Proceedings 10th MEDECOS Conference, April 25 – May 1, 2004, Rhodes, Greece, Arianoutsou & Papanastasis (eds)

Patterns of seed dispersal in three co-occurring *Cistus* species in a *Pinus halepensis* forest of central Greece

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Keywords: *Cistus creticus* L., *Cistus salviifolius* L., *Cistus monspeliensis* L., canopy seed bank, seed dispersal, bradychory, hard-coat seeds, survival traits

ABSTRACT: *Cistus* species are seasonal dimorphic shrubs typical of Mediterranean ecosystems. The majority of them constitute of obligate seeding species, which are early colonizers of disturbed sites, particularly after fire. Patterns of timing of seed dispersal were studied in three co-occurring species of *Cistus*, namely *C. creticus*, *C. salviifolius* and *C. monspeliensis* in a regenerating burnt Aleppo pine forest of Attica, Greece, for a period of one year. From the three species studied, *C. creticus* was found to retain its seeds on its canopy for periods longer than the other two congeneric species. These fruits were formed during previous reproductive periods and they remained on the canopy either closed or opened. Germination experiments showed that seeds of these fruits are exclusively hard-coated seeds. No such mechanism was detected for the other two *Cistus* species studied. Our results suggest that the gradual seed release recorded in *C. creticus* could constitute an efficient risk-reducing trait, which combined with the fact that all seeds remaining on the closed fruits on the plant are hard-coated, could be considered as relevant advantages of the species in the high risk environments it occurs. The other two species seem to be less equipped with such strategies, possessing less staggered mechanisms of both seed release and seed germination.

1 INTRODUCTION

Climatic stress, soil nutrient deficiencies and fire play an important role upon mediterranean ecosystems around the world (Arianoutsou 1998). As a consequence, all biotic communities of these ecosystems have strategies for coping with the above mentioned environmental factors. Plants have mechanisms allowing them to avoid or to endure the direct action of climatic (mainly drought) or soil (nutrient deficiencies) stresses and fire disturbance (Arianoutsou 1998).

The Cistaceae family includes numerous species typical of Mediterranean shrublands (Kazanis and Arianoutsou 1996). The 16 species of the genus *Cistus* are found throughout the Mediterranean region and the Atlantic Islands (Greuter et al. 1984). Many are pioneer species in the post-fire succession in almost all types of Mediterranean communities (Naveh 1974, Arianoutsou and Margaris 1981, Kazanis and Arianoutsou 1996, Arianoutsou 1998, Arianoutsou and Ne'eman 2000). *Cistus* seeds are characterized by the presence of physical dormancy, which in addition to high seed longevity (Troumbis and Trabaud 1986) and small size and mass, allows the formation of persistent soil seed banks (Grime 1989). Most *Cistus* species have massively evolved this adaptation and form persistent banks of hard coated seeds (Thanos et al. 1992) in the fire-prone mediterranean habitats (Trabaud et al. 1997). The genus *Cistus* comprises woody perennial seasonal dimorphic

shrubs, possessing a typical post-fire seeding strategy (Naveh 1974, Arianoutsou-Faraggitaki and Margaris 1982), with seed germination enhanced after fire (Arianoutsou and Margaris 1981) by heat-induced scarification of the hard seed coats (Thanos and Georghiou 1988, Perez-Garcia 1997, Izhaki et al. 2000). Increased post-fire seed germination of *Cistus* spp. has been observed in many field studies (Kazanis and Arianoutsou 1996). Moreover, each year adult *Cistus* individuals release a great amount of seeds, which remain close to the mother plants possessing no special mechanism of distant dispersal (Troumbis and Trabaud 1986, Bastida and Talavera 2002). As for the timing of seed dispersal various patterns have been reported for *Cistus* spp.: either concentrated in late summer-early autumn as for *C. creticus* (Troumbis and Trabaud 1986) or more diffused in time, as in the case of *C. salviifolius* in regions of Peloponnese, Greece (Troumbis and Trabaud 1986) and of *C. ladanifer* and *C. libanotis* in Huelva, SW Spain (Bastida and Talavera 2002).

