Substrate Type and Temperature on Germination Parameters of Butterfly Pea

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SUMMARY. Butterfly pea (*Clitoria ternatea*) is a unique perennial and leguminous plant that produces brightly colored flowers that can be used as a pH-dependent natural food colorant in culinary and cosmetic preparations. Butterfly pea is commonly propagated from seed. Because of the increased interest in its commercial applications, effective production techniques are necessary to ensure consistent and successful commercial production. The objective of this research was to determine the influence of the substrate type and temperature on butterfly pea germination. Two substrate types (rockwool and commercial soilless substrate mix) and three temperatures (70, 80, and 90 °F) were evaluated to determine their effects on germination of butterfly pea seed. Collected and calculated germination data included germination capacity (G), mean germination time (MT), coefficient of variation of the germination time (cv_t), mean germination rate (MR), uncertainty of the germination process (U), and synchrony of the germination process (Z). Differences were observed among substrate temperatures for the MT, cv_t , and MR values, with germination greater at both 70 and 80 °F than at 90 °F. Similarly, significant differences among substrate types were observed for the G, MT, cv_t , and MR values, with germination of seeds in rockwool outperforming seeds in soilless substrate mix. Because of the high priority for successful and uniform germination in commercial plant production operations, the results suggest that commercial germination of butterfly pea would be best in rockwool at 70 °F. Results of this study can be used for the commercial production of butterfly pea, for which propagation from seed is the primary means of plant production.

Originated in the Asian tropics, butterfly pea (*Clitoria ternatea*) is an herbaceous, perennial, leguminous twiner that has become naturalized in the East and West Indies, South and Central Americas, India, and China (Barik et al., 2007; Morris, 2009; Park and Knox, 2016). Flowers are deep blue to purple; when dried, butterfly pea flower extract can be used as a traditional food colorant with a longer shelf life

and greater ease of use than synthetic blue colorants (Siti Azima et al., 2017). Recent health concerns associated with the consumption of synthetic food colorants (Chu et al., 2016; McCann et al., 2007) will likely further popularize the use of butterfly pea in food and beverage preparation, especially in the United States, where recent increased interest in this plant and its applications have already been documented (Simonson, 2016).

Soilless substrate is commonly a mix of organic and inorganic components blended for a particular use or application, and commercial plant production often relies on germination blends or mixtures containing high proportions of peat or coir to

facilitate high water retention characteristics. An alternative substrate, rockwool is primarily composed of basaltic rock heated to ≈ 1600 °F and then spun to create a fibrous mass. Use of rockwool in commercial plant production operations has increased in recent years through the expansion of countries importing rockwool, an increase in the number of crops grown in rockwool, and through the development of a wider range of products containing rockwool (Bussell and McKennie, 2004). This has multiple advantages over soil-based substrates, primarily in the sterility, consistency, and water- and nutrient-holding capacities of the substrate. High temperatures required to manufacture rockwool render it biologically inert and, therefore, free from potential weeds, pests, and diseases that might normally hinder germination. This manufacturing process also renders a very consistent substrate that possesses an ideal air-to-water ratio for horticultural production, especially for seed germination (Allaire et al., 2005).

Butterfly pea is a heavy seeder, with between 6 and 3400 total seeds per plant produced from an assay of 19 varying accessions of butterfly pea. This high seeding rate has led to germination from seed as the primary method of propagation for butterfly pea (Fig. 1) (Morris, 2009). High levels of plant-available water are important for butterfly pea seedlings, with the literature recommending field seeding following a rainfall event (650–1250 mm) and when the possibility for subsequent rain is likely (Collins and Grundy, 2005). More accurately, Das et al. (2017) determined that the inherent moisture content of butterfly pea seeds is 6.8%; although that can be altered to between 5% and 12% with no negative effects, germinability was maximized at 10% seed moisture content. This is often accomplished through scarification or compromise of the seedcoat. Nagar and Meena (2015) found that

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
0.3048	ft	m	3.2808
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
16.3871	inch ³	cm ³	0.0610
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

Fig. 1. Dried butterfly pea seed pods remaining on the vine from the previous year's growth (left). Butterfly pea seeds after being removed from the dried seed pods described (middle). Seven-day-old butterfly pea seedlings in soilless substrate under a vented humidity dome (right).

either physical (abrasion with sandpaper) or chemical (soaking in sulfuric acid) scarification methods can increase germination by 70.5% compared to that of a control (Makasana et al., 2016).

