

RESEARCH ARTICLE

GERMINATION PATTERNS OF SOIL SEED BANKS IN RELATION TO FIRE IN PORTUGUESE LITTORAL PINE FOREST VEGETATION

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ABSTRACT

Germination behavior of maritime pine (*Pinus pinaster* Aiton) forests soil seed banks after fire treatments in controlled laboratory conditions was analyzed. Germination response of all tree and shrub seeds after wildfires in the Leiria National Forest, Portugal, was simulated by treating sample trays of soil seed banks with distinct combinations of: burning time (0 min, 5 min, and 15 min), seed depth (5 cm and 8.5 cm), and presence or absence of ash cover. The design included control samples for null hypothesis testing. During a time span of 20 months after treatments, the maximum number of seedlings observed every 30 day period, their taxonomical identity, the number of destroyed seeds, and the number of non-germinated seeds were analyzed. Six functional species groups, defined by germination response (i.e., germinated, destroyed, non-germinated), were identified using minimum-variance hierarchical clustering and correspondence analysis. In addition, the influence of the three environmental design factors and three functional species groups based on their general germination response were then analyzed by means of general regression models. Clustering and ordination results suggest two obvious main groups in terms of post-fire germination response: 1) seeders—fast-growing pioneer shrubs that respond positively to post-fire germination, and 2) resprouters—slow-growing understory tall-shrubs in which germination is largely depressed. Each of the two main functional species groups were further subdivided into six sub-groups, which are distinct in mean germinated, destroyed, and non-germinated seed values. Burning time and seed depth were highly significant in explaining post-fire germination response, while the presence of ash cover was less significant. Results suggest that pioneer seeder behavior is largely promoted by wildfires that are, in turn, detrimental to slow-growing, late-successional tall shrubs. Forest fire hazard risk management can thus be put into perspective—seeder fuel beds are promoted by wildfire and are themselves highly fire prone, and resprouters are not (due to discontinuous horizontal and vertical structure and less flammable leaf composition).

Keywords: *Pinus pinaster*, post-fire germination, resprouters, seeders, soil seed bank, understory vegetation management

Citation: Santos, L., J. Capelo, and M. Tavares. 2010. Germination patterns of soil seed banks in relation to fire in Portuguese littoral pine forest vegetation. *Fire Ecology* 6(3): 1-15. doi: 10.4996/fireecology.0603001

INTRODUCTION

Fire has shaped Mediterranean ecosystems for millennia (Keeley 1986). Natural wildfires have acted as ever-present evolutionary and ecological processes, and human-caused fires associated with agriculture and grazing have historically been the major factors in defining the Mediterranean landscape. Direct ecological effects on forest and scrub vegetation are the destruction of plants or their aerial parts, consumption of litter and seeds in the soil, and modification of the soil structure near the surface (Keeley 1986). Other well known effects include the direct promotion or inhibition of soil seed bank dormancy or germination. Indirect effects include the elimination of toxic soil compounds, which also interfere with dormancy and germination (Romero 1989). Several processes associated with changed radiation fluxes in the soil (such as changes in carbon, water, organic matter, and nutrient cycles) can also be relevant, thus giving rise to changes in the composition, diversity, and structure of subsequent vegetation. Barnes *et al.* (1997) suggest that interference in geomorphology and soil formation can induce genetic and adaptive character shifts and, therefore, can change plant population processes and ecological succession (Raison 1980, Maia *et al.* 2004). Direct impact of fire on soil seed bank occurs either by destroying non-dormant seeds or by breaking the seed coat of dormant seeds. Differences in species sensitivity to heat and smoke, and also to post-fire environmental conditions (such as presence of ash), change plant composition by altering their relative proportions when compared to similar unburned sites (Auld 1987, Elliott *et al.* 2009).

Fire-adapted plants with persistent seeds use two strategies: 1) profuse germination right after fire by means of generalized dormancy broken by heat, as is the case of shrubs of the legume family (Martin *et al.* 1975, Jeffery *et al.* 1988, Auld and O'Connell 1991, Keeley 1991); and 2) frequent short, intense germina-

tion cycles over an extended period after fire (Keeley 1991).

Plants with a temporary seed soil bank and non-dormant seeds that germinate as soon as environmental conditions are suitable exhibit another type of fire-adapted behavior. This strategy involves stimulation of flowering and fruiting by fire and abundant production of short-lived non-dormant seeds that germinate immediately.

We were interested in analyzing, through floristic analysis of understory vegetation, the role of soil seed bank regeneration in maintaining the balance between seeders and resprouters. The distinction between these fire adaptations were described by Schutte *et al.* (1995) and van der Bank *et al.* (1999). These authors clearly indicated that seeders are fire-prone, profuse producers of small seeds that have variable viability and tend to have short dormancy periods. In turn, resprouters are adapted to fire by means of the resprouting of dormant buds in underground or close-to-ground xylopods, and are low-number large seed producers that are highly sensitive to fire. Higgins *et al.* (2008) describe the seeder strategy as non-persistent semelparous, and the resprouter strategy as persistent iteroparous. In the context of Mediterranean vegetation, seeders are pioneer low shrubs that produce profuse numbers of seeds (*r*-strategy) and have rolled leaves (malacophyllous). This vegetation includes rock rose (Cistaceae), some heath (Ericaceae), brooms (*Cytisus*, *Teline*), and gorses (*Ulex*) (Blondel and Aronson 1999). In Portuguese pine vegetation, resprouters are of late successional stages and mostly hard-leaved (sclerophyllous) and do not produce large quantities of seeds (*k*-strategists). These include oaks (*Quercus*), pistachios (*Pistacia*), privets (*Phillyrea*), and junipers (*Juniperus*). Higgins *et al.* (2008) distinguish the two life-cycle strategies as fire adaptations in relation to seed production and emergence. Pine understory vegetation includes both seeders (non-persistent semelparous) and resprouters (per-

