2004. Dotted vertical bars indicate separation among sites

were cut and weighed every 10 cm, and 0.2*l* aliquots were taken for dry soil-weight determination. For every 10 cm depth, the percentage of soil gravel was obtained by dividing the coarse earth fraction (particles ≥2 mm diam) by the fine earth fraction (<2 mm diam), and soil texture (sand, silt and clay contents) was determined through the pipette method (Gee and Bauder 1986). This method consists of completely dispersing a soil sample in water, and passing it through a set of sieves to separate out the sand fractions. A sedimentation procedure is then used to ascertain the quantities of silt and clay that had passed through the sieves. Because soil particles are more dense than water, they tend to sink, settling at a velocity that is proportional to their size. In the fine earth fraction, 72 determinations were conducted for each parameter.

Soil infiltration measurements

To measure the soil infiltration capacities at sites 1–3, the double-ring infiltrometer test was used (Bower 1986). Briefly, this test consists of driving two concentric open cylinders into the ground (~30 cm), partially filling the rings with water, and then maintaining the water at a constant level while the water in the rings infiltrates into the soil. The support area for each infiltration measurement was the area of the inner ring in the double-ring apparatus, which had a diameter of 30 cm. The volume of water added to the inner ring, to maintain the water level constant, is the volume of water that infiltrates the soil. In April 2003, three tests per site and some 14 measurements per test were conducted. Each test lasted approximately 2–3 h. Before testing, the average water contents (gravimetric measurement, 0–10 cm depth) of soils at sites 1, 2 and 3 were 12.2%, 13.6% and 9.2%, respectively. The most widely used empirical model to describe infiltration is the Horton model given by $f_p = f_c + (f_0 - f_c)\, e^{-kt}$ (Chin 2008), where f_p is the infiltration rate under ponded conditions, also called the infiltration capacity; f_0 is the initial (maximum) infiltration capacity; f_c is the asymptotic (minimum) infiltration capacity ($t \to \infty$); and k is the decay constant.

Soil water content measurements

Soil water content (θ) was measured on sites 1 and 3 under *Q. ilex* trees showing different decline conditions. Three declining trees from site 1 and three healthy trees from site 3 (mean scores of 3.7 and 0.3, respectively) were used (Fig. 1). For each tree, θ was measured at 2 m from the north side of the tree trunk and at 40 and 80 cm depths by Time Domain Reflectometry (TDR) (Tektronic model 1502 C). TDR-probes were constructed manually according to Cubera and Moreno (2007). Each probe was comprised of two 20 cm long stainless steel parallel rods, sharpened at the tip to facilitate their introduction into the soil. Rod diameter was 0.6 cm and the separation between their axes was 3 cm. One rod was connected to a conductor of a low ohm-resistance coaxial cable and the other was connected to the mesh of the cable. All connections were coated with an epoxy resin (Stuers kit EPOFIX®) which acted as an electrical insulator, and held the rods firmly in a parallel position. Soil was drilled as described previously, and for each tree a first probe was placed vertically and buried at 80 cm depth in the undisturbed soil, and then a second probe was placed vertically and buried at 40 cm depth. Efforts were made to ensure maximum contact between the rods and the soil. Coaxial cables 100 and 60 cm long were connected to the first and second probes, respectively. The upper 20 cm lengths of the cables were left unburied, and their endings striped to allow connection with the TDR. A calibration curve of the TDR-probes was done in the laboratory with soil samples collected from the studied area. Monthly measurements of θ started in January 2003 and finished in January 2004.

Additional TDR-probes were installed under potassium phosphonate-treated trees, in order to check the possible influence of θ on the recovery of treated trees. Six *Q. ilex* trees, rated in April 2002 as 3 (first rating), were used. Trees belonged to subgroup (iv) that had been treated in autumn and in spring. In April 2004, trees were grouped into 'better than before treatments' and 'equal or worse than before treatments' (recovery tendency). TDR-probes were installed at 40- and 80-cm depths as described previously, and θ was measured monthly from January 2003 to January 2004.

