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RESEARCH ARTICLE

Effect of heat shock on the germination of seeds of the species *Acacia senegal* L. and *Acacia seyal* Del. from sub-Saharan Africa (Ethiopia)

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Abstract

Aim of the study: Understanding post-fire germination of tree species in arid and semi-arid zones of sub-Saharan Africa.

Area of study: Ethiopian *Acacia senegal* L. and *Acacia seyal* Del. forests.

Material and methods: Seeds were subjected to heat shocks at combinations of four temperatures (60º, 90º, 120º and 150ºC) and three exposure times (1, 5 and 10 minutes). A control was also included, resulting in a total of thirteen treatments. After the application of the heat shocks, the viability of no germinated seeds was assessed after immersion in a Tetrazolium solution. A mixed and a logistic model were used to analyse the influence of heat shock on germination.

Main results: Results showed that germination depended on the species, the heat shock treatment and their interaction. Both species showed similar germination results at temperatures below 90ºC in all exposure times, however, germination in *Acacia senegal* was statistically higher in most of the heat shocks. On the other hand, germination probability decreased in both species, when the exposure time increased, although with a different behaviour. In 1 minute of time of exposure, the germination probability was higher than 60% in the two species throughout the temperature range. However, at 5 minutes of time and temperature smaller than 90°C, the probability of germination was higher than 70% in *A. senegal* and 50% in *A. seyal*. Although germination in both species was impacted by the different heat shocks, non-germinated seeds were viable.

Research highlights: This paper showed, according to these results, that heat shock would negatively influence the regeneration of both species, and especially for *A. seyal*.

Additional keywords: germination, *Acacia*, heat shock, logistic model.

Authors´ contributions: Amelework Kassa carried out the methods section. Celia Herrero summarized the results. Both wrote the manuscript. Then Kassa and Herrero have contributed equally to the paper. Valentín Pando performed the statistical analysis. Felipe Bravo and Ricardo Alía conceived the study and helped to draft the manuscript.

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Introduction

Fire is one of the most important disturbances in forest ecosystems (Bond & Keeley, 2005; Pausas & Keeley, 2009). It causes changes in forest structure, biomass, or species composition because of different mechanisms involving adaptive or post fire responses. Some studies, however, have reported the positive effect of fire in certain ecosystems, due to the subsequent increase in seedling density (Gashaw & Michelsen, 2002; Walters *et al.*, 2004).

Forest fires are a constant threat in arid lands because they endanger ecological niches with high biodiversity (Danthu *et al.*, 2003; Savadongo *et al.*, 2007). The post-fire response of the vegetation depends on many factors, including the pre-fire condition of the species, environmental and ground temperatures, fire intensity, ash concentration, nutrient balance or the animal populations associated with the forest ecosystems (Gashaw & Michelsen, 2002; Núñez *et al.*, 2003).

In arid environments, fire tolerance is identified as one of the main factors that controls the distribution of *Acacia* species (Burke, 2006). Specifically, different adaptive mechanisms to forest fires have been identified. So, among others, heat-shock triggered germination (e.g. hard seed structures in *Fabaceae* family make the seed impermeable to water until being scarified by heat or other abrasive agent), or germination triggered by combustion chemicals (Keeley *et al.*, 2011). On the other hand, seed dormancy, a very common feature in many species of the *Fabaceae* family, derived in many occasions of seed coat structures, is a strategy to ensure survival in adverse conditions (Danthu *et al.*, 2003). Seed dormancy can be interrupted by several methods (Teketay, 1996; Baskin & Baskin, 1998; Tigabu & Odén, 2001) such as heat shock, scarification methods, or ingestion by herbivores or birds (Miller, 1995; Baskin & Baskin, 1998; Razanamandranto *et al.*, 2004).

Previous studies have analyzed the effect of heat shock on seed germination in forest species in arid zones of Africa (Teketay, 1996; Sy *et al.*, 2001; Gashaw & Michelsen, 2002; Danthu *et al.*, 2003; Zida, 2007; Maraghni *et al.*, 2010). In *Acacia* spp., Teketay (1996) tested different heat shocks (60ºC, 80, 100ºC during 15, 30 and 60 minutes) and showed that once the dormancy in leguminous species with hard seed coats was broken, the seeds germinated in a wide range of temperatures. However, temperatures higher than 100ºC and reduced exposure time was not checked by Teketay (1996), that could mimic fast fires, where different temperatures affect seeds for a short period of time. This information is essential for evaluating the effect of fire on forest regeneration, and for knowing the adaptive significance of seed dormancy in relation to forest fires in these ecosystems.

The African savannah is a good example to study those arid and fragile ecosystems to present periodic droughts and the increase of the over-exploitation of forest resources (Malagnoux *et al.*, 2007). In particular, the Sudanese savannah, situated in the northern hemisphere of Africa, is an ecosystem with vast open land and few tree species. It covers 5.25 million km^2, from Senegal to the Ethiopian Highlands. Leguminous species from genus *Acacia, Boswellia* and *Comiphora* are representative of this ecosystem (Danthu *et al.*, 2003; Eriksson *et al.*, 2003; Kulkarni *et al.*, 2007). They provide timber and non-timber products, such as firewood, coal, fodder, wood for fences and agricultural implements, natural gums, frankincense, myrrh and medicines (Loth *et al.*, 2005). Nonetheless, 25-50% of the area is affected by fire, caused by humans or other natural climate factors (Savadogo *et al.*, 2007; Zida, 2007), and therefore it is important to understand the biological and environmental factors that affect seed germination. This knowledge allows us to ensure recruitment (Sy *et al.*, 2001; Gashaw & Michelsen, 2002), or to respond after wildfire disturbances.

