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Effect of high temperatures on seed germination of two woody Leguminosae

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Abstract

Cytisus scoparius and *Genista florida* regenerate after fire by stump-sprouting but also by seed. Seeds of these species were heated to a range of temperatures similar to those registered on the surface soil during natural fires (from 50 to 150 °C) and a range of exposure times (from 1 to 15 min). No germination was observed at high temperatures, ⩾130 °C, when the exposure time was 5 min or more. However, moderate heat treatments (at 70 and 100 °C) significantly increased the rate of germination relative to controls. *Cytisus scoparius* is more favoured by fire action than *Genista florida*, with germination rates slightly greater following 100 °C for 5 min and 130 °C for 1 min than after mechanical scarification.

Introduction

Forest fires have had a great influence on the vegetation dynamics of Southern Europe and in general, all Mediterranean-type ecosystems. It is therefore common for the community which appears after the fire to have a similar composition to that which previously existed (Naveh 1975; Trabaud 1983, 1987; Casal *et al.* 1990; Mazzoleni & Pizzolongo 1990). Most woody species characteristic of these areas are capable of regenerating by sprouting and there are only a few which regenerate solely from seed. Even those species that can regenerate both by seed and vegetative propagation tend to regenerate by sprouting, since it allows them to quickly reoccupy the space (Trabaud 1987).

The two species in this study, *Cytisus scoparius* and *Genista florida*, can be included in the latter group. They are not typical mediterranean shrubs even though they can be found in the Mediterranean Region. Their distribution spans the Eurosiberian Region. *G. florida* tends to be restricted to the Iberian Peninsula, but *C. scoparius* is widespread throughout Europe, even as far as Southern Sweden (Tutin *et al.* 1978). In the province of León (Northwest Spain), where this study was carried out, they usually constitute shrub formations or form part of the undergrowth in communities dominated by *Quercus pyrenaica*. These communities have undergone great human pressure for centuries. Felling, pasture and above all fires, which have all greatly increased over the last few decades, are the major forces (Tárrega & Luis 1990). In most woody species, including *Genista florida* and *Cytisus scoparius*, post-fire regeneration comes from stump-sprouting leading to an autosuccession process (Tárrega & Luis 1988;

Calvo *et al.* 1990). However, seedlings from both species are also found in burnt areas.

Since this study deals with species that are taxonomically close and usually co-exist or occupy the same areas where fires occur, it is of great interest to compare their seeds' response to heat. Heat-stimulated germination has been documented for many species in the chaparral of California (Stone & Juhren 1953; Quick & Quick 1961; Keeley 1987, 1991) which undergoes severe wildfires such as those occurring in the Mediterranean Basin. Intense heat may crack hard seed coats or melt waxy coverings. However, for seeds of most species, a few minutes at temperatures above 120 °C result in decreased germination, and temperatures greater than 150 °C for more than a few minutes are usually lethal (Stone & Juhren 1953; Keeley *et al.* 1985). During wildfires, complex mosaics of spots exposed to a broad range of temperatures have been recorded (De Bano *et al.* 1977; Trabaud 1979). Thus, it appears legitimate to ask if seeds of the two mentioned species have identical or similar heat requirements. Differential heat requirement could have adaptative value because such a mechanism would allow germination of these species over a broad spectrum of micro-sites according to temperatures. Alternatively, uniform heat requirements would cause simultaneous germination of the two species and lead to a competition between the seedlings.

Therefore, the aim of this study was to determine how an increase in temperature at ground level during fire affects seed germination. It must be kept in mind that although sexual reproduction is not quantitatively the most important form of regeneration with regard to biomass contribution, it can be of great interest since it is the only way of conserving the genetic variability of these species.

If there are numerous studies of the influence of fire on seed germination in Mediterranean shrubs (Naveh 1973; Keeley 1977, 1986, 1987; Papanastasis & Romanas 1977; Muñoz & Fuentes 1989; Trabaud & Casal 1989; Trabaud & Oustric 1989a, b; Valbuena *et al.* 1990), only studies of *Ulex europaeus* by Pereiras (1984) and *Cytisus multiflorus* and *C. oromediterraneus* by Añorbe (1988) have been carried out on woody Leguminosae species.

