



Effects of Smoke and Heat-Shock on Germination in Eight Perennial *Reseda* Species (Resedaceae)

Sekiz çok yıllık *Reseda* (Resedaceae) Türünün Çimlenmesi Üzerine Duman ve Sıcaklık Şokunun Etkisi

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ABSTRACT

Reseda is an understudied genus regarding germination ecology. We studied the germination response of eight perennial *Reseda* species to fire related cues by performing a germination experiment including two aqueous smoke treatments (at 1:5 and 1:20 concentrations), one heat shock (100°C for 5 min.) treatment, and two corresponding controls. Smoke slightly decreased germination percentage in *R. aucheri* ssp. *rotundifolia* and *R. minoica*, and the reduction in *R. luteola* was statistically significant ($P < 0.01$). In contrary, smoke promoted germination in *R. coodei*, *R. globulosa*, and *R. orientalis* slightly, and markedly in *R. anatolica* (from 3 to 11%; $P = 0.055$). Heat shock treatment did not affect the germination in three of four species tested, but significantly increased germination percentage in *R. luteola* in comparison to the control ($P = 0.046$). Consequently, there was no consistent germination response to smoke or heat shock in *Reseda* species studied. This study is the most comprehensive study tested fire-related germination of several *Reseda* species and provides the first germination results on seven *Reseda* species. Further studies that include more species and treatments would show a more completed figure for fire-related germination patterns in the genus *Reseda*.

Key Words

Germination, heat shock, *Reseda*, smoke.

ÖZ

Reseda çimlenme ekolojisi bakımından az çalışılmış bir cinstir. Bu çalışmada, sekiz çok yıllık *Reseda* türünün yangınla ilişkili sinyallere çimlenme cevabı, iki sulu duman uygulaması (1:5 ve 1:20 konsantrasyonlarında), bir sıcaklık şoku uygulaması (100°C'de 5 dakika) ve iki kontrol grubu içeren bir çimlenme deneyi gerçekleştirilerek araştırılmıştır. Duman, *R. aucheri* ssp. *rotundifolia* ve *R. minoica*'da yüzeysel bir şekilde, *R. luteola*'da ise istatistiksel olarak anlamlı bir şekilde ($P < 0,01$) çimlenmeyi azaltmıştır. Bunun aksine, duman *R. coodei*, *R. globulosa* ve *R. orientalis*'de yüzeysel olarak, *R. anatolica*'da ise belirgin olarak (%3'den %11'e; $P = 0.055$) çimlenmeyi artırmıştır. Sıcaklık şoku uygulaması test edilen dört türün üçünün çimlenmesini etkilemezken, *R. luteola*'nın çimlenme yüzdesini kontrole göre belirgin olarak artırmıştır ($P = 0.046$). Sonuç olarak, çalışmada incelenen *Reseda* türlerinde duman ya da sıcaklık şokuna karşı tutarlı bir çimlenme cevabı gözlenmemiştir. Bu çalışma, çok sayıda *Reseda* türünün yangınla ilişkili çimlenmesini inceleyen en kapsamlı çalışmadır ve yedi *Reseda* türü için ilk kez çimlenme bulgusu sunmaktadır. İleride daha çok sayıda tür ve uygulama içerecek şekilde yapılacak çalışmalar, *Reseda* cinsinde yangınla ilişkili çimlenme örüntülerini daha kapsamlı bir şekilde ortaya koyacaktır.

Anahtar Kelimeler

Çimlenme, sıcaklık şoku, *Reseda*, duman.

Article History: Received: Aug 10, 2020; Revised: Mar 10, 2021; Accepted: Mar 10, 2021; Available Online: Jun 10, 2021.

DOI: <https://doi.org/10.15671/hjbc.768365>

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INTRODUCTION

The genus *Reseda* L., and in a broad sense Resedaceae family, has drawn relatively less attention regarding the seed science and germination ecology [1]. Among others, *R. lutea* has drawn additional attention as it is an introduced and weed species in Australia [2] and North America [3]. It is known that *R. lutea* and *R. suffruticosa* has physiological dormancy and germinate well at various temperatures between 10 and 30°C [2,4,5]. For *R. lutea*, dark conditions and being buried are better than light conditions and being on the surface for germination [2,5], but there is also a result indicating no effect of light on germination in this species [6]. On the contrary, light conditions break dormancy in *R. luteola* [6]. It is also known that myrmecochory and unassisted dispersal are the main seed dispersal mechanisms in species of *Reseda* genus [1,7].

