Biodivers Conserv (2008) 17:2427–2439
DOI 10.1007/s10531-008-9390-8

ORIGINAL PAPER

Seed and cone diversity and seed germination of *Pinus pinea* in Strofylia Site of the Natura 2000 Network

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Received: 9 November 2007 / Accepted: 1 April 2008 / Published online: 18 April 2008
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Abstract Variation in cone size, seed number per cone, seed potential, seed efficiency, seed morphology and seed germination behavior of *Pinus pinea* and its relation to stand conditions was analyzed. Data were collected from *P. pinea* forest in Strofylia, southern Greece, a forest that belongs to the Natura 2000 European network and the RAMSAR convention and is characterized by the absence of regeneration for many decades. The pine stands found in the area were distinguished into five categories according to a previous study and our observations, regarding stand age, canopy cover and the degree of stress by human pressure. The categories are: (I) young artificial, (II) closed-mature, (III) open-mature, (IV) over-mature stands and (V) highly degraded stands. Cones were collected from all stand types and their morphological characteristics as well as their seed production were measured. Seeds were extracted from the collected cones, measured and their germination behavior was tested. The findings showed that the over-mature and the high degraded stands and to a lesser extent, the closed-mature stands, produced significantly smaller cones with a lower seed potential, a lower number of filled seeds per cone, a greater number of not fully developed seeds and reduced seed morphological characteristics than the young and open-mature stands. However, the seed germination behavior of fully developed seeds was only slightly affected by the stand type.

Keywords Cone variation · Natural regeneration · Nature conservation · Seed efficiency · Seed germination · Seed morphology · Umbrella pine

Introduction

Pinus pinea (Umbrella pine) is a Mediterranean tree species found along the Mediterranean basin. The ecological, aesthetic and economic value of the species and its ability to

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withstand low intensity fires (Tapias et al. 2004) make this species very important for multipurpose forestry and for nature conservation. The species habitat is included in the Annex I of the Directive 92/43 of the European Union.

Strofylia forest, southern Greece, is the main *P. pinea* forest in Greece that represents 80% of the total *P. pinea* forests in the country. Another important *P. pinea* forest, in the Lake Kaiafa, in Peloponnese, has been greatly damaged by wildfire in the last summer. Strofylia forest is located within a wide system of dunes and wetlands that belongs to the RAMSAR convention, as well as to the NATURA 2000 European network and to the Special Protected Areas for birds. However, absence of natural regeneration of *P. pinea* has been observed for many decades, and this seems to contribute to a forest succession and the gradual replacement of *P. pinea* by the species *Pinus halepensis* (Papamichos 1986; Moussouris and Regato 1999). Such a phenomenon occurs in many cases along the Meditteranean basin (Barbero et al. 1998; Tapias et al. 2004). Regeneration absence of *P. pinea* may result from insufficient supply or low quality of the seeds produced by mother trees. However, failure to produce seeds may result from limited pollination or resources, and there is little consensus regarding their relative importance in natural systems (Lawerence 1993; Ramsey 1997; Bazzaz et al. 2000).

P. pinea is considered to be a species easily propagated by seeds (Ganatsas and Tsakaldimi 2007). Cone production begins late, at the age of 15–20 years, compared to the other Mediterranean pines (10–15 years or earlier for *Pinus halepensis, Pinus brutia, Pinus pinaster*) (Thanos and Daskalakou 2000; Tapias et al. 2001; Zagas et al. 2004). The cones ripen in April, 3 years after pollination (the longest maturation period of any pine, Frankis 1999); they are not serotinous and open at low temperatures (28°C or less) (Tapias et al. 2001). As a result, *P. pinea* distribution is reduced by wildfires (Rodrigo et al. 2007), and it seems unlikely that *P. pinea* forests will recover to their pre-fire state; therefore, action is recommended to restore their ecosystems (Perula et al. 2003).

