

POST-FIRE REGENERATION OF HARDSEEDED PLANTS: ECOPHYSIOLOGY OF SEED GERMINATION

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SUMMARY

Hardseeded plants constitute a major component of the postfire flora in Mediterranean ecosystems. In the present study germination mechanisms are investigated in representative fire following species belonging to the families Leguminosae (*Calicotome villosa*, *Spartium junceum*, *Anthyllis hermanniae*, *Scorpiurus muricatus*, *Hippocrepis unisiliquosa*), Convolvulaceae (*Convolvulus elegantissimus*), Anacardiaceae (*Rhus coriaria*) and Cistaceae (*Cistus creticus*, *Fumana thymifolia*). All species are characterised by a primary dormancy due to the water-impermeable (hard) seed coat. Germinability of untreated seeds is generally low (0-20%, *Spartium junceum* 44%). Breakage of dormancy is achieved by mechanical scarification of the seed coat through the technique of seed immersion in boiling water for several seconds (optimal duration: 2-60 s). This laboratory simulation of fire heat results in an impressive enhancement of germination (nearly 100% in most cases). With the exception of *Rhus coriaria* (whose seeds show an additional embryo dormancy released by a subsequent, prolonged imbibition at relatively low temperatures), in all the species tested, thermal scarification is by far the most important factor of germination induction since it results in optimal germinability at a wide temperature range and under various light regimes.

INTRODUCTION

Fire is a natural event in most Mediterranean ecosystems and, although it is considered as a climatic hazard, it is also an environmental element by itself. Therefore it is not surprising at all that many plant species dominating the fire-prone environments of the Mediterranean rim are shown to possess adaptation mechanisms towards fire.

Besides vegetative resprouting, postfire seed germination is of paramount importance for ecosystem regeneration and may be the result of either fire-avoidance or fire-induction mechanisms. Typical representatives of the former case are the two major Mediterranean pine species (*Pinus halepensis* and *P. brutia*). On the other hand, fire-induced seed germination is

one of the most potentially adaptive traits of plants towards fire and in species regenerating exclusively by seeds, seedling recruitment occurs massively only following a fire. Among the fire-induced plants eminent are several rockroses as well as numerous legumes. Most of these species produce seeds with a hard, water-impermeable seed coat, that not only permits the survival of the seed, during a wildfire, but also requires the high temperatures developed by fire for germination induction.

The aim of the present study was to investigate the germination mechanisms and their ecological implications in representative hardseeded fire followers belonging to the families Leguminosae (*Calicotome villosa*, *Spartium junceum*, *Anthyllis hermanniae*, *Scorpiurus muricatus*, *Hippocrepis unisiliquosa*), Convolvulaceae (*Convolvulus elegantissimus*), Anacardiaceae (*Rhus coriaria*) and Cistaceae (*Cistus creticus*, *Fumana thymifolia*).

MATERIALS AND METHODS

Fruits of *Anthyllis hermanniae* were collected in July 1989 in Diomedes Botanical Garden, Haidari, Attica. Seeds of *Fumana thymifolia* and fruits of *Convolvulus elegantissimus* were collected in June 1990 and July 1991, respectively, at Mt Parnes, Attica, at a burned pine forest site, one and two years after the fire, respectively. Fruits of *Rhus coriaria* were collected in March 1993 at Mt Troodos, Cyprus. Fruits of *Scorpiurus muricatus* and *Hippocrepis unisiliquosa* were collected in June 1993, at a burned pine forest in Kapandriti, Attica during the first postfire year. Fruits of *Cistus creticus*, *Calicotome villosa* and *Spartium junceum* were collected in August 1993 in western Crete. The average weight ($n=50$) of the one-seeded fruits of *Anthyllis hermanniae* was found to be 2.3 ± 0.1 mg; the average seed weight ($n=50$) of *Fumana thymifolia* and *Convolvulus elegantissimus* was 2.8 ± 0.6 mg and 11.6 ± 0.4 mg, respectively. The average weight ($n=100$) of 5 seeds of *Cistus creticus* was 3.9 ± 0.1 mg; therefore, the estimated average seed weight was 0.8 mg. The average seed weight ($n=100$) of *Rhus coriaria*, *Scorpiurus muricatus*, *Hippocrepis unisiliquosa*, *Calicotome villosa* and *Spartium junceum* was found to be 12.9 ± 0.2 mg, 9.8 ± 0.3 mg, 3.4 ± 0.1 mg, 7.0 ± 0.1 mg and 8.8 ± 0.2 mg, respectively. With the exception of *Anthyllis hermanniae*, throughout the present work, seeds of all species were used after removal of the surrounding fruit tissues.

