



## Effect of fire on hard-coated Cistaceae seed banks and its influence on techniques for quantifying seed banks

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### Abstract

The impact of fire on hard-coated Cistaceae (*Halimium ocymoides*, *Cistus ladanifer*, and *C. salvifolius*) soil seed banks in a Mediterranean 'maquis' shrubland, and its effect on seed germinability were studied. The study also contrasts the effectiveness of two widely used techniques for quantifying seed banks, the seedling emergence and the physical separation methods, in relation to fire. The null hypothesis that a massive enhancement of physically-dormant Cistaceae seed germination by fire would make use of the time-consuming physical separation technique unnecessary was tested. Fire reduced Cistaceae seed banks in the 0–2 cm deep soil layer by both seed fire-consumption and lethal temperatures, revealed by the significant decreasing of the seed bank density and by the increase of apparently-intact but soft-unviable seeds, respectively. In contrast, no damage was recorded in the 2–5 cm soil layer. A dramatic seed bank depletion (> 90%) in both soil layers was recorded one year after fire in the burnt area, coinciding with a significant increase of seedling density confined to the first post-fire year. The ecological consequences of this massive post-fire seed bank input are discussed. A germinability test revealed that germination of surviving Cistaceae seeds was significantly enhanced in all cases except for the *C. salvifolius* seed bank in the deeper soil layer. However, final germination levels (60–75%) did not correspond to the magnitude of seed bank depletion, especially for *C. salvifolius*, which suggests that other environmental factors not exclusively associated with fire may also be important in softening Cistaceae seeds. Germination enhancement by fire soil-heating was not high enough to reject the physical separation technique, at least in the deeper soil layer. The simultaneous use of both seedling emergence and physical separation is recommended for reliable seed bank estimates when a physically-dormant hard-seeded component can be expected in the soil, as in many Mediterranean ecosystems, regardless of fire occurrence.

**Nomenclature:** Tutin et al. (1964–1980).

### Introduction

Cistaceae is a plant family of shrubs, subshrubs and occasionally herbs, including 8 genera and 165 species with a mainly European north-temperate distribution, especially in the Mediterranean basin (Heywood 1985). *Cistus* and, to a lesser extent, *Halimium* species are an important component in many shrublands in fire-prone summer-dry Mediterranean ecosystems (e.g., 'maquis', 'garrigue'). The 'jaral', one of

the most widespread shrubland types in the western Iberian Peninsula, is entirely dominated by *C. ladanifer*; other *Cistus* (i.e., *C. salvifolius*, *C. crispus*) and *Halimium* (i.e., *H. ocymoides*) species are also well represented (Núñez-Olivera et al. 1995).

Principal Cistaceae shrubs usually behave as obligate seeders after disturbances (Trabaud 1970). These species can accumulate long-lived persistent seed banks in the soil through the production of large yields of impermeable-hardcoated seeds, a seed fea-

ture widely extended in the Cistaceae family (Thanos et al. 1992). Hardseededness imposes a primary (physical) seed dormancy, preventing seed germination (Baskin & Baskin 1989). Persistence of seeds in the soil provides dispersal 'in time', which is considered an adaptation to an environment where disturbances are unpredictable (Rolston 1978), as in the case of fires.

Traditionally, there is the point of view that Cistaceae-dominated shrublands are strongly associated with a regime of recurrent wildfires (Gausson 1970). Many *Cistus* species have been considered as social phytophytes (*sensu* Kuhnholz-Lordat 1938), because they establish massively after fire by seed germination, frequently forming pure stands (Kuhnholz-Lordat 1938, Juhren 1966). This large-scale seedling emergence from soil-stored seeds is usually confined to the earliest post-fire stages (Naveh 1974; Arianoutsou & Margaritis 1981). It is broadly accepted that high temperatures recorded in the soil during a wildfire are the main factor responsible for this germination response, since the thermal shock associated with fire can break down the testa of buried seeds, thus, overcoming physical dormancy (Baskin & Baskin 1989). This idea has been largely supported by many laboratory experiments where Cistaceae seed germination was significantly enhanced by heat treatments simulating those fire-temperatures recorded in the upper soil layers (Vuillemin & Bulard 1981; Troumbis & Trabaud 1986; Thanos & Georghiou 1988; Corral et al. 1990; Thanos et al. 1992; Valbuena et al. 1992; González-Rabanal & Casal 1995). However, the effect of fire on natural buried Cistaceae seed populations has not been analyzed in detail. Laboratory experiments have shown not only that heat can stimulate hard-seed germination, but also it can be lethal for the embryo if the temperature is too high or if heat exposure is too long. What is the real impact of fire on the seed banks of these species? To what extent are buried seeds stimulated for germination or killed by fire? How does fire affect the germinability of Cistaceae seeds with burial depth? Is fire-heat the exclusive factor promoting post-fire seed germination? All these topics remain unknown.

