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Germination of the exotic *Calotropis procera* (Aiton) W.T. (Apocynaceae) in Mexico

Germinación de la exótica *Calotropis procera* (Aiton) W.T. (Apocynaceae) en México

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Abstract

Background: Seed germination strategies are important for exotic species to identify the factors that control seed germination and establishment.

Questions and /or Hypotheses: Temperature and light germination requirements for seeds of *Calotropis procera* do not change neither in its native regions nor in lands where it is exotic. *Calotropis procera* show germination traits that may increase their probability of colonization.

Studied species: *Calotropis procera* is a perennial plant native to some desertic areas in Asia and Africa and now naturalized in America.

Study site and dates: Mature fruits were collected in Oaxaca in September 2019 to test germination parameters. The last experiment was conducted in September 2021.

Methods: We determined the effect of different temperatures, photoblastic response, and loss of viability of seeds kept at laboratory conditions for two years. We performed several sowings under controlled conditions and achieved germinability, *t*50 and the time to germinate.

Results: Seeds were non-dormant, neutral photoblastic and did not lose viability after two years of dry storage. Temperature affected germinability and *t*50. Optimum germination temperature was 30 °C with no germination above 40 °C.

Conclusions: Germination requirements of seeds of *Calotropis procera* studied were similar to those reported worldwide. Though seed germination was affected by maximum mean temperatures, seeds germinated fast and at high percentages under a wide range of temperatures, which together with other attributes, may confer *C. procera* great chances for successful colonization.

Key words: exotic, Oaxaca, photoblastism, temperature, viability.

Resumen

Antecedentes: Las estrategias germinativas de las semillas en especies exóticas son esenciales para identificar los factores que controlan la germinación y el establecimiento.

Preguntas y / o Hipótesis: Los requerimientos germinativos de temperatura y luz de las semillas de *Calotropis procera* no varían en su región nativa ni donde es exótica. *Calotropis procera* muestra características germinativas que pudieran incrementar su probabilidad de colonización.

Especies de estudio: *Calotropis procera* es una planta perenne nativa de regiones desérticas de Asia y África, naturalizada en América.

Sitio y años de estudio: Colectamos frutos maduros en Oaxaca, en septiembre de 2019 para determinar parámetros germinativos. El último experimento se realizó en septiembre de 2021.

Métodos: Determinamos el efecto de diferentes temperaturas, fotoblastismo y pérdida de viabilidad de semillas almacenadas durante dos años en laboratorio, mediante experimentos bajo condiciones controladas. Determinamos germinabilidad, *t*50 y tiempo de inicio de germinación.

Resultados: Las semillas no mostraron latencia, tuvieron fotoblastismo indiferente y mantuvieron su viabilidad después de dos años de almacenamiento. La temperatura afectó la capacidad germinativa y el *t*50. La temperatura óptima fue de 30 °C y no obtuvimos germinación por arriba de 40 °C.

Conclusiones: Los requerimientos germinativos de semillas de *Calotropis procera* estudiadas fueron similares a los reportados en otras partes del mundo. Aunque la germinación fue afectada por la temperatura máxima, las semillas germinaron rápido y en porcentajes altos en un amplio intervalo de temperatura lo que, junto con otros atributos, pudiera maximizar las probabilidades de *C. procera* para una colonización exitosa.

Palabras clave: exótica, Oaxaca, fotoblastismo, temperatura, viabilidad.

Like most native plant species, exotic/invasive species (from now on exotic) may reproduce by seeds or by means of vegetative propagation, so the understanding of the variables which control germination processes is necessary to identify the main habitat conditions that allow the formation of a new individual. In exotic plant species, several traits such as growth rate and competitive abilities have been studied (Pyšek & Richardson 2007). However, the germination requirements which determine the survival and establishment of plant species are of less interest (Udo *et al.* 2017). Seed biology aspects like germination requirements, dormancy and dispersal are important to consider because they may affect the probability of establishment and its invasiveness capacity (Baskin & Baskin 2014, Gioria & Pyšek 2017).

