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# THERMAL TREATMENTS AND GERMINATION RESPONSE OVER TIME OF SEEDS FROM SEROTINOUS AND NON SEROTINOUS CONES OF *PINUS HALEPENSIS* MILL.

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***Abstract***

*Germination values of seeds of Pinus halepensis Mill. at different temperatures, times of exposure and different times of storage have been analysed.*

*Strobila for testing were collected from a natural Aleppo pine seed-forest growing on dune sands in the South of Italy, separately considering young (brownish) and old (greyish) strobila, the latter considered serotinous.*

*Both free seeds, extracted after oven exposure, and seeds that were still encased in intact strobila, were used.*

*Combinations of temperature and time of exposure where selected to simulate the effect of surface fires on the crown and on the soil seed bank.*

*Germinations values were analysed after 1,6 and 24 months of treatment in order to consider the effect of aging, both on soil and crown seed bank.*

*A mixture of moderate temperatures and short exposure confirms a stimulating effect of thermal stress, which increases germination and has been already observed for many Mediterranean species.*

*Only temperatures from 170° and above, even for brief exposure, cause a marked decrease in germination values .*

*Woody strobila protect encased seeds, allowing them to perform excellent germination values at temperatures of 220° and more, with exposure times up to 3' which simulate a rather intense but slow running fire.*

*Seeds from serotinous cones always exhibit the best results.*

*Germination values remain rather stable over time, concluding that soil seed bank is almost unaffected by the passage of running fire and that seeds, still encased in strobila, stay vitality intact even in stands that appear to be dead.*

**Keywords:** crown seed-bank; germination value; serotiny; soil seed-bank; serotinous cones; fire embracing strategy; pyriscence; xeriscence; dual life history**;** strategy**;**

## INTRODUCTION

A number of species exhibit cones or fruits that dehisce (split open) after a fire or under its effect; this trait is known as *serotiny*, commonly accepted to describe the late release of ripe seeds from cones, infructescences or fruits (Lamont *et al.*, 1991).The term, meaning late-occurring, literally denotes a late liberation of seeds and was used for the first time by Michaux (1803) referring to Pinus serotina (Thanos, 2004).

*Sensu stricto* serotiny is the retention of mature seeds in a canopy-stored seed bank with delayed dispersal; this trait is now also called *bradychory* (Thanos, op.cit.) but commonly refers to seed release and dispersal as a result of fire (Lamont *et al.*, 1991) and to cones requiring fire heat to release their seed (Givnish, 1981).

Serotiny is a common trait of species inhabiting a fire-prone environment, enhancing their survival during a fire (Zwolinski, 1990); through serotiny, plants utilise their pensile canopy seedbank and start post-fire re-colonization of burned areas.

Serotiny maximises the number of seeds available for the next generation by storing subsequent seed crops and protecting them from predators and heat (Lamont *et al.*,1999), concentrating many years of seed production into one large seed dispersal event (Tinker & Romme, 1994).

Plants whose reproduction is stimulated by fire can also be defined as fire-recruiters (Polakow *et al.,* 1999).

Part of the serotinous cone opens even in the absence of fire, under warm and dry weather conditions (Nathan *et al.*, 1999).

The ratio of serotinous to non-serotinous cones seems to be related to the frequency of fire, as observed in many other species of genus Pinus.

The level of serotiny should mainly depend on the fire regime; if fires are common, the serotinous cones will dominate; if fires are rare, open, non serotinous cones are more common. Leone *et al.* (1999) found a level of serotiny within the range of 30-70% for an unburned adult stand whereas 70-100% for a young post-fire regenerated stand.

Thanos and Daskalakou (2000) estimated the level of serotiny for a 5-12 year-old burned forest and a 30-50 year-old unburned forest as 95% and 48%, respectively.

A commonly accepted explanation relates serotiny - in the sense of stimulation induced by fire on mature cones to open promptly and to release the mature seeds - to heat which melts the resins that bind the apophyses of the cone scales (Ahlgreen,1974). This assumes that serotinous cones are sealed off by a resin coating which requires high temperatures, usually above 60 °C. before they will open. Such temperatures only occur during a fire.

The movement of the cone scale away from the cone axis on drying is related to the greater shrinkage of ventral tissues than dorsal tissues within the cone scale (Harlow *et al*, 1964 ; Allen & Wardrop, 1964); different degree of shrinkage is confirmed (Leone *et al.*, 1998) by different anatomic features in serotinous and non serotinous cones.

Serotinous strobila exhibit a high resin content, therefore a more efficient sealing of the scales, which presumably allows them to remain closed, even during intense fires (Leone *et al.*, op.cit.).

Cones opening is probably based on the reflex mechanism driven by a gradient in moisture content between scale tissue and the ambient air rather than only simple heating (Nathan & Ne'eman, 2000).

Cones actually open as a response to extremely dry weather and as an adaptation to favourable conditions for dispersal (Nathan *et al*., 1999).

