

Fire as a threatening factor for endemic plants of the Canary Islands

Víctor Garzón-Machado · Marcelino J. del Arco Aguilar ·
Francisco Valdés González · Pedro L. Pérez-de-Paz

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Abstract The endemic flora of the Canary Islands is remarkable but highly vulnerable to environmental changes. Several factors, such as fire, could be threatening. The purpose of this study was to analyze various treatments in endemic threatened species which could have been growing in the understory of the Canary pine forest. We attempted to improve germination through dormancy breaking, and analyze the influence of fire on these plants. Seven physical and chemical treatments were tested: control, mechanical scarification, boiling water, dry heat (50 and 120 °C), smoke and smoked water. Mechanical scarification showed a positive response in germination percentage in two species characterized by hard seed coat, but out of all of the species tested, only one demonstrated a positive response to any of the treatments related to fire. At the temperatures registered on wildfires, neither the seeds situated on the aerial structure nor seeds in seed bank seem to be able to survive and germinate. The increasing incidence of fires through anthropogenic causes could contribute to the current threat status of these species and poorness of the Canary pine forest understory.

Keywords Fire · Seed treatments · Threatened species · Pine forest understory · Canary Islands

Introduction

The Canary Islands are part of one of the most remarkable biodiversity hotspots on the planet (Médail and Quézel 1997; Myers et al. 2000), which include 539 endemic vascular plants comprising approximately 40 % of the native flora and more than 25 % of the total

V. Garzón-Machado (✉) · M. J. del Arco Aguilar · P. L. Pérez-de-Paz
Departamento de Biología Vegetal (Botánica), Universidad de La Laguna,
C/Astrofísico Francisco Sánchez s/n, 38071 La Laguna, Tenerife, Spain
e-mail: vigarma@ull.es

F. V. González
Departamento de Biología Vegetal (Fisiología Vegetal), Universidad de La Laguna,
C/Astrofísico Francisco Sánchez s/n, 38071 La Laguna, Tenerife, Spain

flora (Acebes et al. 2010). This endemic diversity is highly vulnerable to environmental changes, especially disruptive and destructive alterations through human interference (Bramwell 1990). Accordingly, vegetation communities have an important endemic element.

The Canary pine forest is the most widespread plant community throughout the archipelago (Del Arco et al. 2010). It is dominated by the endemic *Pinus canariensis*, which is a species of Mediterranean affinity (Millar 1993) and a possible survivor of a subtropical mountain pine already present throughout the Mediterranean in the Tertiary (Klaus 1989). The Canary pine forest also has a Mediterranean affinity, and in its typical appearance has been described as a poor formation in the understory species (Santos 1983; Pérez-de-Paz et al. 1994). Several factors, including fire, could contribute to this poorness.

Wildfire is an important natural process for shaping Mediterranean climate ecosystems (Trabaud 1997), but the number of fires has increased in recent decades (Malkinson et al. 2011), modifying the floristic composition and threatening biodiversity (Arnan et al. 2006; Syphard et al. 2009). The current fire regime is producing negative effects, especially in the pine forests (Pausas et al. 2008). Human activity has significantly impacted the increase of the frequency and severity of fires (Piñol et al. 1998), which together with the effects of alien species and habitat transformation (i.e., urbanization, deforestation, etc.) are disturbing the biodiversity (Cowling et al. 1996).

Fires are recurrent in the pine forests of the Canary Islands (Arévalo et al. 2001), where *P. canariensis* exhibits several morphological and physiological resistance traits, such as thick bark, thick buds, tall growth habit, deep rooting, longevity and sprouting capability, to name a few (Climent et al. 2004). Nevertheless, some rare understory species do not show these characteristics, and they could be threatened by the incidence of fire. Today, species of different genera, such as *Cheirolophus*, *Convolvulus*, *Crambe*, *Helianthemum* and *Echium*, are primarily relegated to the steep slopes and pockets of developed soils on cliffs and can germinate and grow in the understory of the Canary pine forest in well-developed soils (Garzón-Machado et al. unpubl.).

