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Spider Plant (*Cleome gynandra* L.): An Emerging Weed in the Sweet Corn–Brassica Cropping System

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Abstract: Spider plant (*Cleome gynandra* L.) is an emerging noxious weed, affecting cultivated vegetables in Queensland, Australia. It is a prolific seed producer, forming large seedbanks with variable seedling emergence. A study was carried out to investigate the seed biology of spider plant, focusing on its seed germination ecology, viz., influence of temperatures, illumination conditions, medium salinity, pH, substrate moisture, burial depth, and after-ripening. Freshly harvested seeds were negatively photoblastic and had combinational dormancy. Improved germination was obtained by physical scarification followed by soaking for 16 h, by dry storage for over 6 months, and by the imbibition of gibberellic acid. Maximum germination percentages of 70 to 80% were recorded under constant darkness at alternating day/night temperatures of 20/30 °C, or with 18 to 27 °C constant temperatures. Spider plant showed a broad tolerance to pH but only moderate salt and moisture stress tolerance, since only 42 and 26% germination were observed with 60 mM NaCl and at −0.40 MPa, respectively. Seeds placed on the soil surface did not germinate, however, at a burial depth of 1.0 to 1.5 cm, which resulted in *ca.* 80% seedling emergence. These findings will assist land managers to predict seasonal emergence and will aid in deploying management approaches to control this weed.

Keywords: *Cleome gynandra* L.; vegetable; dormancy; germination; negatively photoblastic; gibberellic acid; after-ripening; temperature; stress

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1. Introduction

Spider plant (*Cleome gynandra* L.), also known as cat's whiskers, is a member of the family Capparaceae [1,2], which is phylogenetically close to the Brassicaceae [3]. Native to Africa, Asia, and the Middle East, the species has been reported to be a significant weed of field and horticultural crops in the Caribbean Islands (Bermuda, Bahamas, Cuba), Central and South America, Central and Northern Europe, China, Japan, Korea, New Zealand, certain Pacific Islands, and Australia [4,5]. It is also an important vegetable crop in many African countries, including Kenya, Tanzania, and Zambia, where it is cultivated as a fresh leafy vegetable [6].

The mode of introduction of spider plant into Australia in unknown; however, it was first recorded as a weed in 1977 by the Queensland Herbarium [7]. The species is now considered to be an emerging weed of cultivated crops in northern Queensland, Australia, with a report of it infesting sugarcane (*Saccharum officinarum* L.) crops dating back to 1987 [8]. More recently, the weed has spread to southeast Queensland (SEQ), possibly through seed carried on farm machinery, and is now commonly found in sweet corn (*Zea mays* convar. *saccharate* var. rugosa), brassica (*Brassica oleracea* var. capitata and *B. oleracea* var. sabauda), beans (*Phaseolus vulguris* L.), and pumpkin (*Cucurbita* spp. L.) crops in the Lockyer Valley, Southern Downs, and Somerset Regions of SEQ. Approximately 60% of all Queensland vegetable crops are grown in these regions [9].

In Australia, the importance of spider plant presently relates to its persistence in the cultivated vegetable production systems (Figure 1a–d) and the difficulty it causes farm managers with its control (Figure 1b–d). The delayed and irregular emergence of the species assists it to escape traditional weed management approaches and can result in many in-crop weed patches (Figure 1d). Persistence of the weed requires additional weed management throughout the growing season; otherwise, it will significantly reduce crop yield through competition, since a single mature plant can reach up to 1.5 m height with a spreading canopy [10]. In addition, spider plant can cause qualitative yield loss, as it can harbour damaging crop pests and diseases, which in turn prevent crop produce being sold interstate due to Australian State quarantine regulations [11]. Moreover, weed biomass can interfere with crop harvest and produce processing. Therefore, knowledge of its seed ecology is important to refine and to extend present management approaches.

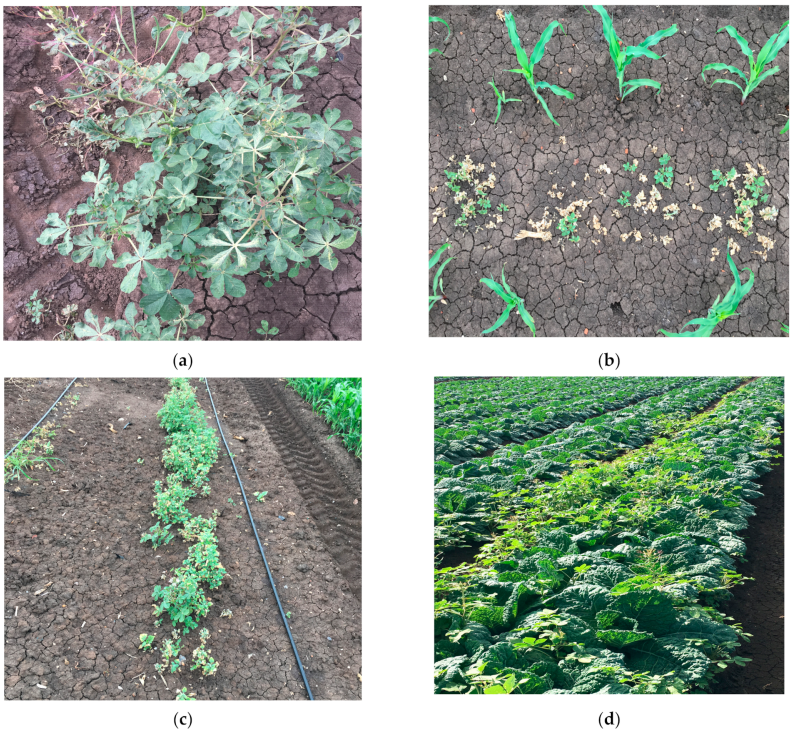

Figure 1. Persistence of spider plant individuals in the Australian cropping systems: (**a**) image of a single spider plant individual; (**b**) seedlings surviving traditional chemical control in a sweet corn crop; (**c**) a developing population along the dripper line; and (**d**) in-crop mature spider plant individuals in a cabbage crop.

The main traits that support spider plant invasiveness are its ability to produce large numbers of seeds [6,12] with variable dormancy [12–14], that allow it to emerge unpredictably and set large seedbanks. However, little is known regarding the environmental requirements that influence this species' germination or establishment within a crop.

The few studies that have been undertaken on the seed biology of spider plant indicate that seeds generally have a poorly characterized dormancy mechanism that requires *ca.* 6 months of natural after-ripening to be overcome [12–15]. Consequently, because of dormancy and an extended longevity of its seeds, the species can form large persistent soil seedbanks [16]. Thus, detailed knowledge of the dormancy mechanism is important to understand the persistence strategies of spider plants in Australian cropping systems. Further research to date regarding seed ecology has been restricted to the assessment of temperature and light on germination. Studies conducted by Ochuodho and Modi [14] showed that the species germinates best at 20/30 °C under constant darkness. However, no studies have been undertaken so far on the impact of soil moisture availability, salinity and pH, or burial depth on the germination and emergence of spider plant. Detailed knowledge of

spider plant seed dormancy mechanisms and germination ecology in response to common environmental factors will help predict seasonal emergence patterns and the creation of better management strategies. To achieve this aim, the specific objectives of this study were to determine the dormancy mechanism of spider plant and to determine the optimal seed germination conditions, with a specific focus on substrate moisture availability, medium, pH, and burial depth on germination of spider plant.