Species in the *Aculiferum* subgenus of *Acacia* genus, which includes *A. senegal*, show a soft seed covering (Danthu *et al.*, 2003). That means that they are sensitive to high fire intensities and prolonged exposure time. Seeds from species in the second subgenus, *Acacia*, which includes *A. seyal*, have a hard and impermeable outer cover that is resistant to high temperature heat shocks (Danthu *et al.*, 2003). *Acacia senegal* L. and *A. seyal* Del., within this ecosystem, are two socioeconomically important species with contrasting seed coat structures. These differences make them suitable to test the hypothesis that species in arid environments, with thick coats, have deeply dormant seeds and that frequency and type of forest fires (e.g. exposure and intensity), influence differently the dormancy of the seeds depending on the seed coat characteristics. To address this hypothesis, the aim of this paper was to assess the effect of different heat shock treatments (differing in temperature and exposure time) on the germination of the *Acacia senegal* and *A. seyal*. The results could provide information of great relevance for sustainable forest management and conservation of the species with better silvicultural practices during the regeneration period or in post-fire regeneration treatments.

Material and methods

Material and Experimental design

Seeds were collected from representative, mature and physically well grown trees in the Rift Valley in Ethiopia in January of 2010. Seeds from *A. senegal* were collected from Langanno (7º26'N-38º47'E), and *A. seyal* from the Abijata-Shala National Park (7º32'N - 38º40'E) in stands with a density of 40 trees/ha by personnel of the Ethiopian Environment and Forest Research Institute (EEFRI). The altitude of both areas ranged from 1550 to 1570 m asl (Argaw *et al.*, 1999). Seeds were kept at a constant temperature (4ºC) in a bag for two months to keep the conditions collected by EEFRI until the time of the experiment.

The seeds were exposed to twelve heat shock treatments, consisting of 4 different temperatures (60ºC, 90ºC, 120ºC and 150ºC) and 3 exposure times (1, 5 and 10 minutes). A control, without heat shock treatment, was considered as an additional treatment. Heat shocks were performed in a hot dry oven. So, the experiment consisted of thirteen experimental treatments (4 x 3 heat shock combinations and the control). The range of temperatures chosen is the standard in this type of studies (Núñez & Calvo, 2000). Regular spacing temperatures is common to facilitate the statistical analysis, while the temperature range includes a lot of possibilities (Trabaud, 1979) contrasted with studies of prescribed fires. Similar temperatures were reached in the soil (Hernando *et al.*, 2000), and in the crown, where the maximum reached temperatures did not exceed the 140ºC (Vega, 2000).

Four replicates (consisting of 25 seeds) for each treatment were established following a randomized complete block design, were the experimental unit was a 9-cm Petri dish with two filter paper discs dampened with sterilized distilled water. In total, the experiment was composed of a total of 2600 seeds (1300 by species and 100 by treatment and species).

The germination test was performed in a germination chamber under constant conditions (30ºC, 80% of relative humidity and 12h light/12h dark of photoperiod) for 45 days, following the International Seed Testing Association [ISTA] rules (Don, 2013; ISTA, 2015) and adapting for dry areas. In the case of *A. seyal* seeds, after the application of heat shock, they were immersed in boiling water to interrupt the dormancy for 24 h (Larsen, 1964; Teketay, 1996).

Every three days, the germinated seeds were counted and removed and the filter paper was moistened. A seed was considered as germinated when the radicle had emerged at least 2 mm from the testa (Come, 1970). From each Petri dish, accumulative germination was determined as the number of seeds germinated during the entire experiment. The proportion of germinated seeds was defined as the accumulative germination with respect to the total for each combination of block and plot (number of seeds germinated/25 seeds).

At the end of the experiment, the viability of no germinated seeds was assessed by counting live and dead seeds after immersion in a Tetrazolium solution (Paynter & Dixon, 1990). This solution is a biochemical method, which determines seed viability and is used by ISTA.

Statistical analysis

The proportion of germinated seeds was transformed into the arcsine of the square root of the proportion of germinated seeds, which is a very common operation for stabilizing the variance of observed data in experiments of this type (Núñez & Calvo, 2000; Herrero *et al.*, 2007). This transformation was used to correct the statistics' assumptions underlying the normality of the linear model (Montgomery, 1997).

A mixed model was applied in order to detect significant differences in the germination at the end of the experiment as a function of species (*Acacia senegal* and *Acacia seyal*), heat shock (13 treatments: control and 12 combination of four temperatures and three exposure time) and the interaction between the two factors. There were 52 observations for each species, for a total of 104. These observations corresponded to the four replicate by each heat shock by the two species (4 replicates*13 heat shocks*2 species=104). The model was expressed as follows [Eq. 1]:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\,\beta_{ij} + \boldsymbol{\varepsilon}_{n\,(ijk)} \qquad \text{[Eq. 1]}$$

where:

y_{ijk}: Arcsine of the square root of the proportion of germinated seeds by species *i* with the heat shock *j* in the replicate *k* (1 to 4); μ: General mean effect; α_i: The effect of the species factor *i* (1= *A. senegal*; 2= *A. seyal*); β_j: The effect of heat shock *j*, (60ºC_1min; 60ºC_5min; 60ºC_10min; 90ºC_1min; 90ºC_5min; 90ºC_10min; 120ºC_1min; 120ºC_5min; 120ºC_10min; 150ºC_1min; 150ºC_5min, 150ºC_10min; control [30ºC_0]); $\alpha\beta_{ij}$: The effect of the interaction between species *i* and heat shock *j*, and $\varepsilon_{n\,(ijk)}$: experimental error, where $\varepsilon_{ijk} \rightarrow N(0, \sigma_i^2)$.