In a recent study, we established the current floristic composition of the vegetation of natural areas (Caldera de Taburiente National Park), including pine forests (Garzón-Machado et al. 2011). In addition, we studied the effect of introduced herbivores (Garzón-Machado et al. 2010), suggesting their contribution to the poverty of this plant community. In the present study, we evaluated the germination rate of several species, which could have been integrated in the natural pine forest understory, to determine the causes underlying the poverty of this plant community.

The aim of this study was to characterize the effects of various treatments on several threatened species associated with pine forests, improve germination through dormancy breaking and analyze the influence of fires on these plants.

Methodology

Plant material

We selected five low-phanerophyte endemic species: *Cheirolophus arboreus* (Asteraceae), *Convolvulus fruticosus* (Convolvulaceae; a low woody climber), *Crambe microcarpa* (Brassicaceae), *Echium webbii* (Boraginaceae) and *Helianthemum cirae* (sp. nov., Santos 1993) (Cistaceae). These species, except *C. fruticosus*, which also grows in El Hierro and Tenerife, are exclusively found in La Palma. These plants primarily grow on steep slopes,

soil-bearing ledges and soil-pockets on the cliffs of pine forests; notably, *E. webbia* appears outside these environments and can be observed sparsely growing in the pine forest. *H. cirae*, a rare local endemism, has only been observed in pine forests. Other species that exceed this habitat (Gómez-Campo et al. 1996; Bramwell and Bramwell 2001) are *C. fruticulosus*, which also grows in coastal cliffs and thermo-sclerophyllous woodland, and *C. arboreus*, *C. microcarpa* and *E. webbia*, which are also observed growing in the evergreen laurel forest.

These species belong to genera with a high percentage of endemism and vulnerability (Fig. 1). Among the selected species, *C. arboreus* is classified as critically endangered (CR) (Bañares et al. 2003) or endangered (Moreno 2008), and *C. fruticulosus* and *C. microcarpa* are vulnerable (Moreno 2008). Although CR, *H. cirae* has been provisionally excluded from the red list (Moreno 2008) because it has not been validly published.

The seeds were collected from as many individuals as possible, each one from a single natural population of La Palma (Table 1; Fig. 1) and stored under laboratory conditions (controlled temperature and low humidity) until further use. To verify seed viability, and utilize seeds with adequate viability, a tetrazolium test (TZ) was performed before applying the different germination treatments.

Treatments

Seven treatments were tested: control, mechanical scarification, boiling water, dry heat (two temperatures), smoke and smoke solution. All necessary laboratory equipment was

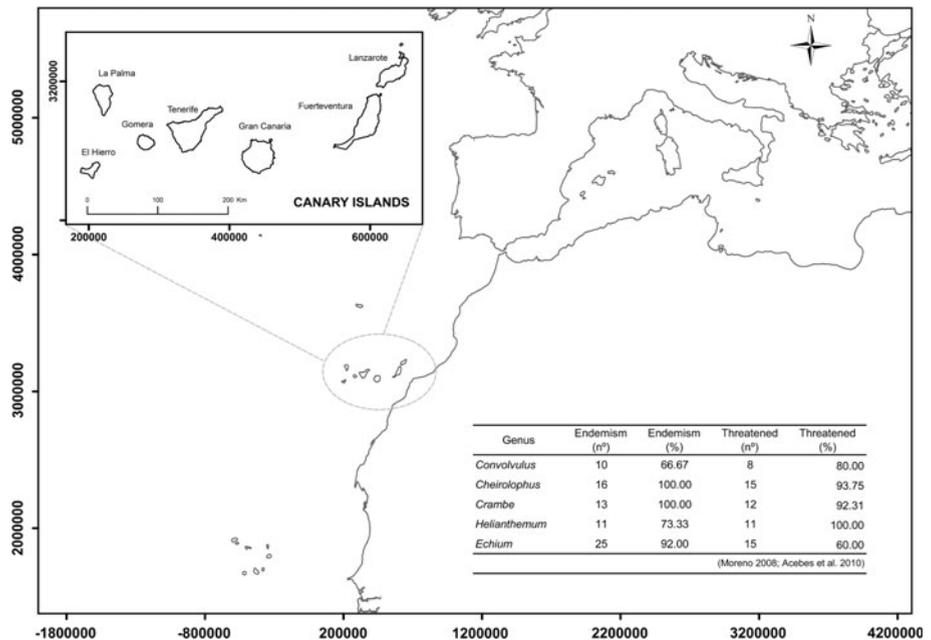


Fig. 1 Geographical location and table showing the high endemism and vulnerability rate of the genera studied

