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Seed germination eco-physiology of *Mikania micrantha* H.B.K

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Abstract. *Mikania micrantha* H.B.K is an invasive alien weed in South China. To help understand the mechanisms of its invasiveness, we studied its seed germination ecophysiology. We found that the optimal temperatures for seed germination were 25, 30, 15/30°C (night/day), and an ambient temperature (24-32°C). Germination percentage (GP) in dark was less than 10%, and it increased with increasing light intensity up to 700 lx. However, GP and germination index (GI) did not change significantly when light was more than 700 and 550 lx, respectively. Germination was enhanced by white, yellow and red light more than far-red, blue, and green light. Light sensitivity and germination of seeds increased with increasing dark incubation time. Newly ripened seeds exhibited innate dormancy that was released by a two-month after-ripening period in dry storage. Except at a storage temperature of -5°C, seed GP decreased when storage time exceeded the after-ripening period, and this decrease was more pronounced from a storage temperature of 4°C to 15°C to ambient 24-32°C. Percentage seedling emergence was lower and slower when seeds were covered deeper in soil, and no emergence occurred when seeds were buried at 1.5 and 1.75 cm in clayey and sandy soil, respectively. With increased soil moisture content (MC) from 8% to about 20%, seedling emergence became increased and quickened. However, an MC greater than 23% reduced emergence. These responses of seed germination to various treatments indicate that this weed is well adapted to environments in South China.

Keywords: Germination; Light; *Mikania micrantha*, Seed; Soil depth; Soil moisture; Soil type; Temperature.

Introduction

Mikania micrantha (hereafter *Mikania*), a member of the Asteraceae, originates in Central and South America. In its native land, it is a component of aquatic ecosystems such as marshes and riverbanks and rarely grows in other habitats (Ye and Zhou, 2001). However, it is very variable in form, and in many areas it is extremely invasive. *Mikania* has been called "Mile-a-minute" and "plant-killer" (Waterhouse, 1994; Ye and Zhou, 2001). The species is widespread throughout tropical Asia, including India, Malaysia, Thailand, and Indonesia, and it has recently been observed in Nepal and Australia. *Mikania* also occurs in Papua New Guinea, the Solomon Islands, the Philippines, Christmas Island in the Indian Ocean, and on Pacific Ocean islands including Fiji and Western Samoa (Evans et al., 2001). Since the 1980s, it has been found in South China. Its distribution is much wider than previously thought (Sankaran et al., 2001).

Mikania is one of the 100 most serious tropical weeds in the world. It is a fast growing perennial creeping vine that colonizes agricultural land and damages tree crops, agroforestry, and multipurpose trees in moist forest zones of Asia, particularly Southeast Asia (Muniappan and Viraktamath, 1993; Waterhouse, 1994) and South China (Huang et al., 2000). The species causes substantial yield losses in agroforestry systems, in tea, oil palm, rubber, teak, and sal (*Shorea robusta*) plantations, as well as in many crops including bamboo, reed, plantains and pineapples. *Mikania* has also invaded natural evergreen, semi-evergreen, and moist deciduous forests, and it is threatening the biodiversity of these ecosystems. At present, no efficient ways are available to control this aggressive weedy species (Holm et al., 1977; Ye and Zhou, 2001).

Mkania has vigorous vegetative growth and a high sexual reproductive capacity (Swarmy and Ramakrishnan, 1987), but it multiplies mainly by seed (Holm et al., 1977). Optimal conditions for seed germination and emergence are often a reflection of the optimal growth conditions for the entire life cycle of a plant species, and the survival and invasion of plants are associated mainly with the mechanisms of seed germination and emergence (Raejmanek and Richardson, 1996). Seed morphology influences seed dispersal, and environmental factors—such as temperature, light, soil moisture content, and soil depth—affect seed germination and seedling establishment (Baskin and Baskin, 1989; Benvenuti et al., 2001). Knowledge about these stages of the life cycle of *Mikania* will help us understand the optimum conditions for its seed germination and seedling establishment, predict its population dynamics, explain how this species spreads to new areas, and enable us to develop methods to control its invasion. However, we do not have such basic information for the species. Thus, we observed the morphological characteristics of *Mikania* seeds, studied its seed germination in different storage

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conditions, incubation temperatures and light regimes, and investigated the effect of soil types, soil moisture level, and depth of burying in soil on seedling emergence.