A variance for each species was determined by the mixed model. On the other hand, Dunnett's test was used to compare the germination of each treatment with the control and Tukey's test was used for multiple comparisons of the treatments. The data were fitted using SAS 9.4 Proc Mixed software (SAS Institute Inc., 2017).

Logistic regression was used to model the germination probability as a function of the exposure time and the temperature at which the seeds were subjected. The logistic model was expressed as follows [Eq. 2]:

$$p = \frac{1}{1 + e^{-z}} \qquad \text{[Eq. 2]}$$

where: *p*: probability of germination, *z*: a linear function of the explanatory variables considered in the analysis.

The coefficients of the z function were obtained using maximum likelihood method. The full model, including independent and quadratic terms, and all reduced models were tested. The residual mean deviation was used to scale the standard errors and test statistics in the fitting process. The models were evaluated using the value of the statistical parameters AIC and –2 log likelihood. In order to choose the best model to explain the germination process, score selection variables was used at the beginning of the process to know the behavior of the variables in the fitting process. After this, other different models were tested to introduce combinations of different variables. Some of the candidate models are shown in Tab. 1. The final model was chosen taking

Table 1. Different candidate logistic models, tested in the fitting process, for *A. senegal* and *A. seyal* species.

Model	Statistical parameters			Variables											
	AIC	SC	-2LL	Intercept	S	T	t	T^2	t^2	T*t	S*T	S*t	S*T^2	S*t^2	S*T*t
1	222.556	292.916	198.556	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
2	244.563	273.880	234.563	**	ns	-	**	-	-	***	-	*	-	-	-
3	203.363	232.680	193.363	**	-	ns	-	-	-	***	-	*	-	-	-
4	233.663	268.843	221.663	**	-	-	ns	-	ns	**	-	ns	-	-	ns
5	226.500	249.953	218.500	***	-	-	-	-	**	***	-	-	-	-	*
6	232.571	267.751	220.571	**	-	-	*	-	-	**	ns	ns	-	-	ns
7	184.017	213.323	174.007	**	ns	-	-	*	-	**	-	-	-	-	ns
8	196.91	226.227	186.911	*	ns	-	-	**	-	-	ns	-	-	-	*
9	240.964	270.280	230.949	**	-	-	*	-	-	**	-	ns	-	-	ns
10	**190.118**	**213.571**	**182.118**	***	**-**	**-**	**-**	*	**-**	***	**-**	**-**	**-**	**-**	*

Note: AIC: Akaike information criterion; SC: Schwarz Criterion; S: Species; T: temperature; t: Time; T^2: Temperature^2; t^2: Time^2; Significant levels: ***: (p<0.001); **: (p<0.01); *: (p<0.05); ns: no significant; - means variables not included in the model. In bold, the chosen model.

account the smallest values of statistical parameters and the best combination of significant variables with a reasonable biological interpretation. The data was fitted using SAS 9.4 Proc Logistic software (SAS Institute Inc., 2017).

Results

The mixed model (Tab. 2) showed that the proportion of germinated seeds depended on the species (Pr>F=0.0004), applied heat shock (Pr>F=<0.0001) and the interaction between both factors (Pr>F=0.0007). So, heat shock treatments in each species showed different impact on the proportion of germination. Germination was not different to the control for *A. senegal* under exposure times of 1 minute for all the temperatures, or for temperatures smaller than 90º for exposure time higher than 1 minute (Figure 1a). However, for temperatures higher or equal to 120ºC and time of exposure higher or equal to 5 minutes, germination was reduced significantly in comparison to the control. For 5 and 10 minutes and temperatures higher than 120ºC, the seeds did not germinate. On the other hand, in the case of *A. seyal*, the germination was reduced respect to the control, in exposure times of 1 minute and temperature equal to 90ºC (Figure 1a), and when the exposure time was higher than 5 minutes for all the temperatures. Tukey's test showed (Figure 1b) that the germination of *A. seyal*, was smaller than *A. senegal* in the most of the heat shock treatments with time exposure higher than 5 minutes. Higher time exposures decreased the germination for all the temperatures in comparison to the same heat shock in *A. senegal*. Tab. 3a showed that, in *A. senegal*, hardly non-germinated seeds were viable. In contrast, *A. seyal* showed a high proportion of viable non germinated seeds, even under high exposure times and temperatures (Tab. 3b). At the highest exposure time (10 minutes) and higher temperatures (120 and 150ºC), there were a different percentages of viable seeds, although the percentage of germination was clearly reduced. The results of control were 59 Germinated and 2 Viable seeds in *Acacia senegal* (G+V=61) and 70 Germinated and 6 Viable in *Acacia seyal* (G+V=76).

Table 2. Type 3 test for mixed effects in the mixed model applied.

Effect	Num df	Den df	F-value	P>F
Species	1	78	13.58	0.0004
Heat shock treatment	12	78	42.38	<0.0001
Species*Heat shock treatment	12	78	3.31	0.0007

Note: Num df, Den df Numerator and denominator degrees of freedom, respectively; F-Value value of the F statistic; Pr>F p-value associated with the previous F-statistic.

