Fire and summer temperatures work together breaking physical seed dormancy

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Fire and high summer soil temperatures can break physical seed dormancy in Mediterranean fire-prone ecosystems. Their independent effect is somewhat recognized but both factors may act together with a synergistic effect yet unknown. This study aims to determine the isolated and combined effects of fire and summer temperatures on the release of physical seed dormancy in Cistaceae species. Fire and summer temperature treatments were applied in a factorial experiment to seeds of 12 species of Cistaceae. Seeds previously exposed or not to a heat shock (fire simulation) were kept for 1 or 2 months at constant or alternating temperatures (summer temperatures simulation). Additionally, I compared the effect of exposing the seeds to a heat shock before or after they had been subjected to the summer temperatures. Heat shock increased germination of all species, but summer temperatures produced different results. When seeds were exposed to summer temperatures after heat shock, germination decreased. This negative effect disappeared when heat shock was simulated at the end of the summer temperatures. Fire and summer temperatures modulate timing of germination in Cistaceae with a joint control on post-fire regeneration. Cycling of sensitivity to physical dormancy release may be the mechanism to explain this fine-tuning, which would ensure germination when environmental conditions are suitable for growth. These results contribute to our understanding of vegetation dynamics and postfire regeneration in Mediterranean ecosystems.

Cistaceae is a plant family widely distributed in the Mediterranean Basin forming vast areas of shrublands, which dominate the landscape after fire occurrence. All species of this family are obligate seeders and consequently their post-fire establishment relies exclusively on seed germination. After fire, Cistaceae germinate massively and their seedlings colonize the new open habitats, which are free of competitors and provided with favourable conditions for establishment.

Cistaceae regeneration associated to fire occurrence is due to the hard coat of seeds that impose physical dormancy. Seeds with physical dormancy cannot germinate because of palisade cell layer(s) that avoids water intake to the seed. Seeds remain dormant until some factor(s) make the seed coat permeable to water. Seeds with physical dormancy show specialized structures in the seed coat like the chalazal plug in Cistaceae. During physical dormancy break, this plug is pushed slightly into the seed and forms a circular opening (oculus) through which the seeds imbibe water. In addition to the unplugging of the seed coat opening, exposure to dry heat also causes cracks in the outer coat layers.

Traditionally, temperature thresholds needed for breaking physical seed dormancy in Cistaceae have been related to fire. However, currently a debate exists about whether fire or summer temperatures are the most appropriate cues for physical dormancy release. The cumulative effect of soil temperatures during summer might provide a similar heat amount to fire and thus, both factors could be equally effective in breaking dormancy. Therefore, the underlying concern is to discern whether the temperature thresholds that break physical seed dormancy have been shaped by fire or physical dormancy has evolved associated to the summer temperatures. According to the summer hypothesis, soil temperatures reached in soil during summer can break physical seed dormancy, especially in bare soils where daily temperatures reach the highest values and fluctuate more widely. These open habitats may be associated to the gaps created after fire but also after other perturbations.

In the Mediterranean Basin, no consistent results have been found for Cistaceae species. While summer treatments had no effect over the seed dormancy of five species (Cistus albidus, C. creticus, C. parviflorus, C. salviifolius and Fumana thymifolia), in other case the most influencing factor on seedling emergence of Cistus albidus was...
the microhabitats opened with fire\textsuperscript{17}. Likewise, unclear results have also emerged for Fabaceae species, other plant family typically with hard seed coats\textsuperscript{1}. In some cases, soil temperature regimes after summer fires play a key role in breaking physical seed dormancy, both in Europe\textsuperscript{19,21} and in Australia\textsuperscript{22–24}. However, in other cases, seeds show dormancy cues bound to temperatures that only occur during fire\textsuperscript{25,26}.