Data analysis

To evaluate the effect of potassium phosphonate injections on *Q. ilex* growth and fruit production, multifactorial ANOVAs were performed considering 'shoot growth' and 'acorn production' as dependent variables, 'injection treatments', 'first score' and 'branch orientation' as factors, and 'tree diameter' as covariate. The Horton infiltration model ($f_p = f_c + (f_0 - f_c)\, e^{-kt}$) was fitted to each individual double-ring test by finding the Horton parameters (f_0; f_c; k) that provide the best fit (least squares) between the measured infiltration capacity (f_p, cm h^{-1}) *versus* time (t, h) and the model. To analyze soil properties among sites, several unifactorial ANOVAs were performed considering 'soil bulk density', 'gravel content', 'sand content', 'silt content', 'clay content', 'f_0', 'f_c' and 'k' as dependent variables, and 'site' as a factor. LSD tests were used to compare average values ($P<0.05$).

Scores of tree condition and θ values were transformed using the arcsine of the square root to obtain normality. To evaluate the effect of potassium

phosphonate injections on tree condition, repeated-measure ANCOVAs were performed using 'transformed scores of tree condition' at different dates as dependent variable (repeated-measure), 'first score' and 'injection treatments' as independent variables, and 'tree diameter' as covariate. To evaluate the effect of θ on tree condition, repeated-measure ANCOVAs were performed using 'θ' at different dates as dependent variable, 'first score' as independent variable, and 'tree diameter' as covariate. Finally, to evaluate the effect of θ on the recovery of treated trees, repeated-measure ANCOVAs were performed using 'θ' as dependent variable, 'recovery tendency' as independent variable, 'tree diameter' as covariate, and 'date' as repeated-measure. For all analyses, the STATISTICA (StatSoft Inc., Tulsa, OK, USA) v.5 program was used.

Results

Isolation and quantification of *Phytophthora cinnamomi*

In November 2002, *P. cinnamomi* was successfully isolated under seven *Q. ilex* trees, from three, four and one soil samples of sites 1–3, respectively. From root samples, only one tree from site 1 showed two positive isolations of *P. cinnamomi*. Colonies of *Pythium* spp. were isolated from rotten roots of trees located at sites 1 and 3. In March 2005, *P. cinnamomi* was isolated from soil under only two trees at site 1, where a density of seven colonies per g of soil was estimated. In November 2008, *P. cinnamomi* was isolated from soil under five trees (sites 1–3), and from rotten roots of two trees (sites 1 and 2). At this time, nine colonies of *P. cinnamomi* per g of soil and 14 colonies of *Py. spiculum* per g of soil were obtained.

Development of visual symptoms

Over the 5 years following the first treatments, no toxicity due to potassium phosphonate was observed. The treatments had no beneficial effect on trees initially scored as 0, 1, or 3 (Fig. 3a, b, d). Over the same period, trees initially scored as 2 and injected in both spring and autumn showed higher decline symptoms than untreated control trees ($P<0.05$; Fig. 3c). Trees initially scored as 4 and injected in