Serotinous species include a number of pine species whose cone scales open and release seeds as fire passes and/or immediately thereafter. *Pinus halepensis* Mill. and other closely related species, such as *Pinus brutia* Ten., are characterized by partial serotiny, where individual trees carry both non-serotinous and serotinous cones (Panetsos, 1981). *Pinus halepensis* Mill. is a post-fire obligate seeder, which regenerates only from seeds in the post-fire environment (Ne'eman *et al.*, 2004) and whose recovery strategy consists of rapid, prolific seedling recruitment immediately after fire, enhanced by serotiny.

Serotinous species are common in low productive sites with limited tree height, which increases the danger that any fire could develop into a canopy fire. Under such conditions, pine species have only a relatively thin bark that does not supply enough heat insulation to the cambium and leads to tree death after wild fire(Ne'eman *et al.* ,op.cit.). *Pinus halepensis* in our study area belongs to the last group.

Seeds stored in serotinous cones represent an abundant seed source that scatters over the ground within days or weeks of the passage of a fire, insuring a gradual release of seeds having different biological values (Saracino & Leone, 1993a & 1993b); seeds profit from improved post-fire conditions (Goubitz *et al.*, 2003) to reach a *safe site condition* (sensu Harper *et al.*, 1965; Harper 1980), for seed germination.

After fire, seeds released from serotinous cones often fall on a favourable, soft seedbed, made up of ashes which provide nutrients; scorched needles cover seeds, with a sort of mulching, which helps in seedling germination and avoids excess predation (Leone *et al.* , 2000).

In this sense, serotiny assists species in the surge of recruitment under temporary conditions of low or reduced competition.

The ability of the Aleppo pine to rapidly reseed and re-colonize an area after a fire gives it an immense advantage over competing species, whose seeds must be blown onto the site from a nearby seed source--if it even exists.

Serotinous cones can remain on the tree crown unopened for years and contain viable seeds that are insulated by the woody scales.

In survival strategy of species, seed storage in the crown, abundant release of seeds after fire, gradual change of the colour of seeds (Saracino *et al.*, 1997), selective and gradual release of the biologically best seeds (Saracino & Leone, 1994) act sinergically to ensure large and vigorous regeneration.

But serotiny is much more than delayed opening of cones: it allows *P. halepensis* (Gill, 1981) to cope with fire-induced disturbances.

Serotiny is just the triggering tool for an abundant seed fall, which insures re-colonization of burned areas; a due emphasis is therefore to be given to seed resistance to thermal shock of fire, as part of a complex "dual life history strategy" of species (Trabaud, 1987), not only to late opening of scales.

Our study mainly aims to investigate how seeds respond to the effects of thermal treatment at different temperatures and times of exposure, on both free seeds, already extracted from serotinous (S) and non serotinous (NS) cones, and on seeds that are still encased in strobila, which surely protect the inside seed from overheating. Seed viability actually is not markedly affected by heating, unless the cone ignites, in which case the seed is killed.

We have also focused our attention on the effect of time after treatments in order to simulate the effects of running fires and their consequences on soil and canopy seed bank over time.

## MATERIAL AND METHODS

### Study area

This study was carried out in the domanial forest "Perronello", a large (3,200 ha) natural *Pinus halepensis* forest growing on alluvial sandy, recently formed dunes facing the Ionian Sea, in the Province of Taranto, Southern Italy (40°30'42" N, 17°01'12" E).

The coastal pine forests grow in a semi-arid Mediterranean type climate, with drought between May and October; irregularly distributed rainfalls reach equinox maxima (absolute maximum in November and relative maximum in March) and Summer minimum values. Mean annual rainfall (535 mm) varies considerably from one year to the other: in some years it is below 400 mm (325 mm in 1935; 337 mm in 1942; 348 mm in 1965; 319 mm in 1970), in others it may exceptionally exceed 900 mm (918 mm in 1972).

Mean annual temperature 15.8 °C; mean minimum temperature 8.2 °C; mean maximum temperature 24.9 °C.

Soil moisture regime is influenced by the yearly course of potential evapotranspiration (PET). The annual PET computed at Marina di Ginosa is 815.2 mm (Macchia *et al.*,1975)

The climate and soil characteristics in the study area show several analogies with those of a pine forest growing on the Algerian high plain, studied by Moravec (1990).

The forest is made up by pure, patchy even-aged *P. halepensis* stands of natural origin. Understory vegetation is represented by evergreen sclerophyllous shrub species (Francini, 1953), mainly *Phillyrea angustifolia*, *Pistacia lentiscus*, *Rosmarinus officinalis*, *Cistus salvifolius*, etc.

The region is typical of fire-prone ecosystems in that many species have a recognized ability to regenerate after fire (Trabaud, 1987). In the last 3-4 decades, fire occurrence has increased in this region as a consequence of land use change, mainly touristic development on the coastal fringe.