However, little is known about variation in seed morphology and quality of *P. pinea* and how these may be modified by the environment or stand conditions. Generally, reports on inter or within population variation in seed characteristics and germination behavior and their correlations with environmental or mother tree variables are limited in the literature (Zhang 1998; Baskin and Baskin 1998), even though it has been reported that stand parameters such as mother tree age (Connor and Lander 1991), crown size (Shearer 1986) and site conditions (Sorensen and Miles 1978; Stoehr 2000) influence seed characteristics of tree species. For example, Court-Picon et al. (2004) found a great variability in seed weight among the different provenances of *P. pinea*. Skordilis and Thanos (1997) and Escudero et al. (2002) also reported a high variability in seed germination of *P. pinea* that depends on environmental conditions and population variability. Some of this variation can be of genetic origin, but much of it is known to be environmental (Mamo et al. 2006).

In this study, we present the research referenced to *P. pinea* forest in Strofylia. The potential connection between the variation in cone, seed potential, seed number per cone, seed efficiency, seed morphological characteristics, seed germination and the stand parameters (age, structure, canopy cover and degradation degree) was studied. The hypothesis was that there would be a significant effect of the ecological conditions of the mother trees on seed morphology and physiology, and thus, the analysis of this effect could contribute to a better understanding of the natural process of forest succession in the area and will help in taking the appropriate silvicultural measures in order to conserve this important species habitat.

Materials and methods

Study area

The *P. pinea* forest of Strofylia is located in the western Peleponnesos, southern Greece. The forest covers a north-south coastal strip with a length of 15 km and an average width of 1.5 km. *P. halepensis* and *Quercus aegilops* are other important tree species of the Strofylia forest (Georgiadis et al. 1990). All the area has been subjected to high touristic pressure resulting in thousands of people visiting the area every summer; however, this pressure is uniformly distributed throughout the area. Overgrazing also contributes to *P. pinea* habitat deterioration. The population of *P. pinea* in the area occupies rather uniform site conditions; the whole area is characterized by flat surfaces lying on sandy-dune formations, at an altitude of a few meters (1–5 m) above sea level. The soils appear to have sandy to loamy sand texture with a high water table level (Papamichos 1986).

Cone and seed data collection

Collection of the cones was carried out in late April of the years 2003 and 2005. Cones were collected from the five different types of stand that are met in the forest (Papamichos and Alifragis 1986): (I) young artificial, (II) closed-mature, (III) open-mature, (IV) over-mature and (V) highly degraded. The young artificial stands were established by the local Forest Service from seedlings produced from seeds of local origin, while the mature stands are considered natural (Georgiadis et al. 1990), even though there is a great debate about the species origin throughout the world (Martinez and Montero 2004). The V category concerns a great part of the forest, mainly the stands along the roads, which is highly degraded due to a high touristic pressure. In this part of the forest, the stands are highly depressed by human activities (picnic, camping, walking, games etc.), as well as by overgrazing. These actions cause severe compaction of the soil surface (Papamichos 1986) which may stress tree physiological development and inhibit both seed germination and seedling growth (Kozlowski 2002). Figure 1 shows the distribution of stand types in the area. In order to document the above stands type category, three plots of 0.1 ha (25 m × 40 m) were selected in each stand type for stand data collection, except in the case of young artificial stands where the plot size was 0.01 ha (10 m × 10 m). All the trees within the plots were measured; the measurements included diameter at breast height (DBH), height (H), stand density and total canopy cover. The age of the trees was estimated based on historical data as well as by tree ring measurements; three tree cores from each stand type were taken and they were transported to the laboratory where their annual rings were determined. Human impact (overgrazing, walking, picnic, games, camping) in each stand type was estimated by visual observations as well as using the available data of the local Forest Service.

