



Seed biology and early seedling developmental traits of *Clidemia hirta*, an invasive species of Sri Lankan rainforests compared to two native species sharing the same habitat

B.R. Chandima P. SAMARASINGHE^{1,2,*}, K.M.G. Gehan JAYASURIYA^{1,2}, A.M. Thilanka A. GUNARATNE^{1,2}, Kingsley W. DIXON³, Mahesh SENANAYAKA⁴

1. Department of Botany, University of Peradeniya, Peradeniya, Sri Lanka.; 2. Postgraduate Institute of Science, University of Peradeniya, Peradeniya, Sri Lanka. 3. ARC Centre for Mine Site Restoration, School of Molecular and Life Sciences, Curtin University, Western Australia. 4. Forest Department Sri Lanka, Ringgold standard institution, Battaramulla, Western Sri Lanka. *Corresponding author's emails: brprabodani@gmail.com; Tel: +94770412912.

(Manuscript received 19 May 2021; Accepted 16 December 2021; Online published 11 January 2022)

ABSTRACT: Comparative studies on invasive species with native species sharing the same habitats are valuable in controlling invasive plants. This study compared seed biology and seedling development traits of two native species, *Melastoma malabathricum* L and *Plectranthus kanneliyensis* Willems, and the invasive species *Clidemia hirta* (L.) D. Don, which share a tropical rainforest habitat in Sri Lanka. In particular, the adaptability of these species to their habitat was assessed. Seed moisture content, dormancy status and desiccation sensitivity were also tested. Seedling growth and survival was observed through greenhouse experiments. Seeds of all the study species germinated >50% in light/dark conditions, but none germinated in complete darkness or under green light. Time taken to reach 50% germination (T_{50}) and final germination percentage suggested that *C. hirta* and *P. kanneliyensis* seeds are non-dormant while *M. malabathricum* seeds are dormant. All study species produced desiccation tolerant seeds. Under all light conditions, seeds of *C. hirta* recorded the highest seed germination and seedling survival percentage, while *P. kanneliyensis* had the highest growth rate. Interspecies difference in photosynthesis efficiency was not observed. These experiments demonstrated that the study species have different strategies to adapt to their shared habitat. Furthermore, higher germination percentage and higher survival may be the key factors that determines the invading ability of *C. hirta*, enabling them to outcompete *M. malabathricum* and *P. kanneliyensis*. Hence, immediate actions must be taken to prevent further invasion of *C. hirta* into disturbed sites in tropical rainforest regions.

KEY WORDS: Invasive species, native species, seed dormancy, seed germination, seedling survival.

INTRODUCTION

Invasion of exotic species has become a major threat to the biodiversity of tropical habitats, including rainforests (Bradshaw *et al.*, 2009; Meyer and Cowie, 2010; Krupnick, 2013). These species affect native species through competition (Levine *et al.*, 2003), change of the edaphic factors (Jordan *et al.*, 2008) and alteration of fire regimes (Antonio, 2000). Invasive species through these effects lower the native biodiversity with a disproportionately negative impact on rare endemic species (Hejda *et al.*, 2009).

Clidemia hirta has been reported as one of the world's 100 worst invasive species (Global Invasive Species Database, 2020). In its native range (lowlands of Central and South America and the Caribbean islands), *C. hirta* is known to be found in open areas but can also invade closed canopy forests in its introduced range (DeWalt *et al.*, 2004a). This species can replace native species that are specialized in inhabiting open sites in rainforests by changing habitat conditions (Wester and Wood, 1977). Moreover *C. hirta* invasion is recorded to alter forest regeneration through alteration of the habitat in many rainforests worldwide (Hawaiian Rainforests [Wester and Wood, 1977], Malaysia [Peters, 2001; Le *et*

al., 2018], Taiwan [Yang, 2001], Fiji [Simmonds, 2009]).

Tropical lowland rainforests of Sri Lanka, which are biodiversity rich ecosystems with many endemic plant species (60–75%) have not escaped from this threat (Kariyawasam *et al.*, 2020; Bambaradeniya, 2002). *C. hirta* was first introduced to Sri Lanka in 1894 through Peradeniya Botanical Gardens (Wijesundara, 2010). In Sri Lanka it is found in the sub-montane areas of wet zone, and occupies open areas in lowland rainforest edges, roadsides and canopy gaps in rainforests rather than in forest understory and is now spreading to higher elevations in the Kandy and Ambagamuwa areas (Wijesundara, 2010).

Although improved management and control practices are crucial to control this invasive plant, while promoting the establishment of native species (Simmonds, 2009), there are no such attempts made so far in Sri Lanka regarding *C. hirta*. It is imperative to gather information on the life history of the invasive species and native species that share the same habitat to plan for the successful management of invasive species. Seed germination is considered as a transition from the most tolerant developmental stage *i.e.* the seed to the most vulnerable stage in plant development, the seedling (Castro *et al.*, 2005). Growth of a new plant is associated



with successful germination and seedling development (Wolny *et al.*, 2018). The timing of germination determines the post-germination conditions experienced by seedlings (Donohue 2003, 2005), including the competitive environment (Forbis, 2010), and thus can strongly affect the growth, fitness and survival of a species and its invasion potential (Donohue, 2005; Donohue *et al.*, 2010; Fenner and Thompson, 2005). The successful invasion of tropical plant species generally depends on different life-history traits through all stages of the life cycle (Chapple *et al.*, 2012; Malíková *et al.*, 2012; Mullah *et al.*, 2014). Invasive species are generally characterized by high seed production, effective seed dispersal mechanisms, high germination success in a wide range of environmental conditions, fast growth, and high phenotypic plasticity of seedlings (Moravcová *et al.*, 2015; Moran *et al.*, 2017). Although there are several studies on seed germination and seedling establishment of invasive species, there are limited comparative studies with native species growing under natural conditions (Gioria *et al.*, 2018). However, comparative studies on germination and seedling development between invasive and native species are crucial in understanding the invasion process to better perform weed risk analysis and control measures (Guido *et al.*, 2017).

Melastoma malabathricum and *Plectranthus kanneliyensis* are the most common native species occupying the same habitat as *C. hirta*. These three species are frequently found in disturbed sites such as roadsides and canopy gaps in the “Sinharaja” and other rainforests in Sri Lanka (Personal observations of BRCPS and KMGGJ). There is only limited information available on seed germination and seed developmental traits of these three species; a study conducted in Malaysia found that *M. malabathricum* seeds require light to germinate, and plants of this species grown under full light conditions displayed higher rate of growth in terms of number of leaves, fruits and seed production compared to partial sunlight (Faravani and Bakar, 2007). Another study conducted in an undisturbed continental tropical forest at Pasoh, Peninsular Malaysia demonstrated that the relative growth rate of *C. hirta* seedlings was significantly higher in gaps and gap edges than in the forest understory (Peters, 2001), but other studies have shown that it can be found in light conditions ranging from full sunlight to 100% canopy cover, demonstrating its high tolerance to shade (Wester and Wood, 1977). A comparative study conducted in Hawaii and Costa Rica reported that survival, growth and habitat distribution of *C. hirta* was greater in introduced areas than in native areas where invasion is facilitated by lack of effective pathogens and predators in the introduced range (DeWalt *et al.*, 2004a,b).

