

Seed morphology and allelopathy of invasive *Praxelis clematidea*

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Abstract

Praxelis [*Praxelis clematidea* (Griseb.) R.M.King & H.Rob.] is an invasive species that infests many agricultural systems globally, such as orchards, rubber plantations, and other economic crops. The purpose of this research was to study seed morphology, germination factors, and allelopathy of aboveground parts of *P. clematidea*. *P. clematidea* seeds are small, light, and possess pappi that allow them to be dispersed easily by wind or animals. Among four *P. clematidea* populations collected from different provinces in Thailand, the size of *P. clematidea* seeds ranged from 2.6 to 3.2 mm in length, 0.6 to 0.7 mm in width, and were 0.4 mm in thickness. The weight of *P. clematidea* seeds ranged from 0.13 to 0.21 mg. *P. clematidea* had about 44 to 48 seeds per head. Seeds germinated over a temperature range of 20 to 30 °C while high (45 °C) and low (10 °C) temperatures reduced germination. Maximum germination occurred when seeds were planted on the soil surface. No seedlings germinated when seeds were planted at a depth more than 1 cm. *P. clematidea* extracts from aerial plant parts at concentrations of 25 and 50% inhibited seedling growth of hairy beggarticks (*Bidens pilosa* L.). Basic knowledge of the seed biology of *P. clematidea* and allelochemicals can help in understanding the invasiveness and in developing management strategies for this weed.

Keywords: germination; invasive plant; morphology; planting depth; praxelis; temperature

Introduction

Invasive species are organisms that are exotic to an ecosystem (Monaco *et al.*, 2002). They spread aggressively and may affect indigenous ecosystems and biodiversity. They cause negative effects on the environment as well as cause harm to the economy, and human health in the plant communities that they invade (Vitousek *et al.*, 1996). These invasions can lead to alteration of ecology such as changes in species composition, changes in nutrient cycling, and increased forest fires (Sakai *et al.*, 2001).

Praxelis [*Praxelis clematidea* (Griseb.) R.M. King & H. Rob.] together with about 13 other species of the *Praxelis* genus belongs to Asteraceae family (King and Robinson, 1987). It has been classified as an annual or short-lived perennial herb to suffrutescent shrub with a height of between 100 and 130 cm. The stem is erect or branched from the base, and has soft pubescent. The leaves are ovate, rounded to cuneately narrowing at the base, irregularly toothed margins with acute to obtuse apex. The flowers are in cylindrical head inflorescences (capitulescences) with conical receptacles. Primary capitulescence is a corymb while secondary capitulescence is a panicle with peduncle length up to 0.7 cm. The involucre are subimbricate with 2-3 seriate bracts, and the phyllaries are deciduous during fruiting stage. The fruit is an obconical achene with bristle pappi (Abbott *et al.*, 2008; Christ and Ritter, 2019). *P. clematidea* is fast growing and can produce numerous flowers and seeds within the first growing season. Seeds are small in size with a pappus that can help them to easily disperse by wind, animals, and farm machinery. *P. clematidea* has high adaptability and survives in a wide range of conditions (Veldkamp, 1999; Holland, 2006).

Praxelis is native to South America, and distributed throughout northern Argentina, southern Brazil, Bolivia, and Paraguay (King and Robinson, 1987). There were two *Praxelis* species found in southern Brazil, *Praxelis clematidea* and *Praxelis missionum* (Christ and Ritter, 2019). Waterhouse *et al.* (2003) previously reported the various habitats of invasive *P. clematidea* in Australia which included roadsides, stream banks, pastures, and also shady undisturbed woodlands. Veldkamp (1999) mentioned the difficulties in controlling *P. clematidea* due to its robust rootstock and long growing and flowering season. In addition to these characteristics, *P. clematidea* can form dense patches that are self-compatible and resistant to fire, all of which have helped to define *P. clematidea* as a high-risk weed in the USA (USDA, 2014). It has been reported as an invasive weed in many countries such as Australia (CRC Weed Management, 2003), China (Waterhouse *et al.*, 2003), USA (Abbott *et al.*, 2008; Gardner and Williges, 2015), and countries in Southeast Asia (Veldkamp, 1999). In Thailand, *P. clematidea* was first reported in 2003 in orchards and rubber plantations under the misidentified name of *Chromolaena* sp. (Plant Protection Research and Development, 2005) due to its similarity in leaf appearance to Siam weed [*Chromolaena odorata* (L.) R.M.King & H.Rob.] (Anantanamane *et al.*, 2013). The biology and ecology of this species have been rarely studied in Thailand. Anantanamane *et al.* (2013) reported that *P. clematidea* had better germination and growth in sandy soil and produced up to 1,400 seeds per plant in the first season.