Pines in the area, repeatedly swept by fires, exhibit an abundant presence of serotinous cones; direct count of serotinous and non serotinous cones, manually picked from adult trees (N=10), confirms that serotinous and non serotinous cones are rather balanced as a total number (1042 S, 1036 NS per tree, as average) but differently placed on crowns; the upper third of crown contains 53% of S, whereas intermediate and lower third contain more than 50% of non serotinous.

From October to December, strobila for this study were picked from 6 adult plants of Aleppo pine of the same size, age and recently wind blown. They were manually picked from the upper, medium and lower third of crowns, mixing them yet distinguishing grey, older strobila, from brown; the former, of chalky appearance, are considered serotinous. A total of 1800 strobila was picked, of which 900 grey and 900 brown.

### *Experimental design and data analysis*

Thermal treatments and germination have been carried out in the seed laboratory of CODRA Mediterranea srl in Pignola (Pz) where all facilities are available for seed testing.

Thermal treatments were carried out both on seeds that were previously extracted from strobila and on seeds that were still encased in them, treating the whole strobila in the laboratory oven.

For seed extraction, strobila were previously dried according to a specific protocol (Tab.1) purposely designed in CODRA laboratories as adapt to *Pinus halepensis* of Southern Italian Regions.

Table 1. Strobila exsiccation parameters

| Type of strobila | Temperature (°C) | Humidity (%) | Time (h) |
|---|---|---|---|
| Brown, non serotinous strobila (NS) | 48 | 20 | 16 |
| Grey, serotinous strobila (S)* | 35 | 50 | 16 |
| | 48 | 20 | 16 |

Thermal treatment, in terms of increasing temperature values and different time of exposure has been carried out according to Tab. 2; values have been purposely chosen to simulate the effect of a running fire, with different rate of spread, on free seeds laying on soil surface or embedded in the first 5 cm soil layer (DeBano, 1989; Sackett & Haase, 1992; Albini *et al.,* 1996).

Table 2. Thermal treatments: Temperature and Exposure time for free seeds

| T °C | Time (minutes) | | | |
|---|---|---|---|---|
| 70 °C | 2' | 5' | 10' | 20' |
| 120 °C | 2' | 5' | 10' | 20' |
| 170 °C | 2' | 5' | 10' | 20' |
| 220 °C | 2' | 5' | 10' | 20' |

Thermal treatments directly on strobila, keeping them constantly separated by colour (brown/grey, i.e. NS/S) were carried out according an experimental design (Tab. 3) aimed to simulate the thermal rise at crown level, during a running fire, on pine trees of an average height of 10-12 meters (Carvalho *et al.* , 2002).

The maximum time of 3' considers the values of rate of spread for a slow fire. Crown torching actually does not ignite cones because the high temperatures are unlikely to last more than 3 minutes.

Table 3. Thermal treatments (Temperature v. Time of exposure) for strobila

| T °C | Time (minutes) | | |
|---|---|---|---|
| 70 °C | 1' | 2' | 3' |
| 120 °C | 1' | 2' | 3' |
| 170 °C | 1' | 2' | 3' |
| 220 °C | 1' | 2' | 3' |
| 270 °C | 1' | 2' | 3' |

Strobila (360, respectively NS and S) were treated according to the above mentioned range of temperatures for different exposure times and subsequently put in the exsiccator for seed extraction.

Thermal treatments were carried out in a laboratory oven, switched on 1 hour before and preheated to a temperature 2°C higher than necessary.

Seeds and strobila, respectively, were placed in baking containers for treatment.

*Percentage of germination*

Germination testing was carried out after 1, 6 and 24 months, on seeds stored at room temperature.

Seeds treated inside strobila were also extracted and stored under same conditions.

Four representative seed lot samples, each containing 100 seeds, were taken from each kind of seeds after the thermal treatment as above described.

Seeds were manually placed in circular, concentric rows on two circular filter papers covered with a transparent dome cover, in a Jacobsen germinator, germination plate temperature-conditioned by means of he water basin below. The water bath was equipped with an automatic temperature control device.

The germination dishes, equipped with a paper wick and a paper substrate, were placed on the germination plate.Through slots in the germination plate the wick reaches the water bath below, thus supplying the required humidity to the paper substrate.

The photoperiod was set at 16 h light and 8 h darkness, light at 1250 Lux.

Each germination test lasted 28 days; first count after 7 days from start, subsequent controls and count each third day.

Seeds were considered germinated when the radicle protruded at least 2 mm.

Germination was then evaluated, reporting the percentage number of seedlings that have developed normally under optimum laboratory conditions.

Each germination test was completed with a control test on seedlots of same size of untreated seeds. The control test, too, was therefore repeated after 1, 6 and 24 months.

An easier evaluation of the results is offered by the following graphs (Figg 1,2) and Table 4, which reports germination values of seeds as before treated.