Research examining the influence of temperature on germination success has been evaluated for butterfly pea; however, conflicting observations of the optimal germination temperature (T_o) appear in the literature. Selvamaleeswaran et al. (2011) found that exposing butterfly pea seed to temperatures of 16, 28, 30, and 35 °C resulted in germinability rates of 56%, 60%, 65%, and 20%, indicating a peak T_o of 30 °C and a drastic reduction in germination at 35 °C. This reduction was likely caused by thermo-inhibition, or the inability of seeds to germinate at high temperatures. Similarly, McDonald (2002) evaluated butterfly pea seed germination at temperatures between 8 and 44 °C, with peak germination between 24 and 28 °C and a rapid decrease in germinability above or below that range. Thermo-inhibition was again observed at the highest imposed temperature treatments (≥ 40 °C). The mean germination rate followed a similar trend, increasing proportionally with an increased substrate temperature from 8 to 28 °C, maximizing at T_o of 32 °C, and then declining between 36 to 40 °C. Differences in observations reported by Selvamaleeswaran et al. (2011) and McDonald (2002) were likely caused by their use of cotton and paper, respectively, as germination media.

Because of the conflicting responses to imposed temperature treatments and a lack of research examining seed germination in commonly used commercial media, research examining the influence of substrate composition and temperature on germination of butterfly pea is needed for commercial plant production. To address this need, six germination metrics were measured and recorded for seed of butterfly pea in two varying common commercial germination media subjected to one of three temperature treatments. Results from this work will provide plant producers and growers with critical information necessary to successfully germinate butterfly pea.

Materials and methods

SEED. Three different trials were performed using the same experimental design: one each per week across the span of 3 weeks beginning on 17 Sept. and ending on 8 Oct. 2018. For each trial, 216 butterfly pea seeds were harvested from a single plant grown in a 100- × 48-ft gutter-connected greenhouse with 30% light-reducing polycarbonate paneling located in Apopka, FL (lat. 28.64°N, long. 81.55°W). On 17 Sept., 24 Sept., and 1 Oct., day 0 for each experimental trial, seeds for each trial were scarified mechanically using a razor blade according to the procedure described by Mackay et al. (2001) before being soaked in deionized water (DI) water for 24 h.

SUBSTRATE TYPE. Two different substrate types were used for the experiment: soilless substrate mix

(30% pine bark, 48% peat, 10% perlite, and 12% vermiculite; Fafard 4P; Sun Gro Horticulture, Agawam, MA) and rockwool (Grodan, Roermond, The Netherlands). On 18 Sept., 25 Sept., and 2 Oct., day 1 for each experimental trial, three 36-count soilless substrate plug trays (1.5 inches long × 1.5 inches wide × 2.25 inches high) in half. The trays were then filled with soilless substrate, and each plug was seeded with a single scarified and soaked butterfly pea seed ≈ 0.25 inch under the soils surface. Three 36-count rockwool cube sheets were also prepared by cutting 98-count rockwool cube sheets (1.5 inch³), soaking them for 30 min in water adjusted to pH 5.5 using a pH down buffering solution (General Hydroponics, Sebastopol, CA), and then seeding with the scarified and soaked butterfly pea seeds. The six substrate sheets were placed in a propagation unit located within a laboratory environment.

SUBSTRATE TEMPERATURE. The propagation unit used in this experiment consisted of a multitier shelved structure (Compact SunLite 3-Tier Garden; Gardener's Supply Co., Burlington, VT), with each of the three shelves equipped with a 7.5-inch vented humidity dome (HydroFarm, Petaluma, CA), a 10- × 20.75-inch heat mat (Vivosun, Shanghai, China), fluorescent lighting (T5; Sunblaster, Langley, BC, Canada), and a mini thermohygrometer (Mondi, Vancouver, BC, Canada) to measure environmental conditions within each dome. A digital thermohygrometer (AcuRite, Lake Geneva, WI) was placed on the exterior of the propagation unit to record ambient environmental conditions, with mean temperatures and humidity of 73 °F (56%) and 73 °F (55.5%), respectively, for trials 2 and 3 (data for trial 1 were unavailable). Heating mats were programmed to maintain substrate temperatures of 70, 80, and 90 °F for the three experimental substrate temperature treatments imposed in the study. Top and side vents of the humidity domes were fully closed to maintain a constant humidity level. Lights were positioned ≈ 2 inches from the top of the dome, providing illumination between 55 and 90 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the outer corners and the center of the substrate sheet, respectively,

throughout the duration of the experiment. Each of the six substrate sheets had ≈ 25 mL of water applied daily using a laboratory wash bottle (Thermo Fisher Scientific, Waltham, MA).

