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Article *in* Ekoloji · January 2011
DOI: 10.5053/ekoloji.2011.7913

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Ekoloji 20, 79, 99-104 (2011)
doi: 10.5053/ekoloji.2011.7913

RESEARCH NOTE

Fire-Related Cues (Heat Shock and Smoke) and Seed Germination in a *Cistus creticus* Population in Southwestern Turkey

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Abstract
This study investigated the relationship between fire-related cues (heat shock and smoke) and the germination behaviour of *Cistus creticus* (Cistaceae) seeds under laboratory conditions. The seeds were obtained from a long-unburned *Pinus brutia* Ten. forest located on the Bozburun peninsula, Muğla, Turkey. Heat treatments were carried out at 80, 100, 120, and 150°C for five minutes. For smoke treatments, seeds were incubated in a liquid smoke solution for 24 hours. Both dry and watered controls were included in the experiment for heat and smoke treatments, respectively. Extra treatments of heat and smoke combinations and a cold stratification treatment were also performed. The treated and untreated seeds were sown into Petri dishes containing agar as a substrate, and the germinations were monitored until the end of the experiment. When compared with the control, the heat shock of 100, 120 and 150°C had significant stimulatory effects on the germination response, whereas 80°C, smoke and cold stratification did not. The results of the heat treatments are in accordance with current literature, but there is still a need for more studies for the comparison of the smoke treatment. In summary, the results indicated that seed germination of the considered population of *C. creticus* is stimulated by moderate and high heat intensities, but not by low heat intensity and smoke. It is important to know the germination response of plant species to fire cues for better management decisions in fire-prone woodland ecosystems.
Keywords: *Cistus creticus*, cold stratification, fire, germination, smoke, thermal shock.

Türkiye'nin Güneybatısındaki bir *Cistus creticus* Populasyonunda Tohum Çimlenmesi ve Yangınla İlişkili İmler (Isı Şoku ve Duman)

Özet
Bu çalışmada *Cistus creticus* (Cistaceae) tohumlarının çimlenme davranışı ile yangınla ilişkili imler (ısı şoku ve duman) arasındaki ilişki laboratuvar şartlarında araştırılmıştır. Tohumlar, Türkiye'nin güneybatısındaki Muğla İli'nde yer alan Bozburun yarımadasında uzun süredir yangına maruz kalmamış bir *Pinus brutia* Ten. ormanından elde edilmiştir. Isı uygulamaları 80, 100, 120 ve 150°C'lerde beş dakika olacak şekilde gerçekleştirilmiştir. Duman uygulamaları için, tohumlar sıvı duman çözeltisinde 24 saat boyunca inkübe edilmiştir. Isı ve duman uygulamaları için, sırasıyla, kuru ve sulu kontroller deneye dâhil edilmiştir. Isı ve duman kombinasyonlarını içeren ek uygulamalar ve bir soğuk katlama uygulaması da yürütülmüştür. Uygulama geçiren ve geçirmeyen tüm tohumlar dolgu maddesi olarak agar içeren Petri tabaklarına yerleştirilmiş ve çimlenmeler deneyin sonunda kadar kontrol edilmiştir. Kontrollerle karşılaştırıldığında, 100, 120 ve 150°C'lik ısı şoklarının çimlenme cevabı üzerine uyarıcı etkisinin olduğu, 80°C'lik ısı şokunun, dumanın ve soğuk katlamanın ise olmadığı saptanmıştır. Isı uygulamaları ile ilgili sonuçlar mevcut literatür ile uyumludur, ancak duman uygulaması ile ilgili sonuçların kapsamlı bir şekilde karşılaştırılabilmesi için daha çok çalışma yapılmasına gereksinim vardır. Özet olarak, sonuçlar ele alınan *C. creticus* populasyonunda tohum çimlenmesinin orta ve yüksek ısı şiddetleri ile uyarılırken, düşük ısı şiddeti ve dumanla uyarılmadığını göstermiştir. Bitki türlerinin yangın imlerine çimlenme cevabının bilinmesi, yangına eğilimli orman ekosistemlerinde daha iyi yönetim kararları verebilmek için önemlidir.
Anahtar Kelimeler: *Cistus creticus*, çimlenme, duman, sıcaklık şoku, soğuk katlama, yangın.

Tavsanoglu C (2011) Fire-Related Cues (Heat Shock and Smoke) and Seed Germination in a *Cistus creticus* Population in Southwestern Turkey. Ekoloji 20 (79): 99-104.