Ten dominant and semi-dominant trees were selected from each stand type; these were randomly selected at a distance of at least 50 m (Dangasuk and Panetsos 2004) and they should bear at least 10 mature closed cones. Five cones per tree were collected in each year (2003 and 2005). In the laboratory, the length and the width (at the widest point) of the cones were measured (Johnson et al. 2003) with vernier calipers. Afterwards, the cones were put under the sun to open; the seeds were extracted and stored at 6°C until further analysis. All cones were completely dissected to determine seed yields per cone (Stoehr 2000). Seed potential (number of fertile scales × 2) was estimated in all collected cones. Then, the seeds were distinguished into filled and not fully developed ones based on their size (length > 12 mm and length < 12 mm, respectively). Damaged or not well-developed

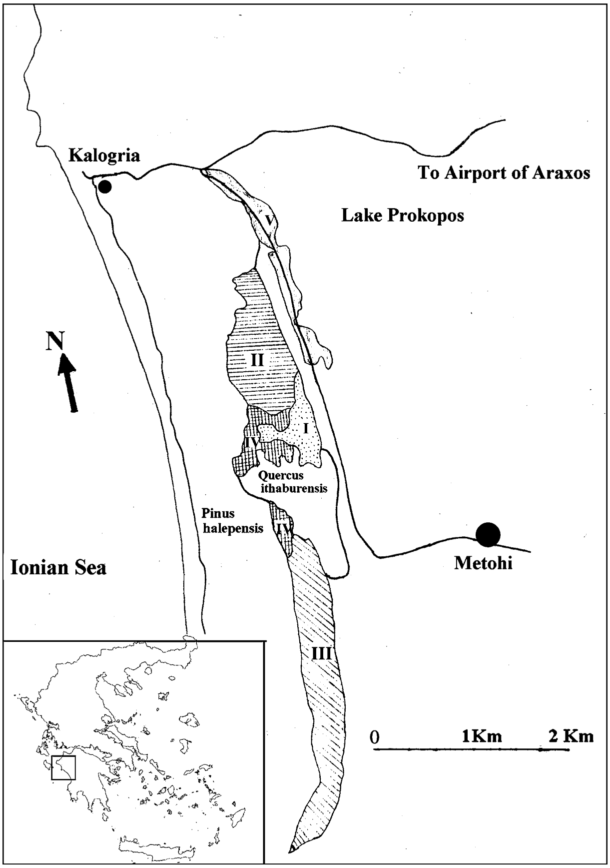

Fig. 1 Map of the area where the distribution of the stand types tested is shown; (I) young artificial, (II) closed-mature, (III) open-mature, (IV) over-mature and (V) highly degraded stands

seeds were recorded as well as the seed efficiency (number of filled seeds/seed potential) (Turgeon et al. 2004). Seeds were immersed in water and any floating seeds were removed. Size of the filled seeds was measured with vernier calipers and their weight was determined individually using an analytical balance (±0.001 mg). Length of wing was measured with vernier calipers. All seed measurements were carried out in a random sample of 5 seeds per cone. However, three replications of ten not-fully developed seeds were subjected to germination test. One month later, none of these seeds had germinated. Cone total dry weight was measured after oven-drying 30 cones per stand type, in 72°C for 48 h, while the total seed weight per cone was separately recorded. In order to estimate the investment on seeds, the total seed weight per cone was computed as percentage of the total cone weight.

For germination tests one hundred (100) filled seeds randomly selected from each stand type (of 2003 collection) were divided into 10 replicates of 10 seeds each. Prior to germination the seeds were dusted with fungicide (Captan) in order to prevent fungal infection. Germination tests were performed with these seed batches in glass petri dishes (diameter 9 cm, lined with two sheets of filter paper and moistened with deionized water). In the petri dishes the seeds were separated from each other in order to reduce the chance of cross contamination by micro-flora. Experiments were carried out in a plant growth chamber under continuous light with a photon flux density of 55–65 $\mu E \cdot m^{-2} \cdot s^{-1}$, where the temperature was kept constant at 20 ± 1°C; this temperature value is suggested from International Seed Testing Association, International Seed Testing Association (1993) for *P. pinea* laboratory germination trials and seedling production in the nurseries. Germination was recorded every two days and was considered complete when no additional seeds germinated. The criterion of germination was radicle protrusion through the seed coat (Skordilis and Thanos 1997; Saeed and Thanos 1998; Raccuia et al. 2004). The measurements taken were: germination energy (the early count), mean time to complete germination (MGT) and the germination percentages (every two days germination and total germination). Germination percentage was calculated as the ratio between the number of germinated seeds at a given time and the number of seeds sown (ISTA 1993). MGT was calculated as follows (Hartmann et al. 1997; Raccuia et al. 2004): MGT = $(N_1T_1 + N_2T_2 + \cdots + N_xT_x)$/total number of seeds germinated, where N_i = number of seeds germinating within consecutive intervals of time and T_i = the time between the beginning of the test and the end of the particular interval of measurement.