Another interesting question is to determine whether the break of physical dormancy after summer temperatures is produced by the cumulative heat reached at the soil surface or by the alternating changes of soil temperatures. The mechanism seems to be highly dependent on the species, although there are not enough works to determine which mechanism is the most widely observed and most works have focused mainly on Fabaceae species or on the effects of alternating temperatures\textsuperscript{22–24}. For instance, both mechanisms, high and highly fluctuating temperatures promoted dormancy break in hard seeds of \textit{Stylosanthes humilis} and \textit{S. hamata} (Fabaceae) during the hot season in northern Australia\textsuperscript{25}. In the case of two \textit{Erodium} species (Geraniaceae) and \textit{Adenanthera pavonina} (Fabaceae), temperature fluctuations were more important than high constant temperatures in overcoming dormancy\textsuperscript{26,27}. However, neither constant high nor alternating temperature treatments were effective in breaking physical dormancy of \textit{Senna marilandica} (Fabaceae)\textsuperscript{28}.

The combined effect of high temperatures of the summer season and the typical high summer temperatures of the Mediterranean Basin\textsuperscript{1}.

In Mediterranean habitats, seed dispersal occurs frequently during the summer, when seeds have to withstand the high soil temperatures before their germination in autumn or winter and eventual wildfire, which typically occurs during the summer\textsuperscript{29}. Cistaceae have frequently extended dispersal times\textsuperscript{30} and thus, the timing of seed release, soon or later in the season, will determine the duration of exposure to high temperatures on soil. When a fire occurs, seeds have been exposed to a heat shock of the very high temperatures reached during the fire, but also to the moderately high summer temperatures occurring at the beginning or at the end of summer season. This involves that seeds could be exposed to the same factors but in different sequences. In the present work, I addressed all these issues by comparing the effects of fire vs. summer, fire plus summer, fire before summer vs. fire after summer, and simulation of summer with different characteristics.

In the Mediterranean region, summer is probably the harshest season for living organisms. The hypothesis tested here is that the temperature thresholds that break physical seed dormancy in Cistaceae have been shaped by fire in combination with high summer temperatures. These thresholds are typically both present during the summer. Therefore, it could be expected to find a synergistic effect of fire heat shock and summer temperatures, which would expect the germination of a greater proportion of seeds when the conditions are favourable for seedling establishment. The main objective of this work was to evaluate the effects of high summer soil temperatures and a short heat shock representative of fire on the release of physical seed dormancy in \textit{Cistus} and \textit{Halimium} species. Specifically, we addressed the following questions: (1) Is physical dormancy released after seed exposure to a heat shock simulating fire temperatures? (2) Has the combined effect of heat shock plus summer temperatures a synergistic effect? (3) Can different length of exposure to summer temperatures change the germination response? (4) Is the accumulative effect of high constant temperatures enough for breaking physical dormancy or are alternating temperatures required? (5) Finally, does the heat shock have different effects on germination response when is produced before or after the summer temperatures?

**Methods**

**Study species and seed collection.** We studied 12 species of the family Cistaceae, belonging to two related genera \textit{Cistus} and \textit{Halimium}. The selected species were \textit{Cistus albidus} L., \textit{C. clusii} Dunnal, \textit{C. ladanifer} L., \textit{Cistus halimifolius} L., \textit{C. monspeliensis} L., \textit{C. populifolius} L., \textit{C. psilosepalus} Sweet, \textit{C. salvifolius} L., \textit{Halimium atriplicifolium} (Lam.) Spach, \textit{Halimium halimifolium} (L.) Willk, \textit{Halimium ocymoides} (Lam.) Willk and \textit{Halimium viscosum} (Willk.) P. Silva. All these species are common and widely distributed in the western Mediterranean Basin\textsuperscript{1}.

Mature capsules were collected from at least 30 individuals for each species between July and August 2016 in the centre of the Iberian Peninsula (Supplementary Data Table S1). Fruits were carried to the laboratory where seeds were extracted and cleaned. Seeds were stored in paper bags until the beginning of the experiments the following November.