The logistic model chosen to explain the effects of *Acacia* species probability of germination, revealed that all variables and their interactions exert significant influences (Pr>χ^2 <0.05) (Tab. 4) on the germination of the species studied. The logistic regression model provided additional information on the differences between species and the influence of the different factors considered in the study on the germination probability. According to the logistic model, the probability of germination in *A. senegal* (Fig. 2) decreased as temperature increased, and this occurs in a similar way for all three exposure times. Germination probability remained above 60% up to 120ºC and above 50% in the

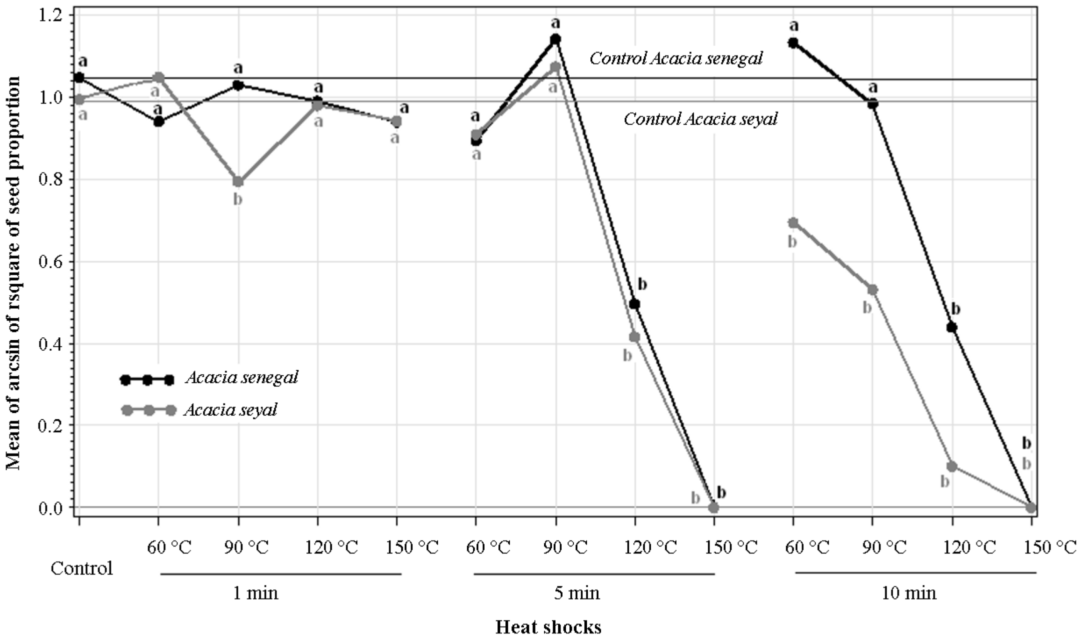

Figure 1a. Mean of the arcsin of the square of seed proportion obtained in the different heat shocks respect to the control (Dunnet's test). Letter "a" shows not significant difference in germination respect to the control; letter "b" shows significant differences respect to the control.

highest temperatures at 1 minute exposure. In *A. seyal* (Fig. 2), the decrease in probability of germination was slightly more pronounced as temperature increased. Although the behaviour was consistent in both species, with exposure time of 1 min, the probability was reduced to 60% at 120°C approximately. While in *A. senegal*, the differences were observed from 60ºC among the exposure times and were pronounced at 90ºC, in *A. seyal*, differences were observed from 60ºC.

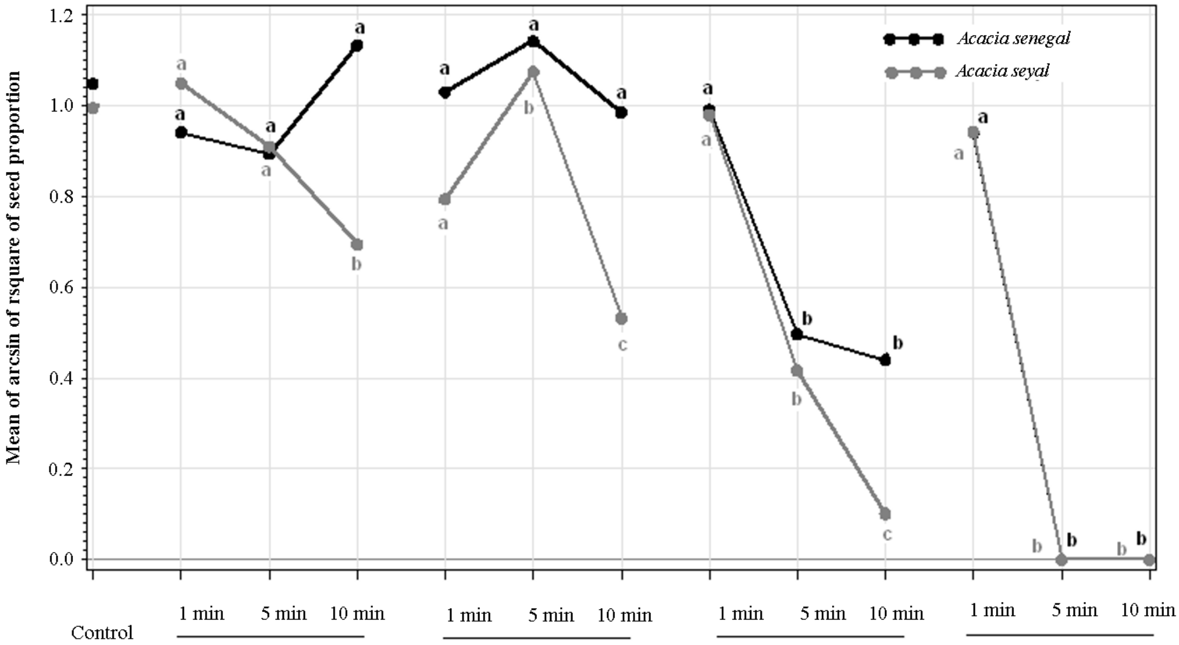

Figure 1b. Mean of the arcsin of the square of seed proportion obtained in the different heat shocks (Tukey's test). Different letters showed significant differences in germination after the heat shocks.