GERMINATION PARAMETERS. Six germination parameters were collected, calculated, and reported throughout the experiment according to the procedures outlined by Ranal et al. (2009). These included the following: germination capacity (G); mean germination time (MT); coefficient of variation of the germination time (cv_t); mean germination rate (MR); uncertainty of the germination process (U); and synchrony of the germination process (Z). G, also referred to as germinability, is characterized as the binary response of whether a fully formed cotyledon is visible above the substrate surface at the end of the observations (germinated/nongerminated). That qualitative value was then converted to a quantitative attribute, percentage, for the entire treatment group for statistical analyses and reporting. MT is the mean of the germination time per day weighted by the number of seeds germinated per data measurement time interval (days). The mean germination time provided a quantifiable assessment of the average amount of time necessary for maximum germination of an experimental group. Use of the weighted mean for this measurement accounts for variance in the number of seeds germinated per time interval. The cv_t was a measurement of the variability in relation to the MT, allowing for additional comparisons independent of the mean germination time magnitude.

Calculated as the reciprocal of MT, the MR quantified germination rate increases and decreases in relation to $1/MT$ rather than MT alone. U was a measurement of the degree of uncertainty associated with the frequency and distribution of germination within an experimental group. Because germination of only one seed can change U, this measurement value quantified the degree of spread of germination as influenced by temporal factors. Conversely, Z represents the degree of overlap that existed during germination and is only produced when two or more seeds finished germination within

the same time interval (Ranal et al., 2009).

EXPERIMENTAL DESIGN. The experiment was organized as a 2×3 factorial in a completely randomized design, with substrate type and substrate temperature assigned as independent variables. Three replicates were cultivated for each treatment combination, one per experimental trial. Substrate temperature, vented humidity dome interior temperature and humidity (percent), and ambient laboratory temperature and humidity (percent) were recorded daily for the portions of trial 1 (20–23 Sept.), trial 2 (26 Sept.–1 Oct.), and trial 3 (3–8 Oct.) when germination was occurring (Table 1). Germination parameters were analyzed based on a collected response and then reported as percentages; a restricted maximum likelihood mixed model analysis was performed using the data collected from the 36 germinated seeds per experimental trial (JMP[®] Pro 14; SAS Institute, Cary, NC) with post hoc mean separation tests performed using Tukey's honest significant difference test and a germination trial with variance within treatment combination replicates defined as the random error term. Statistical tests were considered significant if $P \leq 0.05$.

Results

TEMPERATURE AND HUMIDITY. With some notable exceptions, the substrate temperature, vented humidity dome interior temperature and humidity, and ambient laboratory temperature and humidity were all consistent within expectations because measurements correlated positively with the increasing experimental temperature treatments. Although the mean interior temperature was higher for the 80 °F treatment than the 70 °F treatment for trial 3, this measurement is considered secondary to the substrate temperature, which is a more accurate representation of the experimental treatment being applied. These inconsistencies were likely caused by fluctuations in the activity of the heating mats, which are attached to a controller that uses a thermometer placed in the substrate to regulate activation and duration to maintain the desired substrate temperature.

GERMINATION PARAMETERS. No significant interactions existed between

substrate temperature and type for any of the six germination parameters; therefore, the main effects of substrate temperature and substrate type were analyzed separately as independent factors (Table 2). Differences in germination capacity (G) were observed for substrate type ($P = 0.03$), with rockwool (91.7%) resulting in greater germination compared with the soilless substrate (83.6%). G values of 70 °F (92.6%), 80 °F (88.4%), and 90 °F (81.9%) for the substrate temperature treatment groups indicated no significant difference, but the germination capacity was increased with increasing substrate temperatures (Table 2, Fig. 2).

Similar trends were observed for the MT, cv_t , and MR parameters, with differences noted among substrate temperature and substrate type treatments. The MT of the substrate temperature treatments decreased as the temperature was increased from 70 °F (2.2 d) to 80 °F (1.9 d) to 90 °F (1.7 d) ($P = 0.01$), whereas rockwool (2.1 d) had a slower germination rate than the soilless substrate (1.8 d) treatment ($P = 0.01$). Therefore, a trend between higher MT values and increased G values was noted and further reinforced by the MT values for the substrate temperature $\times$ substrate type interaction effect; the 70 °F rockwool (2.3 d), 80 °F rockwool (2 d), and 70 °F soilless substrate (2 d) experimental treatments had the highest G values at 98%, 92%, and 87%, respectively.