**Fire vs. summer temperatures.** Fire and summer temperature treatments were applied in a factorial experiment in such a way that seeds exposed or not to a heat shock simulating fire temperatures were subject to or not to the different treatments simulating summer temperatures. That is, seeds were exposed to two factors heat shock (with heat shock or without heat shock) and summer temperatures (1 month at constant 50°C or at alternating 50/20°C, 2 months at constant 50°C or alternating 50/20°C). Fire temperatures were simulated by the exposure of seeds to a heat shock of 100°C for ten minutes in an air-forced oven. Although temperatures reached in the soil during fires vary widely we chose this specific temperature and time of exposure, which have been commonly recorded in Mediterranean fire shrublands\textsuperscript{21,22}, because this temperature seemed to be the optimal for breaking seed dormancy of many Cistaceae species\textsuperscript{29}.

Mediterranean climate is characterized by high summer temperatures, which are usually above 40°C. When air temperatures are around 20–25°C, soil temperatures are similar but, when mean of maximum temperatures are around 35°C, soil temperature can reach 60°C\textsuperscript{31}. Summer temperatures were simulated by exposing uninimbred seeds to two long duration dry heat treatments for 1 and 2 months at constant temperature (50°C) or at daily cycles of alternating temperatures (50/20°C) that might be expected to represent current summer temperatures\textsuperscript{22}. Summer treatments were conducted in a refrigerated precision cabinet (JP Selecta Hotcold-UB).

After these treatments, seeds were sown on two sheets of filter paper moistened with 1.2 ml of distilled water, in plastic Petri dishes of diameter 5.5 cm. For germination tests, four replicates of 25 seeds were used for
each species, and seeds incubated at 20°C and 12 h photoperiod, the optimal germination conditions for many Mediterranean species. Petri dishes were laid at random over temperatures and humidity controlled chamber (Model G-21, Ibercex). Germinated seeds were checked and eliminated weekly over the course of 8 weeks. At the end of the experiment, ungerminated seeds were checked for viability using a cut test. Those firm seeds with white endosperm were considered as viable, while mushy seeds with brown endosperm or affected by fungi were considered as inviable. Germination percentages were corrected by viability, i.e., germination percentages were estimated in relation to viable seeds and not in relation to total number of seeds.

### Fires before and after the summer season.

In a second experiment, we tested whether the timing in which seeds were exposed to heat shock, before or after the summer temperatures, affected their germination response. In the first experiment, the 50°C treatment showed highest germination rates than constant 50°C, and length of exposure (1 or 2 months) had almost no effects. Consequently, in the second experiment I subjected seeds to a heat shock (100°C for 10 minutes) and then they were exposed to 50/20°C for one month. In the other treatment, seeds were first exposed to summer temperatures (50/20°C for 1 month) and then were exposed to a similar heat shock. Then seeds were germinated at the same conditions as in the previous experiment.

### Data analysis.

For each species, I used generalised linear models (GLMs) with a binomial error distribution and logit link function to compare final germination among the different treatments. First, I analysed the effects of fire vs. summer temperatures on final germination and seed viability. Since germination without heat shock was very low, I analysed the effects of the different summer temperatures (50 vs. 50/20°C) and different lengths of time (1 vs. 2 months) for the seeds that had been exposed to both heat shock and summer temperatures. Additionally, for the second experiment, the effect of before vs. after summer temperatures heat shock was analysed in the same way.

### Results.

Heat shock simulating fire temperature increased germination of all species, while summer temperatures had the opposite effect by decreasing germination (Table 1, Figs. 1 and 2). Only in the case of *C. ladanifer*, summer temperatures slightly increased the germination of seeds not exposed to heat shock, such as the significant interaction between both factors shown (Table 1). Additionally, significant interactions between heat shock and summer temperatures also emerged for *C. psilosepalus* and *H. atriplicifolium*. In these cases, the decrease of germination produced by summer temperatures was more intense in seeds exposed to heat shock than in non-exposed ones. *C. populifolius* and *H. atriplicifolium* were very sensitive to summer temperatures since the treatment strongly lessened germination (Figs. 1 and 2).

