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Predicting the Establishment and Spread of Siam Weed in Australia: A Test of Abiotic Cues on Seed Dormancy and Germination

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ABSTRACT

Significant infestations of *Chromolaena odorata* are present in the Alice river basin of Townsville and South Johnstone area of Queensland in the north of Australia. This study was undertaken to predict the spread of *C. odorata* by assessing the impact of abiotic cues on dormancy and germination trends. Seeds from wet tropics infestations in a primary and a secondary forest in Australia were germinated in 1.5×0.9 cm deep petri dishes in an incubator at mean temperature 30°C. Petri dishes containing 50 seeds each were set in a Randomized Complete Block Design of 4 treatments and 3 replications. The empirical trajectory of seed dormancy and germination response of *C. odorata* to salinity, smoke, acid and alkalines were investigated. Smoked seeds germinated significantly (p<0.0001) higher in water than in soil. The percentage seed germination in smoke treatment was significantly (p<0.001) higher than in non-smoke conditions. Germination rate in both primary and secondary forests was relatively high at low sodium chloride (NaCl) concentration (0.02, 0.04, 0.06 and 0.08 mol L⁻¹). Seeds from populations in primary forests responded more to salinity than seeds sourced from secondary forests. Alkaline solutions (1 M KOH and 1 M NaOH) significantly reduced seed emergence. This study should provide a useful reference tool to effectively predict the trajectory of *C. odorata* infestations in tropical landscapes.

Key words: Salinity, smoke, invasive plant, primary forest, secondary forests

INTRODUCTION

C. odorata is an invasive plant species with neotropical origins (McFadyen, 1996). Globally, C. odorata presents a serious threat to biodiversity, especially to native species in tropical regions. It is has been reported that Siam weed infestations were first discovered in Bingil Bay and around the Tully river catchment in the 1960s (Wilson, 2003). Considerable infestations of C. odorata exist in Cairns, Cassowary, Charters Towers, Tablelands and Townsville Regional Council forest areas. The C. odorata infestations could overtake farmlands and secondary forest sites (Brooks, 2009). The weed is expected to profusely invade farmlands, including pasture areas and could induce allergic reactions when consumed by grazing cattle and horses. It also has the potential to invade undisturbed forest lands (Tefera et al., 2008) and increase the fuel load in tropical forest fires. Approximately 67 million hectares of bush land was subject to wild forest fires between 2002 and 2003. Significant amounts of prescribed burning also occur each year (Ellis et al., 2004). The Biosecurity Queensland research team established monitoring plots in field trials in a Townsville C. odorata infestation area which was burnt (using aerial incendiaries) in early October 2008

(Brooks, 2009). Data from the pre-burn site included weed size, fuel loads, soil seed banks and soil moisture level. Majority of plants got scorched but not consumed. In a post-fire survey, it was reported that fire controlled young plants. However, larger plants resprouted from the basal section that persisted in the soil after the fire (Brooks, 2009). Approximately, 89% of the seeds were located on the soil surface at burning and a mortality rate of 72.5% occurred leading to significant reduction in the seed bank (Patane et al., 2009). C. odorata also depicts a phytochrome-mediated germination response and this is characteristic of many invasive weeds and that promotes rapid spread to disturbed areas (Erasmus and van Staden, 1986). In addition, Chauhan and Johnson (2008) demonstrated the effects of salt by placing the South African ecotype of C. odorata seeds in dishes containing 5 mL of solution of 0, 25, 50,100, 150, 200 and 250 mM sodium chloride (NaCl). Increase in salinity (Hasanuzzaman et al., 2010) resulted in a significant reduction in the rate of seeds that germinated (Abbad et al., 2004; Al-Ahmadi and Kafi, 2006). Witkowski (2000) showed that majority of the South African ecotype of C. odorata seeds persist for more than a 12 month period. However, the study showed a briefly persisting seed bank from greater seeding depth. Persistent soil seeds serve as key determinant of the length of control operations of eradication campaigns in Australia (Brooks, 2009). C. odorata seeds generally germinate at the start of rainy season (Jeffery, 2010). The growth of seedlings is prolific and seedlings that have germinated early in the rainy season may blossom during the following flowering season in June-July. In Australia, it has be shown that environmental conditions provided exceptionally good circumstances for C. odorata growth over the period 2007-2010, with above average data in all known infestation ecologies (Jeffery, 2010). Specific research focusing on the specific effect of abiotic stress cues on the dormancy and germination of the Australian ecotype of C. odorata is rare. The comparative differences in germination of C. odorata between tropical primary and secondary forests have also not been well documented (RIRDC, 2011). Critical knowledge in the milieu of seed and germination ecology should be very crucial to understand the rapid early growth rate and the exponentially aggressive invasiveness of C. odorata in disturbed ecosystems. This study attempted to define the basis for prediction of infestation and spread of C. odorata by assessing response of seeds to abiotic cues.