2. Materials and Methods

2.1. Seed Collection, Multiplication, and Preparation

Seed biology attributes were studied under controlled conditions (October 2018 to November 2019 and January to June 2020) at The University of Queensland (UQ), Gatton Campus (27.5554° S and 152.3372° E). Mature pods of spider plant were collected from *ca.* 30 randomly selected field-growing plants in a pumpkin crop belonging to a commercial farming business located in the Lockyer Valley, SEQ (27.563770° S; 152.255591° E). Further pods were collected from spider plants growing in a nursery greenhouse at UQ, Gatton Campus (Figure 2a). From both sets of plants, the pods were hand-harvested at maturity (pods were deemed to have reached maturity when their colour had changed from green to green-yellow, *ca.* 60 days after emergence) (Figure 2b). From all pods, seeds were isolated by hand, and sieved to remove extraneous plant material and other inert matter. All collections of seeds made from an individual source were pooled and stored by date of collection.

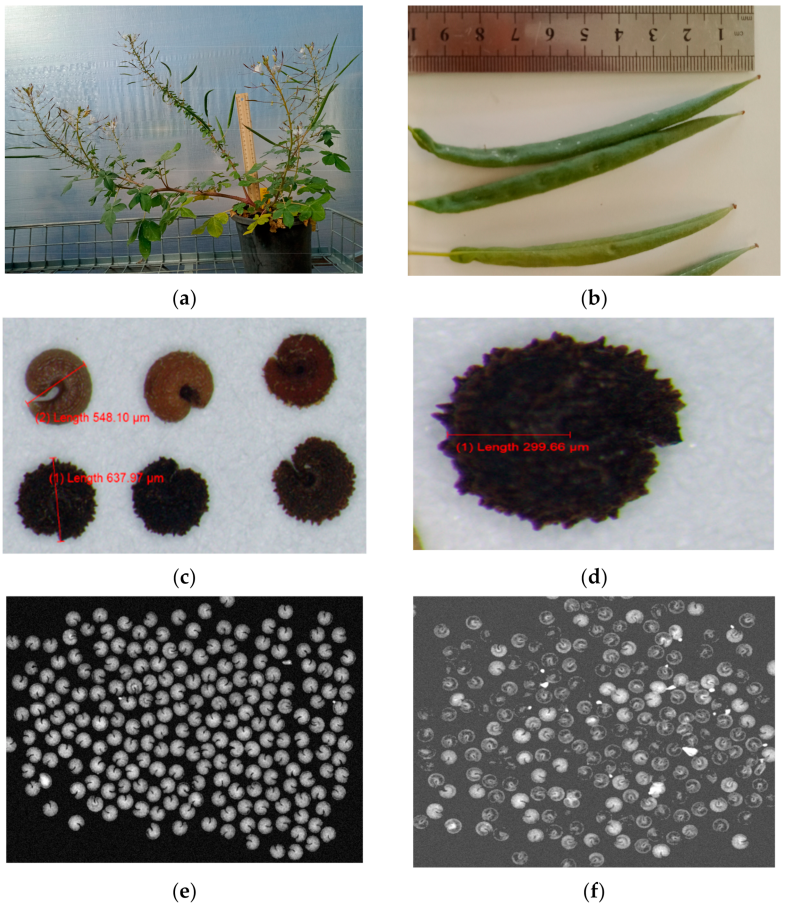

Figure 2. Images of spider plants: (**a**) a single spider plant; (**b**) intact pods (fruits); (**c**) seeds with variable maturity showing different colours; (**d**) an intact black mature seed; (**e**) an X-ray image of these black seeds showing *ca.* 90% seed fill; and (**f**) an X-ray image of brown underdeveloped seeds showing many poorly filled seeds.

Since the colour of the harvested seeds was either black or brown (Figure 2c) the two forms of seeds were observed under a dissecting microscope (Olympus: SZX, Olympus Ltd., Melbourne, Australia) to determine seed coat and embryo fill characteristics by radiography (Faxitron® MX-20 Cabinet X-ray System, Lincolnshire, IL, USA) (Figure 2c–e). For radiography, spider plant seeds (four replicates of 50 seeds) were exposed to 18 Kv for *ca.* 30 s (using two times resolution), and images were captured using DX1.0 software with ImageAssist (Faxitron X-ray LLC, Lincolnshire, IL, USA). The images obtained from the microscopic and X-ray examinations were used to determine the physiological status of the seeds and seed fill percentage. Filled seeds appeared opaque, while unfilled seeds appeared transparent (Figure 2e,f). Once examined, all seed lots were sun-dried for *ca.* 15 days to reduce the moisture content to a constant level of *ca.* 12% (determined by Pfeuffer HE50 moisture meter, Graintec Scientific, Toowoomba, Australia) and stored in paper bags in labelled, airtight glass bottles with silica gel beads in a dedicated seed store, until used for experimentation *ca.* 20 weeks later. Upon close examination, it was observed that the black seeds had a thick, well-developed testa, which was a feature relating to seed age; X-ray radiography showed *ca.* 40% of the brown (or light brown) seeds to be underdeveloped (Figure 2f). Therefore, for all studies reported here, only fully mature black seeds were used (Figure 2e).

2.2. Seed Biology Study

Five sub-experiments were conducted to complete the seed biology study.

General Seed Germination Protocol

Selected for health and uniformity, several black seeds were surface-sterilized by shaking in a sodium hypochlorite (2% NaOCl; *v*/*v*) solution containing two drops of Tween 20 (Labchem, Zelienople, PA, USA) for 120 s, followed by a triple rinse in sterile deionised water, then blotted dry before being used in the germination studies.

All incubator germination tests were performed by placing 50 seeds evenly across the surface of 9 cm diameter plastic Petri dishes, each containing a double layer of Whatman No. 1 filter paper [17,18] moistened with 5 mL of deionised water. Two drops of a fungicide (Pervicure; 1.5 mL L^{-1}) were added to each dish to prevent fungal growth. Petri dishes were then wrapped around their edges with transparent parafilm strips to reduce evaporation and placed into an incubator (Thermoline Illuminated Refrigerator Incubator, TRIL-750, Brisbane, Australia), and germination was observed every 3 days for 21 days. Germinated seeds were removed from the dishes when first observed. To simulate dark conditions, Petri dishes were wrapped in three layers of aluminum foil, and germination was observed in a darkened room under an LED light covered with green cellophane (50% transmittance) which allowed only green light to pass through. Seeds were considered to have germinated when the radicle was at least 2 mm long.

After 21 days, ungerminated seeds were examined by X-ray to check their fill status; then, those seeds were tested for viability using a tetrazolium test [19]. In performing the tetrazolium test, 25 randomly selected ungerminated but filled seeds from each germination test were imbibed in deionised water for a further 18 h (here after h). Seeds were then punctured, and the seed coat further weakened, with a needle placed through the central region of each seed to allow access of tetrazolium solution into the embryo. Seeds were then placed in a 0.5% (*w*/*v*) tetrazolium chloride solution (MP Biomedicals; LLC, Solon, OH, USA) and incubated at 30 °C for 24 h [20]. Tetrazolium staining (indicating metabolically active tissues) was evaluated under a dissecting microscope. Viable seeds were counted as those having much of the root and shoot meristems-stained red. The final viability of a treatment was calculated from the number of seeds that germinated added to the number of dormant seeds that stained red in the tetrazolium test.

