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Effect of Ecological Factors on Seed Germination of Alien Weed *Tridax procumbens* (Asteraceae)

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Author's contribution

The sole author designed, analyzed and interprets and prepared the manuscript

Original Research Article

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ABSTRACT

Aims: To determine the effects of light, temperature, pH, salt stress, moisture content, depth of seed burial, and storage periods on its seed germination of *Tridax procumbens* seeds.

Study Design: The seeds of *T. procumbens* were collected in October - December 2012 from several paddy fields along Thailand. Seeds collected from many randomly selected plants were unsoiled and stored at room temperature until used in the experiments.

Place and Duration of Study: The experiment was conducted in Faculty of Science and Technology, Phranakhon Rajabhat University, between January 2013 and May 2014.

Methodology: To increase understanding of its rapid spread, the influences of light, temperature, pH, salt stress, moisture content, depth of seed burial, and storage periods on its seed germination have been evaluated.

Results: The seeds were obviously stimulated germination by light in *T. procumbens*, with highest germination rate at an alternating under the 12 h light/12 h dark regime, but a few seeds still germinated in the dark. The seeds germinated at a constant temperature in the range of 5–45°C and reached a maximum at 25°C. The seeds germinated over a wide pH range (4–10), with the highest germination rate at 7. The optimum soil moisture content for germination was around 5- 40%. The species was moderately tolerant of salt stress, with NaCl solution concentration > 0.15 mol L⁻¹. The highest seedling emergence occurred for seeds placed from 0 to 2 cm deep, and no seedlings emerged from a 3-cm burial depth.

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The seeds of *T. procumbens* have no apparent dormancy and retain a high viability after room storage for 450 days.

Conclusion: This study has demonstrated that *T. procumbens* are adapted to a wide range of environmental conditions.

Keywords: Seeds; germination; light, temperature; pH; burial depth.

1. INTRODUCTION

Alien, exotic, introduced, non-indigenous, or non-native species is a species existing outside its native distributional region, which has mostly arrived there by anthropogenic activity, either intentional or accidental. Many of these alien can become invasive when they succeed to outcompete indigenous organisms for resources [1,2]. Knowledge of the influence of environmental factors on the seed germination of an alien weed species is consequently important for understanding successful of such a species, as well as determining its management practices [3].

Tridax procumbens L. is native to tropical America in the daisy family (Asteraceae). The species is a short-lived perennial plant with stems 10-60 cm tall with adventitious roots arising at the nodes. This species is assumed to have been introduced to paleotropical region as early as 1900 and later becoming a common weed of arable land [4]. Currently, the species is widespread throughout the tropical and sub-tropical regions as a pioneer species on disturbed land in several countries. In Thailand, the species is habitually abundant in pastures and cultivated areas as well as along the roadsides, waste grounds, on the open gravel banks and rocky shores of rivers [5].

In spite of its wide distribution, little published information is available on the factors affecting the seed germination of *T. procumbens* [5]. The objective of this study was to know the effects of light, temperature, pH, salt stress, moisture content, depth of seed burial, and storage periods on its seed germination of *T. procumbens* seeds. A better understanding of seed germination of the species could assist the progress of successful this alien weed control policy as well as a description of the potential infestation range of the species.

2. MATERIALS AND METHODS

2.1 Seed Collection

The seeds of *T. procumbens* were collected in October - December 2012 from several paddy fields along Thailand. Seeds collected from many randomly selected plants from all 77 provinces in Thailand according to ISTA rules for seed testing [6] were unsoiled and stored at room temperature until used in the experiments.

2.2 Seed Germination

For sterilization, the seeds were soaked with $1gL^{-1}$ of $HgCl_2$ for 1min and then they were rinsed three times with deionized water. Fifty seeds per treatment, replicated three times, were placed into a 9 cm Petri dish with four pieces of filter paper, moistened with 9ml of distilled water or a treatment solution. Under these conditions, the seeds were not submerged but imbibed on the moistened paper. To make sure that the pH value and the

concentration of the liquid in the Petri dishes stayed constant, one day after treatment, the solution was sucked out of each Petri dish and then substituted by the required liquid. The same routine was done every other day after treatment. Each Petri dish was sealed with Parafilm, and then put in a growth chamber with a constant temperature. The germinated seeds with an obvious radicle were counted and removed daily for 10–18 days until no further germination arise for 5 days [7]. The ungerminated seeds were subjected to a simple pressure test to determine their viability [8].