MATERIALS AND METHODS

Study sites description: The study was conducted between June, 2010 and May, 2011. Two major habitats within a primary and secondary forest landscape in the north of the state of Queensland, Australia are considered in this study. The major *C. odorata* weed infestations are restricted to two regions, the adjacent Tully/Johnstone catchments (S 17° 37′ 24.5″ E145° 57′ 05.8″) and another 150 km south in Townsville/Thuringowa (S19° 22′ 22.22″ E 146° 36′ 15.3″) region. The germinability of seeds from the primary (South Johnstone River catchment-SJR) and secondary forest (Townsville Region-TSV) habitats were compared to understand the dynamics of the emergence and early growth ecology of *C. odorata* in disturbed habitats as a function of the abiotic stress tolerance.

Seed collection and storage: Quarantine permission was secured to obtain the matured and dry seeds of *C. odorata* from the laboratories of the Queensland Biosecurity institution in March 2011. These included high quality germplasm sourced from the South Johnstone Region and Townsville Region 18 months earlier. The seeds were stored in the dark and kept under room temperature prior to seed germination tests. Seeds obtained were tightly sealed and secured to prevent accidental dispersal.

Germination tests: Growth chamber tests were carried out in 1.5×0.9 cm deep petri dishes. These were set in an MIR-153 Sanyo Incubator. In each replication, 50 seeds from each habitat were set in petri dishes embedded with Qualitative Advantec filter paper and served with different substances to assess abiotic stress response and tolerance of the seedlings. The effect of salinity stress factors was assessed by placing seeds in dishes containing 5 mL solution (Chauhan and $Johnson,\ 2008)\ of\ 0.02,\ 0.04,\ 0.06,\ 0.08,\ 0.1,\ 0.2,\ 1.0,\ 1.2,\ 1.4,\ 1.6,\ 1.8,\ 2.0,\ 2.5\ and\ 3.0\ m\ L^{-1}$ sodium chloride (NaCl). Abu Ziada et al. (2008) used similar concentrations in tests on Amaranthus species. Smoke used in seed treatment was produced from the burning of a pair of Japanese chopsticks warebashi. In related studies scarification treatments have been used (Alderete-Chavez et al., 2010; Travlos et al., 2007). The smoke was passed through a plastic tube to cool briefly prior to reaching the seeds in a 7.0×9.0 cm closed transparent plastic container. Seeds were exposed to smoke for 10 min to break the dormancy. The treatment time was counted from the moment the seeds were completely engulfed in thick, dark cloud of smoke. The smoked seeds were then set in the petri dishes. The smoke was also dissolved in water to produce smoke solution. The other abiotic stress cues were evaluated as alkaline (1 M KOH, 1 M NaOH and Wood Ash), acid (1 M HCl) and loamy soil. Plain water was set as a control. Also, seed treatments were maintained at a temperature range of 28.5-30.5°C. Fungal growth was controlled with Benlate.

Statistical analysis: The experiment was arranged in a Randomized Complete Block design. There were 4 treatments and 3 replications of each abiotic stress component conducted in 2 rounds of experiments. In the incubator experiment each replication was set on separate shelves as distinctive blocks. The mean number of germinated seeds (emergence at 2 mm radical length) was monitored daily and recorded exactly every 24 h initializing from 1 DAS (day after seed setting) to 14 DAS. Germination data were analyzed for statistical significance by analysis of variance (ANOVA). Regression analysis and ANOVA of germinated seed was determined using JMP 4 Statistical Discovery 2000.