Increased variations were observed within cv_t values as the substrate temperature was increased for the 70 °F (20.4%), 80 °F (27.4%), and 90 °F (37.7%) treatments ($P < 0.01$), whereas the substrate type affected the cv_t values of the rockwool (23.6%) and soilless substrate (33.4%) treatments ($P = 0.01$). This would indicate that lower levels of variation result in higher G values, which again was supported by the cv_t values for the substrate temperature by substrate type interaction effect; the 80 °F rockwool (19.4%) and 70 °F rockwool (19.8%) treatments were the most uniform, followed by the 70 °F soilless substrate treatment (21.1%).

Finally, as the substrate temperature increased, so did the MR values for the 70 °F (0.47 d^{-1}), 80 °F (0.53 d^{-1}), and 90 °F (0.60 d^{-1}) treatments ($P = 0.03$). Substrate type was similarly affected in the rockwool (0.49 d^{-1})

Table 1. Recorded substrate temperature, vented humidity dome interior temperature, and humidity for trials 1, 2, and 3 of the 70, 80, and 90 °F substrate temperature treatments for butterfly pea seeds.

Treatment temp. (°F) ^z	Recorded substrate temp. (°F)			Interior temp. (°F)			Interior humidity (%)		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
70	75.7 ^y	77.4	77.5	75.2	76.6	76.5	66.7	68.8	65.5
80	80.6	80.8	80.6	75.7	76.6	75.6	67.7	71.0	69.2
90	82.8	82.8	83.7	79.2	78.6	77	70.5	69.8	71.2

^z(°F – 32) ÷ 1.8 = °C.

^yMean of the environmental parameters collected daily for trial 1 (20–23 Sept.), trial 2 (26 Sept.–1 Oct.), and trial 3 (3–8 Oct.).

Table 2. Germination parameters for substrate temperature (70, 80, and 90 °F) independent variables, substrate [rockwool (RW) and soilless substrate (SS)] independent variables, and temperature × substrate interaction effects for butterfly pea seeds.

Temp. (°F) × substrate ^z	G (%) ^y		MT (d)		cv _t (%)		MR (d ⁻¹)		U		Z	
	P = 0.77*		P = 0.93		P = 0.15		P = 0.65		P = 0.36		P = 0.17	
70 × RW	98.15	a	2.29	a	19.82	a	0.44	a	0.79	a	0.64	a
70 × SS	87.04	a	2.04	a	21.06	a	0.49	a	0.84	a	0.68	a
80 × RW	91.67	a	2.04	a	19.42	a	0.49	a	0.74	a	0.73	a
80 × SS	85.19	a	1.74	a	35.37	a	0.58	a	1.25	a	0.46	a
90 × RW	85.19	a	1.86	a	31.66	a	0.54	a	1.12	a	0.56	a
90 × SS	78.70	a	1.54	a	43.74	a	0.67	a	1.27	a	0.46	a
Temp (°F)	P = 0.07		P = 0.01*		P < 0.01*		P = 0.03*		P = 0.13		P = 0.19	
70	92.59	a	2.16	a	20.44	b	0.47	b	0.82	a	0.66	a
80	88.43	a	1.89	ab	27.40	b	0.54	ab	0.99	a	0.60	a
90	81.94	a	1.70	b	37.70	a	0.60	a	1.19	a	0.51	a
Substrate	P = 0.03*		P = 0.01*		P = 0.01*		P = 0.02*		P = 0.11		P = 0.12	
RW	91.67	a	2.07	a	23.63	b	0.49	b	0.88	a	0.64	a
SS	83.64	b	1.77	b	33.39	a	0.58	a	1.12	a	0.54	a

^z(°F – 32) ÷ 1.8 = °C.

^yG = mean germination capacity, MT = mean germination time, cv_t = coefficient of variation of the germination time, MR = mean germination rate, U = uncertainty of the germination process, Z = synchrony of the germination process.

*Significance at $P \leq 0.05$; means (n = 36 per experimental trial) within column with the same letter are not significantly different via Tukey's honest significant difference test ($P \leq 0.05$).

Fig. 2. Germinated butterfly pea seedlings in 36-count trays, three each for the rockwool and soilless substrate treatments, for the 70, 80, and 90 °F (21.1, 26.7, and 32.2 °C) substrate temperature treatments on the final day (23 Sept.) of experimental trial 1.

and soilless substrate (0.58 d⁻¹) treatments ($P = 0.03$). Following the same trend as MT, a longer germination time and subsequent lower germination rate exhibited the highest G values; the 70 °F rockwool (0.44 d⁻¹) treatment combination had the lowest germination rate, followed by 70 °F soilless substrate (0.49 d⁻¹) and 80 °F rockwool (0.49 d⁻¹).

