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Germination Biology and Occurrence of Polyembryony in Two Forms of Cats Claw Creeper Vine, *Dolichandra unguis-cati* (Bignoniaceae): Implications for Its Invasiveness and Management

Joshua C. Buru[1*], Kunjithapatham Dhileepan[2], Olusegun Osunkoya[2,3], Tanya Scharaschkin[1]

[1]Earth, Environmental and Biological Sciences, Science and Engineering Faculty, Queensland University of Technology, Brisbane, Australia
[2]Department of Agriculture, Forestry and Fisheries, Biosecurity Queensland, Eco-Sciences Precinct, Brisbane, Australia
[3]College of Marine and Environmental Sciences, James Cook University, Cairns, Australia
Email: *joshuacomrade.buru@hdr.qut.edu.au

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Abstract

Cat's claw creeper vine, *Dolichandra unguis-cati* (L.) Lohmann (syn. *Macfadyena unguis-cati* (L.) Gentry), is a major environmental weed in Australia. Two forms of the weed with distinctive leaf morphology and reproductive traits, including varying fruit size, occur in Queensland, Australia. The long pod form occurs in a few localities in Queensland, while the short pod form is widely distributed in Queensland and northern part of New South Wales. This investigation aimed to evaluate germination behavior and occurrence of polyembryony (production of multiple seedlings from a single seed) in the two forms of the weed. Seeds were germinated in growth chambers set to 10/20˚C, 15/25˚C, 20/30˚C, 30/45˚C and 25˚C, representing ambient temperature conditions of the region. Germination and polyembryony were monitored over a period of 12 weeks. For all the treatments in this study, seeds from short pod plants exhibited significantly higher germination rates and higher occurrence of polyembryony than those from long pod plants. Seeds from long pod plants did not germinate at the lowest temperature of 10/20˚C; in contrast, those of the short pod form germinated under this condition, albeit at a lower rate (reaching a maximum 45% ger-

*Corresponding author.

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mination at week 12). Results from this study could explain why the short pod form of *D. unguis-cati* is the more widely distributed plants in Australia, while the long pod is confined to a few localities. The results have implication in predicting future range of both forms of the invasive *D. unguis-cati*, as well as inform management decisions for control of the weed.

Keywords

***Macfadyena unguis-cati*, Plant Sexual Reproduction, Plant Invasion, Propagule Pressure, Seed Ecology, Woody Vine**

1. Introduction

Plant invasions result in environmental degradation [1], heavy financial costs [2] and loss of biodiversity [3]. Understanding plant traits contributing to invasiveness may thus help in determining the best way to control invasive species [4]. Many biotic and abiotic hypotheses have been proposed to explain why some species become invasive [5]-[7]. While factors such as species-specific traits in determining invasiveness may be important (e.g. high specific leaf area, competitiveness, greater morphological and physiological plasticity than co-occurring native/introduced, but non-invasive species, niche pre-emption, and release from natural enemies in the novel environment) [8] [9], there is an increasing evidence that propagule pressure (size, number of individuals introduced, temporal and spatial patterns of arrival and establishment in a novel ecosystem) plays a proportionally major role in driving invasion success [10]-[12].

Alongside of propagule pressure, the reproductive strategy of invasive plants also plays a significant role at all the stages of the invasion process. Versatility in reproductive strategies ensures variable range of environments in which the invasive plants can proliferate and spread into [6]. Time-to-germination initiation and rate of germination are measurable characteristics that can be used to predict the success of any species in a given environment [13]. Most plant species germinate optimally within a narrow range of environmental conditions, but the ability to germinate under different environmental conditions (*i.e.*, germination plasticity) can be an adaptation to maximize fitness, especially for invasive species in novel environments [6] [12]. An important cue for seed germination is the ambient temperature, especially during period of soil water availability [14]. The interactive effects of temperature and light conditions may also substantially influence germination and thus enhance the survival and establishment of the seedling stage [15], perhaps through provision of synergistic environmental resources.

Additionally, some plant species portray a rare phenomenon of polyembryony (the formation of extranumerary embryos in single seeds; [16] [17]), that has been shown to further increase the propagule pressure of a species in novel environments [18]. Such embryos arise from either apomictic (asexual) or amphimictic (sexual) processes [19]. Occurrence of polyembryony is ascertained through emergence of multiple seedlings from a single seed during germination [20]. Although little is known about the ecological consequences of polyembryony [18], any process that increases the number of individuals to the next generation is advantageous as it adds to the propagule pressure [10]. However, some evidence suggests that polyembryony may be disadvantageous due to competition between polyembryonic siblings from early developmental stages to seedling establishment (eg. [19]). Although polyembryony is widely reported in angiosperms, it is prevalent in only a few families, including Myrtaceae, Cactaceae, Rutaceae, Anacardiaceae and Bignoniaceae [21]. In the family Bignoniaceae, polyembryony has been reported in species like *Handroanthus ochraceus*, *H. chrysotrichus* [22], *Anemopaegma acutifolium*, *A. arvense*, *A. glaucum* and *A. scabriusculum* [20].

Cat's claw creeper vine, *Dolichandra unguis-cati* (L.) Lohmann (syn. *Macfadyena unguis-cati* (L.) Gentry) (Bignoniaceae), a native of the Greater and Lesser Antilles, Mexico, South and Central America to Argentina, including Trinidad and Tobago [23], was initially introduced to Australia as an ornamental plant in the late 1800s, but now a major environmental woody weed [24]. It has also been introduced to other parts of the world, such as southern and central Africa, Asia, North America and parts of Europe, where it is now also classified as an environmental weed [25]. Cat's claw creeper has recently been listed as a Weed of National Significance (WoNS) in Australia [26]. It is also included in the Global Invasive Species Database (GISD) [27].