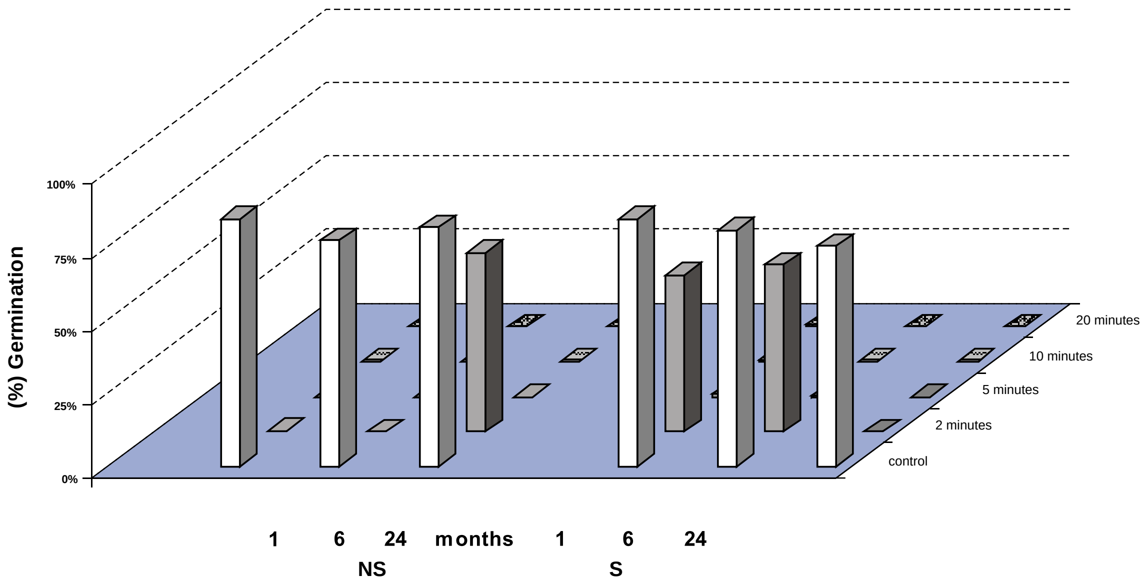


Fig. 1. (upper)

Germination values in % in free seeds extracted from serotinous(gray) and non serotinous (brown) strobila under 170°C and different exposure time

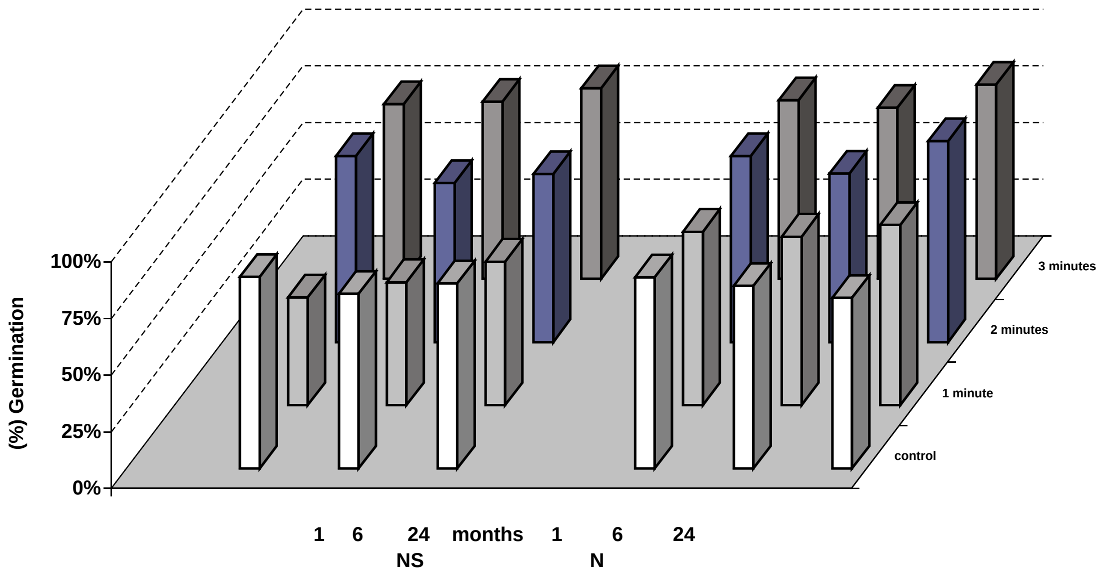


Fig. 2. (lower)

Germination values in % in seeds still encased in serotinous/non serotinous strobila under 170 ℃ for 1,2,3 minutes

Table 4. Germination percentage: test at 1-6-24 months from thermal treatment, respectively on extracted, seeds and seeds still encased in strobila

**AFTER 1 MONTH**

***FREE SEEDS EXTRACTED FROM***

| | ***GREY STROBILA*** | | | | | ***BROWN STROBILA*** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Time of exposure** | | | | | **Time of exposure** | | | |
| **T ℃** | **2’** | **5’** | **10’** | **20’** | **T ℃** | **2’** | **5’** | **10’** | **20’** |
| **70℃** | 73,00 | 76,50 | 74,25 | 71,50 | **70℃** | 68,50 | 59,25 | 51,50 | 61,75 |
| **120℃** | 76,50 | 75,00 | 66,50 | 40,00 | **120℃** | 43,00 | 45,25 | 54,75 | 50,00 |
| **170℃** | 53,25 | 0,75 | 0,50 | 0,50 | **170℃** | 0,25 | 0,00 | 0,00 | 0,00 |
| **220℃** | 0,00 | 0,00 | 0,00 | 0,00 | **220℃** | 0,00 | 0,00 | 0,00 | 0,00 |
| **CONTROL** | | **84,25** | | | **CONTROL** | | | **84,50** | |