The primary aim of this work was to study the phenology of fruit formation and the timing of seed release in three co-occurring *Cistus* species, *Cistus creticus*, *Cistus salviifolius* and *Cistus monspeliensis*. As the experiment was evolving an additional question was addressed which dealt with the exploration of the seed germination characteristics in relation to the time of their release.

2 MATERIALS AND METHODS

2.1 *Study site and species studied*

The study site (Agios Stefanos) is situated at the foothills of Mt. Penteli in the south-eastern part of Attica, Central Greece, at an altitude of 380 m a.s.l. The climate in the region is typical mediterranean with a mean annual temperature of 16 ^{o}C and a mean annual rainfall of 455 mm. The parent rock material of the site is schist. The *Pinus halepensis* forest that was covering the area was burnt in the summer of 1993; hence at the time of the study the study site was at the 7th post-fire year. The vegetation consisted mainly of low seasonal dimorphic woody shrubs (*Cistus* spp., *Sarcopoterium spinosum*, *Callicotome villosa*, *Satureja thymbra*, *Genista acanthoclada*), a few scarce individuals of evergreen shrubs (*Pistacia lentiscus)*, young *Pinus halepensis* saplings and herbaceous species of the Graminae family. The contribution of *Cistus* spp. to the total vegetation cover accounted for a total of 40%. Three *Cistus* species were present in the site [*C. creticus* L., *C. salviifolius* L. and *C. monspeliensis* L.] and they were all studied.

Cistus species are seasonal dimorphic woody shrubs and they form woody, loculicidal capsules that contain numerous small, angled seeds. *C. creticus* is about 100 cm in height, distributed throughout Greece and the Aegean, found in rocky places, dry hill slopes and woodland margins as well as in maquis and garrigue. Its fruits are dark brown, woody, hairy, and thinly villose (Meikle 1977). The capsule is broadly ovoid or subglobose, 6-10 mm long and 6-8 mm wide. *C. salviifolius* is a bushy evergreen shrub distributed throughout the Mediterranean, usually shorter than *C. creticus* and it is found in similar places. It forms globose capsules about 7 mm in diameter, thinly hairy, dark brown, concealed by the persistent, accrescent, papery sepals. *C. monspeliensis* is also a woody shrub about 100 cm in height, distributed throughout the Mediterranean in rocky habitats. Its fruits are subglobose, about 4-5 mm in diameter, dark brown, subglabrous, concealed within the accrescent, papery, persistent sepals (Meikle 1977). Fruit formation for all the three species takes place after the end of the wet period, at the beginning of the dry, warm period. Seed dispersal begins during the dry summer. All three species are rather short living species, their individuals appear a maximum life span of about twenty years (Arianoutsou - Faraggitaki and Margaris 1982, Skourou 2003).

2.2 *Seed dispersal*

In order to study the duration of the period of seed dispersal of the three *Cistus* species, 30 similar sized individuals of every species were selected and marked within the above described area. All plants and fruits were tagged at the onset of the dispersal period (June 1999) with numbered plastic

labels. At the beginning of the study, 185 *C. creticus* fruits, 237 *C. salviifolius* fruits and 617 *C. monspeliensis* fruits were available on the marked plants and thus they were all marked. The fruits were monitored on a two-month basis for one year. On each occasion, 4 types of fruits were recorded: i) those that were produced during the previous reproductive period (year 1998) (last year's-L), ii) those that were produced during the current reproductive period and had not been dispersed yet (closed-C), iii) those that were produced during the current reproductive period and from which seed dispersal had started but not finished (open-O) and iv) those that were produced during the current reproductive period and from which seed dispersal had been completed (empty-E). Last year's fruits were distinguished from those of the current year due to their darker color and lack of sepals.

2.3 *Seed germination*

The quality of the seeds (hard vs. soft seed coated) contained in the fruit-types studied, was studied with preliminary germination tests. Fruit collection took place in October 2000 at the site of Agios Stephanos. At that time, almost all types of fruits were present; *C. creticus* was bearing fruits which had been produced during the previous reproductive period (L) as well as closed (C) and open (O) fruits produced during the current reproductive period. *C. salviifolius* had fruits produced only during the current year (O and C), while *C. monspeliensis* had mainly open fruits with and without seeds. Closed *C. monspeliensis* fruits were too few to be collected since they would not provide enough seeds for the experiment planned. Empty fruits were not collected as they had no seeds to be tested. For each fruit type and for each species, 30 capsules were randomly collected from 3-6 plants selected at random, put in paper bags and transported to the laboratory. Fruits were air-dried and kept in paper bags for a three-month period.