Some invasive weed species become dominant due to their ability to produce biochemicals that native species have never encountered (Callaway and Ridenour, 2004). Allelopathy refers to the chemical interaction between plants, including microorganisms with both stimulatory and inhibitory influences (Rice, 1984), and allelopathic potential of plant species can influence the growth and distribution of other species (Rice, 1984; Inderjit and Keating, 1999). Allelopathy has been suggested as a vital mechanism for invasive species to outcompete native plants (Hierro and Callaway, 2003). Many plants in the Asteraceae family have allelopathic potential, and the activities, types, and amount of allelochemicals differ depending on plant species and plant parts (Chon and Nelson, 2010). In *P. clematidea*, allelochemicals have been reported as groups of sesquiterpenes and monoterpenes in volatile oil extracts from fresh aboveground plant extracts (Wang *et al.*, 2006) and groups of flavonoids from dry and aboveground plant extracts (Maia *et al.*, 2011; Falcão *et al.*, 2013). The allelopathic potential of *P. clematidea* has not been extensively studied. Thepphakhun and Intanon (2016) showed that water extracts of fresh *P. clematidea* leaf tissues had the greatest inhibition of germination and growth of leaf mustard [*Brassica juncea* (L.) Czern.] and rice (*Oryza sativa* L.) compared to extracts from the stems and flowers. Patsai (2011) reported that water extracts from dry leaves of *P. clematidea* inhibited germination and growth of field mustard (*Brassica campestris* var. *chinensis*.), mission grass [*Pennisetum polystachion* (L.) Schult.], wild bush bean [*Phaseolus lathyroides* (L.) Urb.], rice, and mung bean [*Vigna radiata*

(L.) Wilczek.]. To date, no studies have evaluated germination and growth inhibition by the combined aboveground tissues of *P. clematidea* on other plants.

Other factors supporting the invasion of *P. clematidea* remain unknown. *P. clematidea* only propagates by wind-dispersed seeds (Veldkamp, 1999), so seed morphology and germination factors are very important in the distribution and establishment of populations. Greater knowledge of *P. clematidea* biology such as seed morphology, germination factors, and plant allelochemicals may help to understand the invasiveness of *P. clematidea* and to develop suitable management strategies. The objective of this research, therefore, was to study the seed morphology and identification, germination factors, and allelopathy of aboveground parts of *P. clematidea*. The hypothesis of this study was that *P. clematidea* invades rapidly due to its ability to disperse and its allelopathic activity.

Materials and Methods

Praxelis characteristics and seed morphology

Morphology of seed, vegetative, and reproductive stages of *P. clematidea* were recorded. *P. clematidea* seeds were collected before shattering from four different locations in the Northern region of Thailand with no previous report of invasion, an orchard area in Chiang Mai Province (Sansai District, 18.916624°N, 99.017832°E), a rice field in Phetchabun Province (Wichian Buri District, 15.674403°N, 101.19486°E), a cassava field in Kamphaeng Phet Province (Phran Kratai District, 16.735016°N, 99.429345°E), and a maize field in Nan Province (Na Noi District, 18.325981°N, 100.573042°E). *P. clematidea* seeds were cleaned by removing plant residues, air-dried, and kept in paper bags at room temperature until use.

A thousand *P. clematidea* seeds with the persistent pappi from the four different populations were weighed. A total of 100 *P. clematidea* seeds were counted in 10 replications and used to determine seed weight (ISTA, 2016). Seed size was determined by measuring the length (longest length without pappus), width (in the middle of seed), and thickness. A hundred replicate seeds were measured using a stereo microscope (SZX7 Olympus, Tokyo, Japan). The seed surface area was calculated by multiplying the length, width, and thickness. The number of seeds per head was recorded by randomly counting seeds on six heads per plant from five plants in each population.

To identify distinguishing characteristics of *P. clematidea* seeds and dispersal mechanisms from similar species in the same habitat of *P. clematidea*, a vegetation survey of Asteraceae species was conducted in a site of 0.8 ha maize field in Nan Province near areas of *P. clematidea* invasion. A sampling unit was a belt transect of 0.5 x 100 m (50 m²) which was placed across the field in three replications to represent weed floras of the sampling area. There were four dominant Asteraceae species, *P. clematidea*, tropic ageratum [*Ageratum conyzoides* (L.) L.], tall fleabane [*Conyza sumatrensis* (S.F. Blake) Pruski & G.Sancho], and redflower ragleaf [*Crassocephalum crepidioides* (Benth.) S. Moore]. Seeds of the four species were collected, cleaned, and stored in paper bags. Seeds were weighed, and the seed surface area was estimated as previously described. Seeds of *A. conyzoides* were weighed with their persistent pappi while seeds of *C. sumatrensis* and *C. crepidioides* were weighed without pappi due to their nonpersistent pappi. The ratio of seed weight to surface area was calculated.