Materials and Methods

Seed and Seed Size

Seeds used in this study were collected in autumn 2002 from a wild population of *Mikania* in a natural habitat in Dongguan City (22°39´~23°09°02´N, 113°31´~114°15´E), Guangdong Province, China. The population consisted of over 200 individual plants. Immediately after collection, four groups of 1000 seeds were weighed with a digital balance to estimate the average 1000-grain-weight, and four groups of 20 seeds were used to measure seed size (width and length) with scaled rulers. Seed moisture content (MC) was determined according to the Rules of ISTA (1985).

Water absorption and floatation time in distilled water was studied in naturally air-dried seeds with and without pappus. Experiments were carried out with four replicates of 100 seeds each, in transparent plastic bottles that were 5.5 cm in height and 3 cm in diameter, with the bottles kept at 25°C.

Common Methods in Seed Germination and Emergence Tests

Growth chambers with automatic temperature and light controls were used in all germination and emergence experiments. Only black, fully developed seeds were used. Seeds with approximately 10% MC were stored in sealed plastic bags at 15°C for 60 days before use. Seeds were soaked for 10 min in 0.2% sodium hypochlorite, washed with distilled water, and then placed on 0.8-1% agar in petri dishes (90 mm in diameter) covered with lids, with each replicate consisting of 50 seeds. For all tests, four replicates were used per treatment. The test period was 30 days. Germinated seeds were counted in their test conditions once per day (with germination in dark monitored in far red light). Germination temperature was 30°C; light at the soil surface was 1000 lx provided by cool white fluorescent lamps, and photoperiod was 12 h per day, unless described differently in specific tests. Germination and emergence were defined as the appearance of a radicle over 0.5 cm in length. Details of methods were as follows:

Temperature. Temperatures at which germination was tested were constant 5, 10, 20, 25, 30, 35 and 40°C, alternate 15/30°C (night/day), and ambient (24 to 32°C).

Light quality. Seeds were germinated at ambient temperature in white, far-red, red, yellow, blue, and green light at approximately 600 lx. Far-red light was supplied by a 150W medical infrared lamp (650-750 nm, the R/FR ratio was about 0.4), and white, red, yellow, blue, and green lights were provided by 15W fluorescent lamps.

Light intensity. Effects of light intensity on germination were determined at ambient temperature at 0, 100, 200, 300, 550, 700, 850, 950 and 1050 lx provided by cool white light lamps. Light intensity was regulated by changing number of lamps and lamp height.

Light exposure and incubation time. Seeds were incubated at 30°C in dark for 7 or 14 days, then exposed to cool white light at 200 lx for 1 h and at 550 lx for 2 h or 4 days for 12 h a day and then germinated in dark for 30 days.

Storage temperature and length. Seeds were dried on silica gels in desiccators at ambient temperature for 2 days to obtain an MC of about 10%. They were packaged in aluminum foil, and the packages were placed at -5, 4 and 15°C and at ambient temperature for 10, 40, 60, 90, 120, 150 and 210 days. At the end of each storage time, seeds were taken out of these packages, seed samples made as described earlier and tested for germination.

Soil depth. Polyvinyl Chloride boxes (28 cm×17.5 cm×12 cm) were filled with sandy or clayey soil, and their bottoms and sides were covered with aluminum foil to allow light to reach seeds only from above the soil surface. The soils were screened through a 1 mm mesh and sterilized at 105°C for 5 h in an oven. They were then moistened with distilled water to approximately 20% MC. Each box was divided into four sections by black plastic dividers, and one sample of seeds was sown onto each section. The seeds were then covered with sand in the sandy soil box or clay in the clayey soil box at depths of 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5 and 1.75 cm.

Soil MC. Seeds were placed on the bottom of petri dishes and covered by about 0.25 cm of washed, sterilized dry soil. The sandy soil and clayey soil were mixed with distilled water to create water to soil percentages of approximately 8%, 12%, 15%, 18%, 20%, 22%, 25%, 28% and 30% in weight. The dishes were covered with lids and placed in fluorescent light with 550 lx at the soil surface. Distilled water was added to each petri dish every other day to retain the original moisture/soil ratio. Germinated seeds were counted without removing, which would have reduced soil moisture.