sistent iteroparous). Seeders coarsely correspond to pioneer, rolled-leaved many-seed producers, *r*-strategist from initial succession stages; while resprouters correspond to leathery leaved, few-seed producers, *k*-strategists from late successional stages (Blondel and Aronson 1999; Higgins *et al.* 2008). Two distinct parallel life-strategies of regeneration as a response to post-fire conditions was demonstrated to stand for two distinct evolutionary lineages by Pausas and Verdú (2005), Verdú *et al.* (2007), and Paula and Pausas (2008). On the one hand, resprouters represent a lineage in which evolutionary pressure seems to have acted mostly on positive selection of resprouting capacity, whereas post-fire seed survival is largely depressed (Paula and Pausas 2008). On the other hand, propagule-persistent plants (seeders) represent a distinct lineage in which post-fire germination is either not disfavored by fire or enhanced by it (Paula and Pausas 2008). Thus, post-fire seed production and survival seem to be, in general, negatively correlated in resprouters and seeders (Paula and Pausas 2008). Some differences in distinct Mediterranean vegetation biogeographical contexts were also found by Paula *et al.* (2006) between the Mediterranean basin and California, USA. This character-functional distinction suggests a degree of coherence with the paleo-biogeographical origin types identified by Herrera (1992): seeders were neomediterranean with a continental-dry late Tertiary Central-Asia origin, and resprouters were paleomediterranean xeric sub-tropical mid Tertiary autochthonous taxa. Soil seed banks play an important role in post-fire succession of these two distinct fire-functional types.

Maritime pine (*Pinus pinaster* Aiton) forests dominate large stretches of the landscape in Iberian Peninsula, westernmost France, and other smaller areas in western Mediterranean regions. We selected the Leiria National Forest, Portugal, as a case study representative of a long-established maritime pine forest. Records show that this forest has inhibited the inland movement of maritime sand dunes dating

back to the thirteenth century. Also, accurate forestry management data exist from the nineteenth century on, when the forest was largely replanted. Low-fertility sandy soils, persistence of salt-rich spray, and regular wildfires associated with fire-prone understory are also expected to be critical to soil seed bank dynamics, and therefore expected to make the maritime forests sensitive to limiting factors (heat, exposure to radiation, drought). As maritime pine is the ecologically dominant species, its own coherence in relation to general soil seed bank patterns is to be kept in mind. Agee (1998) and Reyes and Casal (2004) suggest that maritime pine is an obligate seeder, depending solely on successful germination from the soil seed bank in post-fire conditions. We wanted to determine if maritime pine follows the same regeneration strategy as dominant fire-prone seeder scrub.

To approach the germination pattern in relation to the issues raised above, an experimental simulation of post-fire soil seed bank behavior was set in controlled conditions. We hoped to evaluate which species survive, are destroyed, or are non-emergent (although apparently not destroyed by fire but with an undetermined viability or dormancy status). The environmental factors experimentally simulated were time of exposure to fire, depth of the simulated soil seed bank, and the presence of ash. Thus, we aimed to focus on two main questions. First, are there groups of plants that are coherent in post-fire germination response? Second, is the later taxonomic-functional information, along with experimental variables, enough to account for a model that explains the germination pattern? The former question is addressed by classification, ordination, and mean tests of germination parameters in groups. If taxonomic identity correlates to germination response, this is suggestive of functional coherence and would be an interesting result in itself, apart from its subsequent use in answering the second question. The latter question is investigated by general regression model analysis.

The functional opposition between sprouters and seeders, which might be apparent in the data from the Portuguese pine vegetation, could put into perspective the parallel evolutionary lineages in Mediterranean fire-adaptation previously recognized by Pausas and Verdú (2005), Pausas *et al.* (2006), Verdú *et al.* (2007), and Paula and Pausas (2008).

METHODS

Study Area

The study area was the Leiria National Forest, on the western coast of Portugal (N 39° 42' 24" to N 39° 53' 00"; W 9° 03' 00" to W 8° 03' 30"), approximately 18 km long and 7 km wide, with a total area of 11 000 ha. The area is composed exclusively of even-aged stands of maritime pine managed for timber and soil protection since the nineteenth century. The climate is winter-rain, summer-dry mesomediterranean oceanic (yearly average temperature of 14°C, mean maximum of the hottest month [August] of 24.4°C, mean minimum of coldest month [January] of 4.9°C, with a dry period of four months [Mesquita and Sousa 2009]). The soils are podzols derived from Pleistocene ancient dunes and formerly active maritime dunes from the Holocene. Understory vegetation is composed mostly of 32 species of shrubs of the families Cistaceae (*Halimium*, *Cistus*), Ericaceae (*Erica*, *Calluna*), Fabaceae (*Ulex*, *Stauracanthus*, *Cytisus*), Empetraceae (*Corema*), Fagaceae (*Quercus*), Anacardiaceae (*Pistacia*), and Myricaceae (*Myrica*) (Table 1). The herb layer is diverse and strongly associated with the dune vegetation.

Field Sampling and Preparation of Simulated Soil Seed Banks

Samples were drawn from the forest strip closest to the sea where human intervention is minimal. First, sterile soil beds were obtained

(i.e., soil samples were collected *in situ* and made seed-free and loose-organic-matter free by flocculation). The soil samples were treated with a saline solution composed of 10 g of sodium hexametaphosphate and 5 g of magnesium sulphate in 200 ml of water per 100 g of soil. Soil samples were stirred with the solution, then washed with running water and sieved, leading to the elimination of all floating lighter materials, including all seeds. For each tray, approximately 14.0 kg of sterile soil was thus prepared. Seeds were collected directly from fruits of taxonomically identified plants. Nomenclature follows the published volumes of Castroviejo *et al.* (1986) and Franco and Rocha Afonso (1971). Seeds were tested for viability by manual compression.