***SEEDS ENCASED IN***

| | ***GREY STROBILA*** | | | | ***BROWN STROBILA*** | | |
|---|---|---|---|---|---|---|---|
| | **Time of exposure** | | | | **Time of exposure** | | |
| **T℃** | **1’** | **2’** | **3’** | **T℃** | **1’** | **2’** | **3’** |
| **70℃** | 82,50 | 87,00 | 73,00 | **70℃** | 60,75 | 53,00 | 76,75 |
| **120℃** | 76,50 | 78,00 | 76,00 | **120℃** | 75,75 | 71,50 | 71,25 |
| **170℃** | 76,50 | 82,00 | 78,75 | **170℃** | 47,50 | 82,00 | 77,00 |
| **220℃** | 81,75 | 78,50 | 83,00 | **220℃** | 71,00 | 70,75 | 76,50 |
| **270℃** | 80,75 | 88,25 | 87,50 | **270℃** | 81,00 | 84,25 | 79,50 |
| **CONTROL** | | **84,25** | | **CONTROL** | | **84,50** | |

***AFTER 6 MONTHS***

***FREE SEEDS EXTRACTED FROM***

| | ***GREY STROBILA*** | | | | | ***BROWN STROBILA*** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Time of exposure** | | | | | **Time of exposure** | | | |
| **T℃** | **2’** | **5’** | **10’** | **20’** | **T℃** | **2’** | **5’** | **10’** | **20’** |
| **70℃** | 69,25 | 63,25 | 73,00 | 85,00 | **70℃** | 58,00 | 58,00 | 52,25 | 55,25 |
| **120℃** | 72,00 | 71,75 | 74,00 | 49,25 | **120℃** | 49,00 | 40,00 | 29,75 | 51,50 |
| **170℃** | 56,75 | 0,25 | 0,00 | 0,00 | **170℃** | 0,00 | 0,00 | 0,00 | 0,00 |
| **220℃** | 0,00 | 0,00 | 0,00 | 0,00 | **220℃** | 0,00 | 0,00 | 1,50 | 0,00 |
| **CONTROL** | | **80,50** | | | **CONTROL** | | | **77,00** | |

**SEEDS ENCASED IN**

| | ***GREY STROBILA*** | | | | ***BROWN STROBILA*** | | |
|---|---|---|---|---|---|---|---|
| | **Time of exposure** | | | | **Time of exposure** | | |
| **T℃** | **1’** | **2’** | **3’** | **T℃** | **1’** | **2’** | **3’** |
| **70℃** | 75,25 | 79,25 | 78,00 | **70℃** | 49,75 | 60,00 | 73,00 |
| **120℃** | 88,50 | 77,75 | 83,25 | **120℃** | 77,75 | 73,25 | 73,00 |
| **170℃** | 74,25 | 74,25 | 75,50 | **170℃** | 54,25 | 70,00 | 78,00 |
| **220℃** | 83,25 | 83,50 | 78,50 | **220℃** | 85,50 | 65,50 | 71,75 |
| **270℃** | 87,75 | 77,00 | 88,00 | **270℃** | 77,00 | 82,75 | 84,25 |
| **CONTROL** | | **80,50** | | **CONTROL** | | **77,00** | |

**AFTER 24 MONTHS**

***FREE SEEDS EXTRACTED FROM***

| | ***GREY STROBILA*** | | | | | ***BROWN STROBILA*** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Time of exposure** | | | | | **Time of exposure** | | | |
| **T℃** | **2’** | **5’** | **10’** | **20’** | **T℃** | **2’** | **5’** | **10’** | **20’** |
| **70℃** | 68,75 | 62 | 69,75 | 82 | **70℃** | 57 | 51 | 62,25 | 63,5 |
| **120℃** | 70,25 | 67 | 64,25 | 36 | **120℃** | 56 | 49 | 35,5 | 52 |
| **170℃** | 61 | 0 | 0 | 0 | **170℃** | 0 | 0 | 0 | 0 |
| **220℃** | 0 | 0 | 0 | 0 | **220℃** | 0 | 0 | 0 | 0 |
| **CONTROL** | | **75,25** | | | **CONTROL** | | | **81,5** | |