INTRODUCTION

Cistus creticus L. (Cistaceae) is a fire-following plant species appearing in abundance after wildfires (Tavşanoğlu and Gürkan 2005). This is, actually, a common property of *Cistus* L. (Roy and Sonié 1992, Schiller et al. 1997), which is an endemic genus for the Mediterranean basin (Guzmán et al. 2009). There is substantial seed dormancy in species of *Cistus* imposed by their hard seed coats which inhibit the uptake of water from the environment (Thanos and Georghiou 1988, Thanos et al. 1992). Therefore, scarification is needed to break the seed dormancy, either by the high temperatures naturally produced by wildfires, or by mechanical

Received: 22.11.2009 / Accepted: 14.01.2011

disturbances such as tilling or ploughing the soil (Troumbis and Trabaud 1986, Pugnaire and Lozano 1997). Consequently, species of *Cistus* germinate in large quantities immediately after a fire in the Mediterranean basin (Thanos et al. 1989, Eshel et al. 2000, Tavşanoğlu and Gürkan 2005).

Although the germination responses of several *Cistus* species to fire cues are well known throughout the Mediterranean basin (Paula and Pausas 2009, Paula et al. 2009), there are just a few studies on that of *C. creticus* (Troumbis and Trabaud 1986, Thanos and Georghiou 1988, Tilki 2008). These studies showed that germination in *C. creticus* was stimulated by fire temperatures, but the intensity of heat treatments that stimulate germination varies from study to study. Moreover, there is still a lack of information on the germination response of the Mediterranean basin species to smoke (Paula and Pausas 2009, Paula et al. 2009), which is of an important role in fire-stimulated germination in other Mediterranean type fire-prone ecosystems (Brown 1993, Keeley and Fotheringham 1998, Thomas et al. 2003). Regarding *C. creticus*, there is just one study showing that smoke-stimulated germination of this species is present (Crosti et al. 2006).

To study the fire-relevant plant traits of the Mediterranean species is of importance due to the changes in the fire regime in the Mediterranean basin (Paula et al. 2009). Therefore, to know the germination response of the Mediterranean species and populations in relation to fire cues may be crucial in the near future for the areas with a changed fire regime. The aim of this study was to test whether seed germination in a *C. creticus* population from southwestern Turkey was stimulated by heat-shock and smoke (fire cues). This study will contribute to our current knowledge on the fire response of this species.

MATERIALS AND METHODS

Study site and seed collection

Fruits of the *C. creticus* were collected in October 2008 from a long-unburned *Pinus brutia* Ten. (Turkish red pine) stand on the Bozburun peninsula, Muğla, Turkey (36°46'35" N, 28°10'57" E, 441 m). The study area was located on limestone bedrock, and has a typical Mediterranean climate with dry summers and wet winters. No sign of any recent surface fire (burned woody material or scorched bark of pine trees) at the collection site was detected, and the individuals of *C. creticus* were mostly located in the gaps through the forest canopy constituted by old (> 80 years) *P. brutia* trees.

Mature fruits were collected from 15 individuals which were randomly selected. After collection, the fruits were taken to the laboratory in nylon bags, and then the fruits were opened by hand to obtain seeds. The seeds were stored in paper envelopes at room temperature and humidity for six months before the germination experiment was conducted. The experiment was conducted from April to June 2009.

Weights of ten replicates of 100 seeds were measured by using the Ohaus Adventurer™ balance with 0.001 g readability before the experiment started. The average (± SE) seed weight of the studied population of *C. creticus* was 0.67 ± 0.01 mg.

Preparation of smoke solution

Preparation of the smoke solution followed the methodology of Jäger et al. (1996). The dry leaves of the *Cistus* spp. were crushed and separated into small pieces. Four replicates of 5 g were separately heated in an oven for 30 min at 190-195°C. After the treatment, 50 mL of distilled water was added to the plant material and let stand for 10 minutes. After that, the liquid was filtrated into a bottle to use as a smoke solution.

The experiment

Heat treatments were carried out in a temperature-controlled oven at 80, 100, 120, and 150°C for five minutes in order to simulate the temporal pattern of the upper-zone soil temperatures during a fire (Trabaud 1979). The process was repeated four times for each treatment to allow four independent replicates (Morrison and Morris 2000). An additional four replicates of seed lots not subjected to any heat treatment were also used, serving as the control for the heat treatments. For the smoke treatment, seeds were incubated in the liquid smoke solution for 24 hours before sowing in the Petri dishes. To see the interactive effects of heat and smoke treatments, extra treatments of 80, 100, 120, and 150°C for five minutes were applied; then, seeds from these treatments were incubated in the liquid smoke solution for 24 hours. These extra treatments were also repeated four times for each one. Four replicates of seed lots were incubated in distilled water for 24 hours without any heat treatment, and these seed lots were used as a watered control for

comparing with the other smoke treatments.