Broadly, the germination parameters reported under different conditions are germinability, mean germination time, synchrony, and days to germination, among others, which provide valuable information about germination behavior. Particularly for exotic plants, the parameters *t*50 and time to germination are important for the invasion process following introduction to new lands as already underlined by Gioria *et al*. (2018) and Wijayabandara *et al.* (2013) which suggested that germination requirements can be used to predict the invasiveness potential of exotic species. Further, other plant attributes commonly reported in exotic species that may contribute substantially to the process of colonization are a flowering period throughout the whole year, high seed production with a high ability for dispersal, non-dormant seeds, and germination of seeds within a short time and under a wide range of conditions reaching high germination percentages (Pyšek & Richardson 2007, Muñoz & Ackerman 2011).

Calotropis procera is a perennial shrub or small tree native to tropical and subtropical Asia and Africa, common to the Egyptian and Iranian deserts (Hassan *et al.* 2015). Its common names in different countries are rubber bush, apple of Sodom, giant milkweed and in Mexico it is scarcely known as *algodoncillo gigante africano*. This species has become naturalized in South America, Central America, Mexico and Caribbean and Pacific Islands (Rahman & Wilcock 1991). In Brazil, some regions of Asia and Africa, and in Australia, *C. procera* is considered an invasive species (Reddy 2008, Fabricante *et al.* 2013, Leal *et al*. 2013, Bufebo *et al.* 2016). In this last country, some studies report the formation of dense thickets in alluvial plains near rivers and its establishment in adjacent pastures is reported, resulting in a decrease of the pasture value and difficulty in grazing (Meadley 1971, Parsons & Cuthbertson 2001). In Mexico, according to the CONABIO webpage of exotic plant species list, ENCICLOVIDA (2022), *C. procera* has been naturalized which, for some authors, is the first step for invasiveness (Palma-Ordaz & Delgadillo-Rodríguez 2014). This species has already been found in the states of Quintana Roo, Yucatán, Chiapas, Oaxaca, and Michoacán (NaturaLista 2022), mostly showing a ruderal habit in xeric environments. Data obtained from different herbaria (FCME, MEXU, and UAMIZ) confirm the presence of this species in these states, being Oaxaca the entity with the highest number of specimens recorded.

Some germination studies done with *C. procera* in other countries have shown that seeds exhibit no dormancy (Sen *et al.* 1968), that are neutrally photoblastic (Sen *et al.*1968, Oliveira-Bento *et al.* 2013), that their optimum temperatures for germination are 30 and 25/35 °C (Oliveira-Bento *et al.* 2013), 30 °C (Taghvaei *et al.* 2015, Menge *et al.* 2016), and 20, 25 and 30 °C (Sen *et al.* 1968). Some seed traits such as number of seeds per fruit and morphometric data have been documented for *C. procera* seeds from other countries (Heneidak & Hassan 2005, Oliveira-Bento *et al.* 2013, Gabr 2014) and more recently from seeds harvested in Mexico (Navarrete-Sauza *et al.* pers. obs.).

In Mexico, there are no formal studies of *C. procera* and due to its occurrence in some states, it is crucial to obtain data about their germination behavior that, together with other studies, contribute to the understanding of their germination dynamics and establishment.

Some plant species considered exotic in different parts of the world vary in their germination requirements as a response to the environment (Fakhr *et al*. 2022, Zhou *et al*. 2021). However, the permanence of some of these germination traits may increase the probability of colonization and spreading of other exotic plant species like *Calotropis procera*. Therefore, the aim of this study was to prove, from several experiments done under laboratory conditions, that germination requirements of temperature and light for *C. procera* seeds collected in Oaxaca, Mexico, do not change neither in its native regions nor in lands where it is an exotic species.