2.3. Seed Germination Studies

2.3.1. Determination of Appropriate Dormancy-Breaking Treatments

Previous authors showed that freshly harvested spider plant seeds have dormancy [13,14]. Thus, a series of experiments were conducted to investigate the effects of some commonly used chemical and physical dormancy-breaking treatments. All tests were carried out on *ca.* 4-week-old seeds and run at 20/30 °C [14] using the germination protocol as described above.

Leaching

An amount of 300 black seeds was placed in each of three glass beakers, each containing 250 mL of deionised water, agitated for *ca.* 120 s, and then left to soak for either 8, 16, or 24 h under room temperature (22 ± 2 °C). The three seeds lots were then triple rinsed by swirling in 100 mL of deionised water before being placed into a germination test. Petri dishes containing seeds without leaching were included as a control.

Gibberellic Acid

The effect of gibberellic acid (GA_3; Sigma-Aldrich, St. Louis, MO, USA) was evaluated by preparing 0.00, 0.60, 1.20, or 1.80 mM GA_3 solutions in deionised water. Seed germination was evaluated by incubating 50 evenly spaced seeds in the Petri dishes containing 5 mL of freshly prepared GA_3 solution. Petri dishes with 5 mL of deionised water were used as a control.

Scarification

The effect of chemical and mechanical scarification was evaluated on black seeds. With chemical scarification, 300 seeds were placed in each of five glass beakers with 30 mL of 1 M sulphuric acid (H_2SO_4), and exposed for either 0, 10, 20, 30, or 40 min. Seeds were removed after each exposure time and triple rinsed by swirling in deionised water for 120 s. Mechanical scarification was imposed by abrading *ca.* 500 seeds between two sheets of 80 grit sandpaper (Makita, New South Wales, Australia). Non-scarified seeds were included as a control.

Dry After-Ripening

The effect of after-ripening was evaluated by storing seeds at 25 ± 1 °C for 6 months and checking seed germination after 2, 4, 6, 10, 12, or 24 months, since previous studies had shown dry storage for *ca.* 6 months could remove dormancy [13,14]. In the present study, a further seed germination test was conducted 24 months after-ripened seeds without GA_3, as residual dormancy was not detected at that time.

2.3.2. Optimal Germination Conditions

Constant Temperature

To determine the effects of constant temperature under a light and dark photoperiod, seeds were incubated, using the general seed germination protocol as described above, on a thermogradient bar (Thermoline, Wetherill Park, New South Wales, Australia). The bar contained 10 equal-sized chambers, each with a different, but constant, temperature, ranging from 9 to 33 ± 2 °C (i.e., 9, 12, 15, 18, 21, 24, 27, 30, or 33 ± 2 °C). The ambient temperature inside each of the chambers was recorded every hour with a data logger (Tinytags, TGP 4017, Hastings Data Loggers, New South Wales, Australia). The experiment was conducted twice with *ca.* 6- and 24-month-old seeds.

Alternating Temperatures

Studies involving alternating day/night temperatures and a single light/dark photoperiod were undertaken in a series of five identical germination incubators (Thermoline Illuminated Refrigerator Incubator, TRIL-750, Brisbane, Australia), each with a cool white fluorescent light producing a photosynthetic photon flux density of *ca.* 100 μmol m^{-2} s^{-1}.

Seeds were incubated at five different alternate temperatures (viz. 10/15, 10/20, 20/15, 25/15, or 30/20 ± 2 °C) under two different photoperiods: complete darkness (24 h dark) or a 12/12 h day/night photoperiod. All other conditions were as described for the germination test conducted under constant temperature. A dark germination test, with an alternating temperature of 30/20 °C, was undertaken for further seed ecology studies on 6-month-old seeds with the addition of 1.20 mM of GA_3 to overcome dormancy.

2.3.3. Seed Germination under Abiotic Stress

Moisture Stress

The effect of moisture stress on seed germination was determined using the previously established optimal germination conditions. To examine the effect of substrate moisture stress on seed germination, aqueous solutions with osmotic potentials of 0.0, −0.1, −0.2, −0.4, −0.8, or −1.6 MPa were prepared with polyethylene glycol 8000 (Sigma-Aldrich, St. Louis, MO, USA) as previously described [21]. These levels of moisture stress were selected as they, except for the lowest value, are likely to be those found in different regions of Australia where this weed may invade.

Salinity Stress

The effect of medium salinity on seed germination was determined under optimal germination conditions by preparing 0, 20, 40, 60, 80, 100, or 120 mM sodium chloride (NaCl) solutions [21]. This range, except for the highest value, represents soil salinity levels found in different soils in Australia where this weed may invade [22]. At the end of the study, ungerminated seeds remaining in the highest salinity stress conditions were rinsed under running water for 10 min, then imbibed in 5 mL of 1.20 mM GA_3 and placed back into the incubator. New germinations were counted every 7th day for 21 days.

pH Stress

The effect of medium pH was studied under optimal germination conditions by using solutions with a pH ranging from 5.0 to 10.0. These pH levels were selected based on the literature, which suggests that the pH of Australian soils varies between 4.0 and 10.0 [23]; thus, this range would represent soil pH levels where this weed may invade. Buffer solutions with pH values of 5.0 to 10.0 were prepared according to the method described by Chachalis and Reddy [24]. A 2 mM solution of MES [2-(N-morpholino) ethane sulphonic acid] was adjusted with 0.1 M hydrogen chloride (HCl) or sodium hydroxide (NaOH) to obtain the solutions of pH 5.0 and 6.0. A 2 mM solution of HEPES [N-(2-hydroxymethyl)piperazine–N–(2-ethane sulphonic acid)] was adjusted with 0.1 M NaOH to obtain the solutions of pH 7.0 and 8.0. Buffer solutions of pH 9.0 and 10.0 were prepared with 2 mM tricine [N-Tris (hydroxymethyl) methyl glycine] and adjusted with 0.1 M NaOH. Unbuffered deionised water (pH 6.4) was used as a control. Other conditions were the same as those described in the standard germination test.

2.3.4. Seed Germination under Variable Burial Depth

Burial Depth

The influence of burial depth on germination was studied in pots (black plastic, 15 cm diameter) contained within a Birkdale greenhouse at the UQ Gatton Campus. Triplicate groups of 25 seeds (6-month-old), pre-soaked with 1.20 mM GA_3 for 72 h, were placed on the soil surface in the pots and then covered with soil to achieve burial depths of 0.0, 0.5, 1.0, 2.0, 2.5, 3.0, or 4.0 cm. Pots were watered daily with an overhead sprinkler to keep the soil at field capacity. Seedlings were considered to have emerged when cotyledons were visible, and the experiment was continued for 45 days after sowing.

2.3.5. Seed Germination After-Ripening
After-Ripening

The effect of after-ripening on germination was assessed following the standard protocol as described above, and for eight-time intervals of after-ripening (i.e., 0, 2, 4, 6, 8, 10, 12, and 24 months after seed harvest).