Effects of temperature and depth on germination

Germination tests were prepared for four *P. clematidea* populations, Chiang Mai (CM), Petchabun (PB), Kamphaeng Phet (KP), and Nan (NN) using the germination method of ISTA (2016). The CM population was used in this study due to its greater germination rate within 2 weeks after seed collection as compared to that of other populations which had germination rates less than 60%. The effects of temperature

and planting depth were conducted in the laboratory and the research field station, respectively, at Department of Agricultural Science, Naresuan University, Phitsanulok, Thailand.

Effect of temperature. Twenty-five *P. clematidea* seeds were placed in a Petri dish (Hycon Plastics, Bangkok, Thailand) and incubated at a constant temperature of 10, 20, 25, 30, or 45 °C to evaluate the response to a wide range of germination temperatures. Petri dishes were placed in a growth chamber with a 12 h photoperiod. Seven days later, germination was counted as positive when the seedlings had a hypocotyl and/or a radical length of at least 2 mm (Intanon *et al.*, 2014). The experiment was performed in a completely randomized design with four replications of each temperature. The experiment was conducted twice, and the means were averaged ($n = 8$).

Effect of planting depth. The method was modified from Peachey and Mallory-Smith (2007). Fifty *P. clematidea* seeds were placed in a 0.2 mm mesh nylon bag in order to avoid the loss of seeds. Seed bags were buried in polypropylene soil tubes in the late rainy season in October 2016 and October 2017. Meteorological data such as ambient temperature, relative humidity, and total precipitation were taken from a provincial weather station, Phitsanulok, Thailand, approximately 15.7 km from the study site (TMD, 2018). There were 28.4 and 28 °C for average ambient temperature, 82 and 82% for relative humidity, and 186 and 128 mm precipitation during October 2016 and October 2017, respectively. The soil tubes were buried at 0, 1, 4, 7, and 10 cm below the soil surface. The soil tubes, 8 cm diameter and 10 cm height, were filled to a height of 6 cm with soil of the Taphan Hin series (Fine-silty, mixed, active, isohyperthermic Ultic Haplustalfs) mixed with soilless medium at a ratio of 1:1 in order to improve drainage. The soil was 28.6% sand, 21.4% silt, and 50% clay and was taken from a field that had no *P. clematidea* infestation. Each soil tube had twelve holes of a 5-mm size at the bottom of the tube to allow contact of the soil in the tube with the field soil. The seed bag was placed in the partially filled soil tubes, then covered with 1 cm of soil to a final depth in the soil tube of 7 cm. The soil was moistened before the tubes were buried in the field. The seed bag was placed at the soil surface and sprinkled with a thin layer of soil (about 2 mm) for the 0 cm depth. The soil tubes were placed in the field and watered throughout the study as needed to maintain adequate moisture. Average soil temperature during the studies was between 26 and 28 °C (TGP-4500 Tinytag Plus 2, Micron Meters, Georgia, USA). Germination counts were recorded 30 d after planting. Germination was counted as positive as previously described. The experiment was performed in a completely randomized design with three replications of each depth. The experiment was conducted twice, and the means were averaged ($n = 6$).

Allelopathic effect on germination and growth of B. pilosa

Praxelis clematidea plants, all aboveground tissues, were collected during flowering from monospecific stands in Wangthong District, Phitsanulok Province, Thailand (16.903787°N, 100.527014°E). The samples were cleaned and dried at 45 °C for 72 h, shredded into small pieces, and ground into a fine powder using an electric grinder and stored in paper bags until use. The seeds of hairy beggarticks (*Bidens pilosa* L.), an Asteraceae weed species were used as the test species due to their high percentage of germination (> 90%) and non-dormant seeds. Seeds of *B. pilosa* were collected from roadsides with no history of herbicide application in Muang District, Phitsanulok Province, Thailand (16.735436°N, 100.194270°E). Seeds were cleaned with distilled water and sterilized with 2.5% sodium hypochlorite for 10 min to prevent fungal contamination, dried, and stored in paper bags until use. This study was conducted in the laboratory at Department of Agricultural Science, Naresuan University, Phitsanulok, Thailand.