Table 1 Data for the seeds used in experiment

| Species | Collection date | N ^o of individuals | % Viability (TZ) |
|----------------------|-----------------|-------------------------------|------------------|
| <i>C. arboreus</i> | Aug'09 | 20 | 73 |
| <i>C. fruticosus</i> | Jul'09 | 25 | 87 |
| <i>C. microcarpa</i> | Sep'09 | 16 | 84 |
| <i>E. webbia</i> | Jun'09 | 320 | 74 |
| <i>H. cirae</i> | Jun'09 | 60 | 96 |

Experiment date: Jul'11–Sep'11; Oct'11–Dec'11 (heat (50 °C) and boiling water treatments)

previously sterilized. In addition, the seeds were soaked in NaClO (1–2 %) for 5 min as an antifungal pretreatment.

Mechanical scarification was induced by the abrasion of the seeds between two sheets of fine-grained sandpaper. For the boiling water treatment, the seeds were immersed in 500 ml of boiling distilled water (100 °C), and subsequently cooled to room temperature for 24 h. The dry heat treatments were performed using an electric oven at 50 °C for 10 min and 120 °C for 5 min. These treatments were selected based on the results obtained in previous studies (Herranz et al. 1998; Moreira et al. 2010). These temperatures are the extremes of the range registered during wildfires in the upper layer of the soil (0–5 cm depth) (DeBano et al. 1977; Trabaud 1979; Bradstock and Auld 1995), where the majority of the seed bank is primarily concentrated (Simpson et al. 1989). The 50 °C treatment was not applied to *C. arboreus* and *C. microcarpa* because there were not enough seeds.

The smoke effect was tested using two different methods: direct smoke and smoked water solution. The direct smoke was generated using an ordinary beekeeper's smoker to burn a mixture of dry and fresh plant material (De Lange and Boucher 1990) gathered from the surrounding vegetation (mainly pine material) (Gilmour et al. 2000). The smoker was used to pump smoke into a closed vacuum chamber containing the seeds for 30 min (De Lange and Boucher 1990). The second smoke treatment was conducted with a smoked water solution. A mixture of dry and fresh plant material was burned in a muffle furnace at 185° for 1 h, and the resulting ashes were immersed in 100 ml of distilled water with vigorous shaking. The resulting solution was decanted and filtered.

Four replicates of 25 seeds per treatment were made. The seeds were sown on two porous cellulose filter papers previously moistened with 3 ml of distilled water in 9-cm Petri dishes. The Petri dishes were incubated in a seed germinator at 25/15 °C under a 16 h light/8 h dark photoperiod. This photoperiod and temperature range was similar to the natural conditions in which these species are exposed. Three milliliters of distilled water was added as needed to maintain constant moisture during the experiments, and the germinating seeds were counted daily over a 60-day incubation period. The germination was measured as the emergence of a 2 mm radicle. The germinated seeds were removed from the dishes.

Statistical analysis

The germination percentages (mean value \pm SE), T_{50} (germination speed) and MGT (mean germination time) were calculated for each species. The T_{50} and MGT were calculated using the formulas previously described in Thanos and Doussi (1995) and Tompsett and Pritchard (1998), respectively. These two parameters were not calculated when the final germination percentage was equal to or less than 5 %.

The non-parametric Kruskal–Wallis test was used to identify the significance of the differences in the germination percentages among treatments because assumptions required for parametric testing could not be met. This test is appropriate for a low number of replicates. The post hoc Conover-Iman test was applied to detect differences ($P < 0.01$) in the comparison between the pairs of treatments. All analyses were performed using XLSTAT (Addinsoft 2012).

