

EFFECT OF SOIL SCARIFICATION ON NATURAL REGENERATION OF MATURE SCOTS PINE (*Pinus sylvestris*) STANDS IN GREECE

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ABSTRACT

The natural regeneration of Scots pine stands at the Sarakatsana location in the Pieria Mountains improved considerably after the removal of the dense ground vegetation followed mechanical soil scarification. The experimental design consisted of replications on scarified and non-scarified soil. The results four years after the experiment was set up showed that regeneration reached 59 seedlings m⁻² in scarified and 7 seedlings m⁻² in non-scarified soil. Soil scarification seems to have had a positive effect on the emergence and survival of naturally regenerated Scots pine. The negligible number of dead seedlings in the first two measurements could be due to the favourable site and/or good climatic conditions. Although the mother stand appears to be in a critical condition due to age and increased necrosis as a result of fungal attacks, there is still considerable potential for regeneration in terms of fructification and seed production. The study findings indicate that there can be new growth of Scots pine forest with the shelterwood method of natural regeneration in combination with soil scarification.

Keywords: Regeneration, Silviculture, Scarification, Scots Pine, Pieria Mt.

1. Introduction

Pure and mixed stands of Scots pine (*Pinus sylvestris* L.) occupy an area of 20.944 ha (Ministry of Agriculture, 1992). As a high altitude species, Scots pine usually forms the tree line in the Greek mountain ranges of Pieria, Vermio, Orvilos, Lailias and Rhodope which constitute the species' Southernmost limits of European distribution (Athanasiadis, 1986; Strid and Tan, 1997). Even though in Greece Scots pine is limited to specific areas, it is of considerable ecological and economic significance, particularly within the context of the global warming phenomenon with its adverse effect on forests (Koski, 1991; Loarie *et al.*, 2009). Climate change make the survival of Scots pine in these areas uncertain because there is no possibility of its shifting to an even higher altitude (Loarie *et al.*, 2009; Mason and Alías, 2000; Bergengren *et al.*, 2011; Groffman *et al.*, 2012; Grace *et al.*, 2002; Rebetz and Dobbartin, 2004; Kullman, 2007).

Many previous studies have shown significant relationships between the succession of natural regeneration and site factors as well as the mother stand (Tegelmark, 1998; Hille and Ouden, 2004; Hallikainen *et al.*, 2007). More specifically, soil scarification has proved to be particularly favourable for other species of the boreal coniferous zone, such as spruce, beech and birch (Huss and Burshcel, 1972; Dohrenbusch, 1997). In their study on the effects of forest fires on Scots pine regeneration, Kuuluvainen and Rouvinen's (2000), findings showed there was significant variation in the understorey tree

population in terms of abundance, quality and spatial pattern. They found that the time factor since the last fire plays a restrictive role on the establishment of new understorey trees. Rodrigo *et al.* (2004) found that Scots pine forests do not follow the direct regeneration pattern after fire but change to other situations mainly because the trees cannot keep their cones closed for years. This partial failure of direct regeneration in combination with climate change needs serious consideration for the regeneration of the Mediterranean Scots pine ecosystems after fire.

Natural regeneration is also affected by ground vegetation, which hinders, firstly, seedling roots coming into contact with moist soil and secondly, their access to nutrients (Béland *et al.*, 2000). In addition, Karlsson and Örländer, (2000) found in their study that the establishment of Scots pine regeneration is significantly affected when soil scarification occurs just before there is a rich seed fall. Hannerz *et al.*, (2002) report that soil scarification carried out in the spring, no later than mid-May creates a good seed bed for that year's seeds. Castro *et al.*, (2002) have analyzed the role of the herbaceous layer as a barrier mechanism that impedes the establishment of woody Scots pine seedlings, rather than acting competitively through biotic interactions. This occurs particularly in Mediterranean environments where, herbaceous ground vegetation loses most of its moisture before the achievement of seedling emergence. Scots pine distribution and regeneration in Mediterranean region is different to that of northern Europe. This distinction is determined by differences in climatic conditions and more specifically drought, which directly affects seedlings establishment (Castro *et al.*, 2004).