Table 3a. Percentage of germinated (G) and viable (V) seeds of *A. senegal* at the range of temperature studied at exposure time of 1, 5 and 10 minutes.

Temperature (ºC)	Time of exposure								
	1 minute			5 minutes			10 minutes		
	G(%)	V(%)	G+V(%)	G(%)	V(%)	G+V(%)	G(%)	V(%)	G+V(%)
60	65	2	67	59	4	63	77	3	80
90	73	4	77	82	0	82	69	6	75
120	69	0	69	23	4	27	20	1	21
150	65	5	70	0	0	0	0	0	0

Note: G (Total of Germinated) and V (Viable) seeds estimated after a Tetrazolium test.

Discussion

This work has analyzed the effects of heat shock treatments on the germination of two important species of arid ecosystems. Both species, under extreme conditions (exposure time of 5 minutes and temperatures of 150ºC) did not germinate, however, the species differed in their behaviour in the other range of temperatures. *A. senegal* was more tolerant to heat shock treatments because showed higher germination than *A. seyal*. This information is essential to improve the sustainable forest management prescriptions to the conservation of these fragile ecosystems in relation to forest fires.

Fire has been reported as a tool for stimulating seed germination (Núñez & Calvo, 2000) that also serves to eliminate competing species (Núñez *et al.*, 2003; Zuloaga-Aguilar *et al.*, 2011). However, under arid conditions, fire characterized by high temperature or prolonged exposure time could cause lower survival or even prevent germination in *Acacia* species (Danthu *et al.*, 2003; Walters *et al.*, 2004; Dayamba *et al.*, 2008; Zuloaga-Aguilar *et al.*, 2011), depending on the seed-coat characteristics.

In this study, the effects of several heat shock treatments on germination were evaluated for *Acacia senegal* and *Acacia seyal* species from the Sudanese savannah in the semi-arid region of Southwest Ethiopia. Our results confirmed that the proportion of germinated seeds in both species was equal to or above 60% at 1 minute exposure to all temperatures. On the other hand, the probability of germination of the two species also decreased when the temperature increased, being more pronounced in *A. seyal*. The logistic model provided information regarding how the probability of germination were affected by differences between the species and the heat shocks considered. Additionally, the model allowed us to introduce quadratic terms into the process in order to test how they would affect germination probabilities. In our case, the square of the temperature proved to be an explanatory variable, indicating the asymptotic limit of the germination capacity of these two species (Tab. 4). Logistic model added scientific advances to the germination in arid environments. Our findings could allow stakeholders to fit germination predictive models to wider areas in Ethiopia. This is a highlight result of this work because logistic model is not a method regularly used in similar studies focused on germination of Ethiopian trees and shrubs.

In *A. seyal*, our results differed from those obtained by Gashaw & Michelsen (2002). These authors observed that the germination was smaller than 30% over the entire range of studied temperatures (20, 60, 90, 120, 150, 200ºC) at 1 and 5 minutes of exposure time. These differences may be related to the characteristics of the scratch method employed [hot water for 24 hours in our study *vs*. 1 to 3 hours by Gashaw & Michelsen (2002)], and the germination conditions [germination chamber with constant conditions of temperature, humidity

Table 3b. Percentage of germinated (G) and viable (V) seeds of *A. seyal* at the range of temperature studied at exposure time of 1, 5 and 10 minutes.

Temperature (ºC)	Time of exposure								
	1 minute			5 minutes			10 minutes		
	G(%)	V(%)	G+V(%)	G(%)	V(%)	G+V(%)	G(%)	V(%)	G+V(%)
60	80	8	88	70	10	80	40	18	58
90	60	33	93	80	11	91	25	46	51
120	70	4	74	20	15	35	5	36	41
150	68	7	75	0	32	32	0	42	42

Note: G (Total of Germinated) and V (Viable) seeds estimated after a Tetrazolium test.

Table 4. Chosen logistic model parameters adjusted for *A. senegal* and *A. seyal* species.

Parameter	Estimate	Standard error	Wald Index	P> χ^2
Independent term	1.73620	0.370200	21.9932	<0.0001
Temperature^2	-0.00007	0.000026	6.3381	0.0118
Time* temperature	-0.00301	0.000714	17.7478	<0.0001
Species*time* temperature	0.00123	0.000685	3.2241	0.0426

and photoperiod *vs*. soil to simulate field conditions]. Therefore, our conditions that did not depend on local conditions (humidity and temperature of the soil). On the other hand, the prolonged scarification methods, allowed us to obtain a greater germination under control treatment, and therefore, a better comparison of these results to the different heat shock treatments. Gashaw & Michelsen (2002) reported no germination under heat shock with temperatures higher than 120ºC -for time of exposure greater than 1 minute- and with temperatures higher than 90ºC -for time of exposure greater than 5 minutes-. They did not consider time exposure higher than 5 min, however, our results showed that if temperature was smaller than 90ºC, the proportion of germination was similar statistically to the control in *Acacia senegal*. The increase of germination with higher exposure time obtained in this paper may be caused by a secondary seed dormancy, as the Tetrazolium test confirmed that some of these seeds were viable (Tab. 3a,b). In this paper, a strengthen statistical analysis was carried out respect the previous researchers (Teketay, 1996; Gashaw & Michelsen, 2002). A linear mixed model, which consider the experimental error in the fitting process, added robustness to the obtained results. In addition, with the Dunnet's test, additional information was obtained about the germination in the treatments respect to the BAU germination (control treatment). These results allowed us to complete the information obtained by Tukey's tests.