The present work forms a part of a more extensive study on the impact of fire on whole seed banks (Ferrandis 1996), where the assessment of soil-stored seeds was carried out by the simultaneous use of a physical separation method and a seedling emergence method. This gave us the chance to compare the effectiveness of these two techniques in relation

to fire. Methods based on physical separation consist of isolating and identifying seeds from soil samples, whereas in seedling emergence methods soil samples are placed under suitable conditions for seed germination, and emergent seedlings are identified and counted as viable seeds. In general, estimates derived by physical separation are higher than those derived by seedling emergence, since the former include non-viable, apparently-healthy seeds, and the latter do not detect viable seeds whose appropriate germination stimuli are not provided (Gross 1990, Manders 1990). Thompson et al. (1997) concluded that methods based on physical separation are costly, time consuming and rather inaccurate. They therefore suggested the use of a standardized seedling emergence method originally designed by Ter Herdt et al. (1996). However, Manders (1990) showed that relatively large hard-coated seeds were easily found by physical separation but achieved low germination levels, escaping detection by seedling emergence. According to this finding, the use of physical separation should be indispensable in studies on the seed banks of many Mediterranean ecosystems where a large Cistaceae, physically-dormant seed component should be expected in the soil. If fire-heat, however, massively stimulated germination of hard-coated seeds, seedling emergence could become effective enough in detecting seeds which survived fire and, consequently, the more tedious physical separation technique could be obviated.

The goals of this study are: (i) to assess the impact of fire on the natural seed banks of Cistaceae species in a Mediterranean shrubland, analyzing the effect on seed survival and germinability, and (ii) to test the null hypothesis that fire makes the use of the physical separation technique unnecessary by the massive heat-enhancement of hard-coated seed germination.

## Methods

### *Study area*

The study area was located in Cabañeros National Park (between the provinces of Ciudad Real and Toledo, west-central Spain), an area of 40 000 ha which pools several well conserved Mediterranean ecosystems typical of the inland Iberian Peninsula. On 15 August 1993 an intense fire affected a dense shrubland (U.T.M. coordinates: 30SUJ7060; altitude: 860 m), totally destroying the above-ground vegetation in an area of around 350 ha. Data from the closest

meteorological observatory (Retuerta, 20 km away) corresponding to a 40 year record period revealed a climate belonging to the temperate Mediterranean type with an annual average temperature of 12.4 °C. Annual rainfall totals 586.4 mm, showing two peaks throughout the year (February and November). High temperature and low rainfall coincidence determines a dry period from June to September, which markedly intensifies during July and August when absolute maximum temperature exceeds 40 °C and rainfall is not over 7 mm a month.

The soil in the area consists of siliceous lithosoils developed by the deposition of fine particles (principally sand and clay) on large stony areas of quaternary quartzites. The current vegetation is the consequence of a regime of recurrent fires in areas originally occupied by *Quercus suber* L. and *Quercus rotundifolia* L. mixed forests. It consisted of a 20–25 year old 'maquis', forming a very dense (plant cover=80–100%) 1.5–2.5 m high shrubland dominated by *Cistus ladanifer* L., *Cistus salvifolius* L., *Halimium ocyroides* (Lam.) Willk., *Erica australis* L., and *Erica arborea* L. Other abundant species were *Arbutus unedo* L., *Phillyrea angustifolia* L., *Lavandula stoechas* L. subsp. *pedunculata* (Miller) Samp. ex Rozeira, and *Erica scoparia* L. Species with a secondary importance were *Cistus populifolius* L., *Chamaespartium tridentatum* (L.) P. Gibbs., *Rosmarinus officinalis* L., and *Thymelaea villosa* (L.) Endl.

#### Seed bank analysis

A stand in the burnt area and another in the adjacent unburnt vegetation (control) were selected on a relatively flat terrain immediately after fire. Soil sampling was carried out two days after fire (summer 1993) in both the burnt stand and the control, and one year after fire (autumn 1994) and two years after fire (autumn 1995) in the burnt stand. In each stand, five 60 cm × 100 cm permanent plots were randomly placed. In each sampling, a 20 cm × 60 cm subplot was excavated from every plot in two successive 0–2 cm (Layer I) and 2–5 cm (Layer II) deep soil layers, each one forming a sample and being studied separately. A 20 cm wide band was left intact between contiguous subplots, so as to avoid soil crumbling after the previous sampling affecting the second one. Samples were carried in plastic bags to the laboratory, where they were completely dried. Big stones and vegetative plant material (i.e., bulbs, thick roots) were removed, and the soil samples were manually homogenized. Every

sample was halved by mass, assigning a half (subsample) to the seedling emergence method and the other subsample to the physical separation method.

In the seedling emergence method, subsamples were spread over a 2 cm layer sterile peat in 30 cm × 40 cm black plastic seedlings trays, and placed in a greenhouse for seedling emergence after being stored in darkness at 4–6 °C for 6 weeks. This previous cold-stratification has been shown to enhance the effectiveness of the method because it promotes germination of many physiologically-dormant seed species (Gross 1990). The recording period lasted 12 months. Thickness of the soil sample layer in trays never exceeded 1 cm. Trays were periodically watered so as to maintain samples constantly wet, and checked every 2 days for seedling emergence. There was no artificial temperature control system in the greenhouse. Emergent seedlings were removed as soon as they were identified. During the summer (July and August), the few seedlings remaining unidentified were transplanted to individual pots and tray watering was interrupted until September.

In the physical separation method, seeds were separated using a binocular microscope after subjecting soil to a dispersal treatment with a sodium hexametaphosphate dissolution (5%) and a washing through a 0.5 mm mesh sieve. In consequence, no species having seeds smaller than this size was accounted for by this technique. An initial attempt to sieve soil through a 0.25 mm mesh size had to be promptly abandoned, since the huge difficulty in detecting and counting the highly abundant tiny *Erica* seeds in samples made it totally time-consuming and inoperative. Criteria for assuming a seed as being viable depended on the type of seed coat. Soft-coated seeds were cut open and the embryo checked for good appearance, especially hydration and colour, whereas hard-coated seeds were initially considered to be viable if they had a healthy external appearance.