Results

Germination percentage

The results of the TZ showed that the viability was higher than 70 % in all species (Table 1). However, the germination percentage in the control for all species was lower (Table 2). Mechanical scarification enhanced germination in all species, except *E. webbii*. This treatment increased eightfold the germination percentage compared to control treatment in *H. cirae*. The effect of temperature was different at 50 and 120 °C. While the control values were not modified at 50 °C, at higher temperatures (120 °C), the germination in *E. webbii*, *C. microcarpa* and *C. arboreus* was reduced. Surprisingly, at 120 °C, the germination rate was increased in *C. fruticosus*. The effect of high temperature through the boiling water also decreased germination, even reaching total inhibition (*E. webbii*, *C. microcarpa* and *C. arboreus*). Similar to the effects of incubation at higher temperatures, boiling also improved germination in *C. fruticosus*. The effects of smoke and smoked water solution were less noticeable. Compared with the control treatment, the smoke treatment increased germination in *C. microcarpa*, while both treatments with smoke and smoked water solution increased germination in *C. arboreus* (Fig. 2; Table 2).

The Kruskal–Wallis analysis was performed for each species separately, obtaining significant differences ($P < 0.01$) between the treatments in all species (Table 3). Treatment with boiling water inhibited germination in *E. webbii*. The post hoc test showed that the others treatments did not significantly vary the germination percentage with respect to the control in this species. Mechanical scarification showed significant differences from the rest of treatments in *H. cirae*. The germination of *C. microcarpa* increased with smoke, but this effect was not significant with respect to the control. The only significant difference was observed between the control and boiling water treatments. Treatment with boiling water completely inhibited germination, as in *C. arboreus*. For this species, high temperatures through boiling water and heat (120 °C) were negatives, decreasing germination to 0 and 2 %, respectively. Finally, the only species that showed a significant increase in germination with the temperature was *C. fruticosus*. The boiling water enhanced the germination percentage to 78 % compared with the 6 % observed in the control treatment. Heat (120 °C) and scarification also showed significant differences as compared with the rest of the treatments.

T_{50}

The T_{50} value represents the time required to reach 50 % germination, which indicates germination speed. The earliest mean time for germination in all species was observed under the control and smoke treatments (Table 2). Conversely, heat (120 °C), boiling water and smoked water demonstrated a delay in T_{50} . The latter treatment showed a T_{50}

Table 2 The effect of the different treatments on the final germination percentage (mean value \pm SE), T_{50} (mean value in days \pm SE) and MGT (mean value in days \pm SE)

