

Seed germination and seedling emergence of the invasive exotic species, *Clausena excavata*

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(With 4 figures)

Abstract

The aims of this study were to evaluate the effects of light and temperature on seed germination and the effect of light on seedling emergence of *Clausena excavata*, an invasive exotic species. Seeds were incubated at constant temperatures (10 to 40 °C), under continuous white light and darkness. Seedling emergence was evaluated under full sun and canopy shade. There was no significant difference between seeds germinated in the presence or absence of light at temperatures between 20 and 35 °C. Seeds maintained at 20 °C germinated more slowly than other temperatures. Independent of temperature, seed germination was much more synchronised under white light than under darkness. In the field, emergence occurred in both environments, but a greater percentage was observed under full sun. Seedling emergence occurred at the same rate and synchronisation index under both conditions. *C. excavata* has a high invader potential in open areas and shaded environments.

Keywords: alien species, light condition, neuter photoblastic, recalcitrant seed, Rutaceae.

Germinação de sementes e emergência de plântulas de uma espécie exótica invasora, *Clausena excavata*

Resumo

Este estudo teve como objetivos avaliar os efeitos da luz e da temperatura na germinação de sementes e o efeito da luz na emergência das plântulas de *Clausena excavata*, uma espécie exótica invasora. O experimento de germinação foi realizado em temperaturas constantes (10 a 40 °C) e mantido na presença e ausência de luz. O experimento de emergência foi realizado sob duas condições de luz, a pleno sol e à sombra. As sementes germinaram tanto na presença como na ausência de luz, nas temperaturas de 20 a 35 °C, sem diferença entre a porcentagem de sementes germinadas nas diferentes temperaturas. Sementes mantidas a 20 °C, em ambas as condições de luz, germinaram mais lentamente em relação às demais temperaturas. Independente da temperatura, na presença de luz, as sementes apresentaram uma germinação muito mais sincronizada do que no escuro. Em campo, a emergência ocorreu em ambos os ambientes, porém em maior porcentagem a pleno sol. A emergência se deu com a mesma velocidade nas duas condições testadas. Quanto à sincronia, as plântulas emergiram igualmente em ambas as condições. Assim, pode-se dizer que *C. excavata* possui sementes fotoblásticas neutras e, apesar da influência da luz na porcentagem de emergência de suas plântulas, o potencial invasivo da espécie é alto, tanto em ambientes com disponibilidade de luz quanto em ambientes sombreados.

Palavras-chave: espécie exótica, condição de luz, fotoblástica neutra, semente recalcitrante, Rutaceae.

1. Introduction

Biological invasions represent one of the greatest dangers to local and global biodiversity (Washitani, 2001). Little is known about invasive species in Brazil (Peterson and Pivello, 2008). These invasions can cause harm on several levels, affecting individuals (e.g. mortality and growth), population dynamics (abundance, population growth and

extinction), the community (abundance of species and diversity) and ecological processes (Parker et al., 1999). In the process of biological invasion, invasive exotic species become dominant in the environment and harm native species and ecosystem functioning, and may even cause the extinction of native species (Pivello, 2007).

Invasive species show the most diverse life forms, from herbs to trees, and are present in various ecosystems (Cronk and Fuller, 1995; Seastedt, 2007).

Vines and lianas are frequently found along the edges of somewhat intact tropical forests, while colonisation of exotic species frequently occurs in clearings (Dislich et al., 2002). However, according to these authors, disturbed areas and small fragments are more susceptible to biological invasions, since many of these species are helophytes, which are more easily established in these environments. Among some of the characteristics that invasive species have and that promote their success as invaders are fast growth, tolerance to environment stress and high tolerance to environment heterogeneity (Sakai et al., 2001).

Disturbances in tropical forests, such as the formation of clearings, have been seen as changes in a habitat. These alterations influence many abiotic factors, such as light availability and presence of undergrowth, which alter environmental temperature and humidity in the canopy gap (Everham III et al., 1996).

According to Baskin, CC. and Baskin, JM. (1988), temperature, light, and soil humidity are the most important environmental factors controlling seed germination and seedling establishment. According to Rejmánek and Richardson (1996), optimal conditions for seed germination and seedling emergence are the same as those for optimal growth of a species, since plant survival and invasion are strongly related to mechanisms of germination and emergence. Seed germination studies are important not only for the useful information they contribute to seed technology, but also for their help in understanding the ecophysiology of plant species (Borghetti and Ferreira, 2004). This knowledge helps explain biogeographic peculiarities of many species (Borghetti, 2005) and predict possible environments susceptible to invasion according to specific attributes of invasive species (Cordell et al., 2002).

