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DORMANCY AND GERMINATION IN CISTUS CLUSII (CISTACEAE): EFFECT OF BIOTIC AND ABIOTIC FACTORS

Jorge CASTRO* & Ana Teresa ROMERO-GARCÍÁ**

SUMMARY

An analysis was made of treatments to promote the germination of Cistus clusii (Cistaceae), attempting to simulate some abiotic factors (thermal shock) and biotic factors (gibberellic hormones and chemical scarification) of the natural habitat of this species. Germination was increased by chemical scarification (such as may occur when seeds pass through the digestive tract of a mammal) and thermal shock (such as may occur during forest fires). In the latter case, both the intensity and duration of the treatment were determining factors.

The study of the germination capacity after a chilling period showed that, in addition to the dormancy imposed by the seed coats characteristic of the family Cistaceae, in this species physiological dormancy occurs at the embryo level. These results complement the currently available data on the characteristic dormancy processes of Cistaceae seeds, for which dormancy other than that imposed by the seed coats has rarely been described.

INTRODUCTION

Cistaceae is a family of shrubs and herbs which are characteristic of dry, sunny habitats, its main diversification centre being the Mediterranean region.
(Proctor, 1978). All taxa in this family present seeds with hard coats (hardseededness, Thanos et al., 1992), considered to be a particular type of primary seed dormancy (Mayer & Poljakoff-Mayber, 1989) that impedes seed germination unless previous scarification occurs.

In a natural environment, seed scarification can occur in various ways, including fire and animal ingestion (Keeley, 1987; Mayer & Poljakoff-Mayber, 1989; Baskin & Baskin, 1989). After a fire, a strong wave of emergence of Cistaceae seedlings often results (Trabaud & Oustric, 1989a; Roy & Sonié, 1992), and thermal shock in the laboratory promotes seed germination in a number of these species (e.g. Trabaud, 1995 and references cited therein). In addition, the passage of Cistaceae seeds through the digestive tract of animals has been shown to increase germination in this family (Malo & Suárez, 1996).

While dormancy imposed by the seed coat has been widely described in Cistaceae, physiological dormancy at the embryo level has been reported only in rare cases in this family (but see Peña et al., 1988 for a suggestion of secondary dormancy). There are two types of physiological dormancy — primary, physiological dormancy acquired while seeds are maturing in the mother plant; and secondary, dormancy provoked in shed seeds by unfavourable environmental conditions (Mayer & Poljakoff-Mayber, 1989). Physiological dormancy seems to be regulated by temperatures of around 0 °C, affecting the production of hormones involved in the germination process (Bewley & Black, 1994; Vleeshouwers et al., 1995). Thus, traditionally, the most common methods used experimentally to break physiological dormancy have been hormonal treatments and seed stratification (Hartmann & Kester, 1983).

In the present study, we analyse various aspects of germination in seeds of Cistus clusii Dunal, a species which, as the rest of the members of Cistaceae, is characterized by hard-coated seeds (Thanos et al., 1992). The objectives of the study were 1) to investigate whether physiological dormancy occurs at embryo level in this species, assuming that in this case a change in the germination percentage after a period of chilling should be expected, and 2) to examine the germination response to treatments intended to simulate common abiotic and biotic factors that act on these seeds in the natural environment. For an abiotic treatment, we applied thermal shock comparable to that produced during a fire, and for biotic treatments, we considered a gibberellic hormone (GA₃) of the type synthesized by embryos, and chemical scarification (sulphuric acid) as may occur in seeds passing through the digestive tract of animals.