Although the uncertainty of the germination process (U) and the synchrony of the germination process (G) responded similarly to treatments, observed trends offer insights into factors influential to the germination of butterfly pea. Lower U values indicated that germination was more concentrated in time; the rockwool and soilless substrate treatments had U values of 0.88 and 1.12, respectively, whereas higher Z values exhibited by rockwool (0.64) as compared with those exhibited by the soilless substrate (0.54) treatment identified a higher degree of germination overlap. Similar trends were observed for

Fig. 3. Germinability (primary axis, clustered columns) of butterfly pea seeds in relation to the recorded substrate temperature (secondary axis, markers) for experimental trials 1, 2, and 3 of the rockwool and soilless substrate treatments and the 70, 80, and 90 °F substrate temperature treatments. $(^{\circ}\text{F} - 32) \div 1.8 = ^{\circ}\text{C}$.

substrate temperatures with U values of 0.82, 0.99, and 1.19 and Z values of 0.66, 0.60, and 0.51, for the 70, 80, and 90 °F treatments, respectively.

Discussion

In the context of seed germination parameters, T_o is defined as the temperature at which maximal G is observed. This is often achieved in the shortest MT and with the highest MR. Reductions in seed G are observed when substrate temperatures fall below [suboptimal ($< T_o$)] or exceed [supra-optimal ($> T_o$)] the T_o at a rate proportional to the absolute value of the difference. This occurs until either the lowest [base substrate temperature (T_b)] or the highest [ceiling substrate temperature (T_c)] temperatures at which germination can proceed are reached, resulting in thermo-inhibition. Although these phenological events and their underlying physiology are well-documented, there is some contest over the definition of T_o , primarily regarding whether it is a distinct temperature or a range of temperatures (Watt and Bloomberg, 2012).

Four of the six germination parameters (G, MT, cv_t , and MR) were optimized when seeds were germinated in rockwool, with more consistent germinability when compared with those germinated in soilless substrate. Our results indicate that rockwool is a superior germination media compared to the soilless substrate mix

for butterfly pea seeds, likely due to superior porosity and water-holding capacity of the rockwool, more consistency due to its manufacturing process, a lack of competitive organisms, or a combination of the three factors (Allaire et al., 2005). This also supports the conclusions of Collins and Grundy (2005) that butterfly pea requires high levels of available water during germination and seedling stages.

Similar trends were observed among treatments for the substrate temperature independent variable, with differences among three of the six germination parameters (MT, cv_t , and MR). Considering the relationship theorized between G values and increasing substrate temperatures, it can be concluded that butterfly pea produces a higher percentage of viable germinated seeds in a more consistent amount of time and at a more consistent rate when substrate temperature is decreased from 90 to 80 to 70 °F (Fig. 3).

This is consistent with T_o values presented in the literature. McDonald (2002) concluded that the T_o was between 24 and 28 °C, with G declining drastically at sub-optimal or supra-optimal temperatures. Although our observed T_o is lower than values reported by McDonald (2002), the temperature at which thermo-inhibition occurred was similar. Differences in T_o values between studies were likely due to the differences in substrates used; McDonald relied on

moist seed-test filter paper in 9-cm petri dishes to achieve germination. In their review, Olle et al. (2012) determined that when Grodan rockwool is saturated and then allowed to drain completely by gravitational pull, the remaining slab will contain 5% rockwool fibers, 15% pore space, and 80% water. The authors concluded that this ratio of water, or nutrient solution, to air promotes vigorous root growth.

Conclusions

Although faster germination and, therefore, lowered MT are often desired to reduce production time in commercial plant cultivation operations, this can lead to uneven or restricted emergence and a lack of cultivation consistency. Because uniformity is a high priority, the results suggest commercial germination of butterfly pea is maximized when the rockwool substrate is used at a substrate temperature of ≈ 70 °F. This is further confirmed by G values for the substrate temperature $\times$ substrate type interaction effects, where the 70 °F rockwool, 80 °F rockwool, and 70 °F soilless substrate treatment combinations exhibited the highest numbers of viable germinated seeds at 98%, 92%, and 87%, respectively. Although substrate temperature and type were both shown to significantly influence the germination success of butterfly pea, the influence of other commercial production parameters not evaluated in this study may be important.

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