Hence, this study was designed to compare seed biology and early seedling developmental traits of the invasive species *Clidemia hirta* (shrub; Family:

Melastomataceae) with the native species *Melastoma malabathricum* (shrub; Family: Melastomataceae) and *Plectranthus kanneliyensis* (herb; Family: Lamiaceae). Species occurring in similar habitats tend to share common seed biological traits (Wang *et al.*, 2014; Wyse and Dickie, 2018; Cross *et al.*, 2019; Phartyal *et al.*, 2019). On the other hand, invasive species can possess some seed biological traits that are different from the native species which cause the invasiveness of these species (Martinez-Ghersa *et al.*, 2000; Cervera and Tabla, 2009; Divisek *et al.*, 2018; Li *et al.*, 2018; Zhang *et al.*, 2020). We hypothesized that the invasive species, *C. hirta* may possess seed biological and seedling traits that facilitate successful competition with the native species that share the same habitat.

MATERIALS AND METHODS

Study species and seed collection

Two native species, *Melastoma malabathricum* and *Plectranthus kanneliyensis* sharing the same habitat with *Clidemia hirta*, a tropical invasive species were selected for the study. Further *C. hirta*, like the native analogue species, produced large numbers of seeds which is common to pioneering, light-demanding species (Tiebel *et al.*, 2018). Fruits were collected from at least ten individuals from each of the study species located in the forest roadsides within the Sinharaja Forest Reserve in Sri Lanka (6.4236°N and 80.3881°E) in 2019. The collected fruits were stored in paper bags and seeds were extracted within 1–2 days. Seeds were air dried for 2 hrs to remove the external moisture and stored in sealed containers. Experiments were initiated within 2–3 days of seed collection.

Seed Germination

Four replicates of 100 seeds per species (total of 1200 seeds) were incubated on moistened tissue papers in petri dishes at 25°C inside an incubator under three types of light conditions: full light (with 12-hour darkness), complete darkness, and green shade (with 12-hour darkness), using an artificial light (cool white fluorescent light). Complete darkness was provided by wrapping petri dishes with aluminium foil, while the green shade condition was provided by wrapping petri dishes with green shade house paper (Lal and Sachan, 2017). Green shade house paper was used in reducing the intensity of photosynthetically active wavelength under artificial light (cool white fluorescent light), and to study the impact of transmitting sub-canopy light on seed germination. Seeds were observed for germination at 3-day intervals for 60 days. Conspicuous radical emergence (> 1 mm) was the criterion used to detect germination. Final cumulative germination percentage and time taken to reach 50% germination (T_{50}) was defined using the germination observations.



Seed dormancy

Information gathered from standard germination under light/dark condition was used to determine whether the seeds retained dormancy. Seeds with $T_{50} \leq 30$ days and final cumulative germination $> 75\%$ were considered as non-dormant. Seeds with $T_{50} > 30$ days and final germination $< 75\%$ were considered dormant.

An experiment was conducted to confirm the dormancy of *M. malabathricum* as it was categorized as dormant according to the above criterion. Four replicates [total of 1600 seeds; 4 (replicates) \times 100 (seeds per replicate) \times 4 (storage treatments)] of the *M. malabathricum* were stored dry for 1, 3, 6 and 12 months at 8 °C and then the retrieved seeds were incubated on tissue paper moistened with distilled water in 9 cm diameter petri dishes at 25°C. Further, another sample containing four replicates of *M. malabathricum* seeds (4 replicates \times 100 seeds per replicate) were incubated on tissue papers moistened with 500 ppm Gibberellic Acid (GA_3). Seeds were checked for germination on 3-day intervals for 60 days.

Seed moisture content

Ten replicates of five seeds each (total of 50 seeds) of each species were weighed to the nearest 0.001g and oven dried at 120°C for 3 hrs. Oven dried seed samples were reweighed and the moisture content was calculated on a fresh mass basis (ISTA, 2018) using the following equation:

$$\text{Seed moisture content} = \frac{\text{Initial mass} - \text{Oven dry mass}}{\text{Initial mass}} \times 100\%$$

Desiccation sensitivity/tolerance of seeds

Desiccation tolerance of seeds of the three study species was identified using the 100-seed-test as described in Pritchard *et al.*, (2004). However, as seeds were small, the total number of seeds used for the experiments had to be increased above 100 to achieve a measurable mass. Therefore, we used 200 seeds instead of 100 seeds. A total of 50 freshly collected seeds were used to determine the moisture content of the seeds of each species using ten replicates as described above. Another sample with two replicates containing 25 fresh seeds each was used to conduct an initial germination test. A third sample of 100 seeds was then desiccated by mixing with an equal mass of silica gel in an air-tight container. At the same time, a fourth sample containing 100 seeds were placed in high humidity in a sealed container to maintain humid conditions. Both desiccated and humid samples were held in an incubator at 25°C. For the desiccated sample, the silica gel was changed, and the seeds were weighed every 1–3 days. When the seeds reached a constant mass at equilibrium, usually after 2–3 weeks, 50 seeds were taken to determine the moisture content. The container holding the moist-stored control seeds was opened every 1-3 days for 1 hour to allow

aeration. Then moist stored sample was also removed and the moisture content of 50 seeds was measured. Germination tests were conducted separately for remaining moist stored and desiccated seeds after dividing the remaining 50 seeds into two replicates (25 seeds each).

Seedling growth and survival

Germinated seedlings were transplanted into seedling trays with four replicates for each species. Seven seedlings were used for each species in each tray. Four plastic trays each (35cm \times 25cm) were maintained under full sunlight, 40% light and green light conditions (20% light). Forty percent light was provided by incubating the trays in a greenhouse (poly-tunnel) with a black shade net calibrated to deliver 40% light. Green light condition was provided by using a green clad poly-tunnel with the green material providing transmitted light at 550 nm (264 LUX). All the seedlings were watered at two-day intervals. Light intensity inside the setup was measured using Victor 1010A Digital Lux Meter at 12.30 pm in 9 days intervals to confirm the expected reduction of the light levels in different light treatments. Seedling height and number of leaves of each seedling were recorded at two-week intervals for 98 days. Percentage seedling survival (number of seedlings remained in the trays at the end of the experiment / after 98 days) of each species under each light condition was determined.