**SEEDS ENCASED IN**

| | ***GREY STROBILA*** | | | | ***BROWN STROBILA*** | | |
|---|---|---|---|---|---|---|---|
| | **Time of exposure** | | | | **Time of exposure** | | |
| **T℃** | **1’** | **2’** | **3’** | **T℃** | **1’** | **2’** | **3’** |
| **70℃** | 75,50 | 79,75 | 85,75 | **70℃** | 63,25 | 75 | 74,25 |
| **120℃** | 74,75 | 83 | 89,75 | **120℃** | 68,75 | 66,25 | 69,75 |
| **170℃** | 79,50 | 88,50 | 85,50 | **170℃** | 63,25 | 74 | 84 |
| **220℃** | 82,25 | 86,75 | 82,50 | **220℃** | 75,75 | 74 | 74,50 |
| **270℃** | 86,50 | 77,25 | 86,75 | **270℃** | 92,25 | 91,25 | 89,50 |
| **CONTROL** | | **72,25** | | **CONTROL** | | **81,5** | |

Seed exposure to temperatures higher than 70℃ causes a decrease in the percentage of seed germination: increasing temperature and time of exposure may lead to the total inhibition of seed germination, as already observed (Habrouk *et al.*,1999). At temperatures below 70℃, seeds from serotinous cones (S) always exhibit better germination percentages than non serotinous (NS), until long exposure times (20 minutes). Performance of good heat resistance is observed not only at short term, but also after 6 and 24 months of treatment: at 70℃, for 20', after 6 and 24 months germination percentage of S seeds results always higher than control. This confirms that moderate temperatures, such as 70℃ in our study, have a stimulating effect on germination for many Mediterranean species, probably through chemical and physical changes in the hard seed coat (Habrouk *et al.*, op.cit.; Habrouk, 2002).

When temperatures increase (120 ℃), germination of S seeds, higher in comparison to NS in shorter treatments, decreases with time of exposure; values < 50% are observed only in long term exposure (20 minutes) whereas the NS seeds decrease is immediate: less than 50% after 2' of exposure. Differences between S and NS seeds persist over time: after 24 months at 120℃ S seeds germination values is >50% until 10', whereas NS seeds pass the threshold of 50% after 5'. At 170℃ only S seeds can resist, even though only for short time (2') after which germination is rather null; NS on the contrary exhibit germination values next to zero. This threshold probably marks the inhibition of germination.

For temperature values of 220℃ no germination in either type of seed was observed.

Synthesizing observations, there is a difference in the germination response to fire between S and NS seeds, with seeds from serotinous cones resulting more tolerant of fire-related factors, mainly high temperature (Ne'eman *et al.*, 2004). This suggests that seeds are physiologically different and that both types contribute to recruitment through different patterns: through *pyriscence*, fire-induced seed release (sensu Lamont *et al.*, 1991), which leads to post-fire recruitment in fire-prone areas and *xeriscence*, drought-induced seed release (sensu Nathan *et al*. 1999), which results in widespread recruitment during a tree's lifetime. Thus, P. halepensis dual life history strategy allows it to adapt to invading open disturbed sites generated both by fire or by other factors of disturbance (Nathan and Ne'eman, 2000).

The response of seeds still encased in strobila that are exposed to thermal treatment is completely different: germination values are rather high and persist with values next to control, even at high temperatures and long times of exposure; here, once again, S seeds exhibit higher performance than NS. Germination values maintain their values over time, after 6 and 24 months, for both types of seeds. Not surprisingly, values of germination of seeds exposed to maximum temperature inside strobila are higher than those at lower temperatures and are higher than control.

High values of germination are actually observed from 70℃ to 270℃, remaining stable over time; in some cases, thermal shock increases germination value. This happens at 270℃, where germination values are more or less the same as free seeds at 70℃.

In addition, 1 and 2' values are sharply higher than control at 270℃ , both in S and NS seeds.

Data confirm that cones of P. halepensis withstand temperatures of 200 ℃ or even 400 ℃ over a short period of time (Martines-Sanchez *et al.*, 1997; Reyes & Casal, 1997; Fraver, 1992) and plausibly allow to state that at 270℃, for such times of exposure, inside temperatures reach values within the range of 65-85℃ (R. Salvatore, unpublished data), which stimulate germination.

*Statistical Analysis*

In order to establish an existing difference among the two control groups of serotinous and non serotinous strobila, a Kolmogorov-Smirnov test for two independent samples was performed at first ($p<.001$), while a ?² test was used for the two distributions of “survival” data ($p< .001$).

We concluded that the two populations of seeds are different in terms of germination attitude both in normal and in unsettled conditions. Moreover, a one-side Z test showed higher germination values for brownish seeds in comparison to greyish ones, therefore suggesting that younger (brownish) seeds are more productive.

The evaluation of thermal treatment factors was made by an ANOVA study of single and interaction effects, upon angular transformation $y = \text{arcsen} \sqrt{p}$ of data frequencies for the three independent sets of observations at one month, six months and two years.

All computations were done in SAS SYSTEM v.8.2 running with Windows XP.

We started the ANOVA study on germination varying four factors (time of fire exposure, temperature, variety of seeds, interaction between time of exposure - temperature). As the four effects turned out to all be significant (p < .0001) we considered two different three-way ANOVA analysis for the two varieties of seeds (S/NS as mentioned) separately (each of them consisted of 120 observations) and more, in every single group of seeds we performed a two-way ANOVA in order to better study the eventual influence of temperature and time of thermal exposure and the resulting interaction effect, taking into account four repetitions per time.

