Taiwania, 54(3): 255-260, 2009

Contrasting Effects of Aqueous Tissue Extracts from an Invasive Plant, *Bidens pilosa* L. var. *radiata*, on the Performance of Its Sympatric Plant Species

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(Manuscript received 8 April 2009; accepted 16 June 2009)

ABSTRACT: *Bidens pilosa* L. var. *radiata* Sch. Bip., a common weed in lowland Taiwan, is listed as one of the twenty most noxious invasive plants in Taiwan. In this study, we examined the effect of aqueous extracts of leaves, stems and roots of the invasive plant on germination and growth of seedlings (estimated by measuring the elongation of hypocotyls and radicals) of the same species and two other sympatric species, *B. bipinnata* and *Ageraturem conyzoides*. The objective of this study was to understand whether the aqueous tissue extracts affected the performance of the target species and whether these effects varied among tissue types and among target species. We found that the germination percentage of seeds of *B. bipinnata* was significantly reduced by root and leaf extracts, that of *B. pilosa* var. *radiata* was also significantly reduced by the application of root extract, while that of *A. conyzoides* was not affected by any of the three tissue extracts. The application of stem and leaf extracts inhibited the elongation of radicals of *B. pilosa* var. *radiata*, consequently, the growth of seedlings of this species was decreased in these two treatments. Though the elongation of hypocotyls was stimulated by leaf extract, the overall growth of seedlings of *B. bipinnata* was not affected by any tissue extract. In contrast, all three extracts stimulated the elongation of hypocotyls and radicals of *A. conyzoides*, consequently, the overall growth of seedlings of this plant was promoted by all three extracts. These results revealed that aqueous extracts from tissue of *B. pilosa* var. *radiata* had differential effect on the emergence and seedling growth of the three target species. The inhibition effect of its root and leaf extracts on the germination of *B. bipinnata* may partially explain the overwhelming dominance of *B. pilosa* var. *radiata* over *B. bipinnata* when they are sympatric.

KEY WORDS: *Ageraturem conyzoides*, aqueous tissue extracts, *Bidens pilosa* L. var. *radiata* Sch. Bip., *B. bipinnata*, invasive plants.

INTRODUCTION

Two exotic *Bidens* species, belonging to Asteraceae, were reported in Taiwan (Peng et al., 1998). *Bidens pilosa* L. var. *radiata* Sch. Bip., a native plant of North America (Peng et al., 1998), was first recorded in Taiwan in 1984 (Wu et al., 2004). It is now widely distributed and listed as one of the twenty most noxious invasive plants in Taiwan (Chang et al., 2003). *Bidens bipinnata*, the other exotic *Bidens* species in Taiwan, is also a native of North America and was first recorded in Taiwan in 1904 (Wu et al., 2004). In contrast to *B. pilosa* var. *radiata*, *B. bipinnata* has become a naturalized plant and mainly distributed in Southern Taiwan (Peng et al., 1998). Probably due to its less competitive than *B. pilosa* var. *radiata*, very few populations of *B. bipinnata* can be found in Taiwan now (personal obs). During a field survey of the distribution of *Bidens* species in Taiwan, we found both species growing sympatrically inside an abandoned farm where *B. pilosa* outnumbered *B. bipinnata*. According to observations made by the owner of the orchard, *B. bipinnata* used to dominate the farm, however, it declined rapidly during *B. pilosa* var. *radiata* invasion. By the time we started the experiment, about 99 % of the ground inside the farm was covered by *B. pilosa* L. var. *radiata* (ca. 40 plants m^{-2}), while only about 20 plants of *B. bipinnata* (covered less than 10 m^2) were found. The question occurred to us, what makes *B. pilosa* L. var. *radiata* outcompete *B. bipinnata*?