2.4. Experimental Design and Statistical Analysis

Petri dishes were randomly allocated to temperature-controlled incubators, and their positions changed twice per week. The final germination was calculated by Equation (1):

$$G = \left[\frac{A}{B}\right] \times 100 \quad (1)$$

where A is the total number of germinated seeds at the end of the experiment + the number of viable seeds obtained by tetrazolium tests and/or X-ray, and B is the total number of seeds tested.

The significance of the means of individual treatment factors (temperature, moisture, salinity, pH, burial depth, and storage) was estimated with one-way Analysis of Variance (ANOVA) using Graph Pad Prism (Version 9.4.1). The interaction between light and temperature was analysed with two-way ANOVA. The assumptions of normality and homoscedasticity of ANOVA model were met. Means were separated using the least significant difference (LSD) test at 95% confidence interval. Treatment means were presented in bar charts with ± two standard errors of means (SEM) using Microsoft Excel. Germination percentages at different moisture stresses or NaCl concentrations were fitted to a two-parameter sigmoidal curve using a simple linear regression model (Equation (2)) using Microsoft Excel:

$$y = a + bx, \quad (2)$$

where y is germination percentage at the osmotic potential of x Mpa or NaCl concentration of x mM. The coefficients of determination (R^2) values were used to determine the goodness of fit to all selected models.

3. Results

3.1. Determination of Appropriate Dormancy-Breaking Treatments

Certain dormancy-breaking approaches used (i.e., applied scarification and scarification followed by leaching for 16 h) could partially overcome physical dormancy (58%; Figure 3b). When further treated to remove physiological dormancy, seeds imbibed in GA_3 solution gave the highest germination percentages (80%) ($p < 0.0001$) (Figure 3a), with germination reduced when the concentration was increased or decreased below this. Chemical scarification using H_2SO_4 for 20 min slightly increased the germination percentage (35%: Figure 3b).

3.2. Optimal Germination Conditions

The findings showed that germination of spider plant seeds was significantly affected by temperature and by light (Figure 4a,b). Although germination was observed over a wide range of temperatures (10/15 to 25/35 °C) and constant day/night temperatures (9 to 33 °C), the results show freshly harvested spider plant seeds germinated best under darkness and constant temperature (Figure 4a). In comparison with aged seeds (24 months old), maximum germination of *ca.* 80% was found using 24 h darkness, although some germination, *ca.* 30%, was observed under a 12/12 h light/dark photoperiod (Figure 4b). With both seed lots, maximum germination of 70 to 80% was obtained at a constant temperature of 18 to 27 °C under complete darkness.

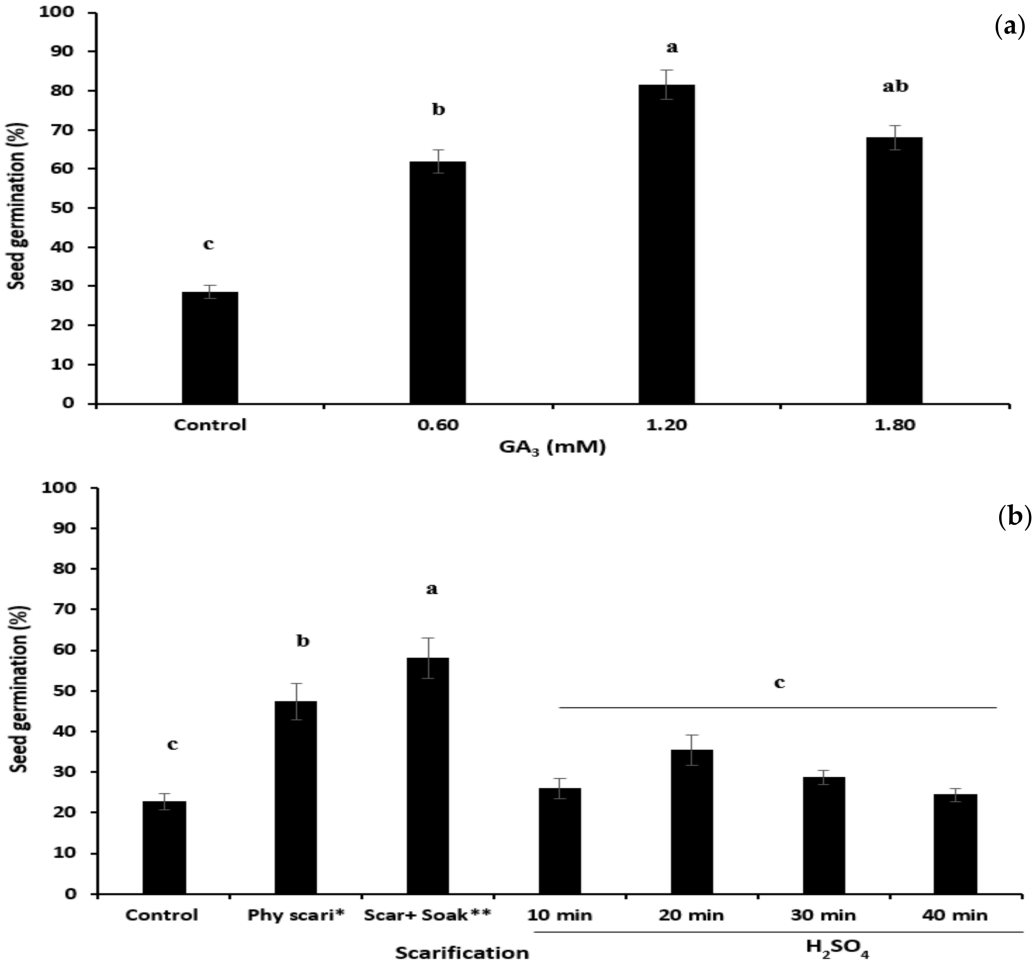

Figure 3. (**a**) Effect of $GA_{3;}$ and (**b**) effect of various physical dormancy-breaking treatments on spider plant (*Cleome gynandra*) seed germination under an alternating (30/20 °C) 12/12 h thermoperiod and 24 h darkness. Seeds were incubated for 21 days prior to assessment. Error bars represent ± two standard errors of the mean for three replicates of 100 seeds. Different letters above bars indicate statistical significance. * Physical scarification; ** Physical scarification + soaking in water for 16 h.

In contrast, a similar significant ($p < 0.001$) difference in germination percentage was found in alternating day/night temperatures when tested under varying temperature regimes (Figure 5). The optimum alternating day/night temperatures recorded for maximum germination (*ca.* 80%) were 20/30 °C, though seeds germinated across all temperature treatments applied, ranging from 10/15 °C to 25/35 °C. However, seed germination was lowest (<20%) at 10/15 °C (day/night) temperatures.

3.3. Seed Germination under Abiotic Stress

3.3.1. pH Stress

No significant treatment effects were detected on spider plant germination in response to the applied pH treatments ranging from 5.0 to 10.0, except for at pH 5.0 and 8.0, where the effects were marginally significant ($p < 0.05$; Figure 6). Maximum (82%) and minimum (62%) germination were obtained at pH 8.0 and pH 5.0, respectively, which depicts that pH may not be a limiting factor for spider plant germination in most of Australia's cultivated soils, as crops are generally not cultivated in soils with pH 5.0.