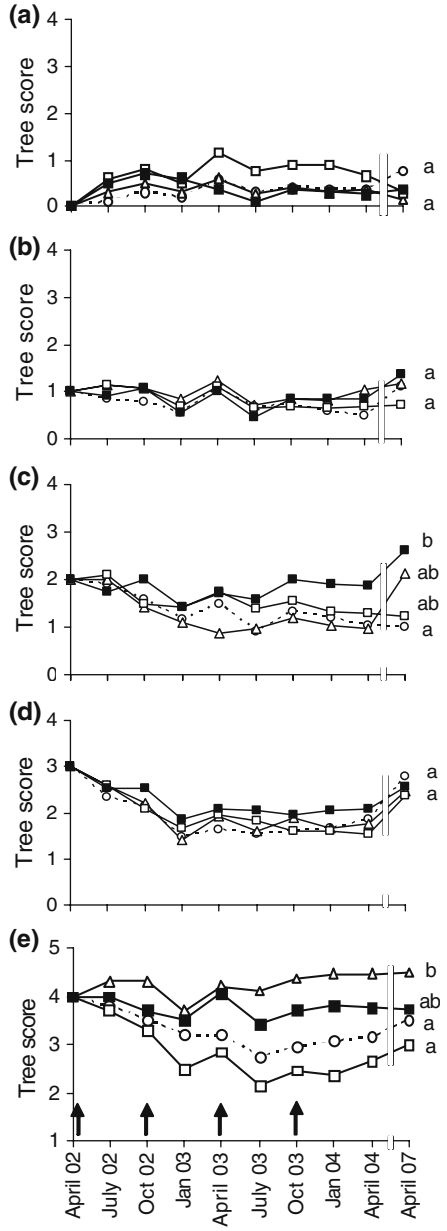

Fig. 3 Symptom development of *Quercus ilex* trees treated with potassium phosphonate injections. Trees were grouped initially according to **a** 0 = healthy trees, **b** 1 = trees showing few decline symptoms, **c** 2 = trees showing moderate symptoms, **d** 3 = trees showing pronounced symptoms, indicating an advanced stage of decline, and **e** 4 = very advanced decline. Trees were injected in spring (□), autumn (△), spring and autumn (■), or left untreated (○) ($N=12$). Arrows indicate treatment dates, and different letters along right-hand y axis indicate significant differences among treatments at $P<0.05$

autumn showed higher decline symptoms than control trees ($P<0.05$; Fig. 3e). If the 240 trees are analyzed together, irrespective of their first score, symptoms in April 2007 among spring-treated trees and autumn-treated trees, or among trees injected twice a year, once a year, and non-injected, were not significantly different ($P>0.10$; data not shown).

By the end of the experiment, ~40% of the untreated control trees and ~50% of the trees injected with potassium phosphonate in spring had improved in health (Table 1). Injections in autumn and injections in spring and autumn resulted in no tree improvement compared with the control. A number of trees declined in health by the end of the experiment, especially those injected in both spring and autumn. Five years after the first treatments, 10% of the control trees had died (Table 1).

Effect of treatments on tree growth and acorn production

In the first year of the experiment, trees injected in both spring and autumn showed a significantly greater shoot growth than control trees ($P<0.05$; Table 2). In the second year, no effect of the treatments on vegetative growth was observed (Table 2). In both 2002 and 2003, average growth of branches oriented to the south was greater than the average growth of branches oriented to the north, east and west (5.4, 4.3, 4.8, 4.7 and 5.0, 3.7, 3.9, 4.2 cm yr^{-1}, respectively; $P<0.05$). No differences in acorn production were observed when control and injected trees were compared (Table 3). During both 2002 and 2003, trees scored as 4 showed less acorn production and shoot growth than healthy and low-symptomatic trees (Tables 2 and 3). In both years, average acorn production of branches oriented to the south was higher than that of branches oriented to the north, east and west (6.7, 4.1, 5.9, 4.1 and 9.0, 4.2, 5.7, 5.7 acorns per branch, respectively; $P<0.05$).

Soil measurements

Mean soil bulk density at site 3 was significantly ($P<0.001$) higher than mean soil bulk densities at sites 1 and 2 (Table 4). Site 2, at which the highest decline incidence and tree mortality was observed, had a higher ($P<0.05$) percentage of gravel than sites 1 and 3. The particle size of soil also varied significantly ($P<0.05$) among sites, although the percentages of clay were similar at all sites (Table 4). Soils at site 2 showed higher maximum and minimum infiltration capacities than soils at sites 1 and 3 ($P<0.05$, Table 4).

In January 2003, the water table was near the soil surface in tubes II and VII (sites 1 and 3, respectively; Fig. 2), and only in these tubes was the water table observed within the top 0.2 m depth during 1 and 2 months, respectively. Water table depths observed during February and March (data not shown) were intermediate to water table depths observed in January and April 2003 (Fig. 2b). From April 2003 to April 2004, the water table level of site 2 was generally below the maximum depth of the tubes, although this situation occurred only from August 2003 to November 2003 at sites 1 and 3.