Laboratory Procedures

Fifty viable seeds of each of the 32 species were placed in sterile soil beds in 35 cm × 51 cm × 10.5 cm metal trays in exactly analogous conditions for 10 design modalities: (Figure 1), time of exposure to fire (0 min, 5 min, and 15 min), depth of the simulated soil seed bank (5 cm and 8.5 cm) and the presence or absence of ash in the four burned modalities. The 50 seeds of each species were placed in groups 1.5 cm apart, in rows. Five trays had the seeds planted 8.5 cm deep, and the remaining five trays had the seeds planted 5.0 cm deep. A total of 20 trays were used because the 10 trials were repeated twice to account for two replicates of each modality (see Figure 1). Using a metal frame with corresponding dimensions that was placed on top of the seed trays, fire was produced by igniting a 35 cm × 51 cm × 10 cm volume of dry and live branches of shrubs taken from the sampling locations. Fires were extinguished by mechanical means (covering them) after 0 min, 5 min, and 15 min, respectively. Ashes were left in the tray in the ash modalities. From the no-ash modalities, ash was removed. Four unburned trays were used as controls for null-hypothesis test-

Table 1. Common names, scientific names, and abbreviations for species analyzed in this study.

| Scientific name | Common name | Abbreviation |
|--|------------------------------|--------------|
| <i>Acacia longifolia</i> (Andrews) Wild | Sydney golden wattle | Acal |
| <i>Artemisia crithmifolia</i> L. | Samphire-leaved southernwood | Arcri |
| <i>Calluna vulgaris</i> (L.) Hull | Heather | Cavlu |
| <i>Cistus crispus</i> L. | Curled leaved rockrose | Cicri |
| <i>Cistus monspeliensis</i> L. | Montpelier cistus | Cimo |
| <i>Cistus salvifolius</i> L. | Sage leaf rockrose | Cisa |
| <i>Coronilla glauca</i> L. | Sea-green | Cogl |
| <i>Corema album</i> (L.) D. Don | Crowberry | Coral |
| <i>Cytisus grandiflorus</i> (Brot.) DC. | Broom | Cytg |
| <i>Daphne gnidium</i> L. | Spurge flax | Dafni |
| <i>Erica arborea</i> L. | Briar root | Erar |
| <i>Erica umbellata</i> L. | Dwarf Spanish heath | Erum |
| <i>Genista triacanthos</i> Brot. | Broom | Gerti |
| <i>Halimium calycinum</i> (L.) K. Koch | Yellow rockrose | Hacal |
| <i>Halimium halimifolium</i> (L.) Willk. | Rockrose | Haha |
| <i>Helichrysum picardi</i> Boiss. & Reut. | Strawflower | Hepi |
| <i>Juniperus turbinata</i> Guss. | Sabina | Jutu |
| <i>Medicago marina</i> L. | Sea medick | Mema |
| <i>Myrica faya</i> Aiton | Faya | Myfa |
| <i>Ononis natrix</i> L. subsp. <i>ramosissima</i> (Desf.) Battand | Bush restharrow | Ona |
| <i>Othantus maritimus</i> (L.) Hoffm. & Link | Sea lilly | Otama |
| <i>Phillyrea angustifolia</i> L. | Evergreen privet | Phan |
| <i>Pistacia lentiscus</i> L. | Mastic | Pisle |
| <i>Rhamnus alaternus</i> L. | Italian buckthorn | Rhal |
| <i>Ruscus aculeatus</i> L. | Butcher's broom | Rusa |
| <i>Smilax aspera</i> L. | Rough bindweed | Smas |
| <i>Spartium junceum</i> L. | Spanish broom | Spaj |
| <i>Stauracanthus genistoides</i> (Brot.) Samp. | Gorse | Stag |
| <i>Ulex jussiaei</i> Webb | Furze | Ujus |
| <i>Ulex europaeus</i> L. ssp. <i>Latebracteatus</i> (Mariz) Rothm. | Gorse | Uleul |
| <i>Viburnum tinus</i> L. | Laurustinus. | Viti |

ing (one for each seed depth, two replicates). Trays were sheltered from rain, but kept under seasonal temperature variations for Lisbon, Portugal—not significantly different from the original location from August 1999 to March 2001—and watered to keep daily moisture approximately constant. Every 30 days for 20 months, emergent seedlings were identified and their numbers per species counted. Afterwards, the remaining seeds were separated from the substratum by flocculation and classified by manual compression into destroyed and non-germinated categories.

Data Analysis

For each species, the maximum percent of germinated seeds in 20 months (GM), the number of destroyed seeds (DT), and the number of non-germinated seeds (NG) were calculated. A first data set of 640 observations (32 species × 20 trays) with the corresponding values of presence of ash (ASH), time of exposure to fire (TIME), depth of simulated soil seed bank (DEPTH), GM, DT, and NG were prepared. Three data matrices of 32 species × 10 modalities were re-calculated with each cell

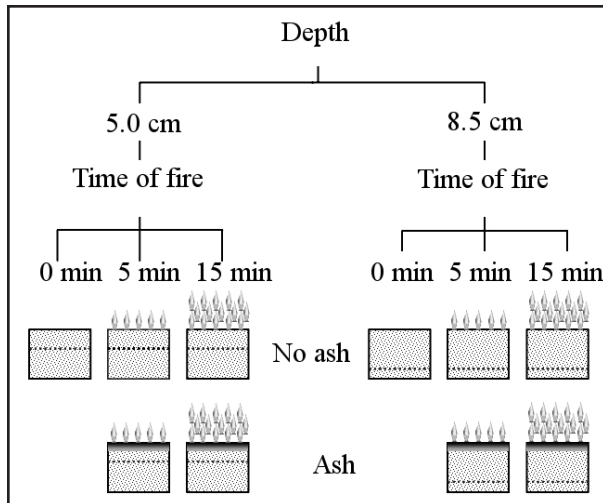


Figure 1. Design of modalities of simulated laboratory fire.