Materials and methods

Seed collection

Fruits were collected in June and July 1990 in the northeast province of León (1°30′ E, 42°65′ N). Neighboring areas were selected for sampling so that climate, topography, soil and age were as similar as possible among sites. The plants were part of an ecosystem dominated by *Quercus pyrenaica* and had a post-fire regeneration age greater than ten years. Thus, the seeds were collected from plants that had been subjected to fire and had recovered.

The pods, once collected, were dried in the open air until they opened, and the seeds fell out. They were then stored in the dark at 4 °C until they were used. this, according to Come (1970), assures the best conservation.

Experimental treatment

Healthy seeds not attacked by insects were selected. Parasite attacks, above all those by Curculionidae, are common on this type of plant. When they partially consume the seeds, they can exert a beneficial action favouring germination by breaking of the tegument. However, the viability of the seedlings can, in some cases, be affected (Añorbe 1988).

Treatments were carried out at the end of September 1990. The seeds were heated to a range of temperatures similar to those registered in the upper layer of the soil during natural fires in which the majority of seeds are usually found (Archibold 1979; Trabaud 1980; Puentes *et al.* 1989). In general, soil temperatures reached during fires are directly related to the biomass of fuel which burns. Peaks soil surface temperatures in rapidly burning chaparral wildfires (California) may reach over 700 °C, but this heat penetrate very little into the soil (De Bano *et al.* 1977) because of the rapid speed of movement associated with these fires, and the low heat conductivity of earth. Peaks and durations of soil temperatures associated with chaparral fires have been studied in detail by De Bano *et al.* (1977). Typical profiles of maximum temperatures with soil depth were considered by these authors for intense, moderate and light burns. In intense fires the maximum temperature at a depth of 2.5 cm may reach 200 °C and in moderate ones 160 °C, at the depth of 5 cm the maximum in intense fires reached only 60 °C for a few minutes. Similar results were recorded by Trabaud (1979) in garrigue fires. The temperatures chosen were 50 °C, 70 °C, 100 °C, 130 °C and 150 °C, with seed exposure periods of 1, 5, 10 and 15 min in each case. The treatments were carried out in an oven simultaneously for both species.

A hundred seeds from each species were used in each treatment. Additional 100 seeds were scarified using a hand file.

Germination tests

Immediately following the treatments, seeds were sown in plastic Petri dishes, 10 cm in diameter, on two layers of filter paper saturated with demineralized water. There were 5 replicates of 20 seeds for each treatment. One hundred non-treated seeds, used as controls, were also laid out under the same conditions.

Once seeds were sown, Petri dishes were randomly placed in a greenhouse. They were periodically re-randomized. The average temperature throughout the experiment was 22 °C during the day and 17 °C night. The dishes were watered using demineralized water in order to keep the filter paper saturated.

Fungus attacks were controlled using a fungicide (Qentosyl 1/1000).

The Petri dishes were checked daily, noting and eliminating the germinated seeds. A seed was considered to have germinated when the radicle had perforated the teguments (Come 1970) and was easily seen. The experiment was continued in this way for 48 days. On this day germination continued but at a lower rate, so daily checking was considered no necessary. Therefore, the dishes were watered and sealed with 'parafilm' and left for another 12 days until 60 days elapsed. At this time the number of germinated seeds was counted.

Some of the germinated seeds, selected at random from the different treatments, were planted in pots and monitored for viable seedling development. Two months after planting, their growth continued normally and their average height was 22.8 cm $\pm$ 1.08 for *Cytisus scoparius* and 5.3 cm $\pm$ 0.63 for *Genista florida*.

Statistical analysis

The results of germination after 60 days were analyzed using an ANOVA. Tukey's test (1949) was used to detect any significant differences ($\alpha = 0.05$) in the comparison between the pairs of treatments. It was previously shown that samples did not separate from the normal distribution using the David *et al.* test (1954), and that their variances were homogeneous using the Cochran test (1941).

