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Institut Méditerranéen d'Ecologie et Paléoécologie (IMEP) - UMR CNRS 6116, LBEM, Université de Provence, Centre de St. Jérôme, Case 421, 13397 Marseille, Cedex 20, France

Summary. Recent economic and social changes in north Mediterranean regions have led to an important rural depopulation. Consequently, meadows developed on abandoned agricultural lands (characterized by high species richness) undergo reforestation. These former fields are mainly colonized by *Pinus halepensis* Miller, which is known to synthetize a wide range of secondary metabolites, among these, some could influence plant succession through allelopathy. The allelopathic potential of *P. halepensis*, was tested against two target species (*Lactuca sativa* L. and *Linum strictum* L.) with aqueous extracts obtained from different organs (root and needle) taking into account the individual age (±10, ±30 and >60 years old). Root and needle extracts affected differently germination and growth of the two target species, the responses varying with concentration of extracts, age and organs tested. The strongest inhibitory effect was observed on the germination and growth of *L. strictum*, exposed to needle extracts of young *P. halepensis* (±10 years old), and root extracts of older *P. halepensis* (>30 years old). These extracts contained several phenolic acids (*e.g.* 4-hydroxybenzoic acid and p-coumaric acid), which are known as allelochemicals and their concentrations vary with age and organ tested. Hence, *P. halepensis* could influence secondary succession through the release of potential allelochemicals in the environment by leaf leachates or root exudates.

Key words. Allelopathy – phenolic compounds – land abandonment – plant succession – ecosystem functioning – Mediterranean ecosystem

Introduction

The Mediterranean region has an intense and ancient agricultural history, but recent rural depopulation has greatly modified land occupation, in north Mediterranean countries, since the beginning of the 19th century (Barbero *et al*., 1990; Lepart and Debussche, 1992; Preiss *et al*., 1997). These changes have led to a strong reforestation through secondary succession, where the abandoned soil is, at the beginning, free of vegetation other than crops or pastures (Gilmore, 1999). Although the different stages of successional dynamics leading to forest ecosystems are well described (Lepart and Escarré, 1983), the functional mechanisms involved are poorly documented or unknown. Moreover, some authors consider that replacement of plant species during succession is partly due to allelopathic mechanisms (Rice, 1984; Pellissier, 1993; Reigosa *et al*., 1999).

In Provence (South-East of France), *Pinus halepensis* Miller (Pinaceae, Pinales), which is a pioneer and expansionist species, colonizes abandoned agricultural land characterized by high biodiversity (Lepart and Debussche, 1992; Tatoni, 1992; Roche and Tatoni, 1995; Quézel, 2000). The result is an almost monospecific young forest which leads to paraclimaxes that delay the establishment of oak forest ecosystems (Quézel and Médail, 2004).

By its richness in secondary metabolites (Pasqua *et al*., 2002; Macchioni *et al*., 2003; Maestre *et al*., 2003; Pasqualini *et al*., 2003), *P. halepensis* could play an important role, in secondary succession through several processes. For example, secondary compounds (terpenoids and/or phenolic compounds) can affect root symbionts and site quality through interference with decomposition, mineralization and humification (Kuiters, 1990; Kainulainen and Holopainen, 2002). They could also influence secondary succession by interspecific competition through allelopathy (Rice, 1984; Lambers *et al.*, 1998). Allelochemicals (mostly phenolic compounds and terpenoids; Rice, 1984; Rizvi *et al*., 1999) can be released by different ways: roots exudation, decomposition of plant organs (*e.g*. litter) or rain leaching (Rice, 1984). Concerning *P. halepensis,* Maestre *et al*. (2003), observed an inhibitory effect of this species on seedling establishment of various species in pine stands suggesting allelopathic effects of litter or root exudates. Moreover, allelopathic potential may be modified by several factors such as the age of the donor plant (Inderjit and Asakawa, 2001).

The aim of this study was to test (1) the allelopathic potential of *P. halepensis*, through its different organ-sources (needles for leaching and roots for exudation), and (2) its ageing at different stages of secondary succession. This would allow us to better understand the secondary succession that takes place in Mediterranean regions undergoing agricultural land abandonment.

Correspondence to: Anne Bousquet-Mélou, e-mail: anne.bousquet-melou @up.univ-mrs.fr

Table 1 Characteristics of study sites and trees of *P. halepensis* (Y: young pines; M: medium-aged pines; O: old pines; Mean age, diameter, height: ± standard deviation and global index (Global indices between -19 and 59 correspond to high fertility for *P. halepensis*)