containing absolute counts of GM, DT, or NG (i.e., the sum of both replicates of each modality). The ten modalities were coded as combinations of the three factors as such: $C = 0, 1$ (presence or absence of ash); $T = 0, 5, 15$ (minutes of time of exposure to fire); and $P = 5, 8.5$ (cm depth of simulated soil seed bank). For example, a modality would be expressed as: C0T5P8.

First, the 32×10 matrices of DG, DT, and NG were classified into dendrogram trees by Ward's hierarchical minimum-variance clustering method, using Euclidian distances in order to approach a species classification in groups of similar response to germination, destruction of seeds, or non-germination. These can be named functional species groups and coded further as the categorical variable SPGROUP. By testing against alternative random trees by a bootstrap procedure ($P < 0.05$), we discarded the NG tree as non-significant in relation to the other 25 trees, keeping only the first two (GM and DT) for an eventual consensus classification of species. Groups obtained by clustering were then tested for significant multiple pair-wise mean differences in GM and DT values, using the *post hoc* ANOVA Newman-Keuls test. This test is used for an uneven number of observations, at a significance level of 0.05.

Correspondence between species (and species groups) and modalities of fire treatment was determined by detrended correspondence analysis (DCA) (Hill 1979). The DCA is complementary to classification and to determinant modalities in the global species similarity pattern of GM and DT response and its correspondence to functional species groups obtained by clustering. The DCA was performed by CANOCO 4.0 software (Microcomputerpower, Ithica, New York, USA; Ter Braak and Smlauer 1998).

To model the response of GM, DT, and NG to the factors TIME, DEPTH, ASH, and SPGROUP, two runs of a general regression model (GRM) were used ($F: P < 0.05$). The GRMs were produced in a general linear model context so that categorical independent variables could be used and also because it allows simultaneous fitting of several dependent variables along with a single *post hoc* analysis of covariance (ANCOVA). Moreover, the link function used was identity, and a normal distribution of errors was assumed (e.g., binomial for the categorical variables). Therefore, each model is, in fact, equivalent to a multiple linear regression (MLR). Such an extension of MLRs allows the calculation of the determination R^2 coefficient, because MLRs are least-square methods and are currently used in ecological studies (Ter Braak 1987, Jongman *et al.* 1995, Neter *et al.* 1996). A first run was made including only TIME, DEPTH, and ASH without using the functional species groups obtained by clustering, i.e., irrespective of taxonomic differences in species germination response. In general, models were largely non-significant ($R^2 < 0.1$; $F: P > 0.05$; models not shown). A second run included the categorical variable for species according to clustering results and Newman-Keuls *post hoc* pair-wise mean tests (SPGROUP). Such categorical variables were then included as predictors in the GRM procedure in a second run as a set of six nominal (binary) variables, A to F. Global goodness of the sub-models (one for each dependent variable: GM, DT, and NG) was ana-

lyzed by R^2 and the model's analysis of variance sum-of-squares values, F ratio, and corresponding probability ($P < 0.05$). Contribution to global fit of each independent variable was drawn from the associated t -statistic ($P < 0.05$). Data manipulation, cluster analysis, GRM, and statistical tests were made using STATISTICA 7 software (StatSoft, Tulsa, Oklahoma, USA). Bootstrap analysis was performed by the pvclust package running in the R environment (Suzuki and Shimodaira 2006).

RESULTS

Setting of Functional Species Groups

The clustering dendrograms by Ward's method, performed three times on each of the 32 × 10 matrices of GM (Figure 2), DT, and NG, produced a classification of species response in germination. Testing of dendrogram trees by bootstrap analysis for reliability (bootstrap probability: $P < 0.05$) selected GM and DT trees as the only significant ones, because the NG tree was not significantly distinct from a random tree. We should note that the non-emergence of seedlings from seeds that were apparently not destroyed is inconclusive be-

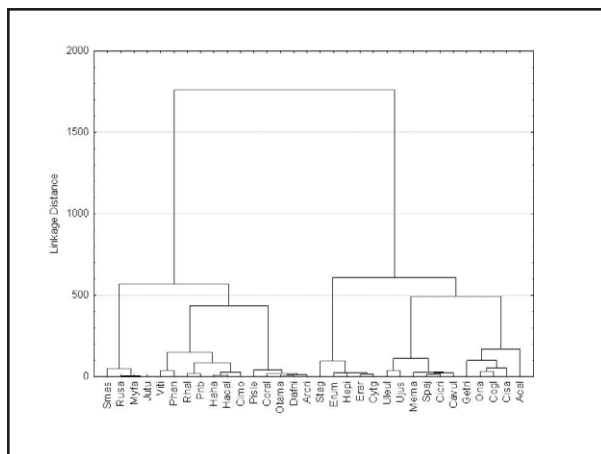


Figure 2. Dendrogram of clustering by Ward's method using Euclidian distances of species 32 × 10 matrix (species by simulated fire modalities) using GM values.

cause 1) either fire treatments inhibited or had no influence on dormancy breaking or germination, or 2) some other not-controlled experimental factors were involved in viability of seeds or dormancy status. Due to the design of the experiment, the reasons for non-emergence are largely unknown. The number of clusters was visually set by coherence of terminal branches and cut approximately at the 300 linking distance value, so that six clusters arise in the GM tree (Figure 2) and five in the DT tree (not shown). Both species classifications are completely coincident. Because significant GM mean differences were found in the first and third GM clusters but not in the DT clusters, we used the six GM clusters as functional species groups as the species classification in subsequent analyses (Table 2). These species groups were used as taxonomic categorical predictors in GRM and for Newman-Keuls (see Table 3 and Table 4 for Newman-Keuls pair-wise mean tests; in DT, the probability for group pair A and C was $P = 0.678609$). Overall pair-wise group mean differences between NG values, if GM groups are applied, are non-significant (see Table 5).