A 10% (w/v) *P. clematidea* extract was prepared in the proportion of 100 g of fine powder of *P. clematidea* in 1,000 ml of distilled water and mixed at 100 rpm for 5 min at 25 °C. The extract was filtered twice through two layers of 0.2 mm mesh cotton cloth and two layers of filter paper (Whatman No.1, Buckinghamshire, UK). Two extract dilutions, 25% (2.5% w/v) and 50% (5% w/v) were prepared by adding distilled water. Distilled water was used as a control. In addition to distilled water, different concentrations of polyethylene glycol 6000 (PEG 6000, Kemaus, New South Wales, Australia) solutions were included as

controls to test for the possible osmotic effects of the extracts. The osmotic potential of each extract concentration was measured with a vapor pressure osmometer (Vapro 5600, Wescor Inc. Logan, UT, USA). Concentrations of 9.5 and 13.1% of polyethylene glycol were included as osmotic potential controls for extract concentrations of 25 and 50%, respectively.

Sixteen seeds of *B. pilosa* were placed in a 9 cm diameter Petri dish lined with one filter paper. From each extract concentration, 5 ml were added to each Petri dish. Petri dishes were placed in a growth chamber at 25 °C with a 12 h photoperiod. After 7 d, germination and root and shoot lengths were measured. Germination was counted as positive as previously described. The experiment was performed in a completely randomized design with three replications of each concentration. The experiment was conducted twice, and the means were averaged ($n = 6$).

Statistical analyses

There were no significant differences based on the Levene's ANOVA test for homogeneity of variances for germination studies which were conducted twice; therefore, data were pooled across studies and means were averaged. At the end of the germination period, the germination percentage was calculated using the following equation:

$$\text{Germination Percentages (\%)} = (\text{Number of germinated seeds}) / (\text{Total number of seeds}) \times 100 \quad (1)$$

Inhibition percentage (IP) in allelopathic study was obtained by using the following equation:

$$\text{IP} = 100 - (\text{T} \times 100 / \text{C}) \quad (2)$$

where T represents response of treatment and C is response of untreated control.

Data of germination percentage, shoot and root lengths, and seed morphology of *P. clematidea* and other weed species were analyzed by ANOVA. Means were separated using Tukey's HSD at $p < 0.05$. All statistical analyses were performed using the program R v. 3.5.0 (R Core Team, 2018).

Results and Discussion

Praxelis characteristics and seed morphology

Misidentification of *P. clematidea* with other related species in the same family has caused inaccurate invasion reports (Corlett and Shaw, 1995; Veldkamp, 1999; Holland, 2006; USDA, 2014). *P. clematidea* was mistaken for bluebroomrape (*Ageratum houstonianum* Mill.), *A. conyzoides*, and *Conoclinium coelestinum* (L.) DC. (Veldkamp, 1999; Abbott *et al.*, 2008). Therefore, *P. clematidea* was characterized at various stages for more accurate identification. In the seedling stage, *P. clematidea* has cotyledon leaves which are kidney-shaped that may dry up and fall off as seedlings mature (Figure 1A). The true leaves of *P. clematidea* are coarsely toothed leaf margins, oppositely arranged along the cylindrical stem, which is covered in short soft hairs (Figure 1B). When plant parts are crushed, they emit an odor similar to cat urine. The leaves of *P. clematidea* have more coarsely toothed margins and more tapered bases than those of *A. conyzoides*, which have smooth teeth along the edges and a rounded leaf base (CRC Weed Management, 2003; Holland, 2006; Abbott *et al.*, 2008).

Praxelis clematidea has a unique characteristic of a lilac to intense blue inflorescence with deciduous bracts at the ends of stems and flowers with clusters of up to 48 florets (Table 1; Figure 1B). The flower of the *A. conyzoides* is pale blue or pale lilac with persistent bracts. The florets of *P. clematidea* are set in a high cone-shaped receptacle while those of *A. conyzoides* are set in a flat receptacle (CRC Weed Management, 2003; Holland, 2006; Abbott *et al.*, 2008). The phyllaries of *P. clematidea* has conspicuous dark striations from the veins (Abbott *et al.*, 2008; Gardner and Williges, 2015).