Seeds were removed from their capsules and pooled in groups corresponding to the types of fruits from which they had originated. Each group was divided into two lots. One lot was left untreated as a control, while the other was subjected to heating at 100^{o}C for 15 min, a combination of conditions which has been proved to promote germination to maximum (Thanos and Georghiou 1988). Thirty (30) seeds from each lot per fruit type were placed in Petri dishes (9 cm in diameter) lined with two filter paper disks and moistened with 6 ml of de-ionized water. Five replicates per fruit type were made. Germination was accomplished in dark, under constant temperature (15 ^{o}C), in controlled temperature cabinets (model BK 5060 EL, Heraeus, Hanau, W. Germany). The criterion for germination was visible radicle protrusion. Measurements were taken in weekly intervals and germinated seeds were discarded after having been counted. Germination tests were considered finished when no more germination was noticed for two weeks. The duration of the experiment was 10 weeks, thus germination was completed within 8 weeks after the initiation of the test.

Seeds that germinated without heat treatment (control) are considered to be soft-coated while those that germinated after heat treatment (treated) are considered to be hard-coated dormant seeds (Thanos et al. 1992).

3 RESULTS

3.1 *Fruit production and seed dispersal*

At the onset of the experiment (June 1999) 617 fruits were available and thus marked on the 30 tagged individuals of *C. monspeliensis*, 237 on the 30 individuals of *C. salviifolius* and 185 at *C. creticus* individuals (Fig. 1). Between successive observations the total number of fruits did not remain constant in all three species studied (Fig.1). This may be due to the fact that a few capsules might have been discarded from the plant, but also to the fact that in periods of fruit formation, the number of fruits was increasing. Fruits that existed in April's 1999 observation and were still on the plant in June 2000 were considered to belong to the previous reproductive period (Last year's).

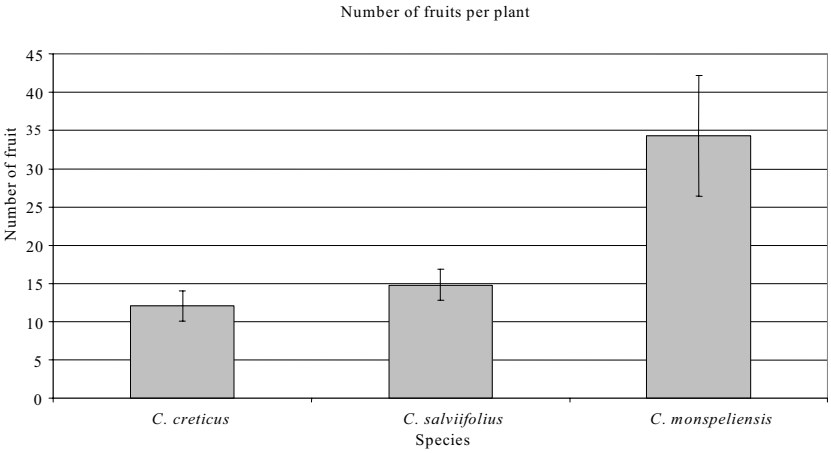


Figure 1. Total number of fruits produced per plant for *C. creticus*, *C. salviifolius* and *C. monspeliensis*, during the reproductive period studied. Values are means from a sample of 30 individual plants per species; bars represent s.e.

C. monspeliensis plants produced the highest number of fruits per plant 34.3 ± 7.9 (mean ± s.e., number of plants marked, n=30) as compared to its congeneric species (Fig.1). Fruit production in *C. monspeliensis* was more or less synchronized with that of the other species studied, being started in late April-early May and accomplished at the beginning of June (Fig. 2a). Rapid seed dispersal started late in June almost simultaneously in all marked individuals (Fig. 2a). As autumn was approaching its end, all capsules were opening; by the end of winter all seeds had been dispersed (Fig. 2a and Fig. 3).