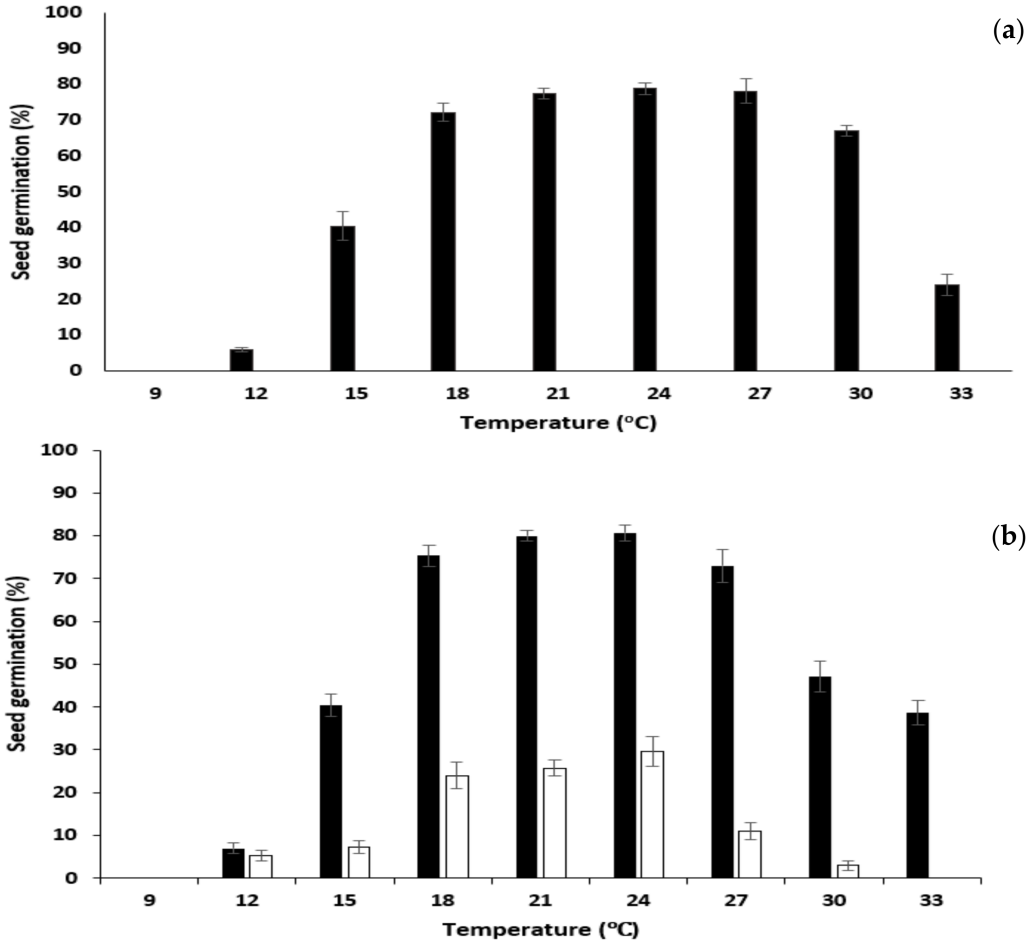

Figure 4. Effect of day/night temperatures (9 to 33 °C) on spider plant (*Cleome gynandra*) seed germination: (**a**) 6-month-old seeds treated with GA_3 (1.20 mM) and (**b**) 24-month-old seeds with GA_3 incubated under a 12/12 h light/dark photoperiod or complete darkness. Incubation occurred for 21 days prior to assessment. Error bars represent ± two standard errors of the mean for three replicates of 100 seeds.

3.3.2. Moisture Stress

A three-parameter negative sigmoid curve corresponding to germination data showed significant treatment effects on spider plant germination in response to different osmotic potentials ($p < 0.001$; Figure 7). A sharp decline in germination was observed with the increase in moisture stress from 0 to −0.8 MPa. Germination was lowest (<10%) at −0.6 MPa, and no germination was observed at −0.8 MPa. The moisture stress that caused a 50% decrease in germination based on the regression model was −0.35 MPa.

3.3.3. Salinity Stress

Salt stress significantly affected spider plant germination ($p < 0.0001$) (Figure 8). A three-parameter negative sigmoid curve fitted to the germination data showed seed germination gradually decreased with the increase in NaCl concentration, with no germination recorded at 100 mM NaCl. The regression equation showed 35 mM NaCl to be the concentration that restricted germination by 50%. Moreover, results obtained from the hydroponic study further depicted that spider plants were susceptible to salinity stress, as young plants did not survive in solutions with >40 mM of NaCl, although seeds with 80 mM NaCl solutions germinated.

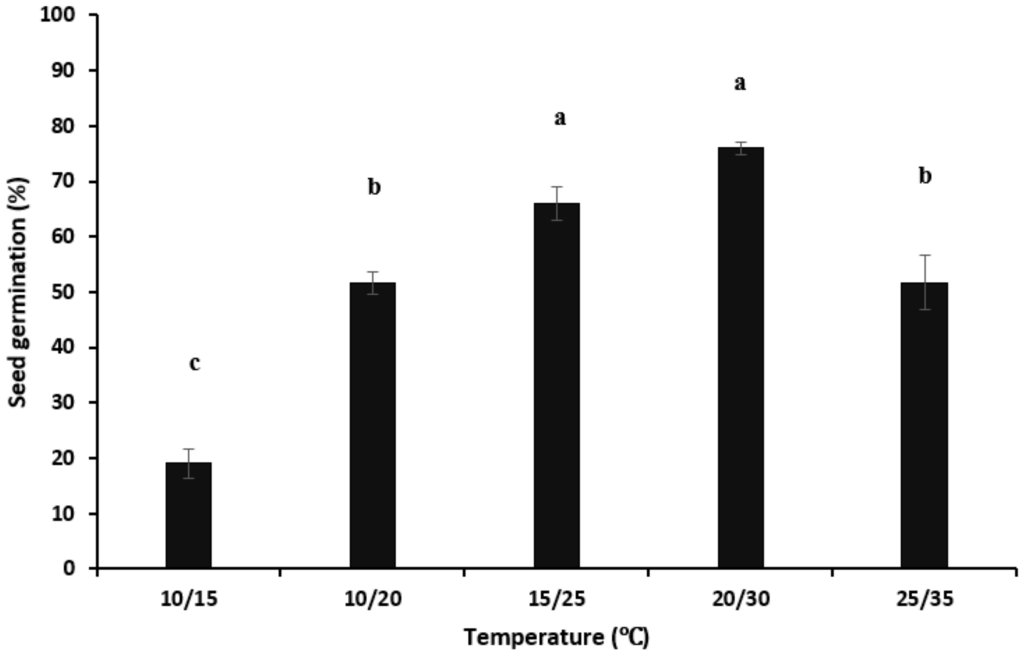

Figure 5. Effect of alternating day/night temperatures (30/20 °C) on the germination of 6-month-old spider plant (*Cleome gynandra*) seeds when incubated under a 12/12 h light/dark photoperiod or complete darkness with GA_3 (1.20 mM). Incubation occurred for 21 days prior to assessment. Different letters above bars indicate statistical significance. Error bars represent ± two standard errors of the mean for three replicates of 100 seeds.