Data from the first sampling (1993) was used to analyze the fire impact on Cistaceae seed banks and to contrast the null hypothesis propounded in relation to quantifying methods. From the second and the third soil sampling (1994 and 1995), only data concerning Cistaceae are shown in the present work, in order to assess the post-fire changes in their buried seed populations. Changes in other taxa in the seed bank are analyzed in detail by Ferrandis et al. (1998).

### *Record of seedlings in the field during the first two post-fire springs*

Seedlings appearing in twenty 20 cm × 60 cm plots in each stand were identified and counted on 1 March and 25 May in 1994. One year later, on 3 March and 30 May 1995, new seedlings in the 20 plots in the burnt stand were counted. In each plot, the highest of the two yearly records was considered for each species so as to minimize any possible error in the estimation owing to seedling mortality between the two yearly annotations.

### *Analysis of fire effect on seed germinability*

Cistaceae seeds isolated by the physical separation technique from burnt and unburnt soil samples collected in summer 1993 and initially considered as being viable by their healthy external appearance, were tested for germination. For each Cistaceae species and separately for every soil layer in each stand, a similar seed number from every subsample were grouped, and ten 20 seed replicates were made if possible. When seed quantity was not high enough, replicate number or size were reduced in order to obtain a suitable replication for statistical analysis. Seeds were germinated in plastic Petri dishes (diam. 7 cm) over 2 layers of filter paper. They were kept saturated with distilled water during the whole experiment, and laid at random on a temperature controlled chamber (model Radiber, AGP-600, 9,000 LUX, ±0.1 °C of precision). Incubation was carried out in darkness at 17 °C. This temperature has been shown to be optimal for seed germination in many *Cistus* species (Vuillemin & Bulard 1981, Thanos & Georghiou 1988), whereas light conditions are irrelevant (Thanos & Georghiou 1988). Radicle emergence was the criterion for deciding if germination had taken place. Recounting of the germinated seeds was done every two days over a period of 52 days. The criterion for finishing the experiment was the absence of new seed-imbibition in the last two weeks. Imbibed seeds for that period which did not germinate were computed, and both imbibed and non-imbibed ungerminated seeds were tested for viability by a tinction with a 2, 3, 5-triphenyl-tetrazolium chloride dissolution (0.1%, 15 hours at 20 °C in darkness; Besnier 1989).

### *Statistical analysis*

In general, Zar's (1984) suggestions were taken into account in the application of tests. Data of seed bank

and seedling records in the field did not fit to a normal distribution, so that comparisons were performed by nonparametric tests at the 0.05 significant level. Hence, seed bank density (i.e., seeds per m<sup>2</sup> in a given soil layer) and seedling density (i.e., seedlings per m<sup>2</sup>) in control and burnt stand were compared by the Mann–Whitney *U* test. Seed bank changes in the burnt stand throughout the two post-fire years (from 1993 to 1995) were analyzed by the Friedman test for related samples, whereas the Wilcoxon paired-sample test was performed to compare seedling density in the burnt stand between 1994 and 1995.

In the germinability test, three parameters were analyzed: final germination level, germination rate expressed by *T*<sub>50</sub> (time, in days, required for manifestation of half of the final germination level), and final percentage of unviable imbibed seeds as a measure of seeds killed by fire-heat. For each parameter, data were submitted to a two-way Anova defined by stand (control, burnt) and soil layer (0–2 cm, 2–5 cm) variables. Normality (Kolmogorov–Smirnov test) and homogeneity of variance (Cochran test) were previously checked. Data of final germination and unviable imbibed seeds, both in percentages, needed an arcsine transformation.

The log-likelihood ratio for contingency tables was used to determine the efficacy of each quantifying seed bank technique in detecting the presence of different-coated (hard or soft) seeds. However, when the expected frequency in a cell was less than 5, Fisher's exact test was used instead.

## **Results**

### *Impact of fire and post-fire changes in the seed banks*

Comparisons between the control and the burnt stand revealed that fire significantly reduced seed bank density of the three woody Cistaceae species in the upper soil layer (Mann–Withney test: *U* = 0.0; *p* < 0.01; Figure 1a), but not in the deeper one (*U* = 10.0; n.s.; Figure 1b).

A gradual reduction of soil seed reserves was recorded for the three species during the two first post-fire years, resulting in a total (i.e., *C. ladanifer*), nearly total (i.e., *C. salvifolius*), or severe (i.e., *H. ocymoides*) seed bank depletion (Figure 1). So, at the end of summer 1994, these species showed a dramatic reduction of their seed banks in both soil layers; this soil seed population reduction was intensified two years after