Calculations for Each Seed Sample

1. Germination percent (GP) = (number of germinated seeds/ total number of seeds) × 100,
2. Germination index (GI) = $\Sigma\ (G_t/D_t)$, summation of mean number of germinated seeds per day for t days,
3. Emergence percent (EP) = (number of emerged seedlings/ total number of seeds) × 100, and
4. Emergence index (EI) = $\Sigma\ (G_t/D_t)$, summation of mean number of emerged seedlings per day for t days.

Statistical Analysis

We used $p<0.05$ as significance level. Percentage values were transformed into arcsin $(P/100)^{1/2}$ for ANOVA. Data were subjected to ANOVA using Excel (Microsoft Inc., 1985-1999, Version 2000) to determine if there were significant treatment effects. LSD was used to test for significant differences among treatment means for significant treatment effects.

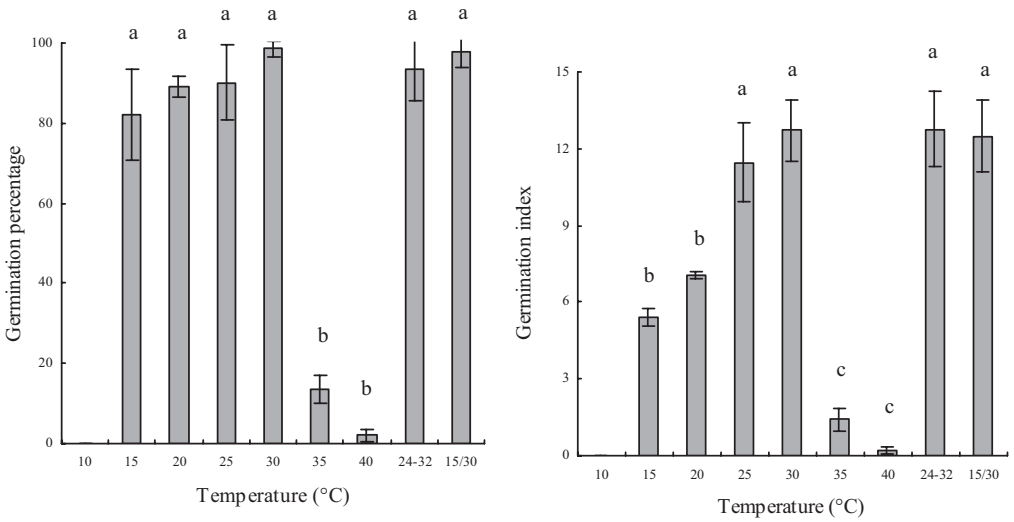

Figure 1. Germination percentage and germination index of *Mikania micrantha* seeds at various temperatures.

Results

Morphological Characteristics of Seeds

Mikania flowers and achenes (hereafter called seeds) were produced from August to next May in South China, but abundant flowering and seed-setting mainly occurred from Sept. to Nov. In natural habitats, a single plant produced up to 4000 viable seeds every year.

Seeds were linear-oblong, four or five-angled, glabrous, and the surfaces were sparingly glandular. Most of the ripe seeds were black or dark brown while a small number were yellowish or brown. Length was 1.85±0.24 mm (mean±SE, n=50) and width 0.42±0.11 mm. The average 1000-grain dry weight of fully ripe seeds was 0.087±0.016 g. Each seed had a terminal pappus of 25 to 38 soft white bristles, and the pappus was 2 to 4 mm long.

Seeds with pappus absorbed two to four times more water than those without pappus when placed on the surface of distilled water. Seeds with or without pappus could float on water for a long time. Even after 7 days, 70-80% of seeds (including germinated ones) with pappus, and 30% to 50% of those without pappus, still floated.

Germination at Various Temperatures

GPs and GIs of *Mikania* seeds were 90-98% and 11-13, respectively, at 25°C, 30°C, 15/30°C, and ambient temperature, and there were no significant differences among these four temperatures for either parameter (Figure 1). In these conditions, germination began after incubation for 3 days and was almost finished 5 days later. Although GPs at 15 and 20°C were not significantly different from those at the above temperatures, GIs were significantly smaller. At 15 and 20°C, seeds began to germinate after incubation for 7 and 4 days and finished about 15 and 10 days later, respectively. At 10, 35 and 40°C, GPs and GIs were significantly lower than those at the other temperatures (Figure 1).