Scarification of seeds was achieved through thermal pretreatment. More specifically, seeds were placed in a small strainer and subsequently immersed in boiling water (100 °C). In order to assess the response of seeds of the different species to various heat quantities, the intensity was maintained stable (temperature was kept constant at 100 °C by use of boiling water) and several durations of immersion were tested. In each case, 25 seeds per treatment were used and eventually transferred to 20 °C, in the dark, where germination experiments were performed.

As a result of an initial screening the optimal boiling duration was determined for each species; this duration was used thereafter prior to the experiments concerning temperature and light effects on germination. In particular, the following treatments were applied: *Hippocrepis unisiliquosa*-3 s, *Anthyllis hermanniae*-10 s, *Scorpiurus muricatus*-10 s, *Spartium junceum*-10 s, *Convolvulus elegantissimus*-20 s, *Calicotome villosa*-30 s and *Rhus coriaria*-60 s.

Germination tests were performed in Petri dishes (7 cm in diameter) lined with two filter papers and moistened with 3 ml distilled water. The criterion of germination was visible radicle protrusion. Germination tests were considered finished when no additional seeds germinated. Each value is the mean from five samples of 20 seeds (25 for *Rhus coriaria*) and \pm numbers (in Tables 2 and 3) represent standard error (SE). T_{50} is the time needed for manifestation of half of the final germination level and is used as a parameter of germination rate.

Germination experiments in the dark were carried out in temperature-controlled plant growth cabinets (model BK 5060 EL, Heraeus, Hanau, Germany). Germination experiments under various light regimes were performed on a temperature- and light-programmable growth bench, model GB48 (Convion, Canada) where temperature was kept constant at 15 ± 1 °C and white light was provided on a 24 hour basis. The white light source consisted of 10 white fluorescent tubes and 48 white incandescent bulbs and had an emission spectrum quite similar to natural daylight. When continuous white light, cW, was filtered through single sheets of Plexiglas filters (or combinations of them), various light regimes were obtained: cR, cB, cFRI and cFRII. More specifically, for the continuous red (cR) and blue light (cB) a single sheet of red or blue, respectively, Plexiglas filter was used; for the continuous intense far red light (cFRI) two blue and one red sheets of Plexiglas filters were used while for the continuous mild far red light (cFRII) three light blue and one light red sheets of Plexiglas filters were used. Finally, seeds were also germinated in complete darkness (D).

RESULTS

The prominent characteristic of all species studied is their water-impermeable, hard seeds. As shown in Table 1, germinability of untreated seeds (control) was generally low (0-20%) with the exception of *Spartium junceum* (44%). Seed softening and therefore breakage of dormancy was achieved by mechanical scarification of the seed coat through the technique of seed immersion in boiling water for a few seconds. After thermal pretreatment seeds were sown for germination at 20 °C, in the dark. Table 1 shows final germination levels of untreated seeds of the different species (at 20 °C in the dark) as well as maximum germination percentages after thermal pretreatment with the corresponding immersion durations given in parentheses. Germination values (represented by symbols in Fig. 1) in the thermal pretreatment curves