MATERIALS AND METHODS

THE SPECIES AND STUDY AREA

Cistus clusii Dunal (Cistaceae) is a shrub reaching 40-70 cm in height, frequently forming part of scrubland vegetation in the western Mediterranean region. It grows in dry, sunny environments, mainly on limestone substrates and in warm habitats, though sometimes reaching altitudes of 1 500 m a.s.l. (Demoly &Montserrat, 1993). This plant is frequent in the southeastern Iberian Peninsula, especially in coastal areas, with moderate levels of both abundance and cover (Martín-Bolaños & Guinea, 1949). The fruit is a woody capsule, 4-8 mm in size,
which opens when ripe, producing 40 to 60 small (about 1 mm thick) seeds released in early autumn. The species is consumed by free ranging-livestock, mainly domestic sheep and goats (Fernández, 1995). Fires are commonly followed by waves of seedling recruitment (personal observation).

For this study, four populations were selected on different hillsides in the province of Granada, southeastern Spain (Table 1). The populations, though relatively close to one another (< 50 km apart), range in altitude from sea level to 1200 m. The climate is of Mediterranean type (Capel-Molina, 1981), but minimum temperatures and precipitation vary according to altitude (Table 1). In all four populations, C. clusii forms part of a scrubland cover on carbonate substrates. Other common species in the study areas include Ulex parviflorus, Rosmarinus officinalis, Thymus spp. as well as Genista umbellata and Stipa tenacissima in the three lower populations.

### TABLE 1

**Location of Cistus clusii populations.**

<table>
<thead>
<tr>
<th>Pop. code</th>
<th>Source Location</th>
<th>Altitude (m)</th>
<th>Mean T. of Jan. (°C)</th>
<th>Prec. (mm)</th>
<th>Date of seed collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Calahonda, Sierra de la Contraviesa 36°43'N, 3°23'W</td>
<td>30</td>
<td>12.4</td>
<td>405</td>
<td>20-VII-94</td>
</tr>
<tr>
<td>2</td>
<td>Cerro Gordo, Almuñécar 36°43'N, 3°47'W</td>
<td>50</td>
<td>12.5</td>
<td>474</td>
<td>11-IX-94</td>
</tr>
<tr>
<td>3</td>
<td>Lanjarón, Sierra Nevada 36°53'N, 3°32'W</td>
<td>600</td>
<td>8.7</td>
<td>511</td>
<td>11-IX-94</td>
</tr>
<tr>
<td>4</td>
<td>Sierra de Cazulas 36°50'N, 3°43'W</td>
<td>1200</td>
<td>-5.7*</td>
<td>783*</td>
<td>05-IX-94</td>
</tr>
</tbody>
</table>

*Note: January is the coldest month. (*), data from a weather station located 180 m higher.*

### SEED COLLECTION AND GERMINATION EXPERIMENTS

In 1994, ripe fruits were pooled from at least 30 plants per population and each pool was split into two groups, stored in darkness in paper bags either at room temperature of 18 to 25 °C (hereafter, “non-stratified treatment”) or refrigerated at a constant temperature of 4 °C (hereafter, “stratified treatment”). Two germination experiments, spanning March and April 1995, were performed in a growth chamber (ASL, ± 0.1 °C) with a photoperiod of 16 h (0600-2200 h) and alternating temperatures of 15 °C during dark periods and 20 °C during light periods. Light was provided by fluorescent tubes emitting a photon flux density of 135 μmol m⁻² s⁻¹ in the PAR range, measured at lamp level using a Li-Cor LI-200 sz pyranometer sensor (Li-Cor Inc., Lincoln, Nebraska, USA). Seeds were placed in glass Petri dishes of 12 cm diameter containing filter paper disks resting on a single layer of 5 mm glass beads (modified from Roy & Sonié, 1992), all materials having previously been sterilized. At the beginning of the experiments, 20 ml of sterilized distilled water was added to each dish, and moisture was replenished as needed (approx. every two weeks). Each Petri dish contained 100 seeds, with 4 replicates per treatment. Dishes were randomly repositioned within the chamber every 5 days. Prior to each experiment, seeds were individually examined under a dissecting microscope, and any damaged or empty seeds were discarded.
Germination, identified as visible radical protrusion, was recorded daily until aprox. 95% of germinable seeds had germinated for each treatment, records being spaced 2-3 days thereafter until complete a 5-week period. The experiments were ended when all treatments registered 10 consecutive days without germination. The germination rate was estimated as $T_{50}$, i.e. the time needed for manifestation of half of the final germination level. In treatments prone to fungal attack, seeds were previously disinfected by immersion in a 1% sodium hypochlorite solution for 10 min, followed by thorough rinsing with sterile distilled water.