Site id	Coordinates	Altitude (m)	Mean age of pines (years)	Mean diameter of pines (cm)	Mean height of pines (m)	Global index
Y1	43°46' 1''N 5°19'49''W	269	9 ± 1	19 ± 2	4 ± 1	26
Y2	43°46'14''N 5°17'45''W	265	12 ± 1	15 ± 1	4 ± 1	30
Y3	43°45'32''N 5°17'45''W	217	11 ± 2	19 ± 2	5 ± 1	17
M1	43°46'00''N 5°19'51''W	272	32 ± 2	33 ± 4	9 ± 1	16
M2	43°45'24''N 5°19'11''W	217	28 ± 5	34 ± 1	12 ± 2	22
M3	43°45'59''N 5°19'44''W	269	34 ± 2	37 ± 5	11 ± 2	27
O1	43°45'50''N 5°19'42''W	248	78 ± 3	58 ± 5	15 ± 2	25
O2	43°46'18''N 5°24'1''W	350	68 ± 3	66 ± 7	16 ± 2	42
O3	43°45'0''N 5°19'40''W	269	73 ± 6	57 ± 8	13 ± 2	29

Material and methods

Sampling sites

In order to evaluate the variability of *P. halepensis* allelopathic potential in relation to different stages of secondary succession, three classes of age, of *P. halepensis* were chosen:

- meadows colonized by dispersed individuals of young *P. halepensis* (±10 years old) and named successional stage "Y".
- a monospecific forest stand of medium-aged *P. halepensis* (±30 years old), recently closed and without understory and named successional stage "M".
- a "mature forest" of old *P. halepensis* (>60 years old) with well-developed understory and named successional stage "O".

Three replicates were selected for each successional stage (Table I).

Sites were selected along the southern hillside of the Luberon Mountains in the Natural Regional Park (South of France), on the basis of similar global index (climatic and topo-edaphic conditions) using a model developed by CEMAGREF (Ripert and Vennetier, 2002). All sites were characterized by deep agricultural soils (> 1 m) of rendosol type according to the "référentiel pédologique" (AFES, 1995), Rendzic Leptosol according to FAO (FAO, 1998) and Rendoll in "Soil Taxonomy" (Soil Survey Staff, 1999), with no slope and a high fertility for *P. halepensis* (Table I).

Material collection

Needles and roots were collected from five individuals at each site. Needles were harvested from the entire tree crown and roots were sampled in close proximity to the pines (diameter < 1.5 m). The samples were stored at –24 °C.

Bioassays

Four principal pathways are known for allelochemicals to be released into the environment: litter decomposition, root exudates, vaporisation into the air, rain and dew transfering leaf compounds to the soil (Rice, 1984). Because some authors have shown that water-soluble compounds are probably most involved in allelophathy (Vyvyan, 2002), natural leachates and root exudates were chosen to be approached in this study.

Needle and root extracts were prepared by soaking 50 g (fresh weight) in 250 ml of distilled water (10 % dry weight as plant material contains 50% moisture). Extracts were prepared at room temperature and left in darkness for 24 hours. Diluted solutions (5 and 2.5 %) were prepared from the mother solution.

Two target species were selected: (1) *Lactuca sativa* L. var. *batavia* (Asteraceae, Asterales), species known for its sensitivity to allelopathic substances and frequently used for bioassays (Molina *et al*., 1991; Chiapusio *et al*., 1997; Chou *et al*.,1998), and (2) *Linum strictum* L. (Linaceae, Linales), present in open environments in Provence calcareous areas (Loisel, 1976), particularly in the first secondary succession stages following abandoned agricultural lands (Tatoni, 1992).

The use of *L. sativa* could demonstrate the possible mechanisms by which *P. halepensis* competes with other species while the results concerning *L. strictum*, which grows in the study area, have more ecological significance (Bousquet-Mélou *et al*., 2005).

Trials were carried out using glass Petri dishes with Whatman® n°4 filter papers (Molina *et al*., 1991; Bong-Seop, 1992; Chiapusio *et al*., 1997; Chou *et al*., 1998). Twenty seeds of each target species were placed in Petri dishes, to which 1ml of extract solution was added. The dishes were then regularly sprayed with an equal volume of the same extract solution until the end of the experiment. Three factors were tested: age (sampling site corresponding to the age of the stand), plant organ (needle or root), and dose (extract concentration). For controls, distilled water was used. Five replicates were used for each combination (age/ organ/ dose). Trials were carried out, during natural photoperiod, at a fixed temperature (20.5 °C ± 1°C), and at 100% relative humidity, approximately. Two variables were analysed for the two target species: germination of seeds (rate and response curve), and length of seedlings (radicle, hypocotyl, and total) 5 days after germination.

Osmotic pressure measurements

To be sure that the inhibitor effects observed on germination rate and growth are not due to osmotic pressures but to allelochemicals of *P. halepensis* (Anderson and Loucks, 1966), osmolality (mOsmol/kg H_2O) of all extracts (10%) was measured with a micro-osmometer from Roebling (Type13/13DR – Autocal).

Apparatus was recalibrated every 10 samples with deionized water and certified solution of 300 mOsmol/kg H_2O (even if no calibration drift was observed).

