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Effects of Gap Size and Soil Chemical Properties on the Natural Regeneration in Black Pine (*Pinus nigra* Arn.) Stands

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ABSTRACT

This paper deals with the problem of black pine (*Pinus nigra* Arn.) stand re-naturalization in the Apennines of Central Italy. Gap cuttings of two different sizes (gap diameter (D) to stand height (H) ratios were 0.75 and 1.0) in age class stands (50 and 90 years old) have been carried out, studying the soil properties and the tree regeneration dynamics in the different gaps 7 years after the beginning of the experiment. The small gaps D:H = 0.75 had the greater concentrations of total phenols, polyphenols and fulvic acids. In contrast, a greater amount of humic acids was detected in the medium gaps D:H = 1. Results indicate that black pine was the best regenerated species in gaps with D:H = 1 and its presence was increasing. Some broadleaves, pubescent oak (*Quercus pubescens*) and holm oak (*Quercus ilex*) particularly and hop-hornbeam (*Ostrya carpinifolia*) and manna-ash (*Fraxinus ornus*) in a subordinate way, were also present in the gaps. These results confirm that the gap cutting system represents an effective tool for the natural regeneration priming and a treatment with low environmental impact which provides suitable condition for the re-naturalization of the conifer monocultures.

Keywords: gap size, forest management, natural regeneration, phenolic compounds, *Pinus nigra*, soil properties

INTRODUCTION

Many forests in Europe have undergone large changes in vegetation composition since forest managers have replaced native hardwood by coniferous species. This phenomenon has been very pronounced in Southern Italy, where excessive exploitation of forests, intensive agropastoral practices and summer forest fires resulted in strong deterioration of Mediterranean forests (Thirgood 1981; Blondel 2006). Consequently, in many places soils have been eroded and have become almost unproductive. The effects of this deterioration have been reinforced by the physical environmental conditions (climate and orography), which have combined with human impact and have not been favourable to the recovery of lands by vegetation (Blondel and Aronson 1995). To avoid ‘‘mineral landscapes’’ (Molinier and Molinier 1971) at the end of this progressive deterioration process, the National Forest Service decided to afforest these denuded areas. After the strong increase in forested surfaces during the 20th century, foresters’ demands for afforestation have evolved to the choice of more productive, summer drought- and fire-resistant types of trees. However, few species can fulfil the requirements of the Mediterranean environment. These requirements are mainly tolerance to summer drought and high amounts of active limestone in soils and the ability to cope with occasional low temperatures in winter. The processes of gap creating and filling are thought to play a central role in species coexistence and regeneration in a variety of forests (Brokaw 1987; Lertzman 1992; Brett and Kilnka 1998; Dalling *et al.* 1998; Nagel *et al.* 2010). Variations in physical environment within gaps provide opportunities for species that could not establish under a closed canopy. Canopy openings as a result of tree falls create an environment different from the adjacent forest (Hubbell and Foster 1986), which influences plant regeneration (Brown 1993). In addition, gap processes partly determine forest structure and play an important role to maintain plant species richness (Denslow 1987; Schnitzer and Carson 2001). Thus, the creation of gaps in forests is an opportunity for the system to change in both species dynamics and ecological processes (Wright *et al*. 1998), by increasing environmental heterogeneity and altering abundances and distribution of abiotic and biotic resources (Denslow 1987), and it has been recognised to have a less severe impact on forest ecosystems than traditional management practices (Dai 1996). Differentiation of the species responses to gap sizes has significant implications for general model of forest dynamics (Canham *et al.* 1990; Marthews *et al*. 2008). It is often assumed that the increased levels of light and nutrients (Hartshorn 1978; Denslow 1980; Riddoch *et al*. 1991; Coates and Burton 1999; Gagnon *et al*. 2003) which occur as gaps are created, act as important factors controlling natural regeneration, which depends upon many other variables, including biological, chemical and physical properties of the seedbed (Paluch 2005). For studies on forest natural regeneration, measuring gap size is an important issue (van der Meer *et al.* 1999) to calculate the rotation cycle, related to regeneration patterns (Denslow 1987) because gap area influence the availability of resource such as light, water and nutrient which are critical for seedling establishment and growth. Thus, natural variation within and among gaps of different size results in highly variable responses in key of forest processes. The importance of small gap-forming disturbances has emerged as a common theme in research on forests dynamic and natural regeneration from a variety of forests worldwide (Lertzman 1992; Gray and Spies 1996; Mclaren and Janke 1996; Jennings *et al*. 1999; Franklin *et al*. 2002; de Römer *et al*. 2007). Gaps created by the natural process are generally small-scale and ephemeral (Spies and Franklin 1989), while in forests where stand-destroying disturbances are rare, the small-scale disturbances that create gaps play key roles on the development of forest structure and on forest floor conditions such as light regime, soil water content and litter decomposition (Brokaw 1987; Lertzman *et al*. 1996; Myers *et al.* 2000).