Each *P. clematidea* floret produces one seed. The *P. clematidea* seed has an angular shape, hairy on the seed surface, and the seed color turns from brown to black during ripening (Figure 1C). Seed thickness from

the 4 populations was 0.4 mm and no differences were found among populations ($p > 0.05$) (Table 1). Seed weight, length, and width were somewhat different among populations. Seed weight and length were greatest in the Nan population which were 0.2 mg and 3.2 mm, respectively, and seed width was greatest in Phetchabun population at 0.7 mm (Table 1). Seed size and seed surface can be used to distinguish between *P. clematidea* and *C. odorata*. Seeds of *C. odorata* were narrower than *P. clematidea* with 0.2 to 0.8 mm width, 3 to 5 mm length, and without hairs on seed surface (Groenewegen *et al.*, 2017). *P. clematidea*'s pappus is longer than the length of its seed, which can be used to identify the species. A previous study reported *P. clematidea* had about 15 to 40 bristles of pappus while the similar species, *A. conyzoides*, had only 5 bristles (CRC Weed Management, 2003).

The number of *P. clematidea* seeds per head ranged from 44 to 48 seeds among the four populations of Thailand. Corlett and Shaw (1995) reported that *P. clematidea* in Hong Kong had about 50 to 65 seeds per head. Small-seeded species are able to produce many more seeds than large-seeded species (Moles *et al.*, 2005). Important characteristics that make weeds successfully disperse and establish in new areas are seed characteristics such as a high number of seeds, a small seed size, and seed hair. *P. clematidea* can invade new areas easily because the plant can reach maturity in 30 to 35 d after emergence and produce the first seed set within 45 to 50 d after emergence (Anantanamane *et al.*, 2013). Flowers of *P. clematidea* can be produced year round, and the seeds are not dormant. Due to these characteristics of high productivity, *P. clematidea* has been reported as an invasive plant in many countries in Asia and in Australia (Veldkamp, 1999; CRC Weed Management, 2003; Waterhouse, 2003) and was characterized as an invasive alien species in Thailand (TH-BIF, 2018).

Praxelis clematidea had the smallest ratio of weight to surface area when compared to *A. conyzoides*, *C. sumatrensis* and *C. crepidioides* (Table 2). The smaller ratio of *P. clematidea* means that the seeds of *P. clematidea* persists longer in the air compared to other three species. The small seed size of *P. clematidea* with a persistent pappus can help extend dispersal distance via wind. A small seed with low weight to surface area was reported to result in a slow descent rate (Meyer and Carlson, 2001). However, in addition to wind, seeds of *P. clematidea* can be distributed by water, animals, and birds (CRC Weed Management, 2003).

Table 1. Seed morphological characteristics of four populations (Pop) of *Praxelis clematidea* collected from four Thailand Provinces, Chiang Mai (CM), Petchabun (PB), Kamphaeng Phet (KP), and Nan (NN)

Pop	Weight ^a (mg)		Length (mm)		Width (mm)		Thickness (mm)		Seeds per head	
CM	0.13	± 0.001 c	2.79	± 0.01 bc	0.65	± 0.02 b	0.4	± 0.01 ns ^b	48	± 1.2 ns
PB	0.14	± 0.001 c	2.83	± 0.02 b	0.73	± 0.01 a	0.4	± 0.03	46	± 1.1
KP	0.17	± 0.005 b	2.55	± 0.11 c	0.65	± 0.02 b	0.4	± 0.03	44	± 1.2
NN	0.21	± 0.003 a	3.20	± 0.20 a	0.64	± 0.03 b	0.4	± 0.02	- ^c	

^a Different letter between populations denote significantly differences (Tukey's HSD, $p < 0.05$). Data are shown as mean ± SE. ^b Not significant. ^c Data for number of seeds per head are not available for the Nan population.

Table 2. Seed weights, areas, and ratio of weight to surface area of *Praxelis clematidea* and other invasive Asteraceae weeds in Nan Province, Thailand

Species	Weight ^a (mg)		Surface area (mm ²)		Weight:surface area (mg mm ⁻²)
<i>Praxelis clematidea</i>	0.208	± 0.003 b	0.798	± 0.071 a	0.261
<i>Ageratum conyzoides</i>	0.107	± 0.003 c	0.375	± 0.032 b	0.286
<i>Conyza sumatrensis</i>	0.032	± 0.003 d	0.091	± 0.009 c	0.354
<i>Crassocephalum crepidioides</i>	0.223	± 0.006 a	0.698	± 0.051 a	0.320

^a Different letters between species denote significantly differences (Tukey's HSD, $p < 0.05$), $n = 10$. Data are shown as mean ± SE.