Over the last thirty years fungal attacks have caused dieback of Scots pine in the Pieria mountains (Diamandis and de Kam, 1986; Diamandis and Perlerou, 2003). The increasing necrotic phenomena along with the inability of old growth stands to regenerate naturally are of major concern the local Forest Service.

The aims of the present study were twofold: (1) to determine whether the treatment of soil scarification has a positive effect on natural regeneration in comparison to non-scarified soil in old and low density Scots pine stands; and (2) whether the spatial position of the disseminated seeds affects the emergence and the survival of the natural regeneration of Scots pine in the Pieria Mountains.

2. Material and methods

2.1. Experimental site and treatments

Our study site was in the Pieria Mountains at a location known as Sarakatsana (Figure 1), which is part of the state owned forest (40°16', 25'N, 22°12,51'E, alt. 1700 m a.s.l, slope 20% and the North aspect).

The mother stand had: mean breast height diameter (dbh) 71.3 cm, mean height 22.7 m, mean height to the base of live crown 8.5 m and mean green crown length 14.3 m. The number of stems were 60 per ha, the basal area was 29.2 m² ha⁻¹ and the under bark stocking volume was 233.04 m³ ha⁻¹. From the stumps of recently cut neighbouring trees, the age of the stand was estimated to be around 130 years. Ground cover degree was found at 0.17 and the canopy closure at 0.20. The mean projection of the crowns of the mother stems was 27.9 m². The cover of the herbaceous layer in the non-scarified soil was 98% while one year after scarification the respective ground cover was 15%. The species of the herbaceous vegetation in order of importance for each treatment were as follows:

Scarified soil: *Pinus sylvestris* (5%), *Festuca nigrescens ssp. nigrescens* Lam. (4%), *Deschampsia flexuosa* L. (4%) and other species with a smaller (<1%) participation.

Non-scarified soil: *Vaccinium myrtillus* L. (30%), *Deschampsia flexuosa* (20%), *Festuca nigrescens ssp. nigrescens* (15%), *Luzula luzuloides* (L.) (5%), *Myosotis sylvatica* Ehrh. (5%), *Cardus sp.* (5%), *Hydracium sp.* (2%), *Campanula sparsa* Friv. (2%) and other species with a smaller (<1%) participation.

Climatically, the area belongs to the mountainous zone with conditions that are similar to those of central Europe, i.e. there is a transition from Mediterranean to sub Mediterranean –temperate continental climate. Geologically, the area belongs to the Serbo-Macedonian zone, occupied by siliceous –Cristal schist rocks (gneiss) and soils of low productivity (Papamichos, 1979).

The experiment is organized in two treatments (scarified and non-scarified soil). Every treatment on the soil had dimensions of 10 x 40m with three replications (Figure 2).