Moreover, the results of the viability of seeds improved our knowledge about the consequences of fire in arid species. The germination after fire depended on the intensity of heat shock and also limited to seed survival. Heat shocks could not scarify the integument of the seeds without resultant lethal damage to their embryos. Our findings showed that they could retain viability instead of dying. In these sense, further analysis of Germinated+Viable seeds and Germinated given Germinated+Viable might be useful. In this regard, in *A. seyal*, dormancy would be a strategy or an adaptation to environmental perturbations. This result was agreed with Danthu *et al.* (2003), who observed

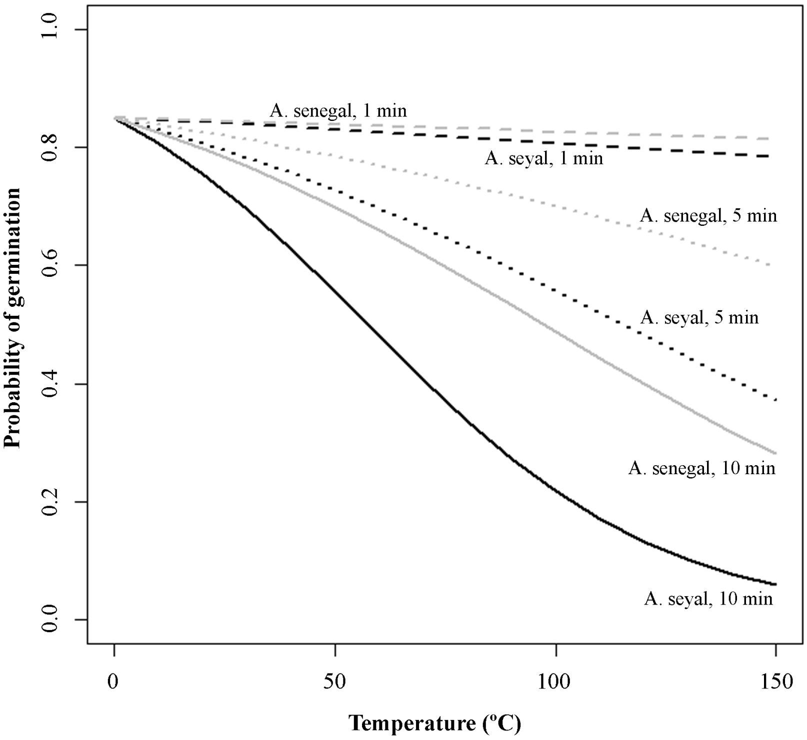

Figure 2. Logistic model for the probability of germination after different heat shock treatments in *A. senegal* and *A. seyal*.

that the percentage of viable seeds at temperatures between 232-241°C was about 80%.

On the other hand, in *Acacia senegal*, our results agreed with those obtained by Danthu *et al.* (2003), who obtained germinations higher than 90% in this species at two fire intensities. The first intensity simulated by Danthu *et al.* (2003) consisted of fuel load about 100g/m^2 that reached temperatures over 154-160°C at 1 m above the ground. The second, 500g/m^2 that reached 93-99°C at 3 m above ground. Therefore, our results could be similar than those obtained in studies that evaluate germination at 1 m above ground.

In many species, a similar germination under moderate heat shock treatments has also been reported. A threshold of temperatures/times of exposures were found in species adapted to forest fires, such as *Genista florida* (Tárrega *et al.*, 1992), *Pinus sylvestris* (Núñez & Calvo, 2000), *C. striatus, G. berberidea* and *G. triacanthos* (Rivas *et al.*, 2006), and *Pinus pinaster* (Herrero *et al.*, 2007).

The differences found in this paper among the two species, reflect an important evolutionary process under arid conditions, related to seed coat characteristics and dormancy of seed. The successful establishment of seedlings depends on long-term seed survival and dispersion strategies (Gashaw & Michelsen, 2002; Danthu *et al.*, 2003; Eriksson *et al.*, 2003; Zida, 2007). In this paper, results showed the seed survival of *A. seyal* after application of heat shock treatments. Although *A. senegal* showed higher germination after different heat shocks, *A. seyal* showed poor results in the treatments respect to the control, but viable seeds after heat shocks. It could be related to a secondary seed dormancy, but more studies would be needed to confirm this fact as it would depend on the scarification method applied. Previous researchers such as Teketay (1996), Danthu *et al.* (2003) and Villalobos *et al.* (2002) showed that chemical scarification with Sulphuric acid or mechanical treatment improved the germination under high temperatures.

In this study, one only seed lot origin from each species was used in order to target the germination in the different heat shock treatments with respect to the control. In this sense, one of the limitations of this study was the lack of assessment the intra-specific variation on the tolerance to heat-shock treatments. However, the pre-germination treatments allowed us to obtain a higher germination under control conditions. These treatments could not affect the results because they mostly addressed changes respect the control for the heat-shock treatments.

The combination of knowledge about the biology of a species allowed us to understand the functional characteristics and the evolution of a plant in different regions and ecosystems (Paula *et al.*, 2009). Models could simulate changes in plant community composition due to different scenarios. In fire-prone ecosystems, changes in fire regime may be more relevant to climate change (Paula *et al.*, 2009) which may affect the appearance biological, physical and chemical ecosystem, depending on the intensity, duration and the degree of destruction of the fire (Ursinoa & Rulli, 2010). In this sense, our results are very important for the best regeneration management or for the post fire regeneration treatment because, if an intense forest fire happens, additional treatments (e.g. seeding, planting, or selviculture treatments to increase the regeneration) could be beneficial due to the fire damage on the existing seed bank.