To date, the germination patterns in only two species of *Reseda* has been tested with regards to fire-related cues such as heat shock and smoke. Germination in *R. lutea* is not stimulated by moderate- or high-intensity heat shocks [8,9], but a slight increase in germination occurs in low-intensity heat shocks [9]. Similarly, *R. luteola* also found not to respond to heat shocks [8]. Smoke effect on germination has only once tested for any *Reseda* species, and this study shows that germination of *R. lutea* is inhibited by aqueous smoke application [9]. Nitrate, sometimes considered as another fire cue since its level increase in the soil in early post-fire environments [10], is also known to stimulate germination in *R. lutea* and *R. luteola* [6].

Our knowledge of the seedling establishment patterns of *Reseda* species in post-fire environments is also limited. There are records of successful post-fire seedling emergence in *R. phyteuma* [7,11,12] and *R. luteola* [13], but no seedling of *R. lutea* was found in burned sites [14]. As far as we know, there is no other record of the presence of *Reseda* species in burned areas.

Our study aimed to test the germination response of several *Reseda* species to fire related cues, heat shock and smoke. Considering *a priori* information about the seedling establishment of some *Reseda* species in post-fire environments, we expected that germination in some species we tested in the study will improve after we apply fire-related cues to seeds. For this purpose, we performed a germination experiment including

one heat shock and two aqueous smoke treatments on eight *Reseda* species naturally grown in Turkey.

MATERIALS and METHODS

Taxa

Reseda is the largest genus of the Resedaceae family, with approximately 65 species mainly distributed in the Mediterranean basin and southwestern Asia [15]. The species of *Reseda* are annual or perennial herbs and occur in limestone or gypseous soils and arid or semi-arid environments [15]. The Resedaceae family is located in the order Brassicales according to APG IV system [16], and the genus *Reseda* consists of 4 subgenera (Neoreseda, Glaucoreseda, Luteola) and three sections (Leucocoreseda, Reseda, Phyteuma) [17]. This subgeneric classification is based on morphological differences, petal structures, number of carpels, placental positioning, seed size, and testa ornamentations [18,19]. This classification takes its final shape according to molecular data [20].

Reseda is the only genus of the Resedaceae family in Turkey, including 18 indigenous taxa of which ten are endemic, whereas nine of them found only in Turkey [17,18,21-33]. The taxa included in the current study reflect and represent the variety of subgeneric classification of the *Reseda* species in Turkey.

Seed collection, storage, and preparation

Fruits of eight *Reseda* species were collected from different locations in Turkey between September 2013 and April 2015 (Table 1). The collections were conducted before the time of seed dispersal for each species. Fruits were collected from min. 10 individuals for each species. All these species are perennial herbs, and five are of conservation importance as they have been assigned to various threat categories by IUCN (Table 1). Fruits had been stored at room temperature and moisture in a dark place until the germination experiment was conducted in December 2015. Before the experiment, seeds were separated from fruit parts by hand and using sieves of different mesh sizes. Voucher specimens of plant taxa were deposited in the Hacettepe University Herbarium (HUB), Ankara, Turkey.

Preparation of smoke solutions

Aqueous smoke solutions were prepared by using the procedure described in Jäger et al. [34]. Four replicates of dry plant material composed of *Quercus coccifera* le-

Table 1. The list of *Reseda* species included in the study. The location (Loc; latitude, longitude, and altitude) and the date (Date) of seed collection, IUCN threat category (TC), phytogeographic region (Phyto; Med: Mediterranean, E.Med: Eastern Mediterranean, M.Med: Mountain Mediterranean, Ir-Tur: Irano-Turanian, Eux: Euxine hinterland, Cosm: Cosmopolitan), seed surface ornamentation (Surf; p: papillose, r: rugose, rl: rugulose, s: smooth), and seed size (SSize) are given for each taxa.