Repeated-measure ANCOVAs showed significant differences of θ under trees with different decline ratings (Fig. 4a, c, e), at 40- and 80-cm depths ($P=0.01$ and $P=0.04$, respectively). In the same way, analysis performed with θ values measured under injected trees with different rates of recovery from decline (Fig. 4b,d,f) showed that θ values under trees

Table 1 Effect of potassium phosphonate injection treatments (60 trees per treatment) on *Quercus ilex* trees, by comparing tree health ratings of April 2002 and April 2007

Injection treatments	Trees better than before treatments		Trees worse than before treatments		Dead trees
	(n)	(%)	(n)	(%)	(n)
Control	26	42.5	18	30.0	6
Spring	31	51.3	11	17.9	3
Autumn	19	31.6	17	28.9	8
Spring and autumn	17	28.2	23	38.5	11

Table 2 Shoot growth (cm) of *Quercus ilex* trees treated with potassium phosphonate injections

Injection treatments	Yr	Tree score[a] before treatments					Average[b]
		0	1	2	3	4	(*N*=60)
Control (*n*=12)	2002	5.1	5.0	4.4	5.0	3.3	4.7 a
Spring (*n*=12)	2002	5.0	4.2	5.6	5.2	4.5	4.9 a
Autumn (*n*=12)	2002	5.0	6.4	3.8	4.1	3.3	4.7 a
Spring and autumn (*n*=12)	2002	6.4	5.7	6.1	5.7	3.4	5.6 b
Average[b] (*N*=48)	2002	5.4 y	5.3 y	5.0 y	5.0 y	3.7 x	
Control (*n*=12)	2003	4.2	4.6	4.2	4.5	3.3	4.3 a
Spring (*n*=12)	2003	4.2	4.1	3.4	4.8	3.6	4.0 a
Autumn (*n*=12)	2003	4.9	5.6	4.8	4.4	3.0	4.7 a
Spring and autumn (*n*=12)	2003	4.8	4.5	3.8	4.8	3.3	4.3 a
Average[b] (*N*=48)	2003	4.5 y	4.7 y	4.0 xy	4.6 y	3.3 x	

[a] 0 = healthy trees (crown transparency <5%); 1 = trees showing few decline symptoms, i.e., necrotic leaves, defoliation and dead branches (crown transparency 6–20%); 2 = trees showing moderate symptoms (21–40%); 3 = trees showing pronounced symptoms, indicating an advanced stage of decline (41–60%); and 4 = very advanced decline (>60%)

[b] Averages followed by a common letter do not differ statistically at $P=0.05$

which recovered from decline were higher than the θ values under trees which did not recover from decline, at both the 40- and 80-cm depths ($P<0.001$).

Discussion

Trunk injections of potassium phosphonate were not found to alleviate symptoms of decline in *Q. ilex* trees, confirming previous observations (Porras et al. 2007; Tuset and Sánchez 2004). Trees initially showing moderate decline symptoms continued to decline after injections in both spring and autumn. Although injections in spring resulted in a higher percentage of trees improving in health and in fewer dead trees in comparison with the control trees, the benefits were not significant for any category of tree decline. Trunk injection with phosphonates is partic-

Table 3 Acorn production (fruits per branch) of *Quercus ilex* trees treated with potassium phosphonate injections

Injection treatments	Yr	Tree score[a] before treatments					Average[b]
		0	1	2	3	4	(*N*=60)
Control (*n*=12)	2002	8.3	4.6	6.1	6.3	5.2	6.3 a
Spring (*n*=12)	2002	5.0	6.9	3.6	4.5	5.0	5.0 a
Autumn (*n*=12)	2002	9.5	4.4	5.6	4.4	0.4	6.0 a
Spring and autumn (*n*=12)	2002	4.0	6.8	5.3	2.2	0.0	4.6 a
Average[b] (*N*=48)	2002	6.7 y	5.7 y	5.2 y	4.3 xy	2.6 x	
Control (*n*=12)	2003	8.3	8.5	5.8	4.1	3.6	6.1 a
Spring (*n*=12)	2003	6.9	8.6	6.2	5.3	2.1	5.8 a
Autumn (*n*=12)	2003	9.7	7.5	5.8	8.1	5.6	7.3 a
Spring and autumn (*n*=12)	2003	9.3	7.3	7.7	4.6	0.6	5.9 a
Average[b] (*N*=48)	2003	8.6 y	8.0 y	6.4 xy	5.5 xy	3.0 x	