One experiment was conducted to test the existence of physiological dormancy, and the other to evaluate the impact on germination exerted by treatments simulating the abiotic and biotic factors mentioned above.

**Experiment 1. Test of physiological dormancy**

We analysed the germination of the stratified and non-stratified seeds for the four populations. Four replicates per treatment and population were used, as described above. At the time of the experiment, stratification had lasted six months for populations 2, 3 and 4, and eight months for population 1. The seeds were disinfected in all cases.

**Experimental 2. Effect of abiotic and biotic factors on germination**

Non-stratified seeds from population 1 were used, and the following treatment groups were tested:

1. Dry heat in an oven at $100 \pm 2$ °C for 10, 20, 30 and 60 minutes (treatments Th10 to Th60). Seeds were not disinfected.
2. Dry heat in an oven for 10 min at 80, 100, 120 and 140 °C (treatments K80 to K140). Seeds were not disinfected. For treatments K100 and Th10 a single set of four replicates was used, as they represent the same conditions.
3. Immersion of the seeds in a gibberellic acid solution (GA3) at 50, 100, 200, 400 and 800 ppm for 24 h (treatments G50 to G800), keeping the temperature constant at 25 °C. Seeds were disinfected before immersion in the hormone.
4. Immersion of the seeds in concentrated sulphuric acid for 5, 10 or 15 min (treatments S5, S10 and S15, respectively), keeping temperature of the solution constant at 25 °C. Seeds were immediately rinsed with sterile distilled water, and subsequently disinfected.
5. Controls: we used four additional dishes containing non-disinfected seeds for groups 1 and 2 or disinfected seeds for groups 3 and 4.

The heat treatments fit the range of time and temperature commonly reached by the heat waves at around 2 cm in soil depth during fires in Mediterranean shrublands (Trabaud, 1979; Whelan, 1995), thereby simulating an abiotic factor common in the habitat of the species. Chemical scarification with sulphuric acid erodes the seed coats, increasing their permeability and consequently enhancing germination (Hartmann & Kester, 1983), as happens with seeds passing through the digestive tracts of animals (Mayer & Poljakoff-Mayber 1989; Baskin & Baskin 1989). Thus, this procedure can be considered to simulate of seed consumption by livestock, a biotic factor affecting *Cistus clusii* seeds.

All statistical analyses were carried out with non-parametric tests. Since the sample size was four, the Mann-Whitney test (U values) was used when three or
fewer treatments were involved; for more than three treatments, we used the Kruskall-Wallis test (H values), following Fowler & Cohen (1990). Values are means ± 1SD. T50 values were calculated in days.

RESULTS

EXPERIMENT 1. TEST OF PHYSIOLOGICAL DORMANCY

Chilling affected the seeds from different populations in different ways (Fig. 1). Population 1 revealed no significant differences between the two batches of seeds (8.0 ± 5.7 for stratified seeds and 9.5 ± 2.9 for non-stratified, U = 10.5, p > 0.05). However, in population 2 the germination percentage of the stratified seeds was lower than that of the non-stratified seeds (1.5 ± 1.0 and 15.3 ± 3.4 respectively, U = 16, p < 0.05), as in population 3 (6.2 ± 0.9 versus 15.3 ± 2.9, U = 16, p < 0.05). Nevertheless, in population 4 (located at the highest altitude; see Table I) the situation reversed, with the stratified batch having the higher germination percentage (11.2 ± 0.9 versus 7.5 ± 1.7, U = 15.5, p < 0.05).

