The effects of constant temperatures, light and seed quality on the germination characteristics of *Agave americana*

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Abstract. The influence of constant temperatures, light and seed quality on *A. americana* seed germination was investigated with the objective of detailing the environmental control for germination in this species. The lower and upper temperature limits for germination (radicle emergence) and seedling production in the light (12 h d⁻¹) were between 11°C and 16°C, and 36°C and 40°C, respectively. Maxima for both these growth parameters were evident at temperatures between 21°C and 31°C, and the minimum mean time for germination (MTG) occurred at 26°C. Reducing photoperiod to ca. 1 min white light per day («nominal dark» treatment) did not significantly affect the level of germination (radicle emergence) or seedling production, but MTG was significantly increased. Incubation in «complete darkness» (green light used for scoring) resulted in lower germination and seedling production at 26°C compared to both nominal darkness and light conditions, and MTG was increased in comparison to the light treatment. MTG at 26°C was progressively increased with lowered seed quality induced by accelerated aging under seven environmental conditions; a negative linear relationship was observed between the probit percent of germination and log-MTG. The results indicate that temperature, light and seed quality influence the germination response in *A. americana*.

Key words: *Agave americana*, century plant, light, seed aging, seed germination rate, temperature.

Resumen. Se estudió el efecto de temperaturas constantes y de la calidad de la luz y las semillas en la germinación de las semillas de *A. americana*, con el objetivo de entender el control ambiental de la germinación en esta especie. Los límites inferiores y superiores para la germinación (emergencia de la radícula) y para la producción de plántulas en luz (12 h d⁻¹) fue de entre 11°C y 16°C, y de entre 36°C y 40°C, respectivamente. El máximo para ambos parámetros de crecimiento se encontró a temperaturas de entre 21°C y 31°C, y el tiempo mínimo medio para la germinación (MTG= TMG) fue a los 26°C. El reducir el fotoperíodo a ca. de 1 min de luz blanca por día (tratamiento de «obscuridad nominal») no afectó significativamente el nivel de germinación (emergencia de la radícula) o la producción de plántulas, pero la TMG aumentó significativamente. La incubación a «obscuridad completa» (usando luz verde para registrar los datos) resultó en una menor germinación y producción de plántulas a 26°C cuando se comparó tanto con los tratamientos de obscuridad nominal como con los de luz, y el TMG se incrementó en comparación con el tratamiento con luz. EL TMG a 26°C aumentó progresivamente cuando se redujo la calidad de las semillas con un envejecimiento acelerado en siete condiciones ambientales; observándose una relación lineal negativa entre el porcentaje probit de germinación y el logaritmo del TMG. Los resultados indican que la temperatura, la luz y la calidad de las semillas afectan la respuesta germinativa de *A. americana*. (traducido por los editores especiales).

Palabras clave: *Agave americana*, maguey americano, luz, edad de semillas, tasa de germinación de semillas, temperatura.

INTRODUCTION

Agave americana L. appears to have originated in North-East Mexico, but its varieties are cultivated in several regions of the world mainly in «Mediterranean» climates (Gentry, 1982). Although primarily propagating through bulbils and runners, flowering and subsequent seed production occasionally occur. In NE Mexico, flowering coincides with summer rain (June–September) (Gentry, 1982).

Environmental conditions for seed germination in *Agave* and *Manfreda* are known for a few species. *Manfreda virginica* (L.) Rose (= *Agave virginica*) seed require a short period of cold stratification to facilitate germination at

higher temperatures between 18°C and 28°C (Baskin and Baskin, 1971). In contrast, there is no evidence of seed dormancy in three other species, *A. deserti* Engelm., *A. lechuguilla* Torr. and *A. parryi* Engelm. (Freeman, 1973, 1975; Freeman *et al.* 1977; Jordan and Nobel, 1979). Lower and upper temperature limits for 50% germination in these species are ca. 13°C and ca. 32°C respectively (Freeman, 1973, 1975; Nobel, 1977; Jordan and Nobel, 1979). Light does not appear to be a necessary stimulus for germination in some *Agave* and *Manfreda* species: both *M. virginica* (Baskin and Baskin, 1971) and *A. deserti* (Jordan and Nobel, 1979) seeds germinate equally well in the light and the dark.

As well as environmental conditions, germination rate is also dependent on seed initial quality (viability). Thus, a negative linear relationship has been observed between the probit percent of germination and the mean time for germination for seeds of a number of crop species (Ellis and Roberts, 1980). Seed viability can be manipulated using accelerated aging techniques in which seed temperatures and moisture contents are carefully controlled. Such techniques can be used not only to quantify the germination rate–viability relationship, but also to estimate the effects of short-term exposure to high temperatures, similar to those encountered at the soil surface in arid lands, on seed quality.