Re-naturalization of coniferous monoculture represents one of the main objectives of silviculturists also in many others European Country where in the past a lot of planta-

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Table 1 Stand characteristics of the experimental area located in artificial black pine stands in the Plaia Mountain, Central Apennines (Italy).

Area code	Altitude (m)	Exposure	Stand age (years)	Mean height (m)	D/H	Slope inclination (°)
1	830	O	50	14.1	1	17
2	830	O	50	13.9	0.75	17
3	840	O	90	18.2	1	25
4	850	O	90	17.3	0.75	24

tion have been introduced in denuded areas even in substitution of natural broadleaved stands (Heitz and Rehfuss 1999; Mosandl and Kussner 1999; Kenk and Guehne 2001; Malcolm *et al.* 2001; Diaci 2002; Zerbe 2002; Page and Cameron 2006; Dekker *et al.* 2007). These actions are justified on an economic and ecological point of view to obtain population with major functional and structural stability (von Lupke and Spellmann 1999; Utschig 1999).

Among the silvicultural practices proposed for the re-naturalization of conifer's afforestations, those related to the opening in the canopy cover have gained a great importance (Mercurio 2005, 2009).

The creation of gaps reproduces natural disturbance (Watt 1947; Pickett and White 1985; Whitmore 1982; Oldeman 1990; Runkle 1991; Coates and Burton 1997; Franklin *et al.* 2002; St-Denis *et al.* 2010), and consents to increase the heterogeneity of the habitat, the composition and the structural complexity of the population. The gap creation has been proposed as silvicultural practice for black pine stands whether to regenerate itself and/or other species. Preto (1983) experimented gaps with size no more than 300 m^2. Cucchi (1984) created in a black pine stand (Northern Apennine) gaps with dimension of 200-400 m^2 to favour the *Fagus* and *Abies* tree regeneration. In addition, Bernetti (1995) sustained that gaps should be done only to conserve the *Pinus* plantation. Malcom *et al.* (2001) suggested that the optimal dimension of opening to favour the regeneration of *Pinus laricio* are gaps with D:H in the range of 1-2.

Gugliotta *et al.* (2006) showed that in a *Pinus laricio* stand the creation of large gaps (1500 m^2) favours the affirmation of *Pinus laricio*; differently, the restoration of native tree species (i.e. beech and silver fir) may be favoured in smaller gaps (380 m^2). Thus, if the objective is the re-naturalization of coniferous monoculture, the gap opening may have a double meaning:

a) To create favourable conditions for affirmation and regeneration of the tree species.
b) To favour dynamic affirmation processes of other species that has already been established.

Understanding of how the regeneration of different tree species may be associated with the environmental conditions and soil properties that change between gaps and closed canopy, among gaps of different sizes and within individual gaps is important for silvicultural management (Gray and Spies 1996; Coates and Burton 1999). Although studies of gap dynamic processes are common in other forest ecosystems worldwide (Dahir and Lorimer 1996; Kneeshaw and Bergeron 1998; Parish and Antos 2004; Splechtna *et al.* 2005), gap processes have received little research attention in these forests.