Figure 1. — Germination percentage of stratified and non-stratified seeds for Cistus clusii populations. Bars indicate standard deviation. Comparisons within populations were made with a Mann-Whitney test (ns = non significant; * = p < 0.05).
The germination percentage remained very low unless treatments to promote germination were used. No significant differences in germination percentage were found between the disinfected \((9.5 \pm 2.9)\) and non-disinfected \((6.5 \pm 3.1)\) control treatments \((U = 12.5, p = 0.19)\), and \(T_{50}\) values were also similar \((18\) and \(19\) days, respectively). The percentage of seeds that died during the course of the experiment, was < \(1.5\) \%, except for treatment \(S_{15}\) (Table II).

**TABLE II**

*Germination percentages and deaths of the treatments applied to Cistus clusii seeds.*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination (%)</th>
<th>(T_{50}) (days)</th>
<th>Death (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T_{h10})</td>
<td>31.8 ± 3.4*</td>
<td>18</td>
<td>0.5 ± 1.0</td>
</tr>
<tr>
<td>(T_{h20})</td>
<td>55.5 ± 2.4*</td>
<td>14</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td>(T_{h30})</td>
<td>55.2 ± 1.0*</td>
<td>10</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>(T_{h60})</td>
<td>47.8 ± 4.6*</td>
<td>12</td>
<td>1.3 ± 1.5</td>
</tr>
<tr>
<td>(K_{80})</td>
<td>14.0 ± 2.0*</td>
<td>17</td>
<td>0.5 ± 1.0</td>
</tr>
<tr>
<td>(K_{100})</td>
<td>31.8 ± 3.4*</td>
<td>18</td>
<td>0.5 ± 1.0</td>
</tr>
<tr>
<td>(K_{120})</td>
<td>93.2 ± 2.9*</td>
<td>10</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td>(K_{140})</td>
<td>71.5 ± 2.5*</td>
<td>10</td>
<td>1.0 ± 1.4</td>
</tr>
<tr>
<td>(G_{50})</td>
<td>7.2 ± 1.7*(^{mn})</td>
<td>10</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>(G_{100})</td>
<td>4.8 ± 1.7*(^{mn})</td>
<td>10</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>(G_{200})</td>
<td>9.2 ± 2.6*(^{mn})</td>
<td>11</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>(G_{400})</td>
<td>7.2 ± 1.0*(^{mn})</td>
<td>10</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>(G_{800})</td>
<td>9.5 ± 3.0*(^{mn})</td>
<td>10</td>
<td>1.0 ± 1.2</td>
</tr>
<tr>
<td>(S_{5})</td>
<td>41.2 ± 14.3*</td>
<td>5</td>
<td>0.1 ± 0.4</td>
</tr>
<tr>
<td>(S_{10})</td>
<td>63.2 ± 6.55*</td>
<td>4</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td>(S_{15})</td>
<td>49.8 ± 19.2*</td>
<td>5</td>
<td>15.5 ± 12.8</td>
</tr>
</tbody>
</table>

**Note:** The germination percentages are compared with the percentage obtained for the controls according to the Mann-Whitney test \((ns = non\ significant; * = p < 0.05)\). The \(\pm\) value represents the standard deviation. \(Th\), heat of seeds at \(100\) °C for \(10\) to \(60\) min; \(K\), heat of seeds for \(10\) min at \(80\) to \(140\) °C; \(G\), immersion of seeds in gibberellic acid at \(50\) to \(800\) ppm; \(S\), chemical scarification by immersion of seeds in sulphuric acid for \(5\) to \(15\) min.