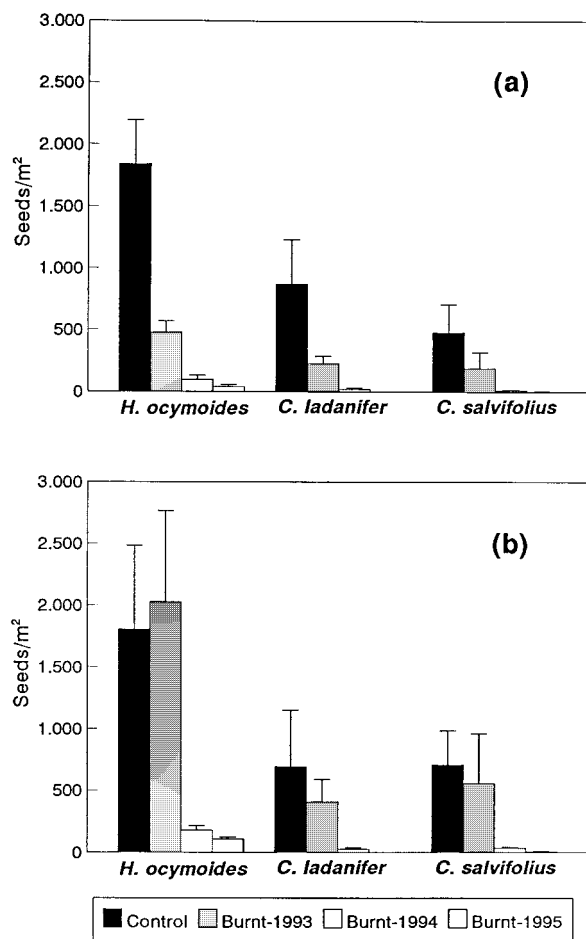


Figure 1. Seed bank density (seeds  $m^{-2}$ ) of Cistaceae species recorded in (a) 0–2 cm and in (b) 2–5 cm deep soil layers in the control (August 1993) and in the burnt stand (August 1993, September 1994, and September 1995). Bars on columns represent the standard deviation.  $n = 5$  in all cases.

fire, in September 1995 (Friedman test: 0–2 cm deep layer:  $\chi_r^2 = 12.05$ ;  $p < 0.01$ ; 2–5 cm deep layer:  $\chi_r^2 = 8.40$ ;  $p < 0.05$ ; a multiple comparison revealed significant differences among the three years in both soil layers).

#### Post-fire seedling emergence in the field

Germination of hard-coated Cistaceae seed banks was enhanced by fire, as denoted by the significantly higher emergent seedling (plants younger than 1 year) density of these species recorded during the first post-fire spring (1994) in the burnt stand than in the control stand (Mann–Whitney test:  $U = 3.0$ ;  $p < 0.001$ ; Table 1).

Table 1. Average seedling density (seedlings  $m^{-2}$ ;  $\pm$  standard deviation) of Cistaceae species in the first (1994) and in the second (1995) post-fire springs.  $n = 20$  in all cases.

	1994		1995
	Control stand	Burnt stand	Burnt stand
<i>H. ocymoides</i>	6.2 ( $\pm 2.5$ )	180.8 ( $\pm 38.3$ )	10.0 ( $\pm 2.4$ )
<i>C. salvifolius</i>	4.1 ( $\pm 1.6$ )	35.0 ( $\pm 14.3$ )	0
<i>C. ladanifer</i>	2.5 ( $\pm 1.4$ )	73.3 ( $\pm 22.6$ )	4.1 ( $\pm 1.6$ )
Totals	12.9 ( $\pm 3.6$ )	289.2 ( $\pm 43.04$ )	14.1 ( $\pm 2.2$ )

This massive post-fire seed germination, however, was restricted to the first year after fire. So, seedling density of woody Cistaceae species was significantly reduced during the second post-fire spring (1995) with respect to the previous year (Wilcoxon test:  $z = 3.92$ ;  $p < 0.001$ ; Table 1).

#### Effect of fire on the germinability and viability of hard-coated seeds

Germination of *H. ocymoides* and *C. ladanifer* seeds isolated by the separation method was promoted by fire: final germination level was significantly higher for seeds extracted from the burnt soil samples than from the control (Figure 2a,b). In contrast, germination of *C. salvifolius* was only enhanced by fire in seeds contained in the upper soil layer, but not for those in the deeper one (Figure 2c). Germination rate, expressed by the  $T_{50}$  parameter, did not show variation by fire in any species (Figure 2).

For the three woody Cistaceae species, the number of unviable imbibed seeds was significantly higher in the upper soil layer of the burnt stand than in the other three cases (two-way Anova; Figure 2), where this class of seed was scarce. Non-imbibed seeds of the three species showed a high level of viability in both soil layers of both stands, exceeding in all cases 90% (Figure 2).

#### Effect of fire on techniques for quantifying seed banks

A complete list of the estimation of seed bank composition by both the separation and the emergence techniques is shown in Table 2. In soil samples from unburnt stand, the effectiveness of each method depended on the seedcoat type. In both control soil layers, the separation technique detected a higher quantity of hard-coated seeds (separation

Table 2. List of species and seeds recorded in the analysis of the whole soil seed bank contained in samples collected immediately after fire (summer 1993). Estimates by seedling emergence (Em.) and by physical separation (Sep.) are given for each soil layer (I= 0-2 cm; II= 2-5 cm) in the control and in the burnt stand. Seed coat type: s= soft; h= hard; u= unknown. Seed size classes: 1=  $\varnothing > 1$  mm; 2=  $1 \text{ mm} > \varnothing > 0.5$  mm; 3=  $0.5 \text{ mm} > \varnothing > 0.25$  mm. Dead seedlings= seedlings in the emergence method which died before being identified. Species 1 and 2= seeds in the separation method which could not be identified.