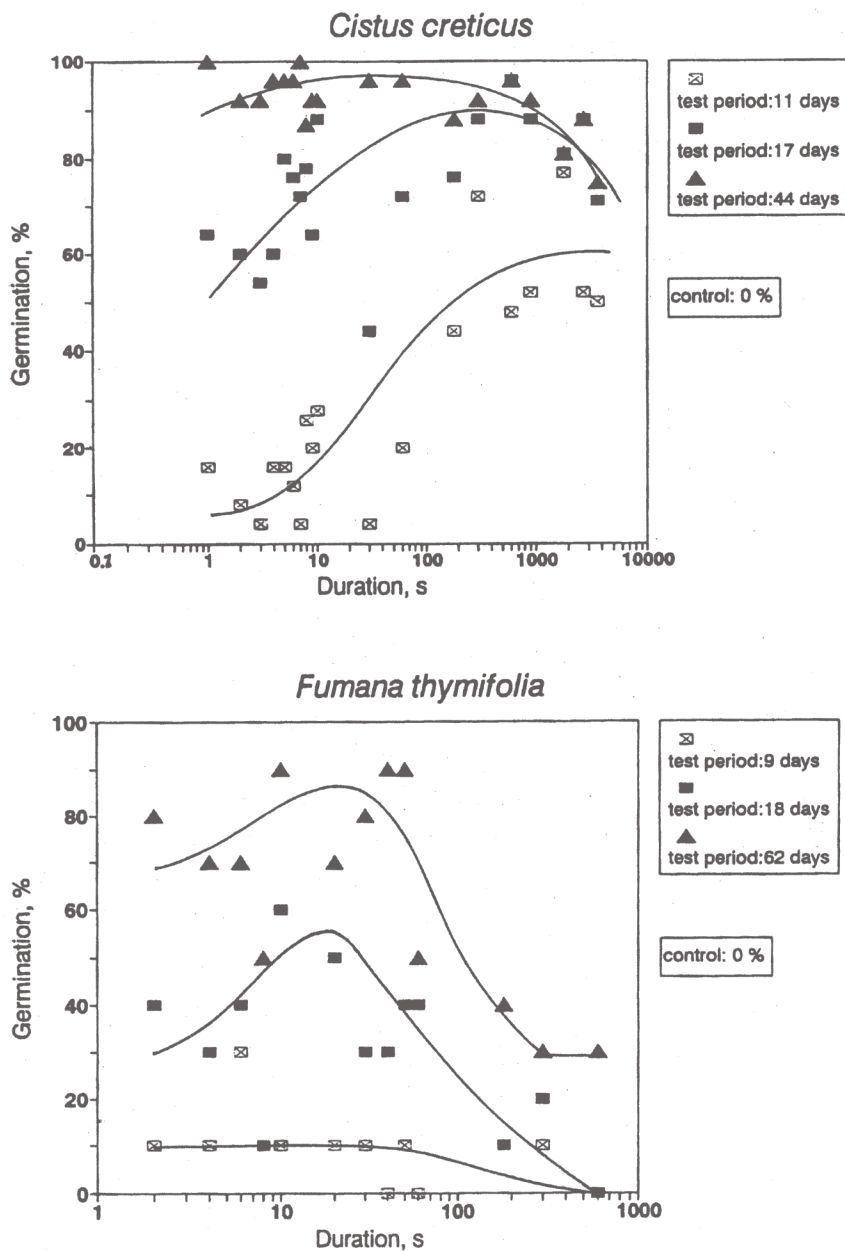


Figure 1. Dark germination at 20 °C after immersion in boiling water for various durations, in two hardseeded species of Cistaceae.

Table 1. Final germination of untreated and thermally pretreated seeds at 20 °C in the dark.

Plant species	Germination (%)	
	Untreated seeds	Thermally pretreated seeds
<i>Hippocrepis unisiliquosa</i>	20	96 (3 s)
<i>Convolvulus elegantissimus</i>	0	80 (3 s)
<i>Calicotome villosa</i>	8	100 (5 s)
<i>Scorpiurus muricatus</i>	0	100 (5 s)
<i>Spartium junceum</i>	44	100 (5 s)
<i>Cistus creticus</i>	0	100 (7 s)
<i>Anthyllis hermanniae*</i>	0	64 (10 s)
<i>Fumana thymifolia</i>	0	90 (40 s)
<i>Rhus coriaria**</i>	0	100 (60 s)

* final germination percentage after thermal pretreatment is certainly higher than that presented since no correction for sound seeds was made

** values represent water-permeable (imbibing) seeds (instead of germinants)

correspond to measurements taken at three different times during the experimentation period. Fig. 1 shows the thermal pretreatment curves for *Cistus creticus* and *Fumana thymifolia*. A considerable promotion of germination was similarly obtained in all cases, with the exception of *Rhus coriaria*, the seeds of which possess an additional embryo dormancy and require a further treatment; therefore, for the latter species only the imbibition incidents were recorded (judged by the visible swelling of seeds). In almost all cases, final measurements in the curves include a range of constant high germination (optimal duration range) and a range where germination decreases rapidly with prolonged exposure to heat due to loss of seed viability (lethal range). In several cases a range of suboptimal germination, at rather short heating durations, was also observed. In this last range, immersion periods are not sufficient to break dormancy in the entire seed population. These results suggest that seeds in a given population are, to a certain degree, heterogeneous in their hardcoatedness variable.