Clausena excavata Burm. f. – wampee, *clausena curry* – is a semideciduous tree that is native to India, Southeastern Asia and the Philippines. Height reaches between 6 and 9 m, with irregular and open canopy. Flowers are whitish and small, arranged in terminal bunches of 20-30 cm length. Fruits are shiny rose ellipsoid drupes, of 7-10 mm in length, sweetish succulent pulp, and 1 or 2 (Lorenzi et al., 2003) non-dormant seeds (Ng, 1980 apud Baskin, CC. and Baskin, JM., 1998). Medicinal properties of its leaves, bark and roots are known (Wu et al., 1998). In Brazil, its natural regeneration was related as predominant in a recovered forest (Vieira and Gandolfi, 2006), indicating its invasive potential. This species is known in Australia as a weed that is common in Christmas Island vegetation (Green et al., 2004) and as an invasive species in Hawaii (Space and Imada, 2004).

The aims of this study were to evaluate the effects of light and temperature on seed germination of *Clausena excavata* under laboratory conditions and the effect of light on seedling emergence under natural conditions, in order to understand the invasive capacity of this species.

2. Material and Methods

Clausena excavata seeds were harvested from mature fruits from 20 plants located in a recovered forest in Iracemápolis, SP, Brazil (22° 35' S, 47° 31' W), in January 2006. This area is a heterogeneous reforestation, with 19 year old native and exotic species. The climate according to Köppen (1948) is Cwa (humid subtropical climate characterised by hot humid summers and dry winters) with annual precipitation between 1,100 and 1,700 mm (Oliveira, 2004).

After harvest, fruits were washed, the pulp removed under tap water, and seeds manually separated at the Photomorphogenesis Plant Laboratory (UNESP/Rio Claro). For the germination tests, four replicates of 25 seeds were placed inside clear or black plastic gerbox containers lined with filter paper soaked in 10mL distilled water for white light and darkness treatments. To maintain moisture, distilled water was added as needed. White light treatments were carried out with two 15 W fluorescent daylight lamps with photon fluence rate of 32.85 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at seed level. Experiments were carried out in germinators (FANEM model 347-G and MARCONI model MA 403) at 5 °C (± 1 °C) increments from 10 to 40 °C. Water content of *C. excavata* seeds was 50.78% as determined according to Rules for Seed Analysis (Brasil, 1992), after drying 100 recently harvested seeds at 105 °C for 24 hours. According to Chin (1989), seeds with this water content at dispersal period are classified as recalcitrant seeds.

Germinated seeds were counted and removed at 24 hour intervals. Seeds were considered to be germinated when the primary root reached a minimum length of 1 mm (Brasil, 1992). Seeds incubated in the dark were observed under a dim green safelight (Amaral-Baroli and Takaki, 2001). Monitoring was interrupted when no germination was observed for five consecutive days.

Seedling emergence of *C. excavata* was evaluated under direct sunlight and under canopy shade at the Experimental Garden (UNESP/Rio Claro) in February 2006. Under full sun, the mean R:FR ratio was 1.22, mean air temperature was 30.5 \pm 0.8 °C, and mean soil temperature was 28.5 \pm 0.5 °C. Under canopy shade, the mean R:FR ratio was 0.23, mean air temperature was 24.8 \pm 0.4 °C, and mean soil temperature was 24.4 \pm 0.2 °C. Air temperatures were measured using a maximum and minimum thermometer in each environment, and soil temperatures were obtained by burying a thermometer in the plant substrate.

For seedling growth experiments, four replicates of 40 seeds were sown at a depth of 1 cm in black plastic bags (15 \times 30 cm) containing a soil-humus mixture (1:1) and kept in either full sun or canopy shade. Experiments were watered daily except on rainy days. Seedling emergence was observed daily for 45 days, finishing nine (sun) and ten (shade) days after the last emergence. Seedlings were considered emerged when the stem was free from the substrate, since this species presents hypogeal germination.

Percent (%) and rate (\bar{v}) of germination and emergence as well as frequency (f_j) and synchronisation index of germination

(U) were calculated according to Labouriau (1983) for each temperature in full sun or shade. Thus, $\bar{r} = 1/\bar{t}$, in which \bar{r} : germination and emergence rate; \bar{t} : mean time of germination and emergence; $\bar{t} = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i}$, in which n_i : number of germinated seeds and seedlings emerged in time t_i , t_i : time from experiment initiation and the i^{th} observation (day), k : last time of seed germination and seedling emergence; $f_i = \frac{n_i}{\sum_{i=1}^k n_i}$, in which n_i : number of germinated seeds and emerged seedlings in time t_i , k : last time of seed germination and seedling emergence; $U = -\sum_{i=1}^k f_i \log_2 f_i$, in which f_i : relative frequency of seed germination and seedling emergence, k : last time of seed germination and seedling emergence.