Therefore, we accept, for the purpose of analysis, that the six functional species groups as defined by germination behavior are those in Table 2. Mean GM, DT, and NG of groups are those in Table 6. The most striking differences are those between A and C and the remaining groups, having a very low number of germinated seeds corresponding with the highest number of destroyed seeds. In clustering, group B joins {A, C} at a high similarity value and consistently has also relatively low value of GM. The D, E, and F groups are ranked with much higher number of germinated seeds and with lower number of destroyed seeds. At a high linking distance in the dendrogram, the combinations of {A, C, B} and {D, E} emerge as clearly separate. The number of non-emergent seeds is inconclusive. Results are suggestive of a straightforward correspondence with resprouter and seeder behavior, respectively.

Table 2. Functional species groups in relation to germination behavior.

| A | B | C | D | E | F |
|----------------------------|-------------------------------|-------------------------------|----------------------------------|----------------------------|----------------------------|
| <i>Smilax aspera</i> | <i>Viburnum tinus</i> | <i>Pistacia lentiscus</i> | <i>Stauracanthus genistoides</i> | <i>Ulex latebracteatus</i> | <i>Genista triacanthos</i> |
| <i>Ruscus aculeatus</i> | <i>Phillyrea angustifolia</i> | <i>Corema album</i> | <i>Erica umbellata</i> | <i>Ulex jussiaei</i> | <i>Ononis ramosissima</i> |
| <i>Myrica faya</i> | <i>Rhamnus alaternus</i> | <i>Otanthus maritimus</i> | <i>Helichrysum picardii</i> | <i>Medicago marina</i> | <i>Coronilla glauca</i> |
| <i>Juniperus turbinata</i> | <i>Pinus pinaster</i> | <i>Daphne gnidium</i> | <i>Erica arborea</i> | <i>Spartium junceum</i> | <i>Cistus salvifolius</i> |
| | <i>Halimium halimifolium</i> | <i>Artemisia crithmifolia</i> | <i>Cytisus grandiflorus</i> | <i>Cistus crispus</i> | <i>Acacia longifolia</i> |
| | <i>Halimium calycinum</i> | | | <i>Calluna vulgaris</i> | |
| | <i>Cistus monspeliensis</i> | | | | |

Table 3. Newman-Keuls pair-wise multiple mean test for GM in species groups. No tests had values for $P > 0.05$.

| SPGROUP | C | F | E | D | B |
|----------------|----------|----------|----------|----------|----------|
| C | --- | | | | |
| F | 0.000017 | --- | | | |
| E | 0.000008 | 0.000089 | --- | | |
| D | 0.000022 | 0.000022 | 0.000054 | -- | |
| B | 0.003721 | 0.000008 | 0.000022 | 0.000009 | --- |
| A | 0.000015 | 0.000020 | 0.000017 | 0.000008 | 0.000022 |

Table 4. Newman-Keuls pair-wise multiple mean test for DT in species groups. Values for $P > 0.05$ are marked with an asterisk (*).

| SPGROUP | C | F | E | D | B |
|----------------|------------|----------|----------|----------|----------|
| C | --- | | | | |
| F | 0.000017 | --- | | | |
| E | 0.000008 | 0.000009 | --- | | |
| D | 0.000022 | 0.000022 | 0.000020 | --- | |
| B | 0.000288 | 0.000008 | 0.000022 | 0.000009 | --- |
| A | 0.678609 * | 0.000020 | 0.000017 | 0.000008 | 0.000171 |

Table 5. Newman-Keuls pair-wise multiple mean test for NG in species groups. Values for $P > 0.05$ are marked with an asterisk (*).

| SPGROUP | C | F | E | D | B |
|---------|-------------|-------------|-------------|-------------|-----------|
| C | --- | | | | |
| F | 0.191 155 * | --- | | | |
| E | 0.730 582 * | 0.333 161 * | --- | | |
| D | 0.567 983 | 0.379 209 * | 0.854 029 * | --- | |
| B | 0.724 216 * | 0.259 701 * | 0.773 523 * | 0.884 571 * | --- |
| A | 0.000 020 | 0.000 009 | 0.000 008 | 0.000 017 | 0.000 022 |

Table 6. GM, DT, and NG mean values and standard errors (SE) by species groups listed in the order that they were entered into the GLM

| SPGROUP | GM mean | GM SE | DT mean | DT SE | NG mean | NG SE | n |
|---------|---------|---------|---------|---------|---------|---------|-----|
| C | 9.68000 | 1.42890 | 39.9800 | 1.35842 | 0.34000 | 0.75583 | 100 |
| F | 43.5500 | 1.42890 | 3.83000 | 1.35842 | 2.62000 | 0.75583 | 100 |
| E | 35.7250 | 1.30440 | 13.1416 | 1.24006 | 1.13333 | 0.68998 | 120 |
| D | 27.6300 | 1.42890 | 21.4300 | 1.35842 | 0.94000 | 0.75583 | 100 |
| B | 15.4428 | 1.20764 | 33.1214 | 1.14808 | 1.43571 | 0.63879 | 140 |
| A | 0.73750 | 1.59756 | 40.7625 | 1.51876 | 8.50000 | 0.84505 | 80 |