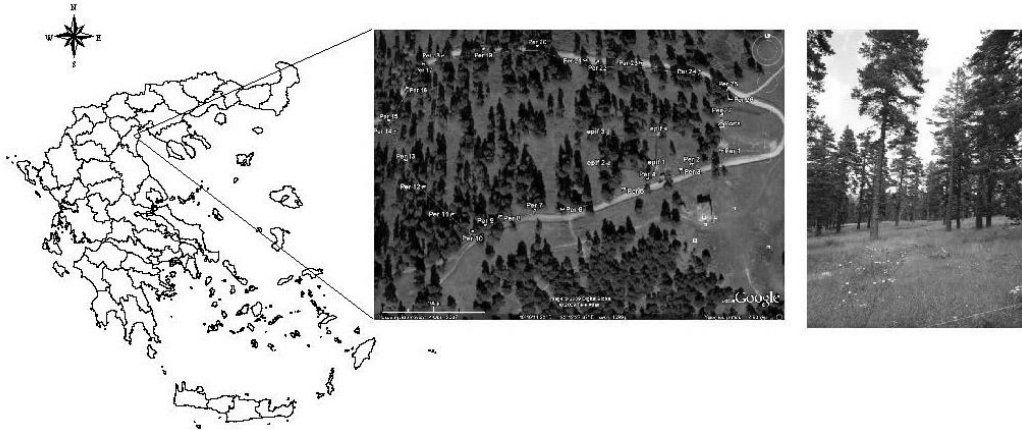


Figure 1. Map of the experimental plot of Scots pine at Pieria Mt. (location Sarakatsana).

Scarification was carried out in the autumn 2008 and comprised the mechanical removal of the surface organic cover, i.e. revealing the mineral soil parallel with the contours. The scarificator, a small bulldozer Caterpillar D7, created strips 2 m wide and 15 cm deep. An experimental area of 6.6 ha was fenced off with wire mesh to provide protection against livestock grazing.

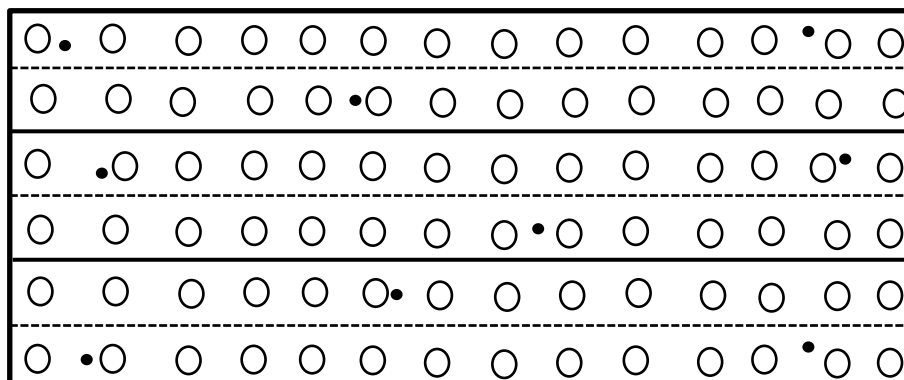


Figure 2. Layout of treatment plots and traps, black circles indicate seed traps 1 m², white circles indicate sample plots where seedlings were measured 1 m², dashed lines separate treatments (scarified/non-scarified soil) full bold horizontal lines separate repetitions.

2.2. Weather data

Meteorological data such as air temperature, precipitation and relative humidity were obtained from an electronic weather station that had been operated some years prior to the experiment 500 m Northeast from the study area (alt. 1670 m a.s.l, <http://meddman.agro.auth.gr/index.php?stationID=7>).

Temperature and precipitation were used to construct the climatic diagram for the vegetative periods 2009 - 2011 (Figure 3). The mean monthly precipitation in the vegetation period exhibits variations. Over the two year period, there was a sharp reduction in precipitation from 816 mm in 2009 to just over half (435 mm) in 2011. Figure 3 also shows that while there was no hot and dry period in 2009 but there was one for approximately one month in both 2011 and 2012, a phenomenon that is not normally

observed at such altitudes or in such ecosystems. The 2011 hot and dry period was succeeded the autumn season with monthly rainfalls that did not exceed 63 mm however, in 2009 and 2010, respective rainfall exceeded 270 mm each year. The same decreasing trend was recorded for mean monthly relative humidity in the vegetation period where it gradually decreased from 77.1% in 2009 to 74.1% and to 64.4% in 2011 and 2012.