In order to test the rapidity of germination of both species, the percentage of seeds which germinated after 10, 20, 30 and 40 days, with respect to the total number of germinated seeds (after 60 days) was calculated for each treatment.

Results

Germination percentages

The percentages of germination after 60 days are shown in Figures 1 and 2.

In neither one of the species did germination take place at a temperature of 130 °C or 150 °C when the exposure time was 5 min or more, although the seeds are capable of absorbing water and swelling (their increase in size was clearly seen).

The germination percentages of *Genista florida* were very low at any temperature for 1 min duration, except for 150 °C. As temperatures were

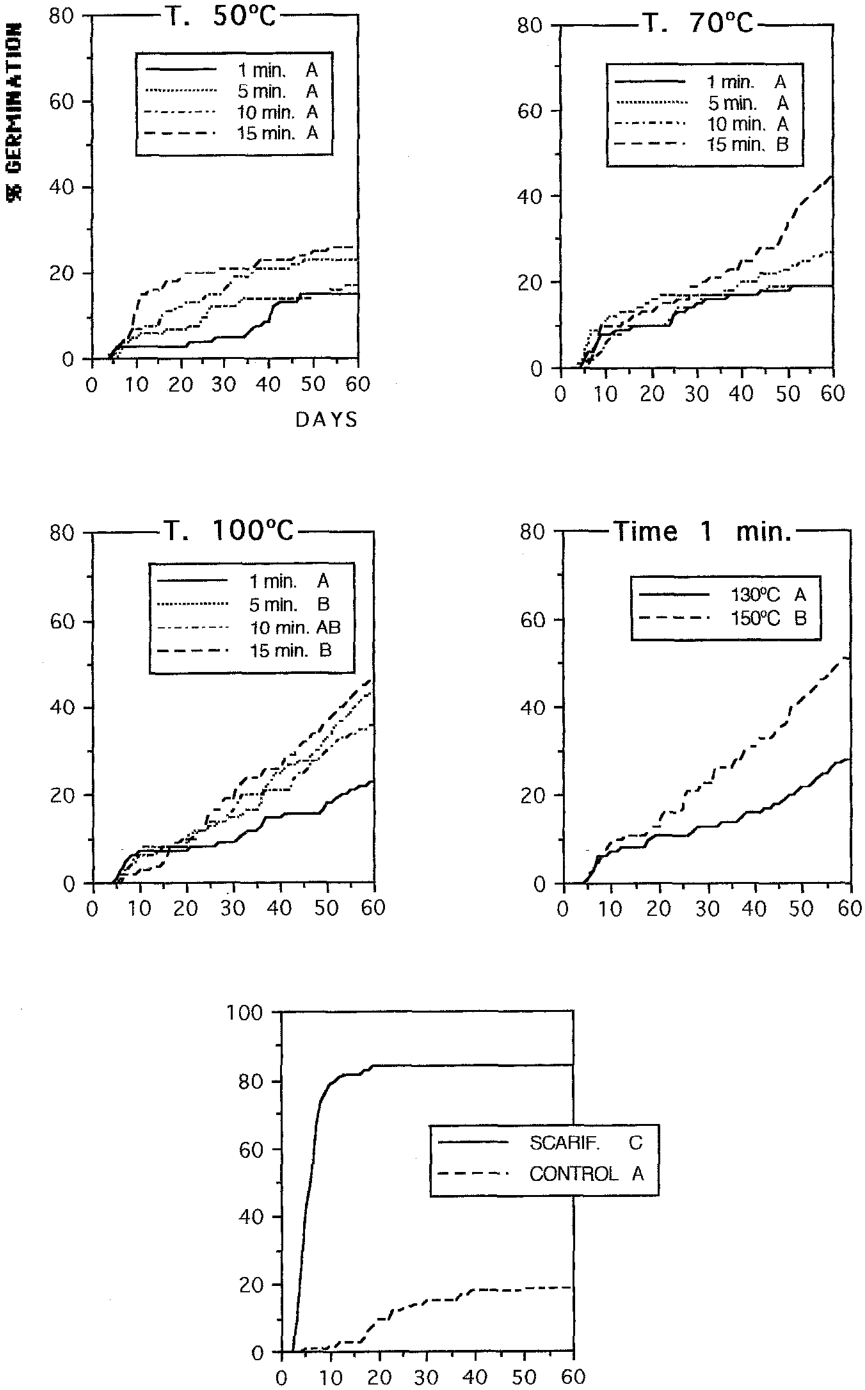

Fig. 1. Germination dynamic in *Genista florida* seeds for each heat treatment, comparing with scarified and control seeds. A same letter following the type of treatment indicates that no significant differences were detected (P = 0.05) between the treatments in the species.