Ordination of Germination Responses in Relation to Treatments

Mean and standard deviation values of germinated (GM) and destroyed (DT) seeds are shown in Table 7. In general, the presence of fire corresponds to lower values of germinated seeds when compared to non-fire modalities, irrespective of depth and presence of ash. For any period of time, with or without presence of ash, deeper seeds seem to have greater percentages of emergence. A protection effect of a thicker layer of soil could justify this, particularly in the case of species of which seeds are very sensitive to destruction. In turn, the figures seem to suggest that in some species, a balance between protection and dormancy-breaking by fire is dependant on depth and on species group. An almost opposite pattern between groups {A, B, C} and {D, E, F} is evident. In the first group, high destruction and

low seedling emergence is found, and in the second, the pattern is the opposite. Clearly, the most fire-sensitive groups are A and C. Joint interpretation of Table 7 and the DCA ordination in Figure 3 seems to suggest that the same general pattern of species of groups {A, B, C} is associated with the absence of fire or low fire modalities, as indicated by their location in the left two quadrants. Some other tendencies of positive co-variation are also suggested by the DCA in Figure 3. Axis 1 seems to rank species by decreasing fire sensitivity, changing to fire-prone in germinating as you move from left to right. In the left quadrants, where fire-sensitive species groups are found, group B seems to correspond to higher emergence rate (GM) if seeds are deeper (8.5 cm). Group B is also closer to the center of the diagram near group D, which exhibits intermediate behavior. More fire-prone species groups and more intense fire treatments occur in the right quad-

Table 7. Mean \pm SD values of germinated (GM) and destroyed (DT) seeds, by functional species group (FSG) and by modalities of fire treatment.

| FSG | | C0T0P5 | C0T0P8 | C0T5P5 | C0T5P8 | C0T15P5 | C0T15P8 | C1T5P5 | C1T5P8 | C1T15P5 | C1T15P8 |
|-----|----|-----------------------|----------------------|-----------------------|----------------------|----------------------|----------------------|-----------------------|----------------------|----------------------|----------------------|
| A | GM | 9.00 ± 12.14 | 5.75 ± 8.81 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | DT | 8.75 ± 10.96 | 6.75 ± 5.12 | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 | 99.75 ± 0.50 | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 |
| B | GM | 74.57 ± 24.22 | 83.00 ± 18.64 | 0.00 ± 0.00 | 73.14 ± 13.14 | 0.00 ± 0.00 | 4.71 ± 11.54 | 0.00 ± 0.00 | 73.42 ± 17.19 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | DT | 15.4 2 ± 19.48 | 7.71 ± 5.08 | 100.0 0 ± 0.00 | 22.57 ± 14.25 | 100.00 ± 0.00 | 95.28 ± 12.47 | 100.0 0 ± 0.00 | 21.42 ± 14.48 | 100.00 ± 0.00 | 100.00 ± 0.00 |
| C | GM | 93.20 ± 5.26 | 91.20 ± 5.44 | 0.00 ± 0.00 | 5.00 ± 11.18 | 0.00 ± 0.00 | 0.80 ± 1.78 | 0.00 ± 0.00 | 3.40 ± 7.60 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | DT | 4.80 ± 3.42 | 7.40 ± 6.22 | 99.20 ± 1.78 | 93.6 ± 14.31 | 100.00 ± 0.00 | 99.20 ± 1.78 | 100.00 ± 0.00 | 95.40 ± 10.28 | 100.00 ± 0.00 | 100.00 ± 0.00 |
| D | GM | 87.6 0 ± 8.73 | 92.20 ± 11.03 | 91.20 ± 8.04 | 91.00 ± 8.03 | 0.60 ± 1.34 | 10.00 ± 22.36 | 89.80 ± 8.87 | 90.20 ± 9.90 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | DT | 4.80 ± 3.03 | 2.00 ± 1.22 | 8.80 ± 8.04 | 7.80 ± 6.72 | 99.40 ± 1.34 | 90.00 ± 22.36 | 10.20 ± 8.87 | 5.60 ± 6.10 | 100.00 ± 0.00 | 100.00 ± 0.00 |
| E | GM | 94.50 ± 4.13 | 88.16 ± 12.17 | 91.00 ± 8.48 | 81.16 ± 21.05 | 0.00 ± 0.00 | 83.00 ± 13.49 | 94.16 ± 8.90 | 89.00 ± 10.88 | 0.83 ± 2.04 | 92.66 ± 4.54 |
| | DT | 4.33 ± 2.06 | 4.66 ± 2.42 | 6.83 ± 6.94 | 12.33 ± 10.76 | 100 ± 0.00 | 16.83 ± 13.22 | 3.66 ± 4.27 | 7.66 ± 6.40 | 99.16 ± 2.04 | 7.33 ± 4.54 |
| F | GM | 67.2 ± 36.64 | 68.6 ± 39.01 | 93.6 ± 6.80 | 96.6 ± 3.43 | 96.6 ± 3.04 | 88.4 ± 22.63 | 96.2 ± 5.54 | 98.6 ± 1.51 | 76.2 ± 24.30 | 89 ± 21.81 |
| | DT | 9.80 ± 6.72 | 3.60 ± 4.92 | 6.40 ± 6.80 | 3.40 ± 3.43 | 3.20 ± 2.68 | 10.20 ± 19.51 | 3.80 ± 5.54 | 1.40 ± 1.51 | 23.80 ± 24.30 | 11.00 ± 21.81 |