The purpose of this paper is to assess whether black pine stands requires a specific canopy gap size for regeneration comparing seedling establishment in various sizes of canopy gaps.

Specific objectives were to (i) describe gap and soil characteristics; (ii) assess the status of tree regeneration in relation to gap size; and (iii) compare the relative abundance and species composition of potential successors to assess the dynamic of forest composition.

MATERIALS AND METHODS

Experimental sites

The study area was located in artificial black pine stands in the Plaia Mountain, Introdacqua (AQ), Italy (Central Apennine) (42° 01′ N; 13° 54′ E) at 830-850 m a.s.l. with a slope inclination of 17-25° (**Table 1**).

This area is characterized by mean annual precipitation of 746 mm, summer precipitation of 110 mm. Mean annual temperature is 11.8°C, mean temperature of the coldest month is 1.9°C, and mean temperature of the warmest month is 21.9°C. Soils, classified as Lithic Hapludoll (Soil Survey Staff 1998), are moderately deep (30-40 cm) and derived from calcareous bedrock. The natural forest vegetation is dominated by *Quercus pubescens* Willd. Following field observations the presence of insect population: *Haematoloma dorsatum*, *Matsucoccus pini*, *Leucaspis pusilla*, *Pissodes notatus*, *Tomicus piniperda*, *Traumatocampa pityocampa*, indicate the precarious healthy state of these black pine stands.

During late spring of 2000 the following were created:

-2 circular gaps removing all trees in a 50-year-old stand; the gap diameter (D, measured between stems edges) to the tree height (H) ratios for the two gap sizes were 0.75 and 1;

-2 circular gaps removing all trees in a 90-year-old stand; the gap diameter (D, measured between stems edges) to the tree height (H) ratios for the two gap sizes were 0.75 and 1.

Each gap was paired with an adjacent under canopy cover site located 25–30 m from the edge of the gap.

Natural regeneration

Thirteen circular sub-plots of 3.14 m^2 (100 cm radius) were established inside each gap. One sub-plot was positioned in the center of each gap and the others were located at one-third of the radius starting from the centre to the gap edge along the cardinal directions. All seedlings inside each sub-plot were identified by species and total height was measured. Census of seedlings in each sub-plot was conducted at the end of the summer 2002 and 2007.

Soil sampling and analysis

The changes in soil chemistry were studied in the 2007. Soil samples were collected in summer from the middle area of gaps and the adjacent forest sites. Three soil samples were taken from each site.

Undisturbed soil samples were taken by using a steel core sampler of a 100-cm^3 volume (5 cm in diameter and 5 cm in height). Prior to the soil analysis, all the soil samples were air-dried and sieved (<2 mm).

Particle-size analysis was carried out by the hydrometer method using sodium hexametaphosphate as a dispersant (Bouyoucos 1962); pH was measured in distilled water (solid:solution ratio 1: 2.5) with a glass electrode; organic carbon was estimated by the Walkley-Black procedure (Nelson and Sommers 1982), and it was converted to organic matter by multiplying the percentage of carbon by 1,72; total N was measured by the Kjeldahl method (Bremner and Mulvaney 1982) and cation exchange capacity (CEC) was determined by using the barium chloride-triethanolamine method (Mehlich 1953). Humic substances were extracted with 0.1 N NaOH (1: 10, w/v); the suspension was shaken for 16 h at room temperature and centrifuged at 5000 rpm for 30 min; the extract was dialysed in Wisking tubes against distilled water at pH 6.0. Subsequently, the solution was filtered through a column of Amberlite IR 120 H^+. The fractionation of humic substances was carried out as follows: aliquots of extracts were acidified to pH 2.0 with dilute H_2SO_4; the humic acids precipitated were removed by centrifugation, while the fulvic acids corresponded to the supernatants (Bettany *et al.* 1980). The C content of humic and fulvic acids was determined by dichromate oxidation (Nelson and Sommers 1982). Phenols were extracted with distilled water as this is

Table 2 Selected soil chemical properties in medium (1-3) and small (2-4) gaps and in the adjacent under canopy cover sites (5-8).