Germination in Various Light Intensities

Less than 10% of the seeds germinated in the dark. GIs increased with increase in light intensity from 0 to 550 lx, and GPs increased from 0 to 700 lx (Figure 2). There were no significant differences among GIs above and including 550 lx, or among GPs above and including 700 lx (Figure 2).

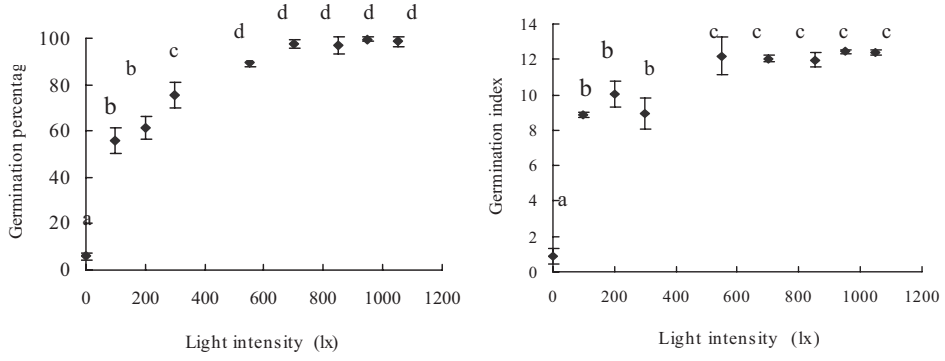

Figure 2. Germination percentage and index of *Mikania micrantha* seeds at different light intensities.

Table 1. Germination percentage (GP %) and germination index (GI) of *Mikania micrantha* seeds in various light qualities.

Light quality	Far-red	Red	Yellow	White	Blue	Green
GP (%)	30.00 ± 5.0[b]	82.50 ± 10.50[a]	80.50 ± 11.70[a]	75.50 ± 5.26[a]	32.00 ± 4.32[b]	24.50 ± 8.85[b]
GI	3.05 ± 0.58[b]	9.70 ± 1.05[a]	9.62 ± 0.65[a]	8.75 ± 0.71[a]	3.36 ± 0.24[b]	2.69 ± 0.98[b]

Values in each row with the same letters are not significantly different at p=0.05 in LSD test.

Table 2. Effect of a short period of white light on seed germination percentage (GP %) of *Mikania micrantha*, as influenced by duration of incubation, light intensity, and duration of light exposure.

Days of incubation	Light intensity (lx)	Light exposure duration	GP (%)
7	0	0	10.00 ± 5.00[a]
7	200	1 h	38.50 ± 10.26[d]
7	550	2 h	49.33 ± 1.15[d]
7	550	4 days (12 h/d)	83.00 ± 5.29[bc]
14	0	0	10.50 ± 5.03[a]
14	200	1h	72.00 ± 1.58[b]
14	550	2 h	77.78 ± 6.94[b]
14	550	4 days (12 h/d)	92.50 ± 5.29[c]

Values in the last column with the same letters on right corner are not significantly different at p=0.05 in LSD test.

Germination in Various Light Qualities

GPs and GIs were not significantly different among red, yellow and white light or among far-red, blue and green light (Table 1). However, GPs and GIs were significantly higher in the former than in the latter three light conditions.

Germination after Dark Incubation and Light Exposure

GPs were almost the same for seeds incubated in the dark for 7 and 14 days without light exposure, but they were significantly lower than the GPs in other treatments (Table 2). After a 7-day incubation, GPs of seeds exposed to 200 lx for 1h and to 550 lx for 2 h did not differ significantly, but both were significantly lower than the GP of seeds exposed to 500 lx for 4 days. These trends were the same for seeds incubated for 14 days. With light exposure, GPs were higher after incubation for 14 days than for 7 days and significantly so at light exposures of 200 lx for 1 h and 550 lx for 2 h (Table 2).