D. unguis-cati regenerates both asexually (vegetatively) by production of a large number of golf-size subterranean tubers as well as sexually with production of numerous papery seeds [24] [28]. In forest landscapes infested by *D. unguis-cati*, the weedy vine smothers the tree canopies and its biomass can build to a point where it collapses the tree canopy structures [29]. At the same time, it may create thick mats on forest floors that choke out any existing low vegetation and hamper seedling recruitment [24]. This growth pattern transforms natural habitats into monospecific stands and may result in loss of floral biodiversity and changes in soil biota and chemistry [30] [31].

In Australia, two morphologically and phenologically distinct forms of *D. unguis-cati* occur [32]. These forms have been informally referred to as long pod (LP) and short pod (SP) plants based on their average (+SE) fruit length at maturity (LP plant: 70.024 ± 2.35 cm; SP plant: 30.089 ± 8.96 cm). The LP and SP forms have been shown to carry an average of 120 ± 10.67 and 60.89 ± 23.17 seeds per pod at maturity, respectively. Seeds of both forms are two-winged, papery and flattened/oblong in shape, 10 - 18 mm long, 4.2 - 5.8 mm wide. The average seed biomass is not significantly different between the forms of *D. unguis-cati* (mean seed biomass for LP plant: 16.6 ± 0.65 gm and for SP plant: 15.65 ± 0.83 gm) [32]. The SP plant is the more prevalent form in Australia invading more than 2000 km stretch of coastal lands of South East Queensland (SE QLD) and northern New South Wales (NSW), while the LP plant occurs only in a few isolated localities in SE QLD (Dhileepan K, per. observation; [33]). In the literature, the SP plant is also the form that is widely referred to in the entire introduced range of the weed [33] [34]. As LP plant occurs only in few localities of southeast Queensland, it does not appear to be as invasive as the SP plant form, but the cause for this difference in level of prevalence between the two plant forms is not known. Thus it is natural to start the search for differences in prevalence between the two forms in its invaded range by examining their seed biology.

Seed germination dynamics of both forms of *D. unguis-cati* have not been adequately studied in Australia or elsewhere. The only study on the seed bank ecology of the SP plant form [35] found it to have low seed longevity, usually less than 12% and 1% at 1 year for soil-surface (<1 cm depth) and buried seeds (5 cm depth), respectively. The same study also inferred occurrence of ~40% polyembryony due to emergence of multiple seedlings from single seeds [35]. However, as their study was only on SP plant, it was not known whether the same frequency of polyembryony occurs in the LP plant. In addition, Vivian-Smith and Panetta [35] did not confirm the presence of polyembryony using established methods (e.g., radicle emergence and seedling separation) nor did it differentiate between the different classes of polyembryony. Interestingly a study from its native range mentioned that *D. unguis-cati* did not exhibit any polyembryony [20].

The aims of this study were to 1) determine whether there are differences in seed germination behaviour of two form of *D. unguis-cati*, documenting the range of environmental resources of temperature and photo-regime over which seeds of LP and SP plants will germinate; 2) confirm if polyembryony occurred in both forms of *D. unguis-cati*; and 3) if yes, test for differences in the frequency and classes of polyembryony between the two forms of the weed.

2. Materials and Methods

2.1. Acquisition and Storage of Seeds

Seeds of the long pod (LP) and the short pod (SP) forms of *D. unguis-cati* were collected during the fruiting months of 2013 from various sites around the greater Brisbane area in South East Queensland, Australia. Seeds of SP plants were obtained from the following infestation sites: South Bank (27˚55'S, 153˚01'E), Ipswich Forest Reserve (27˚32'S, 152˚42'E), Chelmer (27˚47'S, 152˚58'E), Bardon (27˚30'S, 152˚41'E) and Boonah (27˚60'S, 152˚41'E). Seeds of LP plants were collected from Carindale (27˚30'S, 152˚41'E), Bardon (27˚30'S, 152˚41'E) and Sherwood (27˚30'S, 152˚59'E). Fewer populations of LP plants were sampled because this form occurs only in a few sites in SE QLD; also flowering (and consequently fruit formation) does not occur every year [32]. Seeds were collected from dried fruits (pods) just before dehiscence of the pod to ensure maturity of the seeds [24]. Once collected, seeds were stored for two weeks at room temperature in paper envelopes that were placed in containers with silica gel to ensure they were kept dry before germination assay. For the purposes of this experiment, seeds collected from various sites were bulked per pod form of *D. unguis-cati*, though we recognise that there could be differences in germination behaviour between individual plants and amongst sites for a given plant pod form.