Taxon	Date	Loc	TC	Phyto	Surf	SSize (mm)
<i>R. alba</i> L.	Apr'14	37°51'46" N, 27°14'54" E 5 m	LC	Med	p	1.0-1.5 × 1.0
<i>R. anatolica</i> (Abdallah & de Wit) Snogerup & Snogerup	Jun'13	40°57'30" N, 33°40'21" D 1027 m	LC	Eux	r	2.0-2.2 × 1.5-2.0
<i>R. aucheri</i> Boiss. ssp. <i>rotundifolia</i> (Kotschy ex Müll. Arg.) Rech. f.	Sep'13	37°42'46" N, 41°24'41" E 500-700 m	VU	Ir-Tur	rl	0.8 × 0.5
<i>R. coodei</i> Hub.-Mor.*	Jul'14	37°24'10" N, 34°18'41" E 1500 m	CR	M.Med	r	2.5-3.0 × 2.0
<i>R. globulosa</i> Fisch. & C. A. Mey	Jul'14	40°45'36" N, 41°47'74" E 670 m	EN	Ir-Tur	s	2.0 × 1.0
<i>R. luteola</i> L.	Jun'13	40°57'30" N, 33°40'21" E 1027 m	LC	Cosm	s	1.0 × 0.5-0.8
<i>R. minoica</i> Martín-Bravo, P. Jiménez- Mejías	May'14	36°46'98" N, 34°28'00" E 330 m	EN	Med	r	1.5-1.8 × 1.0-1.5
<i>R. orientalis</i> (Müll. Arg.) Boiss. ^Ω	Apr'15	37°2'46" N, 36°8'00" D 70 m	VU	E.Med	r	2.0-2.2 × 1.5-2.0

* Endemic to Turkey; ^Ω rare species.

aves, and small twigs (5 g for each replicate) were grounded into small pieces, placed in metallic containers, and heated in a temperature-controlled oven between 190-195°C for 30 min. Before placed in the oven, metallic containers were covered with an aluminum foil. In this way, containers were able to keep the smoke generated from the burnt plant material. After the treatment, 50 ml of distilled water was added to and mixed with the plant material in the container, and then was left for 10 min. This solution was filtered into a bottle to obtain the stock smoke solution. Later, one ml stock solution was diluted in 5 and 20 ml of distilled water to prepare lower concentrations of smoke solutions that will be used in the experiment (1:5 and 1:20 smoke solutions).

The germination experiment

For all species included in the study, a group of seeds placed into Eppendorf tubes including aqueous smoke solutions for 24 h (four replicate of 25 seeds for each smoke treatment). Another group of seeds were placed in the tubes including distilled water to serve as the control for smoke treatments. For heat shock treatment, we only used four species in which enough seeds were available, and a group of seeds placed into alumi-

num pockets were subjected to 100°C for 5 min. in a temperature-controlled oven. Heat shock treatments were separately applied to four replicates of 25 seeds for each species. Four replicates of non-treated seeds were also included in the study to serve as a control group for the heat shock treatment.

After the treatments were applied, seeds were sown into Petri dishes containing agar as a substrate. Then, the dishes were placed in a temperature- and light-controlled cabinet at 20°C under 12:12 h photoperiod (dark/light) conditions (Fig. 1). These incubation conditions are within the suitable range of many *Reseda* species [2,4,5]. Germinations were daily checked until germinations became infrequent in any treatment, then once for two or three days. In each check, germinated seeds were counted and removed from the Petri dishes. At the end of the experiment (40 days after the incubation was initiated), a cut test was performed on the seeds remained in Petri dishes to determine their viability. Seeds with firm, white, and healthy embryos were recorded as viable, and ones including rotten embryos as non-viable. Seeds without an embryo (i.e., empty seeds) were also recorded.