Materials and methods

Study species. *Calotropis procera* (Aiton) W.T. (Apocynaceae) is a perennial plant with a flowering period that lasts the whole year in Mexico. It is shrubby in most cases, but small trees that do not exceed four meters high can also be found. The flowers, when pollinated, produce green sub-globose/ovoid follicles. The fruit opens longitudinally through a dehiscence line that completely exposes the seeds at maturity (Hassan *et al.* 2015, Navarrete-Sauza *et al.* unpub. work). Each follicle has up to 500 seeds and each one has a tuft of silky hairs (collectively called "coma") that facilitates their dispersion by wind but may also be dispersed by water or by animals (Parsons & Cuthbertson 2001, Hassan *et al.* 2015). It is a drought-resistant and salt-tolerant xerophytic species that can grow easily in xeric habitats, survive in adverse climatic conditions, and develop in poor soils (Galal *et al.* 2015). It reproduces mainly sexually (Lottermoser 2011, Galal *et al.* 2015), but also asexually through half stumps, root suckers, and root cuttings (Hassan *et al.* 2015).

Collection site. Information of the collection sites in the state of Oaxaca, Mexico, was obtained from data extracted from the National Herbarium (MEXU) and from the coordinates provided by Dr. Leonardo O. Alvarado-Cárdenas. Other herbaria were examined to register coordinates, but no specimens were found in most cases, or just a few not considered because their collection sites were already within MEXU data.

Fruit collection was made alongside roads and highways in an approximately 55 km trajectory. Because it is a ruderal plant and does not distribute abundantly in any particular area, the route represented the population. All along this trajectory the same type of vegetation was observed (tropical deciduous forest; Torres-Colín 2004). The harvest was done in September 2019 and began in the municipality of San Cristóbal (16° 26' 16.09" N; 95° 31' 28.13" W), continued at some points within the urban area of Tehuantepec (16° 19' 28.69 N; 95° 14' 27.72" W) and ended on the La Ventosa-Salina Cruz highway (16° 12' 13.47" N; 95° 18' 47.44" W), in the municipality of Santo Domingo Tehuantepec. Climatic conditions reported an annual temperature variation between 20 and 30 °C, with a maximum mean temperature of 35 °C, a minimum annual precipitation of 600 mm and a maximum of 1,000 mm (INEGI 2010).

A total of 78 fruits were collected, mature seeds were extracted from the fruits and seeds were mixed and stored inside paper bags at room temperature until germination experiments were performed.

Germination experiments. Initial germination experiment.- To test germinability of fresh seeds, six replicates of 50 seeds each were sown fifteen days after seed harvest in Petri dishes with 1 % bacteriological agar (Bioxon) and put inside a germination chamber (Lab-Line model 844L, Melrose Park, Illinois) at 25 °C with a 12-h photoperiod. Germination was considered once the radicle emerged from the testa and the experiment lasted 10 days.

Temperature and light treatments.- To determine the effect of temperature and the photoblastic response under a 12-h photoperiod and under complete darkness conditions, six replicates of 50 seeds for each treatment were sown in Petri dishes of 10 cm diameter with 1 % bacteriological agar (Bioxon) and put inside six germination chambers (Lab-Line model 844L, Melrose Park, Illinois), each one with a different temperature going from 20 to 45 °C every 5 °C. Germination chambers had a 12-h photoperiod, and the experiment was followed for 19 days. For experiments under 12-h photoperiod, Petri dishes were put inside a transparent plastic bag and checked daily. For complete darkness experiments, Petri dishes were covered with two layers of aluminum foil and were checked until the end of the experiment. A seed was considered germinated once the radicle emerged from the testa. The experiment lasted 19 days. Germination capacity, the time to reach 50 % germination ($t50$) and the time to initiate germination (lag time) were the data obtained from this experiment. Cumulative germination curves were obtained with SigmaPlot (v. 11.0).