Germination without heat shock was very low (Figs. 1 and 2) and consequently, the effects of the different summer temperatures (50 vs. 50/20°C) and different times (1 vs. 2 months) were analysed just for the seeds that had been previously exposed to heat shock. Overall, different regime of temperatures affected the germination responses more than duration of treatments (Table 2). The negative effect of summer temperatures on seed germination was stronger after constant 50°C than alternating 50/20°C. In the cases of *C. ladanifer*, *C. psilosepalus*, *H. atriplicifolium* and *H. halimifolium* the different summer temperatures had similar effects. Different time of exposure to summer temperatures only had a significant effect for *C. laurifolius*, *H. halimifolium* and *H. ocymoides* (Table 2), with lower germination after one month than after two months of treatment (Figs. 1 and 2).

Finally, the timing in which seeds were exposed to heat shock, before or after the summer temperatures, was determinant of the germination response of all species except *C. ladanifer* who showed very high germination in any case (Table 3). Exposure to heat shock after summer temperatures improved germination profusely (Fig. 3).

### Table 1.

Results from GLM for main effects of fire and summer temperature treatments and their interactions on final seed germination of the studied Cistaceae species.

| Fire x Summer | Fire | Summer | p |
|---------------|------|--------|---|
|                | X²  |        | P | X²  |        | P |
| Cistus albidus | 50.477 | <0.001 | 12.787 | <0.001 | 0.364 | 0.546 |
| Cistus chamaejasme | 49.274 | <0.001 | 10.834 | 0.001 | 0.092 | 0.762 |
| Cistus ladanifer | 325.653 | <0.002 | 9.539 | 0.002 | 19.111 | <0.001 |
| Cistus laurifolius | 101.235 | <0.001 | 25.574 | <0.001 | 1.903 | 0.168 |
| Cistus monspeliensis | 38.380 | <0.001 | 17.512 | <0.001 | 0.171 | 0.680 |
| Cistus populifolius | 10.170 | 0.001 | 8.997 | 0.003 | 2.080 | 0.149 |
| Cistus psilosepalus | 110.336 | <0.001 | 119.446 | <0.001 | 3.958 | 0.047 |
| Cistus salviifolius | 74.054 | <0.001 | 3.799 | 0.051 | 3.194 | 0.074 |
| Halimium atriplicifolium | 46.080 | <0.001 | 37.079 | <0.001 | 21.720 | <0.001 |
| Halimium halimifolium | 166.490 | <0.001 | 35.038 | <0.001 | 3.768 | 0.052 |
| Halimium ocymoides | 109.385 | <0.001 | 23.958 | <0.001 | 3.361 | 0.067 |
| Halimium viscosum | 72.607 | <0.001 | 26.827 | <0.001 | 1.515 | 0.218 |
Discussion

Heat shock by itself was a key factor promoting seed germination of all studied species, which concurs with the massive germination found in Mediterranean shrublands after fire, and with the results of other laboratory experiments. In the cases of *C. albidus* and *C. clusii*, although germination increased after heat shock it did not reach 50% despite the high viability of their seeds. These results may lead us to think that these species need higher temperatures for breaking dormancy, but most studies report a high variability of temperature thresholds.
for breaking of physical dormancy\cite{14,38-43}. Such variability may be explained as a mechanism of diversification in relation to different fire intensities experienced by seeds at the soil surface, as well as variations in burial depth\cite{33}.

Physical dormancy contributes to maintenance of long-lived soil seed banks, where seeds persist while environmental conditions are unfavourable for establishment. These long-lived soil seed banks confer long-term persistence for the species\cite{44} and a bet-hedging strategy, which spreads the risk of extinction\cite{34,45}. In fire-prone ecosystems, conditions for seedling establishment are particularly favourable just after fire and the temporal window for seedling establishment is usually short\cite{32,46}. Consequently, many Mediterranean plants produce seeds that are released from dormancy only after being exposed to fire-related factors, such as heat\cite{47-49}. In species with physical dormancy, once this type of dormancy is broken it cannot be reversed\cite{8}. Additionally, the embryo is usually non-dormant within the impermeable seed coat and seeds will be ready to germinate when the water

Figure 2. Germination percentages (mean ± standard error) of *Halimium* species at the different summer temperature treatments without heat shock or previously exposed to heat shock (100 °C for 10 minutes). Control seeds were not exposed to summer temperatures (0 months in white). Summer temperature treatments consisted in the storage at constant 50 °C or alternating 50/20 °C for one month (grey) or two months (black).