Germination after Storage at Various Temperatures

GP of freshly harvested seeds was only about 10%. GPs increased at all four storage temperatures as storage time increased from 0 to 60 days (Figure 3). As the storage time increased from 60 to 210 days, GPs remained similar for seeds stored at -5°C, but they were lower at the other three storage temperatures. Such a decrease in GP with storage time became more pronounced with increasing storage temperatures from 4°C to 15°C and 24-32°C (Figure 3).

Seedling Emergence in Various Soil Depths

EP (98.5%) and EI (16.42) were highest for seeds placed on the surface of both clayey and sandy soils (Figure 4). However, they were higher in sandy than in clayey soil at other depths and decreased as soil depths increased. No seedlings emerged from 1.5 cm in clay or 1.75 cm in sand.

Seedling Emergence in Various Soil MC

Seedlings began to emerge after 3 days both in the clayey and sandy soil. EP and EI increased with increasing soil MC from approximately 8% to 20%, and they changed little with MC from 20.19% to 22.37% in the clayey and from 18.33% to 22.62% in sandy soil (Figure 5). EP and EI decreased as the soil MC increased from 22.37% to 29.21% in clayey soil and from 22.62% to 26.50% in sandy soil. Below an MC of 12%, EP and EI were not significantly different between clayey and sandy soil, but at an MC of 10%-20%, they were higher in clayey than in sandy soil while at an MC of above 23%, they were higher in sandy than in clayey soil (Figure 5). The optimum soil MC for emergence was approximately 18-23% in clayey soil and 20-23% in sandy soil.

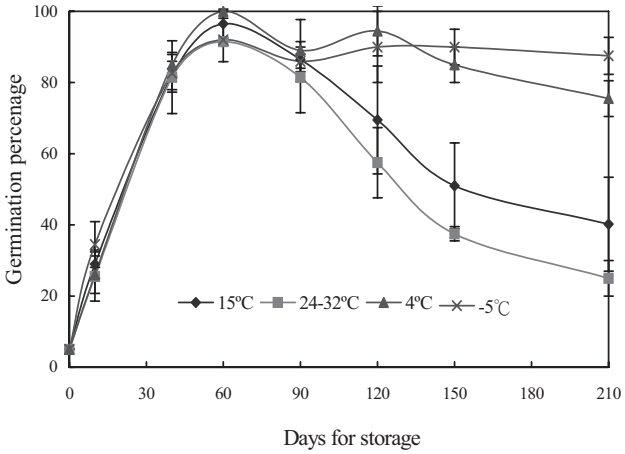

Figure 3. Effect of storage at various temperatures on seed germination.

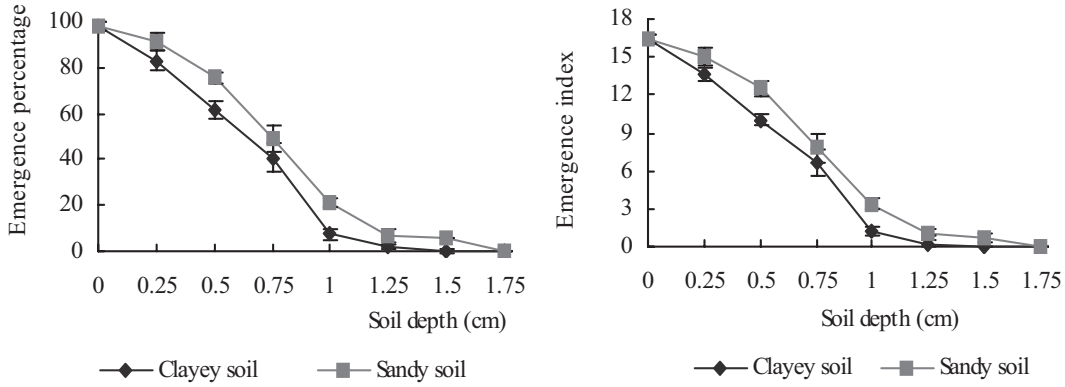

Figure 4. Effect of soil depth on seedling emergence.