Not only the mechanisms by which *B. pilosa* var. *radiata* displaces *B. bipinnata* are unexplored, factors contributing to the success of *B. pilosa* var. *radiata* in Taiwan are poorly understood. Allelopathy, the plant-plant interaction mediated by chemicals released from the plant, is known to play a significant role in affecting community structure, including plant dominance and succession (Muller, 1966). Recently, more and more studies have reported that part of the success of invasive plants may be their novel allelopathic effects (Bais et al., 2003; Hierro and Gallaway, 2003; Prati and Bossdorf, 2004; Dorning and Cipollini, 2006; Cipollini et al., 2008). Stevens and Tang (1985) reported that root exudates of *B. pilosa* inhibited seedlings growth of some crop species. Accordingly, is it possible that *B. pilosa* var. *radiata* also exerts allelopathic effects on *B. bipinnata*? Because of difficulties in separating allelochemical effects from resource competition, it is extremely difficult to demonstrate allelopathy effect in the field (Bais et al., 2003). Instead, many researches have successfully shown the potential for allelopathy in invasive plants using tissue extracts (Lawrence et al., 1991; Roberts and Anderson, 2001; Bais et al., 2003; Hierro and Callaway, 2003; Doring and Cipollini, 2006; Cipollini et al., 2008). A similar approach was also applied in this research to study the effects of the invasive plant on its sympatric species.

Ageraturem conyzoides, an annual herb originally from tropical America, is also a common weed in Taiwan and is often found growing sympatically with the invasive plant in Northern Taiwan (pers. obs.). Is the performance of this species also affected by the invasive plant? This is the second question we would like to answer in this study. If the invasive plant exerts differential effects on its sympatric plants, it will have significant potential in altering community structure in Taiwan.

The objective of this study was to understand whether the aqueous tissue extracts from *B. pilosa* var. *radiata* affected the performance of its sympatric plants and whether the effects varied among tissue types and among target species. To achieve the objective, we applied aqueous tissue extracts from the invasive plant to seeds of target species and then measured seed germination and seedling growth of these species.

MATERIALS AND METHODS

Methods used by San Emeterio et al. (2004) were adopted to mimic the leachates of *B. pilosa* var. *radiata* produced in the field and to test its allelopathic potential on target species.

B. pilosa var. *radiata* were harvested in Juy of 2005 from an abandoned farm in Madou Town (120°15' E, 23°10' N), Tainan County, SouthernTaiwan. The farm, ca. 1500 m^2, was originally established for the cultivation of shaddock, while had been abandoned for at least one year before we stated conducting the experiment. At the time of the plant harvesting, no more shaddock could be seen, and the farm was densely covered by other plant species, mainly *Basella alba* L., *B. bipinnata, B. pilosa* var. *radiata*, *Chloris barbata* Sw., *Ipomoea cairica* (L.) Sweet, and *Passiflora suberosa* Linn. Among them, *B. pilosa* var. *radiata* was the most dominant species covering about 99 % of the ground inside the farm (ca. 40 plants m^{-2}).

Plants of *B. pilosa* var. *radiata* harvested from the farm were separated into leaf, stem, and root components. Each component was first cleaned to remove the dirt, air-dried and then ground into powder by a mechanical grinder. Five gram of each ground component was dissolved into a 5 L of distilled water by 12 hours of continuous blending. The resulting solution was then subjected to centrifugation (3000 rpm for 15 min for root, 3500 rpm 20 min for stem and leaf components) for the separation of supernatant and residues. The supernatant was collected and its water potential and pH value determined with a osmometer (WP4-T, Decagon Devices, Inc., USA) and a pH meter (TS-2, syntex), respectively. The effect of extract from each component, prepared as above, of *B. pilosa* var. *radiata* on seed germination rate and the growth of seedlings were examined.

Seeds of *B. pilosa* var. *radiata* and *B. bipinnata* were also collected from the abandoned farm on July 2nd, 2005. Though *Ageraturem conyzoides* was not found in the farm, it is often found growing sympatrically with *B. pilosa* var. *radiata* in Northern Taiwan (pers. obs.). Accordingly, we also examined the effect of extracs of *B. pilosa* var. *radiata* on this species. Seeds of *A. conyzoides* were collected on the campus of National Taiwan University, Taipei, Taiwan, 2005.