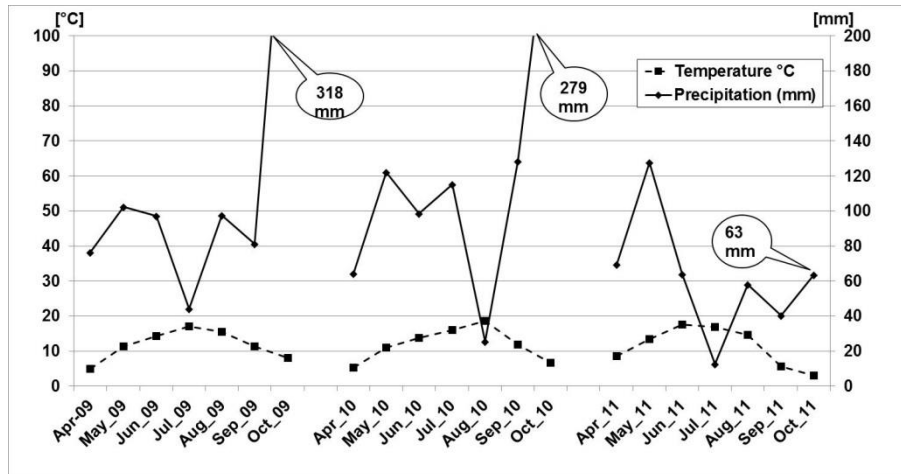


Figure 3. Climatic diagram of GAUSSEN – WALTER for the first (three) vegetation periods after scarification at the Sarakatsana location on the Pieria Mountain, 1700 m a.s.l (source: <http://meddman.agro. auth.gr/index.php?stationID=7>)

2.3. Natural regeneration data

Within each treatment plot, 16 circular sample plots were placed out systematically and their centers were marked with poles (Figure 2). The diameter of each plot was 1.128 m (area 1 m²). The number of live and dead seedlings was measured each summer following scarification (2009) until the end of 2012.

Seed traps of 1 m² each were set along three replications (3 traps for each replication) (Figure 2). The seed fall has been measured every year between 2010 and 2013.

2.4. Statistical analysis

Mixed ANOVA (GLM) with repeated measures was used to analyze and compare differences between groups (soil treatments, replications) and differences within groups (time of measurement) $p=0,05$ was chosen as the cut-off level for statistical significance (Page *et. al.*, 2003). The ANOVA general model was:

$$Y_{ij} = \mu + b_i + c_i + w_j + \varepsilon_{ij}$$

where Y_{ij} =observed quantity of seedlings, for group j in block i , μ = general mean, b_i = effect of soil treatment i , c_i = effect of repetition i , w_j = effect of time j , and ε_{ij} = random error. Results of the ANOVA are shown as $[F(df1;df2)=value, p=value \text{ when } p<0.05]$, where $df1$ = degrees of freedom for groups and $df2$ =degrees of freedom for random variation. All statistical analyses were done with SPSS, Release 17.

3. Results

3.1 Regeneration

3.1.1. Seed production

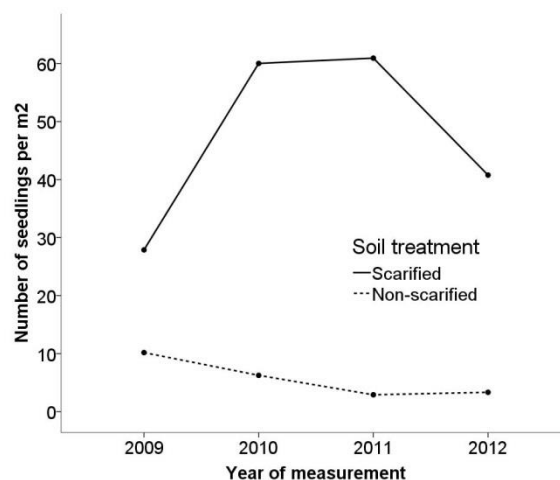
After the seedtraps were set up, a total of eight seedfall collections were carried out - two for each year (Table 1). The seedfall for 2011 was satisfactory in comparison to the other years. The 2011 germination test of seedfall showed that the germination ability was 29.6%, empty seeds 57.2% and dead seeds 13.2%. For 2013 the germination ability of the seeds was 66.3%, 29.1% were empty and 4.1% dead.