[a] 0 = healthy trees (crown transparency ≤5%); 1 = trees showing few decline symptoms, i.e., necrotic leaves, defoliation and dead branches (crown transparency 6–20%); 2 = trees showing moderate symptoms (21–40%); 3 = trees showing pronounced symptoms, indicating an advanced stage of decline (41–60%); and 4 = very advanced decline (>60%)

[b] Averages followed by a common letter do not differ statistically at $P=0.05$

Table 4 Soil characteristics of three sites in which *Quercus ilex* trees show different incidence values of decay. Within rows (each parameter), a common letter indicates no significant difference at $P=0.05$ between sites

	Site 1 (incidence[a] = 32%)	Site 2 (incidence[a] = 96%)	Site 3 (incidence[a] = 11%)
Soil bulk density (mg m^{-3})	1.35 a	1.47 a	1.70 b
Gravel (%)	44.8 a	64.3 b	42.7 a
Sand (%)	67.3 b	58.4 ab	48.6 a
Silt (%)	21.9 a	29.2 a	44.2 b
Clay (%)	10.8 a	12.4 a	7.2 a
f_0 (cm h^{-1})[b]	39 a	145 b	32 a
f_c (cm h^{-1})[b]	18 a	79 b	13 a
k (min^{-1})[b]	0.44 a	1.04 a	0.16 a

[a] Percentage of *Q. ilex* trees with symptoms of decay

[b] Horton model parameters indicate maximum infiltration capacity (f_0), minimum infiltration capacity (f_c), and infiltration decay factor (k) (Chin 2008)

ularly effective for the control of *Phytophthora* diseases of tree crops (Diczbalis et al. 2004; Guest et al. 1995; Opoku et al. 2007; Whiley et al. 1995). However, the control of *P. cinnamomi* and the control of oak decline should clearly be differentiated. Nowadays, *Q. ilex* stands recover from decline after long periods of rain, and when management practices that cause root damage, soil degradation, and lack of natural regeneration are minimized (Tuset and Sánchez 2004; A. Solla, unpublished). Since *P. cinnamomi* has been reported as the main factor of oak decline in Portugal (Moreira et al. 2000) and Spain (Brasier 1996; Brasier et al. 1993; Gallego et al. 1999; Sánchez et al. 2006), and potassium phosphonate injections have been found to alleviate decline in *Q. ilex* trees in which *P. cinnamomi* was isolated (Fernández-Escobar et al. 1999), numerous land owners applied this treatment to their oaks. Desperation due to widespread mortality and lack of other control measures often leads to potassium phosphonate treatment initiation even if no *Phytophthora* isolations have been made. Our results will help Iberian foresters to reconsider the extensive use of potassium phosphonate treatments, even if *P. cinnamomi* is present in soil or in *Q. ilex* roots, and will encourage scientists to find alternative solutions. Nevertheless, further studies should examine if higher rates of potassium phosphonate are appropriate.