2.2. Experimental Design

Seed germination: Seeds were physically screened to ensure they were firm and intact. Those that appeared not to have viable embryonic content and/or damaged by insects were not included in the germination assays. Seeds were sterilised by soaking in 1% sodium hypochlorite (NaOCl) for 5 minutes, then rinsed in water for 3 minutes [14]. Sterilised seeds were placed in 15 cm diameter petri dishes lined with 2 - 3 layers of 15 cm Whatman filter paper (No. 1) moistened with distilled water. Thereafter they were exposed to varying temperature regimes in growth chambers (model ADAPTIS A1000; Conviron Ltd., USA) at the Queensland University of Technology (QUT) in Brisbane, QLD-Australia. Germination conditions were set to 1) cool (10/20˚C), 2) moderate (15/25˚C), 3) warm (20/30˚C) and 4) hot (30/45˚C) temperature regimes for 12 hours at each alternate temperature. Seed germination took place in light/darkness conditions (12-hour photo-period) or constant 24 hours darkness. When a 12-hour photo-period was applied, the higher temperature corresponded with presence of light. Most of the temperature regimes followed the conditions applied by Vivian-Smith and Panetta [35] and also reflect the night-day temperature fluctuations experienced by the focal species in its range in SE QLD. Other treatments, consisting of germination at constant room temperature (25˚C) with 5) 12 hour light/darkness, or 6) 24 hour darkness were added. Fifteen (15) replicates of 20 seeds each were used for each pod form in each treatment. Germination data were recorded every seven (7) days for 12 weeks after which the assay was terminated due to logistic reasons, and because no more appreciable germination was observed. After the 12th week, the ungerminated seeds were checked and found to be mostly rotten with no visible viable embryo; testing the embryo of ungerminated seeds confirms loss of viability, except for those in the low temperature (10/20˚C). A dim light was used to examine the seed germination in the continuous darkness treatment.

Each seed was considered to have germinated with the emergence of one or more radicles [15]. Total germination percentage was calculated from the total number of germinated seeds divided by the total number of seeds. Germination rate index (GRI) was calculated following the Equation (1) of Maguire [36],

$$\mathrm{GRI} = \left(\mathrm{N_i}/\mathrm{D_i}\right), \tag{1}$$

where N_i represents germinated seeds on the ith day and D_i is the number of days from the commencement of the germination assay to the ith day (see also [14]). GRI was determined at six and 12 weeks for each treatment. Cumulative mean germination data (%) were plotted against time (weeks) from which the following indices were extracted: time-to-initiation of germination (T_1), time to 50% (T_{50}), and time to maximum germination (65% (T_{65})) in this study (see [13]).

2.3. Estimation of Occurrence of Polyembryony

During germination, if only one radicle emerged, seeds were considered mono-embryonic, and if with two or more radicles, then polyembryonic [20]. Total percentage polyembryony was calculated from the proportion of seeds showing two or more radicles at germination for a given treatment. Seeds showing polyembryony were grouped into classes (twins, triplets and quadruplets) depending on the number of radicles that emerged during germination [19].

2.4. Fate of Polyembryonic Seedlings

The consequence of polyembryony in the two forms of *D. unguis-cati* was further determined through seedling establishment [19] [20]. After germination, polyembryonic siblings with at least one pair of leaves were taken out of the germination petri-dishes and transferred into 20 cm plastic pots filled with locally available commercial soil (Osmocote Multi-Purpose Potting Mix with trace elements). Seedlings were left to grow in a light environment (range: 60 - 250 µmol·m^{-2}·s^{-1}) over a 1 year period to ascertain if the individual seedlings would establish independent of other siblings, and whether each sibling would develop separate roots and tubers or not.

2.5. Data Analysis

Germination percentage data were arcsine transformed before analysis to improve normality of residuals. Analysis of variance (ANOVA) was used to test the effects of light, temperature regimes, and pod form of *D. unguis-cati*, *as well as* their interactions on germination indices and occurrence of polyembryony. The form of *D. unguis-cati*, light, and temperature regimes were used as fixed effects on the ANOVA model. A Tukey HSD

post-hoc test was performed to assess the germination and polyembryony differences between the temperature treatments. When no significant interactions were detected, a Pearson's χ^2 statistical test was used to compare the frequency of polyembryonic seeds of LP and SP plant forms. All statistical tests were carried out at $\alpha < 0.05$ using the *R* statistical program on R version 3.1.0 [37].

3. Results

3.1. Temperature and Light Effects on Time-to-Start and Rate of Germination

At all the temperature regimes, the seeds of the short pod (SP) plant form showed rapid germination whilst those of the long pod (LP) plant was gradual and slower (**Table 1**; **Figure 1**). Across the temperature range and photoperiod cycles tested, it took seeds of SP plants an average of 11.5 days to initiation of germination (T_1), except for the cool 10/20˚C regime where up to 28 days was required to T_1 (**Table 1**). It took seeds of LP plants significantly longer period (an average of 20.2 days) to initiation of germination (T_1) across temperatures, other than 10/20˚C in which no germination was recorded (**Figure 1(a)**). Note that the inhibitory effect of low temperatures (10/20˚C) on germination was more pronounced on seeds of the LP than those of SP plants, since the former did not germinate at all at this temperature. When the experiment was terminated, seed germination in SP at 10/20˚C had reached about 42.9% (**Figure 1(a)**). However, the gradient of the curve indicate that had the experiment continued, more seeds would have germinated with time, and therefore a higher total germination percentage could have been attained at this temperature too.

Time to 50% germination (T_{50}) was also significantly lower for seeds of SP plants (range: 14 - 34 days) when compared to those of LP plants (range: 30 - 84 days) across the temperature cycles tested (**Table 1**). Light did not have any significant effect on T_1, T_{50} or T_{65} for both LP and SP plants at 20/30˚C (**Figure 1(c)**), but that was not the case at constant 25˚C for LP plants (**Figure 1(d)**). Light had a significant germination effect on T_{50} on LP plants at 25˚C, but not on seeds of SP plants. At 25˚C, it took seeds of LP plants under light condition, 30 days to reach T_{50} but >84 days to reach T_{50} in constant darkness (**Table 1** and **Figure 1(d)**). In contrast, at 25˚C constant condition, T_{50} value was the same for seeds of SP plants (14 - 16 day), irrespective of the light conditions.