RESULTS AND DISCUSSION

of salinity on germination: The rate of seed germination of C. odorata was significantly (p<0.0001) influenced by increase in salinity level. Seed germination reduced between 0.1-1.0 mol L^{-1} NaCl and percentage seed germination was low at >1 mol L^{-1} NaCl (Fig. 1). At higher concentration (0.1-1.0 mol L⁻¹ NaCl), percentage germination at 8 days was, 0.1 mol L⁻¹ (48, 32%), 0.2 mol L⁻¹ (10, 12%) and 1.0 mol L⁻¹ (10, 8%) for primary and secondary forests in parenthesis, respectively (Fig. 1). The seed emergence was also generally delayed 5-8 days (Fig. 1). The mean percentage of germinated seeds was significantly lower as salinity level increased; $0.02 \text{ mol } L^{-1}$ (73, 48%; Fig. 2a), $0.04 \text{ mol } L^{-1}$ (78, 46%; Fig. 2b), $0.06 \text{ mol } L^{-1}$ (60, 44%; Fig. 2c) and 0.08 mol L^{-1} (68, 40%; Fig. 2d) at 8 days. Germination rate in both primary and secondary forests was highest at low salinity concentrations of NaCl-0.02 mol L⁻¹ (Fig. 2a) and 0.04 mol L⁻¹ (Fig. 2b). Initial seed germination began earlier (2 days after seeding), at lower salinity levels 0.02 mol L⁻¹- $0.06 \text{ mol L}^{-1} \text{ NaCl concentrations (Fig. 2a-c)}$. The rate of emergence was significantly delayed by slight increases in the NaCl concentration (Fig. 2a-d). Seeds did not germinate in higher NaCl concentration treatments (2.0-3.0 mol L⁻¹). However, seeds that were previously exposed to high NaCl concentrations for 14 days, could germinate when they were reintroduced into plain water. Also, primary forest (Townsville-TSV) seeds in 0.08 NaCl concentration, emerged 3 days (Fig. 2a) while germination in 0.2 mol L⁻¹ NaCl solution, started 7 days (Fig. 1). The secondary forest

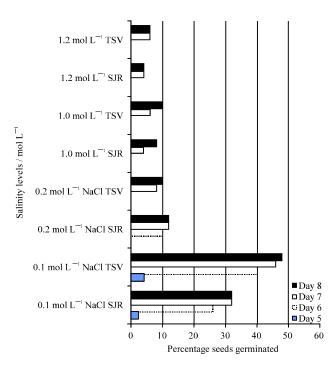


Fig. 1: The cumulative mean percentage of seeds that germinated in various concentrations of sodium chloride (NaCl) solution as compared between the primary forest in Townsville region (TSV) and the secondary forest in South Johnstone River catchment (SJR)

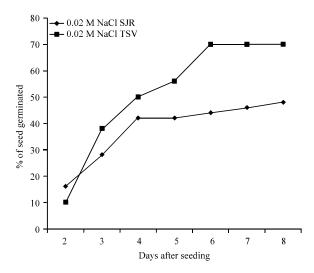


Fig. 2a: Daily mean percentage of non-smoked seeds that germinated in 0.02 mol L⁻¹ of sodium chloride (NaCl) solution tested for seeds from the primary forest in Townsville region (TSV) and the secondary forest in South Johnstone River catchment (SJR)

(South Johnstone-SJR) in 0.08 mol L^{-1} NaCl germinated 2 days (Fig. 2a) and 6 days in 0.2 mol L^{-1} NaCl (Fig. 1). Strong saline solution appeared to have a preservative effect on the germinal constitution of viable seeds. The seeds from the primary forest responded better to salinity conditions than seeds sourced from secondary forest. In a related study, increasing salt

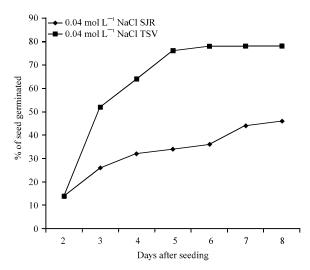


Fig. 2b: Daily mean percentage of non-smoked seeds that germinated in $0.04 \text{ mol } L^{-1}$ of sodium chloride (NaCl) solution tested for seeds from the primary forest in Townsville region (TSV) and the secondary forest in South Johnstone River catchment (SJR)