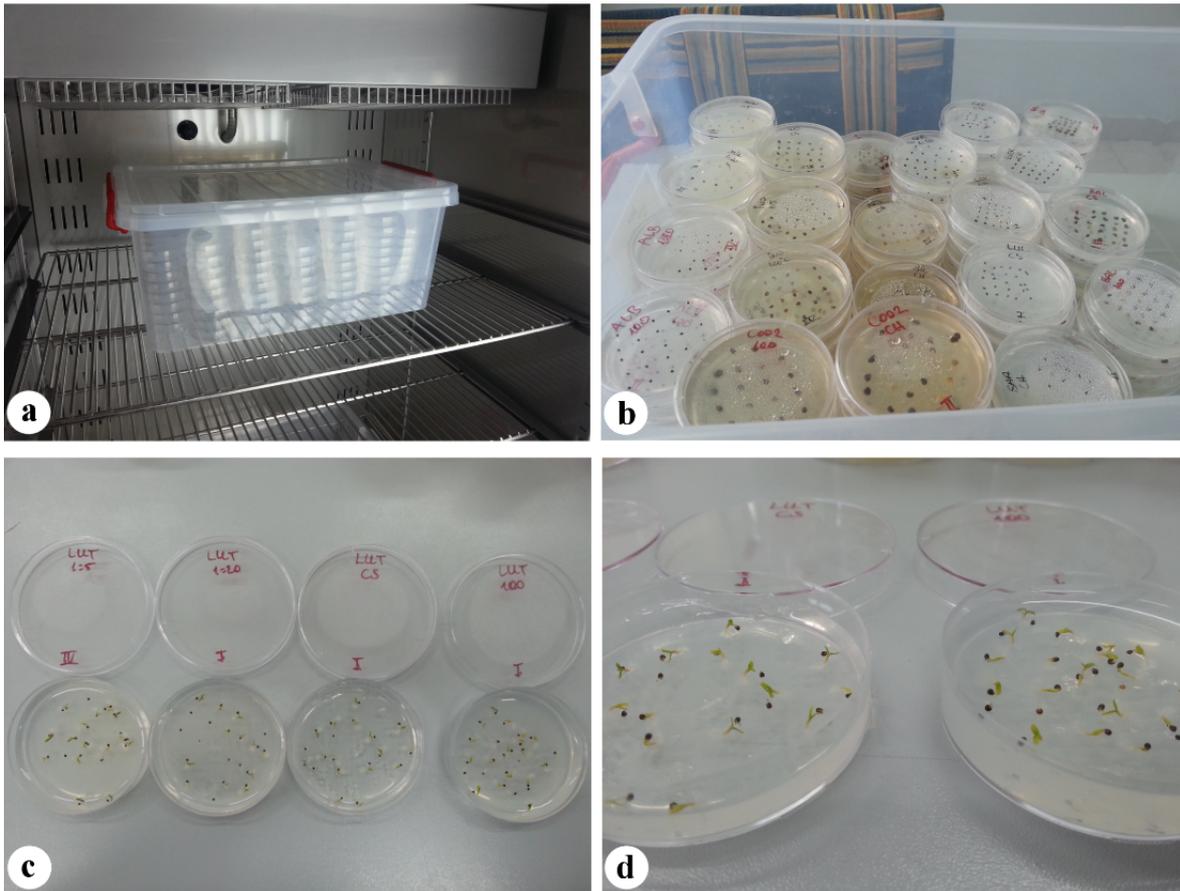


Figure 1. The germination experiment. The climate cabinet where incubation conditions were set (a), Petri dishes included the sown seeds (b, c), and the germinated *R. luteola* seeds (d) are shown.

Data analysis

For each replicate Petri dish, empty seeds were removed from the data to correct the initial seed number for each replicate. For each species, the final germination of each treatment was compared with their corresponding control (smoke control, CS or heat shock control, CH) using the analysis of deviance (a generalized linear model). In this analysis, we considered the germinated versus non-germinated seeds in each replicate, and therefore assumed a binomial error distribution [35,36]. The statistical analysis was performed on R software [37].

RESULTS and DISCUSSION

Germination in *Reseda* species included in the study were variable in the control, as three had very low germination (< 4%, i.e., high dormancy level), one had very high germination (> 85%, i.e., low dormancy level), and others were found in-between these extremes (Table 2).

Germination percentage slightly improved in *R. anatolica*, *R. coodei*, *R. globulosa*, and *R. orientalis* in, at least, one of the smoke treatments. However, these improvements were not statistically significant (Table 2), but the more pronounced increase in *R. anatolica* (from 3 to 11%) was critically significant ($P = 0.055$, Table 2). On the other hand, the germination in remaining *Reseda* species were negatively affected from the smoke treatments (*R. aucheri* ssp. *rotundifolia*, *R. luteola*, and *R. minoica*), and the reduction in germination percentage was statistically significant in *R. luteola* ($P = 0.007$ in 1:5, and $P = 0.006$ in 1:20 treatment; Table 2). Heat shock treatment did not affect the germination in three of four species tested (Table 2). On the other hand, heat shock application significantly increased germination percentage in comparison to the control in *R. luteola* ($P = 0.046$; Table 2).