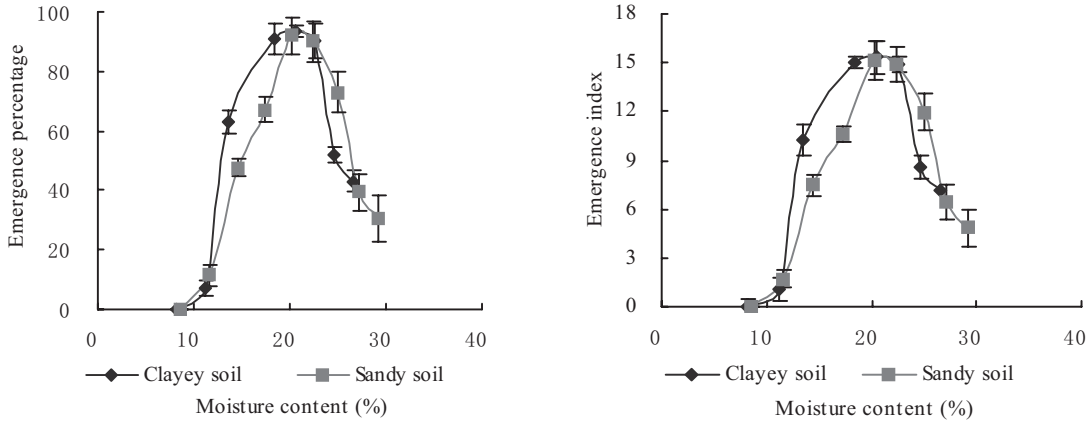

Figure 5. Effect of soil moisture content on seedling emergence percentage and emergence index.

Discussion

Mikania produces a copious amount of seeds, which is one of the reasons this species has great invasive potential. The seed is very light and has pappuses that facilitate dispersal by wind. The pappus enables seeds to cling to the clothing of humans, the skin of animals and other surfaces. When the seeds fall on the surface of water, the pappus helps prevent them from sinking and thus facilitates dispersing the seeds by runoff water. It helps the seed absorb water beneficial for seed germination. Therefore, the pappus is of ecological and physiological importance.

Although the optimal temperatures for germination of the seeds were 25, 30, 15/30 (night/day), and an ambient temperature of 24~32°C, some seeds can germinate at 15 and 35°C. This relatively broad temperature range for seed germination may be one reason this invasive weed is distributed so widely in tropical and subtropical areas. Temperature fluctuation enhances seed germination for some plant species (Benvenuti et al., 2001), but in our study alternate temperatures did not significantly improve seed germination.

The seeds had innate dormancy as fresh seeds germinated poorly and dry storage improved germination considerably. Storage for 60 days at -5, 4, 15°C, and at an ambient 24~32°C gave the highest germination. Therefore, the seeds need about two months for dormancy to be completely released. This result supports the viewpoint by Hu and Pavl (1994) that *Mikania* seeds require a period of after-ripening to come out of dormancy. As in many other species, this dormancy release may be related to the degradation of seed germination inhibitors (Baskin and Baskin, 1989).

Storage temperature is one of the most important factors affecting seed longevity. When storage time was longer than 60 days, the higher the storage temperatures, from 4 to 15 and 24~32°C, the faster the GP decreased in our study. This temperature effect is due to the fact that high temperature causes high metabolism (Robert, 1973).

In South China, most seeds of this weed mature from Sept. to Nov. and germinate the next spring and early summer. Our results show that after storage for 7 months at 15°C and 24~32°C, germination was still over 40 and 20%, respectively, and much higher at 4 and -5°C. These are within the temperature ranges in the natural habitats of this weed. Therefore, from maturation to germination temperature alone would not kill all the seeds in nature.

Seeds of this invasive plant are light sensitive, germinating much better in light than in dark. Hu and Pavel (1994)