Flash heating of the seeds increased the germination percentage in all the cases tested (Table II). When the heat was kept constant at \(100\) °C and the exposure time was varied (treatments \(Th\)), the germination percentage increased in all cases with respect to control. There were differences according to the exposure time \((H = 12.52, p = 0.006)\). The maximum germination was registered with exposure times of \(20\) and \(30\) min (Table II), which gave similar values \((55\%)\). At \(10\) min, germination increased only slightly \((32\%)\), while at \(60\) min the germination percentage \((48\%)\) declined significantly in comparison with that recorded at \(20\) and \(30\) min \((U = 15.5, and U = 16, respectively; p < 0.05)\). Heating for \(10\) min at variable temperatures also promoted germination (treatments \(K\), Table II), with differences between the exposure temperatures \((H = 14.26, p = 0.003)\). The highest
The germination percentage resulted from heating at 120 °C (93 %), increasing steadily from 80 °C. However, at 140 °C the percentage decreased (71 %).

The gibberellic hormones did not raise the germination percentage in any of the concentrations tested (Table II). Nevertheless, seeds germinated faster, with $T_{50}$ values of 10 to 11 against $T_{50}$ of 18 for the control treatment, suggesting that the seeds did indeed respond to the hormone. On the other hand, the sulphuric-acid treatments significantly boosted the percentage and rate of germination (Table II). The acid had a scarifying effect by eroding the seed coats, often exposing the cotyledon. The replicates varied widely (reflected in the standard deviation), probably owing to the treatment procedure, since a slight delay in rinsing can cause a considerable variation in the intensity of the treatment because of the highly erosive nature of the acid.

**DISCUSSION**

**TEST OF PHYSIOLOGICAL DORMANCY**

As stated above, hardseededness is a common feature in Cistaceae, and other types of seed dormancy have rarely been reported. However, Peña et al. (1988) suggested the existence of secondary dormancy at embryo level for *Halimium halimifolium* seeds (Cistaceae). The present study shows that processes of physiological dormancy may also be present in *Cistus clusii* seeds, given the variation in the germination percentage after a chilling period in three of the populations.

A seed without dormancy will germinate, if no other impediment is present, as soon as they find suitable conditions of temperature and humidity, which in the Mediterranean regions typically occurs in spring or early autumn. Physiological dormancy is considered a mechanism that impedes germination during periods when conditions favour germination but not seedling establishment. This type of dormancy appears to be governed by changes in membrane fluidity occurring at temperatures of around 0 °C, affecting the production of hormones involved in the germination process (Bewley & Black, 1994; Vleeshouwers et al., 1995). Thus, in a natural environment, primary physiological dormancy (dormancy entered while seeds are maturing) will prevent germination of seeds during autumn, when seedling establishment during subsequent days is improbable due, for example, to freezing. A period of chilling gives rise to the dormancy-relieving process. In a natural environment this means the end of dormancy during the winter months, allowing seeds to germinate the following spring, an appropriate season for seedling establishment. Even if dormancy is lost before the end of winter, germination will be prevented as low winter temperatures will maintain seed metabolism at minimum until temperatures rise the following spring. Therefore, primary physiological dormancy appears to be more important in populations located at higher altitudes, where winter temperatures are low, whereas in places located at lower altitudes (and therefore with mild winters) primary dormancy may be absent. In population 4, located at the highest altitude and thus with lowest winter temperatures (Table I), the germination percentage increased after a chilling period, suggesting that processes of primary physiological dormancy operate in the seeds of this population. In fact, several studies on the germination capacity of
populations distributed along an altitudinal gradient have shown that seeds from the coldest (highest) sites have a higher degree of dormancy than do seeds from the lowest places, and respond positively to chilling (e.g. Meyer & Kitchen, 1994).

In population 1, chilling had no significant effect, although, in populations 2 and 3, chilling did decrease the germination percentage. Thus, in the latter case, stratification appears to induce secondary dormancy at the embryo level. This situation has been also described for species distributed along an altitudinal gradient — that is, in populations located at medium or lowest altitudes, chilling either causes seeds to enter a process of secondary dormancy or does not change the germination percentage (Meyer & Kitchen, 1994; Meyer et al., 1995). Therefore it seems that C. clusii seeds are endowed with mechanisms at embryo level of either primary or secondary dormancy.