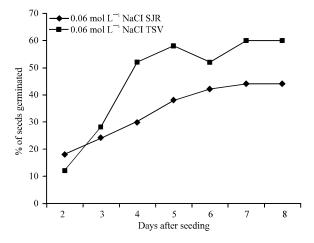


Fig. 2c: Daily mean percentage of non-smoked seeds that germinated in 0.06 mol L⁻¹ of sodium chloride (NaCl) solution tested for seeds from the primary forest in Townsville region (TSV) and the secondary forest in South Johnstone River catchment (SJR)

concentrations were found to have negatively affected germination in *C. odorata*. In a study on the rate of seed germination of *C. odorata*, there was a mean reduction of approximately 50% seed germination at a concentration of 171.6 mM sodium chloride solution (Chauhan and Johnson, 2008). Seeds from primary forest responded better to salinity conditions than seeds sourced from secondary forest. At 0.04 mol L⁻¹ NaCl solution, the mean seed germination was significantly (p<0.0001) highest at 80% for primary forest (Fig. 2c). Optimum salinity levels for the germination ranged between 0.02-0.06 mol L⁻¹ NaCl solution. The percentage mean germination for primary forest ranged between 60-78% and secondary forest 44-48% (Fig. 2b-d). The potential for rapid spread and infestation has been predicted for the primary forests in the coastal and sub-coastal

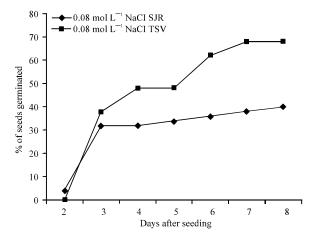


Fig. 2d: Daily mean percentage of non-smoked seeds that germinated in 0.08 mol L⁻¹ of sodium chloride (NaCl) solution tested for seeds from the primary forest in Townsville region (TSV) and the secondary forest in South Johnstone River catchment (SJR)

areas of Queensland, New South Wales, the Northern Territory and Western Australia (Brooks, 2009).

Records on the salinity tolerance of *C. odorata* for the South Johnstone and Townsville regions are previously unknown. However, this study identifies the potential complexities of salinity and weed invasiveness that might combine to predispose native forests in Queensland coastal areas to increased *C. odorata* infestations. Significant populations from the primary forests near the Mossman River, Innot Hot Springs, Innisfail and Townsville areas, are expected to spread to adjoining secondary forests if current eradication strategies fail to adequately inhibit the dispersal.

Effects of smoke on germination: Smoked seeds germinated significantly (p<0.0001) better in water than in soil. Smoke improved germination better than plain water (control), in both primary and secondary forests (Fig. 3). The mean seed germination, was significantly (p<0.002) delayed under soil conditions in both forest types, when compared with smoke solution only conditions. Smoke condition generally seemed to facilitate early seed germination but overtly delay the germinability of seeds sourced from secondary forests as opposed to pattern in primary forest. This might explain the direct impact of habitat disturbance on colonization and rapid succession trajectory exhibited by C. odorata in disturbed habitats, in this case, the secondary forest of Townsville. Non-smoked seeds in soil started germinating 4-5 days after seeding (Fig. 4a). Generally, smoking the seeds appeared to speed up the process of germination thus terminating dormancy significantly. Smoked seeds in soil germinated from 3-4 days after seeding (Fig. 4b). Daily germination rates show that, by 8 days, seeds from secondary forests (72%) had better germination success than seeds from the undisturbed primary forests (64%) in smoke solution (Fig. 5a). Relatively, smoke solution improved germination rate for secondary forest seeds better than under non-smoked conditions. The germination rate for secondary forest was 63% under nonsmoked condition (Fig. 5b). In Australia, seed germination is known to occur at the start of the rainy season (Parsons and Cuthbertson, 2001; Patane et al., 2009). Incidentally, the fire season in Queensland starts from the winter to spring prior to the rainy season. Water was shown to have facilitated germination in non-smoked seeds substantially. By 8 days, the percentage germination

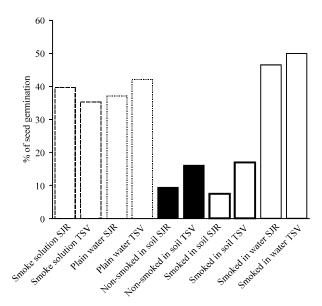


Fig. 3: The cumulative mean percentage of seeds that germinated in various conditions of smoked and non-smoked seeds, mediated and non-mediated soil as investigated between the primary forest in Townsville region (TSV) and the secondary forest in South Johnstone River catchment (SJR)