C. salviifolius plants produced 14.8 ± 2 (mean ± s.e, number of marked plants, n=30) fruits per plant (Fig. 1). Fruit production covered a period between May to late June (Fig. 2b). Seed dispersal took place between September and April, peaking in winter months (Fig. 2b). At the end of April, when pollinated flowers were developing into fruits of the current year (C), a few fruits from the last year (L) with some seeds were still remaining on the plant (Fig. 2b and Fig. 3). These fruits formed a poor, transient canopy seed bank [0.57 fruits per plant, corresponding to 1.9 fruits / m^2 of ground area], which does not last on the plant for more than two months (Fig. 3).

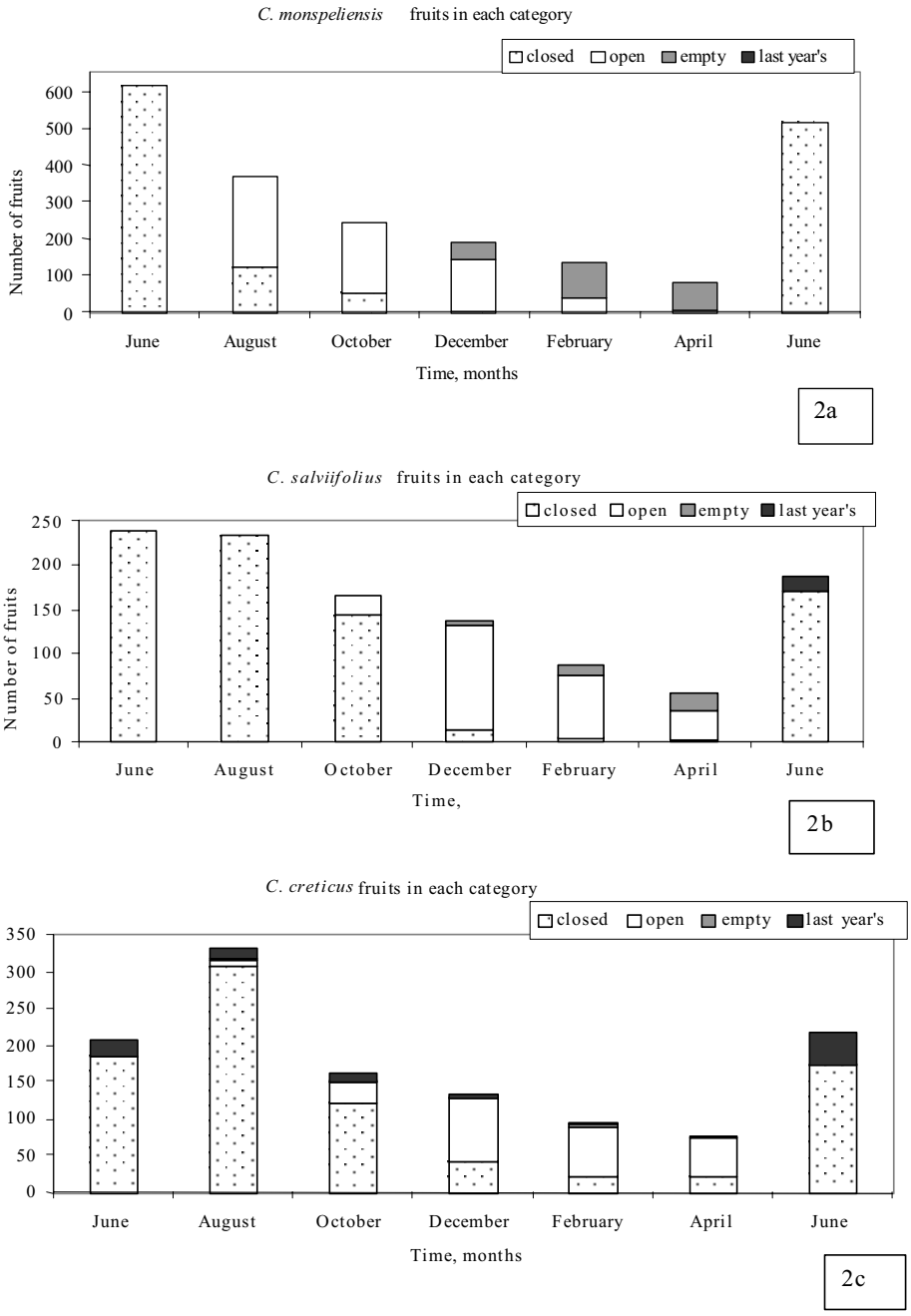


Figure 2. Number of open, closed, empty and last year's fruits recorded on the 30 marked individuals *of Cistus monspeliensis* (2a), *Cistus salviifolius* (2b) and *Cistus creticus* (2c). Records were taken on a two-month interval for the period of one year. At the onset of the experiment 617, 237 and 185 fruits were existing on the individuals of *C. monspeliensis*, *C. salviifolius* and *C. creticus* respectively and were thus marked.