The rate (speed) of germination estimated by Maguire's germination rate index (GRI) showed significantly higher germination rates for seeds of SP plants than LP plants at all temperature regimes (**Figure 2**). The magnitude of the difference in germination rate between the two pod forms was also greater at 6 weeks compared to 12 weeks, especially for the moderate (15/25˚C) and warm (25˚C and 20/30˚C) temperature regimes (**Figure 2(a)**, **Figure 2(b)** and **Table 2**). At 6 weeks, GRI values for all interaction effects (pod form x light; pod form x temperature, light x temperature, and form x light x temperature) were significant ($P < 0.05$; **Table 2**), suggesting that GRI response values for seeds of each plant pod form varied significantly depending on light and/or

Table 1. Effect of temperature cycles and light regimes on the time (days) to initiation of germination (T1), time to 50% (T50) and time to final (65% (T65)) germination for two forms of *D. unguis-cati*. These data were extracted from cumulative mean germination percentage curves plotted as a function of time. LL: Low light = 24 hour or constant darkness; HL: high light = 12 hour photoperiod; SP: short pod plants; LP: long pod plants. Data from the 30/45˚C temperature regime were not included in the table because germination incidents were minimal.

	Time (days) to % germination					
	T_1		T_{50}		T_{65}	
Treatment	**SP**	**LP**	**SP**	**LP**	**SP**	**LP**
10/20˚C + LL	28	N/A	>84	N/A	>84	>84
15/25˚C + LL	21	28	34	>84	45	>84
20/30˚C + HL	7	28	30	58	70	>84
20/30˚C + LL	7	28	22	61	42	>84
25˚C + HL	3	10	16	30	27	>84
25˚C + LL	3	7	14	>84	77	>84
Mean response	11.5	20.2	>33.3	>63.4	>57.5	>84

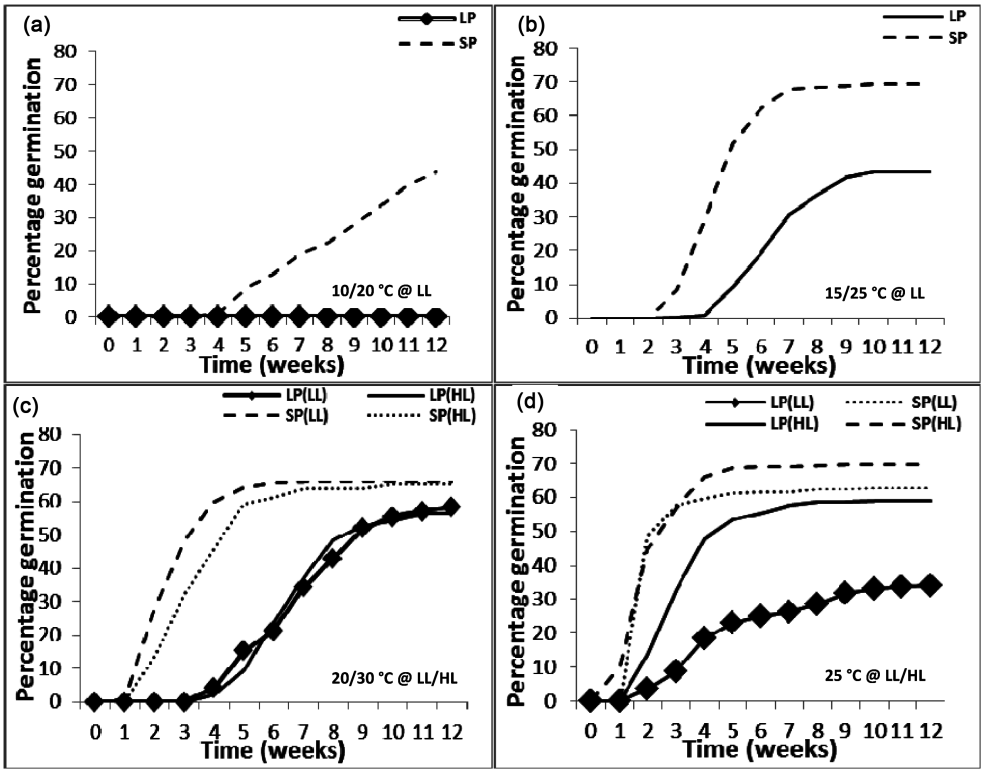

Figure 1. Cumulative mean germination percentage as a function of time for two forms of *D. unguis-cati* seeds (long pod (LP) and short pod (SP) plants at different cycles of 12 hour low/high temperatures and photoperiod. LL: Low light = constant darkness; HL: High light conditions = 12 hour photoperiod. (a): 10/20˚C with constant darkness; (b): 15/25˚C with constant darkness; (c): 20/30˚C with both constant darkness and light conditions; (d): room temperature (25˚C) with both constant darkness and light conditions. Cumulative germination curves for the 30/45˚C temperature regime were not included because of insignificant germination incidents.