| Species | Germination (% \pm SE) | | | | | | |
|--------------------------|--------------------------|------------------|------------------|------------------|-----------------|-------------------|-------------------|
| | Control | Smoke | Scarification | Heat (120 °C) | Heat (50 °C) | Smoked water | Boiling water |
| <i>C. arboreus</i> | 47 \pm 9.29 | 63 \pm 10.75 | 64 \pm 7.66 | 2* \pm 2.00 | NO | 62 \pm 3.83 | 0* \pm 0.00 |
| <i>C. fruticosus</i> | 6 \pm 2.00 | 5 \pm 2.52 | 33* \pm 1.91 | 45* \pm 1.91 | 3 \pm 1.91 | 1 \pm 1.00 | 78* \pm 4.76 |
| <i>C. microcarpa</i> | 25 \pm 10.37 | 44 \pm 11.77 | 29 \pm 1.00 | 10 \pm 2.00 | NO | 20 \pm 5.16 | 0* \pm 0.00 |
| <i>E. webbia</i> | 55 \pm 9.57 | 51 \pm 10.75 | 28 \pm 7.83 | 31 \pm 1.00 | 54 \pm 8.71 | 55 \pm 9.43 | 0* \pm 0.00 |
| <i>H. citrae</i> | 12 \pm 5.88 | 6 \pm 1.15 | 97* \pm 1.00 | 19 \pm 3.78 | 6 \pm 3.46 | 10 \pm 4.16 | 4 \pm 1.63 |
| T_{50} (days \pm SE) | | | | | | | |
| <i>C. arboreus</i> | 13.32 \pm 4.34 | 9.75 \pm 2.10 | 11.35 \pm 1.79 | NC | NO | 33.85* \pm 1.14 | NC |
| <i>C. fruticosus</i> | 8.33 \pm 4.34 | 5.66 \pm 3.00 | 19.00 \pm 3.13 | 9.43 \pm 0.94 | NC | NC | 5.41 \pm 1.05 |
| <i>C. microcarpa</i> | 14.37 \pm 5.78 | 11.18 \pm 1.28 | 13.25 \pm 2.83 | 19 \pm 5.72 | NO | 21.67 \pm 4.80 | NC |
| <i>E. webbia</i> | 5.62 \pm 0.66 | 5.81 \pm 0.37 | 8.87 \pm 3.74 | 11 \pm 1.22 | 5.83 \pm 0.49 | 7.14 \pm 1.18 | NC |
| <i>H. citrae</i> | 6.50 \pm 2.36 | 4.62 \pm 1.32 | 1.88 \pm 0.82 | 25.62 \pm 3.74 | 2.88 \pm 1.83 | 11.50 \pm 6.11 | 21.66 \pm 16.41 |
| MGT (days \pm SE) | | | | | | | |
| <i>C. arboreus</i> | 21.66 \pm 3.82 | 16.17 \pm 2.93 | 17.80 \pm 1.80 | NC | NO | 30.10 \pm 2.00 | NC |
| <i>C. fruticosus</i> | 6.87 \pm 4.02 | 7 \pm 3.51 | 17.46 \pm 7.22 | 11.98 \pm 0.57 | NC | NC | 7.52 \pm 0.92 |
| <i>C. microcarpa</i> | 17.79 \pm 7.58 | 12.08 \pm 1.10 | 19.09 \pm 3.29 | 24.25 \pm 6.37 | NO | 25.47 \pm 5.46 | NC |
| <i>E. webbia</i> | 8.91 \pm 1.11 | 7.87 \pm 0.81 | 12.07 \pm 5.91 | 14.76 \pm 3.85 | 8.14 \pm 1.15 | 13.77 \pm 3.09 | NC |
| <i>H. citrae</i> | 6.33 \pm 2.59 | 5.12 \pm 1.32 | 4.21 \pm 0.83 | 28.23 \pm 2.93 | 3.18 \pm 1.63 | 12.66 \pm 5.55 | 16.62 \pm 12.86 |

The results were obtained after 60 days. When germination was $\leq 5\%$, the T_{50} and MGT were not calculated NC. NO treatment was not performed because of low seed numbers

The pairwise comparison of the treatment with its corresponding control was significant (* $P < 0.01$)

