Industrial Crops & Products 108 (2017) 113–120

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Research Paper

Dual allelopathic effects of subtropical slash pine (*Pinus elliottii* Engelm.) needles: Leads for using a large biomass reservoir

Kelly Cristine da Silva Rodrigues-Corrêa[a], Gelson Halmenschlager[a], Joséli Schwambach[b], Fernanda de Costa[a], Emili Mezzomo-Trevizan[a], Arthur Germano Fett-Neto[a,*]

[a] *Plant Physiology Laboratory, Center for Biotechnology and Department of Botany, Federal University of Rio Grande do Sul (UFRGS), P.O. Box CP 15005, Brazil*
[b] *University of Caxias do Sul, Institute of Biotechnology Caxias do Sul, RS, Brazil*

A R T I C L E I N F O

Keywords:
Pinus elliottii
Seasonality
Growth
Germination
Litter
Substrate

A B S T R A C T

Pinus elliottii Engelm. (slash pine) is distributed along the maritime coast of Southern Brazil, where it shows invasive pattern and typical allelopathic features. Large quantities of needle litter are produced by pine trees, a biomass that is little explored in areas where this species is alien. Little is known about the dynamics of needle and litter phytochemical interactions, particularly in subtropical environments. To elucidate the full range of needle and litter allelopathic potential, the effects of litter (superficial and deep) and seasonally harvested fresh slash pine needles stored for different times were evaluated against lettuce, tomato and cucumber seeds and seedlings. Increasing concentrations (0%, 1%, 2%, 4%, and 8% w/v) of hot and cold aqueous extracts of needles and litter affected in different ways target plant development. Growth and germination inhibition were directly related to the highest extract concentrations (regardless of the season and mainly in hot water extracts) of needles. On the other hand, stimulatory effects of litter extracts on lettuce growth were observed. Growth and germination of cucumber and tomato were not affected by pine litter as substrate when compared to rice husk. The presumable high polarity and thermal stability of slash pine leaf biomass allelochemicals and their transient toxic effect or growth promoting impact suggest potential applications of this largely available biomass both as a biological herbicide and growth substrate in plant propagation.

1. Introduction

Native from the Northern Hemisphere, *Pinus* is one of the most widely distributed genera throughout different climate regions of the globe, growing either as native or alien species, even in extreme habitats (Rodrigues-Corrêa and Fett-Neto, 2012). Despite the high economic value currently attributed to pine wood and oleoresin (Rodrigues-Corrêa et al., 2012) there is increasing concern about the aggressive potential of invasiveness displayed by *Pinus* species, especially those cultivated out of their native range of distribution (Richardson et al., 2008; Rolon et al., 2011). These species are dispersed by wind and there is notably low plant diversity observed in most understories of pine plantations (Kato-Noguchi et al., 2009). This latter feature has been considered an important trait of allelopathic interference.

The term "allelopathy" was coined by Molisch in 1937 as a chemical reciprocal interaction established among plants (including microorganisms) sharing the same site by means of the release of secondary metabolites, named allelochemicals (Rice, 1984). For the most part, these metabolites are derived from the shikimic acid or isoprenoid pathway and their biosynthesis can be modulated by biotic and abiotic stresses (Nascimento and Fett-Neto, 2010), including seasonal-related changes (Sartor et al., 2013). Allelopathy studies may range from sterile assays (Aryakia et al., 2015) to soil (Corrêa et al., 2008; Sharma et al., 2016) and field tests, being a complex biological phenomenon to ascertain in several circumstances due to issues of solubility, release mechanisms and stability of bioactive compounds (Scognamiglio et al., 2013). Often the use of complementary methods provides more informative data.

The allelopathic effects of soil leachates, green needles and litter extracts of *Pinus* spp. on germination and seedling growth aspects of wild and crop species have been evaluated in natural and cultivated pine stands and have proven to be stimulatory or inhibitory (Lodhi and Killingbeck, 1982; Kil and Yim, 1983; Nektarios et al., 2005; Akkaya et al., 2006; Machado, 2007; Alrababah et al., 2009; Sartor et al., 2009; Kato-Noguchi et al., 2011; Rolon et al., 2011; Valera-Burgos et al., 2012) exhibiting, in some cases, autotoxicity (Garnett et al., 2004; Fernandez et al., 2008; Zhu et al., 2009; Monnier et al., 2011). Studies on potential dual allelopathic effects of *Pinus elliottii* Engelm. (slash

* Corresponding author.
E-mail address: fettneto@cbiot.ufrgs.br (A.G. Fett-Neto).

http://dx.doi.org/10.1016/j.indcrop.2017.06.019
Received 23 March 2017; Received in revised form 15 May 2017; Accepted 7 June 2017

pine) on other plant species are lacking, particularly considering seasonal and temporal variables.

Slash pine is one of the main fast-growing commercial trees introduced in Southern Brazil for both timber and oleoresin production (Rodrigues et al., 2008). In the last years, the environmental impacts of pine silviculture expansion in this region has received special attention due to its distribution along the maritime coast in previously impacted areas, which are under intense winds. Furthermore, field trials have shown a reduction of the aquatic macrophyte richness in slash pine vicinity compared to those exhibited by non-pine stands in wetland ecosystems (Rolon et al., 2011). Moreover, the sparse vegetation under slash pine cultivated forests could be the result of physical limitations, such as shading and heavy litter accumulation, besides allelopathic activity. Litter from *Pinus* stands represents a considerable stock of biomass. *P. taeda*L. in Southern Brazil yields approximately 7 tons of litter.ha^{-1}.year^{-1} (mostly from needles) (Piovesan et al., 2012). The use of pine litter for landscape mulch is a profitable business in North America, also contributing for the removal of excess biomass accumulation, thereby contributing to forest fire prevention (Susaeta et al., 2012). However, the impact of needle and litter biomass on other plant species growth and development remains a matter of controversy, particularly in areas outside its native range of distribution. The aim of this work was to evaluate the allelopathic activity of both needles and litter from slash pine growing as an exotic species in subtropical climate and identify new potential uses for this large biomass stock from pine plantations.