C. creticus produced the lowest number of fruits during the period of study as compared to the two other species (Fig. 1). Production of fruits in this case lasted for a longer period as compared to that of the two other species (Fig. 2c). An average of 12.4 ± 4.8 (mean ± s.e, number of marked plants, n=30) fruits per plant (Fig. 1) were produced from the end of May to the beginning of July (Fig 2c). Seeds from the current production's fruits were dispersed gradually from August throughout the whole year, together with seeds that had remained in capsules produced during the previous reproductive period (last year's) (Fig. 2c). The majority of fruits opened in winter while approximately half of the capsules (48%) were discarded from the plant during September (Fig. 2c). At the beginning of the new reproductive period a number of open and closed fruits (1.47 fruits per plant corresponding to 17.6 fruits / m^2 of ground area) still containing seeds remained on the plant, forming a significant canopy seed bank (Fig. 2c).

The duration of occurrence of each specific fruit type in the three studied species is illustrated in detail in Fig. 3. It is clear that *C. creticus* shows a different pattern from the other two species studied.

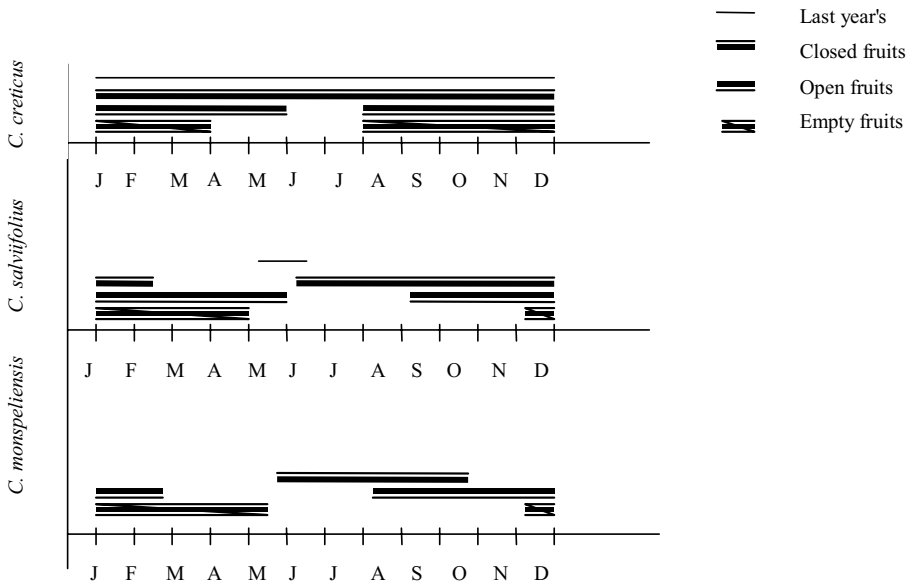


Figure 3. Occurrence of all fruits categories in the three species studied for a period of one year observations.

Results from Kruskal-Wallis analysis of variance test (Statistica 6.0, STATSOFT) support the evidence that *C. creticus* has a different seed dispersal behavior from that of *C. salviifolius* and *C. monspeliensis* as it is expressed from the types of fruits formed and retained by each species (Fig. 4). There is a strong significant difference (p=0.0002, H=23.92, n=118, df=5) (Sokal and Rohlf 1995) in the numbers of the four types of fruits retained on *C. creticus* plants between successive observations (Fig. 2c) whereas differences observed in the patterns of the other two species (Figs 2a,b) were not significant.