also found that the seeds required light for germination. In addition, we found light-sensitivity of the seed increased with increasing incubation time in dark. This implies that living seeds of this invasive plant buried in moist soil would increase in light sensitivity and would germinate when they received some light due to disturbances. Furthermore, in our study the seeds germinated significantly better in white, yellow, and red light than in far-red, green, or blue light, as reported for some other weed species (Kiatsoonthorn and Tjitrosemito, 1992; Bell et al., 1999). Thus, *Mikania* seeds may not germinate well under a plant canopy where the FR/R ratio is high (Frankland, 1981). The mechanism of red/far-red light regulating seed germination via phytochrome system is well understood (Cone and Kendrick, 1986). Finally, we observed that a clayey soil depth of 1.5 cm or a sandy soil depth of 1.75 cm completely inhibited emergence. Therefore, *Mikania* seeds buried by soil may not germinate. Overall, these results indicate that this invasive weed is adapted to open light conditions or disturbed habitats for its germination and establishment. Such environmentally induced photosensitivity of seeds is an adaptation mechanism to ensure that seeds will germinate in sites where the probability of seedling establishment is high (Cone and Kendrick, 1986; Benvenuti et al., 2001). These responses have very important ecological consequences for the formation of a soil seed bank (Baskin and Baskin, 1989).

In this study, soil types influenced EP and EI. Sandy soil has lower particle density and larger particle size than clayey soil. Therefore, more light and air would penetrate deeper into sand than into clay. This may have resulted in the better seed germination and emergence in sandy than in clayey soil at the same soil depth. This plant has small seeds that contain only a small amount of food reserves, and thus the seedlings will not emerge if seeds are buried too deep, as we observed.

Soil MC is an important factor for seed germination and seedling emergence in nature. In this study, the optimum soil MC for emergence of *Mikania* seedlings was 18-23% in clayey and 20-23% in sandy soil. This indicates seed germination and emergence of this plant are dependent on high water availability. *Artemisia ordosica* seeds can germinate at 1.7% MC and reach highest germination at 4.9% MC in sand (Huang and Gutterman, 2000). These results compare with 9% and 20%, respectively, for *Mikania* seeds in our study. In South China, the rainy season occurs in late spring and early summer, and temperatures during these periods are mild, which would favor the germination and seedling establishment of this species. Although light and temperature should favor germination in late autumn and early winter, low soil MC caused by low rainfall would limit seed germination of this weed.

We also found that soil MC higher than the optima inhibited seed germination of this plant. This may be due to insufficient oxygen in the soil and explain why it is rarely found in swamps or other wet habits. Similar results have been reported for many crops and wild plants (Huang and Gutterman, 2000). When seeds of this weed germinated in soil with MC less than 20%, seedling emerged better in clayey soil than in sandy soil. This may be due to more water in the clayed soil than in sandy soil. However, in soil with an MC greater than 23%, seedling emerged better in sandy soil than in clayey soil, which may be due to the sandy soil being more permeable to air than the clayey soil when soil moisture is high.

In conclusion, the seed germination responses of *Mikania micrantha* to various treatments in our study indicate that this weed is well adapted to environmental conditions in many tropical and subtropical areas. This may be one of the important reasons it often invades and dominates plant communities in open areas of South China.

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假澤蘭種子萌發的生理生態學特性研究

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假澤蘭是華南地區危害嚴重的外來雜草。本文對其種子的生理生態特性進行研究以探討其入侵機制，結果表明：種子萌發的適宜溫度是 25～30ºC，15/30ºC 和室溫（24～23ºC）。種子在黑暗中的萌發率低於 10%，光強由 0 升至 700 勒克斯時，發芽狀況逐漸變好，當光強分別達到 700 和 550 勒克斯後繼續增大，發芽率和發芽指數不再有明顯變化。種子在白光、黃光和紅光下的發芽情況比在遠紅光、藍光和綠光下要好。種子在黑暗中浸泡的時間越長，光敏感性也越強；浸泡後的種子移至光下萌發，光量越大，發芽率越高。剛成熟的種子有休眠現象，經乾燥後熟後可解除。在室溫 和 15ºC 貯藏超過 90d，或在 4ºC 下貯藏超過 120d，發芽率顯著下降，在室溫和 15ºC 時下降速度比 4 ºC 和 -5 ºC 時要快。出苗率隨土壤深度的增加而降低，當土壤深度在粘土和沙土中分別超過 1.5 cm 和 1.75 cm 時，出苗率均為 0。當土壤濕度由 8% 上升至 20%，出苗率逐漸升高，當濕度超過 22%，出苗反而受到抑制。假澤蘭種子萌發實驗結果表明它對華南地區有很強的適應性。

關鍵詞：萌發；光照；假澤蘭；種子；土壤深度；土壤濕度；土壤類型；溫度。