Chemical analyses

Instrumentation. Analyses were performed using a Hewlett-Packard GC6890 (coupled to a HP 5973N Mass Selective Detector) equipped with a HP5MS capillary column (30m × 0.25mm × 0.25µm - J&W Scientific). Sample volumes were injected in a splitless mode for 1min with an ALS 7683 Automatic Injector. Purge flow was set to 30ml/min for qualitative study (50ml/min for quantitative one). Helium (99.995%) was used as carrier gas. A constant flow of 1ml/min was set throughout the run for both qualitative and quantitative analyses. For qualitative study, oven temperature was initially programmed at 70 °C, ramped to 270 °C at a rate of 5 °C /min, where it remained for 10min. A second program for quantitative analyses was developed: oven temperature initially set at 50 °C was increased to 220 °C at a rate of 5 °/min where it remained for 6min. Injector temperature and MSD transfer line heater was held to 250 and 280 °C, respectively. The mass spectrometer parameters for EI mode were: ion source, 230 °C; MS quadrupole, 150 °C; electron energy, 70eV; Electron Multiplier Energy, 1100-1200V; data were acquired in scan mode from 40 to 500amu for qualitative analyses and in "Selected Ion Monitoring" mode for quantitative analyses.

Chemical supplies and quality

Methylene chloride, ethyl acetate and acetonitrile HPLC grade were obtained from SDS (Peypin, France). Water of HPLC quality for plant extraction and chemical procedure was obtained from a Milli Q system (Millipore St-Quentin, France). Derivatization reagent, N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) for qualitative study was purchased from Fluka (Sigma-Aldrich). Methylation reagent (methyle iodure, 99.5% purity), internal standard (3-chloroanisole, >97% purity), methanol HPLC grade, phenolics and aliphatic acids, phase transfer catalysts (tetrahexylammonium bromure - THAB, <99% purity, and tri-n-butylmethylphosphonium polymer bound - 1,4mmol Cl^-/g resin - TBMP) used for quantitative analysis were obtained from Sigma-Aldrich. Sodium chlorure and potassium dihydrogenophosphate of analytical grade were provided by Prolabo (VWR, France).

Qualitative analyses

Qualitative studies of chemical components were carried out on root and needle extracts of *P. halepensis.* For each analysis, three randomly-chosen extracts at 10% (used in bioassays) were mixed to be analysed.

The solutions were extracted three times with 25ml ethyl acetate. The three fractions were gathered and concentrated to dryness with rotary evaporator. The residue was suspended in 1ml of methylene chloride, and transferred to a 5ml vial. The procedure was repeated twice and the solution was evaporated to dryness under a stream of helium to remove residual water.
A volume of 200µl of acetonitrile and 200µl of BSTFA+1%TMCS was added to residue to produce trimethylsilyl derivatives. The solution was incubated at 70 °C for 1 hour, let to cool, filtrated and subjected to GC-MS.

Quantitative analyses

Quantitative analyses were performed on extracts of needles and roots of young and old pines. Analysis protocol for this quantitative study was adapted from Fiamegos *et al*. (2004) who developed an extraction-derivatization technique of phenolics *via* Phase Transfer Catalysis (PTC). The methodology was improved to fit phenolics and aliphatic acids (or diacids) methylation and extraction. The method was tested for 2 or 3 compounds of each chemical family: aliphatic acids (palmitic and stearic acids), aliphatic diacids (succinic and azelaic acids), simple phenols (catechol and pyrogallol), acetophenones (acetovanillone and acetosyringone), phenolic acids (4-hydroxybenzoic, protocatechuic and gallic acid) and cinnamic acids (p-coumaric, caffeic and sinapic acids). Chemical compounds were chosen on the basis of their occurrence in *P. halepensis* (qualitative analysis) or chemical interest to follow their capability to be methylated under these conditions before compound screening enlargment. Because of the methylation technique, the quantified derivatives may include several allelochemicals.

Stock solutions of compounds were prepared at 1mg/ml dissolving 25mg pure standard in 25ml deionized water (or 50:50 (V/V) methanol/water for less soluble compounds). Adequate dilutions of stock solutions were made to carry out calibration curves. A buffer solution 1M KH_2PO_4 was prepared by dissolving 13.60g of salt in 100ml deionized water and adjusted to pH 8.0 with diluted NaOH. THAB 0,1M in dichloromethane (217.3mg in 5ml) and internal standard at 100µg/ml in dichloromethane were also prepared.

The procedure occured in 35ml-Pyrex® tubes (Bibby Sterilin Ltd) equipped with PTFE screw caps: to 10ml extract solution was added a stirring bar, 500 µl buffer solution pH 8.0, 50mg TBMP, 100µl THAB 0.1M, 50µl internal standard, 850µl dichloromethane and 100µl methyle iodure. Tubes were hermetically sealed, and heated at 80 °C during 1hour to permit the methylation of compounds. After cooling, solutions were saturated with NaCl and shaked vigorously. After phase separation, organic layer was sampled, filtered onto 0.45µm filter syringe, and analysed by GC-MS.

The same procedure was applied to mixtures of adequate standard solutions to construct calibration curves. Methylated compounds were quantified relative to internal standard.