Photosynthetic efficiency

The photosynthetic efficiency as quantum yield was taken for the seedling growth and survival experiment using a Fluoro-pen (FP 100, Photon System Instruments) on seedlings, 90 days after germination. For each species, three randomly selected leaves from each seedling from 4 replicates were used to determine the average quantum yield for each species (Maxwell and Johnson, 2000).

Data analysis

Germination time courses were generated by fitting Logistic 4- parameter sigmoidal curves to the germination data. Seed germination parameters were determined from the fitted curve. Percentage seedling survival, growth rate under the various light conditions, mean height and mean no. of leaves per plant were analyzed using a two-way ANOVA. Tukeys' mean separation was used to determine the significant differences between treatments. Data analysis was conducted using MINITAB statistical software (Minitab Inc., State College, Pennsylvania, USA).

RESULTS

Seed germination

Seeds of all the study species germinated $> 50\%$ in the light/dark condition (Table 1, Fig. 1). The highest germination percentage was recorded for *C. hirta* (95%) and the lowest for *M. malabathricum* (53.7%). No species

**Table 1.** Seed germination parameters and seed moisture content of the three study species.

Species Name	Cumulative average germination %			T ₅₀ (days)	Moisture Content (Fresh mass basis%)	Storage behaviour
	Full Light	Complete darkness	Green light			
<i>Clidemia hirta</i>	95.0±10.0 ^A	0.0±0.0	0.0±0.0	28.8	6.3±2.5 ^A	DT
<i>Melastoma malabathricum</i>	53.7±6.3 ^{AB}	0.0±0.0	0.0±0.0	35.0	7.9±1.5 ^A	DT
<i>Plectranthus kanneliyensis</i>	77.5±9.6 ^B	0.0±0.0	0.0±0.0	24.4	13.2±7.7 ^A	DT

Different superscripts letters indicate the statistical difference between species. DT, Desiccation tolerant.

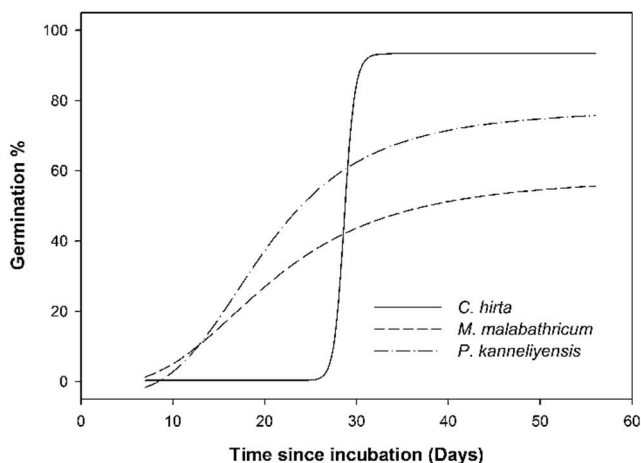


Fig 1. Cumulative germination of *Clidemia hirta*, *Melastoma malabathricum* and *Plectranthus kanneliyensis* seeds incubated at 25°C in light/dark (12/12 hr) condition. Four-parameter logistic sigmoidal curves were fitted to determine the germination behavior.

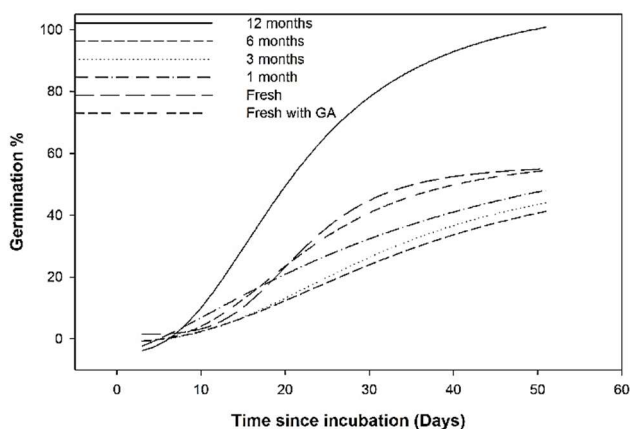


Fig 2. Germination of *Melastoma malabathricum* seeds stored at 8°C for different time periods (12, 6, 3, 1 and 0 [fresh, control] months) and of the fresh seeds treated with Gibberellic acid (GA₃) at 25°C under light/dark (12/12 hr) conditions. Four-parameter logistic sigmoidal curves were fitted to determine the germination behavior.

germinated in the complete darkness and green light conditions. T₅₀ of *P. kanneliyensis* and *C. hirta* seeds was < 30 days while T₅₀ of *M. malabathricum* was > 30 days.

Seed dormancy

As T₅₀ of *C. hirta* and *P. kanneliyensis* seeds were < 30 days and final cumulative germination was > 75 % at

the end of the experiment (by 50 days), seeds of these two species were considered as non-dormant. Although the T₅₀ of *M. malabathricum* was slightly higher than 30 days, final cumulative germination at the end of the experiment (by 50 days) was 55%. Although incubation on tissue papers moistened with 500 ppm Gibberellic Acid (GA₃) did not enhance the germination of *M. malabathricum* seeds, almost all the seeds germinated (100%) after they were stored in dry conditions for 12 months at 8°C and incubated on tissue papers moistened with distilled water (Fig. 2). Since germination has improved after employing dormancy breaking treatment, seeds of *M. malabathricum* were categorized as dormant.

Seed moisture content

The highest average fresh mass was recorded in *P. kanneliyensis* seeds, while the lowest was in *C. hirta*. Similarly, *P. kanneliyensis* and *C. hirta* recorded the highest and the lowest dry mass, respectively. Seed moisture content (MC) of all the study species was <15 %, with the highest MC recorded in *P. kanneliyensis* (13.2%) and the lowest recorded in *C. hirta* (6.3%) (Table 1). However, statistical differences were not observed among species (F=2.5, P=0.358).

Seed desiccation sensitivity/tolerance

C. hirta showed high germination percentage even after desiccation storage compared to the fresh treatment, which is characteristic for desiccation tolerant species. Although *M. malabathricum* and *P. kanneliyensis* seeds germinated after desiccation storage they showed lowered germination percentage compared to the fresh treatment. This result may support greater tolerance of *C. hirta* to desiccation than the two native species. However, when the germination progression curves of study species were compared with the standard germination progression curves by Pritchard *et al.*, (2004), all the species were identified as desiccation tolerant (Fig. 3).