After germination, the germinated seeds were removed from the petri dishes and planted in Quick-pots trays (cell depth 75 mm and cell volume 75 ml) filled with sand. The trays were put in another plant growth chamber under similar conditions and the plants watered with deionized water as needed. When the seed cell dropped we measured the number of cotyledons as well as the cotyledon length.

Statistical analysis

In order to determine the differences in cone and seed traits, among the stand types studied, ANOVA analysis was conducted (SPSS software; SPSS Inc., Chicago, IL. USA) and the Waller–Duncan criterion was used for comparison of the means. Distribution was tested for normality by Kolmogorov-Smirnov criterion and the homogeneity of variances was tested by Levene's test. The percentages were transformed to arsine square root values, before analysis (Snedecor and Cochran 1988; Norusis 1994).

Results

Stand conditions

Despite the similar site conditions, the *P. pinea* stands present great differences in their characteristics (Table 1). They differ in their age; based on the tree ring measurements, the stands were distinguished into three categories; (a) mature stands (approximately 120-years old) that have entered the late phase of maturity, (b) over-mature stands (over 135-years old) that form an open woodland and (c) artificial young stands (25-years old) that cover a relative small area of the forest. Apart from the age differences, the stands greatly differ in their canopy cover. Thus, based on the canopy cover the mature stands were distinguished into two categories: a) closed stands (70–80% canopy cover) and b) open stands (50–60% canopy cover). While, the highly degraded stands, which are also mature stands, are approximately 120 years old, and have a lower canopy cover (30–50%) and stand density (30–50 trees per hectare). However, the five stand types met in the area are all even-aged and the mean diameter (DBH) and height (H) of their trees are shown in the Table 1.

Table 1 Characteristics of the studied *P. pinea* stands

Stand type	Stand type code	Stand structure	Age (years)	Stand density (N/ha)	Canopy cover (%)	DBH (cm)	H (m)	Important human impact
Young artificial	I	Even-aged	25	1800–2000	80	22.9 (0.68)	7.9 (0.08)	Overgrazing
Closed-mature	II	Even-aged	120	100–140	70–80	55.6 (2.54)	18.3 (0.49)	Overgrazing
Open-mature	III	Even-aged	120	70–80	50–60	57.8 (2.63)	17.6 (0.38)	Overgrazing
Over-mature	IV	Even-aged	135+	30–50	30–50	88.1 (3.11)	15.2 (0.44)	Overgrazing
Highly degraded	V	Even-aged	120	30–50	30–50	48.6 (3.23)	16.3 (0.62)	Picnic, camping, walking, games, overgrazing

Values in DBH and H are the mean and the standard error of the mean (in parenthesis)