Statistical analyses

Differences in germination rate were carried out using a chi-square test (Scherrer, 1984). Differences in size (radicle, hypocotyl, total) according to age, dose or plant organ were tested using one-way or two-way ANOVA. Osmotic pressure of our extracts were compared with a threshold value (0.5atm) with a mean comparison with theoretical mean test. Pearson correlation was performed between osmotic pressure and germination rate. Previously, normality and homoscedasticity were tested by Shapiro-Wilks' and Bartlett's tests, respectively. Statgraphics® (version 2.1) was used as software for all statistical analyses.

Results

Bioassays

L. sativa – The overall mean germination rate was 99%, and there was no significant effect of factors on this parameter ($0.68 < \chi^2 < 3.18$; $0.365 < p < 0.879$). The seed germination of this species was not depressed.

Concerning needle extracts, mean length of radicles was significantly longer for all treatments compared to control (one-way ANOVA, $13.06 < F < 171.30$, $p < 0.05$; Fig. 1). This stimulating effect was even greater with increasing age of pines, and more generally, with increasing dose of extracts (Tukey test; Fig. 1). Moreover, needle extract had no or little effect on hypocotyls growth (Tukey test; Fig. 1).

Concerning root extracts, older pines (>30 years) inhibited growth and caused necroses of radicles at the extract concentrations of 5 and 10% (one-way ANOVA, $12.40 < F < 91.67$; $p < 0.05$; Fig. 1). For the lowest extract concentration, a significant stimulation of root growth was observed (Tukey test; Fig. 1). Similarly, root extracts stimulated generally the

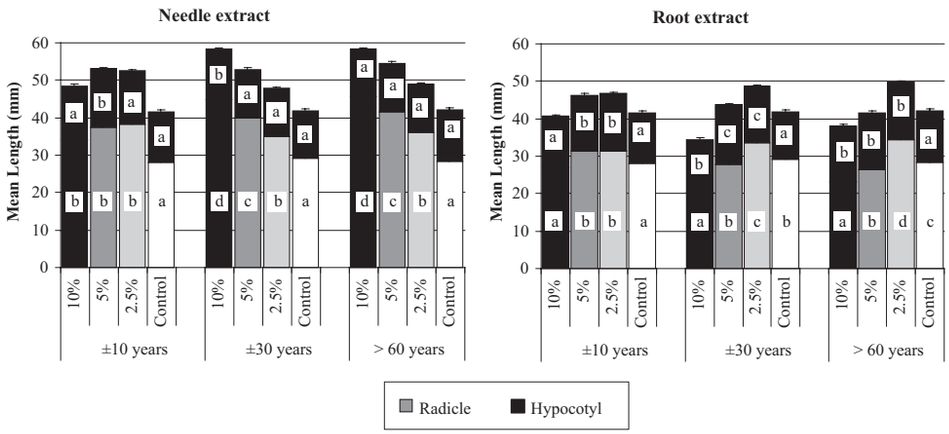

Fig. 1 Mean length of radicles and hypocotyls (and confidence interval at 95 %) of *L. sativa* as a function of *P. halepensis* age, organ nature (root or needle), and extract dose. Tukey's tests with regard to differences between doses are also presented for each organ (radicles in normal style and hypocotyls in italics). Values not different at $\alpha = 5\%$ are denoted with the same letter

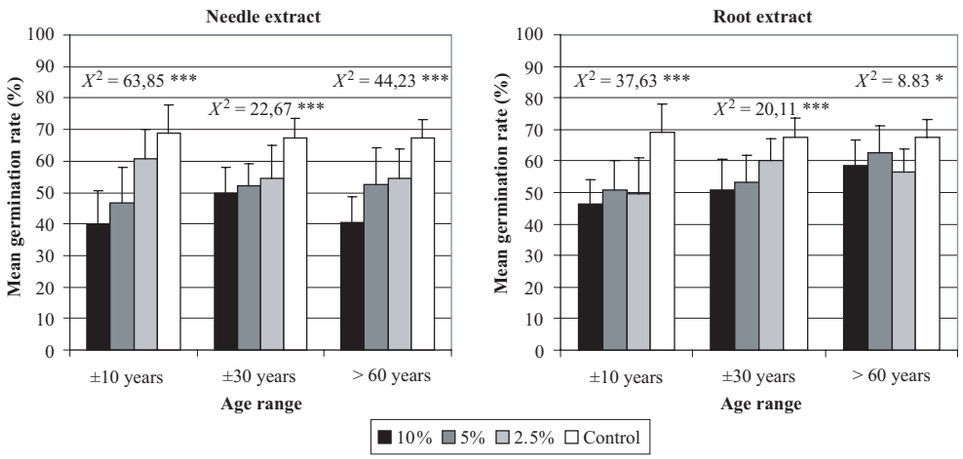

Fig. 2 Mean germination rate of *L. strictum* (and confidence interval at 95 %) as a function of *P. halepensis* age, organ nature (root or needle), and extract dose. Chi-square tests on germination rate are also given (chi-square value and p: * significant at 0.05; ** significant at 0.01; ***: significant at 0.001)

growth of hypocotyls (one way ANOVA, $4.22 < F < 78.56$; $p < 0.05$; Fig. 1).

L. strictum - Root and needle extracts had significant effect on the germination of this species (chi-square test, $p < 0.05$; Fig. 2).