Treated and untreated seeds were sown in Petri dishes containing agar as a substrate. Each dish contained 50 seeds, evenly spaced, and there were four replicate dishes for each treatment (200 seeds for each treatment in total). The dishes were then placed in a germination cabinet and incubated at 20.0°C (± 0.5°C) in darkness. To evaluate the effect of cold stratification on germination, four replicates of untreated seeds were sown in Petri dishes containing agar as substrate and incubated at 4°C for three weeks, and then, were placed in the germination cabinet.

The germinations were checked under a stereomicroscope every three or four days until the end of the experiment. Germinating seeds were counted and removed from the Petri dishes at every check. Radicle emergence was the criterion for scoring germination. The experiment was finalized on the 75th day of the incubation period when no germination was observed for four days in any of the treatments.

Data analysis

Germination mean percentage and mean germination rate after heat, smoke and cold stratification treatments were compared to the germination in the corresponding controls by one-way analysis of variance (ANOVA). The germination rate was estimated as T'_{50} (number of days for 50 % of the number of seeds germinated) rather than T_{50} (number of days until 50 % of the initial seeds had germinated) (Rosselló and Mayol, 2002) due to the percentage of germination in most of the treatments was lower than 50%. Difference among germination percentages of the treatments was tested by using the Tukey HSD test. Before using ANOVA, data normality and homoscedasticity was tested by using the Shapiro-Wilk test and Levene test, respectively. The statistical tests were performed using XLSTAT version 2009.3 (Addinsoft™).

RESULTS

The germination percentage among heat treatment and control groups was significantly different (F= 12.0, d.f.= 4, P= 0.0001), and multiple comparisons showed that germination percentages after 100°C, 120°C and 150°C heat treatments were significantly higher than those of 80°C and the dry control (P < 0.05, Fig. 1). Neither germination mean percentage (±SE) after smoke treatment (3.0% ± 1.3) nor that after cold stratification (5.5% ± 1.0) was significantly different from the corresponding controls (P > 0.05, watered control: 5.5% ± 1.7, dry control: 4.5% ± 1.7). None of the germination percentage of heat + smoke treatment combinations was significantly different from that of the corresponding heat treatment (P > 0.05).

Most of the germinations occurred between the first and the fourth weeks after sowing in all the treatment and control groups (Fig. 2). However, the germination rate (T'_{50}) was significantly different among the heat treatment and control groups (F= 7.5, d.f.= 4, P= 0.002). This difference was mainly due to the earlier germination in the control and the later germinations in the heat treatment groups (Fig. 3A). On the other hand, no significant difference was found between the smoke treatment and the corresponding control (P > 0.05, Fig. 3B). Cold stratification had a significantly slower germination rate than the control due to the three-week extra incubation period at cold temperatures (F= 31.3, d.f.= 1, P= 0.003, not shown in the figure). None of the T'_{50} values of heat + smoke treatment combinations was significantly different from that of the corresponding heat treatment (P> 0.05, not shown in the figure).

DISCUSSION

It is well known that fire temperatures positively affect the germination of *Cistus* (Trabaud and Oustric 1989, Keeley and Baer-Keeley 1999, Chamorro-Moreno and Rosúa-Campos 2004) by breaking the physical dormancy imposed by their hard-coated seeds (Thanos and Georghiou 1988, Thanos et al. 1992). In accordance with the literature on the germination behaviour of the genus, the results of the present study indicated that germination of the considered population of *C. creticus* was stimulated by moderate (100°C 5') and higher (120°C 5' and 150°C 5') heat intensities, but not by a lower (80°C 5') one. Although the germination rate (T'_{50}) was lower in the control and 80°C 5' treatment than higher heat intensity treatments, this result is difficult to discuss since very low germination percentages was observed in both.

According to the previous studies in relation to the effect of dry heat on the germination of *C. creticus*, moderate and high heat intensities result in substantial increases in germination percentage (Thanos and Georghiou 1988, Keeley and Baer-

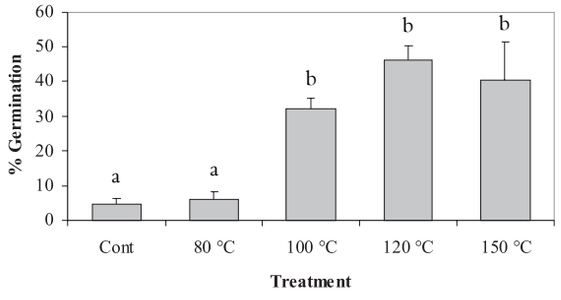

Fig. 1. Germination percentage of the heat treatment groups and the dry control (Cont). The values are means of four replicates, and the error bars are +SE of the mean. Different letters over bars indicates significant ($p < 0.05$) difference between groups.