higher and exposure time longer germination percentages were higher. The differences were only statistically significant with respect to the control for treatments of 70 °C for 15 min, 100 °C for 5 and 15 min and 150 °C for 1 min. In all these cases germination was greater than 44%. At 100 °C for 10 min the germination percentage was only slightly lower than that observed at 100 °C for 5 min (36% and 44%, respectively) although not statistically different. Scarified seeds showed a significantly higher rate of germination than all the other treatments.

In *Cytisus scoparius*, though the germination percentages for all treatments, with the exception of 50 °C for 5 min, were greater than those of the control seeds, no significant differences were detected in those placed at 50 °C for any length of time, 70 °C for 1 or 5 min, and those at 100 °C for 1 min. No significant differences between the mechanically scarified seeds and those placed at 70 °C for 15 min, 100 °C for 5, 10 and 15 min, and at 130 °C for 1 min were observed. Germination rates were slightly greater than those of scarified seeds only when placed at 130 °C for 1 min (79%) and 100 °C for 5 min (75%). The treatments at 70 °C for 10 min and 150 °C for 1 min resulted in intermediate germination percentages, which were statistically different both to control seeds and to scarified seeds.

If both species are compared, using a two-way analysis of variance, no significant statistical differences were observed in the germination rates of the control seeds nor in the mechanically scarified seeds. For any heat treatment with temperatures $\geq$70 °C, the germination rate was greater in *Cytisus scoparius* than in *Genista florida* with the exception of 150 °C for 1 min. However, only the differences in the seeds placed at 70 °C for 15 min, at 100 °C for 5 min and at 130 °C for 1 min were significant.

Germination dynamics

The percentages of germination for each treatment as well as that registered in the control seeds are presented in Figures 1 and 2.

The germination of *Genista florida* scarified seeds began three days after planting and finished after 19 days. Those of *Cytisus scoparius* finished germinating after 25 days. The percentages of germination were 84% and 74% respectively. The control seeds and those placed under heat treatments germinated more slowly and continuously throughout the study period. Germination began and reached its peak later in *Cytisus scoparius* than in *Genista florida* in almost all cases including control seeds.

These results were confirmed when germination percentages of each species in each treatment were analyzed by 10 day periods (Table 1) with respect to the total germinated seeds. For any 10 day period, the germination percentage was always greater in *Genista florida* than in *Cytisus scoparius*, except after the 40th day. On the whole, *G. florida* seeds germinated more quickly than those of *C. scoparius*. Thus, the germination maximum for *G. florida* took place during the first 30 days with percentages of germination varying between 90% for the 70 °C-5 min treatment and 33% for the 50 °C-1 min treatment. In the case of *C. scoparius*, after 30 days, the percentages of germination varied between 50% for the treatment at 150 °C-1 min and 13% for 70 °C-15 min.

The germination percentage of *Genista florida* seeds placed at 50 °C increased with increasing exposure time (Fig. 1). This effect was still observed with temperatures of 70 °C and 100 °C, though patterns were not as clear.