In a pioneer experiment, we found that seeds of *B. pilosa* var. *radiata* and *B. bipinnata* were easy to be infected by fungus. To prevent fungus infection, seeds of these two species were sterilized in NaOCl (5% v/v), and then washed with distilled water before being sowed. Twenty five seeds of each tested species were placed on filter paper in a petri dish (diameter × height = 90 mm × 15 mm). A 4 ml of extract from each component was added to the filter paper in each Petri dish. Distilled water was used as control. There were four replicates for each treatment and resulting in 48 Petri dishes in total. These Petri dishes were then transferred to a growth chamber setting at 25 ℃/20 ℃ for day/night temperature, a relative humidity of 70%, and a 12/12 hr light/dark regime. The photosynthetic photon flux density (wavelengths at 400- 700 nm) at the light period was about 100 μmol m^{-2} s^{-1}.

During the experiment, the solutions in each Petri dish evaporated gradually. To avoid possible effect caused by changes in solution concentration, we replaced the filter papers, added solution and carefully transferred seeds (or seedlings) onto the new plates on day 10 of the experiment.

Seed germination was recorded everyday for 17 days. Seeds were considered germinated when radicles could be observed by naked eyes (according to Reddy and Singh, 1992). All three species are epigeous and on the seedlings regions for radical and hypocotyl can be easily identified (Fig. 1). The effect of extract on the growth of seedlings was assayed by measuring the length of radicle and hypocotyl of the germinating seeds. For each tested species, lengths of radicle and hypocotyl of seedlings were measured on a single day when its seeds had reached the maximal germination rate, i.e., on day 8 for *B. pilosa* var. *radiata*, day 15 for *B. bipinnata*, and day 7 for *A. conyzoides*. These two parameters were not measured on seedlings with only radicles.

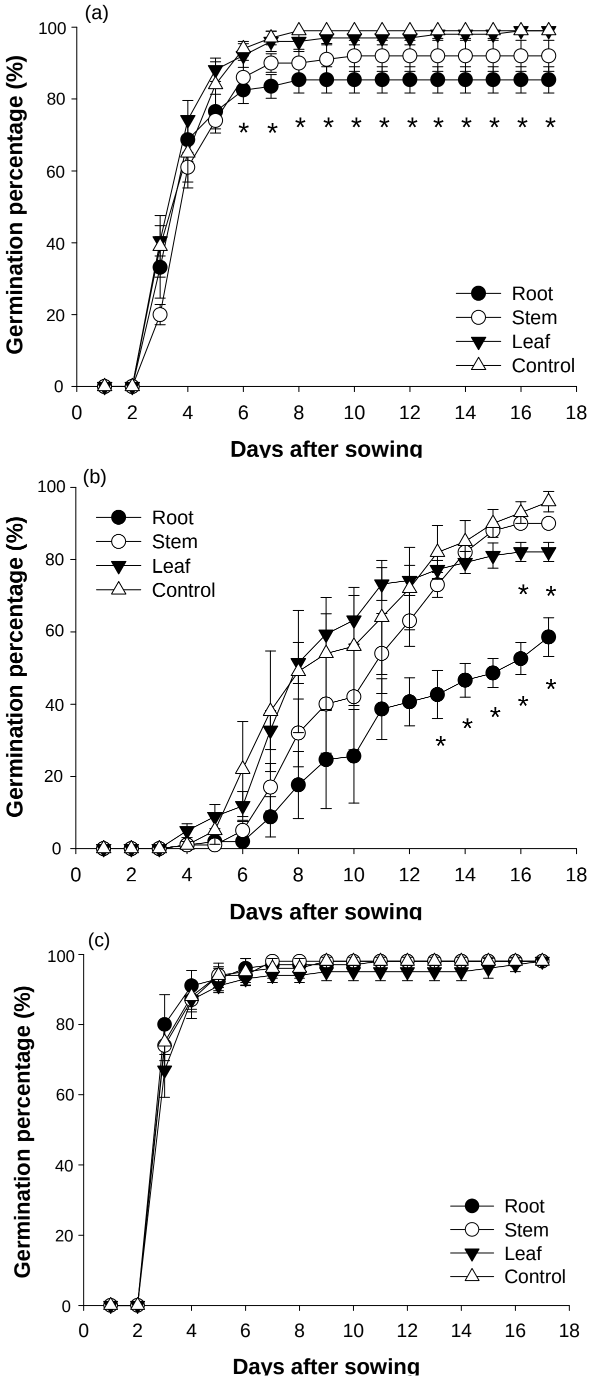

Fig. 1. Effect of aqueous extracts of roots, stems, and leaves of *Bidens pilosa var. radiata* on the germination of (a) *B. pilosa* var. *radiata*, (b) *B. bipinnata*, and (c) *Ageratum conyzoides*. A star sign indicates a significant difference from control (p <0.05, t-test, n = 4).