Soil sites	**pH (H_2O)**	**pH (KCl)**	**Organic Carbon %**	**Organic matter %**	**$CaCO_3$ ppm**	**K_2O ppm**	**N %**	**P_2O_5 ppm**	**C/N**	**CEC (cmol(+) kg^{-1} dry soil)**	**Textural Class**
1	7.1 (0.4) ab	6.0 (0.2) a	3.18 (0.4) d	5.46 (0.4) d	0	397 (4.0) a	4.2 (0.5) bc	7.2 (1.0) a	7.5	36.4 (0.9) c	SF
2	6.9 (0.2) b	5.9 (0.4) a	3.21 (0.3) d	5.52 (0.2) d	0	310 (3.0) c	4.0 (0.6) c	4.7 (0.5) b	8.0	35.9 (1.2) c	SF
3	7.0 (0.5) ab	5.9 (0.3) a	3.18 (0.2) d	5.29(0.4) d	0	382 (4.0) a	4.6 (1.0) a	6.8 (0.9) a	6.6	36.7 (0.9) b	SF
4	7.3 (0.2) a	6.2 (0.4) a	3.16 (0.3) d	5.44 (0.2) d	0	350 (4.0) b	4.2 (1.0) b	4.5(0.3) bc	7.5	36.6 (1.0) b	SF
5	6.9 (0.4) b	6.1 (0.3) a	3.45 (0.1) c	5.96 (0.5) c	0	259 (3.0) d	3.9 (0.9) c	4.2 (0.3) c	8.8	36.5 (0.8) b	SF
6	6.8 (0.5) b	6.1 (0.3) a	3.79 (0.3) b	6.52 (0.3) b	0	336 (2.0) b	4.5 (0.5) a	4.5(0.5) bc	8.4	38.7 (0.8) a	SF
7	6.9 (0.2) b	6.1 (0.2) a	3.94 (0.4) b	6.78 (0.2) b	0	225 (4.0) e	4.3 (0.7) b	4.4 (0.5) c	9.1	38.5 (0.9) a	SF
8	6.9 (0.3) b	5.9 (0.1) a	4.25 (0.5) a	7.90 (0.5) a	0	199 (3.0) f	4.4 (0.6) ab	4.6 (0.4) b	9.6	39.9 (1.2) a	SF

Numbers in parenthesis denote the standard deviation of the mean n= 6. Means in the same column with the same letter are not significantly different at $P \leq 0.05$.

Table 3 Distribution of humic substance fractions and phenolics in gaps of different size small (2-4) and medium (1-3) and in the adjacent under canopy cover sites (5-8). HC= humic carbon; FC= fulvic carbon.

Soil sites	**HC (%)**	**FC (%)**	**HC/FC**	**Total Phenol (g TAE g-1 dry soil)**	**Polyphenol (g TAE g-1 dry soil)**
1	2.16 (0.1) d	1.70 (0.3) c	1.27	350 (4.0) f	69 (1.0) c
2	2.49 (0.3) c	1.26 (0.2) d	1.97	450 (50) d	78 (0.9) b
3	2.10 (0.2) d	1.80 (0.1) c	1.16	395 (4.0) e	71 (1.0) c
4	2.80 (0.3) b	1.31 (0.1) d	2.14	450 (30) d	85 (0.8) a
5	3.17 (0.1) a	2.05 (0.3) ab	1.55	850 (4.0) c	77 (1.1) b
6	3.22 (0.2) a	2.11 (0.2) a	1.50	1250 (5.0) b	84 (1.0) a
7	3.10 (0.3) a	2.12 (0.3) a	1.46	1345 (6.0) a	77 (0.9) b
8	3.20 (0.3) a	1.96 (0.1) b	1.63	1377 (4.0) a	81 (1.0) ab

Numbers in parenthesis denote the standard deviation of the mean n= 6. Means in the same column with the same letter are not significantly different at $P \leq 0.05$.