	Seed size	Seed coat type	Control				Burnt stand			
			Layer I		Layer II		Layer I		Layer II	
			Em.	Sep.	Em.	Sep.	Em.	Sep.	Em.	Sep.
<i>Agrostis tenerrima</i>	3	u	1	-	-	-	2	-	1	-
<i>Aira caryophylla</i>	3	s	10	-	3	-	-	-	-	-
<i>Aiopsis tenella</i>	3	u	5	-	10	-	-	-	-	-
<i>Anarrhinum bellidifolium</i>	3	s	1	-	-	-	1	-	-	-
<i>Anthoxanthum aristatum</i>	2	s	1	-	3	-	-	-	-	-
<i>Arabidopsis thaliana</i>	2	s	15	-	4	-	-	-	-	-
<i>Asterolinon linum-stellatum</i>	2	s	21	48	4	12	-	1	-	1
<i>Briza minima</i>	2	u	-	-	-	-	1	-	-	-
<i>Conyza canadensis</i>	3	s	1	-	-	-	-	-	-	-
<i>Coincya hispida</i>	1	s	-	-	-	-	1	1	-	-
<i>Centaurium maritimum</i>	3	s	-	-	1	-	-	-	-	-
<i>Chamaespartium tridentatum</i>	1	h	-	-	-	-	-	-	-	1
<i>Cistus ladanifer</i>	2	h	38	261	9	208	55	78	36	122
<i>Cistus salvifolius</i>	1	h	6	143	14	212	26	57	18	167
<i>Crassula tillaea</i>	3	s	-	-	1	-	-	-	-	-
<i>Crucianella angustifolia</i>	2	s	3	-	-	-	-	-	-	-
<i>Epilobium tetragonum</i>	1	s	-	-	-	-	-	-	1	-
<i>Erica sp.</i>	3	s	61	-	92	-	659	-	1062	-
<i>Galium parisiense</i>	2	s	1	-	-	-	-	-	-	-
<i>Gnaphalium luteo-album</i>	2	s	1	-	-	-	-	-	-	-
<i>Gymnostyles stolonifera</i>	1	s	-	-	1	-	-	-	-	-
<i>Halimium ocymoides</i>	2	h	23	552	32	541	82	143	86	608
<i>Herniaria cinerea</i>	3	s	-	-	1	-	-	-	-	-
<i>Jasione montana</i>	u	u	-	-	-	-	2	-	-	-
<i>Juncus buffonius</i>	3	s	3	-	1	-	-	-	-	-
<i>Lavandula stoechas</i>	1	s	24	75	1	4	-	-	-	-
<i>Logfia minima</i>	3	s	27	-	1	-	-	-	-	-
<i>Misopates orontium</i>	1	s	1	-	-	-	-	-	-	-
<i>Ranunculus sp.</i>	u	u	17	-	4	-	-	-	-	-
<i>Rubus ulmifolius</i>	1	s	1	-	-	-	-	-	-	-
<i>Rumex acetosella</i>	1	s	20	41	10	75	12	35	18	33
<i>Sagina apetala</i>	3	s	1	-	-	-	-	-	-	-
<i>Sanguisorba minor</i>	1	s	18	50	-	17	-	-	-	-
<i>Sonchus oleraceus</i>	2	s	5	-	-	-	-	-	-	-
<i>Spergularia rubra</i>	3	s	2	-	-	-	-	-	-	-
<i>Trifolium glomeratum</i>	2	h	-	2	-	10	-	-	-	-
<i>Tuberaria lignosa</i>	2	h	-	-	-	-	2	-	-	-
<i>Vulpia myuros</i>	2	s	159	41	5	10	-	-	-	-
Gramineae			8	1	5	-	3	-	-	-
Dead seedlings (emergence method)				5		1		5		
Species 1 (separation method)								2		
Species 2 (separation method)				5		10				1