Having determined the optimal immersion duration for each species, germination was investigated at various constant temperatures in the dark (Table 2) and under various continuous light regimes at 15 °C (Table 3). Due to the limited number of seeds the role of light on germination of *Spartium junceum* and *Hippocrepis unisiliquosa* was not investigated. For the same reason germination of *Fumana thymifolia* seeds in the dark or in the light was not

Table 2. Final dark germination (%) of thermally pretreated seeds of the different species in various constant temperatures. The \pm values represent standard error (SE) and the numbers in parentheses are T_{50} values (in days). 1:*Spartium junceum*, 2:*Calicotome villosa*, 3:*Anthyllis hermanniae*, 4:*Hippocrepis unisiliquosa*, 5:*Scorpiurus muricatus*, 6:*Convolvulus elegantissimus*

Temp(°C)	1	2	3	4	5	6
5	98.0 \pm 2.0 (15.8)	100 (17.0)	82.1 \pm 3.8 (34.4)	97.6 \pm 1.5 (15.5)	95.0 \pm 1.6 (13.8)	67.7 \pm 5.0 (12.9)
10	99.0 \pm 1.0 (7.1)	99.0 \pm 1.0 (8.3)	84.0 \pm 4.2 (28.2)	100 (6.3)	96.0 \pm 1.9 (5.9)	88.1 \pm 4.7 (4.9)
15	99.0 \pm 1.0 (4.1)	100 (5.0)	87.8 \pm 4.0 (19.1)	98.9 \pm 1.1 (4.9)	97.0 \pm 1.2 (4.2)	84.7 \pm 4.7 (2.5)
20	97.0 \pm 2.0 (3.4)	99.0 \pm 1.0 (3.3)	88.4 \pm 4.9 (25.9)	100 (3.7)	99.0 \pm 1.0 (2.1)	90.6 \pm 2.6 (2.5)
25	4.0 \pm 2.9	98.0 \pm 1.2 (3.7)	66.8 \pm 2.9 (38.4)	100 (4.6)	100 (1.8)	87.3 \pm 3.8 (2.6)
30	2.0 \pm 2.0	8.0 \pm 2.6	0	98.8 \pm 1.2 (10.5)	99.0 \pm 1.0 (2.7)	73.1 \pm 7.1 (1.9)

studied. Nevertheless, a previous work (Doussi and Thanos, 1993) has shown that germination of *Fumana thymifolia* is relatively opportunistic in regard to temperature and light. This is illustrated by its broad temperature range (with only a decrease of both final germination percentage and germination rate at 5 and 30 °C) and its ability to germinate even under the highly adverse, far red illumination. An important deviation from the present work was that breakage of dormancy had been achieved by abrasion with sandpaper, a somewhat more artificial method of seed softening than the immersion in boiling water which is simulating fire heat closer. A detailed investigation of *Cistus creticus* germination was not carried out either, since previous findings (Thanos and Georgiou, 1988) have already demonstrated the opportunistic germination behaviour of thermally pretreated (exposed to dry heat) *Cistus creticus* seeds. Results from the "boiling water treatment" of the present study fully confirm, as expected, the existence of seed coat impermeability and the promotive effect of heat shock on *Cistus creticus* germination.

The results of Tables 2 and 3 show the lack of temperature- or light-mediated control of germination. A slight exception is shown by the 3 perennial Leguminosae species: dark germination at 30 °C was inhibited in *Anthyllis hermanniae*, *Calicotome villosa* and *Spartium junceum*; in the latter species germination was also inhibited at 25 °C. When ungerminated

Table 3. Final germination (%) of thermally pretreated seeds in darkness and under various continuous light regimes, at 15 °C. The \pm values represent standard error (SE) and the numbers in parentheses are T_{50} values (in days). 1:*Anthyllis hermanniae*, 2:*Calicotome villosa*, 3:*Scorpiurus muricatus*, 4:*Convolvulus elegantissimus*