Table 1. Seed production for each seed trap in seeds m⁻²

	1	2	3	4	5	6	7	8	9	mean
2010	40	79	33	26	49	56	105	52	87	59
2011	107	135	90	80	100	109	211	110	94	115
2012	11	1	7	2	1	4	0	3	4	4
2013	48	55	42	39	38	47	112	39	44	52

3.1.2 Live seedlings

The analysis showed that the number of live seedlings in scarified soil follows a different evolution pattern in time compared to those in non-scarified soil. In the first year (2009), there was a mean value of 28 seedlings m⁻² in scarified and 10 seedlings m⁻² in non-scarified soil. In the second year (2010) the mean number of scots pine seedlings increased from 28 to 60 seedlings m⁻² in scarified soil but decreased from 10 to 6 seedlings m⁻² in non-scarified soil. In the third year (2011), the number of seedlings remained at the same level (61) and in the fourth year (2012) the mean decreased from 61 to 41 seedlings m⁻² in scarified soil, while in non-scarified soil from 6 seedlings m⁻² it continued to decrease to 3 seedlings m⁻². (Figure 4). There was a statistically significant main effect of soil treatment in Scots pine live seedlings [F(1;69)=189.06; p<0.001]. There was also a significant interaction effect between soil treatment and time of measurement [F(2.53;174.84)=40.28; p<0.001] and graphically depicted by the non-parallel curves in Figure 4. The values for the F criterion for the time effect but also for the time x soil treatment interaction resulted from the correction of the degrees of freedom of the F criterion multiplied by the Huynh-Feldt factor E (0.845) as the Mauchly's sphericity test was statistically significant [W=0.591; X² (5)=35.63 p<0.001] (Girden, 1992).

**Figure 4.** Effect of treatment and time of measurement in the mean values of live seedlings m⁻²

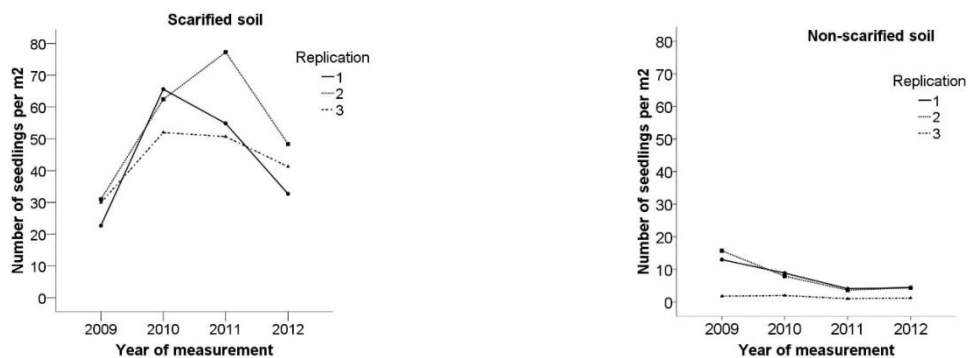
Taking into consideration the effect between subjects factor (replication), the number of live seedlings in the scarified soil follows the same as previous pattern in time compared to non-scarified soil. In scarified soil there was an increase in the regeneration measurements for the four years in all three replications. More specifically, in 2009 regeneration went up from 23 to 31 seedlings m⁻²; in 2010 from 52 to 66 seedlings m⁻²; in 2011 from 51 to 77 seedlings m⁻²; and in 2012 from 33 to 48 seedlings m⁻². Natural regeneration was less in non-scarified soil than in scarified with statistically significant differences in measurements for the four years (Table 2). More specifically, there was variation in natural regeneration in 2009 from 2 to 16 seedlings m⁻², in 2010 from 2 to 9 seedlings m⁻², and in 2011 and 2012 from 1 to 4 seedlings m⁻².