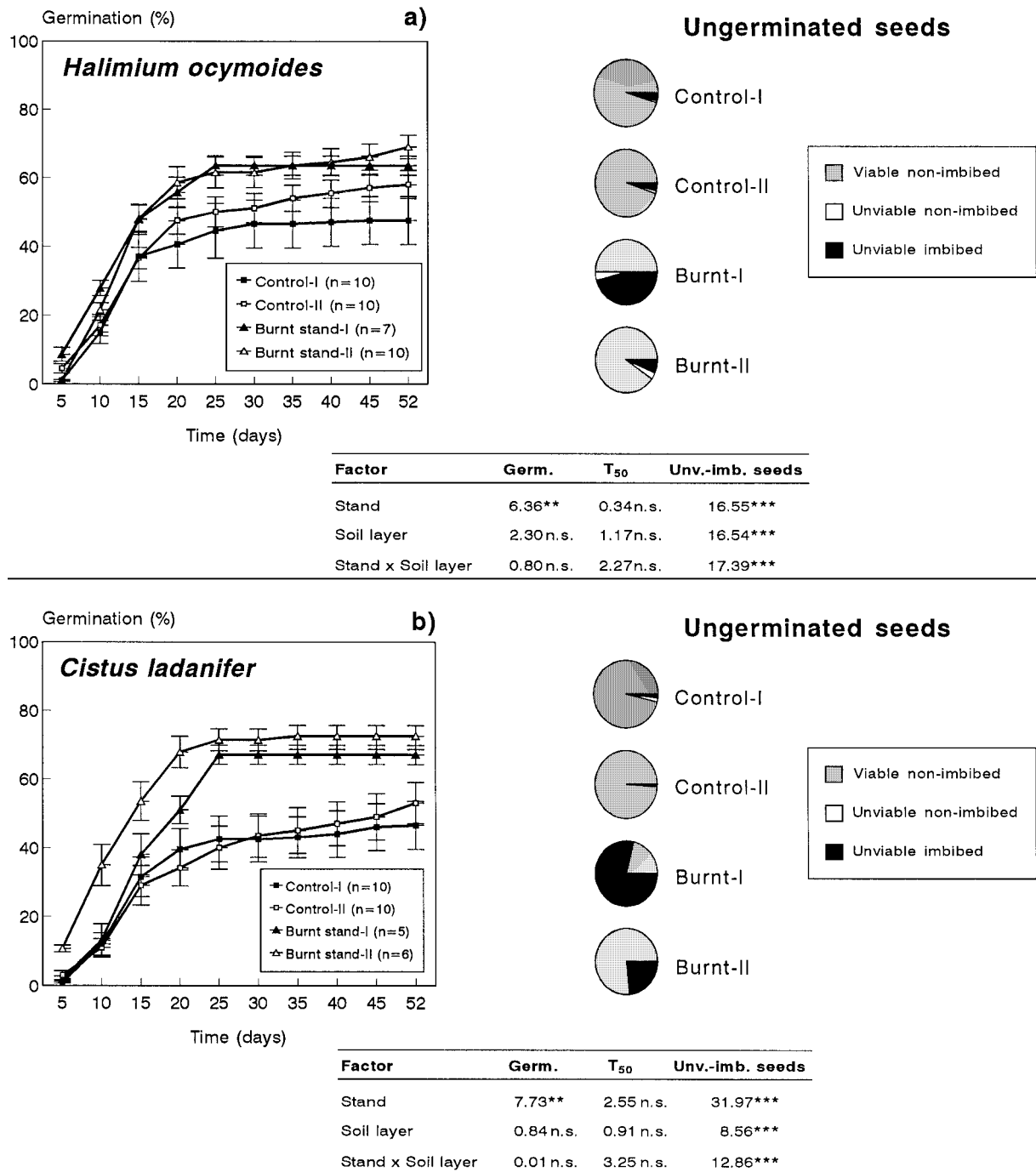
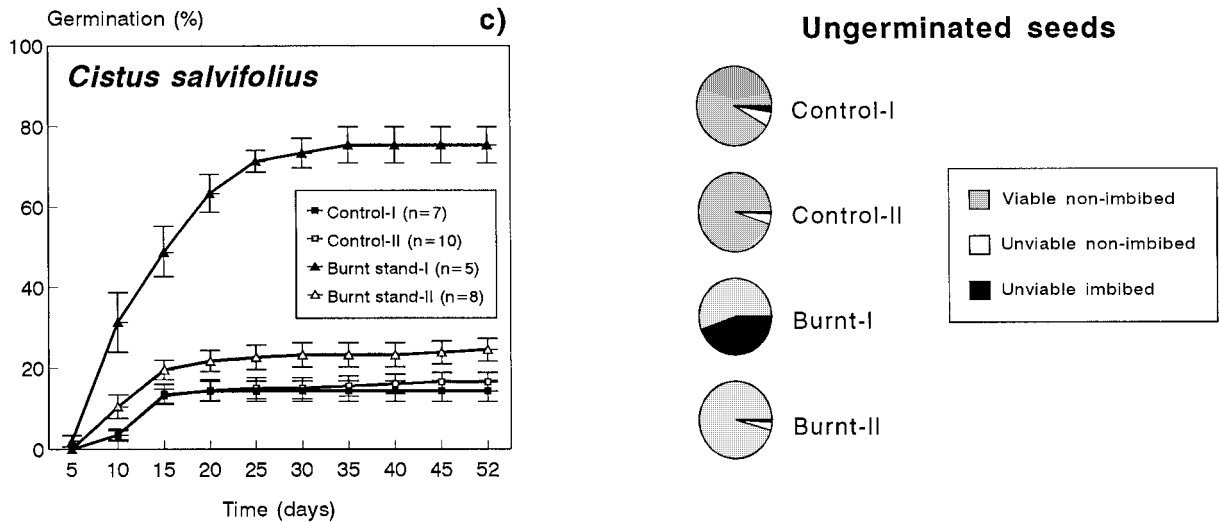


Figure 2. Germinability test on seeds isolated from soil samples for (a) *H. ocymoides*, (b) *C. ladanifer*, and (c) *C. salvifolius*. The figure shows the curves of cumulative average germination ( $\pm$  standard error) for seeds from each soil layer (I= 0–2 cm; II= 2–5 cm) in the control and in the burnt stand, as well as the viability/imbibition stage (in percentages) of seeds remaining ungerminated at the end of the test. Furthermore, F ratio for every factor in the two-way Anovas performed on final germination, T<sub>50</sub>, and unviable imbibed seed percentage, respectively, is shown in a table. Significance level: n.s.= non-significant; \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ .



Factor	Germ.	T <sub>50</sub>	Unv.-imb. seeds
Stand	77.10***	0.28 n.s.	4.03*
Soil layer	43.92***	0.39 n.s.	6.22**
Stand x Soil layer	69.03***	0.18 n.s.	4.57*

Figure 2. Continued.

method/emergence method ratio= 14.3 in Layer I, and 17.6 in Layer II), whereas the number of soft-coated seeds was slightly higher in the emergence technique (emergence/separation= 1.4 in Layer I, and 1.1 in Layer II; Table 3). The emergence method, however, detected a much higher species richness than the separation technique in the upper soil layer. The difference became non-significant in the deeper soil layer due to a reduction in the presence of softseeded species (Table 3).