Germination was depressed but not totally suppressed. Indeed, mean germination rate varied between 40 - 60% with *P. halepensis* extracts. Control had the highest mean germination rate (67 - 69%) all along the experiment (Fig. 3). Delayed germination that was later with increasing dose of extracts, was observed in particular for needle extracts of young pines.

Needle extracts had a significant, but not uniform, effect on growth of *L. strictum* seedlings (one-way ANOVA, $14.12 < F < 205.95$; $p < 0.05$; Fig. 4). An important inhibitory effect, on both radicles and hypocotyls growth, was observed with needle extracts of young pines for the highest concentration. With most of combinations, an intermediate effect was observed: the needles extracts induced an inhibitory effect on radicle growth, but concomitant with a stimulative effect on hypocotyl growth (Tukey's tests; Fig. 4). Finally, a stimulatory effect was observed only for the older pines (>30 years) at the lowest concentration.

Roots extracts had a significant, but not uniform, effect on growth of *L. strictum* seedlings (one-way ANOVA, $9.18 < F < 69.76$; $p < 0.05$; Fig. 4). Generally, root extracts exhibited an inhibitory effect on radicle growth concomitant with a stimulative effect on hypocotyl growth except for the lower concentrations of the young pines extracts (2.5 - 5%; Tukey's tests; Fig. 4). Necroses of radicles were also observed with root extracts of older pines (>30 years).

Osmotic pressure measurements. The mean osmotic pressure of aqueous extracts (10%) varies from 0.35 to 0.69atm (Fig. 5). In our study, only the extract from roots of young pines (10%) have an osmotic pressure significantly higher than 0.5atm (mean comparison with theoretical mean) (Anderson and Loucks, 1966). Therefore, we show that there is no significant correlation ($R^2 = 0.0863$, $p > 0.05$) between osmotic pressure and germination rate (Fig. 6).

Chemical analyses. Qualitative analyses of chemical components confirmed the presence of numerous phenolic compounds such as benzeneacetic, 4-hydroxybenzoic, vanillic, veratric, syringic and p-coumaric acids in *P. halepensis* organs (Appendix 1). Other non-phenolic acids such as lactic, succinic, palmitic acids were also observed.

The composition, with regard to several major compounds, differed between root and needle extracts (e.g. succinic and benzeneacetic acid; Appendix 1).

Quantitative analyses present significant differences between the various extracts analysed (Fig. 7): acetosyringone, 4-hydroxybenzoic acid, and acetovanillone are significantly higher in extracts of young pines needles and

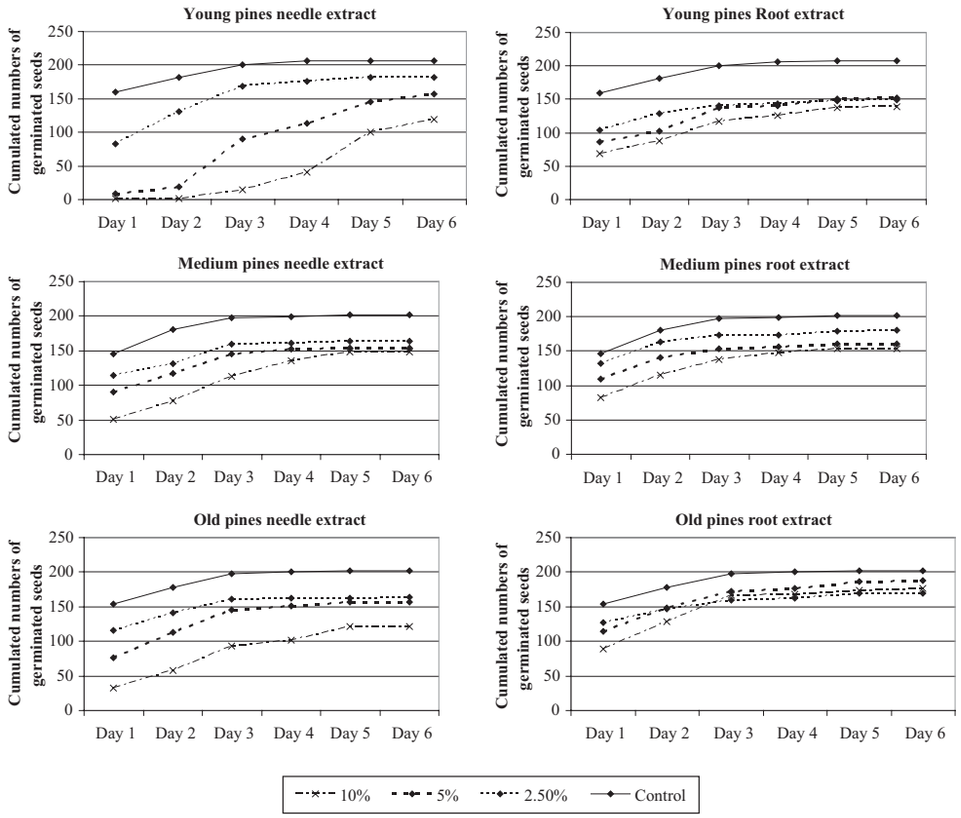

Fig. 3 Germination response curves of seeds of *L. strictum* as a function of *P. halepensis* age, organ nature (root or needle), and extract dose