Table 2. Distribution of Scots pine seedlings m^{-2} on scarified and non-scarified soil for the three replications (mean values)

	Repetition I				Repetition II				Repetition III			
	Scarified soil		non-scarified		Scarified soil		non-scarified		Scarified soil		non-scarified	
	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	dead
2009	23**	2	13**	0	31**	0	16**	0	30**	0	2**	0
2010	66**	1	9**	0	63**	0	8**	0	52**	0	2**	0
2011	55**	2	4**	0	77**	2	4**	0	51**	0	1**	0
2012	33**	2	4**	0	48**	6	4**	0	41**	0	1**	0

** Denotes statistical significant differences at $\alpha=0,05$ between scarified and non-scarified soil in the four years

Figure 5 shows that there are no significant differences among the three replications $F(2;69) = 2.75$; $p=0.071$, but there was significant interaction between time of measurement and replications $F(5.07; 174.84)=3.48$; $p<0.001$. In addition, it can be seen that the time of measurement x soil treatment x repetition interaction was significant $F(5.07; 174.84)=3,48$; $p<0.001$, depicted by the non-parallel curves in Figure 5. The values for the F criterion for the two and three way interactions resulted from the correction of the degrees of freedom of the F criterion multiplied by the Huynh-Feldt factor E (0.845) as the Mauchly's sphericity test was statistically significant [$W=0.591$; $X^2(5)=35.63$; $p<0.001$] (Girden, 1992).

**Figure 5.** Effect of treatment, replication and time of measurement of live seedlings m^{-2} in mean values

3.1.3 Dead seedlings

The number of dead seedlings in scarified soil followed a different evolution pattern in time compared to non-scarified soil. More specifically, in Figure 6, in 2009 and 2010 there was less than 1 m^{-2} of dead seedlings in scarified soil and 0 m^{-2} in non-scarified. In 2011 and 2012, there were 3 m^{-2} of dead seedlings in scarified soil, while in non-scarified it remained the same at 0 m^{-2} . There was a significant main effect of soil treatment on dead seedlings [$F(1;69)=49.14$; $p<0.001$]. There was also a significant main effect of time of measurement on dead seedlings [$F(1.96;135.32)=19.85$; $p<0.001$]. There was significant interaction between time of measurement and soil treatment [$F(1.96;135.32)=18.79$; $p<0.001$], indicated by the non-parallel lines in Figure 6. The values for the F criterion for the time effect but also for the time x soil treatment interaction resulted from the correction of the degrees of freedom of the F criterion multiplied by the Huynh-Feldt factor E (0.654) as the Mauchly's sphericity test was statistically significant [$W=0.144$; $X^2(5)=131.38$ $p<0.001$].

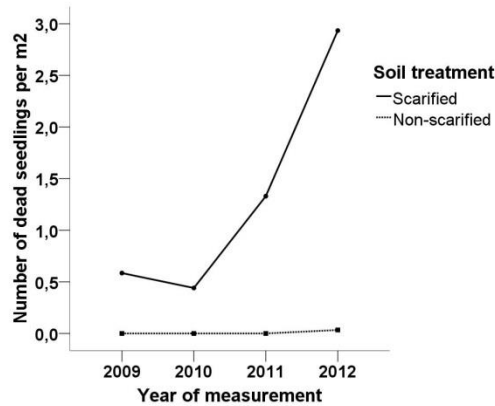


Figure 6. Effect of treatment and time of measurement of dead seedlings per m² in mean values

It can be clearly seen in Figure 7 that there were no dead seedlings in the non-scarified soil in the time period of the study. However, the same figure shows that there was an increase in dead seeds in scarified soil in these four years. More specifically, in 2009 and 2010 the dead seedlings from 0 m⁻² rose to 2 m⁻² and 1 m⁻² respectively; whereas in 2011 and 2012 from 0.5 m⁻² they reached 6 m⁻² and 5 m⁻² respectively. These measurements were statistically significant for the entire time period. More specifically, there was a significant interaction effect among replications [F(2;69) = 8.77; p<0.001], a double interaction effect of time of measurement and replications [F(3.9; 135.32)=10.23; p<0.001] and a triple interaction effect among time of measurement, replications and soil treatment [F(3.9; 135.,32)=10.7; p<0,001]. The values for the F criterion for the time effect but also for the time x soil treatment interaction resulted from the correction of the degrees of freedom of the F criterion multiplied by the Huynh-Feldt factor E (0.654) as the Mauchly's sphericity test was statistically significant [W=0.144; X²(5)=131.38 p<0.001].