EFFECT OF ABIOTIC AND BIOTIC FACTORS ON GERMINATION

The natural germination percentage of C. clusii seeds proved to be around 10% (in contrast to 30-40% reported by Thanos et al., 1992). However, germination increased with treatments that broke the seed coat.

The maximum germination percentage (93%) resulted from thermal shock, supporting similar results for other Cistaceae taxa, especially those belonging to the genus Cistus, in which germination is promoted by thermal shock at around 80-150 °C (Thanos & Georgiou, 1988; Trabaud & Oustric, 1989b; Roy & Sonié, 1992; González-Rabanal & Casal, 1995; Salvador & Lloret, 1995). This increased germination percentage after thermal shock in C. clusii also supports earlier data by Thanos et al. (1992), and, furthermore, shows that both the temperature and the exposure time are determining factors in stimulating seed germination, as in other Cistus species (Trabaud & Oustric, 1989b). At a fixed time of 10 min, the germination percentage rose as the temperature approached 120 °C, although at higher temperatures (140 °C) the percentage fell, as some seeds were apparently killed by the heat. At a constant temperature of 100 °C, increased exposure time once again boosted the germination percentage to a threshold at which the seeds appeared to die (60 min). The optimal exposure times and temperatures coincide with those measured for the fire-caused heat at a soil depth of around 2 cm (e.g. Whelan, 1995). This may explain the observed wave of germination of C. clusii seeds after fires, as occurs in other species of the genus Cistus (Trabaud & Oustric, 1989a; Roy & Sonié, 1992). Fluctuations resulting from daily heating and cooling of the soil are considered responsible for the break-down of the coat in hard-coated seeds (Baskin & Baskin, 1989). In this sense, fire-caused heat can be considered an extreme example, provided that seeds lie at an appropriate depth in the soil. Pugnaire & Lozano (1997) have reported fieldwork in which the germination of C. clusii seeds was promoted after fire, although they considered soil disturbance to be a more important factor. This study complements those results, but emphasizes the importance of fire-caused heat, suggesting that soil disturbance could have the effect of moving large numbers of seeds into positions within the soil which provide temperatures conducive to germination.

In the treatments simulating biotic factors, gibberellic acid (GA₃) did not increase the germination percentage. However, the germination was faster, indicating that the hormone has an effect on germination but penetrates only those seeds that are not dormant, i.e. seeds with coats sufficiently damaged to enable substances to pass through them.
Chemical scarification by seed immersion in sulphuric acid promoted seed germination up to values of 63%, and reduced the germination rate to values very close to those reported for mechanical scarification (using sandpaper) with other Cistaceae species (Thanos & Georghiou, 1988). Seed-coat erosion by sulphuric acid is a common method of promoting the germination of seeds with hard coats (Hartman & Kester, 1983). The higher germination percentage after chemical scarification may be advantageous for hard-coated seeds that are eaten by vertebrates, converting the animal into a quantitative and qualitative dispersion vector by virtue of scarification during the digestive processes (Quinn et al., 1994; Malo & Suárez, 1995; Ibañez & Passera, 1997; Campos & Ojeda, 1997). In this sense, livestock (sheep and goats) consume Cistus clusii plants (Fernández, 1995), possibly increasing the germination percentage of mature seeds. Indeed, Malo & Suárez (1996) have reported that deer are efficient dispersers of Cistus ladanifer seeds, and that the germination percentage increases after the seeds pass through the animals' digestive tracts. This may be extrapolated to other Cistaceae taxa eaten by other ungulates, and Cistaceae hardseededness could be related to animal consumption.

Our results indicate that the dormancy of C. clusii seeds is complex, and that mechanism of physiological dormancy may operate. Nevertheless, physical dormancy imposed by the seed coat largely controls germination, and therefore scarifying treatments raise the germination percentage. Thus, indirectly, our data suggest that animals can play an important role in the germination of this taxa. Similar studies in other Cistaceae species could expand current knowledge concerning dormancy and its role in this family.

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