Seedling growth and survival

The average light intensities of full light, 40 and 20% light treatments were 39.6, 16.5 and 7.1 $\mu\text{molS}^{-1} \text{m}^{-2}$, respectively. The 40% light treatment had an average of 42% light, while the 20% treatment averaged 18% light. Light significantly affected the height of seedlings (F=73.06, P < 0.001). The height of seedlings of all the species under 20% light condition were significantly lower compared to that of the other two higher intensity

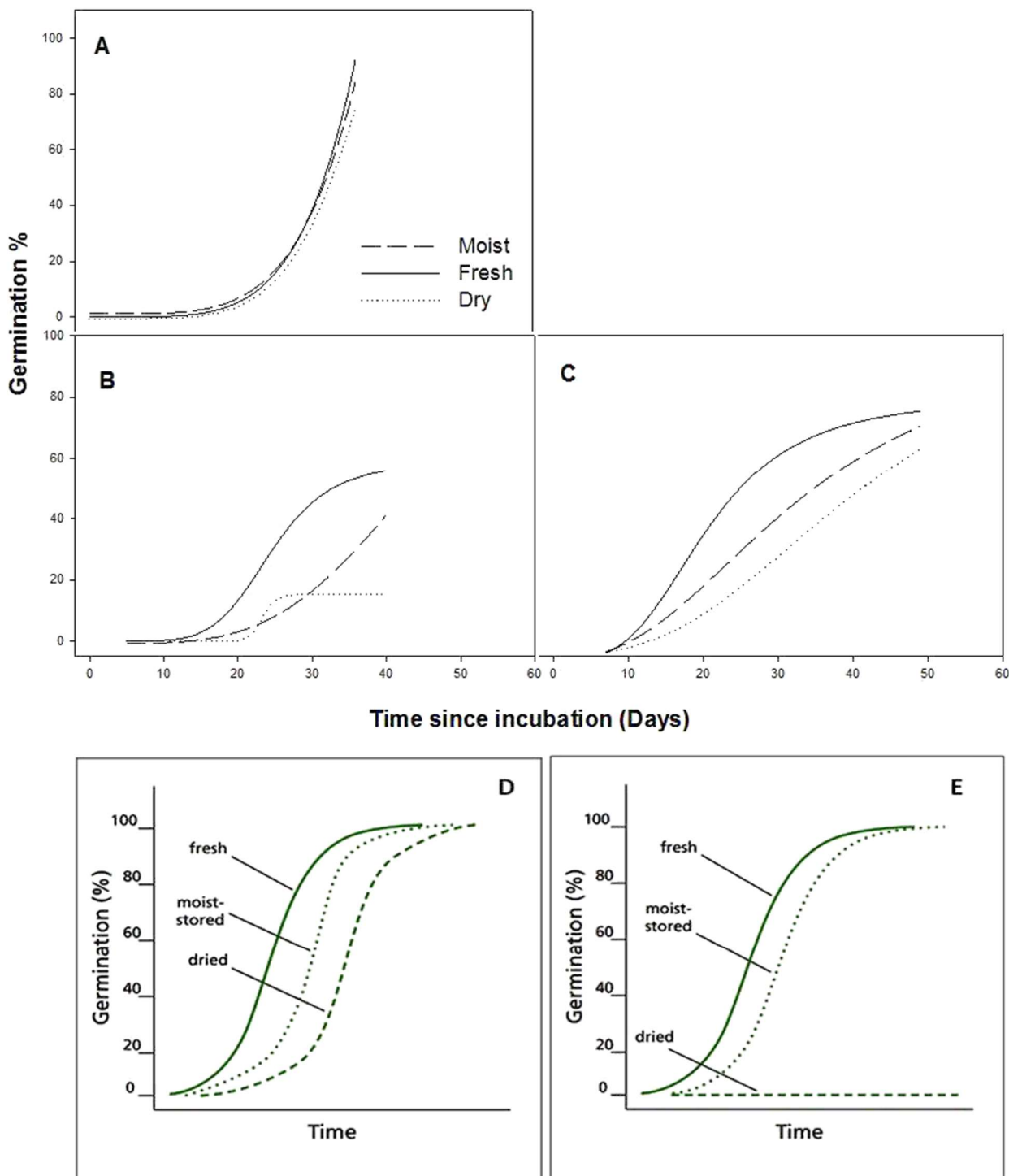


Fig 3. Germination progression curves for fresh, moist stored and dry stored seeds of *Clidemia hirta* (A), *Melastoma malabathricum* (B) and *Plectranthus kanneliyensis* (C) at 25°C in light/dark conditions (12 hr/ 12 hr), and the standard germination progression curves for (D) orthodox and (E) recalcitrant seeds as prepared by Pritchard *et al.*, 2004). Logistic four parameter curves were fitted to determine the germination pattern

light treatments. Further, significant difference in the mean height was observed within treatments ($F=73.06$, $P=0.000$) as well as within species ($F=4.19$, $P=0.025$). Significant differences in the mean number of leaves were

observed within treatments ($F=28.19$, $P=0.001$) as well as within species ($F=7.76$, $P=0.002$). *P. kanneliyensis* had the highest mean growth with the highest mean height (14.7, 15.3 and 9.2 cm, respectively in full, 40 and 20%



light) and the highest mean number of leaves (17, 11.3 and 9.8, respectively in full, 40 and 20% light), whereas *M. malabathricum* had the lowest growth with mean height (1.5 and 0.9 cm, respectively in full and 40% light) and mean number of leaves (6.7 and 4.5, respectively in full and 40% light) under all light treatments, except under the 20% light where *C. hirta* had the lowest growth rate (0.35 cm height and 3.8 leaves) (Fig. 4). However, when considering the effect of the interaction between light treatments and species, there was no significant effect ($F=1.4$, $P=0.259$, $F=1.24$, $P=0.317$).

There was a significant effect on percentage seedling survival observed within treatments ($F=4.69$, $P=0.017$) as well as marginal significance within species ($F=3.26$, $P=0.052$). However, no interactive effect was observed between the two variables ($F=0.71$, $P=0.595$). For all three species, the highest seedling survival was observed in full light condition, while lowest seedling survival was observed in 20% light condition. *C. hirta* had the highest survival rate and *M. malabathricum* had the lowest survival rate regardless of light intensity.