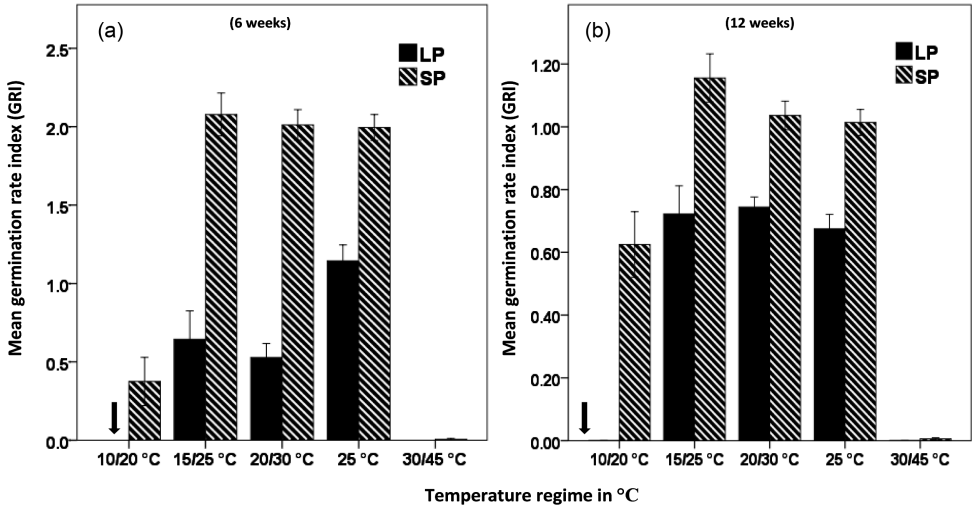

Figure 2. The effects of temperature on germination rate index (mean ± s.e.m) on two forms of *D. unguis-cati*, long pod (LP) and short pod (SP) plants. (a) Germination rate index at 6 weeks since start of germination; (b) Germination rate index at the end of the germination assay, *i.e.*, 12 weeks since start of germination. Data for light and darkness levels were combined at each temperature regime because they were not significantly different. Arrows indicate no germination incidents by LP plants at 10/20˚C.

temperature conditions. In contrast at 12 weeks, only the interaction effect of plant pod form x light was significant ($F_{4, 218} = 9.05$; $P < 0.0001$) (**Table 2**), implying a greater role of light than temperature regime on this germination index.

3.2. Occurrence and Frequency of Polyembryony in LP and SP

Polyembryony occurs in both plant forms of *D. unguis-cati* as demonstrated by emergence of two or more radicles from single seeds during germination (**Figure 3(a)**). However, there was a significant difference in the frequency of polyembryony between LP and SP plants ($\chi^2 = 71.730$, df = 1, $p < 0.002$). SP plants displayed a significantly higher frequency of polyembryony than LP plants (SP plant: 38.52% ± 2.74%; LP plant: 4.68% ± 1.13%) (see **Figure 4** and **Figure 5**). The polyembryonic seedlings lack any connections and were easily separated from each other, with each shoot system detaching with a corresponding radicle or root system (**Figure 6**).

Table 2. Summary results of ANOVA showing effects of plant form of *D. unguis-cati*, temperature and light regimes on the germination rate index (GRI) and total germination %. Significant effects are shown in bold.

Source of variation	df	GRI (6 weeks)		GRI (12 weeks)		Germination %	
		F-ratio	*P*-value	F-ratio	*P*-value	F-ratio	*P*-value
Form	1	174.148	**<0.0001**	102.664	**<0.0001**	82.604	**<0.0001**
Light	4	136.222	**<0.0001**	193.577	**<0.0001**	2.287	0.132
Temp	1	0.299	0.585	0.068	0.794	115.657	**<0.0001**
Form * Light	4	27.126	**<0.0001**	9.053	**<0.0001**	0.005	0.942
Form * Temp	1	5.164	**0.024**	2.368	0.125	9.732	**<0.0001**
Light * Temp	2	3.27	**0.04**	1.186	0.307	5.334	**0.005**
Form * Light * Temp	2	3.201	**0.043**	2.236	0.109	0.023	0.977
Error	218						
Total	234						

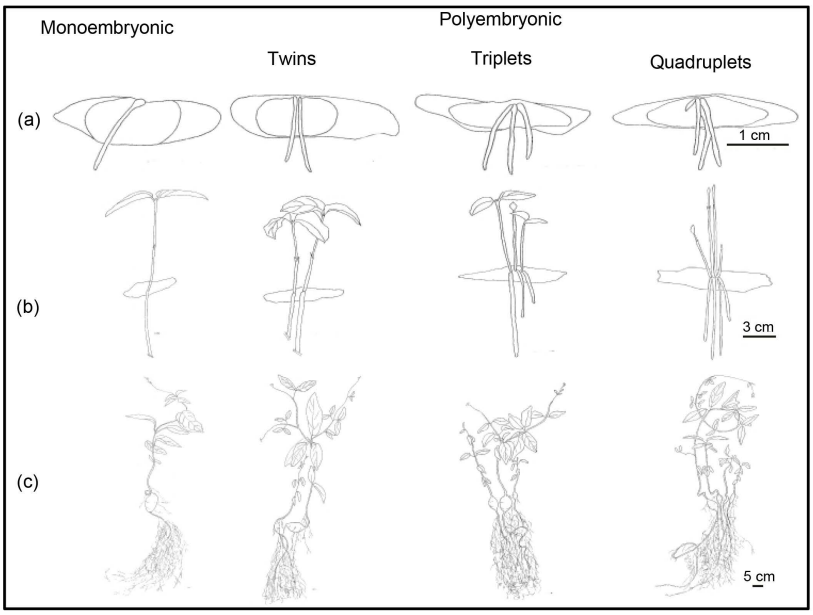

Figure 3. Illustrations of monoembryonic and polyembryonic seedlings of *D. unguis-cati* at different stages of development. Top row (a): Seeds showing emergence of radicles one week since start of germination. Middle row (b): Seedlings at 4 - 6 week since start of germination. Bottom row (c): One year old seedlings, with polyembryonic seedlings clearly showing independent development of tubers and root systems (Illustrations by Tanya Scharaschkin).