In the burnt soil samples, there was also a remarkable difference in the number of seeds detected by each method in both soil layers. In spite of the decrease in the separation/emergence ratio for hard-coated seeds (1.7 in Layer I, and 6.4 in Layer II), the massive presence of small-seeded *Erica sp.* seedlings in trays of the emergence technique substantially increased differences in detecting soft-coated seeds between methods (emergence/separation= 18.2 in Layer I, and 31.8 in Layer II; Table 4). The removal of many softseeded species from the seed bank by fire (Table 2), eliminated significant differences in the species richness detected by both techniques depending on the seedcoat type (Table 4).

## Discussion

### Effect of fire on seed banks

Fire moderately affected Cistaceae seed banks. The fire-heat insulating nature of the soil (De Bano et al. 1977) in combination with the protection provided by the presence of an impermeable palisade layer with strongly lignified cell walls in the testa (Corral et al. 1989) should prevent embryo death by fire in Cistaceae species, particularly in the 2–5 cm deep soil layer. At this soil depth, neither a seed bank reduction (seed fire-consumption) nor an increase of apparently-intact but soft-unviable seed number (fire-heat damage) were detected, in contrast to the 0–2 cm deep soil layer. However, fire damage was not dramatic at this depth: around 750 viable seeds m<sup>-2</sup> was counted in this soil layer after the passage of fire.

Seed survival is crucial in maintaining populations of these obligate seeders with short-distance seed dispersal (Troumbis & Trabaud 1986), because they rely solely on seed banks to replace populations after the passage of fire. Cistaceae plant recovery was accomplished by a massive seedling emergence closely confined to the first post-fire year, in agreement with



Table 3. Contingency tables for the number of seeds and species encountered in the control soil samples by seedling emergence and physical separation according to the seed coat type. The corresponding statistic ( $G = \log$ -likelihood ratio) is also given. Significance level: n.s.= non-significant; \* =  $p < 0.05$ ; \*\*\* =  $p < 0.001$ .

Seeds			Species				
Method	Seed coat type		Totals	Method	Seed coat type		Totals
	Hard	Soft			Hard	Soft	
(a) Layer I							
Emergence	67	376	443	Emergence	3	21	24
Separation	958	255	1213	Separation	4	5	9
Totals	1025	631	1656	Totals	7	26	33
$G = 577.06^{***}$			Fischer's exact test *				
(b) Layer II							
Emergence	55	128	183	Emergence	3	14	17
Separation	971	118	1089	Separation	4	5	9
Totals	1026	246	1272	Totals	7	19	26
$G = 278.44^{***}$			Fischer's exact test; n.s.				

most studies in the Mediterranean basin (Naveh 1974; Arianoutsou & Margaris 1981). Naveh (1974) imputed the decrease of *Cistus sp.* seedling emergence in subsequent years to drought and an increase in the competition intensity, which would inhibit germination. The results, however, show that such temporal post-fire seedling emergence patterns were caused by a severe, nearly total, seed bank depletion, as hypothesized by Purdie (1977) for several legumes and other species in a dry sclerophyll forest in Australia.

Seed bank output by predation and pathogen attack is negligible in *Cistus sp.*, and seed loss by erosion is only prominent for those seeds lying on the soil surface in slope terrains (Troumbis & Trabaud 1987). Thus, the confinement of Cistaceae species recruitment to the early post-fire stages is due to the dramatic depletion of soil seed reserves after massive seed germination promoted by some factor/s related to fire. In fire-prone ecosystems, this strategy must provide an ecological advantage to these heliophylous (Kurrnholtz-Lordat 1938) obligate seeders with long-term permanent seed banks if seedlings have a long enough time to reach maturity and replace soil seed stocks (two years for *H. ocymoides* and *C. salvifolius*, three for *C. ladanifer*, in the study site; unpublished data). This, however, can be fatal for population persistence under a regime of too short inter-fire periods. Trabaud (1992) recorded a local extinction of *C. mon-*

*speliensis* and a dramatic decrease in population size of *C. salvifolius* with an experimental inter-fire period of 2–3 years, whilst 6 years did not reduce their presence.

The germinability test showed that fire directly enhanced seed germination in the three Cistaceae species. This supports the conclusions made from seed-heating treatments in the laboratory (e.g., Vuillemin & Bulard 1981; Valbuena et al. 1992), from seedling emergence records from heated soil samples (Trabaud 1980), and also from seedling emergence records in the field during the early post-fire succession stages (Juhren 1966; Naveh 1974, Arianoutsou & Margaris 1981) that fire-heat is one of the most important natural trigger factors. However, final germination levels achieved by isolated seeds from burnt soil samples (generally < 70%, never > 75%) did not correspond to the magnitude of seed bank depletion registered one year later (> 90%, except for *H. ocymoides* in the upper soil layer), even if dead seeds were taken into account. The most noteworthy case was the *C. salvifolius* seed bank contained in the deeper soil layer, which suffered a dramatic depletion (inter-year reduction= 93.4%) despite the fact that seed germination was not promoted (final germination= 24.5%). Other environmental factors involved in hard-coated seed softening apart from fire-heat are: high temperatures by sunshine and diurnal temperature fluctuation

Table 4. Contingency tables for methodology in relation to seed coat type in the burnt soil samples. Symbols are equal to those in Table 3.