Table 2. Mean germination percentages (\pm SE) in smoke (1:5 and 1:20) and heat shock treatments (100°C 5') and corresponding controls (CS and CH) for each *Reseda* species included in the study. The significance of the comparison of germination percentage in each treatment with the corresponding control is given (ns, not significant; † 0.10 < P < 0.05; *P < 0.05; **P < 0.01). "-" means that the treatment could not be applied due to the lack of enough number of seeds.

Taxa	Smoke experiment			Heat shock experiment	
	CS	1:20	1:5	CH	100
<i>R. alba</i>	1.0 \pm 1.0	2.0 \pm 1.1 ns	1.0 \pm 1.0 ns	0	1.0 \pm 1.0 ns
<i>R. anatolica</i>	3.4 \pm 2.2	2.0 \pm 1.2 ns	10.7 \pm 3.7 †	1.2 \pm 1.2	2.1 \pm 1.2 ns
<i>R. aucheri ssp. rotundifolia</i>	50.4 \pm 12.3	31.0 \pm 7.4 †	40.0 \pm 7.0 ns	55.2 \pm 6.5	50.0 \pm 9.6 ns
<i>R. coodei</i>	1.6 \pm 1.4	5.3 \pm 1.2 ns	5.7 \pm 1.2 ns	-	-
<i>R. globulosa</i>	35.4 \pm 7.5	37.4 \pm 8.9 ns	45.9 \pm 7.0 ns	-	-
<i>R. luteola</i>	91.0 \pm 3.3	76.2 \pm 2.1 **	76.9 \pm 8.2 **	86.9 \pm 1.5	95.6 \pm 0.2 *
<i>R. minoica</i>	58.0 \pm 8.7	51.6 \pm 5.6 ns	35.5 \pm 14.3 ns	-	-
<i>R. orientalis</i> ^{&}	10.7 \pm 10.7	33.0 \pm 4.5 ns	-	-	-

& Two replicates were used.

As far as we know, we obtained the first germination results for all the species we included in the study, except *Reseda luteola* [6,8]. Moreover, our study provides the first results of the germination response of the studied species to smoke. Germination inhibition by smoke was previously found in *R. lutea* [9], and our findings on *R. aucheri ssp. rotundifolia*, *R. luteola*, and *R. minoica* support this result. On the other hand, our results also show that germination in some *Reseda* species can be improved by the application of aqueous smoke solutions. Our study indicates that there is interspecific variability in *Reseda* regarding the germination response to smoke.

Our record for *R. luteola* is the first one for heat shock-improved germination in any *Reseda* species so far tested [8,9]. However, this result contradicts with that of Luna et al. [8], in which found no germination response of *R. luteola* to various heat shock temperatures. This difference may be due to the different light conditions applied in two studies (full dark in [8], and 12:12 h pho-

toperiod in the present study). Indeed, *R. luteola* germinate only in light conditions, but not in darkness [6]. Although we detected a substantial variability in dormancy levels among the studied species, we will not speculate about their dormancy status since we only included one temperature and light condition in our study.

In conclusion, there was no consistent germination response to smoke or heat shock in *Reseda* species studied, but we observed a substantial variation among species. Our study is the most comprehensive germination study tested germination properties of several *Reseda* species in relation to fire-related cues (i.e., heat shock and smoke) and provides the first germination results on seven *Reseda* species. However, more detailed studies including more species and treatments are required to fully understand the germination patterns in the genus *Reseda*.

Acknowledgments

We thank B. Özüdoğru, G. Zare, A. Dođru-Koca, Ç. Özenirler, H. Altınözlü, Ö. Çam, C.B. Kapçak, Z.Z. Acar, H. Gülcan, Y. Ilki, and Şinasi and Şeyda Çilden for their help in fieldworks between 2013 and 2015. The fieldwork of the study was financially supported by the Hacettepe University Scientific Research Unit (project no: 013D04601003). The laboratory experiment was performed at the Functional Ecology Lab., Hacettepe University. This study was part of E. Çilden's Ph.D. dissertation submitted to Hacettepe University. The authors declare that they have no conflict of interest.

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