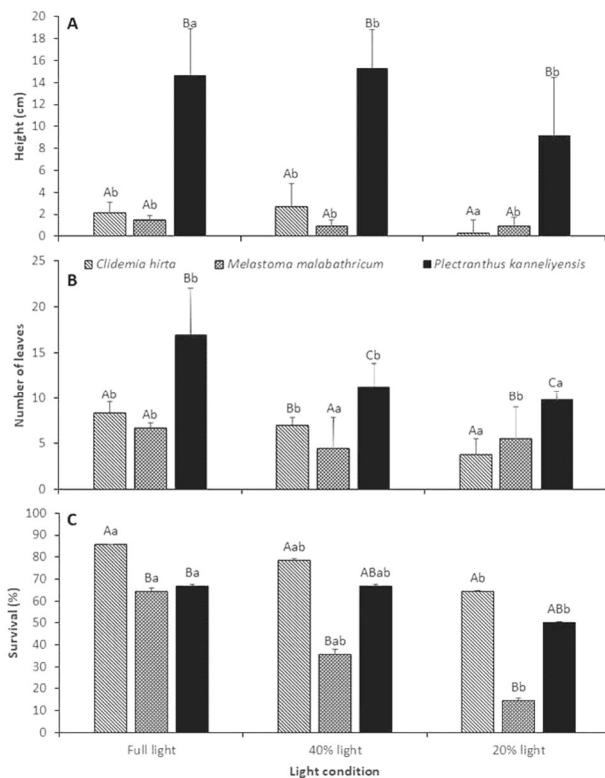


Fig 4. Mean seedling height (A), number of leaves (B) and survival rates (C) of studied species grown under different light treatments. Error bars are + SE. Different uppercase letters indicate the statistical difference between species within the same treatment and different lowercase letters indicate the statistical difference between treatments within the same species.

Photosynthetic efficiency

Significant difference in quantum yield of photosynthesis was observed between light treatments

($F=5.07$, $P=0.015$). However, no differences were present between species ($F=0.68$, $P=0.519$). The highest photosynthetic efficiency was observed in *M. malabathricum* under full light conditions, while the lowest was observed in *P. kanneliyensis* under 20% light conditions (Fig. 5). In all the species, seedlings under full light conditions had higher plant photosynthetic efficiency while 20% light condition had lower plant photosynthetic efficiency.

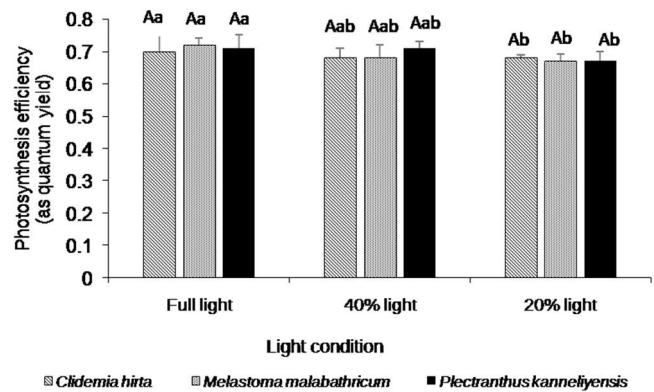


Fig 5. Photosynthetic efficiency of studied species grown under different light treatments. Error bars are + SE. Different uppercase letters indicate the statistical difference between species within the same treatment and different lowercase letters indicate the statistical difference between treatments within the same species.

DISCUSSION

The invasive species *C. hirta* had the highest seed germination and the highest seedling survival in all the conditions tested. The higher competitive ability of invasive species has been assigned to higher germination rate (Xu *et al.*, 2019), survival rate (Cervera and Parra-Tabla, 2009) and higher environmental plasticity (Cano *et al.*, 2008). *C. hirta*, similar to the two native analogue species studied, produced large numbers of seeds resembling R strategy species (Zerbe, 2001) which is common to pioneering, light-demanding species (Tiebel *et al.*, 2018). However, at the end, more *C. hirta* plants remained presumably due to an intrinsically higher germination and seedling survival rates. Interestingly, although none of the seeds of the test species (*C. hirta* and two native species) germinated under low light conditions (20% light and full dark), significant numbers of seedlings and plants of these species survived under low light conditions except for *M. malabathricum* under 20% light conditions. Prior to this study, this phenomenon was not reported. Even under low light conditions the highest survival of seedlings was observed in *C. hirta*, reflecting its high plasticity (Cano *et al.*, 2008). This phenomenon is ecologically significant for early succession light demanding species. As their germination strategy indicates, seeds of these species germinate only in full



light conditions in forest gaps or in disturbed sites. When these species initiate succession, other less light demanding species establish paving the way for the establishment of mid-succession and finally to late-succession species (Bazzas and Pickett, 1980, Finegan, 1984). However, the survival ability of *C. hirta* under low light conditions may help it to persist until reproduction and soil seed bank formation to continue to next generation in a new forest gap. Further, early succession light demanding species must have the ability to produce a seed bank as they have to wait for a forest gap to be created. Lack of germinability of the *C. hirta* seeds under low light conditions appears to be the main reason why *C. hirta* is rarely seen under an intact forest canopy (DeWalt *et al.*, 2004a, Wester and Wood, 1977). However, Waddell *et al.*, (2020) reported that *C. hirta* can be seen in highly shaded parts of the rainforest in Malaysian Borneo, but these plants were smaller compared to those in open areas.

Pioneer, light-demanding species are expected to have the ability to produce a long-lived seed bank (Tiebel *et al.*, 2018). Except *M. malabathricum*, the other two study species produce non-dormant seeds. However, all the three species require light to germinate. Thus, *C. hirta* and *P. kanneliyensis* seeds may reside in the soil until they receive requisite light conditions to trigger germination. *M. malabathricum* seeds may stay in the soil until they receive the required dormancy breaking treatment and then until they receive the requisite light conditions. Further, seeds of all the tested species are tiny (< 0.01 g of dry mass) and almost spherical in shape (data not shown). The size and shape of seeds of the three species allow these to be readily incorporated into the soil seed bank (Yu *et al.*, 2007). Moreover, our study showed that seeds of all the three study species tolerate desiccation. This is another significant character of species producing soil seed banks (Tweddle *et al.*, 2003). However, as shown in Fig. 3, higher desiccation tolerant ability of *C. hirta* compared to the two native species indicated its invasive potential.

Application of GA₃ did not increase germination of *M. malabathricum* indicating that a deeper dormancy process may be involved. Nevertheless, 100% germination was observed for *M. malabathricum* after dry storage for 12 months at 8 °C. Dry storage did alleviate dormancy of *M. malabathricum* seeds. Thus, seeds of *M. malabathricum* were categorized as dormant. In addition, *M. malabathricum* seeds have developed embryos (Subramanyam, 1948) hence morphological or morphophysiological dormancy is most likely absent in this species. According to the literature, seeds of Melastomataceae have permeable seed coats (Baskin and Baskin, 2014). Thus, the dormancy class of this species was identified as physiological dormancy. Since GA₃ application did not improve the percentage germination, this indicates that the seed may have deep physiological

dormancy. Based on these results, higher germination (~100%) of *M. malabathricum* could be expected after 12 months dry storage. Further, after 12 months storage, seeds germinate in synchrony reaching 50% germination within 20 days. Due to higher germination and a lower T₅₀ value, it could be assumed that dry stored *M. malabathricum* could out-compete *C. hirta* seeds and seedlings. Thus, *M. malabathricum* can be used as a replacement native species following *C. hirta* removal. However, the characteristics of seedlings resulting from desiccated seeds of *M. malabathricum* will be needed for evaluation of this species as a candidate to out-compete *C. hirta*.