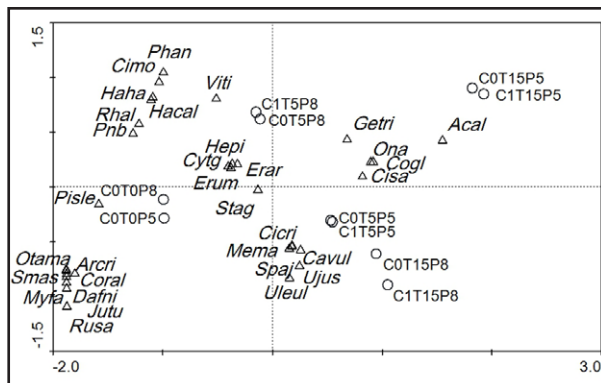


Figure 3. DCA ordination, axis 1 and 2 of 32×10 GM matrix (species by simulated fire modalities). Cumulative percentage variance explained: axis 1: 56.4%; axis 2: 70.0%.

rants. Group E seems to correspond to moderate pyrophytes with regard to longer fire time but with deeper seed beds. One could hypothesize that in group E, emergence is favored by

fire but excessive heat periods could be lethal. Extreme pyrophytes seem to correspond to group F, where longer fire times and shallower seed beds are found.

Models of Germination Response vs. Post-Fire Treatments

In the GRM, the introduction of taxonomic variability by means of functional species groups greatly increased the model's performance. Previous runs with only TIME, DEPTH, and ASH as explanatory variables yielded a poor model ($R^2 = 0.15$; not shown). The GLM model summary in Table 8 shows that fairly good models for GM and DT are obtained when categorical independent variable SPGROUP (species group) is added ($R^2 = 0.64$ and $R^2 = 0.65$, respectively). To confirm the assumption of normality of residuals, a

Table 8. Summary of GLM results for dependent variables GM, DT, and NG: whole model R^2 ; and whole model vs. residual sum-of-squares test.

| | Multiple R | Multiple R^2 | Adjusted R^2 | SS model | df model | SS residual | df residual | F | P |
|-----------|-----------------|-------------------|-------------------|-------------|-------------|----------------|----------------|----------|------|
| GM | 0.780 859 | 0.609 741 | 0.604 794 | 201292.2 | 8 | 128 835.0 | 631 | 123.2345 | 0.00 |
| DT | 0.813 138 | 0.661 194 | 0.656 898 | 227237.0 | 8 | 116 440.0 | 631 | 153.9276 | 0.00 |
| NG | 0.444 005 | 0.197 141 | 0.186 962 | 8851.6 | 8 | 36 048.2 | 631 | 19.3676 | 0.00 |

normal probability plot of raw residuals was performed (for GM as an example, see Figure 4). The sub-model for non-germinated (NG) remains poor, as expected ($R^2 = 0.18$).

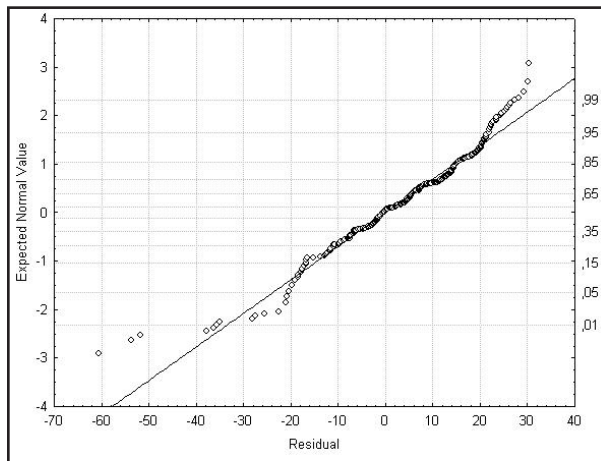


Figure 4. Normal probability plot of raw residuals for the GM sub-model.

The t -statistic probabilities for GM show that the presence of ASH seems to be the only non-significant treatment variable ($P = 0.59$), although ASH is borderline significant ($P = 0.04$) in the DT sub-model (Table 9). Significance in NG is found in ASH and DEPTH, although the model has a very low fit. In GM, the TIME regression coefficient is negative, which suggests inverse relationship (depression) of fire with germination. DEPTH has a positive coefficient, meaning that the depth of the soil bank provides protection against fire. Otherwise, in DT, TIME, and DEPTH coefficients have inverse signs compared to those of GM, which also suggests a direct relationship of destruction with presence of fire. With SP-

GROUP, C has a large value and negative sign (-12.44), meaning that its larger value in the model will reduce GM. The same applies to A, which did not enter the model due to colinearity with the remaining SPGROUP variables. Group B's value is lower (-6.68), but confirms the DCA (Table 8) interpretation of belonging to the fire-sensitive group (in the quadrants of the DCA). On the other hand, D, E, and F have positive values in the GM sub-model and negative ones in DT. These are the fire-prone species groups. Group B is positive in the DT sub-model, again coherent with the fire-sensitive group. The ranking of absolute values of the significant regression coefficients is consistent with the position of the fire-sensitive and fire-prone groups already suggested by DCA. Finally, the contradictory influence of ASH on the model is a bit surprising, as several authors presuppose its influence in the protection or inhibition mechanisms of seed emergence in pine-dominated ecosystems (e.g., Gonzalez-Rabanal and Casal 1994, Escudero 1997, Reyes and Casal 2004).