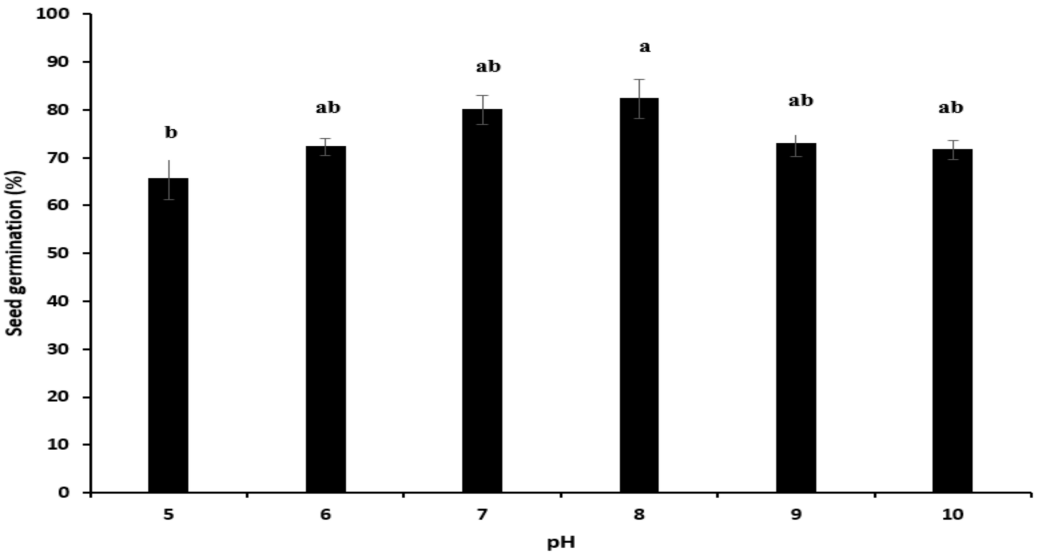

Figure 6. Effect of medium pH on the germination of 6-month-old spider plant (*Cleome gynandra*) seeds incubated under a 12/12 h, 30/20 °C thermoperiod or complete darkness with GA_3 (1.2 mM). Incubation was for 21 days prior to assessment. Different letters above bars indicate statistical significance. Error bars represent ± two standard errors of the mean for three replicates of 100 seeds.

3.4. Seed Germination under Variable Burial Depth

The findings showed that burial depth had a significant effect on seed germination ($p < 0.001$). Seeds placed on the soil surface did not germinate, whereas the highest (74%) seedling emergence was observed from the seeds buried at 1 cm, followed by 1.5 cm (71%) (Figure 9). Only 10% of seedlings emerged from a depth of 2.5 cm, while no germination was observed beyond 2.5 cm depth. Removal of *ca.* 2.0 cm topsoil from the treatment pots (seeds buried at 3.0 and 4.0 cm depth) showed a normal emergence pattern.

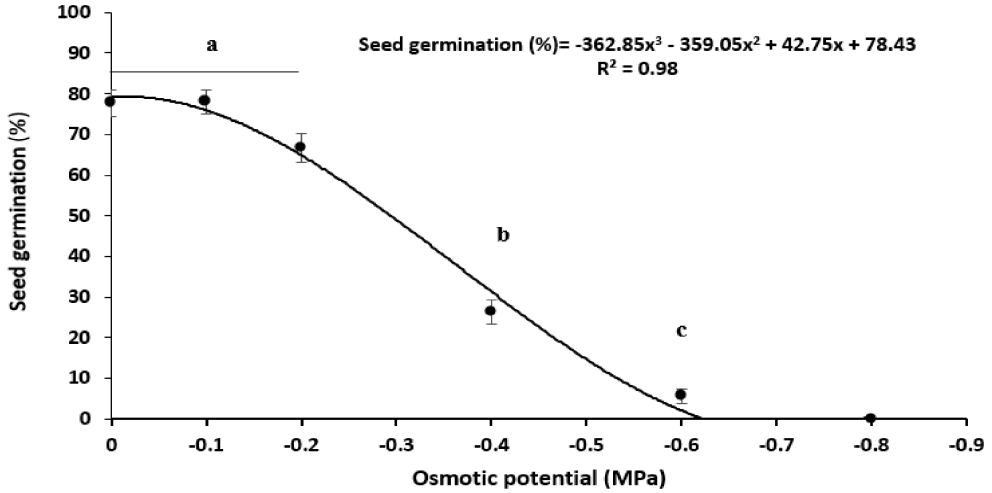

Figure 7. Effect of moisture stress on the germination of 6-month-old spider plant (*Cleome gynandra*) seeds incubated under a 12/12 h, 30/20 °C thermoperiod or complete darkness with GA_3 (1.2 mM). Incubation was for 21 days prior to assessment. Different letters above bars indicate statistical significance. Error bars represent ± two standard errors of the mean for three replicates of 100 seeds.

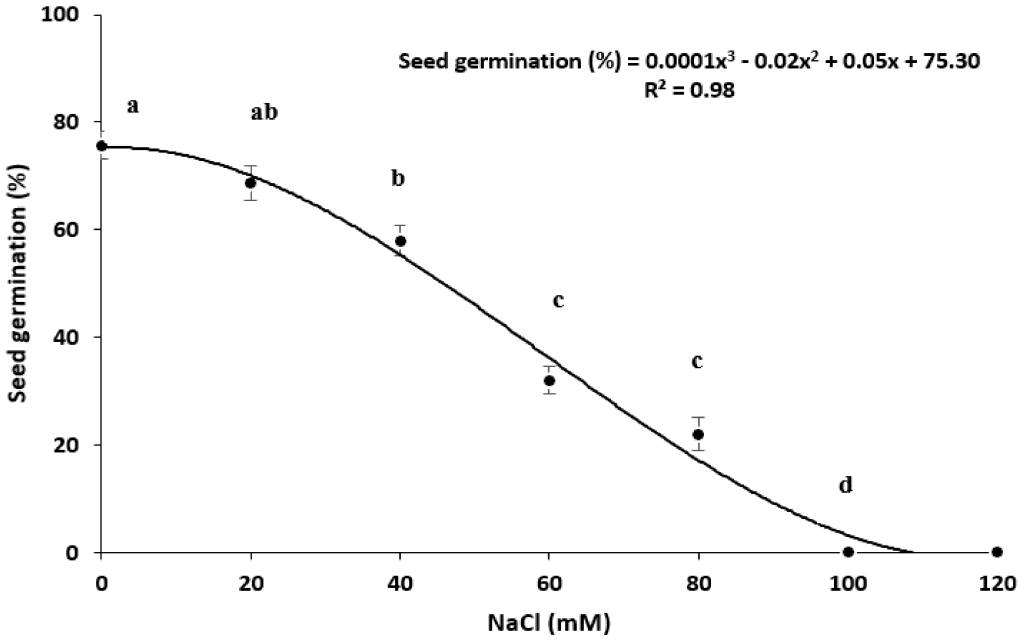

Figure 8. Effect of salinity stress on the germination of 6-month-old spider plant (*Cleome gynandra*) seeds incubated at a 12/12 h, 30/20 °C thermoperiod or complete darkness with GA_3 (1.2 mM). Incubation was for 21 days prior to assessment. Different letters above bars indicate statistical significance. Error bars represent ± two standard errors of the mean for three replicates of 100 seeds.