Cone size and seed production

Statistical analysis showed that there is quite a high variation in cone morphological characteristics. Cones from the young artificial stands are significantly greater and heavier than those of the other four stand types (Table 2). In contrast, the over-mature and the degraded stands produce significantly smaller cones. Also, the cones of the young and open-mature stands have significantly higher seed potential values accompanied by a higher number of filled seeds per cone (98.7 and 92.9 seeds per cone, respectively), than that of the other three stand types. Seed efficiency is also high in these stand types. Total seed weight per cone is high in the young stands and low in the highly degraded and over-mature stands. Seed investment is relatively high in open-mature and young artificial stands, and low in the closed-mature and over-mature stands. It must be noticed that the number of seeds per cone is fairly constant in the young and open-mature stands, while in the over-mature stands as well as in the highly degraded stands the cones exhibit high variability in seed number per cone (Table 2). The low seed efficiency of over-mature stands is due to the fact that a great number of seeds are not fully developed; 14.7 seeds per cone were not fully developed (26.8% of the total number of seeds). However, the percentage of empty seeds was low in all cases and varied from 3.51 to 6.49%, with no significant differences between the stand types. Based on the overall cone characteristics it seems that the young artificial stands achieve the best results, having larger and heavier cones with high seed potential, seed efficiency, total seed weight and number of filled seeds, followed by the open-mature stands.

Seed morphological characteristics

Cone size variation resulted in seed morphological variability; the smaller cones produced by the over-mature and degraded stands included significantly smaller seeds. As a result statistically significant differences were found among the stand types tested in seed morphometric characteristics (Table 3). The average seed length was found to be significantly greater in the young artificial stands (17.7 mm), followed by mature (closed and open) stands (16.7, 16.4 mm, respectively), while the degraded and over-mature stands produced the shortest seeds (15.9 and 16.0 mm, respectively). However, the seed width did

Table 2 Cone morphological characteristics and seed production

Stand type	Cone length (mm)	Cone width (mm)	Seed potential	Number of filled seeds per cone	Min-Max	Number of not fully developed seeds per cone	Percentage of empty seeds (%)	Seed efficiency (%)	Total cone weight (g)	Total seed weight per cone (g)	Seed investment (%)
Young artificial	91.1(2.1)a	73.0(1.0)a	109.3(1.8)a	98.7(2.3)a	65–121	2.2(0.6)c	4.89(0.3)ns	90.0(1.2)a	162.5(8.1)a	57.1(3.0)a	35.1(0.7)ab
Closed-mature	83.3(1.4)b	67.9(0.9)b	94.5(1.2)b	71.3(1.9)b	48–94	5.7(1.2) b	3.78(0.1)ns	75.6(1.9)bc	127.2(4.9)b	39.3(1.6)bc	30.9(0.5)c
Open-mature	83.8(1.2)b	67.9(0.8)b	108.0(1.7)a	92.9(1.9)a	63–115	2.2(0.3)c	5.33(0.8)ns	85.9(1.0)ab	118.5(5.0)b	43.9(1.6)b	37.3(0.6)a
Over-mature	76.8(1.5)c	62.8(1.0)c	93.8(1.7)b	65.2(3.1)b	18–105	12.4(0.8)a	3.51(0.6)ns	68.0(2.4)c	117.1(6.4)b	36.4(2.8)bc	30.2(0.8)c
Highly degraded	74.6(1.5)c	62.4(0.8)c	84.0(2.2)b	69.3(3.3)b	32–91	6.0(0.9)b	6.49(1.0)ns	82.0(2.8)ab	101.2(4.8)c	33.6(2.1)c	32.8(0.9)bc

Values are the mean and the standard error of the mean (in parenthesis) of 100 cones (50 for each year per stand type), except for the total cone weight and total seed weight per cone where N = 30

Values within the same column followed by a different letter are significantly different ($P < 0.05$, Waller–Duncan test)