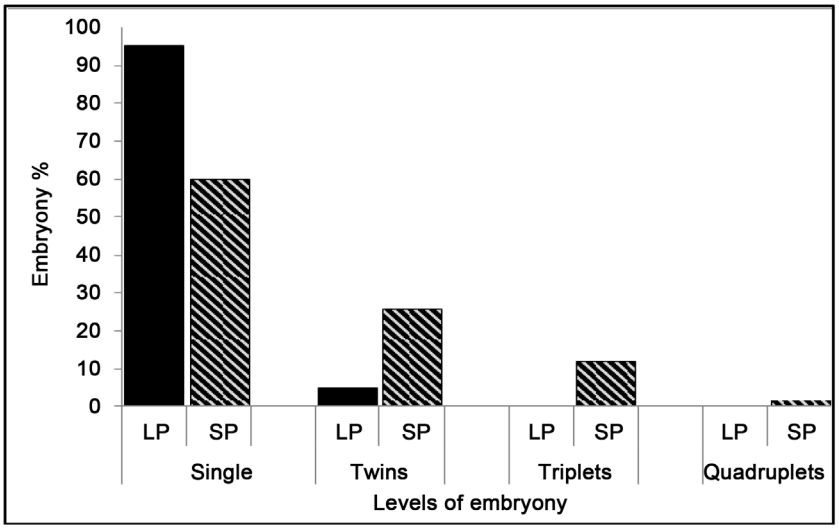

Figure 4. Frequency of different classes of polyembryony in the two forms of *D. unguis-cati*, long pod (LP) and short pod (SP) plants. Only twin seedlings were observed in LP polyembryonic seeds whereas SP also had triplet and quadruplet seedlings. Illustrations represent seedlings between 4-6 weeks since emergence of radicle.

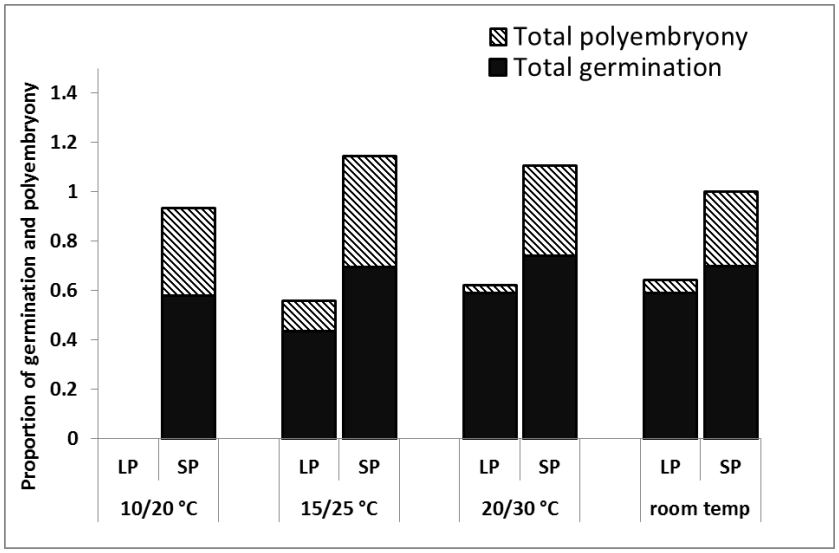

Figure 5. Comparison of the proportion of germination percentage (darkercolor) and polyembryony percentage (diagonal lines) of LP and SP plants for the same temperature regimes: 10/20˚C, 15/25˚C, 20/30˚C and room temperature (25˚C). Differences in polyembryony frequency between temperature regimes were not significant within each form of *Dolichandra unguis-cati* (F-value = 0.902, df = 1, p value = 0.345).

3.3. Classes of Polyembryony in LP and SP

Three classes (twin, triplets and quadruplets) of polyembryonic seedlings were observed in this study (**Figure 3**). In all, single (60.4%; N = 628/1040), twin (25.5%; N = 265/1040), triplet (11.7%; N = 122/1040) and quadruplet (2.01%; N = 21/1040) seedlings were observed in SP plants (**Figure 4**). In LP plant form, all polyembryonic seedlings were twins, and constituted only 4.68% (N = 42/897) of germinated seeds (**Figure 4**).