Means were analyzed by unpaired t-test (double-tailed, alpha = 0.05) to identify significant difference from control.

RESULTS

The water potential of extract from each component was close to 0 MPa (data not presented), which was greater than the threshold value believed to affect seed germination (approximate -0.25 MPa, L. San Emeterio et al., 2004). The pH value was 7.3 of root extract, 5.6 of stem extract, 5.3 of leaf extract, and 6.8 of control.

Almost all of the seeds (99 %) of *B. pilosa* var. *radiata* germinated after being incubated in distilled water for 8 days (Fig. 1a). The final germination percentage of seeds of *B. pilosa* var. *radiata* was significantly reduced by application of root extracts, 39 % reduction, in contrast, that was neither affected by stem extract nor by leaf extract.

After being incubated in distilled water for 17 days, 97% of seeds of *B. bipinnata* germinated (Fig. 1b). In compared to control, the final germination percentage was significantly reduced by root and leaf extracts, 36 and 15% reduction, respectively. Application of stem extract also reduced seed germination percentage, however, the effect was not significant.

In contrast to those of *B. pilosa* var. *radiata* and *B. bipinnata*, the percentage of seed germination of *Ageratum conyzoides* was not affected by any of the three tissue extracts (Fig. 1c).

In *B. pilosa* var. *radiata*, the overall growth of new tissue was reduced by stem and leaf extracts (Fig. 2a). However, these extracts exerted differently on the elongation of hypocotyls and radicals. Both stem and leaf extracts significantly stimulated the elongation of hypocotyl while inhibited that of radical. In contrast, root extract neither affected the elongation of hypocotyls nor that of radicals.

Though the overall growth of seedlings of *B. bipinnata* was not affected by any tissue extract, the elongation of hypocotyls was stimulated by leaf extract (Fig. 2b).

In contrast to that of *B. pilosa* var. *radiata* and *B. bipinnata*, the growth of hypocotyls and radicals of *A. conyzoides* was significantly stimulated by all three tissue extracts. Consequently, the overall growth of seedlings of this species was significantly increased by all three tissue extracts of *B. pilosa* var. *radiata* (Fig. 2c).

DISCUSSION

Results from this study demonstrated that the aqueous extracts from tissues of *B. pilosa* var. *radiata* had differential effect on the emergence and seedling growth of its same species, *B. bipinnata* and *A. conyzoides*.

The responses of seed germination and seedling growth to the extracts were not consistent. Tissue extracts showing significant influence on seed germination (Fig. 1)