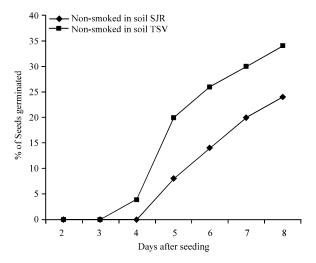


Fig. 4a: Daily rate percentage of non-smoked seeds that germinated in non-mediated soil for seeds obtained from the primary forest in Townsville region (TSV) and the secondary forest in South Johnstone River catchment (SJR)

was 72 and 63% for primary and secondary forests, respectively (Fig. 5b). There was no clear difference between primary (76%) and secondary (72%) forests with respect to smoked seeds that germinated in water (Fig. 5c). Under certain conditions, the weed is highly flammable, however; fires cannot easily be started in dense *Chromolaena* infestations (16 plants m⁻², 100% frequency, = 3 m tall) because of the lack of fine fuels such as grasses and herbs (Goodall and Zacharias, 2002). There is thus, a likely potential for matured seeds in dense

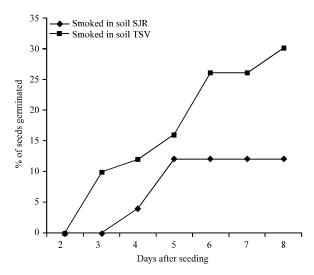


Fig. 4b: Daily rate percentage of smoked seeds that germinated in non-mediated soil measured for seeds taken from the primary forest in Townsville region (TSV) and the secondary forest in South Johnstone River catchment (SJR)

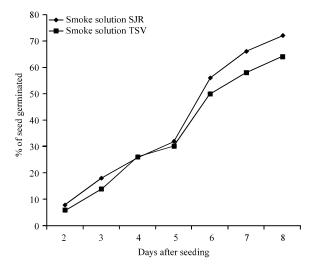


Fig. 5a: Daily rate percentage of non-smoked seeds that germinated in smoke solution, examined for seeds sourced from the primary forest in Townsville region (TSV) and the secondary forest in South Johnstone River catchment (SJR)

infestations to survive partial forest fires and remain dormant till the onset of the next rainy season. The results of the smoke and water treatment tests suggest that under conditions of appreciable rainfall coming soon after a forest fire, the potential for rapid seed germination, establishment and subsequent spread within secondary forests and by extension disturbed habitats like farmlands (croplands and pastures), might be significantly high.

Effects of acid and alkali on seed germination: Potassium hydroxide (KOH 1 M) and sodium hydroxide (NaOH 1 M) both significantly (p<0.001) delayed seed germination when compared with

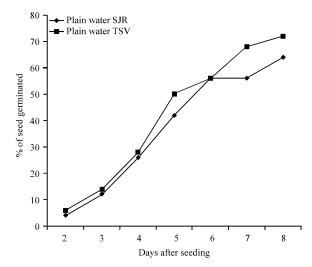


Fig. 5b: Daily rate percentage of non-smoked seeds that germinated in plain water (control), investigated for seeds collected from the primary forest in Townsville region (TSV) and the secondary forest in South Johnstone River catchment (SJR)

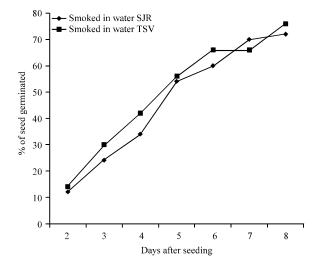


Fig. 5c: Daily rate percentage of smoked seeds that germinated in plain water (control), assessed for seeds obtained from the primary forest in Townsville region (TSV) and the secondary forest in South Johnstone River catchment (SJR)

the effect of other environmental stress cues (smoke, water and soil) on germination success. Wood ash did not generally support seed germination (Fig. 6). Seeds could only germinate in wood ash after the level of alkaline concentration in ash was reduced (Perry, 2011). Seeds from the primary forest performed better in the alkaline solution when compared with the seeds from secondary forests. Hydrochloric acid (HCl 1 M) did not support the germination of the *C. odorata* seeds. The rate of germination was only 2% (Fig. 6). Unlike seeds that were exposed to high NaCl concentrations that germinated after reintroduction into plain water, the seeds in acid and alkaline solutions showed no germinability after the change to plain water.