Seeds			Species				
Method	Seed coat type		Totals	Method	Seed coat type		Totals
	Hard	Soft			Hard	Soft	
(a) Layer I							
Emergence	165	673	838	Emergence	4	4	8
Separation	278	37	315	Separation	3	3	6
Totals	443	710	1153	Totals	7	7	14
$G = 476.63^{***}$			Fischer's exact test; n.s.				
(b) Layer II							
Emergence	140	1081	1221	Emergence	3	3	6
Separation	898	34	932	Separation	4	2	6
Totals	1038	1115	2153	Totals	7	5	12
$G = 1820.33^{***}$			Fischer's exact test; n.s.				

(Rolston 1978, Baskin & Baskin 1989), overwintering (Baskin & Baskin 1989), and alternating humidity and drought during summer (Trabaud & Oustric 1989). The effect of these factors could be amplified in recently burnt, bare, soils, where thermal diffusivity may be higher due to the increase of the mineral fraction and the reduction of soil porosity caused by the elimination of the organic matter (De Bano et al. 1977). Christensen & Kimber (1975) pointed out that hard seeds remaining dormant after fire could have suffered however a debilitation at any coat region (i.e., hilum, micropyle, or at a non-specialized zone) which would facilitate the subsequent softening by another factor. Such a mechanism could satisfactorily explain the post-fire depletion of the non-stimulated *C. salvifolius* seed bank recorded in the 2–5 cm deep soil layer. Although emergence success from this depth may be low, the absence of any other dormancy mechanism beyond the seed coat impermeability in Cistaceae species (Thanos & Georghiou 1988; Corral et al. 1990) would induce seed germination if some of these factors produce seedcoat degradation.

Thick-coatedness may be selected by fire because it provides embryo protection and heat-induced germination. Thanos et al. (1992) postulated a seed ecology syndrome for Cistaceae species where the co-occurrence of hard-coatedness and other seed features would give them a considerable ecological advantage in fire-prone Mediterranean ecosystems. However, this does not mean that Cistaceae species are oblig-

ate pyrophytes. Trabaud (1995) has pointed out that these species are not exclusively dependent on fire, but are plants occupying open disturbed areas with a scarce vegetative cover and without the presence of aggressive competitors. It is probable that hardseededness is favoured under any regime of unpredictable recurrent disturbances, regardless of the nature of the disturbance, because it can simultaneously provide (i) embryo protection, (ii) seed persistence in the soil (Thompson & Booth 1993), and (iii) an efficient dormancy mechanism (if alone) for the quick detection of suitable conditions for seedling establishment in a warm dry-summer Mediterranean climate. To what extent Cistaceae behave as pyrophytes rather than opportunistic invaders, or vice versa, cannot be discerned in the present work. Although fire-heat promoted seed germination, post-fire environmental conditions not exclusively related to fire also had to play an important role. The influence of thermal shocks on Cistaceae seed germination has been extensively analysed. However, the effect of other agents different from fire-heat (i.e., high summer temperatures, temperature fluctuations, alternate soaking and drying, freezing and thawing) on seed softening should be investigated in detail, as well as seedling establishment patterns in disturbed but unburnt areas. This would determine the real degree of dependence on fire of these widespread species in the Mediterranean basin.

### *Effect of fire on techniques for quantifying seed banks*

The effectiveness of the physical separation technique was totally limited by seed size. An initial attempt to isolate all seeds larger than 0.25 mm from soil samples had to be promptly abandoned, since detecting and counting tiny seeds, especially of *Erica sp.*, by microscope was totally time-consuming and inaccurate. This result is in agreement with the general finding (Gross 1990; Manders 1990; Thompson et al. 1997) that physical separation is very tedious and inoperative for the study of whole seed banks. Since a 0.5 mm sieve was finally used, the seedling emergence method was necessary for detecting many soft-coated small-seeded species in the control samples. However, the unburnt seed bank had a prominent hard-coated Cistaceae component that was highly viable (over 90% in all the three species), easily detectable by the microscope due to seed size in combination with shape and colour, but physically dormant (50–80%). The emergence technique was very ineffective in detecting it. In consequence, the simultaneous use of both techniques should be used in studies on the whole seed bank in ecosystems not submitted to a recent fire where the presence of a high hard-coated component can be suspected, as in the case of many Mediterranean ecosystems (Ferrandis 1996).

Although thermal shock improved the effectiveness of the seedling emergence method in detecting hard seeds, germination promotion by fire was not high enough to accept our null hypothesis, especially in the 2–5 cm deep soil layer. It may be due to a less intense thermal scarification at this soil depth, which would emphasize the importance of post-fire environmental factors in Cistaceae germination. Thus, the physical separation method cannot be obviated. However, the use of the seedling emergence technique would also be necessary, due to the high presence of tiny *Erica sp.* seeds in the burnt soil samples, which escape detection by physical separation. In spite of the elimination of most relatively small soft-seeded species from the seed bank, a high number of *Erica sp.* seeds survived fire and its germination was enhanced by some factor related to fire (Ferrandis et al. 1998), so that a much higher number of *Erica sp.* seedlings was recorded in the burnt than in the unburnt soil samples. Therefore, the simultaneous use of both techniques remains necessary for reliable estimates of the whole seed bank, despite the fire effects.

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