Light requirements of the study seedlings were species dependent. As expected, the highest seedling survival of all the species was observed in full sunlight. Seedling survival of *C. hirta* under 20% light conditions is similar to seedling survival of *M. malabathricum* under full sunlight. On the other hand, *P. kanneliyensis* seedlings survived under full light and 40% light conditions equally well. Further, even under 20% light conditions, seedling survival of this species was more than 50%. Even though neither *C. hirta* nor *P. kanneliyensis* seeds germinated under low light conditions, if seedlings were shaded after the emergence, they could survive and grow under low light conditions. Survival of *M. malabathricum* was reduced to half of that in full light conditions when grown under 40% light conditions, showing that it requires high-light conditions (around 1720 LUX, resembling a large forest gap or disturbance).

Under all light conditions, *P. kanneliyensis* had the highest growth with the highest height and number of leaves. Thus, seedlings of *C. hirta* and *P. kanneliyensis* have evolved two different strategies to survive under low light conditions. *C. hirta* had higher survival but slower growth rate in comparison to *P. kanneliyensis* under 20 % light conditions. On the other hand, although seedling survival was low in *P. kanneliyensis* compared to that of *C. hirta* at all light conditions, *P. kanneliyensis* have a higher growth rate which is almost equal to that under full light conditions. Similar to *C. hirta*, the growth rate of *M. malabathricum* is low under all the tested light conditions; however, there were no differences in growth rate of *M. malabathricum* in full light, 40 and 20% light. Thus, seedling growth of the three study species exhibit different strategies to altered light availability. However, photosynthesis efficiency of the three species was not significantly different under all light conditions with all species showing reduced photosynthesis efficiency with reduced light availability. Only a slight reduction of photosynthetic efficiency due to reduction of light intensity is indicative of the high shade tolerance ability of seedlings of these species.

Our experiments showed that three study species have different strategies that enabled them to adapt to their



habitat, although they share the same disturbed habitats in tropical lowland rainforests. Among the two native study species, *M. malabathricum* has more specific light requirements, where it can only successfully grow under full light conditions and is likely to be a light specialist. On the other hand, *C. hirta* is a 'light' generalist. In combination with germination responses, seed dormancy, seedling survival and growth indicate that the three study species are exhibiting functional differences. However, functionally *P. kanneliyensis* and *C. hirta* appear to be ecological analogues as they only show differences in seedling responses to low light conditions and rate of germination. However, the higher germination rate and higher seedling survival under any light conditions of *C. hirta* compared to *P. kanneliyensis* may be a key to the higher invasiveness of the former.

The current study identified the ability of *C. hirta* seeds to produce a soil seed bank as it has orthodox storage behaviour and light requirements for germination. However, a soil seed bank study must be conducted to confirm this. Alternatively, high germination percentages can serve as an escape from unfavorable conditions in the seed bank (Xu *et al.*, 2019). Medeiros (2004) reported that *C. hirta* develops a seed bank that persists for at least three years. Further, this study found that high germination rates and higher seedling survival under any light conditions enable *C. hirta* to migrate into and persist with the other two native species. However, given the low mortality rate under any light condition it is predicted that *C. hirta* will outcompete the two native species in disturbed sites. Thus, *C. hirta* could replace these two species in canopy openings and alter growing conditions within such openings. Le *et al.*, (2018) showed that *C. hirta* cannot grow under a closed forest canopy. However, our study showed that although seeds of *C. hirta* could not germinate under low light conditions, seedlings that have already established could grow under low light at a similar rate to full light conditions. *C. hirta*, in its native range occupies a wide range of habitats; forest edges, roadsides, trails, streams and disturbed areas as scattered plants occasionally as thickets (Wester and Wood, 1977). However, Smith (1992) has reported that in the absence of natural controls and fast-growing competitors, it can form dense monotypic thickets even under forest canopies in Hawaii. Smith (1992) also found that rapid growth and its prolific seed production with over 100 seeds per fruit, has been identified as key invasive traits in *C. hirta*.

As outlined above, *C. hirta* seedlings established in canopy gaps can survive even after the canopy closes and produce abundant seeds. This would enable development of the soil seed bank of *C. hirta* and may allow the species to be able to establish in all canopy gaps thereby replacing native gap colonizers. Further, this species can then invade the understorey through this method, leading to greater impacts on intact native ecosystems. Higher seedling survival and higher rate of germination under

any light conditions seems to increase the invasive potential of this species. Thus, management is needed to remove this species from open areas of the rainforests ahead of invasion into other parts of the ecosystem. The best control method is mechanical removal prior to seed production. However, even after mechanical removal of the plants, the soil seed bank must also be depleted. As *C. hirta* seeds are photoblastic, repeated soil tillage undertaken sequentially could deplete the germinable fraction from the soil seed bank. Seedlings that escape from tillage could then be mechanically removed. As tillage may also disadvantage other native species, restoration of appropriate native species assemblages will also be necessary.

CONCLUSION

Our study highlighted that *Melastoma malabathricum*, *Plectranthus kanneliyensis* and *Clidemia hirta* have different survival strategies to adapt to their shared habitat in tropical lowland rainforests. *C. hirta* had more advantageous strategies including higher germination and low mortality in comparison to the two native species. Those strategies allow *C. hirta* to successfully invade open sites of the forest. Since *C. hirta* has become a major threat to the regeneration of native plants in tropical lowland rainforests world-wide, eradication of *C. hirta* through mechanical removal and restoring these biologically rich ecosystems with native species is vital to decrease the invasion into high biodiverse areas.

ACKNOWLEDGMENTS

We thank Forest Department of Sri Lanka for granting the permission to collect seeds and for the field assistance and National Research Council of Sri Lanka (grant number NRC 17-095) for the financial support.