3.2 *Seed germination*

Germination experiments showed significant differences in the quality of seeds existing in the various fruit types. It is evident that each species has a certain percentage of hard and soft-coated seeds in each fruit type. Fig. 5 shows the percent germination (as 100% we consider the total number of seeds subjected to the experiment, including viable and non-viable seeds) for each species per fruit type and the corresponding T_{50} of germination. High fraction of the seeds did not germinate at all, due to fungal contamination which occurred during the experiment. All possible pairs of groups of seeds from the various fruit types were tested by the Mann-Whitney U-test (Statistica 6.0, STATSOFT) to detect statistically significant differences in their germination behavior in relation to the fruit type they had been derived from. The results are presented in Table 1.

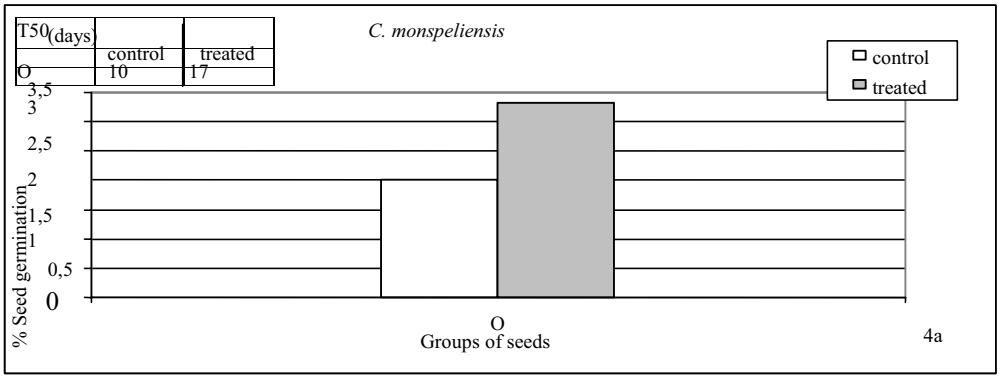
Table 1. Mann-Whitney U-test results concerning differences in germination percentages between treated (t) and non-treated (c) seeds from open (O) closed (C) and last year's (L) fruits for the three species studied. Ho:there is no difference between the tested seeds). Sample size was 5 replicates of 30 seeds per seed type tested, for each species.

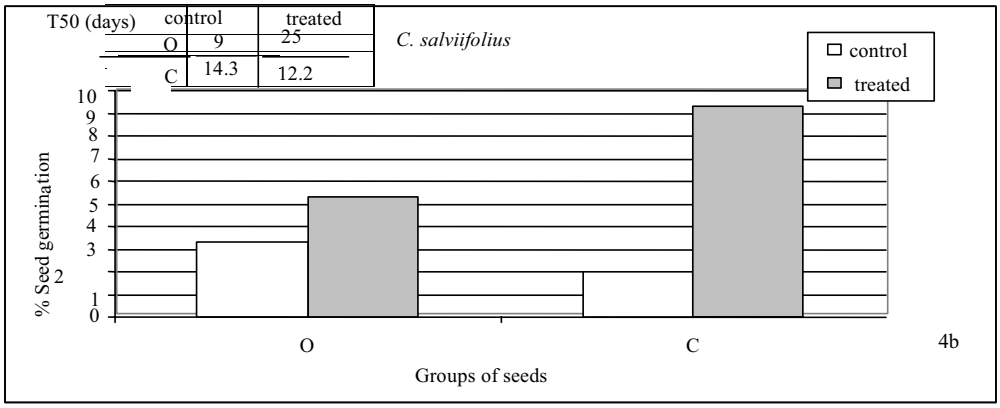
Species	Seed's types tested	O_c-O_t	C_c-C_t	L_c-L_t	O_c-C_c	O_c-L_c	C_c-L_c	O_t-C_t	O_t-L_t	C_t-L_t
C. creticus	U	7.5	8	0	9	0	0	10.5	12	9.5
	p	0.29	1	0.009	0.46	0.009	0.009	0.68	0.92	0.53
C. salviifolius	U	10	1.5	-	11	-	-	6.5	-	-
	p	0.6	0.02	-	0.75	-	-	0.21	-	-
C. monspeliensis	U	8.5	-	-	-	-	-	-	-	-
	p	0.4	-	-	-	-	-	-	-	-

C. monspeliensis seeds from open fruits are both hard and soft-coated at almost equal proportions (p=0.4) (Table 1). However, soft-coated seeds seem to germinate faster (T_{50}=10 days) than the hard-coated ones (T_{50}=17 days) (Fig. 4a).