Table 2. Results from GLM for main effects of different summer temperatures (50 vs. 50/20 °C), time (1 vs. 2 months) and their interactions for seeds that had been previously exposed to heat shock.
is available24. Although in fire-prone habitats, the release from dormancy is usually related to the heat produced during fires6, other cues can act. So, the rupture of physical dormancy can also occur naturally by high summer temperatures or continuous daily fluctuating temperatures50. In this way, Ferrandis et al.51 found that the direct effect of fire was the main responsible for seed germination in three species (C. ladanifer, C. salviifolius and H. ocymoides). However, final germination levels (around 70%) did not correspond to the magnitude of seed bank depletion (>90%). Authors suggested that other environmental factors not exclusively associated to fire, such as temperature fluctuation, might also be involved in softening Cistaceae seeds.

Contrary to fire, in the present study, summer temperatures did not increase germination as expected, but had a significant negative effect on it. In previous works, summer temperatures caused no effects or positive effects on seed germination of Cistaceae species16,17, but these generalized negative effects have not been previously documented. Consequently, the longer and hotter summers may produce negative consequences for regeneration of some plant species. Without simulated fire, seeds showed very low germination levels and summer temperatures caused little effect. However, the negative effect of summer temperatures was much more evident for seeds that had been previously exposed to heat shock. Summer temperatures did not decrease seed viability (Supplementary Data Table S2). The loss of germination after summer temperatures was related with a higher proportion of unimbibed seeds, which may indicate the lack of physical dormancy release. Consequently, one possible explanation for the negative effects of summer temperatures is that Cistaceae may show sensitivity cycling to physical dormancy-break such as it has been described in species of Convolvulaceae and Fabaceae52–55.

Table 3. Results from GLM for main effects of the timing of exposure to heat shock, before or after the summer temperatures, on final seed germination of the studied Cistaceae species.

| Species          | Chi-Square | P     |
|------------------|------------|-------|
| Cistus albidus   | 36.893     | < 0.001 |
| Cistus clusii    | 50.352     | < 0.001 |
| Cistus ladanifer | 1.054      | 0.305 |
| Cistus laurifolius| 6.379     | 0.012 |
| Cistus monspeliensis | 83.970   | < 0.001 |
| Cistus populifolius | 15.908   | < 0.001 |
| Cistus pilossepalus | 28.338   | < 0.001 |
| Cistus salviifolius | 261.677  | < 0.001 |
| Halimium atriplicifolium | 42.568 | < 0.001 |
| Halimium halimifolium | 57.560  | < 0.001 |
| Halimium ocymoides | 25.064   | < 0.001 |
| Halimium viscosum | 66.380   | < 0.001 |

Figure 3. Germination percentages (mean ± standard error) of studied species when were exposed to a heat-shock (100 °C for 10 minutes) before or after the summer temperature treatments (1 month at 50/20 °C). Cal: Cistus albidus, Ccl: Cistus clusii, Cla: Cistus ladanifer, Cla: Cistus laurifolius, Cmo: Cistus monspeliensis, Cpo: Cistus populifolius, Cps: Cistus pilossepalus, Csa: Cistus salviifolius, Hat: Halimium atriplicifolium, Hha: Halimium halimifolium, Hoc: Halimium ocymoides, Hvi: Halimium viscosum.