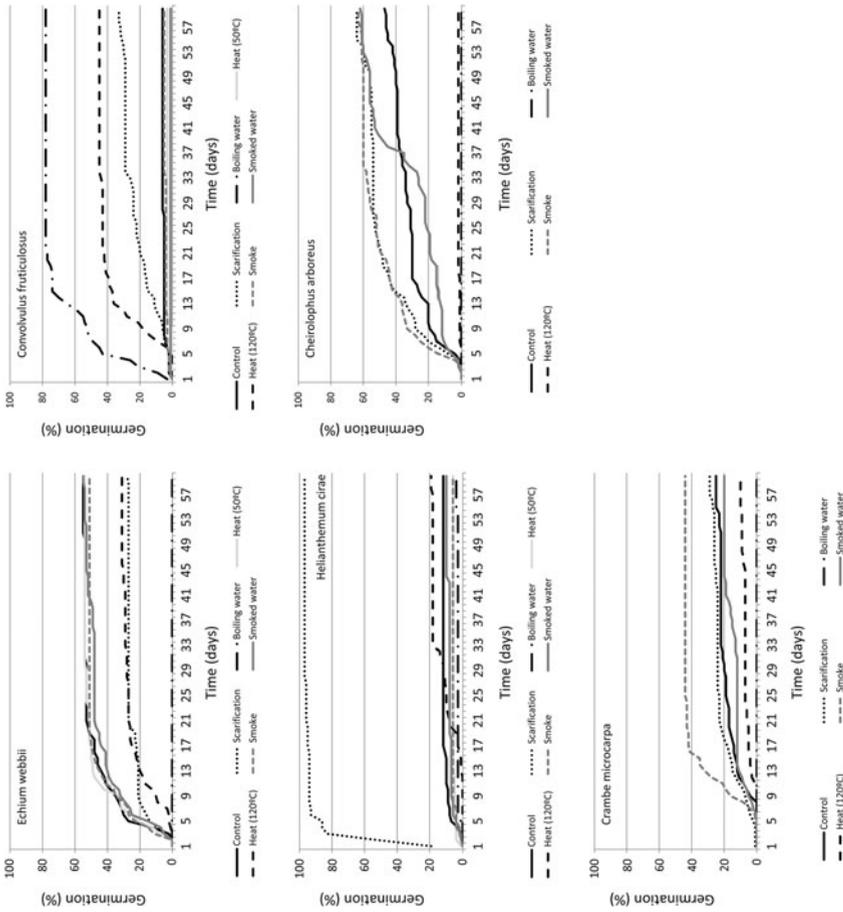


Fig. 2 Cumulative germination percentages for each treatment

Table 3 Results of Kruskal–Wallis analysis for germination percentage, T_{50} and MGT

| Species | %Germination | | T_{50} | | MGT | |
|----------------------|--------------|----------------|----------|----------------|----------|----------------|
| | χ^2 | <i>P</i> value | χ^2 | <i>P</i> value | χ^2 | <i>P</i> value |
| <i>C. arboreus</i> | 17.010 | 0.000* | 8.650 | 0.018 | 7.566 | 0.040 |
| <i>C. fruticosus</i> | 23.363 | 0.000* | 9.275 | 0.032 | 4.178 | 0.405 |
| <i>C. microcarpa</i> | 14.510 | 0.003* | 3.858 | 0.452 | 3.932 | 0.434 |
| <i>E. webbia</i> | 15.293 | 0.005* | 7.788 | 0.168 | 13.096 | 0.008* |
| <i>H. cirae</i> | 15.298 | 0.003* | 13.664 | 0.013 | 10.562 | 0.081 |

Significant difference (* $P < 0.01$) among treatments

value of 33 days in *C. arboreus*. Mechanical scarification in *H. cirae* showed the lowest value of T_{50} .

The Kruskal–Wallis test applied to the data for the T_{50} (Table 3) did not show significant differences among the treatments.

MGT

With regard to the mean germination time, heat (120 °C) and smoked water showed the highest values, with 12–28 and 13–30 days, respectively. The lowest values (3–6 days) were obtained in *H. cirae* with smoke, scarification and heat (50 °C) (Table 2). The MGT values were higher in *C. microcarpa* and *C. arboreus*. The Kruskal–Wallis test showed no significant differences, except for *E. webbia*, but not with regard to the control (Table 3).

Discussion

The germination of these five species in the absence of treatment is heterogeneous, with the best results observed in *E. webbia* and *C. arboreus*. The other three species showed low germination percentages despite the high viability.

Four of the five species were not positively influenced under smoke or thermic treatments; only *C. fruticosus* was positively affected under the strongest thermic conditions. Smoke and smoked water did not affect the germination percentage of any species. Our results are consistent with the previously published results of other authors who studied various species distributed in Mediterranean environments (Buhk and Hensen 2006; Rivas et al. 2006; Gómez-González et al. 2008). The germination of Mediterranean species against smoke exposition is diverse (Keeley and Fotheringham 2000). Moreira et al. (2010) observed a positive response in eight of the thirty Mediterranean basin species studied (Primulaceae, Ericaceae and Lamiaceae); however, Buhk and Hensen (2006), who worked with twenty-one Mediterranean species, did not observe any response to smoke exposition. In any case, there is no direct relationship between the germination induced by smoke and fire-prone ecosystems (Bradshaw et al. 2011).

There was no effect under moderate heat treatment (50 °C) for any species. This temperature can easily be obtained during extreme summer heat waves in Mediterranean ecosystems (Keeley and Fotheringham 2000; Bradshaw et al. 2011). Therefore, this temperature is not necessarily tied to fire environments.