the most realistic extractant in allelopathic studies (Kaminsky and Muller 1977, 1978). Thirty grams of dry weight sample were mixed in 200 mL distilled water and shaken at 75 rev min^{-1} for 20 h at room temperature. Water suspension was filtered through Whatman's No. 1 paper. Total water-soluble phenols were determined by using the Folin-Ciocalteus reagent, following the method of Box (1983), tannic acid was used as standard and the concentration of water-soluble phenolic compounds was expressed as tannic acid equivalents (μg TAE g^{-1} dry soil). Tannins were determined by adding the casein to the solution (1 mg mL^{-1}) and shaking the extracts additionally for 3 h. Casein is insoluble in water and binds polyphenols such as tannins (Seigler *et al.* 1986). After centrifugation and filtration (Whatman's No. 1 paper) the solution was again treated with Folin-Ciocalteus reagent and absorbance measured.

Statistical analysis

Soil data were subjected to analysis of variance (ANOVA) and treatment means were compared using the Student-Newman-Keul's test (Sokal and Rolhf 1995); seedling density data were subjected to the non-parametric Mann-Whitney U test (Zar 1999), using Statistica Software (Statsoft®, Inc. Tulsa, OK, USA).

RESULTS

No significant differences in soil physical characteristics, not only between gaps and under canopy cover sites, but also between gaps of different size were detected (**Table 2**). On the contrary, the data evidenced significant differences in soil chemical properties and organic matter trend, among gaps of different size and under canopy cover sites.

Soil pH was neutral in each site analyzed; under canopy cover sites, a greater amount of organic matter compared to the gaps was observed. No significant differences in organic matter content between the gaps of different sizes were detected (**Table 2**). The value of C/N ratio was higher under canopy cover sites compared to the gaps. Regarding the gaps, the highest value of C/N ratio was detected in the small gaps in the black pine stand of 50 years. The amount of P and K were higher in the gaps compared to the adjacent under canopy cover sites. The amount of total phenols and polyphenols varied among small (soil sites 2-4), medium gaps (soil sites 1-3) and under canopy cover sites (soil sites 5-8). The higher concentration was detected in soil under canopy cover sites, particularly in site 8. The gaps 2 and 4 had the greater concentrations of total phenols and polyphenols (**Table 3**).

Within under canopy cover site, the greater amount of humic and fulvic acids compared to the gaps was observed (**Table 3**). A great amount of humic acid was detected in gaps 1 and 3, a great amount of fulvic acids was detected in gaps 2 and 4.

The density of black pine seedlings was greater compared to the other species, even if the broadleaves were well established as shown by higher values of seedling mean height (**Figs. 1, 2**). Among the broadleaves pubescent oak (*Quercus pubescens* Willd.) and holm oak (*Quercus ilex* L.) were the species more representative, while hop-hornabeam (*Ostrya carpinifolia* Scop.) and manna-ash (*Fraxinus ornus* L.) were less present.

DISCUSSION

Environmental conditions, seed yield, pathogens and seedbed condition may potentially affect the seed germination, seedling emergence and establishment (Telewski and Jaffe 1981; Ogasawara and Ishibashi 1986; Gong *et al.* 1991; Futai and Nakai 1993; Zeng *et al.* 1996; van der Meer *et al.* 1999). Results from this observation indicated that black pine seedling density and growth (older than 1 year) increased significantly with increasing gap size. The establishment succeeded in medium gaps (D:H = 1) but failed in small gaps (D:H = 0.75) and under canopy cover sites. The micro-site conditions observed in this experiment may contribute to this trend. The amount of light received on the forest floor is directly related to the size of the canopy opening (van der Meer *et al.* 1999; Zhu *et al.* 2003).

In this study the better black pine seedlings establishment observed in medium gaps (area code 1) are likely to be the combined consequences of increased light with litter quick decomposition. Increasing the gap size, the competition for moisture and nutrient from surrounding mature trees in the gap area may decrease favouring seedling growth in the gaps (Madsen and Larsen 1997). According to Wang *et al.* (1998), regeneration in forest is controlled by above (light availability) and below (natural organic matter trend) environmental conditions.