2.3 Effect of Light

To determine the influence of light on seed germination, the seeds were incubated under darkness, in comparison with those under the 12h light/12 h dark regime. To exclude light in the treatment with darkness, the Petri dishes were subsequently covered in black polyethylene and the seeds were incubated in a dark chamber. Incidental light was received only during the initial wetting and germination counts. Fifteen days after, the ungerminated seeds in the dark were transferred to light/dark conditions to determine whether the dark condition adversely affected seed germination [5].

2.4 Effect of Temperature

The seeds were incubated in growth chambers with constant temperatures of 5°C, 15°C, 25°C, 35°C and 45°C, respectively. Eighteen days after, the ungerminated seeds from each dish treated with 5°C and 45°C were incubated at 25°C for 5 days to continue the germination experiment.

2.5 Effect of pH

The seeds were exposed to HCl or NaOH aqueous solutions of pH = 4 (10^{-4} mol L⁻¹ HCl solution), pH = 6 (10^{-6} mol L⁻¹ HCl solution), pH = 8 (10^{-6} mol L⁻¹ NaOH solution), and pH = 10 (10^{-4} mol L⁻¹ NaOH solution), respectively, in comparison with the seeds treated with deionized water as the control (pH=7).

2.6 Effect of Soil Moisture Content

Sterilized and rinsed sand was added in each 9cm Petri dish. Different soil moisture contents (5%, 10%, 20%, 30%, and 40%) were achieved by adding deionized water at 2.5g, 5g, 10g, 15g, and 20g, respectively. Vaporized water was added to the Petri dishes every day.

2.7 Effect of Salt Stress

The effect of salt stress was determined in NaCl aqueous solutions of three concentrations $(0.05 \text{mol } L^{-1}, 0.10 \text{mol } L^{-1}, \text{ and } 0.15 \text{mol } L^{-1})$, in comparison with the control (deionized water). Eighteen days after, the ungerminated seeds from each dish treated with NaCl solution were incubated with deionized water for 5days to continue the germination trial.

2.8 Effect of Seed Burial Depth on Seedling Emergence

The effect of the seed burial depth on the seedling emergence of *T. procumbens* was studied in a greenhouse by placing 50 seeds in soil within 15cm-diameter plastic pots. The seeds were located on the soil surface (0 cm) or covered to depths of 1, 2, and 3cm with

soil. The soil was autoclaved and passed through a 0.3 cm sieve before the pots were filled and the experiment commenced. The pots were watered as required to preserve enough soil humidity. The seedlings were considered to have emerged when a cotyledon could be seen and the experiment was finished when no further emergence was documentation for a continuous 27days interval. At the end of the research, the seeds that were buried at the deepest depth, that is, 3cm were retrieved and spread on the soil surface to determine whether they would germinate. The ungerminated seeds were subjected to a simple pressure examination with tweezers to conclude whether the seeds were still viable [9,10].

2.9 Dormancy and Longevity

The seed germination experiments were conducted at 0 day, 150 days, 300 days and 450 days after seed collection and storage in room conditions.

2.10 Data Analysis

Experimentations were performed in entirely randomized propose. Treatments of each experiment were replicated three times, and each experiment was performed twice. Each duplicate for each treatment was positioned randomly inside the growth chamber and rearranged daily. In the temperature investigation, each Petri dish was placed randomly in different growth chambers with their respective temperature regimes. Information was checked to prove normality and homogeneity of variance. One-way ANOVA was employed to test the differences of the data from the experiments with the procedure of SPSS (version 18) data analysis package was used for the statistical analyses.

3. RESULTS AND DISCUSSION

3.1 Light Effect on Seed Germination

The seed germination of *T. procumbens* was obviously stimulated by light with highest germination rate at an alternating under the 12h light/12h dark regime (P < 0.01), however but some seeds still germinated in the dark (Fig. 1). The fact that seed germination arises mostly in the light indicates that the seedling emergence of *T. procumbens* in the field would be favored by the occurrence of seeds on the soil surface. Untilled systems are known to create such an environment [5,11].