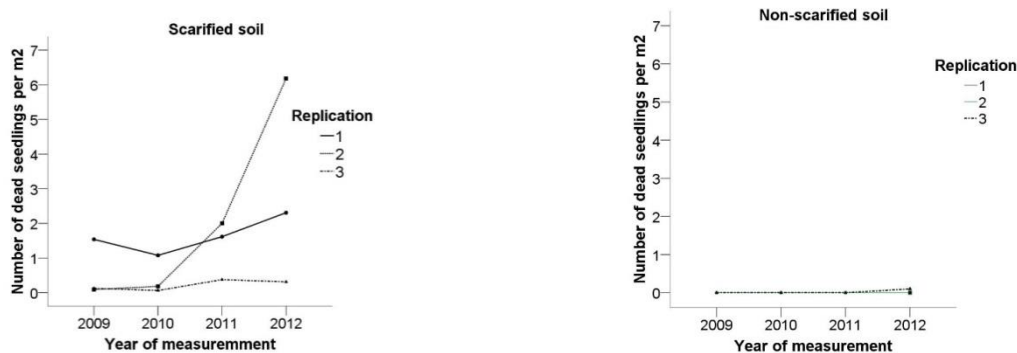


Figure 7. Effect of treatment, replication and time of measurement of dead seedlings m⁻² in mean values

The difference in evolution patterns in the number of dead seedlings is sharper in the second replication than in either the first or the third. The reason for this is due to the fact that there is less protection from the mother stand. The mean canopy closure in the three replications are 45%, 5% and 27% respectively.

4. Discussion

The Scots pine forest at the Sarakatsana location has a number of particular ecological and silvicultural characteristics, which make it warrant attention. These include: it is located in the southernmost limits of its geographic distribution in Europe, its structure which is one-storey, even aged and of low density; its critical health status because of dieback effects and the absence of natural regeneration.

The old growth Scots pine forest at the Sarakatsa location in the Pieria Mountains is around 130 years old and although it has potential, there is an absence of regeneration for a couple of important reasons. Intensive grazing in the past and present and the thick-dense ground vegetation layer of *Vaccinium myrtillus* prevent natural regeneration.

These results from the large and statistically significant difference of living Scots pine seedlings between scarified and non-scarified soil but as well as from the regeneration observed on the sides of the forest roads.

The experimental area was fenced off to exclude livestock grazing, which is well-known to have been extensive in these forests for the past decades. Those that are not grazed present a small increment (Zamora *et al.*, 1999). Significant seed losses have also been observed due to birds (Nystrand & Granström, 1997). Fencing of the study area coupled with the application of soil treatment seem to have had a positive effect on the emergence, density and early evolution of natural regeneration.

Therefore, soil scarification is clearly favourable for the establishment of Scots pine, provided that it is carried out at the appropriate time of year, namely, early autumn in a full seed year prior to seedfall, as was the case in this study. The combination of soil treatment and favourable weather conditions resulted in two years of good seed production, which had the effect of the successful emergence and establishment of regeneration. The number of live seedlings after four measurements taken in four years in the scarified soil exhibit statistically significant differences between and within the main effects of subjects and their associated interactions. This can be explained by the different amount of seed production of the mother trees, the spatial position of the replications (relief, stand density) which received different amounts of seeds from side seed rain, and finally the weather conditions during the vegetation period from April to October (Figure 2). There was a gradual but marked decrease in the relative humidity during the vegetation period (April – October), which from 77.1% in 2009 fell to 74.1% in 2011 only to exhibit a further large drop to reach 64.4% in 2012. In addition, during the vegetation period there was a large fluctuation in the mean monthly precipitation (Figure 3). In 2011, there was almost a 50% reduction in precipitation in comparison to the two previous years. This hot and dry period in 2011 was followed by autumn rainfalls that did not exceed 63 mm.