No differences in germinated *Cytisus scoparius* seeds were observed for exposure at 50 °C for any period of time. Germination in all cases was not significantly different from the control. Differences were first observed at 70 °C. Seed germination was lowest at 1 min and highest at 15 min exposure times. Intermediate germination percentages were observed at 5 and 10 min of exposure. At 100 °C germination was much greater when exposed for more than 5 min although no clear differences are observed in these three graphs. Nor were many differences observed between the seeds placed at 130 °C and 150 °C for 1 min in the first few days, although at the end of

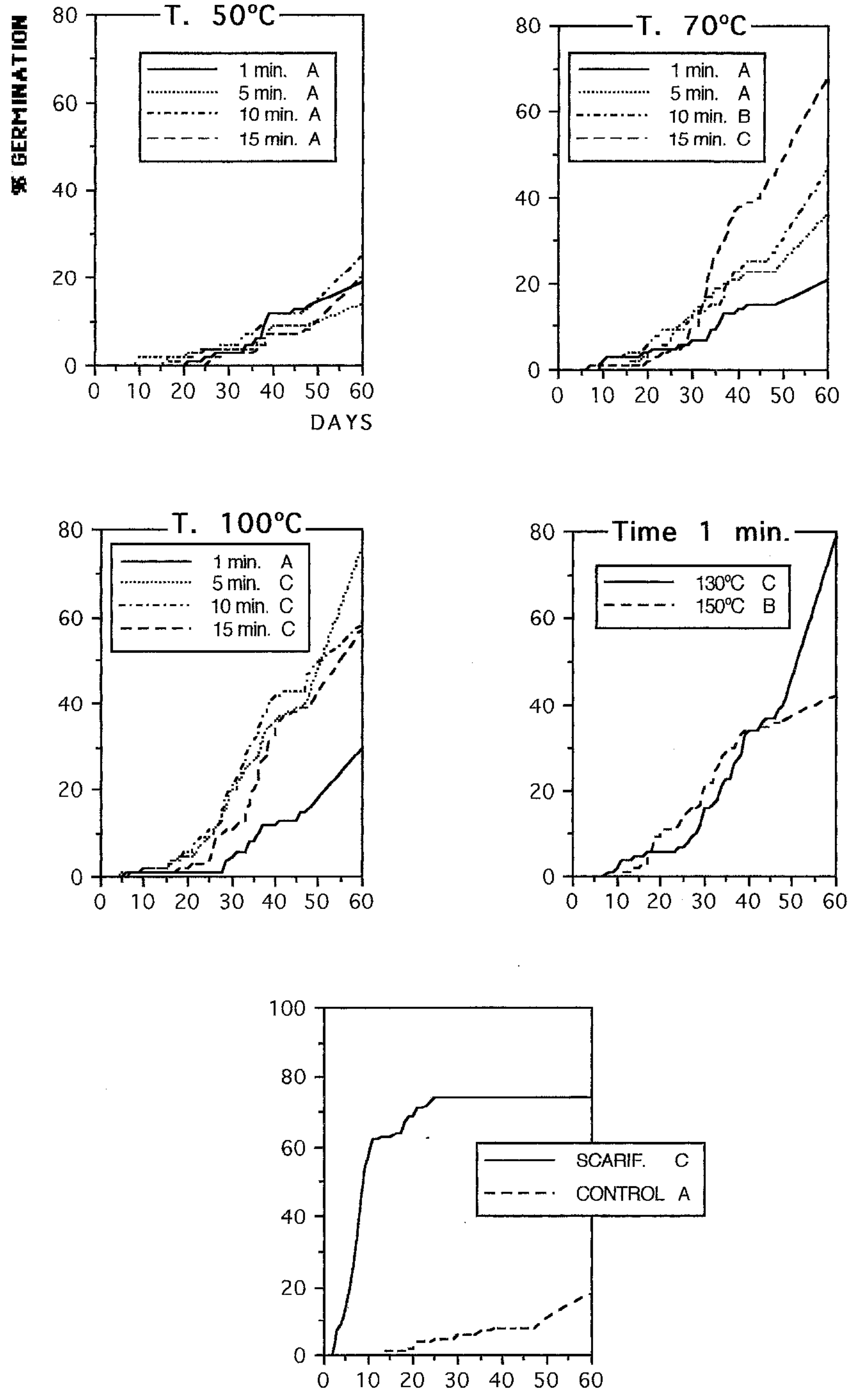

Fig. 2. Germination dynamic in *Cytisus scoparius* seeds for each heat treatment, comparing with scarified and control seeds. A same letter following the type of treatment indicates that no significant differences were detected (P = 0.05) between the treatments in the species.