Here we report on the germination response of *A. americana* seed to a variety of constant temperatures and the presence of light. We also consider the effects of seed aging on the germination response. Our objectives were to quantify the environmental control of germination, with a view to optimising the viability test conditions, and to determine the relationship between seed quality and germination, as a first step towards understanding the longevity characteristics of *A. americana* seeds.

MATERIALS AND METHODS

All studies were performed on a single seedlot of *A. americana* purchased in 1994 from Ellison Horticultural Ltd, Nowra, Australia. Seeds were equilibrated at 15°C and 15 % relative humidity, hermetically sealed in a foil-laminate bag and stored at 15°C until used.

For germination, 25 seeds were sown in 9 cm diameter Petri dishes on 1 % (weight/volume) agar-water, with 2 to 4 replicates per treatment temperature. Temperatures ranged from 11 ± 2°C to 40 ± 2°C. Light was applied for 12 h d⁻¹ at a photon fluence rate of ca. 15 µmol m⁻² s⁻¹ from Warm White fluorescent tubes. For the «nominal dark» treatment, dishes were wrapped in aluminium foil and seed germination was assessed daily under laboratory conditions (ca. 1 min d⁻¹ at ca. 10 µmol m⁻² s⁻¹). For the «complete dark» treatment, foil-wrapped dishes were opened and germination assessed, over a 5 min period, under dim green light (Pritchard and Manger, 1990).

Germination was assessed as radicle emergence to ca. 2 mm outside the seed. Seedling production was also assessed as the development of the cotyledonary arch, as previously observed in *M. virginica* (Baskin and Baskin, 1971). Germination rate (radicle emergence) was estimated as the mean time for viable seeds to germinate:

Mean time to germinate, days = $\sum(Dn)/\sum n$ where n is the number of seeds which germinate on day D , and D is the number of days from the beginning of the germination test (Ellis and Roberts, 1980).

Prior to accelerated aging, seeds were equilibrated at 21°C for at least 2 weeks over saturated salt solutions of LiCl, CaCl₂ · 6H₂O, NaI, NaBr · 2H₂O and NH₄Cl. Average seed equilibrium relative humidities after treatment, as determined by a Michell S-4020 dew point hygrometer (Michell Instruments Ltd., Cambridge), were 18%, 38%, 43%, 58% and 76% respectively. After equilibration, seeds

were hermetically sealed in aluminium foil laminate bags and placed at either 40°C (58% and 76 % r.h.) or 60°C (all r.h. treatments) for aging.

Germination data were subjected to t-test statistics after arc-sine transformation. The relationship between germination rate (MTG) and probit percent germination was analysed using the GLIM statistical package.

RESULTS AND DISCUSSION

Influence of temperature on germination in the light

The effects of constant temperature on germination (radicle emergence) and seedling production in the light (12 h d⁻¹) are shown in Figure 1. Maximum responses occurred at temperatures from 21°C to 31°C. Extending the temperature by about 10°C above or below the limits of this range resulted in little germination; upper and lower temperature limits for growth and development were approximately 40°C and 11°C respectively. Germination rate was slowest at 16°C, with a mean time for germination (MTG) of 33 d (Fig. 1). As temperature increased to 26°C, MTG was reduced more than three-fold to ca. 10 d. In contrast, a further 10°C increase in temperature, to 36°C, resulted in a more than 50 % lengthening of MTG compared to the 26°C treatment (Fig. 1).

Similar responses to temperature have been observed in the seeds of three other agave species, *A. lechuguilla*, *A. parryi* and *A. deserti*, which also have a natural distribution around the Chihuahuan desert region (Freeman, 1973, 1975; Freeman *et al.*, 1977; Jordan and Nobel, 1979); none of these species, including *A. americana*, appear to exhibit seed dormancy. In contrast, *Manfreda virginica*, which has a generally more easterly and northerly distribution within the USA from about 30° N to 40° N, require several weeks of low-temperature after-ripening for maximum rate and percentage germination (Baskin and Baskin, 1971).

Sensitivity of germination to light

When seed exposure to light was reduced from 12 h d⁻¹ to approximately 1 min d⁻¹ (nominal dark) neither total germination nor seedling production at 26°C was significantly affected (Table 1). However, nominal dark treatment resulted in a significant lengthening of MTG, by 56 %, to 15.4 d. Incubation of seeds in complete darkness caused about a 2- to 4-fold decrease in germination and seedling production compared to the nominal dark and light treatments, and MTG was increased over the light treatment by 36 % (Table 1).