Seed viability. To determine the time seeds of *C. procera* may maintain their viability under dry laboratory conditions (20 ± 2 °C), seeds after collection were stored for two years. During this period, four sowings were conducted to test seed germinability. The first was performed two months after collection (November 2019) and the second in January

2020. The initial experimental design considered a sowing every two months for two years following the same procedure, but due to the COVID-19 global pandemics we performed only two more experiments, one in September 2020 –when seeds were one year old– and the last experiment was conducted in September 2021 –when the seeds were two years old–. Experiments were done with six replicates of 50 seeds each placed inside a germination chamber (Lab-Line model 844L, Melrose Park, Illinois) with a 12-h photoperiod and at a constant temperature of 30 °C. The experiment lasted 10 days. Once every experiment was conducted, the final germination percentage was obtained to assess if seeds were losing their viability through time while kept under laboratory conditions.

Statistical analyses. Temperature and light treatments.- A two-way ANOVA with a multiple comparison test (Tukey) was performed using the GraphPad Prism (v. 6) program to determine if there were significant differences in the germination percentages obtained under each treatment. Prior to the analysis of variance, an arcsine transformation of the germination percentages was performed to normalize the data (Sokal & Rohlf 1995).

To obtain the germination parameters of *t*50 and lag time for each temperature, a curve fitting to a sigmoid model was used ($y = a + b/ (1 + exp(-(x - c)/d))$) in the Table Curve 2D program (v. 5.01). From the fitted curve we obtained the *t*50 (the day in each fitted curve where we got 50 % germination) and the lag times (time to germination of the first seed). Subsequently, one-way ANOVAs were performed for each parameter between temperatures in the GraphPad Prism program to determine significant differences among them. This analysis was not performed for the darkness treatment because germination was checked until the end of the experiment, so there is no time-related data to compare among treatments.

Seed viability.- A one-way ANOVA test with a multiple comparison test was performed to determine the loss of viability through time of seeds stored under room conditions (20 ± 2 °C). Germination percentages were arcsine transformed to normalize the data (Sokal & Rohlf 1995). The analysis was performed in the GraphPad Prism program to determine significant differences between treatments (*i.e.,* sowings at different periods of time).

Results

Fruit collection. We obtained a total of 28,401 seeds from the collected fruits. The mean number of seeds per fruit collected was 364 ± 86 SD. The maximum number of seeds in a fruit was 481, while the minimum was 95.

Initial germination experiment. Calotropis procera seeds showed no primary dormancy. The seed sowing was performed only 15 days after collection and germination percentage reached a maximum of 98.7 % one week after the sowing.

Temperature and photoblastic response on germination. Germinability.- Germination was obtained at 20, 25, 30 and 35 °C under both light conditions. In contrast, germination at the two highest temperatures (40 and 45 °C), under both light treatments, was nil (Figure 1). The optimum temperature for germination was 30 °C at a 12-h photoperiod, where *C. procera* seeds got the highest and the fastest germination (Table 1). The germination percentage obtained at 30 °C under a 12-h photoperiod and at 25 °C under complete darkness was 99 %, the highest percentage obtained, while the lowest was 19.33 % at 35 °C under complete darkness (Figure 2).

The two-way ANOVA with multiple comparison test resulted in significant differences in germination among temperatures ($P < 0.001$; $F_{(3, 40)} = 62.49$), between light treatments ($P < 0.001$; $F_{(1, 40)} = 52.32$) and among the interactions ($P < 0.001$; $F_{(3, 40)} = 57.94$). We only found significant differences at 35 °C in the germination of *C. procera* seeds between both light treatments at the same temperature. Likewise, we only obtained significant differences between 20 and 30 °C in the germination under a 12-h photoperiod, which represent the lowest speed and the lowest percentage of germination, and the highest speed and the highest percentage of germination, respectively. Regarding the comparison between the seeds that germinated in darkness treatment, the difference between those exposed to a temperature of 35 °C and all the other temperatures is noticeable. The germination percentage under this condition, as already mentioned, was the lowest of the whole experiment with only 19.33 %. The same analysis shows that there are also significant differences in germination under darkness conditions between those exposed to 20 and at 25 °C. In this experiment it is observed that seeds at 20 and 25 °C under darkness treatment had a higher germination percentage than those that were in a 12-h photoperiod (Figure 2).