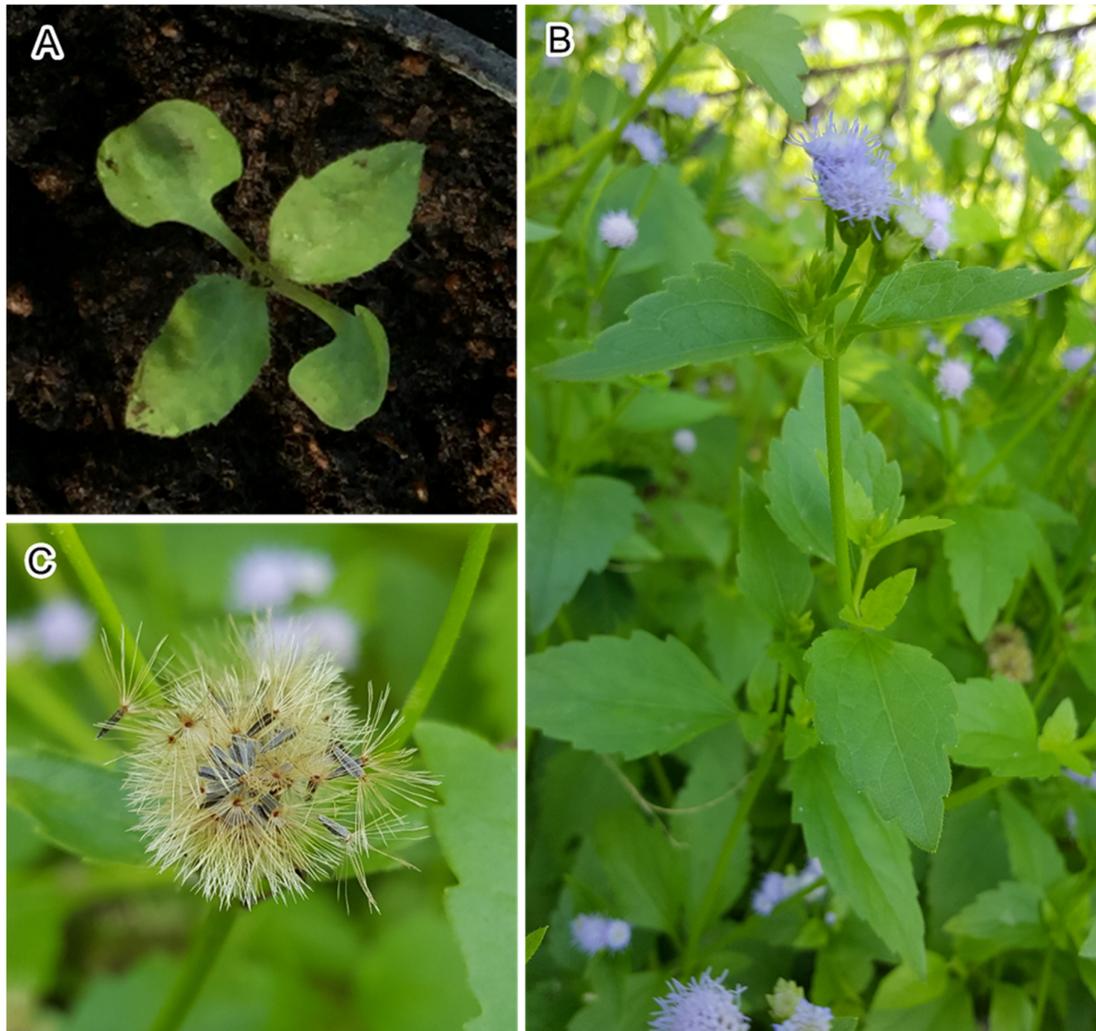


Figure 1. Morphology of *Praxelis clematidea*; seedling (A), leaves and inflorescences (B), and seeds (C)

Effects of temperature and depth on germination

Praxelis clematidea can germinate at various temperatures. To determine the effect of temperature, *P. clematidea* seeds were incubated at 10, 20, 25, 30, and 45 °C. The results showed that the seeds germinated at 20, 25, and 30 °C, but no germination was observed at 10 and 45 °C (Figure 2.). Many tropical and subtropical plants require relatively high temperatures to initiate growth meanwhile relatively high temperatures (≥ 35 °C) inhibit germination of some plant species such as crofton weed [*Ageratina adenophora* (Spreng.) R. M. King & H. Rob.] (Lu *et al.*, 2006), *C. crepidioides* (Chen *et al.*, 2009; Yuan and Wen, 2018), and horseweed (*Erigeron canadensis* L.) (Yuan and Wen, 2018). The fact that *P. clematidea* seeds were able to germinate over a temperature from 20 to 30 °C allows it to grow all year round in Thailand as well as in tropical and subtropical region elsewhere.