Although indicated against oak decline, potassium phosphonate capsules are actually sold as fertilizers and not as fungicides (http://www.fertinyect.com). Trees treated twice a year showed increasing shoot growth during the first year after treatments, although this effect was not maintained in the second year. Results are in accordance with previous research evaluating *Q. ilex* shoot growth after potassium phosphonate treatments (Cordón et al. 2001), but not with those of Fernández-Escobar et al. (1999), who observed greater growth during the second and third years after treatments compared with control trees. No improvement in acorn production was observed, and this fact should be taken into account, since acorns are one of the most important forest products of *Q. ilex* dehesas. To the best of our knowledge, quantification of *Q. ilex* shoot growth and acorn loss due to decline (30% and 60%, respectively, if comparing healthy with very declined trees) is described here for the first time. This information would be useful to estimate the ecological and economical impacts caused by decline, if compared properly with incidence and intensity data available from previous surveys (Sánchez et al. 2002, 2003; Tuset and Sánchez 2004; Vivas et al. unpublished). The differential growth obtained depending on branch orientation agrees with recent measurements on *Q. ilex* trees, which show surprisingly that the canopy radius was higher on the south and west sides of the trunk than on the east and north sides, irrespective of the site aspect (Montero et al. 2008). It would have been interesting in the present study to analyze represen-

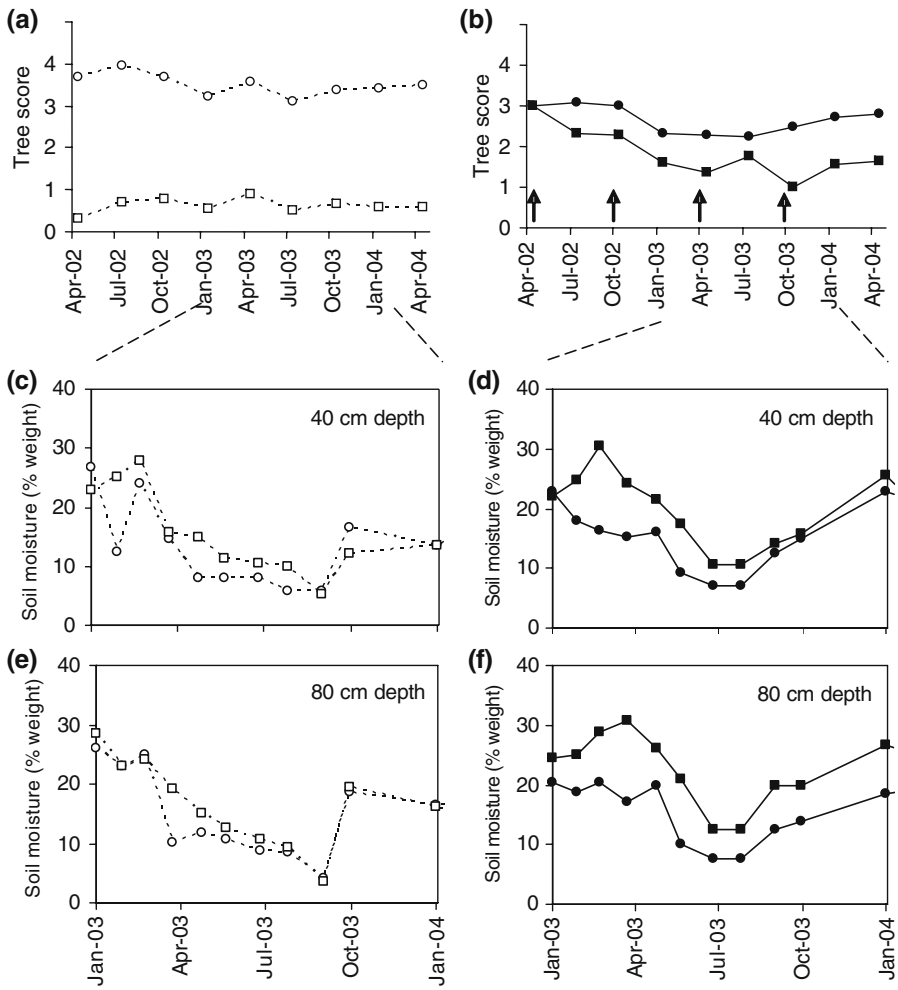

Fig. 4 Symptom development of *Quercus ilex* trees treated with potassium phosphonate injections, and soil moisture content values under these trees, grouped into: (**a**, **c**, **e**) untreated declined (○) and untreated healthy (□) trees; and (**b**, **d**, **f**) treated trees with better health than before treatments (■) and treated trees with a similar health condition as before treatments (●). Average soil moistures were obtained under three trees per group, at 40- and 80-cm depths (**c**, **d** and **e**, **f**, respectively). Arrows indicate treatment dates. Tree scores: 0 = healthy trees (crown transparency ≤5%); 1 = trees showing few decline symptoms; 2 = trees showing moderate symptoms (21–40%); 3 = trees showing pronounced symptoms; and 4 = very advanced decline (>60%)