Table 1. Percentages of germinated seeds every ten days with regard to the total of germinated seeds in each treatment.

	Treatments															
	50 °C				70 °C Exposing time (minutes)				100 °C				130 °C	150 °C	Sc.	C.
	1	5	10	15	1	5	10	15	1	5	10	15	1	1		
G. florida																
10 days	20	29	30	42	42	58	22	22	30	14	17	6	25	18	94	11
20 days	20	41	52	73	53	84	37	29	30	18	22	21	39	25	100	53
30 days	33	71	69	81	79	89	56	42	39	34	47	40	46	45	100	79
40 days	60	82	91	88	89	89	74	55	65	57	58	55	57	61	100	95
C. scoparius																
10 days	0	0	8	0	9	0	4	1	3	3	3	2	2	0	78	0
20 days	0	14	8	0	19	8	13	3	3	7	10	3	7	21	93	12
30 days	16	28	20	15	33	33	26	13	17	27	36	19	20	50	100	35
40 days	63	64	48	35	67	58	50	56	40	48	72	58	43	81	100	47

the study period the germination percentage was clearly greater at 130 °C.

Discussion and conclusion

In both species germination percentages for seeds not submitted to any treatment (control) were very low, although similar or somewhat superior to that observed in similar species (5% in *Cytisus oromediterraneus* and 12% in *C. multiflorus*, according to Añorbe 1988). The ability of these species to stump-sprout enables them to decrease their dependency on reproduction by seed. Therefore their germination percentages are usually inferior to that of species which only reproduce sexually, such as *Rosmarinus officinalis* (Trabaud & Casal 1989) and *Cistus ladanifer* and *C. laurifolius* (Lopes 1988; Valbuena *et al.* 1990). Trabaud and Oustric (1989a, b) obtained low germination rates in non-treated seeds from *Cistus albidus*, *C. monspeliensis* and *C. salvifolius* (between 2% and 7%). However, the percentages registered in mechanically scarified seeds showed that most seeds are capable of germinating when the tegument is broken. A similar scarification process could be carried out naturally by animals through digestion or direct and indirect scratching.

The lowest percentages of germination in *Cytisus scoparius* could be a source of error in short term tests. After 45 days only 8% of the control seeds had germinated as opposed to 18% of *Genista florida*. However, after 60 days the values are similar in both species.

Both temperature and exposure time at any given temperature affected the number and rate of germination. High temperatures, ⩾130 °C, together with long exposure time (⩾5 min), turned out to be fatal for the embryo in both species and no germination was observed. However, such temperatures are rarely registered in the soil at a depth of one centimetre. Between 1 and 5 cm temperatures generally vary between 110 °C and 40 °C (De Bano *et al.* 1977; Trabaud 1979). Heat treatments at 50 °C did not significantly increase the rate of germination. However, with increasing temperature, i.e. 70 °C or 100 °C, germination percentages increased significantly relative to controls. For a given temperature, the lowest germination percentage and the greatest delay before germination corresponds to the shortest time exposure.

Cytisus scoparius is clearly more favoured by fire action than *Genista florida*, with germination percentages generally higher but not significantly different than those of the mechanically scarified seeds. At moderate temperatures a long exposure time is required for optimal germination (70 °C for 15 min). However, as temperature increases above 70 °C, optimal germination occurs at shorter exposure times (at 100 °C the optimum is 5 min, at 130 °C falls to 1 min). Either way, at 100 °C for 10 and 15 min, although germination rate drops slightly, the difference with regard to scarified seeds are not significant and the values remain greater than the control.

Despite the risk involving the extrapolation of laboratory results to field conditions, this study suggests that the rise in soil temperature normally accompanying fires favours seed germination in both species and above all *Cytisus scoparius*. However, the different temperatures and time exposures to which the seeds were submitted in the course of time and at different micro-environment offer them a whole range of germination possibilities. The broad temporal distribution of germination allows them to select more adequate environmental conditions in each case and favouring the reoccupation of the soil. The good sprouting capacity, together with the germination stimulation caused by heat action, allows *Cytisus scoparius* to quickly reoccupy burnt sites and to colonize vast areas.

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