The effect of soil depth on germination was studied by burying seed bags at 2 mm, 1 cm, 4 cm, 7 cm, and 10 cm below the soil surface. The germination of *P. clematidea* seedlings decreased with increasing burial depth (Figure 3.). Germination was 77% for seeds placed on the soil surface and 18% for seeds placed 1 cm below the soil surface. No *P. clematidea* seed germination was observed when seeds were placed at a depth of 4 cm or greater. Planting depth affects seed dormancy and survival (Peachey and Mallory-Smith, 2017). In light-

sensitive species such as lettuce (*Lactuca sativa* L.), a daylight exposure could induce seed germination at 2-mm depth below the soil surface but did not affect germination at 6-mm depth (Woolley and Stoller 1978). In some species, seeds could enter dormancy when buried near the soil surface such as wild proso millet (*Panicum miliaceum* L.) (Calosi *et al.*, 1986) and redroot pigweed (*Amaranthus retroflexus* L.) (Omami *et al.*, 1999). No seedlings of *A. houstonianum* emerged from seeds places more than 2-mm depth below the soil surface (Lamsal *et al.*, 2019). The seedling emergence of coatbuttons [*Tridax procumbens* (L.) L.] decreased drastically with increasing burial depth due to limited light penetration (Vanijajiva, 2014). This study suggests that *P. clematidea* could be controlled by covering the seed with at least 4 cm of soil.

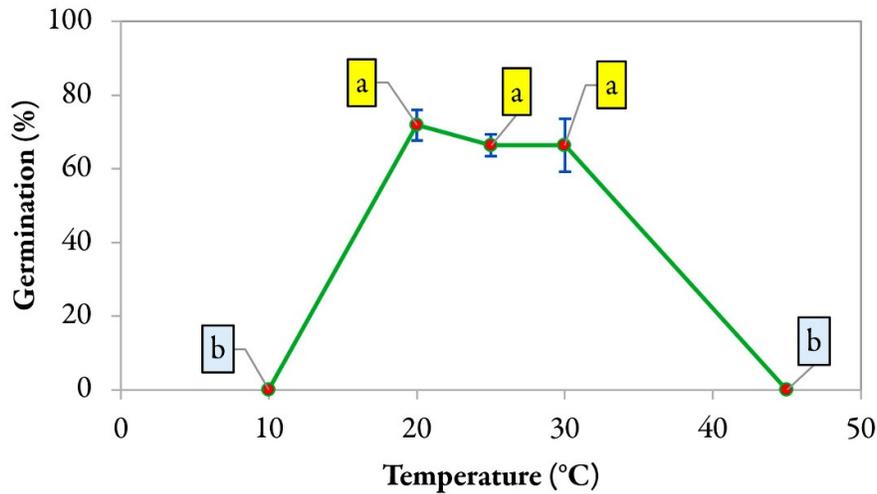


Figure 2. Effect of temperature on germination of *Praxelis clematidea* seeds. Vertical bars represent SE of the means, $n = 8$. Different letters indicate significant differences (Tukey's HSD, $p < 0.05$)

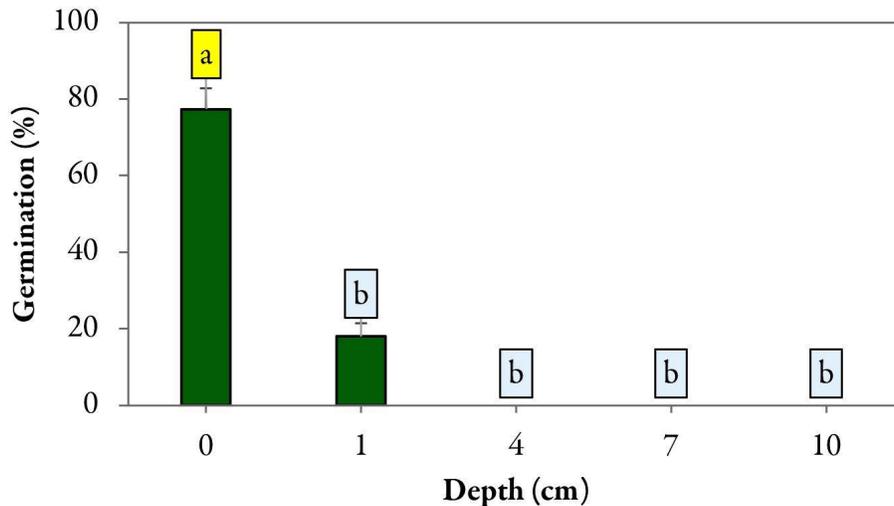


Figure 3. Effect of planting depth on germination of *Praxelis clematidea* seeds. Vertical bars represent SE of the means, $n = 6$. Different letters indicate significant differences (Tukey's HSD, $p < 0.05$)