Germination and emergence data were not normal so the Kruskal–Wallis test was used, and differences among groups were analysed by Nemenyi test ($\alpha = 5\%$). Rate and synchronisation index data were analyzed using a two-way Analysis of Variance (ANOVA) followed by Tukey test ($\alpha = 5\%$) to compare means (Zar, 1999).

Percent, rate of seedling emergence and synchronisation index for each environment, were analyzed by t test to compare means (Zar, 1999).

3. Results and Discussion

Clausena excavata seeds germinate as much in full sun as in the shade at temperatures between 20 and 35 °C, with no significant differences regarding percent germination at different temperatures. Percent germination was high, on average above 98%, for all treatments (Figure 1a). Due to an absence of germination below 20 °C and above 35 °C, cardinal temperatures were determined to have a minimum between 15 and 20 °C and a maximum between 35 and 40 °C. For germination to occur in *C. excavata* seeds a minimum temperature a little above that required by most tropical species is needed, which according to Borghetti (2005) is between 10 and 15 °C.

This germination characteristic of indifference to light allows *C. excavata* seeds to be classified as photoblastically neutral. The possibility of seed germination with little or no light allows the species to occupy microhabitats in the forest or even in soil covered with a layer of stones or an accumulation of leaves (Mercier and Guerreiro-Filho, 1990).

Seeds kept at 20 °C, under both light conditions, germinated more slowly than at other temperatures ($P = 0.000$). Germination rate was strongly and positively affected by light at temperatures between 25 and 35 °C ($P = 0.000$) (Figure 1b). Consequently, the optimal temperature for this species is between 25 and 35 °C, at which greater germination and lower mean germination time were observed regardless of light (Figure 2).

Under suitable moisture conditions, the germination process of recalcitrant seeds occurs quickly (Berjak et al., 1984; Baskin, CC. and Baskin, JM., 1998). In nature, these

seeds should germinate rapidly even inside fruits or right after dehiscence, with no formation of soil seed bank (Farnsworth, 2000). This occurs because these seeds are considered intolerant to desiccation, losing viability when dehydrated to water content below a specific critical level (Bewley and Black, 1994).

Regarding synchronisation of the germination process of *C. excavata*, it was observed that regardless of temperature ($P = 0.908$), seed germination in full sun was more synchronised than that in the shade ($P = 0.003$).

Light insensitivity was also observed in *Leucaena leucocephala* (Lam) de Wit. seeds germinated under white light and darkness, with 76% seed germination (Souza-Filho, 2000). *L. leucocephala* is also an exotic species and is considered an invasive species in many parts of the world (Richardson, 1998; Shine et al., 2003; Espíndola et al., 2005). As reported by Richardson (1998), *L. leucocephala* is among the most planted exotic tree species and amongst its characteristics are fast growth, multiple uses and exceptional adaptation to diverse environmental conditions.

Another tree species that is considered invasive in some regions of the world is *Psidium guajava* L. (Richardson, 1998; Shine et al., 2003; Space and Imada, 2004). In Brazil, where this species is native, its regeneration is intense in open habitats (Lorenzi, 1998). *P. guajava* seeds have the

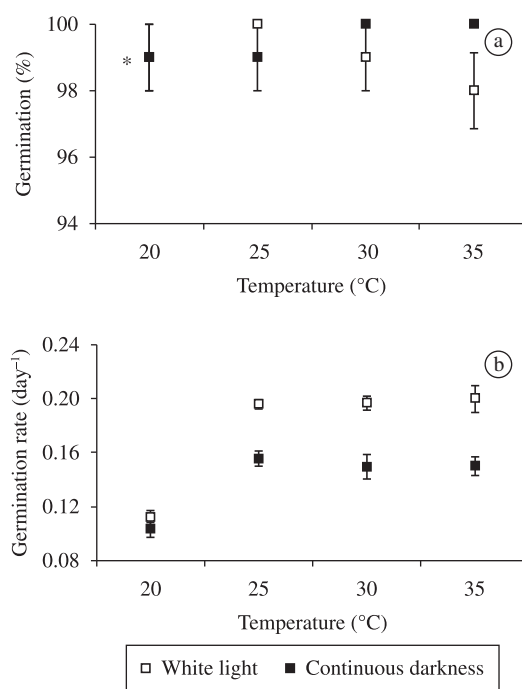


Figure 1. Mean germination a) percentage and b) rate of *Clausena excavata* seeds under white light and continuous darkness. Vertical bars indicate standard error of means. *At 20 °C the mean germination percentage was exactly the same under both light conditions. The seeds did not germinate at tested temperatures below 20 °C and above 35 °C.