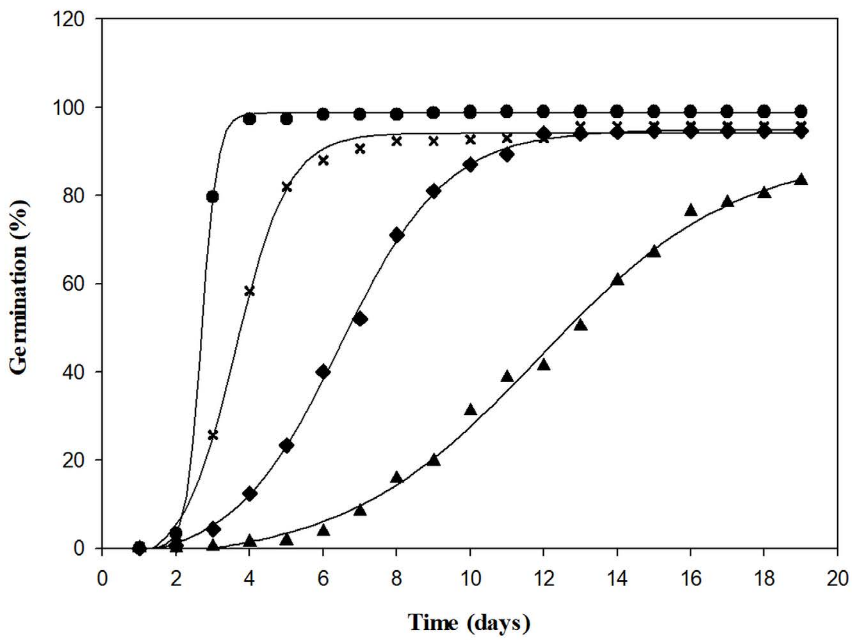

Figure 1. Cumulative germination percentage of *Calotropis procera* seeds fitted to sigmoid curves at four temperature treatments under a 12-h photoperiod. Where: ▲ is 20 °C treatment, ♦ is 25 °C treatment, ● is 30 °C treatment and × is 35 °C treatment.

Table 1. Germination parameters (germinability, *t*50 and lag time) of *Calotropis procera* seeds. Different letters indicate significant differences among treatments ($P < 0.05$).

Temperature treatment	Germinability under 12-h photoperiod (% ± SD)	*t*50 (± SE)	lag time (days)
20 °C	83.34 ± 4.63 *a*	13.87 ± 0.52 *a*	2.7 *a*
25 °C	94.34 ± 2.04 *b*	7.09 ± 0.52 *b*	1.09 *b*
30 °C	99 ± 0.54 *b*	2.53 ± 0.52 *c*	1.18 *b*
35 °C	95.67 ± 1.60 *b*	4.08 ± 0.52 *d*	1.03 *b*

*t*50 and lag time.- The one-way ANOVAs performed to compare each treatment resulted in significant differences for all parameters (Table 1). In the case of *t*50, it is observed that at 30 °C the seeds got the shortest time to reach the 50 % of cumulative germination (3 days). On the other hand, at 20 °C, the seeds took approximately 14 days to reach 50 % of cumulative germination, the highest time obtained. This parameter showed significant differences among each temperature.

The analysis of the lag time shows that only germination at 20 °C is significantly different from the other temperatures (Table 1). As observed, at this temperature the lag time is at least two times higher than at any other temperature, which means that the seeds of *C. procera* germinate faster at higher temperatures.