3.4. Seedling Establishment of Polyembryonic Siblings

Separated polyembryonic seedlings were able to develop and establish individually when transplanted into

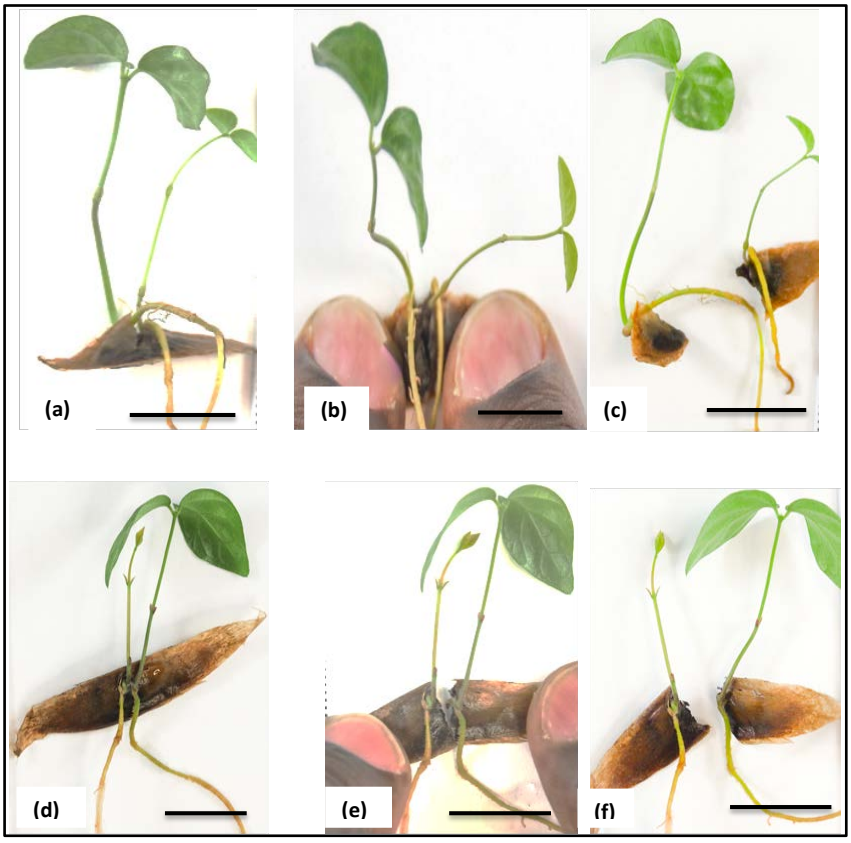

Figure 6. Multiple seedlings emerging from a single seed of *Dolichandra unguis-cati* four weeks since start of germination; (a)-(c): short pod (SP) and (d)-(f): long pod (LP). Intact seeds showing emergence of twin seedlings (a) (d); (Partial separation of seeds into two halves to separate the two seedlings from the same seed (b) (e); Complete separation of seedlings emerging from the same polyembryonic seed (c) (f). Scale bars represent 1 cm.

growth media; they developed their own root system with tubers (**Figure 3(c)**). When left intact and transferred to growth media, polyembryonic siblings still developed individually with independent root and tuber systems. We also observed that for each set of polyembryonic siblings, one seedling was more robust and had a larger subterranean tuber than that of other siblings (**Figure 3**).

3.5. Polyembryony and Germination Rates of LP and SP Plants

This study established that SP plants exhibited higher germination indices and higher frequency of polyembryony than LP plants, irrespective of temperature regimes (**Figure 5**). There were no significant interaction effects of temperature and/or light regime on the occurrence, frequency or classes of polyembryony (temperature x plant form: F-ratio = 0.594, df = 1, p = 0.443; light x plant form: F-ratio = 0.021, df = 1, p = 0.886), implying that these environmental resources did not influence the dynamics of polyembryony. Nonetheless, both LP and SP plants showed their highest frequency of polyembryony (15.9% and 41.2%, respectively) at 15/25˚C (**Figure 5**). At 30/45˚C, only a few incidents (0.3%) of germination (and no incidence of polyembryony) were observed for SP plants, and none at all for LP plants.

4. Discussion

Our results indicate that, on average, there are significant differences in the seed germination responses of LP and SP plants of the invasive *D. unguis-cati* under varying environmental resources of temperature and light regimes. Seeds of SP plants exhibits higher mean value as well greater variation in its germination niche (from

cool to warm temperatures) than those of LP plants (warm temperatures only) (**Figure 1** and **Figure 2**). The polyembryony results confirm previous findings by [35] of occurrence of multiple seedlings from single seeds following germination in *D. unguis-cati*. However, the two forms of *the* invasive woody vine exhibit different frequency of polyembryony: about 40% in SP plants and a much lower frequency (often <5%) in LP plants. It will be worthwhile to investigate if the same phenomenon of polyembryony is observed in other *D. unguis-cati* invaded ranges around the globe, as it will shed more light on the mechanisms of the weed invisibility patterns via its propagule pressure.

4.1. Predicting Invasiveness Potential of the Two Forms of *D. unguis-cati*

Our results suggest that germination niche requirements of the SP plants are broader, non-specific, while the LP plants germinate optimally only under warmer temperature conditions (20/30˚C and 25˚C; **Figure 1**; **Table 1**). Flexible germination cues enhance the invasive capacity of plants by enabling them to spread and establish in novel climatic conditions of recipient communities [38] [39]. SP plants had a much higher germination rate (compared to LP plants) at the cooler temperature regime of 10/20˚C. Equally, although of low frequency, seeds of SP plants showed evidence of germination incidents at hot, 30/45˚C temperature regime while there was no germination at all for LP plants under this scenario. These wider germination amplitudes may indicate greater resilience in seeds of SP plants, and may suggest a potential for this form of *D. unguis-cati* to spread further both into the cooler state of NSW and Victoria as well as into the warmer/hotter areas of Australia (e.g., Northern Territory and western QLD), especially under a climate change scenario. In general, the rapid germination behaviour of seeds of SP plants (**Figure 1**) is typical of invasive species [38] [40]. Whilst the longevity of seeds of SP plants is low (<12% by 1 year) after dispersal [35], its rapid germination under a wider range of temperatures may confer a fitness advantage in terms of seedling establishment and spread.