LITERATURE CITED

- Bambaradeniya, C.N.B.** 2002. The status and impacts of alien invasive species in Sri Lanka. *Zoos Print J.* **17(11)**: 930–935.
- Baskin, C.C. and J.M. Baskin.** 2014. *Seeds: ecology, biogeography and evolution of dormancy and germination.* 2nd ed. Academic Press, San Diego, USA. 1600 pp.
- Bazzas, F.A. and S.T.A. Pickett.** 1980. Physiological ecology of Tropical Succession: A comparative review. *Annu. Rev. Ecol. Syst.* **11(1)**: 287–310.
- Bradshaw, C.J., N.S. Sodhi and B.W. Brook.** 2009. Tropical turmoil: a biodiversity tragedy in progress. *Front. Ecol. Environ.* **7(2)**: 79–87.
- Cameron, T.C., H.J. Wearing, P. Rohani and S.M. Sait.** 2007. Two-species asymmetric competition: effects of age structure on intra- and interspecific interactions. *J. Anim. Ecol.* **76(1)**: 83–93.
- Cano L., J. Escarré, I. Fleck, J.M. Blanco-Moreno and F.X. Sans.** 2008. Increased fitness and plasticity of an invasive species in its introduced range: a study using *Senecio pterophorus*. *J. Ecol.* **96(3)**: 468–476.



- Castro J., R. Zamora, J.A. Hodar and J.M. Gómez.** 2005. Ecology of seed germination of *Pinus sylvestris* L. at its southern, Mediterranean distribution range. *For. Syst.* **14(2)**: 143–152.
- Cervera J.C. and V. Parra-Tabla.** 2009. Seed germination and seedling survival traits of invasive and non-invasive congeneric *Ruellia* species (Acanthaceae) in Yucatan, Mexico. *Plant Ecol.* **205(2)**: 285–293.
- Chapple D.G., S.M. Simmonds and B.B. Wong.** 2012. Can behavioral and personality traits influence the success of unintentional species introductions? *Trends Ecol. Evol.* **27(1)**: 57–64.
- Cross, A.T., M.D. Barrett, S.R. Turner, K.W. Dixon and D.J. Merritt.** 2019. Seed-dormancy depth is partitioned more strongly among habitats than among species in tropical ephemerals. *Aust. J. Bot.* **66(3)**: 230–242.
- D'Antonio, C.M.** 2000. Fire, plant invasions, and global changes. In: Mooney H. A. and Hobbs R. J. (eds.). *Invasive species in a changing world*. Island Press, Washington, DC, USA. 65–93pp
- DeWalt, S.J., J.S. Denslow and J.L. Hamrick.** 2004a. Biomass allocation, growth and photosynthesis of genotypes from native and introduced ranges of the tropical shrub *Clidemia hirta*. *Oecologia* **138(4)**: 521–531.
- DeWalt, S.J., J.S. Denslow and K. Ickes.** 2004b. Natural-enemy release facilitates habitat expansion of the invasive tropical shrub, *Clidemia hirta*. *Ecology* **85(2)**: 471–483.
- Divisek, J., M. Chytrý, B. Beckage, N.J. Gotelli, Z. Lososova, P. Pyšek, D.M. Richardson and J. Molofsky.** 2018. Similarity of introduced plant species to native ones facilitates naturalization, but differences enhance invasion success. *Nat. Commun.* **9(1)**: 4631.
- Donohue, K.** 2003. Setting the stage: phenotypic plasticity as habitat selection. *Int. J. Plant Sci.* **164(S3)**: S79–S92.
- Donohue, K.** 2005. Niche construction through phenological plasticity: life history dynamics and ecological consequences. *New Phytol.* **166(1)**: 83–92.
- Donohue, K., R.R. de Casas, L. Burghardt, K. Kovach and C.G. Willis.** 2010. Germination, post germination adaptation, and species ecological ranges. *Annu. Rev. Ecol. Evol. Syst.* **41(1)**: 293–319.
- Faravani, M. and B.B. Bakar.** 2007. Effects of light on seed germination, growth pattern of the straits *Rhododendron (Melastoma malabathricum* L.). *J. Agric. Biol. Sci.* **2(3)**: 1–5.
- Fenner, M.K., M. Fenner and K. Thompson.** 2005. *The ecology of seeds*. Cambridge University Press.
- Finegan, B.** 1984. Forest succession. *Nature* **312(5990)**: 109–114.
- Forbis, T.A.** 2010. Germination phenology of some Great Basin native annual forb species. *Plant Species Biol.* **25(3)**: 221–230.
- Gioria M., P. Pyšek and B.A. Osborne.** 2018. Timing is everything: does early and late germination favor invasions by herbaceous alien plants? *J. Plant Ecol.* **11(1)**: 4–16.
- Global Invasive Species Database** 2020. Downloaded from http://www.iucngisd.org/gisd/100_worst.php on 22-12-2020.
- Guido, A., D. Hoss and V.D. Pillar.** 2017. Exploring seed to seed effects for understanding invasive species success. *Perspect. Ecol. Conserv.* **15(3)**, 234–238.
- Hejda, M., P. Pyšek and V. Jarošík.** 2009. Impact of invasive plants on the species richness, diversity and composition of invaded communities. *J. Ecol.* **97(3)**: 393–403.
- International Seed Testing Association (ISTA).** 2018. *International Rules for Seed Testing*, Bassersdorf, Switzerland, pp 278.
- Jordan, N.R., D.L. Larson and S.C. Huerd.** 2008. Soil modification by invasive plants: effects on native and invasive species of mixed-grass prairies. *Biol. Invasions* **10(2)**: 177–190.
- Kariyawasam, C.S., L. Kumar and S.S. Ratnayake.** 2020. Potential Risks of Plant Invasions in Protected Areas of Sri Lanka under Climate Change with Special Reference to Threatened Vertebrates. *Climate* **8(4)**: 51–71.
- Krupnick, G.A.** 2013. Conservation of tropical plant biodiversity: what have we done, where are we going? *Biotropica* **45(6)**: 693–708.
- Lal, N. and P. Sachan.** 2017. Effect of different visible light wavelengths on seed germination and photosynthetic pigment contents in *Vigna unguiculata* (L.)Walp. *Indian J. Biol.* **4(2)**: 132–136.
- Le, C., K. Fukumori, T. Hosaka, S. Numata, M. Hashim and T. Kosaki.** 2018. The distribution of an invasive species, *Clidemia hirta* along roads and trails in Endau Rompin National Park, Malaysia. *Trop. Conserv. Sci.* **11**: 1–9.
- Li, L., L. Ren, Y. Bai, D. Lecain, D. Blumenthal and J. Morgan.** 2018. Seed traits and germination of native grasses and invasive forbs are largely insensitive to parental temperature and CO₂ concentration. *Seed Sci. Res.* **28(4)**: 303–311.
- Malíková, L., O. Mudrák and J. Klimešová.** 2012. Adventitious sprouting enables the invasive annual herb *Euphorbia geniculata* to regenerate after severe injury. *Ecol. Res.* **27(5)**: 841–847.
- Marambe, B., C. Bambaradeniya, D.K. Pushpa Kumara and N. Pallewatta.** 2001. Human dimensions of invasive alien species in Sri Lanka. In J.A. McNeely (ed.), *The great reshuffling, human dimensions of invasive alien species*. pp 135–145. IUCN Publications Services Unit, Cambridge, UK.
- Martinez-Ghersa, M.A., C.M. Ghersa, R.L. Bench-Arnold, R.M. Donough and R.A. Sanchez.** 2000. Adaptive traits regulating dormancy and germination of invasive species. *Plant Species Biol.* **15(2)**: 127–137.
- Maxwell, K. and G.N. Johnson.** 2000. Chlorophyll fluorescence - a practical guide. *J. Exp. Bot.* **51(345)**: 659–668.
- Medeiros, A.C.** 2004. Phenology, reproductive potential, seed dispersal and predation, and seedling establishment of three invasive plant species in a Hawaiian rain forest. Doctoral dissertation, Louisiana State University, USA.
- Meyer, W.M. and R.H. Cowie.** 2010. Invasive Temperate Species are a Threat to Tropical Island Biodiversity. *Biotropica* **42(6)**: 732–738.
- Moran, E.V., A. Reid and J.M. Levine.** 2017. Population genetics and adaptation to climate along elevation gradients in invasive *Solidago canadensis*. *Plos One.* **12(9)**: e0185539.
- Moravcová, L., P. Pyšek, V. Jarošík and J. Pergl.** 2015. Getting the right traits: reproductive and dispersal characteristics predict the invasiveness of herbaceous plant species. *Plos One.* **10(4)**: e0123634.
- Mullah, C.J.A., K. Klanderud, Ø. Totland and D. Odee.** 2014. Community invasibility and invasion by non-native *Fraxinus pennsylvanica* trees in a degraded tropical forest. *Biol. Invasions* **16(12)**: 2747–2755.
- Phartyal, S.S., S. Rosbakh, C. Ritz and P. Poschlod.** 2019. Ready for change: seed traits to the high adaptability of mudflat species to their unpredictable habitat. *J. Veg. Sci.* **31(2)**: 331–342.
- Peters, H.A.** 2001. *Clidemia hirta* invasion at the pasoh forest reserve: an unexpected plant invasion in an undisturbed tropical forest 1. *Biotropica* **33(1)**: 60–68.