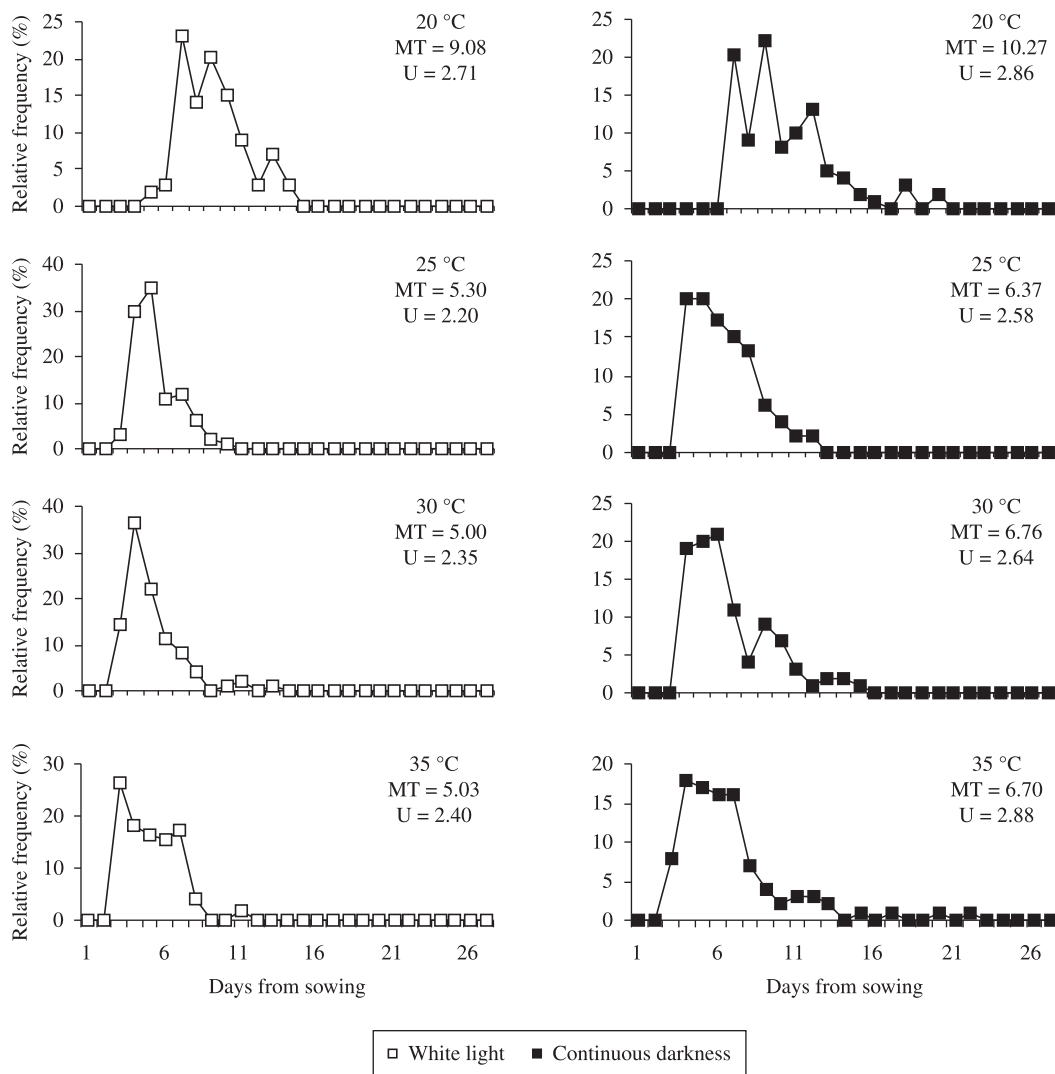


Figure 2. Polygons of relative frequency of seed germination of *Clausena excavata* at different temperatures under white light (□) and continuous darkness (■). MT = mean time of germination (days). U = synchronisation index of germination (bits).

characteristic of colonising gaps and open habitats. Seed germination of this species occurs under white light at temperatures in the range of 20 to 35 °C, but in darkness only where there is oscillation of temperatures (Sugahara and Takaki, 2004). For these authors, seeds of this species are induced to germinate not only by light but also by oscillation in temperatures that occur in open areas or small gaps, when seeds are covered with soil.