3.2 Temperature Effect on Seed Germination

Seeds of *Tridax procumbens* began germinated after one days of experiment and finished in 15days. The seeds did not germinate at the extreme low and high temperatures of $<5^{\circ}$ C and $>45^{\circ}$ C regime (P < 0.01) (Fig. 2). The present results indicate that *T. procumbens* might be able to emerge throughout the year in Thailand, as well as in tropical and subtropical regions elsewhere. The temperature range of 15–35°C favored a higher germination rate.

3.3 pH Effect on Seed Germination

The seeds of *T. procumbens* germinated over the pH range of 4–10, but the germination rate was higher in the pH range of 6–7 (Fig. 3). This data indicates that *T. procumbens* is tolerant to both acidic and alkaline conditions (P < 0.01). Similarly, the seeds of many alien weed species germinate over a wide range of pH values [9,12,13]. The seed germination of *T.*

procumbens over such a broad pH range indicates that the soil pH might not be the limiting factor for the germination of this species in most soil types. In Thailand, *T. procumbens* grows in strongly acidic soil and in alkaline soil. It is assumed that *T. procumbens* seeds are able to survive in sea water and that the plant could grow on seashore areas, as well as in strong alkaline soils, because the seeds were able to germinate at a pH of 10.

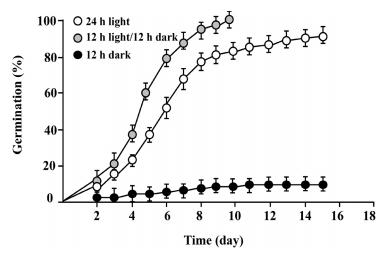


Fig. 1. Seed germination curve of *Tridax procumbens* under a dark and 12 h light/12 h dark cycle. Vertical bars indicate standard errors of the mean

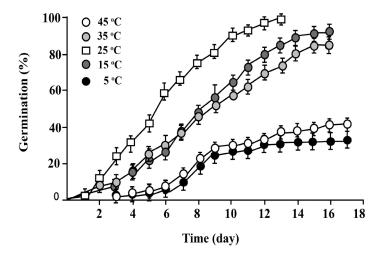


Fig. 2. Seed germination curves of *Tridax procumbens* under different temperatures. The seeds were incubated in a growth chamber under a 12 h light/12 h dark cycle. Vertical bars indicate standard errors of the mean

3.4 Effect of Soil Moisture Content

The germination processes with the treatments of 10–40% moisture content were all similar to each other (P < 0.01) (Fig. 4). The cumulative germination rate of the treatment with the 5% moisture content was ~82 %. The result suggest that *T. procumbens* is well adapted to the dry-land cropping systems where it is a problem, including no-till systems.

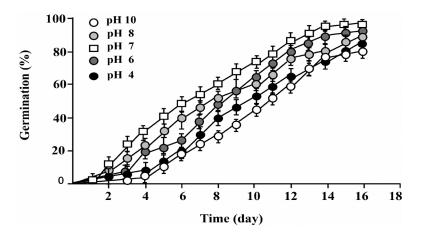
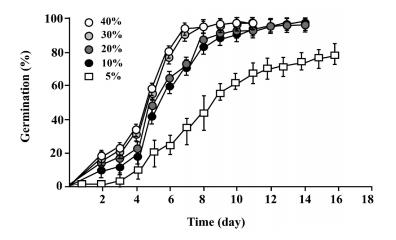
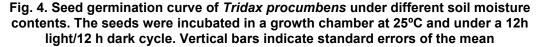


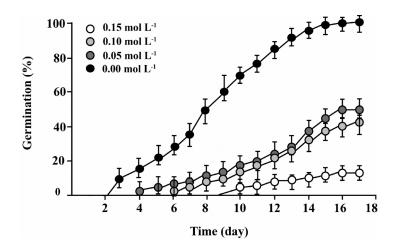
Fig. 3. Seed germination curves of *Tridax procumbens* under different pH conditions. The seeds were incubated in a growth chamber at 25°C and under a 12hlight/12h dark cycle. Vertical bars indicate standard errors of the mean

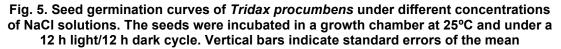




3.5 Effect of Salt Stress

The NaCl solution with different concentrations significantly reduced the cumulative germination (Fig.5). Only~12% of the seeds germinated under the treatment with 0.15mol L⁻¹ NaCl. However, after two days of retreating the seeds with deionized water, the cumulative germination was as high as 86.45±1.62%, indicating that the seeds were not adversely affected by the 0.15 mol L⁻¹NaCl solution (P < 0.01). Similar to Chauhan and Johnson [5] found that salinity also influenced the germination rate of the species. Increasing salinity level to caused reduction in germination rate.