3.5. Seed Germination After-Ripening

After-Ripening

The post-harvest dry storage period had a significant ($p < 0.0001$) influence on seed germination, where an increase in germination from 22 to 84% was observed over the 24-month storage (Figure 10). The findings also showed that freshly harvested spider plant seeds were strongly dormant, where a minimum *ca.* 10-month storage was required to obtain >80% germination.

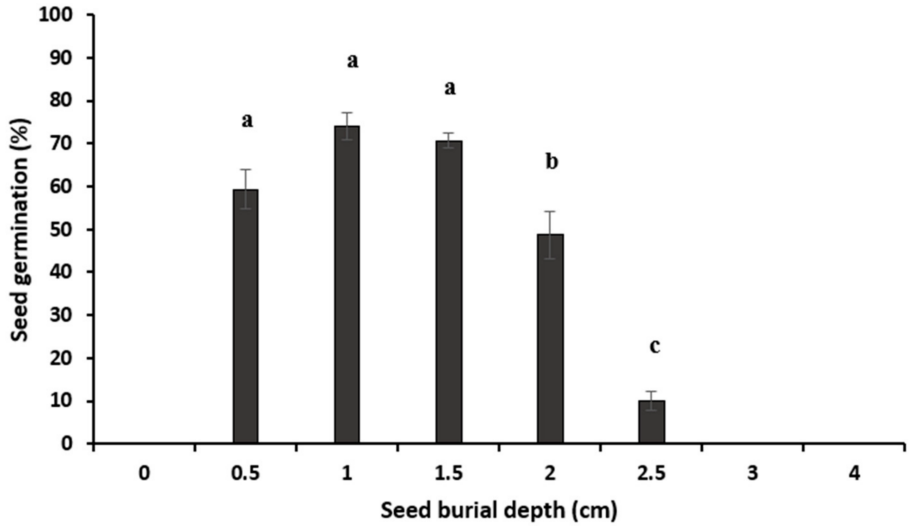

Figure 9. Effect of burial depth (cm) on the emergence of 6-month-old spider plant (*Cleome gynandra*) seeds under ambient temperature and photoperiod. Seeds were pre-soaked with GA_3 (1.2 mM) for 72 h under room temperature (24 ± 2 °C). Different letters above bars indicate statistical significance. Error bars represent ± two standard errors of the mean for three replicates of 100 seeds.

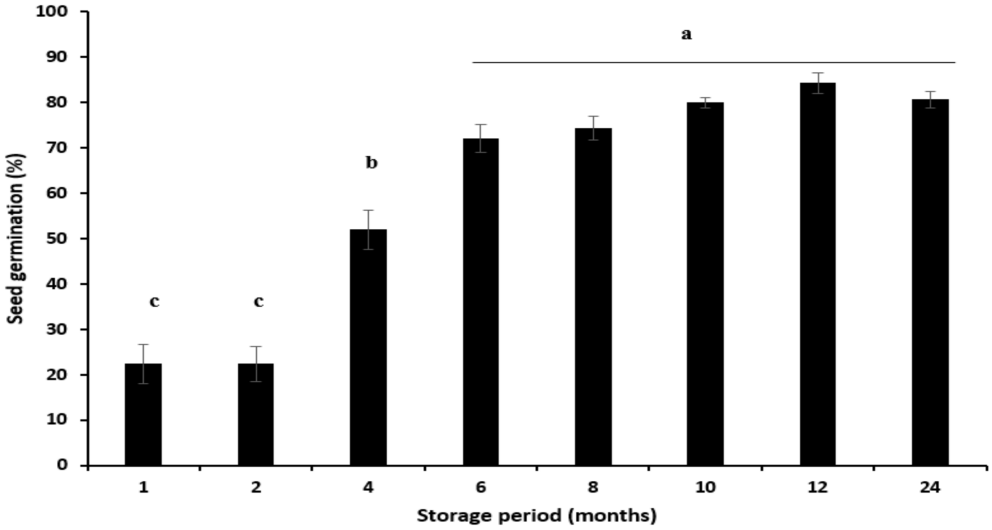

Figure 10. Effect of dry after-ripening on spider plant (*Cleome gynandra*) seed germination under an alternating (30/20 °C) 12/12 h thermoperiod and darkness. Seeds were incubated for 21 days prior to assessment. Different letters above bars indicate statistical significance. Error bars represent ± two standard errors of the mean of three replicates of 100 seeds.

4. Discussion

4.1. Determination of an Appropriate Dormancy-Breaking Treatment

Freshly harvested spider plant seeds gave low germination under a range of thermo- and photoperiods (Figure 3a,b), demonstrating evidence of primary innate dormancy, which is in line with the findings of Boonsong [13] and Chweya and Mnzava [6]. These forms of seed coat-mediated dormancy have been illustrated by many authors [25–28]. For example, Mekenian and Willemsen [29] demonstrated that a germination inhibitor present in the seed coat is responsible for dormancy in wild radish (*Raphanus raphanistrum* L.), whereas Sozzi and Chiesa [30] showed that rupturing seed coat by pricking followed by acid scarification improves germination in caper (*Capparis siposa* L.). This study suggests that seed dormancy in spider plants could be partially removed by physical scarification followed by leaching for 16 h (Figure 3), indicating the presence of seed coat-mediated

physical dormancy. Moreover, authors argued that an intact seed coat can favour dormancy because of its impermeability to water and/or gases, by physically restricting expansion of radicles, or by containing or supplying inhibitors to the embryo [25,28,31,32]. In addition, given the experiment was conducted at 20/30 °C, this high temperature may also have enhanced degradation of the seed tissues [25]. This degradation could promote energy supply to the embryonic axis and may accelerate the diffusion of water, oxygen, carbon dioxide, and inhibitors.

In this approach, seed germination was improved when treated with GA_3 (Figure 3a). Gibberellic acid is known to break or reduce dormancy in a wide range of seeds, including weeds [33–35]. Multiple researchers have reported that GA_3 is associated with breaking seed dormancy by mobilising endosperm reserves, by enhancing the multiplication of embryonic tissue [36,37], and by mediating the phytochrome responses to red and far-red light [38]. Thus, the application of GA_3 in the dark substitute's the light requirements for germination of many Australian native Asteraceae [34]. Consequently, in this study, the imbibition of seeds in GA_3 significantly removed dormancy. The effect of GA_3 on dormant seeds indicates that endogenous gibberellins play an important part in spider plant germination. Findings of this study imply that the delayed patchy emergence of spider plants in cultivated vegetable crops is related to the presence of combinational dormancy in freshly shattered seeds.

4.2. Optimal Germination Conditions

The maximum seed germination under 24 h darkness is in line with a previous finding that some populations of spider plant can be negatively photoblastic [39]. Similar findings have been reported in Asian/African mustard (*Brassica tournefortii* Gouan.) [40], watermelon (*Citrullus vulgaris* Thunb.) [41], and purple passionflower (*Passiflora incarnata* L.) [42]. This study has shown that germination was highest (*ca.* 80%) under 24 h darkness compared to 12/12 h photoperiod at both constant and alternating day/night temperatures (Figures 4 and 5). This response to light exposure could be explained as an adaptation trait of spider plant, since mature pods scatter seeds on the soil surface. Similar high germination under 24 h darkness was reported in parthenium weed (*Parthenium hysterophorus* L.) by Williams and Groves [43] and Pandey and Dubey [44]; however, their studies used seeds pre-treated with light.