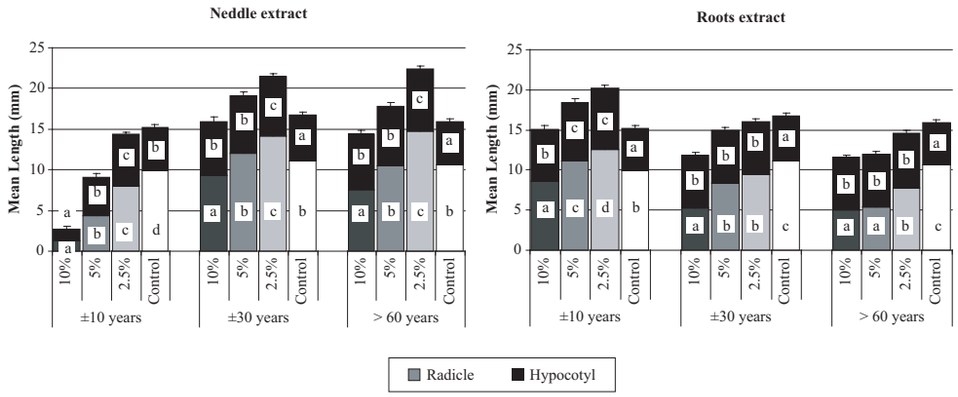

Fig. 4 Mean length of radicle and hypocotyl (and confidence interval at 95 %) of *L. strictum* as a function of *P. halepensis* age, organ nature (root or needle), and extract dose. Tukey's tests with regard to dose effects are also presented for each organ (radicles in normal style and hypocotyls in italics). Value not different at $\alpha = 5\%$ are denoted with the same letter

sinapic acid is significantly higher in extracts of young pines needles and old pines roots (one-way ANOVA, $3.88 < F < 41.95$; $p < 0.05$; Tukey tests, $p < 0.05$).

Discussion

Allelopathic potential of P. halepensis

P. halepensis extracts affected differently germination and growth of the two target species according to the combination of the three parameters tested (dose of extracts, plant organs and age of trees). Similar effects on germination and growth due to *Medicago arborea* L. (Fabaceae, Fabales) have already been observed on *L. strictum* and *L. sativa* (Bousquet-Mélou et al., 2005). The germination rate of *L. sativa* is not affected by allelochemicals of *P. halepensis* while the germination of this species was depressed in other studies (Molina *et al*., 1991; Chiapusio *et al*., 1997; Chou *et al*.,1998).

According to Anderson and Loucks (1966), extracts used in this study have an osmotic pressure no greater than 0.5atm (except for young roots extracts). So, effects observed on germination and growth can be due to allelochemicals of *P. halepensis*.

Our results showed three types of responses for the target species. The first effect observed is a total inhibition of both radicle and hypocotyl growth. This effect is

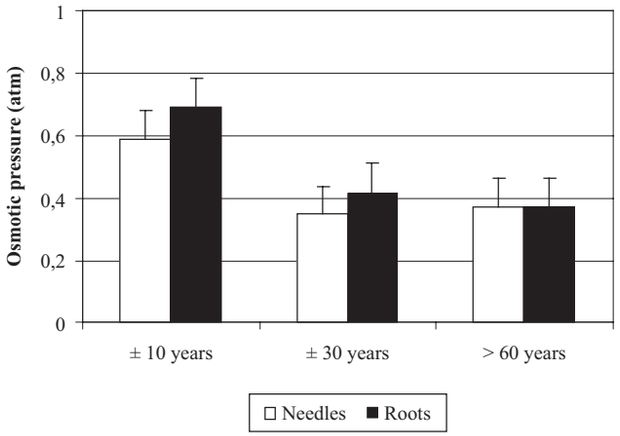

Fig. 5 Mean osmotic pressure (and confidence interval at 95 %) of *P. halepensis* needles and roots extracts (10% dry weight) for the three ages tested.

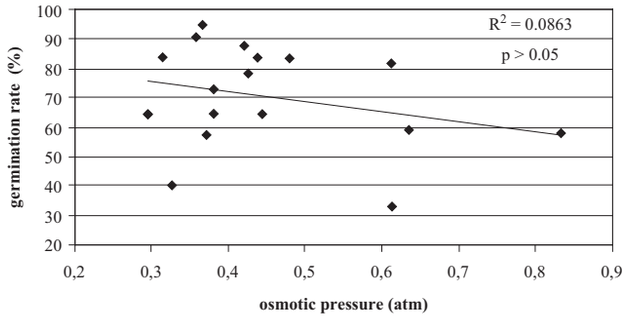

Fig. 6. Relation between osmotic pressure and relative germination rate (germination rate/control germination rate)