Light regime	1	2	3	4
D	87.8 \pm 4.0 (19.1)	100 (5.0)	97.0 \pm 1.2 (4.2)	84.7 \pm 4.7 (2.5)
cW	92.7 \pm 2.2 (14.9)	98.0 \pm 1.2 (5.5)	99.0 \pm 1.0 (8.9)	92.1 \pm 3.8 (8.2)
cR	82.5 \pm 7.8 (21.7)	100 (5.6)	99.0 \pm 1.0 (5.5)	90.3 \pm 3.9 (2.8)
cB	82.7 \pm 2.9 (20.2)	100 (5.2)	100 (4.9)	79.5 \pm 1.9 (4.7)
cFRI	89.4 \pm 4.1 (16.6)	99.0 \pm 1.0 (5.1)	98.0 \pm 1.2 (4.7)	75.9 \pm 8.6 (5.5)
cFRII	93.7 \pm 1.6 (15.1)	100 (6.3)	99.0 \pm 1.0 (5.4)	90.0 \pm 2.9 (5.5)

seeds of *Spartium junceum* and *Calicotome villosa* were transferred to 20 °C an impressive manifestation of germination took place suggesting that no secondary dormancy had been imposed. However, in the case of *Anthyllis hermanniae*, germination after transfer to 15 °C, was considerably low (about 25%), possibly the result of seed ageing and/or necrosis (Fig. 2).

In the case of *Rhus coriaria*, germination behaviour is somewhat different. Apart from the water-impermeable (stony) endocarp, seeds of this species are also characterised by an innate embryo dormancy so that no germination occurs following coat softening. Therefore, the induction of germination requires two separate pretreatments: an initial one to overcome coat impermeability and a subsequent one to promote further development of the apparently immature embryo. In the present study the corresponding treatments were immersion in boiling water for 60 s and subsequent stratification (chilling at 5 °C) for 1, 2 and 3 months. Before being transferred to 5 °C, seeds had remained at 15 °C for 1 month. After these initial pretreatments seeds were transferred for germination at 20 °C, in the dark. Although chilling enhanced germination, *Rhus coriaria* seeds did not seem to have an absolute stratification requirement since a prolonged imbibition at relatively cool temperatures (10, 15 °C) can induce a similar promotion of germination (Fig. 3).

DISCUSSION

The postfire positive feedback of the Mediterranean ecosystems is brought about chiefly by a vigorous and prolific regeneration through both seed germination and resprouting (e.g. Thanos et al., 1989). In all types of Mediterranean ecosystems fire stimulates germination of

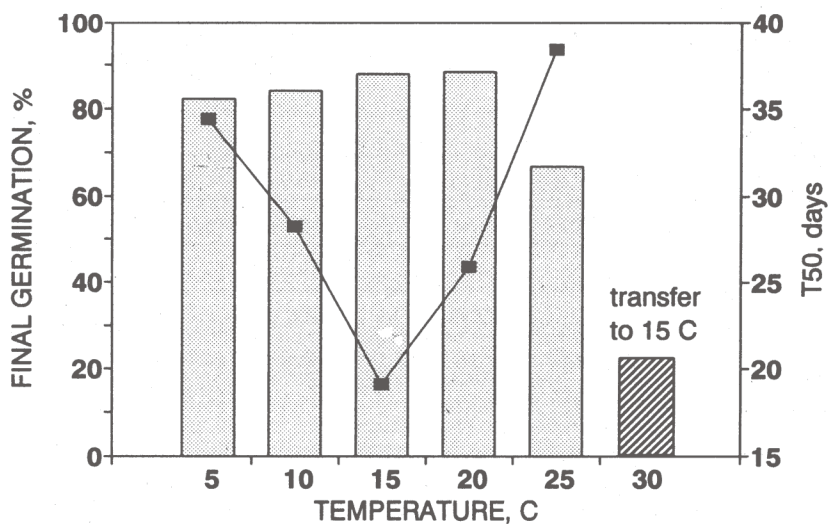


Figure 2. Dark germination of *Anthyllis hermanniae* seeds in various constant temperatures after immersion in boiling water for 10 seconds. Bars represent final germination percentages while filled squares are T₅₀ values (in days).