In all cases in which the effects were significant, a post-hoc Duncan test was used as a tool for determining which means are different from each other.

A further study was carried out for the germination power of seeds extracted from strobila and directly exposed to thermal treatment.

Analogously to the previous case, data were transformed and used for a two-way ANOVA with four replications on 60 observations and a Duncan test was also carried out in all cases in which the means turned out to be statistically different.

In table 5 the ANOVA and Duncan test results are reported respectively for the three periods of time considered in the analysis and for seeds directly exposed to treatment.

Table 5. ANOVA seeds extracted from greyish and brownish strobila at different times and temperatures of exposure

**THERMAL TREATMENT ON EXTRACTED SEEDS FROM**

(**1 month**)

| | **SEROTINOUS CONES** | | | **NON SEROTINOUS CONES** | | |
|---|---|---|---|---|---|---|
| **Variable** | df | F | P | df | F | P |
| Minutes (M) | 3 | 53.77 | <.0001 | 3 | 26,72 | 0,9028 |
| Temperature (T) | 2 | 486,08 | <.0001 | 1 | 396,70 | 0,0078 |
| Minutes X Temperatures (M x T) | 6 | 31,51 | <.0001 | 3 | 296,87 | 0,1268 |
| Duncan (T) | **(70) (120) (170)** | | | **(70) (120)** | | |
| Duncan (M) | **(2) (5,10) (20)** | | | **(2,5,10,20)** | | |

(**6 months**)

| | **SEROTINOUS CONES** | | | **NON SEROTINOUS CONES** | | |
|---|---|---|---|---|---|---|
| **Variable** | df | F | P | df | F | P |
| Minutes (M) | 3 | 118,17 | <.0001 | 3 | 1,42 | 0,2517 |
| Temperature (T) | 2 | 134,96 | <.0001 | 2 | 692,34 | <.0001 |
| Minutes X Temperatures (M x T) | 6 | 109,52 | <.0001 | 6 | 5,08 | 0,007 |
| Duncan (T) | **(70) (120) (170)** | | | **(70) (120) (220)** | | |
| Duncan (M) | **(2) (5,10,20)** | | | **(2,5,10,20)** | | |

(**24 months**)

| | **SEROTINOUS CONES** | | | **NON SEROTINOUS CONES** | | |
|---|---|---|---|---|---|---|
| **Variable** | df | F | P | df | F | P |
| Minutes (M) | 3 | 48,35 | <.0001 | 3 | 2,49 | 0,0846 |
| Temperature (T) | 2 | 387,23 | <.0001 | 1 | 13,10 | 0,0014 |
| Minutes X Temperatures (M x T) | 6 | 40,99 | <.0001 | 3 | 4,41 | 0,0131 |
| Duncan (T) | **(70) (120) (170)** | | | **(70) (120)** | | |
| Duncan (M) | **(2) (5,10,20)** | | | **(2,5,10,20)** | | |

In all cases (observation time 1, 6 and 24 months), both the single effects "minutes", "temperature" and their interaction are equally highly significant for serotinous cones; furthermore, the Duncan test reports similar behaviour in terms of homogeneity inside the subgroups of means over time. The results show, then, an identical germination attitude of S seeds even after two years, while non NS appear to be irregular, in fact only one effect (temperature) is always significant while interaction comes out just in the last period and the duration of treatment does not influence germination at all. Duncan's test sets of means match themselves in all cases.

In table 6 the ANOVA and Duncan test results are reported respectively for the three periods of time considered in the analysis but for seeds exposed to treatment inside the cones.

Table 6. ANOVA seeds encased in cones at different times and temperatures of exposure