tative fine root samples before and during several years after the treatments, and to correlate the obtained values with the values of shoot growth.

Injection treatments had no beneficial effect on trees initially scored as 3 (Fig. 3d), although some treated trees grouped into 'better than before treatments' showed considerable symptom alleviation (Fig. 4b), probably due to the higher soil water content (θ) observed. The physiological status of the host under conditions of low θ would probably have affected the distribution of phosphonate in the tree and thus the concentration confronting the pathogen at the infection court, although this hypothesis needs to be scientifically supported. Concentrations of phosphonate in roots of *Persea americana* and *Durio* sp. varied significantly depending on tree phenology and environmental conditions (Diczbalis et al. 2004; Whiley et al. 1995). Moreover, the efficacy of potassium phosphonate would depend on the amount applied and taken up in relation to plant size, and the tree species (Hardy et al. 2001; Shearer et al. 2006). In the present study, an application of 3–5 capsules each with 225 ml means that one tree was treated with 16.1–26.8 g of H_3PO_3 (active ingredient). In Australia, where there has been extensive experience with the treatment of dieback of forest trees by *P. cinnamomi*, it is recommended to inject between 50 and 200 gl^{-1} of H_3PO_3 in amounts equivalent to 1 ml per cm of tree circumference. For trees of 35–55 cm diameter, this amounts to 5.5–34.6 g of the active ingredient (phosphorus [phosphonic] acid), which is within the range used here. The efficacy of potassium phosphonate is also strongly influenced by the *in planta* phosphate concentrations (Guest and Grant

1991). The phosphate concentrations in Australian soils are among the lowest in the world (Olsen P values of approximately 5 mg Pkg^{-1} soil), which is why Australian plant species are highly sensitive to phytotoxicity by potassium phosphonate. In contrast, phosphate concentrations in European soils are generally much higher and it is most likely that the thresholds for phytotoxicity and the efficient concentration of potassium phosphonate are markedly higher in European plant species in comparison with Australian plant species. Measurements undertaken by Moreno and Obrador (2007) in several *Q. ilex* forests close to the study area provided Olsen and leaf content values of P of 4.6–11.8 mg Pkg^{-1} soil, and 0.34–0.64 mg Pg^{-1} DW, respectively. Although these values are lower than others reported for *Q. ilex* forests in Europe (Moreno and Obrador 2007), it seems that more of the chemical needs to be applied on our trees than on the Australian trees to obtain an effective response against decline. Finally, it should be taken into account that the recommendations followed in Spain and Portugal (3–5 injections per tree) would probably not lead to a uniform distribution of potassium phosphonate in the root system. Potassium phosphonate is transported up- and downwards, but horizontal movement of substances is limited in plants. In Australia, it is usually recommended to apply one injection with 20 ml per 20 cm of circumference. With a tree of 35–55 cm diam, this would mean 5–9 injections, which would substantially increase the efforts and costs of the treatments.