Our results indicate that although black pine seeds are clearly able to germinate in the small gaps even under

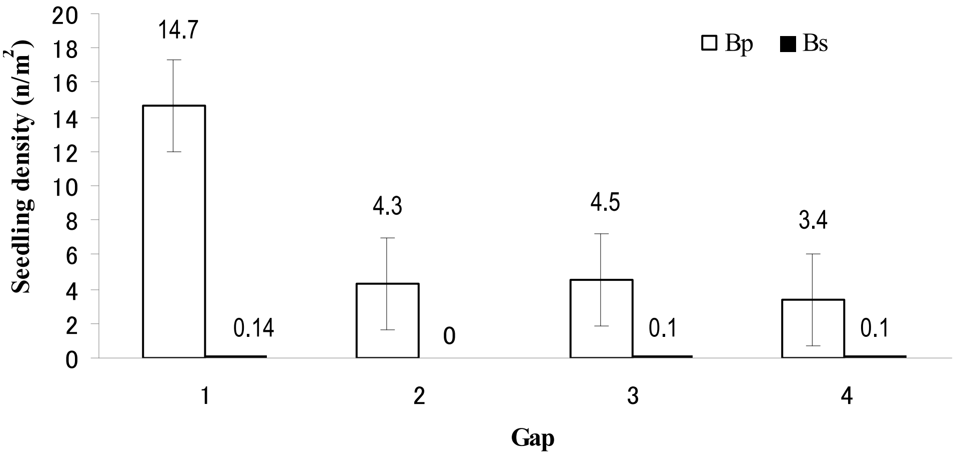

Fig. 1 Variation of black pine (Bp) and broadleaves (Bs) seedling density (seedling number/m^2) in the period 2002-2007. The bars represent the standard error of the means (n= 300 for black pine; n = 70 for broadleaves). Bars with the same letter above indicate no significant difference according to Tukey's test ($P < 0.05$).

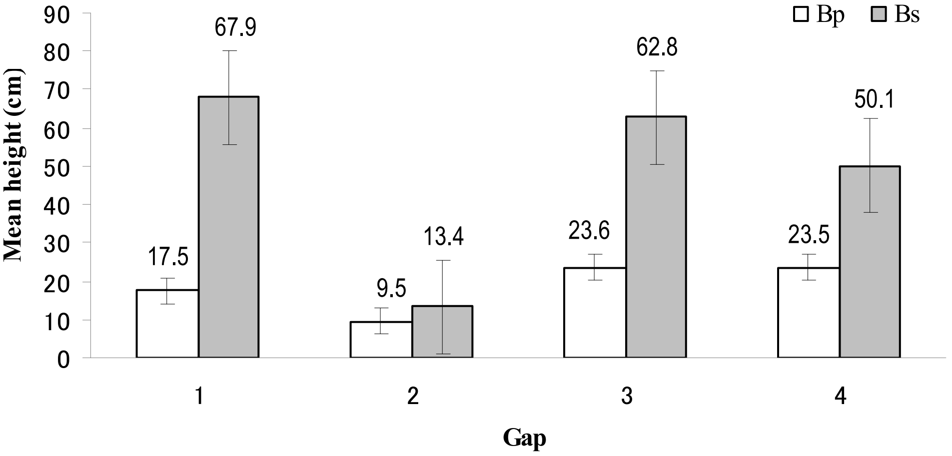

Fig. 2 Variation in the mean height (cm) of black pine (Bp) and broadleaves (Bs) seedlings in the period 2002-2007. The bars represent the standard error of the means (n= 300 for black pine; n = 70 for broadleaves). Bars with the same letter above indicate no significant difference according to Tukey's test ($P < 0.05$).