| THERMAL TREATMENT ON SEEDS STILL ENCASED IN CONES (1 month) | | | | | | |
|---|---|---|---|---|---|---|
| | SEROTINOUS CONES | | | NON SEROTINOUS CONES | | |
| **Variable** | df | F | P | df | F | P |
| Minutes (M) | 2 | 2,23 | 0.1196 | 2 | 6,02 | 0.0048 |
| Temperature (T) | 4 | 4,29 | 0.0050 | 4 | 8,23 | <0.0001 |
| Minutes X Temperatures (M x T) | 8 | 2,21 | 0.0445 | 8 | 6,19 | <0.0001 |
| Duncan (T) | **(70,220,270) (70,120,170) (70,120,170,220)** | | | **(120,170,220) (270) (70) (70,170) (270) (120,220)** | | |
| Duncan (M) | **(1) (2) (3)** | | | **(1) (2,3)** | | |
| **(6 months)** | | | | | | |
| | SEROTINOUS CONES | | | NON SEROTINOUS CONES | | |
| **Variable** | df | F | P | df | F | P |
| Minutes(M) | 2 | 2,23 | 0,1189 | 2 | 5,34 | 0,0083 |
| Temperature (T) | 4 | 5,63 | 0,0009 | 4 | 14,79 | <.0001 |
| Minutes X Temperatures (M x T) | 8 | 2,02 | 0,0655 | 8 | 6,66 | <.0001 |
| Duncan (T) | **(120,220,270) (70,170) (70,220)** | | | **(70) (170) (120,220) (270)** | | |
| Duncan (M) | **(1) (2) (3)** | | | **(1,2) (3)** | | |
| **(24 months)** | | | | | | |
| | SEROTINOUS CONES | | | NON SEROTINOUS CONES | | |
| **Variable** | df | F | P | df | F | P |
| Minutes(M) | 2 | 6,84 | 0,0026 | 2 | 3,67 | 0,0333 |
| Temperature (T) | 4 | 1,10 | 0,3673 | 4 | 30,93 | <.0001 |
| Minutes X Temperatures(M x T) | 8 | 3,23 | 0,0055 | 8 | 2,92 | 0,0102 |
| Duncan (T) | **(70 , 120 ,170,220,270)** | | | **(70,120) (270) (170,220) (120) (270) (70,170,220)** | | |
| Duncan (M) | **(1) (2,3) (1,2) (3)** | | | **(1,2) (3) (1) (2,3)** | | |

Germination for treated seeds encased in greyish cones (S) shows an irregular behaviour with respect to the influencing factors in analysis. In fact, it is determined by an interaction between "temperature" and "minutes" only in the first and last periods of observation, by the single action of "temperature" in the first two periods, while "minutes" are important only in the last case.

On the contrary, NS seeds reflect an identical situation in the three periods because all factors are determining for germination . In particular, the highest temperature always produces excellent outcomes, as in the other case (seeds from serotinous cones), where a decrease occurs in the intermediate time of heating.

## DISCUSSION AND CONCLUSION

Results confirm the crucial role of serotinous cones to produce a prolific release of seeds, enabling a successful re-colonization of burned areas.

If post-fire regeneration depends on canopy-stored seeds in serotinous and, much less, on non-serotinous cones, much of its success depends on the inherent capacity of seeds to overcome heating produced by fire front passage.

Our data fit with similar studies (Goubitz 2001; Goubitz *et al.*, 2003; Goubitz *et al.*, 2004): percentage of germination of NS seeds is higher in the no-fire (control) than in the post-fire scenario; in the post-fire scenario, S seeds, on the contrary, always germinate better than seeds from NS cones.

In general, the germination performance of NS seeds is rather less than S ones.

These results indicate that S seeds are more tolerant of fire-related factors (Ne'eman *et al.* 2004) and support the notion that P. halepensis has a *dual life history-strategy* of a post-fire obligate seeder as well as an invading species in the absence of fire (Goubitz *et al.* , 2004).

Seeds from serotinous and non serotinous strobila seem actually physiologically different, at least in terms of resistance to thermal shock, and contribute to recruitment through different patterns: through *pyriscence*, fire-induced seed release (sensu Lamont *et al*., 1991), in fire-prone areas and *xeriscence*, drought-induced seed release (sensu Nathan *et al*. 1999), which results in widespread recruitment during a tree's life. Thus, P. halepensis has adapted to invading open disturbed sites generated both by fire or by other factors of disturbance (Nathan & Ne'eman, 2000).

No doubt, actually, that woody, resin-closed strobila strongly influence post-fire regenerative success of the Aleppo pine, which depends on the inherent ability of its seeds to tolerate fire severity (Sannikov 1994), but also on the position of these seeds (cones vs. soil bank) during the fire event. Free seeds in the soil bank are more exposed to heating than seeds protected inside cones, both serotinous and non serotinuos ones (Fraver 1992); Keeley and Zedler (1998) suggest that lower site productivity, by lowering maximum plant height, can increase the probability of crown fire and therefore select for serotiny.

Seeds, still encased in serotinous strobila, are heat resistant and therefore maintain intact vitality even in stands apparently dead after fire event.

They represent, in our opinion, a relevant trait of *fire embracing strategy*, in which plants invest little to survive fire; it involves traits that enhance flammability (e.g. large amounts of non self pruned necromasse and empty cones on dead branches) and use fire to cue seedling establishment in the post-fire environment through serotinous cones (Schwilk & Ackerly, 2001; Ne'eman *et al*., 2004).

Good seed resistance to thermal stress inside woody strobila, which act as a very efficient insulator, is consistent with and supports this interpretation in the stands of our study site, and suggests that pine recruitment after fire mainly depends upon the amount of seeds stored in cones and able to insure efficient post fire recruitment.

Easy and abundant regeneration after fires, delayed germination of seeds and seedling density, which is significantly affected by the cover of burnt trees and time passed since fire (Ne'eman & Perewolotsky, 2000; Leone & Lovreglio, 2005), point to the conclusion that the best thing to do in the post fire scenario of Aleppo pine stands is (Dafis, 1991) to avoid dangerous interference with the natural processes based on pyriscence.

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