Our results support the hypothesis that the soil water status plays a major role in *Q. ilex* decline. Low water availability for the tree root system of a *Q. ilex* tree is expected in soils at site 2 (decay incidence of 96%) because of the higher percentage of gravel, higher infiltration capacities, and greater depth to the water table, especially during the summer, in comparison with the soils of sites 1 and 3. In the Netherlands, *Q. robur* trees were found to be most declined on sites with a strongly fluctuating water table (Oosterbaan and Nabuurs 1991). In the Iberian Peninsula, *Q. ilex* decline was associated mostly with shallow soils, with low water retention and subjected to severe drought (Moreira et al. 2000; Moreira and Martins 2005), as observed at site 2, although some studies report the association of oak decline with soils in valleys, with low infiltration, and exposed to a combination of drought and waterlogging conditions (Sánchez et al. 2002, 2003; Tuset et al. 1996), as at site 1. Decline of *Q. ilex* has also been observed at sites where *P. cinnamomi* was not isolated (Brasier et al. 1993; Moreira and Martins 2005; Sánchez et al. 2003; Tuset et al. 1996; Tuset and Sánchez 2004); e.g. Romero et al. (2007) reported only three out of eight declined stands with positive isolations of *P. cinnamomi*. In a recent survey undertaken in the same province as the current study, *P. cinnamomi* was confirmed at only 21 out of 48 declining *Q. ilex* stands (Vivas et al. unpublished), and other factors such as soil compaction and excessive NH_4 content due to overgrazing, soil hydromorphy, and mycorrhizae presence are currently under study. In Dehesa de Santa Amalia no additional pests or pathogens involved in the decline syndrome were observed, and the decline was probably not caused by *P. cinnamomi* in the first place, as the pathogen was isolated from roots of only three out of nine trees, but by water stress. It should be stressed that *P. cinnamomi* was rarely isolated from site 2, where the highest decline incidence and tree mortality was observed, and where the presence of gravel, soil infiltration capacities, and water table depth values were significantly higher than at the other sites. Not all treated trees were tested for the presence of the pathogen since the main objective of the study was to test potassium phosphonate injections for the control of decline. Furthermore, and given the size of most decayed dehesas, if all declined trees were suggested to be tested against *Phytophthora* spp. prior to treatments, this control approach would be economically impractical.

The complexity of the decline syndrome requires that multidisciplinary research is undertaken, and this study was a first approach. We showed for the first time soil infiltration data on declined *Q. ilex* stands. Rawls et al. (1993) provided generalized estimates of f_0 varying from 21 to 90 cm h^{-1} for US soils ranging from fine sandy clay to standard turfed agricultural soil, and Chin (2008) provided values of f_0 up to 127 cm h^{-1} for loamy sand soils of Florida. The f_0 value obtained for site 2 (145 cm h^{-1}), probably as a consequence of the high gravel content, indicates the high capacity of infiltration of some of the soils in which *Q. ilex* trees grow. Wide-scale application of the Horton model is limited because of the dependence of the parameters on specific soil and moisture conditions, but on a low scale would help to achieve a better understanding of soil water movement. Piezometers enabled us to estimate the

duration of waterlogging and to observe differences in the water table depths between sites, although the results were not conclusive since the tubes were not deep enough during the summer period. Finally, our soil bulk density data do not support the hypothesis that *Q. ilex* decline is associated with compacted soils (Cubera et al. 2009). Therefore, long-term experiments are recommended, in which the water table levels and θ are measured during alternating drought and wet periods and related to tree symptoms, tree physiological parameters (e.g. leaf water potentials and rates of photosynthesis; Cubera and Moreno 2007; Moreno and Cubera 2008) and the amount of *P. cinnamomi* colonies in the soil.

In conclusion, care must be taken when recommending potassium phosphonate or other *P. cinnamomi* treatments, even when the pathogen is isolated from soil. Extensive application of potassium phosphonate in the Iberian *Q. ilex* trees would be possible only if additional research is undertaken, including (i) trials to test higher rates of phosphonic acid and more injection points per tree, (ii) studies that quantify phosphonate uptake in trees with different water status, and (iii) studies focused on the physiological status of the trees at the time of the injection, all in relation to the effectiveness of the treatments. Treatments in agricultural environments in which irrigation and fertilization are controlled should be clearly differentiated from treatments in forest environments, especially at sites in which soils are shallow or in Mediterranean areas in which θ is limiting. Finally, the influence of soil water content on host-pathogen interactions deserves further research, and pathologists should include the soil water content as an additional variable that may influence the effectiveness of therapy treatments.

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