According to cycling of sensitivity to physical dormancy-break, dormant seeds can cycle between two states: insensitive and sensitive seeds to physical dormancy break (insensitive ↔ sensitive)56. Insensitive seeds are unable to respond to the dormancy-breaking treatment opposite to sensitive seeds, which can do it56. Previously to cycling sensitivity, some works proposed a cycling of dormancy between seeds with physical dormancy and non-dormant seeds (PY ↔ ND)57,58. However, species with physical dormancy cannot cycle between dormant and non-dormant, because the process of physical dormancy loss is irreversible. That is, once a slit or an opening is formed in the seed coat, a rescaling of this opening would not seem possible56.
In a similar work to this, Hagon and Ballard\(^9\) made seeds of *Trifolium subterraneum* permeable by percussion and then germinated at 20 °C obtaining high germination percentages. Authors stated that permeability of seeds was reversed when seeds were stored dry at 5% relative humidity after percussion. However, when percussed seeds were stored at high relative humidity previously to drying at low relative humidity, germination was high. This happened because the palisade layer in the lens of the seeds stored at high humidity had slits through its entire width. However, when seeds were kept at low humidity, the palisade layer had slits but they did not penetrate through its whole width. The authors concluded that the permeability induced by percussion could be reversed by manipulation of relative humidity. According to Jayasuriya *et al*\(^{16}\), these results can also be explained from the sensitivity cycling approach. From this perspective, percussion may not have made the seeds permeable, but it might have increased the sensitivity of seeds to dormancy release at 20 °C.

We would need additional work to conclude securely that Cistaceae species show sensitivity cycling to physical dormancy breaking, but here I present the first report of it. When the second experiment finished, I checked the ungerminated seeds that remained as unimbibed hard seeds. I observed a significant higher proportion of hard seeds when heat shock was given before summer than when heat shock was given after summer temperatures (Supplementary Data Fig. S1), supporting the explanation of sensitivity cycling to physical dormancy breaking. Zupo *et al*\(^{29}\) also found that seeds of *C. albidus* exposed to fire plus summer temperatures decreased germination in comparison to seeds only exposed to fire, but results were not explained. Probably, these results can also be explained in terms of sensitivity cycling. The underlying idea is that fire by itself cannot break physical dormancy but improve the sensitivity of seeds to an additional cue which definitively breaks dormancy. When after heat shock seeds are kept at moderately high temperatures (e.g., 40 °C) for a few hours, a generalized increase of germination was registered. Consequently, timing of fire, at the beginning or at the end of the summer season, determines the promotion or inhibition of Cistaceae germination. A fine-tuning between summer temperatures and timing of fire must control germination of Cistaceae. If fire happens at the beginning of the summer, high summer soil temperatures and low moisture content must lead seeds of Cistaceae to an insensitive state since the environment conditions are not the appropriate for germination. However, when fire happens at the end of summer, previously to autumn rains, it can act as a key signal triggering germination. Additionally, for many species, the combined effect of summer plus fire temperatures was higher than the isolated effect of fire. This synergic effect of summer plus fire temperatures could improve the opportunities of establishment. Summer temperatures by themselves do not break physical dormancy but modulate the response to fire.

These findings can help to take decisions for effective fuel management treatments such as in the case of prescribed burnings. Burnings before summer could reduce germination of all studied species except *Cistus ladanifer*, which could thrive in absence of competitors. This species forms widespread continuous shrublands very poor in species because it produces phytotoxic active compounds that inhibit the development of other plants\(^{68,69}\) and the resulting landscape accumulates large amounts of standing biomass that produces fine dry fuel, thus increasing the risk of fire. On the contrary, prescribed burnings at the end of the summer season could favour germination of all studied species, which would lead to rich and heterogeneous shrublands and therefore, to reduced fire risk.

In conclusion, in the case of the studied *Cistus* and *Halimium* species, the timing of germination must be controlled by fire but also by summer temperatures and probably by available water after fire\(^{42}\), which ensures...
seedling emergence under favourable conditions for establishment, such as low levels of competition and high availability of resources in postfire environments. However, we should be cautious and avoid generalizations for other genera of Cistaceae such as Fumana, Helianthemum or Tuberaria, since fire and summer temperatures might have played different roles in their evolution and physical dormancy may have diverse origins. The onset of the Mediterranean-type climate regions in the Neogene-Quaternary brought summer high temperatures and a regime of recurrent fire, and consequently they may have worked simultaneously as evolutionary pressures modulating the suitable time for germination of Cistus and Halimium species in the Mediterranean. Studies like this will help us to reach a better understanding on the dynamics and responses of natural species under the current situation of global warming.

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