Seed viability.- The results indicate that seeds of *C. procera* kept under dry room conditions (20 ± 2 °C) did not reduce or lose their viability after two years (Table 2). The one-way ANOVA test showed no significant differences in germination obtained through time ($P = 0.1864$; $F_{(3, 20)} = 1.263$) with germination percentages higher than 98 %.

Discussion

Initial germination experiment. According to the high germination percentage obtained in the first experiment, fifteen days after collection, seeds of *C. procera* showed no primary dormancy, which is in accordance with research from species in other countries where it is either a native or an exotic species (Sen *et al.* 1968, Leal *et al.* 2013, Bebawi *et al.* 2015, Taghvaei *et al.* 2015), probably meaning that the different environmental conditions experienced by the seeds during their development have not affected their dormancy behavior. *Calotropis procera* seeds are non-dormant in agreement with other exotic species (Gioria *et al.* 2018, Xu *et al.* 2019) like *Asphodelus fistulosus* L. (Guerrero-Eloísa 2017) but differs from others that have shown dormancy (El-Keblawy & Al-Rawai 2006, Wijayabandara *et al.* 2013, Ozaslan *et al.* 2017).

Some authors have suggested that seed dormancy is a common attribute of exotic species which may result in the formation of a soil seed bank (Benech-Arnold *et al.* 2000, Fenner & Thompson 2005, Redwood *et al.* 2019). For example, *Lantana camara* L., a widespread exotic species, showed a deep physiological dormancy (Wijayabandara *et al.* 2013), *Ulex europaeus* L. possesses physical dormancy (Udo *et al.* 2017), and Xu *et al*. (2019) and Ozaslan *et al*. (2017) reported that seeds of introduced *Plantago virginica* L. populations and two *Physalis* L. species showed dormancy, respectively. Also, the germination results obtained by Díaz-Segura *et al*. (2020) with *Leonotis nepetifolia* (L.) R. Br. suggest a physiological dormancy. Contrary to these species, data obtained for *C. procera* show high germination percentages for fresh seeds during the first seven days after sowing. However, in a study conducted in India, Amritphale *et al.* (1984) found that one of three sympatric populations showed dormancy. This may be linked to the fact that this population had the lowest seed size and could be explained by a maternal effect induced by different environmental conditions (Campbell *et al.* 2015). In a simulation of adaptive traits done with several weed species, Martínez-Ghersa *et al*. (2000) found no clear relationship between germination traits and dormancy. Concerning how this may contribute to the success in exotic species has been poorly studied (Xu *et al.* 2019).

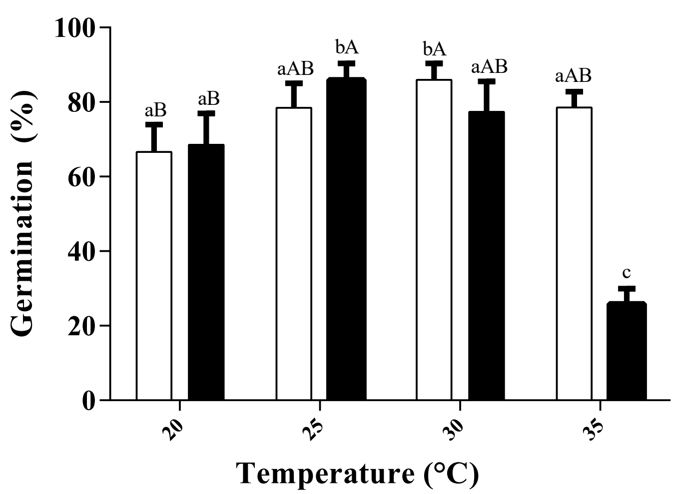

Figure 2. Final germination percentage of *Calotropis procera* seeds at four different temperatures. Black bars indicate complete darkness treatment and white bars indicate the 12-h photoperiod treatment. Different lowercase and capital letters indicate significant differences ($P < 0.05$) among treatments.

Table 2. Germinability of seeds of different ages of *Calotropis procera*. No significant differences were found among seed ages ($P < 0.05$).