In the field, seedling emergence of *Clausena excavata* was observed under both test environments. Direct sunlight presented greater results ($P < 0.05$), with all seeds generating a seedling. Emergence under canopy also occurred with great success (Table 1). Although light influenced the final percentage of seedling emergence in *C. excavata*, the potential occurrence of this species is high under both direct sunlight and shade, confirming results obtained with laboratory controlled conditions.

The emergence rate of seedlings was the same for the two environments ($P = 0.776$). In full sun, emergence first occurred after the 17th day and finished (reached maximum 100%) on the 36th. In the shade, emergence first appeared after 18 days and terminated (90.6%) on the 35th. As can be noted on polygons of relative frequency (Figure 3), seedling emergence occurred in a heterogeneous way under both light conditions and presented the same synchronisation index (Table 1).

At the end of the experiments, all *C. excavata* plants had survived under both treatments. The same success was not observed in *Tecoma stans* L. Juss. ex. Kunth under shade, where only 1.5% of emerged seedlings survived, whereas under direct sunlight seedling survival was 96.9%. Although *T. stans* seeds are indifferent to light conditions for germination, recruitment of their seedlings only occur in open areas, forest edges and gaps (Socolowski et al., 2008).

Table 1. Characteristics of seedling emergence of *Clausena excavata* on two tested environments (Env.).

Env.	Start of emergence (days)	End of emergence (days)	Emergence (%) [*]	Rate (day ⁻¹) [*]	Synchronisation index U (bits)
Sun	17	36	100.0 ± 0.0 ^a	0.0429 ± 0.001 ^a	3.10 ^a
Shade	18	35	90.6 ± 1.2 ^b	0.0424 ± 0.001 ^a	3.30 ^a

Means followed by the same letter, inside each evaluated parameter, are not significantly different by *t*-test. ^{*}Mean values ± standard error of mean.

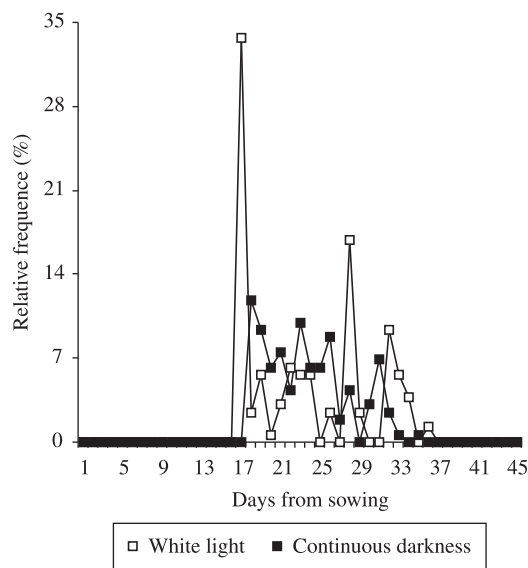


Figure 3. Relative frequency of seedling emergence of *Clausena excavata* under full sun (□) and shade (■) at Experimental Garden (UNESP/Rio Claro).

According to Fine (2002), shade intolerance observed in *T. stans* is common to most invasive exotic species; consequently, these species rarely inhabit environments with low light availability, such as tropical forests. In Brazil, *T. stans* is considered an invasive species (Ziller, 2001). In other areas, such as Hawaii, this species is very invasive in disturbed areas causing serious problems (Space and Imada, 2004).

While studying the effect of disturbance intensity on *Lantana camara* L. invasion, Duggin and Gentle (1998) observed that seed germination, survival and initial seedling growth were significantly and positively correlated with disturbance intensity. In other words, as light availability increased so did germination, survival and growth, which in turn, increased the reproductive success of the species. Canopy shade provided an effective barrier against invasion of *L. camara* (Duggin and Gentle, 1998), contrary to what was observed for *C. excavata*.

Myers et al. (2005) studied the behaviour of *Alliaria petiolata* (M. Bieb) Cavara and Grande, an invasive species in the United States, under several light conditions and observed that invasion of this species occurs at low and high light availability. This plasticity of response

to different levels of light is a factor that can increase invasion success.

During emergence of *C. excavata* seeds, the cotyledons remained below the soil surface, characterising germination as hypogeal. In this case, the cotyledons function only as a reserve organ and photosynthesis does not take place. When the cotyledons remain on the new shoot and are brought above the ground, germination is epigeal. In this case, photosynthesis does take place; however, the cotyledons are vulnerable to herbivores and pathogens since they are exposed (Bazzaz and Pickett, 1980).

In conclusion, seeds of *C. excavata* are capable of germination and recruiting new seedlings under different light conditions, which allows this species to inhabit diverse environments. These characteristics may contribute to the successful propagation of this species.

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