After the successful emergence of the Scots pine natural regeneration in the first two years (Figure 5) and despite the fact that 2011 was a good seed production year in terms of the number of seeds produced (Table 1) over half (57,2%) of the disseminated seeds found to be empty after a laboratory germination test. This meant that there were far fewer new seedlings in 2012, and which had an increased mortality due to the dry summer and autumn periods of the previous year. In the non-scarified soil, there was a progressive decrease in the number of live seedlings, which showed a different evolution pattern to those in scarified soil. The dry weather conditions in both 2010 and 2011 negatively affected the dead organic matter and ground vegetation of the soil surface which meant that the Scots pine seeds which had reached the forest floor were not able to develop enough root in order to penetrate the thick ground vegetation layer and reach the mineral soil.

In the years 2010 and 2011, there was an increased number of dead seedlings, particularly in scarified soil (Figures 6 & 7). In the non-scarified soil, dead seedlings were not measured as they were next to impossible to detect in such dense ground vegetation.

The small number of dead seedlings that was measured during the first two measurements may be due to favorable environment, in terms of good site, stand and weather conditions, which enhanced the seedlings regeneration emergence and survival. However it may also be due to the difficult summer conditions prevailing those two summers which may result in large number of seedlings have been decaying and having disappearing until the next annual regeneration record.

There were similar findings in other comparable research. According to a study by Apatsidis (1977), soil treatment in the form of circular plots ensures favourable germination conditions for black pine seeds as well as the survival of a sufficient number of seedlings. Zagas (1990), reports that in Scots pine stands, in the Rhodope Mountains, natural regeneration was possible for all site qualities with the exception of those found at the lowest site quality, provided that all the appropriate silvicultural treatments were

applied, such as the removal of ground vegetation. In their study on black pine stands Vergos *et al.*, (1994) reported that the establishment and survival of natural regeneration in aged and poor site quality black pine stands was significantly enhanced by soil that had undergone some kind of treatment.

González-Martínez and Bravo, (2001) in their work on the density and structure of natural regeneration of Scots pine in the high basin of the Ebro River report that the ground vegetation is the natural barrier that prevents the Scots pine seeds to come into contact with the mineral soil and creates strong competition with the seedlings, also grazing changes the vegetation composition and has a negative impact on the establishment and development of seedlings. They conclude that fencing of stands under regeneration is the only reliable long-term measure to ensure the development of natural regeneration. Moreover, in their study on the regeneration of Scots pine, Béland *et al.*, (2000) found that after four years in low-intensity shelterwood cut (mother stand with 200 stems ha⁻¹) in scarified soil, there were more than 80,000 ha⁻¹ seedlings, there in clear cut and scarified soil up to 7,000 seedlings ha⁻¹ were found. They also referred to the important role that the mother stand plays in the number of seeds reaching the forest floor. Thus, the denser the mother stand, the greater the number of seeds that reach the scarified soil. There is also a smaller invasion of the competitive ground vegetation, resulting in prolonged regeneration and small mortality of seedlings.

5. Conclusions

The conclusions that can be drawn from this study are: The condition of the mother stand is critical in terms of health status, but fortunately there is still considerable regeneration potential such as the number of parent trees and fruiting capacity. All Scots pine stands in the Pieria mountains with these specific structural characteristics should be progressively fenced and regenerated by the shelterwood method, combined with soil scarification. It is important to carry out the soil treatment in autumn, after a full seed year and when weather conditions are favourable.

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