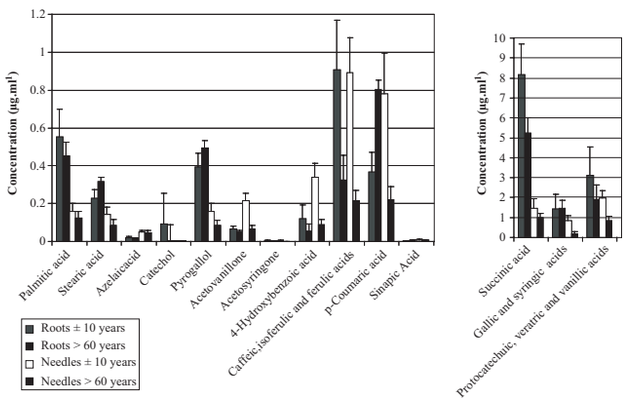

Fig. 7 Quantitative comparison of the allelochemicals studied between needles and roots extracts (10% dry weight) for young and old pines

observed with needle extracts of young pines at the highest concentration. Inhibitory effects are generally observed with high concentrations of chemical components in allelopathic studies (Rice, 1984). Phenolic compounds present in our extracts such as 4-hydroxybenzoic, vanillic, syringic, gallic and p-coumaric acids have already been found in needles of *P. halepensis* (Pasqualini *et al*., 2003), *P. rigida* Mill, and *P. densiflora* Siebold & Zucc. (Bong-Seop, 1992). Other compounds, such as benzoic acid, cinnamic, ferulic and caffeic acids have been found in other pine species (Mirocha *et al*., 1966 *in* Quayyum *et al.,* 1999; Rice, 1984; Bong-Seop, 1992). All these phenolic compounds are known from literature as potential allelochemicals (Rice, 1984; Bong-Seop, 1992). Particularly, p-coumaric acid has a significant effect on the growth of roots and above-ground organs of *Linum usitatissimum* L. (Ray & Hastings, 1992).

Quantitative analyses show differences between non-inhibitory (needles of old pines and roots of young pines) and inhibitory extracts (needles of young pines and roots of old pines). The higher concentrations of acetosyringone, 4-hydroxybenzoic acid, acetovanillone and sinapic acid in young pines needles extracts could explain their inhibitory effect. Moreover, sinapic acid is also present in higher concentration in extracts of old pines roots. This compound could explain the inhibitory effect also shown by old pines roots extracts.

The second effect observed is an inhibition of radicle growth associated with a stimulation of hypocotyl growth. These concomitant inhibitory and stimulatory effects may be interpreted either as the result of a direct molecular alteration or as a specific growth re-orientation related to allelochemical stress avoidance. The latter hypothesis is supported by the previous finding of stress-induced growth redistribution through the increase of shoot/root ratio from seedling of *Arabidopsis thaliana* (Brassicaceae, Brassicales) (Pasternak *et al*., 2005). Changes in the hormonal homeostasis consecutively to the inhibition of polar auxin transport were suspected to be involved in this redistribution of mitotic signals. However, in both cases, molecular alteration or specific physiological response, root architecture impairment may compromise the long-term survival of the seedlings. Indeed, as previously reported, the success of the re-establishment of some Mediterranean species greatly depends on the well-structured root systems of seedlings for an efficient resource capture (Green, 2005).

The last type of response is a simultaneous stimulatory effect on radicles and hypocotyls growth generally observed with the lower concentrations used. This response is a well known allelopathic property. Indeed, low concentration of allelochemicals can have a stimulating effect on target species growth (Rice, 1984). The stimulation of radicle growth is particularly observed for *L. sativa* seedlings. This may be due to a lesser sensitivity of this species compared to *L. strictum* as observed in other studies (Reigosa *et al*., 1996; Hong *et al*., 2004; Nektarios *et al*., 2005).

Consequences of potential allelopathy of P. halepensis on the secondary succession

In our study, the germination of only *L. strictum* was affected by compounds present in the tested tissues, in particular those in needle extracts of young pines. This result is particularly interesting as *L. strictum* is found in the wild in the early successional stages following agricultural

Appendix 1 Chemical compounds found in root and needle extracts of *P. halepensis*. RT: retention time (minutes) of compounds in the column. CAS: Identification number of Chemical Abstract Society