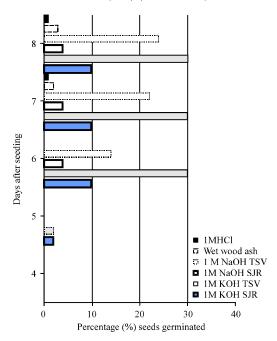


Fig. 6: The cumulative mean percentage of seeds that germinated in 1 M concentration of potassium hydroxide (KOH), sodium hydroxide (NaOH) solution and wood ash, as compared between the primary forest in Townsville region (TSV) and the secondary forest in South Johnstone River catchment (SJR)

The effect of acid and alkaline substances on C. odorata in the study area is not previously been investigated. It has been reported that the prevailing stagnant water and highly acidic soils could suppress the growth and spread of C. odorata (McFadyen, 1996). In addition, there have been noted changes in soil phosphorus and acid phosphatase activity immediately following forest fires (Vazquez et al., 1993). The effects of forest fires on mineralized soil in north Queensland is related to soil heating, combustion and inculcation of ash into the topsoil. Ashes contain compounds which are very alkaline with a pH value of 10 to 12. These compounds may be harmful at higher rates, especially in soils that are already alkaline. Approximately, 80 to 90% of wood ashes are watersoluble mineral matter; high rates can cause salts to build up in soils resulting in increased plant toxification. This study underscored the importance of focusing on early growth stages of the plant. This was confirmed in a related study by Lawes and Panetta (2004). It is presented in this study that initial exposure of mature viable seeds to ash and acid phosphates after a major forest fire, may serve to inhibit early and or rapid seed emergence. This is likely to occur especially among sparsely dense C. odorata populations. However, if the ash and acid load on a site reduces in successive growth seasons as a result of surface leaching, there will likely be the occurrence of higher germination success in response to declining acid or alkaline concentrations. The Tropical Weeds Operational Committee was established in 2005 to provide strategic direction for the Siam weed (C. odorata). Eradication Program which commenced in 1995 (Maher et al., 2008). The total cost for the eradication of C. odorata was \$ 1,533,485 by 2010 (Wickes and Burley, 2008). An economic cost-benefit analysis estimated the combined benefits to agriculture and the environment from eradicating C. odorata over the next thirty years would be 8-\$21 billion (Jeffery, 2010). Besides the potential economic loss, the spread of C. odorata represents a serious threat to native

biodiversity. The Queensland Biosecurity Team reported an increase in the spread of C. odorata core surveillance area. The core surveillance area is the area that included all recorded C. odorata infestations located by GPS mapping and a prescribed dispersal buffer of 200 m. This area included the Johnstone River catchment and Townsville-Thuringowa areas measuring (2538 ha) and 5522 ha (506 ha), respectively. The area under control is shown in parenthesis (Patane et al., 2009). The increase in the ecological and geographical range of C. odorata poses a serious risk to the survival of native biodiversity of the primary forests of Queensland. In a study on the influence of heat on germination and emergence Travlos and Karamanos (2007) reported that heat may impact on germination positively. The study area is prone to fire annually and this trend may help to promote C. odorata spread. The response of the seeds to salinity, smoke, acid and alkaline conditions as demonstrated in this study, shows that (1); seeds dispersed directly to moderately saline soils near the coastal areas might profusely sprout in disturbed habitats namely secondary forests with sparse tree canopy and increased light intensity (2); seeds that may be exposed to smoke but not consumed in pre-spring forest fires, might depict higher germinate rate and result in significant infestations when appreciable amounts of rains follow the fire season (3); concurrently, the accumulation of strongly alkaline wood ash produced from forest fires might delay initial seed emergence but actually promote germination success when subsequent rains reduce the alkalinity levels of ash compounds. The results also show that seeds sourced from primary forest infestations performed better than those from the highly disturbed secondary forests. C. odorata is a pioneer species that grows also in secondary forest succession. It exhibits strong invasive characteristics and a profuse vegetative growth pattern (Hoevers and M'Boob, 1996). The infestations in both habitats have a historical life-span of 18 years. It appeared that plants in primary forests might have become more acclimatized to fertile soils and acquired growth vigor. Further research is needed to predict C. odorata establishment and spread especially to establish the interaction between biomass build-up, fire and germination trajectory (Ilori et al., 2011).

CONCLUSION

This study identified primary forests as potential sources of more viable seeds that may be dispersed to adjoining secondary forest sites, plantation croplands, prairie ranges and pastoral fields. Combined with the presence of a higher fuel load, primary forests might be well considered as predisposing zones for the infestation of $C.\ odorata$. It is important to understand the genetic basis for the trajectory of seed germination chronicled in this study. This is necessary to determine the weed's biological and ecological traits and the conditions essential for early seedling establishment, growth sequence, competitive ability, seed production and dispersal trends. Such knowledge should be useful to understand and improve the current control strategies for $C.\ odorata$ seeds in disturbed forest ecologies in Australia and other tropical areas where infestations are located.

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