The higher frequency of polyembryony in seeds of SP plants could also allow it to proliferate successfully in a variable environment in contrast with those of LP plants (**Figure 4** and **Figure 5**). Thus for the same number of initial seeds introduced in a new environment, there is a greater likelihood that more SP rather than LP plants would establish. Considering that twins, triplets and quadruplets occur in polyembryonic seeds of SP plants, while only twins occur in those of LP plants (and even more so at a lower frequency), it is safe to assume that a higher propagule pressure would be exerted by SP than LP plants upon introduction. Polyembryony may also increase invasiveness potentials by the bet-hedging strategy, which ensures that at least one individual seedling from a polyembryonic seed survives [41] [42]. Although mature LP fruits are known to have twice as many seeds per fruit as SP fruits [32], higher germination and polyembryony exhibited by SP plants are likely to increase propagule pressure leading to SP plants being the more invasive than LP plants. Propagule pressure has previously been correlated with plant invasiveness [43]-[45]. Nonetheless, caution is needed in the interpretation of this finding because the polyembryony phenomenon may not necessarily be adaptive as it puts siblings into direct competition for environmental resources [18] [42].

Some evidence suggests that polyembryony reduces seed germinability significantly [19], but our results do not support this position. SP plants consistently had higher germination rates than LP plants at all temperature regimes considered, despite seeds from SP plants having a higher frequency of polyembryony. Similarly, SP plants showed significantly higher frequency of polyembryony than LP at the same temperature regimes. Temperature did not significantly affect expression of polyembryony, suggesting a genetic basis for the phenomenon [46], but both LP and SP appeared to show relatively higher polyembryony frequencies at 15/25˚C (their optimal growth condition) than at other temperature regimes. Our preliminary observations of 1-year old siblings from polyembryonic seeds indicate equal survival rates, but slower mean growth rates per individual as the number of siblings increase (**Figure 3**; JC Buru, unpublished data). However, it remains to be seen how differences in germination and polyembryony rates will translate to initial biomass gain per individual, and ultimately offspring fitness.

4.2. LP and SP: Should They Be Treated as More than One Species?

The differences in germination dynamics and frequency of polyembryony between SP and LP plants lends further credence to suggestions that the two forms of *D. unguis-cati* could be two extremes of the same species or even different species [33]. Anecdotal evidence suggests that the predominant form of *D. unguis-cati* in the native range is similar in appearance to the form being referred to as LP plants in Australia (Dhileepan K. personal

comm.). So how did the SP plant form become the "chosen one" in the invaded range of Australia? The differences in the invaded range distributions of the two forms could be a product of colonization event, with the LP form being a more recent arrival in Australia compared to the SP form. There may also be an underlying genetic basis responsible for the observed pattern [47]. Though largely unproven and hence suggests scope for more work, the prevalence of the SP plant form in SE QLD may potentially be a classic case of 1) evolutionary, though geologically recent, whole genome duplication followed by diploidization during which genes are lost/modified/rearranged [48] [49], and/or 2) release from natural enemies in the novel environment; both scenarios have the tendency to increase competitiveness, niche pre-emption, and ultimately spread and distribution [5] [50] [51]. Interestingly, an earlier study conducted within the native range found that *D. unguis-cati* did not exhibit any polyembryony [20]. This is in sharp contrast to what we have observed in SP plants (40%), but is closer to our results for LP plants (<5%). Whether the two forms are different species, products of two independent introductions from the native range of the weed, or arose from (auto-/allo-) polyploidy remains to be determined. Only a comprehensive phylogenetic study of the different forms of the species with other members of the *Dolichandra* genus, and comprising detail work on chromosome number (karyotype), level of polyploidy (a trait known to correlate significantly with polyembryony [20] [47]), as well understanding the interplay of their breeding systems (sexual vs. asexual (e.g., apomixis) variation (see [28]), will help clarify the status of the weed (but see [34]) both in its native and invaded ranges.

5. Conclusions and Recommendations

The current study is one of the first to report on the comparative germination rates of the two forms of *D. unguis-cati* in Australia. Further germination assays could involve other conditions such as different moisture thresholds and seed burial and retrieval experiments to explore the extent and contribution of above environmental cues to variation in invasiveness of the two plant forms. To ascertain the ecological consequence (fitness) of polyembryony on *D. unguis-cati*, future studies should consider comparing growth rates of mono- and polyembryonic embryonic seedlings in intra- and inter-specific competition. Due to the vast differences in the germination behaviour (this study), floral [26] and leaf morphological/physiological traits of the two forms of *D. unguis-cati* ([33]; Buru J, unpublished data), different control strategies should be considered for these two forms. Currently, the same chemical and biological control strategies are used to manage the two forms, thus potentially compromising the efficacy of these control options. Leaf-feeding biocontrol agents have been developed, inadvertently only tested on SP plants [26] [52] [53], but applied to both LP and SP plants. Hence whether these agents will equally work on leaf of the LP plant is unknown. The need for additional pod or seed eating biocontrol agents was highlighted by [28]. In light of the current study findings in which we have demonstrated potential roles of propagule pressure and polyembryony as drivers of spread of *D. unguis-cati*, indeed the search for fruit-pod and seed attacking biocontrol agents as additional arsenals would be appropriate for both forms of the weed, but perhaps more so for the SP plants, the more prevalent form of the weed in Australia.

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