Age of seeds	Germinability (% ± SD)
1 month	98.67 ± 0.82 ***a***
4 months	99 ± 0.84 ***a***
1 year	100 ± 0 ***a***
2 years	99.33 ± 0.52 ***a***

Any kind of dormancy may help seeds to persist in the soil seed bank for longer periods, but at present there is no investigation available concerning a soil seed bank for *C. procera* in Mexico. Bebawi *et al.* (2015) suggest that seeds of *C. procera* from northern Queensland, Australia, may form a short-term persistent soil seed bank. It is possible to consider that the formation of a persistent soil seed bank may not be a strategy for *C. procera* in Mexico because flowering and fruiting period lasts almost all the year, so numerous and viable seeds are being incorporated continuously into the soil and a fast germination may promote an early uptake of resources from the soil representing an advantage in xeric environments.

Effect of temperature on germination. Many ecophysiological studies have suggested that temperature plays a crucial role in the detection of the appropriate time for seed germination being the most determinative environmental signal (Baskin & Baskin 2014). Some authors have evaluated the germinative response of exotic species in a temperature gradient (*e.g.,* Vieira *et al*. 2010, Ozaslan *et al*. 2017).

For *C. procera* seeds, a similar germination response to temperatures has been reported from countries where it is a native or an exotic species (Labouriau & Valadares 1976, Taghvaei *et al*. 2015, Menge *et al*. 2016). Seeds from the Iranian deserts showed that the optimum temperature for seed germination was 30 °C, germination rate was slower at 20 °C and the germination rate increased with temperature until the optimum was reached, then it decreased (Taghvaei *et al*. 2015). Besides, these authors reported germination percentages of 100 % in a temperature gradient from 20 to 40 °C, and here we obtained a germination above 94 % from 25 to 35 °C. However, the results they obtained at the maximum temperature for germination (high percentages at 40 °C) differ from ours because our seeds did not germinate at 40 and 45 °C. This differential germination may be due to the temperatures that seeds experience during development in the mother plants (Roach & Wulff 1987). Accordingly, in Iranian deserts the mean maximum temperature is 35 °C, reaching over 40 °C at least four months in a year, contrasting with the mean temperature of the collection sites in Oaxaca, which is 30 °C with a maximum average of 35 °C, but not reaching 40 °C.

Also, Menge *et al*. (2016) mentioned that in nine different sites in Australia where water stress and temperature upon germination was tested, the maximum summer temperatures were lower than 40 °C so seeds barely germinated at this temperature and did not germinate at 45 °C, with an optimum temperature for germination at 30 °C. Also, Labouriau & Valadares (1976) studied germination from 17 to 37 °C with seeds from Northeastern Brazil and obtained that their seeds had low germination at temperatures near 20 °C, the germination rate and percentage increased with temperature until the optimum temperature for germination was reached (30 °C) and germination decreased at temperatures higher than the optimum until germination was nil, though no information from mean temperatures in the study site was provided.

With respect to the germination parameters *t*50 and lag time, Soltani *et al*. (2015) mention that *t*50 is the best parameter to compare germination rate, representing the time for 50 % of germinated seeds, and together with lag time, Gioria & Pyšek (2017) mention that a fast germination is an expected characteristic of exotic species compared with their native congeners. The importance of both parameters in exotic plants lays upon the need to understand its timing for germination as a strategy to establish themselves in new or invaded areas (Gioria *et al*. 2018), but unfortunately none of these two parameters have been reported in other studies with *C. procera*. Nevertheless, they have been reported for other exotic species (Tinoco-Ojanguren *et al*. 2016, Guido *et al.* 2017, Hao *et al.* 2017, Song *et al*.