- Pritchard, H.W., C.B. Wood, S. Hodges and H.J. Vautier.** 2004. 100-seed test for desiccation tolerance and germination: a case study on eight tropical palm species. *Seed Sci. Technol.* **32(2)**: 393–403.
- Simmonds, H.W.** 2009. The biological control of the weed *Clidemia hirta* D. Don., in Fiji. *Bull. Entomol. Res.* **24(3)**: 345–348.
- Singhakumara, B.P., R.S.P. Uduporuwa and P.M.S. Ashton.** 2000. Soil Seed Banks in Relation to Light and Topographic Position of a Hill Dipterocarp Forest in Sri Lanka. *Biotropica* **32(1)**: 190–196.
- Smith, C.W.** 1992. Distribution, status, phenology, rate of spread, and management of *Clidemia* in Hawaii. Alien plant invasions in native ecosystems of Hawaii: management and research. University of Hawaii Cooperative National Park Resources Studies Unit, Honolulu. 241–253.
- Subramanyam, K.** 1948. An embryological study of *Melastoma malabathricum* L. *J. Indian Bot. Soc.* **27**: 11–19.
- Tiebel, K., F. Huth and S. Wagner.** 2018. Soil seed banks of pioneer tree species in European temperate forests: a review. *iForest* **11(1)**: 48–57.
- Tweddle, J.C., J.B. Dickie, C.C. Baskin and J.M. Baskin.** 2003. Ecological aspects of seed desiccation sensitivity. *J. Ecol.* **91(2)**: 294–304.
- Waddell, E.H., D.S. Chapman, J.K. Hill, M. Hughes, A. Bin Sailim, J. Tangah, and L.F. Banin.** 2020. Trait filtering during exotic plant invasion of tropical rainforest remnants along a disturbance gradient. *Funct. Ecol.* **34(12)**: 2584–2597.
- Wang, Y., L. Lai, L. Jiang, P. Zhuang, Y. Zhang, Baskin, J.M. and C.C. Baskin.** 2014. Geographic variation in seed traits within and among forty-two species of *Rhododendron* (Ericaceae) on the Tibetan plateau: relationship with altitude, habitat, plant height and phylogeny. *Ecol. Evol.* **4(10)**: 1913–1923.
- Wijesundara, S.** 2010. Defining invasive alien species. Invasive alien species in Sri Lanka - strengthening capacity to control their introduction and spread, 1–6.
- Wyse, S.V. and J.B. Dickie.** 2018. Taxonomic affinity, habitat and seed mass strongly predict seed desiccation response: a boosted regression trees analysis based on 17539 species. *Ann. Bot.* **121(1)**: 71–83.
- Wester, L.L. and H.B. Wood.** 1977. Koster's curse (*Clidemia hirta*), a weed pest in Hawaiian forests. *Environ. Conserv.* **4(1)**: 35–41.
- Wolny, E., A. Betekhtin, M. Rojek, A. Braszewska-Zalewska, J. Lusinska and R. Hasterok.** 2018. Germination and the early stages of seedling development in *Brachypodium distachyon*. *Int. J. Mol. Sci.* **19(10)**: 2916.
- Xu, X., L. Wolfe, J. Diez, Y. Zheng, H. Guo and S. Hu.** 2019. Differential germination strategies of native and introduced populations of the invasive species *Plantago virginica*. *NeoBiota* **43**: 101–118.
- Yang, S.Z.** 2001. A new record and invasive species in Taiwan, *Clidemia hirta* (L.) D. Don. *Taiwania* **46(3)**: 232–237.
- Yu, S., M. Sternberg, P. Kutiel and H. Chen.** 2007. Seed mass, shape, and persistence in the soil seed bank of Israeli coastal sand dune flora. *Evol. Ecol. Res.* **9(2)**: 325–340.
- Zhang, J., E. Siemann, B. Tian, W. Huang, and J. Ding.** 2020. Differences in seed properties and germination between native and introduced populations of *Triadica sebifera*. *J. Plant Ecol.* **13(1)**: 70–77.
- Zerbe, S.** 2001. On the ecology of *Sorbus aucuparia* (Rosaceae) with special regard to germination, establishment and growth. *Pol. Bot. J.* **46(2)**: 229–239.