DISCUSSION

Although the scope of our study was limited in terms of biogeography and ecological representation, the results are suggestive of two clearly opposed behaviors in relation to germination and contribution of the soil seed bank, that of seeders vs. resprouters. Distinction of seeder and resprouter functional strategies as fire adaptations were described by Schutte *et al.* (1995) and van der Bank *et al.* (1999). Clearly, these life-strategies corre-

Table 9. Parameter estimates of GLM, for dependent variables GM, DT, and NG. Par.= regression coefficients (parameters); SE = standard error; t-statistic for regression coefficients; *P* = probability for 95 % confidence level (*P* < 0.05).

| Effect | Intercept | ASH | TIME | DEPTH | SPGROUP | | | | |
|--------------------|-----------|---------|----------|---------|----------|----------|----------|---------|---------|
| | | | | | C | F | E | D | B |
| GM Par. | 20.8322 | 0.6289 | -1.6602 | 2.2813 | -12.4476 | 21.4224 | 13.5974 | 5.5024 | -6.6847 |
| GM SE | 2.6377 | 1.1982 | 0.0978 | 0.3765 | 1.3000 | 1.3000 | 1.2096 | 1.3000 | 1.1406 |
| GM <i>t</i> | 7.8979 | -0.5249 | -16.9698 | 6.0583 | -9.5751 | 16.4789 | 11.2413 | 4.2327 | -5.8604 |
| GM <i>p</i> | 0.0000 | 0.5998 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| DT Par. | 24.1641 | 2.3195 | 2.0627 | -2.4948 | 14.6024 | -21.5476 | -12.2359 | -3.9476 | 7.7438 |
| DT SE | 2.5076 | 1.1391 | 0.0930 | 0.3580 | 1.2359 | 1.2359 | 1.1499 | 1.2359 | 1.0844 |
| DT <i>t</i> | 9.6363 | 2.0363 | 22.1787 | -6.9691 | 11.8154 | -17.4350 | -10.6405 | -3.1942 | 7.1412 |
| DT <i>p</i> | 0.0000 | 0.0421 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0015 | 0.0000 |
| NG Par. | 5.0037 | -1.6906 | -0.4026 | 0.2135 | -2.1548 | 0.1252 | -1.3615 | -1.5548 | -1.0591 |
| NG SE | 1.3952 | 0.6338 | 0.0517 | 0.1992 | 0.6876 | 0.6876 | 0.6398 | 0.6876 | 0.6034 |
| NG <i>t</i> | 3.5862 | -2.6675 | -7.7795 | 1.0721 | -3.1336 | 0.1820 | -2.1279 | -2.2611 | -1.7554 |
| NG <i>p</i> | 0.0004 | 0.0078 | 0.0000 | 0.2841 | 0.0018 | 0.8556 | 0.0337 | 0.0241 | 0.0797 |

spond to distinct evolutionary investments in seed production and physiological adaptation to wildfires, as demonstrated throughout by Paula and Pausas (2008), Pausas, Keeley and Verdú (2006), Verdú *et al.* (2007), and Pausas and Verdú (2005). These two character syndromes were termed neomediterranean and paleomediterranean elements by Herrera (1992) and relate to the origins of Mediterranean flora in the Tertiary: either continental central Asian elements (seeders) or autochthonous sub-tropical xeric flora (resprouters). These two life-cycle strategies are fire adaptations to seed production and emergence (Higgins *et al.* 2008). Our results are suggestive of such distinction being present in understory vegetation of burned pine forests and, to a large extent, conditioning vegetation response and subsequent composition and structure. Our results in Portuguese pine vegetation fire response are suggestive of some degree of coherence with the evolutionary lineages emphasized by Paula and Pausas (2008) and Verdú *et al.* (2007), the ecological functional groups of van der Bank *et al.* (1999) and Blondel and

Aronson (1999), and the paleo-biogeographical groups of Herrera (1992). The consistently distinct and largely coincident groups that issued from classification of the germinated and destroyed seed data seem to support such putative relationship.

Within these two large categories, analysis of raw mean data and DCA suggests that a group's behavior varies between extremes of non-pyrophyte and pyrophyte types, but also according to seed bed depth influence and time of fire. Fire-sensitive groups range from major destruction of seeds irrespective of depth (groups A and C) to some emergence capacity if seeds are located deeper (B). On the other extreme lies the F group with large emergence (and low destruction rate) of near-surface seed beds. In general, the more fire sensitive a subgroup is, the more it can benefit from deeper positioning, for a given fire-time. Conversely, if the limit of destruction is not exceeded, seeders seem to respond in inverse fashion. In short, among each of the two groups, varying degrees of fire sensitivity or susceptibility correlated to taxonomy can still be found between

each group's extremes. As with DCA, if one focuses on modalities of fire and ash treatment using formal predictive models, we could conclude that depth and fire time seem to act as relevant environmental controls interacting with functional species groups' sensitivity to fire.

Although many authors have strongly suggested that there is a relationship between ash cover and germination (e.g., Reyes and Casal 2004), we were not able to find one. This result may indicate that experimental simulations may not be comparable to real field conditions.

A practical consequence is that dominance of fire prone species groups, {D, E, F}, in the

end, including rockroses, brooms, and small heaths, is expected over sclerophyllous paleo-mediterranean tall-shrubs (also mesophytic forest shrubs, e.g., {A,B,C}) if fire regime is intense and in short periods. The pine tree, which is the ecologically dominant plant, seems to align with the fire-sensitive group but with some emergence capacity if seeds are fairly protected by deeper soil layers (i.e., group B). The major ecological issue is that our results suggest that succession in Portuguese pine forests is drawn back to fire-prone pioneer stages by regular fires and kept that way for long periods.

ACKNOWLEDGEMENTS

We would like to thank colleagues Octávio Ferreira and Susana Gomes, forest managers at the Leiria National Forest, as well as José Campos for advice in statistics. Research was supported by the projects PAMAF Project # 8165 and AGRO 283 at the National Forest Research Station supported by the Ministry of Agriculture, Fisheries, and Rural Development; and Project PARLE-D 'promotion of integrated management and disease control of maritime-pine' supported by the Foundation for Science and Technology of the Ministry of Science and Superior Education.

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