The seed germination of spider plant generally increased with temperature (except for 25/35 °C), while the maximum germination percentage (*ca.* 80%) was recorded at 20/30 °C alternating day/night temperatures (Figure 5). This is consistent with previous findings [15,39]. However, Bohringer, et al. [15] obtained only 25% germination under 24 h darkness at 30 °C. A strong reason for low germination found in their study under high temperature and complete darkness could be associated with the age of the seeds, since their research used 6-month-old seeds. In contrast, the seed lot used by Ochuodho and Modi [39] was 12 months old. Thus, the longer storage period after harvest might have alleviated innate dormancy through natural after-ripening processes as well as by allowing immature embryos to reach maturity. To compare, this approach used GA_3 to minimise the effects of physiological dormancy, which could be a strong reason for increased germination observed across all temperature ranges (discussed earlier in Section 4.1). The capability of spider plants to germinate under a broad temperature range shows the potential for year-round weed infestation.

4.3. Seed Germination under Abiotic Stress

The germination of spider plant was not affected by the soil pH ranging from 5.0 to 10. Many weed species are found capable of germination over a wide range of soil pH values [18,41,45–48]. However, some species such as large crabgrass [*Digitaria sanguinalis* (L.) Scop.] and tropical signal grass [*Urochloa subquadripara* (Trin.) R.D.Wenster] were reported to be sensitive to pH values below 6.0 [49,50]. Results of this study imply that the

soil pH of the Australian cultivated cropping system should not be a limiting factor for spider plant germination if other requirements are met.

Findings of this study show that spider plant does not germinate well under low levels of soil moisture (Figure 7), which suggests that the species favours a moist environment for germination. The germination pattern observed in the field was mostly in areas with perpetually high soil moisture (Supplementary Materials), which supports this finding. Similarly decreasing germination patterns in response to increasing osmotic stress have been reported in other weed species such as goosegrass (*Eleusine indica* L.), where germination gradually reduced at −0.6 MPa and no germination occurred at −0.8 MPa [51]. In pigweed (*Portulaca oleraceae* L.), seed germination progressively decreased between −0.1 and −0.8 MPa, and complete inhibition was reported at −1.0 MPa [47]. Additionally, in parthenium weed (*P. hysterophorus*), complete germination inhibition was reported below −0.52 MPa osmotic potential at 27 °C [18]. Thus, irrigation or available moisture due to precipitation stimulates the emergence and growth of in-crop spider plants throughout the crop growing period.

Spider plants also showed low germination under moderate salt concentrations (Figure 8). An extension of the salinity tolerance study demonstrates that, although seeds germinate in a substrate with up to 80 mM of NaCl, the young seedlings do not survive in growing media containing more than 40 mM of NaCl; however, Kulya et al. [52] showed that the species can tolerate 75 mM of NaCl. Similar reduction or inhibition of germination under salt stress was also observed in Benghal dayflower (*Commelina benghalensis* L.), where 10 mM NaCl solution reduced *ca.* 50% seed germination, and complete inhibition was observed with 80 mM NaCl solution [53]. In wild radish (*Raphanus raphanistrum* L.), the NaCl concentration required for 50% germination reduction was found to be 150 mM, depending on the temperature, among four different Japanese lines [54]. The NaCl concentration required for 50% inhibition in turnip weed was *ca.* 80 mM [48]. This study suggests spider plants could spread and establish in moderately saline soils, which also indicates that they could infest a wide range of field and horticultural crops, as these are generally grown in soils with low salinity. Nevertheless, seed germination will be affected by the crop management practices, particularly the use of inputs (plant nutrients, irrigation) that can alter the availability of soil pH, moisture, and salinity.

4.4. Seed Germination under Variable Burial Depth

The depth of a seed in the soil is important, since it determines whether the conditions required to initiate germination can be met. Germination of spider plant seeds was highest with 1.0 cm burial (Figure 9), which aligns with the previous findings that the species is predominantly negatively photoblastic, since the penetration of light is generally limited to the first few millimetres of the soil [55]. In a different study, Taylor and Revell [56] showed that burial can markedly alter the temperature and relative humidity to which seeds are subjected as the depth is increased. Hence, in this study, burial at 1.0 to 2.0 cm may have buffered the temperature and contributed to retaining a higher moisture content than those on the soil surface. Nutt [57] also disclosed that 1.0 cm burial increased rates of softening in common bird's foot (*Ornithopus sativus* Brot.) and suggested that this softening justifies that burial could alter seed moisture.

This research also showed that germination decreases significantly beyond 2.0 cm soil depth. Several similar studies have also demonstrated that seed germination decreases with increasing soil depth, although the rate is species specific [42,58,59]. However, empirical studies evaluating the fate of weed seeds over the soil volume found no consistent relationship between seed mortality and burial depth [60,61], except that seeds close to the soil surface may die faster [62–64] or slower [65].

4.5. Seed Germination After-Ripening

Dry after-ripening for over 6 months was required to achieve more than 70% seed germination (Figure 10). A similar study in Colombia, where spider plant seeds were

planted for 13 consecutive months, showed that seeds could be dormant for up to 5 months after maturity. Active germination started at 6 months and reached over 85% after 9 months of storage [12]. However, Boonsong [13] reported 90% seed germination after a 3-month storage. The same study also identified prolonged dormancy as well as delayed germination as major constraints in commercial cultivation of spider plants in Kenya. These works indicate that spider plant has an after-ripening period, which is common in many commercial crops [66] such as tomato (*Lycopersicon esculentum* L.) [67], tobacco (*Nicotiana tabacum* L.) [68], and capsicum (*Capsicum annum* L.).

5. Conclusions

This study has shown that spider plant seeds are generally negatively photoblastic and are capable of germination under a wide range of climatic conditions, including a broad range of temperatures. Seeds have both physical and physiological dormancy, which was removed by a combination of aging and imbibition of GA_3. Hence, an after-ripening of over 6 months is recommended to improve the germination rate. The strong dormancy observed might be an important survival strategy for spider plant in Australian cultivated cropping systems. The species germinated under a wide range of medium pH values, which indicates that soil pH should not restrict seedling emergence. However, moderate moisture stress could substantially reduce seed germination, which indicates that additional weeding may be required in areas with high soil moisture. Maximum seed germination was observed at a depth of 1.0 to 1.5 cm, while no germination was recorded on the soil surface. Thus, seedbank accumulation could be managed by adopting a stale or false seedbed technique, particularly in spring. However, under a real farming scenario, the emergence of spider plant will depend on the interaction of many biotic and abiotic factors that can influence dormancy or promote seed germination and seedling emergence. To date, no scientific research was carried out to understand seed ecology of spider plant in the Australian context. Hence, this study will improve our knowledge of some of the important ecological features of the species and to manage its establishment in cultivated crops.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13051430/s1, Figure S1: Images of field observations showing patches of spider plants germinated in areas with high soil moisture.

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