Several models have predicted an increase in the frequency and severity of natural disturbances such as forest fires, windthrows, etc., combined with significant drought episodes and an overall increase in temperature as a consequence of climatic change (IPCC 2007). In the Sudanese savannah, this is exacerbated by intense grazing, which creates temporal and spatial heterogeneity in the fuel load (Savadogo *et al.*, 2007). Our findings showed new data of germination in different fire simulations which could improve the silvicultural strategies designs. Therefore, it would be necessary to avoid prescribed burning in these areas as a method to control the vegetation or to improve the grazing quality in the regeneration areas of these forests, as they could cause a reduction in germination of the two species. Also, control of fuel in these areas would be necessary to reduce the probability of ground forest fires that would also affect the possibility of regeneration of these species. The logistic model could also be included in integrated models of silviculture and forest fires incidences to improve the sustainable management of these species.

Conclusions

In this paper the germination of two species of *Acacia* (*A. senegal* and *A. seyal*) from sub-Saharan Africa has been studied after applying different heat shocks. Differences have been found between the two species in their reaction to these treatments. In the two cases, under extreme conditions (exposure time of 5 minutes and temperatures of 150ºC) the two species did not germinate. But the species differed in their behaviour in the other range of temperatures. *A. senegal* was more tolerant to heat shock treatments because showed higher germination than *A. seyal*. However, *A. seyal* showed viable seeds after heat shocks. Non-germinated seeds were viable after heat shocks which is an important finding for the soil bank of this species. Furthermore, the logistic regression model showed the square of temperature as an explanatory variable, indicating the asymptotic limit germination capacity of these species, as well as the interaction between species exposure time and temperature. This information could serve to enhance forest regeneration by developing vegetation management strategies based on prescribed fires.

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References

Argaw M, Teketay D, Olsson M, 1999. Soil seed flora, germination and regeneration pattern of woody species in an Acacia woodland of the Rift Valley in Ethiopia. J Arid Environ 43: 411-435. https://doi.org/10.1006/jare.1999.0532

Bond WJ, Keeley JE, 2005. Fire as a global 'herbivore': The ecology and evolution of flammable ecosystems. Trends Ecol Evol 20: 387-394. https://doi.org/10.1016/j.tree.2005.04.025

Baskin CC, Baskin JM, 1998. Seeds. Ecology, Biology, and Evolution of Dormancy and Germination. Academic Press, San Diego, USA. 666 pp.

Burke A, 2006. Savanna trees in Namibia-Factors controlling their distribution at the arid end of the spectrum. Flora - Morphology, Distribution, Functional Ecol Plants 201(3): 189-201. https://doi.org/10.1016/j.flora.2005.06.011

Come D, 1970. Les obstacles a la germination. EDN Masson, Paris, France. 162 pp.

Danthu P, Ndongo M, Diao M, Thiam O, Sarr A, Dedhiou B, Vall AOM, 2003. Impact of bush fire on germination of some West African acacias. Forest Ecol Manag 173: 1-10. https://doi.org/10.1016/S0378-1127(01)00822-2

Dayamba SD, Tigabu M, Sawadogo L, Oden PC, 2008. Seed germination of herbaceous and woody species of the Sudanian savanna-woodland in response to heat shock and smoke. Forest Ecol Manag 256: 462-470. https://doi.org/10.1016/j.foreco.2008.04.051

Don R, 2013. ISTA Handbook on Seedling Evaluation, 3rd Edition. ISTA, Zurich, Switzerland. 130 pp.

Eriksson I, Teketay D, Granström A, 2003. Reponse of plant communities to fire in Acacia woodland and a dry Afromontane forest, southern Ethiopia. Forest Ecol Manag 177: 39-50. https://doi.org/10.1016/S0378-1127(02)00325-0

Gashaw M, Michelsen A, 2002. Influence of heat shock on seed germination of plants from regularly burnt savanna woodlands and grasslands in Ethiopia Plant Ecol 159: 83-93. https://doi.org/10.1023/A:1015536900330

Hernando C, Guijarro M, Díez C, 2000. Régimen térmico en troncos de Pinus pinaster: aplicación a las quemas prescritas. In: Actas de la Reunión sobre Quemas Prescritas. Lourizán 1998. Vega JA, Vélez R (eds.). pp. 75-89. Sociedad española de Ciencias Forestales, Lourizán.

Herrero C, San Martín R, Bravo F, 2007. Effect of heat and ash treatments on germination of Pinus pinaster and Cistus laurifolius. J Arid Environ 70: 540-548. https://doi.org/10.1016/j.jaridenv.2006.12.027

IPCC, 2007. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. EDN. Cambridge University Press, United Kingdom and New York. 996 pp.

ISTA, 2015. International Rules for Seed Testing Vol. 215, Introduction, i-1-6 (10). The International Seed Testing Association, Bassersdorf. https://doi.org/10.15258/istarules.2015.10

Keeley JE, Pausas JG, Rundel PW, Bond WJ, Bradstock RA, 2011. Fire as an evolutionary pressure shaping plant traits. Trends Plant Sci 16(8): 406-411. https://doi.org/10.1016/j.tplants.2011.04.002

Kulkarni MG, Sparg SG, Staden JV, 2007. Germination and post-germination response of Acacia seeds to smoke-water and butenolide, a smoke-derived compound. J Arid Environ 69: 177-187. https://doi.org/10.1016/j.jaridenv.2006.09.001

Larsen E, 1964. Germination response of Acacia seeds to boiling. Austral For Res 1: 51-53.

Loth PE, De Boer WF, Heitkonig IMA, Prins HHT, 2005. Germination strategy of the East African savanna tree Acacia tortilis. J Tropical Eco 21: 509-517. https://doi.org/10.1017/S026646740500252X

Malagnoux M, Sène EH, Atzmon N, 2007. Los bosques y el agua. Bosques, árboles y agua en las tierras áridas: un equilibrio delicado. Unasylva 229: 24-29.