2. Material and methods

Bioassays were carried out to evaluate the effect of extract concentration, season of harvest, postharvest storage time, and litter of slash pine needles on initial development of *Lactuca sativa* L. (lettuce). The effects of pine needle litter as substrate material on seed germination and seedling growth of *Cucumis sativus* L. (cucumber) and *Solanum lycopersicum* Lam. (tomato) were also evaluated.

2.1. Plant material

Fresh (green) needles and litter (top 20 cm) were collected from a 24 year-old slash pine plantation (near the city of Mostardas, RS, Brazil; 31°06′25″S, 50°55′16″W) and tested against lettuce seedlings and seeds. Fresh needles were collected in the middle of each season and stored in darkness under controlled temperature (25 °C). Immediately after harvest, 7 d, 14 d, 21 d and 28 d after storage, needles were frozen until the beginning of the experiments.

2.2. Extracts

Increasing concentrations (1%, 2%, 4%, and 8% w/v) of hot and cold aqueous extracts were prepared with fresh needles or litter collected from a slash pine stand. The different plant materials were macerated with 100 mL of boiling distilled water (at 100 °C at the beginning of the extraction, then later kept at room temperature) (hot extracts) or at room temperature (25 °C) (cold extracts) for 24 h. After this period, the extracts were filtered and immediately used in the bioassays. Initial and final pH values and water potential of extracts were recorded. Values of pH were measured with a potentiometer and water potentials were determined by densitometry.

2.3. Growth assays

One hundred seedlings (seeds showing 1 mm of expanded radicle) were distributed in Petri dishes (10 seedlings per dish) containing 6 mL of distilled water (control) or increasing concentrations of slash pine aqueous extracts. At the end of six days, the length of hypocotyls and radicles were measured. Petri dishes were sealed with plastic film and kept under controlled light (45 μmol photons m^{-2} s^{-1}) and temperature (25 °C).

2.4. Sand assays

The effect of fresh needles (FN) incorporated to an artificial substrate (sand previously washed and sterilized) was also tested in concentrations of 4% and 8%. Pure sand was used as control treatment. Lettuce seedlings were cultivated for 30 d, being watered by alternating distilled water and 0.1x MS nutrient solution (Murashige and Skoog, 1962). Ten replicates of 10 plants each were evaluated. At the end of this period, plants were collected and had their fresh and dry (at 65 °C) weights measured.

2.5. Pine needle litter as substrate

The influence of needle litter as cultivation substrate on germination parameters (mean germination time – MGT; germination percentage, both after 5 d), dry weight (DW after 15 and 30 d), height (after 30 d) of tomato and cucumber seedlings was tested in comparison with rice husk substrate. For the latter species numbers of leaves and flowers (after 30 d) were also recorded. Needle litter or rice husks were mixed with vermiculite and commercial top soil at a 2:1:1 ratio. Five replicates of 10 plants each were evaluated.

2.6. Germination assays

One hundred lettuce achenes, herein referred to as seeds, (20 per dish) were set to germinate in different concentrations of extracts (6 mL) or distilled water for six days. The radicle emergence (Baskin et al., 2004) was the germination criterion evaluated every 24 h. To confirm their viability, the seeds that did not germinate until the last day of experiment were subjected to tetrazolium test. The Germination Speed Index (GSI) was calculated according to Maguire (1962).

2.7. Statistical analyses

Data evaluation was performed by using analyses of variance (ANOVA) followed by Tukey or Dunnett's C (for non-parametric Welch ANOVA) tests. A $P \leq 0.05$ was used in all cases. Experiments were independently repeated twice.

3. Results

The various concentrations of aqueous extracts of slash pine affected early growth of *L. sativa* in different ways. In general, litter extracts promoted whole plant growth (Table 1) and had no effect on GSI (data not shown), whereas extracts obtained from slash pine needles that had been stored for different times inhibited growth (Fig. 1, Table 3), besides causing a delay in germination (Table 2).

The most conspicuous inhibitory effect of fresh slash pine needle extracts on lettuce length was observed in radicles (Fig. 1) compared to hypocotyls (Table 3), especially in higher extract concentrations (4% and 8%). Moreover, regardless of the water temperature used as extractor and season considered, the inhibitory effect also seems to be enhanced by storage time. Overall, extract concentration dependence of growth inhibition was apparently less pronounced in fresh needles, particularly in the winter and spring extracts (Fig. 1). In the latter season, even growth stimulatory effects were observed (see below). A sharp reduction in radicle length was observed mainly after the 14th day, even at lower concentrations of the extracts made with boiling water (Table 4). In the experiments using solid substrate, growth inhibition was also observed in plants grown in pots containing fresh needles incorporated in sand (Fig. 2), in which case hypocotyl growth was strongly inhibited.

Lettuce germination inhibition was observed in the most