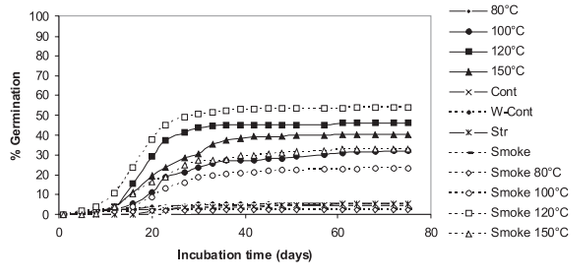

Fig. 2. Percentage of cumulative germination during 75 days after sowing in relation to different treatment and control groups. Str is cold stratification, W-Cont is watered control, and Cont is dry control. Solid lines and solid signs indicate the heat treatments and the corresponding control, and dashed lines and open signs shows smoke, smoke + heat treatments and the corresponding control.

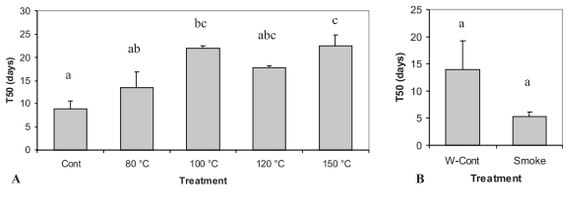

Fig. 3. Mean T'50 values (number of days for 50 % of the number of seeds germinated) of the treatment groups and the controls. The legend is as in Fig. 1 and Fig. 2.

Keeley 1999, Tilki 2008). However, contrary to the present study, it was found that low ones also improve seed germination (Troumbis and Trabaud 1986, Tilki 2008). There is just one study reported that moderate heat intensity does not affect germination (in *C. creticus* ssp. *corsicus*; Thanos et al. 1992). In fact, the variability of germination response to heat treatments among populations of the same species might be common in the Mediterranean basin (e.g. Cruz et al. 2003, Pérez-García et al. 2003), and the differences in the fire regime, climatic conditions, or other environmental factors may be responsible for this variation.

Smoke did not have a significant effect on the germination response when compared with the watered control. Moreover, it is apparent that the stimulatory effect of heat and smoke treatment combinations arose from the effect of the heat treatments alone. This is a result in contradiction with that of Crosti et al. (2006) showing an improvement in germination percentage after smoke treatment, which is the only study on the effect of smoke on germination of *C. creticus*. However, there are contradictory results on the smoke-induced germination when other *Cistus* species are considered. The studies showed that germination of different species of *Cistus* was stimulated by smoke (Pérez-Fernández and Rodríguez-Echeverría 2003) and some were not (Buhk and Hensen 2006, Reyes and Trabaud 2009, Moreira et al. 2010). Moreover, the studies mentioned have different results with regard to the same species (*C. salviifolius*) as well. For the genus *Cistus*, more studies are needed to show if there are differences among species or among populations of the same species in germination response to smoke.

Neither positive nor negative effects of the cold stratification for three weeks was found in the germination percentage. This result can be expected since the seed coat in *Cistus* spp. has already become permeable after the fire, and optimal germination can be obtained at several temperature combinations (Thanos et al. 1992). A similar result has also been reached; Olmez et al. (2007) stated that the control treatment had the highest germination percentage against different durations of cold stratification in *C. creticus*. This lack of stratification requirement seems to be a constant feature for other Mediterranean species as well (Giménez-Benavides et al. 2005, Luna et al. 2008).

As in other low-altitude Mediterranean pine woodlands, fire is an ecological component of *Pinus brutia* forests, and consequently post-fire recovery of these forests is a relatively rapid process (Tavşanoğlu and Gürkan 2009). However, success of the post-fire recovery of *C. creticus*, as an obligate seeder species without any resprouting ability, depends mostly on the formation of the soil seed bank during

fire-free periods. Although there is a decrease in the number of seeds produced (Tavşanoğlu 2010) and a depletion of viable seeds in the soil seed bank (Ne'eman and Izhaki 1999, Clemente et al. 2007) with successional time in the *Cistus* species, the presence of reproductive individuals in the studied forest will prevent this depletion for *C. creticus* in the long term. This, along with the stimulation of germination by fire temperatures, will allow *C. creticus* to persist in the fire-prone vegetation.

The present study demonstrated that germination of the considered *C. creticus* population is stimulated by fire temperatures, but not by smoke and cold stratification. It is important to know the germination response of plant species to fire cues, not only to predict their germination behaviour after fire, but also for better management decisions in fire-prone woodland ecosystems.

ACKNOWLEDGMENTS

I thank the anonymous referee for their comments that improved the manuscript.

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