It is known that high temperatures crack the hard coats of some seeds, allowing water to filter into the embryo activating germination (Herranz et al. 1998). However, if high

temperature is applied for too long or if the seeds have a soft coat, heat can negatively affect the embryo, drying it to lethal levels (Reyes and Trabaud 2009). The seeds of *C. microcarpa*, *C. arboreus* and *E. webbi* were extremely sensitive to boiling water treatment. Moreover, these seeds were not stimulated under high heat (120 °C). Accordingly, these seeds seem not to be adapted to intense fires. Similar negative effects of high temperature were obtained for other Mediterranean species (Pérez-García and González-Benito 2006; Luna et al. 2007; Reyes and Trabaud 2009; Moreira et al. 2010).

Conversely, high temperatures had a positive effect on *C. fruticulosus*. Convolvulaceae and other taxa characterized by hard seed coat are often heat-stimulated, which has also been interpreted as a trait to ensure post-summer germination in dry habitats (Keeley and Fotheringham 2000). These results are consistent with the current distribution of *C. fruticulosus*, mainly restricted to dry and warm habitats (Gómez-Campo et al. 1996; Del Arco et al. 2006).

The scarification treatment had a positive effect on the germination percentage in species with hard seed coats, such as *H. cirae* and *C. fruticulosus*, as scarification makes the seed coat more permeable to water. This treatment positively affected other Mediterranean species, including *Helianthemum* (Robles and Castro 2002; Pérez-García and González-Benito 2006) and *Convolvulus* (González-Martín 1998; Jayasuriya et al. 2008). Scarification increased the germination percentage in *C. microcarpa* and *C. arboreus* as compared with the effects of high temperature treatments or its effect was similar (in *E. webbii*).

During a fire, temperatures at the soil surface can reach over 1,100 °C, decreasing to 150 °C at 5 cm beneath the mineral soil (DeBano 2000). For the plants studied, at these temperatures, neither the seeds situated on aerial structures nor seeds in the seed bank seem to be able to survive and germinate. Only a chance of survival could exist for *C. fruticulosus*. This species seems to use a similar mechanism as that of *Chamaecytisus proliferus* (Leguminosae) or *Cistus symphytifolius* (Cistaceae) (Pérez-García et al. 2008), which are two characteristic species of the pine forest understory (Del Arco et al. 2010). Both of these species have a hard seed coat whose impermeability maintains dormancy. The coat is broken or weakened during a fire, allowing water penetration, breaking the dormancy, and thus stimulating germination. Regarding germination, this is the only positive relationship we could deduce between fire and the five species studied.

Conclusions

In the Canary Islands, wildfire could be a natural agent for shaping the pine forest ecosystem in the past, and undoubtedly it should be considered the principal long-term perturbation influencing the Canary pine forest (Climent et al. 2004). However, the time intervals between successive wildfires could be lengthy, such as in other Mediterranean ecosystems (Trabaud 1997). The frequency of fires has risen since the arrival of the human population to the islands (approximately 2,000 years ago). In fact, the number of fires has quadrupled from 1970 to 2010 by anthropogenic cause (ISTAC 2012).

Although the Canary pine has the capacity to resprout after a fire (Otto et al. 2010), most of the species of the understory do not have this ability. The destructive role of fire is obvious, killing individuals and plants population (Buhk et al. 2007). Thus, a large proportion of the future composition of the plant community depends on the seed bank (Parker and Kelly 1989). This study shows that seeds of these endemic species lose their capacity

to germinate after fires and the repeated effects of fires could have caused their current disappearance within the pine forest understory for the depletion of the seed bank.

Species with hard seed coats, such as *C. symphytifolius* and *C. proliferus*, are among the few that could germinate (Pérez-García et al. 2008), and therefore, they persist. Consequently, the understory poorness of the pine forest could be related to the increased frequency of fires. This is a fact, which added to the strong effect of introduced herbivores in the Canary Islands, is seriously threatening endemic plants and consequently the biodiversity. In addition, frequent fires could facilitate the expansion of alien species, as in other Mediterranean ecosystems (Keeley et al. 2005).

In the light of this study, we recommend conservation actions for threatened species, through applying treatments that improve germination, and we call for caution in asserting that fire enhances the Canary pine forest community (Arévalo et al. 2001), as the wildfire return interval for the Canary pine forest has not been determined.

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