ns: non significant differences

Table 3 Seed morphological characteristics of the five stand types studied

Stand type	Seed morphological characteristics					
	Length (mm)	Width (mm)	Weight (g)	Wing length (mm)	Number of cotyledons	Cotyledons' length (cm)
Young artificial	17.7(0.1)a	9.2(0.1)ns	0.81(0.02)a	6.6(0.5)a	12.0(0.3)a	4.23(0.30)ns
Closed-mature	16.7(0.2)b	9.1(0.1)ns	0.76(0.04)a	6.5(0.2)a	11.3(0.4)ab	3.70(0.07)ns
Open-mature	16.4(0.2)bc	9.2(0.2)ns	0.76(0.04)a	6.5(0.1)a	11.3(0.4)ab	4.06(0.20)ns
Over-mature	16.0(0.15)c	9.1(0.2)ns	0.66(0.08)b	5.6(0.3)b	10.1(0.5)b	3.99(0.15)ns
Highly degraded	15.9(0.3)c	8.6(0.2)ns	0.64(0.08)b	5.4(0.4)b	12.5(0.4)a	3.79(0.16)ns

Values are the mean and the standard error of the mean (in parenthesis). Values followed by a different letter are significantly different ($P < 0.05$, Waller–Duncan test). N = 250

not exhibit any significant differences among the stand types tested and the average width ranged from 8.6 to 9.2 mm.

Seed weight was fairly constant in the young artificial and mature stands (0.81–0.76 g), and it was significantly lower in the over-mature and high degraded stands (0.66 and 0.64 g, respectively).

The length of wing was found to differ between the stand types and ranged from 5.4 to 8.2 mm. Seeds of mature and young stands bear significantly longer wings (mean values 6.5 and 6.6 mm, respectively) than the over-mature and degraded stands (5.6 and 5.4 mm, respectively).

The number of cotyledons per seed was found significantly lower in over-mature stands (10.1) while it did not exhibit differences among the other stand types (11.3–12.5). Also, the cotyledon length seems to remain fairly constant (3.7–4.2 cm) among the stand types tested.

Seed germination

The germination pattern of the seeds tested was not affected by the stand type. Thus, seed germination started on the 8th day after sowing in all cases and it was completed within 4 weeks; after that day no seed germination was observed. The final seed germination percentage was high in all cases and varied from 78 to 98.3%, meaning that all the types of stand produce seeds of high germination capacity (Fig. 2). The seeds of the open-mature stands showed the maximum germination capacity (98.3%) while the seeds of the over-mature stands the minimum one (78%); this percentage was significantly lower than that of the other stand types. The maximum seed germination peak appears 12 days after the beginning of the test.

Seed germination speed did not significantly differ between the stand types tested; the mean time to complete germination ranged between 12.6 and 15.4 days (Fig. 3). The stand type which had most delayed germination was the over-mature stands, while the most rapid germination was observed in open-mature stands.

Discussion

The type of *P. pinea* stands studied, significantly affects the cone and seed morphological characteristics. The highly degraded and the over-mature stands produce the smallest cones with the lowest total seed weight and the shortest and the lightest seeds than the other stand

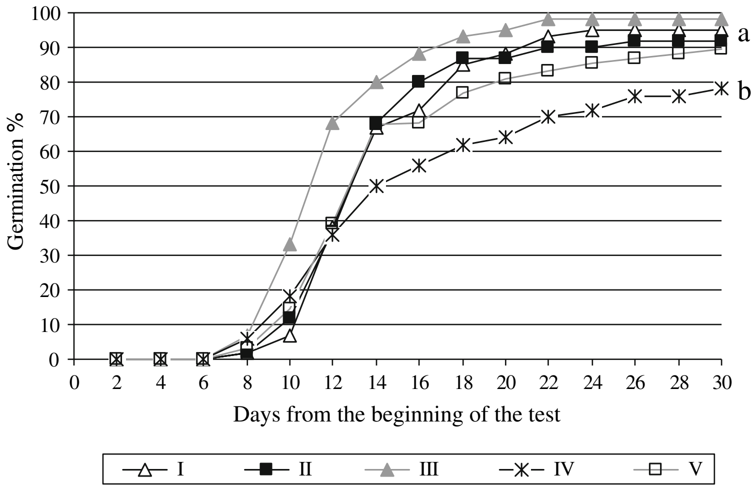

Fig. 2 Seed germination curves of *P. pinea* of the five stand types studied; (I) young artificial, (II) closed-mature, (III) open-mature, (IV) over-mature and (V) highly degraded stands. Error bars are not shown because the std. error was very low. Different letters (a, b) show significantly differences ($P < 0.05$, Waller–Duncan test)