2017) and may be important to evaluate germination and successful establishment in exotic species because it indicates which conditions suit best for a fast germination. *Calotropis procera* seeds started to germinate quickly after sowing and *t*50 decreased as temperatures increased until the optimum was reached, and was negatively affected by temperatures lower than 25 °C and higher than 35 °C. This emphasizes the importance for *C. procera* seeds to be constantly exposed to temperatures above 20 and below 40 °C to have a higher probability for a fast germination at high percentages which could maximize a successful establishment.

Photoblastic response. A neutral photoblastic response under the temperature regime was obtained in accordance with results obtained by other authors (Sen *et al*. 1968, Amritphale *et al*. 1984, Oliveira-Bento *et al*. 2013, Taghvaei *et al*. 2015). Moreover, Leal *et al*. (2013) reported that germinability was not influenced by five levels of light intensity. The neutral photoblastic response reported in all studies done may indicate that the differential maternal environment might not be influencing this response.

Other exotic species have shown a neutral photoblastic response. For example, Vieira *et al*. (2010) found that seeds of *Clausena excavata* Burm. f. is neutrally photoblastic within a temperature range from 20 to 35 °C, Guerrero-Eloísa (2017) reported a neutral photoblastism for *Asphodelus fistulosus* sown under different conditions, Díaz-Segura *et al.* (2020) report neutral photoblastic seeds of *Leonotis nepetifolia* under five different light intensities and Nešić *et al*. (2022) suggested that *Symphyotrichum lanceolatum* (Willd.) G.L. Nesom seeds germinated in higher percentages under light, but a significant percentage may germinate under darkness.

Here, for *C. procera*, we report that germination percentage for seeds incubated under complete darkness was higher at temperatures below 30 °C, though with no significant differences compared with seeds incubated under a 12-h photoperiod. At 35 °C we observed a decrease in the germination percentage under darkness treatment being the lowest percentage obtained in all treatments. In Egypt, Galal *et al*. (2015) obtained that 30 °C under complete darkness are the optimum conditions for germination of *C. procera* seeds as they recorded the highest germination percentage, so with their results they concluded that seeds, once dispersed, must be buried because burial lowers surface temperature. The aforementioned is in accordance with the study conducted by Bebawi *et al.* (2015), where seeds that were not buried in the soil had the lowest germination percentages. This shows the effect that the interaction between light and temperature may cause in the germination response.

Following the environmental conditions to which *C. procera* seeds are exposed in the collection site in Oaxaca, we propose that, if they remain on the surface, they will be more exposed to sunlight and high temperatures that might inhibit or restrain germination, but seeds buried within the first centimeters under the soil may be able to germinate because burial ameliorates extreme temperatures and desiccation. If enough moisture is available, neutral photoblastism may allow seeds of *C. procera* to germinate either on the soil surface if temperatures do not exceed 35 °C, or if they are buried a few centimeters under the soil.

Seed viability. Our findings are in accordance with those obtained by Sen *et al*. (1968), who also found that germination did not change for two-years old seeds kept under laboratory conditions. However, in Australia, Bebawi *et al.* (2015) mention that *C. procera* seeds buried on two kinds of soil lost their viability completely in two years.

In conclusion, *C. procera* seed germination requirements of the Oaxaca population studied are similar to those reported worldwide, where 30 °C is the temperature recorded for optimum germination. Though seed germination was affected by the maximum mean temperature, seeds germinated fast and at high percentages under a wide range of temperatures, which together with attributes such as an extended flowering and fructification (Navarrete-Sauza *et al*. pers. observ., Labouriau &Valadares 1976, Leal *et al.* 2013), asexual propagation (Hassan *et al.* 2015), great number of wind-dispersed seeds produced per fruit (Navarrete-Sauza *et* al. pers. observ.), ability to grow in disturbed and arid environments, and phytotoxic characteristics (Kaur *et al.* 2021) may confer *C. procera* great chances for a successful colonization of new arid and semiarid environments in Mexico. Further studies of *C. procera* are required to better understand its establishment, growth, and reproduction, but also to explain the effects of its presence in Mexico, or to explore its potential uses.

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