Allelopathic effect on germination and growth of B. pilosa

Praxelis clematidea can be found as a dominant species or in monospecific stands. This has led to the hypothesis that *P. clematidea* produces allelochemicals which allow it to outcompete other nearby species. To evaluate this hypothesis, seeds from *B. pilosa* were treated with aerial plant extracts of *P. clematidea*. A preliminary study of the osmotic effects of *P. clematidea* extracts showed that the highest PEG concentration of 13.1% (equal to osmotic potential of *P. clematidea* at 50% concentration) did not significantly affect the germination and seedling length of *B. pilosa* compared to the untreated control ($P > 0.05$). Therefore, it suggested that the observed effects of *P. clematidea* extracts of 25 and 50% concentrations on *B. pilosa* would be due to allelopathy and not osmotic stress. After 7 d, the results showed allelopathic potential of *P. clematidea* extracts to suppress seedling growth of *B. pilosa* (Table 3). At 25% concentration, root and shoot length of *B. pilosa* were reduced by 48 and 31%, respectively, compared to the untreated control. At 50% concentration, root and shoot length of *B. pilosa* were reduced by 76 and 62%, respectively, compared to the untreated control. The root growth of *B. pilosa* was more sensitive to the allelochemicals of *P. clematidea* compared to shoot growth. Abnormalities were observed in that there were twisted hypocotyls and oxidation and discoloration of the root cap at extracts 25 and 50% concentrations. Miller (1996) stated that seedling growth, especially root growth, is more sensitive to allelochemicals than is germination. Previous studies have shown that volatile oil of *P. clematidea* can inhibit the growth of lettuce and field mustard (*Brassica rapa* L. cv. gr. 'Caixin'), and fungi (Wang *et al.*, 2006). The mechanism of action of *P. clematidea* aerial extracts to suppress germination of other species is unknown. In general, allelochemicals affect many physiological processes in plants such as cell division and elongation, membrane permeability, oxidative and antioxidant systems, enzyme synthesis and metabolism, protein and nucleic acid synthesis (Cheng and Cheng, 2015). Allelochemicals that inhibit seed germination can be related to the inhibition of plant metabolism by decreasing activity of enzymes involved in early stages of seed germination such as α -amylase, isocitrate lyase, glucose-6-phosphate dehydrogenase, glucose-phosphate isomerase, and aldolase (Gniazdowska and Bogatek, 2005).

Table 3. Effect of *P. clematidea* extracts on germination and growth of *B. pilosa* (BIDPI)

Concentration ^a	Germination ^b (%)		Root length (cm)		Shoot length (cm)	
0	90.6	± 2.68 ns ^c	2.1	± 0.08 a	1.3	± 0.07 a
25%	91.7	± 3.49	1.1	± 0.12 b	0.9	± 0.09 b
50%	71.9	± 10.43	0.5	± 0.10 c	0.5	± 0.07 c

^a Concentration of aerial extracts of *P. clematidea* by water. Percentages are on w/v basis. ^b Different letters between concentrations denote significant differences (Tukey's HSD, $p < 0.05$), $n = 6$. Data are shown as mean \pm SE. ^c Not significant.

Conclusions

Results of this study confirmed that seed morphology of *Praxelis* can be used to distinguish between related species such as *Ageratum* spp. and *Chromolaena* spp. *P. clematidea* germination occurred in a temperature range of 20 to 30 °C, and at a soil depth of up to 1 cm. Furthermore, *P. clematidea* had characteristics, such as a small seed with a pappus, prolific seed production, no seed dormancy period, and allelopathic effects that help increase its distribution in the environment. It is predicted that this weed has the ability to invade most of the area of Thailand under a range of moisture and soil conditions as long as seed remains on the soil surface. Effective *P. clematidea* control should be undertaken at an early stage before flowering by using integrated weed control methods such as tilling plus a PRE or early POST herbicide. Decreasing the *P. clematidea* seed bank should be added to management programs. The grower should clean

tools and machinery well to prevent the spread of *P. clematidea* to new areas. Plant identification and more information on the biology and ecology of *P. clematidea* would be useful in preventing the spread of this species.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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