Maraghni M, Gorai M, Neffati M, 2010. Seed germination at different temperatures and water stress levels, and seedling emergence from different depths of Ziziphus lotus. South African J Botany 76: 453-459. https://doi.org/10.1016/j.sajb.2010.02.092

Miller MF, 1995. Acacia Seed Survival, Seed-Germination and Seedling Growth Following Pod Consumption by Large Herbivores and Seed Chewing by Rodents. African J Ecol 33: 194-210. https://doi.org/10.1111/j.1365-2028.1995.tb00797.x

Montgomery DC, 1997. Design and Analysis of Experiments, fourth ed. Wiley, New York, USA. 478 pp.

Núñez MR, Calvo L, 2000. Effect of high temperatures on seed germination of Pinus sylvestris and Pinus halepensis. Forest Ecol Manag 131: 183-190. https://doi.org/10.1016/S0378-1127(99)00211-X

Núñez MR, Bravo F, Calvo L, 2003. Predicting the probability of seed germination in Pinus sylvestris L. and four competitor shrub species after fire. Ann. Forest Sci. 60: 75-81. https://doi.org/10.1051/forest:2002076

Paula S, Arianoutsou M, Kazanis D, Tavsanoglu Ç, Lloret F, Buhk C, Ojeda F, Luna B, Moreno-Ojeda F, Luna B, *et al.*, 2009. Fire-related traits for plant species of the Mediterranean Basin Ecol 90(5): 14-20. https://doi.org/10.1890/08-1309.1

Pausas JG, Keeley JE, 2009. A burning story: The role of fire in the history of life. BioScience 59: 593-601. https://doi.org/10.1525/bio.2009.59.7.10

Paynter BH, Dixon KW, 1990. Seed viability and embryo decline in Geleznowia verrucosa Turcz. (Rutaceae). Scientia Horticulturae 45:149-157. https://doi.org/10.1016/0304-4238(90)90077-R

Razanamandranto S, Tigabu M, Neya S, Odén PC, 2004. Effects of gut treatment on recovery and germinability of bovine and ovine ingested seeds of four woody species from the Sudanian savanna in West Africa. Flora 199: 389-397. https://doi.org/10.1078/0367-2530-00167

Rivas M, Reyes O, Casal M, 2006. Influence of heat and smoke treatments on the germination of six leguminous shrubby species. Internat J Wildland Fire 15: 73-80. https://doi.org/10.1071/WF05008

SAS Institute Inc., 2017. SAS/Stattm user's guide. Relase 9.4. Cary, N.C, USA. 432 pp.

Savadogo P, Savadogo L, Tiveau T, 2007. Effects of grazing intensity and prescribed fire on soil physical and hydrological properties and pasture yield in the savanna woodlands of Burkina Faso. Agriculture, Ecosyst Environ118: 80-92. https://doi.org/10.1016/j.agee.2006.05.002

Sy A, Grouzis M, Danthu P, 2001. Seed germination of seven Sahelian legume species. J Arid Environ 49: 875-882. https://doi.org/10.1006/jare.2001.0818

Tárrega R, Calvo L, Trabaud L, 1992. Effect of high temperatures on seed germination of two woody Leguminosae. Vegetatio 102: 139-147. https://doi.org/10.1007/BF00044730

Teketay D, 1996. Germination ecology of twelve indigenous and eight exotic multipurpose leguminous species from Ethiopia. Forest Ecol Manag 80: 209-223. https://doi.org/10.1016/0378-1127(95)03616-4

Tigabu M, Odén PC, 2001. Effect of scarification, gibberellic acid and temperature on seed germination of two multipurpose Albizia species from Ethiopia. Seed Sci Technol 29: 11-20.

Trabaud L, 1979. Etude du comportement du feu dans la Garrige de Chene kermes apartir des temperatures et des vitesses de propagation. Ann Sci Forest 36 (1): 13:38. https://doi.org/10.1051/forest/19790102

Ursinoa N, Rulli M, 2010. Combined effect of fire and water scarcity on vegetation patterns in arid lands. Ecol Mod 221: 2353-2362. https://doi.org/10.1016/j.ecolmodel.2010.06.018

Vega JA, 2000. Resistencia vegetativa ante el fuego a través de la historia de los incendios. In: La Defensa Contra Incendios Forestales: Fundamentos y Experiencias; Vélez R (Ed.), pp. 4.66-4.85. Mc Graw Hill Interamericana de España, S.A.U. Madrid.

Villalobos AE, Peláez DV, Bóo RM, Mayor MD, Elia OR, 2002. Effect of high temperatures on seed germination of Prosopis caldenia Burk. J Arid Environ 52: 371-378. https://doi.org/10.1006/jare.2002.1004

Walters M, Midgley JJ, Somers MJ, 2004. Effects of fire and fire intensity on the germination and establishment of Acacia karroo, Acacia nilotica, Acacia luederitzii and Dichrostachys cinerea in the field. BMC Ecol 4: 3-13.

Zida D, 2007. Impact of forest management regimes on ligneous regeneration in the sudanian savanna of Burkina Faso. Doctoral thesis. Faculty of Forest Sciences Department of Forest Genetics and Plant Physiology. Swedish University of Agricultural Sciences, Umea, Finland.

Zuloaga-Aguilar S, Briones O, Orozco-Segovia A, 2011. Seed germination of montane forest species in response to ash, smoke and heat shock in Mexico. Acta Oecologica 37: 256-262. https://doi.org/10.1016/j.actao.2011.02.009