RT	COMMON NAME	SCIENTIFIC NAME	CAS
	Aliphatic acids		
6.43	Lactic acid	Propanoic acid, 2-hydroxy-	50-21-5
6.50	Malonic acid, diethyl ester	Propanedioic acid, diethyl ester	105-53-3
7.93	Levulinic acid	Pentanoic acid, 4-oxo-	
10.45	Butyric acid, 4-hydroxy-	Butanoic acid, 4-hydroxy-	591-81-1
12.45	Succinic acid	Butanedioic acid	110-15-6
15.71	Non identified (m/z=314)		
28.36	Palmitic acid	Hexadecanoic acid	57-10-3
31.87	Stearic acid	Octadecanoic acid	57-11-4
	Terpenes		
9.79	Verbenone	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-	80-57-9
10.16	Borneol	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-,endo-	507-70-0
14.74	Myrtenoic acid	Bicyclo[3.1.1]hept-2-ene-2-carboxylic acid, 6,6-trimethyl-	19250-17-0
	Phenolic acids		
10.62	Benzoic acid	Benzoic acid	65-85-0
11.91	Benzeneacetic acid	Benzeneacetic acid	103-82-2
19.90	4-Hydroxybenzoic acid	Benzoic acid, 4-hydroxy-	99-96-7
20.14	4-hydroxybenzeneacetic acid	Benzeneacetic acid, 4-hydroxy-	156-38-7
22.95	Vanillic acid	Benzoic acid, 4-hydroxy-3-methoxy-	121-34-6
23.28	Protocatechuic acid	Benzoic acid, 3,4-dihydroxy	99-50-3
24.20	Veratric acid	Benzoic acid, 3,4-dimethoxy-	93-07-2
25.75	Syringic acid	Benzoic acid, 4-hydroxy-3,5-dimethoxy-	530-57-4
27.10	Gallic acid	Benzoic acid, 3,4,5-trihydroxy-	149-91-7
	Cinnamic acids		
17.91	Cinnamic acid	2-Propenoic acid, 3-phenyl-	140-10-3
26.15	Ferulic acid	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-	537-98-4
26.42	p-coumaric acid	2-Propenoic acid, 3-(4-hydroxyphenyl)-	501-98-4
26.51	Hydrocinnamic acid, p-hydroxy-(α or β)-methyl	Benzenepropanoic acid, 4-hydroxy-(α or β)-methyl-	35456-48-5 6739-21-5
29.34	Isoferulic acid	2-Propenoic acid, 3-(3-hydroxy-4-methoxyphenyl)-	25522-33-2
30.23	Caffeic acid	2-Propenoic acid, 3-(3,4-dihydroxyphenyl)-	501-16-6
	Alcohols		
10.13	Phenylethyl alcohol	Benzeneethanol	60-12-8
	Phenolic alcohols		
18.60	p-Tyrosol	Benzeneethanol, 4-hydroxy-	501-94-0
20.25	Vanillyl alcohol	Benzenemethanol, 4-hydroxy-3-methoxy-	498-00-0
21.79	Homovanillyl alcohol	Benzeneethanol, 4-hydroxy-3-methoxy-	2380-78-1
24.09	Dihydroconiferyl alcohol	Benzenepropanol, 4-hydroxy-3-methoxy-	2305-13-7
	Cinnamic alcohols		
15.10	Cinnamyl alcohol	2-Propen-1-ol, 3-phenyl	4407-36-7
15.44	Salicylic alcohol	Benzenemethanol, 2-hydroxy-	90-01-7
23.55	p-Coumaric alcohol	Phenol, 4-(3-hydroxy-1-propenyl)-	20649-40-5

(continued)

Appendix 1. **(Continued)**

RT	COMMON NAME	SCIENTIFIC NAME	CAS
	Others		
34.35	Dehydroabietic acid	1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-(1R,4aS,10aR)-	1740-19-8
26.00	Non identified (m/z=267)	Non identified (m/z=267)	

land abandonment and not in older stages (Tatoni, 1992). Germination (rate and germination delay) as well as growth, of this species are affected by allelochemicals of *P. halepensis*. These results show that *P. halepensis* could limit the establishment of other species by reducing interspecific competition (Reigosa *et al.,* 2000), or by affecting the growth of species already present in the environment. These phenomena could as well facilitate the establishment of seedlings and growth of *P. halepensis* in its early stage (Reigosa *et al*., 1999).

The results showed variations in allelopathic effects due to age and organ of the donor plant as previously reported (Rice, 1984; Inderjit and Asakawa, 2001). Indeed, germination and growth of *L. strictum* were strongly affected by needle extracts of young pines and roots extracts of older pines. So, potential allelopathic effects of *P. halepensis* could be expressed mainly through natural leachates in the early stage following the establishment of this tree (Vyvyan, 2002) whereas, in later successional stages, they could be expressed mainly through root exudates, which significantly increase with tree age. Some authors believe that root exudation, a phenomenon still poorly investigated, could be very important directly or indirectly in allelopathic processes (Callaway & Aschehoug, 2000).

P. halepensis affected germination and growth of the two target species in laboratory experiments. Even if Inderjit and Callaway (2003) and Bais *et al*. (2003) have shown that allelochemicals remain adsorbed in toxic concentrations in soil several authors have shown that their activity could be affected by various soil factors. For example Kobayashi (2004) and Keech *et al.* (2005) have shown that allelochemicals activy could be mediated by numerous soil factors (soil texture, organic and inorganic matter, moisture and organisms). Moreover, allelochemicals have the potential to be important regulators of carbon and nutrients cycling in coniferous forests (Kainulainen and Holopainen, 2002).

The different allelopathic effects between species could change interspecific competition and then mediate plant successional dynamic. Further field and laboratory experiments, on other wild species such as pre-forest and forest species (specially oaks), soils and under different light conditions, among others, are needed in order to demonstrate the complex interactions that occur in abandoned agricultural lands.

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