canopy cover sites, the seedlings apparently require a minimum canopy gap size D:H = 1 in order to survive and develop into seedling, confirming the view that black pine as other *Pinus* species is light-demand tree species (Ogasawara 1986; Murai *et al.* 1992) and also that coniferous species may survive in soil where the mineralization of organic matter prevails. Plant recruitment plays a central role in plant population and dynamic communities (Nathan and Ne'eman 2004). The early recruitment including germination, seedling growth and establishment is particularly important in vegetation dynamic and forest regeneration (Nathan and Muller-Landau 2000). These processes can be influenced by several ecological parameters (e.g. light, nutrients, water availability, soil organic matter, understory vegetation or predation) but also by the successional position of the species within the community (Broncano *et al.* 1998; Brienen *et al.* 2010). Moreover, those ecological factors may influence synergically plant regeneration but only few studies deal with plant recruitment and regeneration taking into account the concomitant effect of several parameters (Broncano *et al.* 1998; Utsugi *et al.* 2006; O'Brien *et al.* 2007). The most studied parameters influencing germination rate and seedlings establishment are environmental parameters such as light (Fuchs *et al.* 2000; Sonohat *et al.* 2004) and/or litter (Jensen and Gutekunst 2003; Janecek and Jan Lepš 2005). Chemically mediated interferences (allelopathy) are less studied even if they can influence plant recruitment by regulating inter and intraspecific interactions (Weir *et al.* 2004). The compounds produced by plants, such as phenol compounds or terpenoids, could inhibit the establishment of seedlings (Gallet 2010). Recent studies suggest the implication of this phenomenon in the establishment of invasive species (Bais *et al.* 2003; Vivanco *et al.* 2004) and in the dynamics in arid environments (Vila and Sardans 1999; Karageorgou *et al.* 2002). 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 *et al.* 2003) and they also can influence interspecific competition (Fernandez *et al.* 2006), and intra-inter-specific interaction.

The purpose of this study was to examine phenol toxicity as a functional process implicated in the natural regeneration.

In our study the different concentration of water soluble phenolic compounds may be the cause of the different black pine regeneration in the gaps of different size. We found in the gaps with D:H = 1 a minor amount of phenols compared to the other sites and this justify the major establishment of black pine seedlings (**Fig. 1**). The higher amount of phenols in gaps with D:H = 0.75 can result in an increase of phytotoxicity with inhibition of *Pinus* seedlings growth or delayed in germination that limit offspring also of the species from which they are produced (Falik *et al.* 2003). Toxicity has been observed in numerous conifer forests such as *Abies alba* Mill. (Becker and Drapier 1985), *Picea abies* L. Karst. (Pellissier 1994), or *Picea mariana* Mill. (Mallik and Newton 1988) but also in Mediterranean area with shrub species such as *Cistus albidus* L. (Robles *et al.* 1999) and *Cistus ladanifer* L. (Alias *et al.* 2006). For these reasons, allelopathy has been argued as a cause of conifer regeneration failure (Mallik 2003).

Allelopathy plays also an important role in forest ecosystem dynamic of different species (Caboun 2006), partially explaining the success or failure of seedling establishment and survival (Pellissier and Souto 1999) of broadleaves in coniferous forest. The present results suggest that phytotoxicity could be considered as one component of the complex interactions existing on regeneration failure in forest. Indeed we have observed that phenol toxicity limits (i) germination rate in earlier succession stage and (ii) seedling growth in late succession stage. Toxicity is coherent with low regeneration observed in black pine stands, and also with a late succession stage of the secondary succession. *Pinus* by its own allelochemicals and other factors generated by itself (such as litter) can greatly limit its natural regeneration and even more the regeneration of other species. Conclusive establishment of an allelopathic interaction is often a complicated issue, as the biological activities of putative allelopathins are governed by a number of different factors. Although it is expected that donor-plant allelopathins carried through the soil will undergo various abiotic and biotic processes before making contact with the roots of receptor plants, their ultimate effect is to inhibit the growth or functions of that receptor (An *et al.* 2001; Muscolo *et al.* 2005; Muscolo and Sidari 2006). The great amount of phenols in the different gaps explains, at least partially, the lack of the broadleaves species regeneration.

CONCLUDING REMARKS

The conclusion of this study have immediate implication for growth prediction of black pine, suggesting that the gap cutting system represents an effective instrument for the natural regeneration priming and a low environmental impact treatment for the re-naturalization of the conifers' monocultures.

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