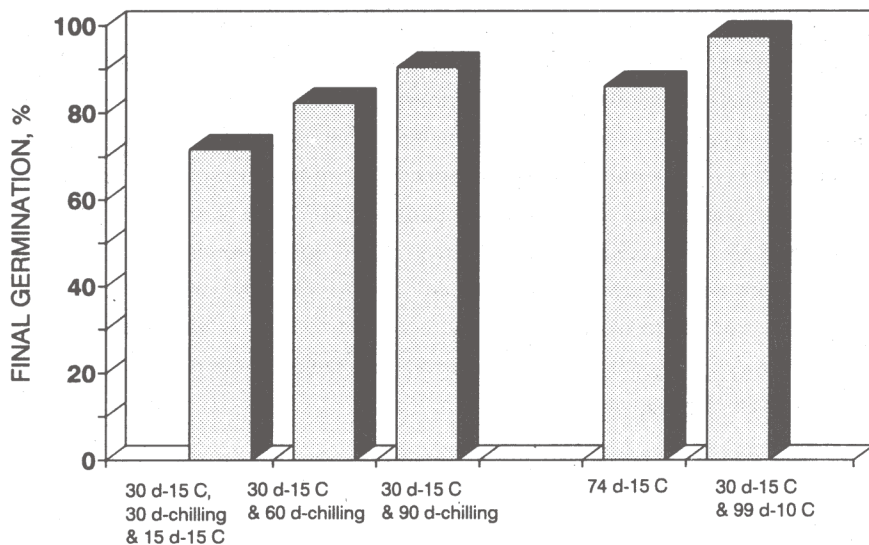


Figure 3. Dark germination of *Rhus coriaria* seeds after immersion in boiling water for 60 seconds. Bars represent final germination percentages, at 20 °C, after application of various pretreatments.

many species (Arianoutsou and Margaris, 1981). The postfire environment is likely to furnish safe sites for the germination of seeds and the establishment of seedlings. It may, in fact, provide the only opportunity for offspring recruitment to certain species (a "regeneration niche"). Plants in fire-prone regions may therefore be expected to have evolved characteristics that allow them to take advantage of the predictable prevalence of favourable conditions (increased availability of water, light and nutrients and thus decreased competition) after each fire. The seed bank in the canopy or in the soil can perceive a fire incident by different means: heat generated by fire respectively induces seed dispersal or satisfies the scarification requirement of seeds of certain "hardseeded" species. Hardseededness has been described for many fire-following plant species, including legumes, Cistaceae (Thanos et al., 1992), Anacardiaceae and Convolvulaceae. The impermeability of the seed coat prevents germination, until a selective damage to the lens or some other region of the seed coat permits imbibition. Fire appears to be the most important factor in overcoming seed coat impermeability in many communities. A secondary role is attributed to the combination of exposure to high temperatures and wide temperature fluctuations through the heating effect of solar radiation on the surface layers of dry soils (insolation) coupled with night-time cooling. Immersion in boiling water is a fast, easy method of seed softening and, most important, safe for both the seeds and the person who applies the treatment. More often, abrasive or acid scarification results in destruction of the seed coat integrity on a substantial proportion of the seed surface. Scarified seed is more vulnerable to deterioration than heat treated seed that ruptures at the lens and mimics a natural occurrence. Heat treatment is thus a preferred method of breaking hardseededness. The route by which water penetrates into the seed after it is naturally softened needs to be examined. In the case of *Rhus coriaria*, a slit with radially oriented cracks, at the point of radicle emergence, can be seen after thermal pretreatment. In the rest of the species, application of heat for a short period may induce mechanical stresses which cause fracture of the palisade cells at the lens, not visible on the external seed surface.

Heterogeneity in the hardseededness variable of the seeds in the species studied is a considerable ecological advantage since germination can, more or less, be manifested after a wide range of fire regimes. Annual legumes (*Scorpiurus muricatus*, *Hippocrepis unisiliquosa*) seem to be characterised by a more opportunistic germination pattern (broader temperature range) compared to perennial legumes (*Anthyllis hermanniae*, *Spartium junceum*, *Calicotome villosa*). This disparity might be explained on the basis of contrasting life habits, since in the former species a more aggressive (virtually weedy) germination behaviour is evidently advantageous. In the case of *Rhus coriaria* a possible scenario would attribute the two-fold requirement for germination induction to a strategy of timing germination of seeds well into the spring season after having experienced the heat shock of a fire and the low winter temperatures. In a recent work (Izhaki, Lahav and Ne'eman, 1992) *Rhus coriaria* was reported,

for the first time, as a postfire facultative seeder. However, an alternative mode of coat softening might be the passage through the digestive track since the fruits of this species are known to be dispersed by frugivorous birds.

In conclusion, all species investigated in this work (with the exception of *Rhus coriaria*) show an overall opportunistic strategy of seed germination, in close agreement to their eminent role in the postfire regeneration process of Mediterranean ecosystems.

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