3.6 Burial Depth Effect on Seedling Emergence

The seedling emergence of *T. procumbens* was about 96% when the seeds were placed on the soil surface. The seedling emergence decreased drastically at a depth of 1 and 2cm and to zero under a 3cm soil layer (P < 0.01) (Fig. 6). Similarly, many weed species found the highest seed germination was when the seeds were placed on the soil surface [8,14]. There are numerous probable clarifications for the lack of appearance from seeds buried at deeper depths. Light penetration is usually inadequate to the first few millimeters of the soil [10,14,15] and seeds deeper in the soil would not receive light. Because of the requirement for light by the species, limited light penetration is possibly the major explanation for low emergence of buried seeds. In addition, small-seeded species might have inadequate carbohydrate reserves restricting them to shallow emergence [11]. Therefore, in general the need for light, decreased seedling emergence because of increased seed burial depth also could be related to the seed size of *T. procumbens*. This study suggests that weedy *T. procumbens* could be controlled successfully by covering the seed with at least 3 cm of soil [16,17].

3.7 Effect of Seed Dormancy and Longevity on Germination

The fresh seeds showed ~98% germination, and the 450days old seeds showed retain high viability about ~90% germination (P < 0.01). Viability of *T. procumbens* seeds remained high until the end of the experiment (Fig. 7). This fact indicates that *T. procumbens* seeds were orthodox. This result indicates that most of *T. procumbens* seeds can survive in field conditions for the following year. Similarly to many researchers who found a significantly germination rate in many alien weed species when the seed was stored at ambient temperatures more than 1 year [18,19].

3.8 Effect of Ecological Factors

There is significant importance in identifying alien species characters answerable for recruit achievement, for understanding mechanisms of succession and to design risk-assessment

procedures that support protective and early finding processes of organization [1]. This study indicates that *T. procumbens* seed is capable of germinating over a range of environmental conditions; however, the actual level of germination depends on a number of ecological factors and seed conditions. It is thought that the seeds are able to disperse over long distances by both wind and water because of the pappus attachment [5]. Seedling emergence could be prevented by coverage of about 3 cm of soil and seed production could be prevented by destroying the plants before the pappus appears. Therefore, the ability to germinate well in all environments offers *T. procumbens* the opportunity to spread to new environments and succeed as a weed if it is able to grow and produce well under such conditions. Studies at other life stages are needed to evaluate the potential of *T. procumbens* to become a serious weed.

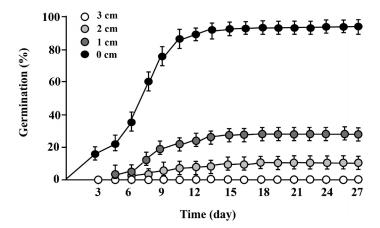


Fig. 6. Seed germination curves of *Tridax procumbens* under different burial depth. The seeds were incubated in a growth chamber at 25°C and under a 12hlight/12 h dark cycle. Vertical bars indicate standard errors of the mean

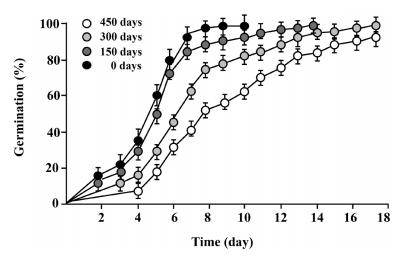


Fig. 7. Seed germination curve of *Tridax procumbens* after different storage periods. The seeds were incubated in a growth chamber at 25°C and under a 12 h light/12 h dark cycle. Vertical bars indicate standard errors of the mean

4. CONCLUSION

The present study has shown that *Tridax procumbens* is adapted to germinate at dispersal time under a wide range of environmental conditions commonly found in the tropics and subtropics, which partially explains its successful infestation in Thailand agricultural fields. As germination was effectively stimulated by light and seedling emergence was optimal at the soil surface. The species can be managed by burying their seeds below the maximum depth of emergence by a tillage operation and subsequently using shallow tillage operations to avoid bringing back the seeds onto the soil surface.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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