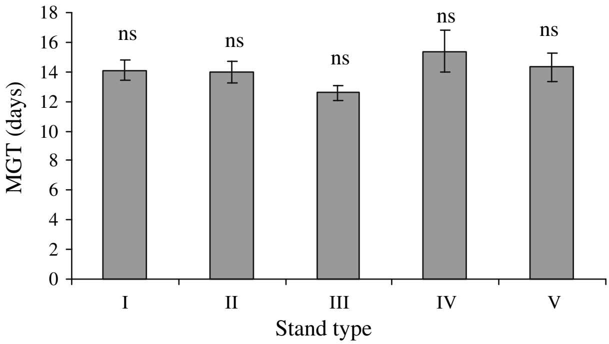

Fig. 3 Mean time to complete germination (MGT) of *P. pinea* seeds for each of the 5 stand types studied. ns: non significant differences ($P > 0.05$). Vertical lines over each bar represent ± Std. error values

types. Moreover, the over-mature stands produce the lowest seed efficiency. Seed weight variability was recorded also by Court-Picon et al. (2004) for several *P. pinea* provenances as well as by Escudero et al. (2002) for the Spanish *P. pinea* populations. The theory that under limited resources availability, a plant may allocate the available resource to the production of fewer larger seeds or many smaller ones (Harper et al. 1970) seems to be true in the case of highly degraded and closed-mature stands.

The greater seed potential and the greater number of filled seeds per cone are produced in young and open-mature stands. Most important is the finding that there is a great reduction in the number of filled seeds per cone in over-mature, high degraded and closed-mature stands and the percentage of not-fully developed seeds in these stands goes up to 26.8%. The not-fully developed seeds are of significantly lower dimensions and without germination ability. Their physiological development stopped before maturation, maybe due to the low capability of energy and nutrient translocation to seed tissues. This low translocation in reproductive organs can occur when the plants are stressed or when resources are limited (Bazzaz et al. 2000); it may also be attributed to aging of reproductive structures of the trees.

The greatest and heaviest cones with the greatest total seed weight as well as the longest seeds are produced in the young artificial stands. Seed investment is relatively high in the open-mature and young artificial stands and low in the closed-mature and over-mature stands. However, seed investment in *P. pinea* is very high (30–37%) compared to other Mediterranean pines (e.g. 3–6% in *P. halepensis* and 5–9% in *P. brutia,* Thanos et al. 1995).

In contrast to other conifer species (Wittwer et al. 1997; Stoehr 2000) the cones of *P. pinea* have a fairly constant number of seeds per cone and a high percentage of sound seeds. In this study, this is true in the case of young and mature stands (ca. 95% sound seeds per cone) when the stands grow under normal conditions. Where the trees are stressed, such as in the case of high degraded stands or when the trees enter the over-mature phase (senescence), their reproductive output seems to be reduced since the cones produce a lower number of sound seeds and many not fully developed seeds. However, even in these stands, only a very low percentage (up to 6.49%) of seeds was found to be empty. A low percentage of aborted seeds was recorded for *P. pinea* cones originating from many Mediterranean regions (Court-Picon et al. 2004).

The seeds bear a very short wing which is significantly longer in young and mature stands. From a functional point of view, the wings in all cases are too small to affect seed dispersal process and additionally they drop very easily from the seeds. The number of cotyledons was significantly reduced in over-mature stands compared to the other stand types. This can be explained as an adaptation of old trees to a low degree of nutrient allocation to fruit tissues. However, the length of cotyledons remained constant in all stand types at the end of the cotyledon development stage.