The germination of hard coated seeds from the closed *C. salviifolius* fruits is significantly different ($p \leq 0.05$) from that of the soft-coated seeds of the same fruit type (Table 1), as closed fruits contain more hard-coated, dormant seeds. Seeds of both types from open fruits exhibit almost equal germination percentages (p=0.6), but the hard-coated seeds seem to need more time (T_{50}=25 days) than the soft-coated (T_{50}=9 days) in order to germinate (Fig. 4b).

From the preliminary germination experiments performed it was evident that seeds remaining on *C. creticus* plants from the previous reproductive period -forming a canopy seed bank- are exclusively hard-coated (Fig. 4c). Seeds collected from the open capsules produced during the current year showed almost equal germination percentages between hard and soft-coated seeds (p=0.29) (Table 1). The same observation applies to seeds from closed capsules produced during the current year (p=1) (Table 1). Germination of last year's hard-coated seeds is significantly lower ($p \leq 0.05$) from that of hard-coated seeds both from open and closed fruits produced during the current year (Table 1). No clear pattern was detected for all the other seed types tested. However, more experimentation is needed in this direction.





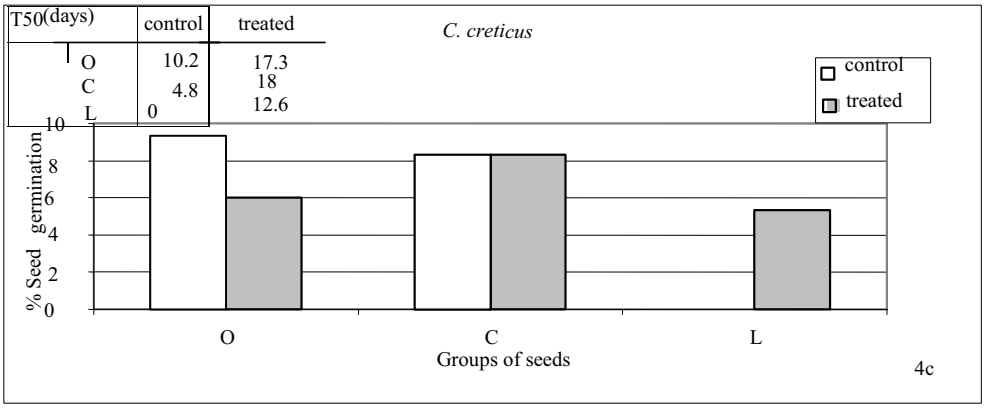


Figure 4. Germination percentages and the corresponding T_{50} for seeds collected from open (O) closed (C) and last year's (L) fruits. Hard coated seeds germinated in treated samples while soft-coated germinated without treatment (control) (percentages were estimated without excluding non-viable seeds). a: *C. monspeliensis*, b: *C. salviifolius*, c: *C. creticus*. The treatment was heating of seeds at 100 ^{o}C for 15 min. Number of seeds per treatment: n=150

4 CONCLUSIONS

Our results suggest that in the case of *C. creticus* delayed dispersal might be an additional adaptation in the fire-prone environment, possibly compensating the lack of resprouting ability. On the contrary, *C. salviifolius* that has the alternative of resprouting (Naveh 1974, pers. observ.) does not really invest resources in forming a canopy seed bank. As far as *C. monspeliensis* is concerned, the high heat requirements for seed coat scarification (Trabaud and Oustric 1989) that it presents, protects its seeds from destruction in case of a wildfire.

The staggered seed release pattern shown by the three species studied and especially *C. creticus* could offer the plant a seed pool additional to its soil seed bank. Gradually, this canopy seed pool will be transferred to the soil seed reserve in a bradychorous way [*bradychory* is a term suggested by Thanos (1999) describing the delay in the opening of pines cones hence delayed seed dispersal] so as species adaptive fitness is capitalized and quite probably maximized, reducing thus the risk of failure in germination and establishment.

ACKNOWLEDGEMENTS

This work, being part of Mrs. P. Skourou Ph.D., was partly supported by a fellowship from the Greek National Fellowship Foundation and partly by the E.U. Project ModMED (EU, DG R&D, ENV4-CT97-0680).

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