Small changes in the germination parameters of *A. americana* seed over an approximate 10³ difference in daily light exposure level between the nominal dark and light treatments indicates that the seeds are relatively insensitive to light within this specific intensity range (Table 1). Small or insignificant changes in germination over this order of magnitude change in lighting are typical of many seeds, for example some members of the Cruciferae (Ellis *et al.*, 1989) and Fagaceae (Pritchard and Manger, 1990). Similarly, *M.*

FIGURE 1. The effect of temperature on germination percentage and mean time to germinate (MTG) is shown for *A. americana* seeds. Germination was assessed as radicle emergence (●) and seedling production (○). MTG values (■) are for radicle emergence only. Bars represent one s.d.

virginica seeds were considered to be light-insensitive (Baskin and Baskin, 1971), as were the seeds of *A. lechuguilla* (Freeman, 1973), *A. parryi* (Freeman, 1975) and *A. deserti* (Jordan and Nobel, 1979). However, the data presented here indicate that *A. americana* seeds are light sensitive at very low light levels (Table 1). Indeed, it appears from the complete dark germination data that about half the seed population have an obligate requirement for light to germinate, but that this effect can be saturated with very short exposures to white light equivalent to a photon dose of approximately $0.5 \text{ mmol m}^{-2} \text{ d}^{-1}$. Similar sensitivities to light have been observed in the seeds of other species, including *Nasturtium officinale* R. Br. and *Rorippa palustris* (L.) Besser (Ellis *et al.*, 1989).

Effect of seed aging on germination characteristics

Figure 2 shows the effects of storage time at 60°C on the germination progress curves of *A. americana* seeds which had been equilibrated to 18% r.h. before storage. From an initial germination of approximately 70%, viability was reduced to about 50% and 20% after 9 d and 21 d storage respectively. MTG at 26°C increased by 27% as aging was extended up to three weeks, from 8.8 d in the control seeds to 11.2 d in the aged seeds (Fig. 2). Analysis of the effects of seed quality on germination characteristics was developed further through an assessment of the relationship between probit percent germination and MTG for seeds maintained under seven different storage conditions. Figure 3 shows that, for the combined data sets, there was a significant negative linear relationship ($P < 0.001$) between germination

TABLE 1. Effect of light on percentage germination (radicle emergence), mean time to germinate (MTG) and percentage seedling production for *A. americana* seeds at 26°C. Data represent mean values with s.d. in parentheses.

Conditions	Germination		Seedling production
	%	MTG (d)	%
12 h d ⁻¹	69 (4) ^c	9.9 (0.7) ^{bc}	59 (6) ^c
Nominal dark	78 (9) ^a	5.4 (2.5) ^c	64 (17) ^a
Complete dark	40 (11) ^{ac}	13.5 (1.5) ^b	14 (14) ^{ac}

Data within columns with the same letter are significantly different: ^a $P < 0.1$; ^b $P < 0.02$; ^c $P < 0.01$

FIGURE 2. The influence of storage time at 60°C on germination progress at 26°C is shown for *A. americana* seeds. Seeds were pre-equilibrated to 18% r.h. and then aged for 0 (●), 6 (○), 9 (■), 15 (□), 21 (▲) and 39 d (△). Mean germination times, in days ($\pm$ s.d.), are given for each treatment.

percentage and log-MTG, such that a seedlot with 50% germination is predicted to have a MTG of 9.9 d.

Soil surface temperatures in the region of 60°C have been recorded in agave growing regions in the USA (Turner *et al.*, 1966). Our data indicate that exposure of *A. americana* seeds to such elevated temperatures will only be tolerated for a few days before there is an appreciable fall (*ca.* 20%)

in viability (Fig. 2). In contrast, the germination of *A. lechuguilla* seed exposed to the same temperature for 7 d was not significantly different from that of control seeds (Freeman, 1973). This might be indicative of inter-specific variation in the longevity characteristics of *Agave* seed, which is worthy of further investigation.

Our data on the relationship between germination percentage and MTG (Fig. 3), suggests the possibility of using germination rate characteristics to identify differences in vigour between seedlots with similar germination capabilities. Indeed, these differences would probably increase if temperatures which were sub-optimal for germination rate were used in the germination test, for example 21°C. However, spatial variations in temperature do occur in incubators. Thus, the use of a single temperature to screen seedlots for vigour differences is not recommended.

FIGURE 3. The relationship between germination percent (probability scale) and mean germination time (log scale) at 26°C is shown for *A. americana* seeds aged by seven methods. Seeds were either pre-equilibrated to 58% (●) and 76% r.h. (○) and then stored at 40°C, or pre-equilibrated to 18% r.h. (■), 38% r.h. (□), 43% r.h. (▲), 58% r.h. (△) and 76% r.h. (▼) and maintained at 60°C. The fitted line (probit germination = $6.608 - 6.647 \log \text{MTG}$) coefficient of determination, R^2 , of 0.70 was significant at $P < 0.001$. The dashed line indicates a MTG value of 9.9d for a seed population with 50 % viability.

CONCLUSIONS

The germination response of the commercial seedlot of *A. americana* used in this study was shown to be dependent on temperature, light and seed quality, as affected by storage treatment. Optimal environmental conditions for germination (total and rate of radicle emergence) and seedling production have been identified as being 26°C in the light (12 h d⁻¹) and the use of these conditions is recommended for germination testing in this species.

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