However, although there is a great variability in seed morphology, the seed germination behavior showed slight differences among the stand types studied. The final seed germination capacity was found high in all cases and varied from 78.0 to 98.3% and the mean time to complete germination ranged between 12.6 and 15.4 days. Based on both germination speed and ability, the best germination performance was achieved by the seeds of open-mature stands; thus, these stands do present the optimum seed quality in the study area. This shows that an open canopy helps trees to develop their maximum reproduction ability. The over-mature stands produce seeds with the lowest germination capacity and the most delayed germination. This can be explained by the great age of the trees (the life-span of *P. pinea* rarely exceeds 150 years, Frankis 1999). Stammatopoulos (1995) reported a very low germination capacity (9.76%) for old *Abies borisii regis* stands in the Parnitha national park, Attica, central Greece which greatly contributed to the absence of forest regeneration. Also, stand degradation has to some extent a similar effect on seed attributes. Highly degraded stands produce a lower number of seeds per cone than mature stands but their seed germination capacity is high.

Compared to the findings of other studies, the germination capacity of *P. pinea* from Strofylia forest is quite a lot higher than that reported by Escudero et al. (2002) for four Spanish populations of *P. pinea* and from that reported by Skordilis and Thanos (1997) for a Greek population (Attica). It can be mentioned that Escudero et al. (2002) found that population variability was the main source of variation in germination response, even though further analysis of a possible influence of environmental conditions was not carried out.

However, despite the high germination ability, the decrease of the seed production in the degraded, over-mature and closed-mature stands observed in Strofylia forest may contribute to the absence of natural regeneration of *P. pinea* that has been reported for many decades. This fact, in combination with an extremely high seed production of *P. halepensis* in the area seems to greatly contribute to a succession in the forest and the gradual replacement of the *P. pinea* by the species *P. halepensis* (Papamichos 1986; Moussouris and Regato 1999). Also, Tapias et al. (2001) report a much higher regeneration ability of *P. halepensis* in Spain than *P. pinea*. Thus, the risk of replacement of over-mature and degraded *P. pinea* stands *by P. halepensis* exists and demands emergent appropriate silvicultural measures (Moussouris and Regato 1999), in order to safeguard the *P. pinea* habitats. Such measures could be regeneration cuttings in the mature stands and protection of the area from overgrazing (Dafis 1986); canopy cover should be reduced to up to 30–40% in order to enhance seed production as well as to improve understorey microclimatic conditions (Ganatsas 1993; Boydak 2004). However, further knowledge on the specific conditions for species regeneration (Thanos et al. 2000) and seedling survival is necessary (Calama and Montero 2005). Karlsson (2000) reported cone and seed production about five times higher in seed trees of *Pinus sylvestris* after release cuttings. According to Shearer (1986), seed morphology may be affected by crown size as there is a close relationship between crown size and seed cone production.

Conclusions

Within population, the variation in cone and seed characteristics and seed germination behavior of *P. pinea* is correlated with environmental or mother plant variables (stand age, canopy cover, site conditions). These findings agree with the results reported for other tree species (Zhang 1998; Baskin and Baskin 1998; Stoehr 2000). When stands are stressed by human pressure, as in the case of high-degraded stands or when stands pass to over-mature phase, as in the case of over-mature stands, their reproductive capacity is reduced. Thus, the risk of replacement of the above *P. pinea* stands with the *P. halepensis* stands increases. In these cases appropriate silvicultural measures are needed in order to safeguard the *P. pinea* habitats included regeneration cuttings and protection of the area from overgrazing. The high light demands of the species should be taken into account when applying regeneration cuttings. The principles of close to nature silviculture should be applied in order to naturally regenerate the *P. pinea* stands, and help the maintenance of the valuable habitats of *P. pinea*.

Aknowledgements We would like to thank the Director of the Laboratory of Forest Protection of the Aristotle University of Thessaloniki, Prof. S. Markalas for making available the laboratory facilities. This research was partly financed by the Life Project 2002/Nat/GR/8491 of the European Union. Authors would like to thank the two anonymous referees for their helpful comments on an earlier version of the manuscript.

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