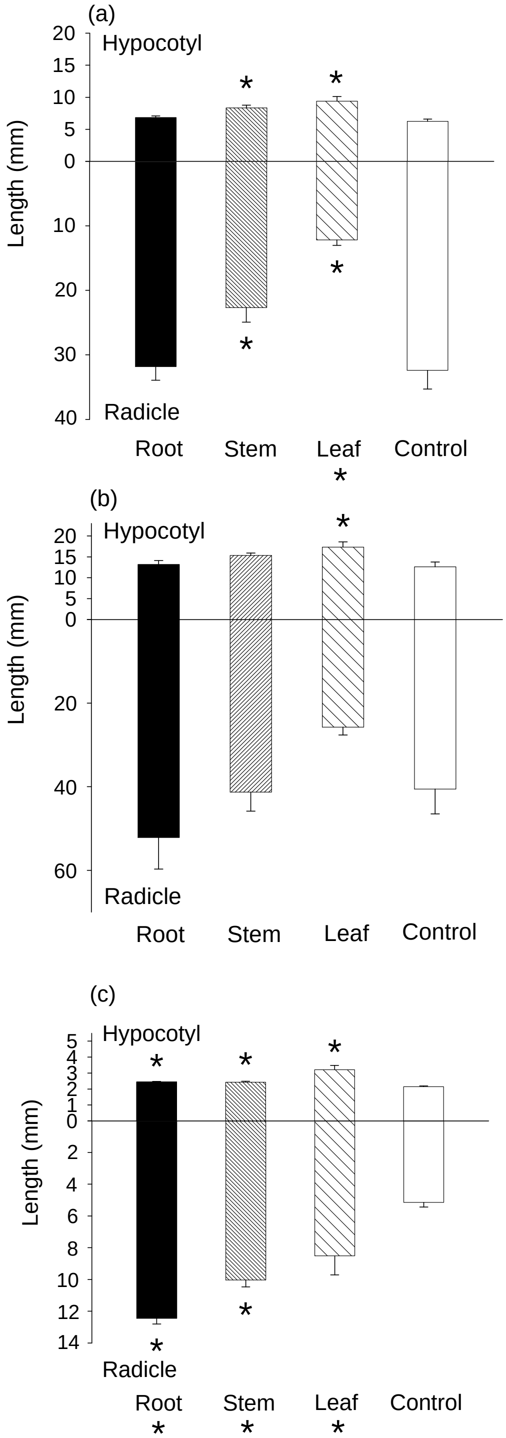

Fig. 2. Effect of aqueous extract of roots, stems, and leaves of *Bidens pilosa var. radiata* on the seedling growth of (a) *B. pilosa* var. *radiata*, (b) *B. bipinnata*, and (c) *Ageratum conyzoides*. Bars stand for standard errors. The star signs above x axis, below x axis, and right beside the names of the treatments indicate significant differences of hypocotyl, radicle, and total seedling length from control, respectively ($p < 0.05$, t-test, n = 4).

did not necessarily exhibit the same level of effect on seedling growth (Fig. 2). For instance, seed germination percentage of *B. bipinnata* was significantly reduced (Fig. 1b), whereas the growth of hypocotyl, radicle and total length of seedling of the same species were not affected (Fig. 2b) by the extracts of root of *B. pilosa* var. *radiata*. In *A. conyzoides*, tissue extracts significantly enhanced the growth of its seedlings (Fig. 2c), however, germination percentage was not affected (Fig. 1c). These results suggested the existence of allelopathic effect, however, the influence of allelochemicals on seed germination and seedling growth may work through different mechanisms. The differential response of germination and seedling growth to tissue extracts was also found in other studies. A study has shown that *B. bipinnata* whose radicle length seemed to be more sensitive to pH value than seed germination (Reddy et al., 1992). In addition, San Emeterio et al. (2004) also found that seedling growth of three pasture species was more sensitive to allelochemiclas than seed germination.

In general, hypocotyls displayed more consistent response to tissue extracts than radicals (Fig. 2). The growth of hypocotyls was mostly stimulated by tissue extracts. In contrast, the response of radicals to tissue extracts varied among species. The elongation of radicals of *B. pilosa* var. *radiata* was inhibited by leaf and stem extracts, that of *B. bipinnata* was not affected, while that of *A. conyzoides* was enhanced by all three extracts. The study by San Emeterio et al. (2004) also showed that shoot extract from *Lolium rigidum* stimulated aerial growth of seedlings of three pasture species. In that study, the authors also demonstrated that allelochemicals displaying a stimulatory effect at low concentrations may exhibit an inhibitory effect at higher concentrations (San Emeterio et al., 2004).

Self-inhibiton was observed in *B. pilosa* var. *radiata*. Seed germination percentate of *B. pilosa* var. *radiata* was lowered by its own extract of leaves. Radicle growth, which was crucial to early establishment, was also inhibited by the extracts of stems and leaves. The influence may be even intensified under restricted water conditions (Kobayashi, 2004; San Emeterio et al., 2004). This self-inhibition potential could reduce possible future intra-species competitions by interfere with the growth of other individuals at an early stage.

Allelochemicals produced in the tissues of plants may enter soils as leachates, root exudates or during tissue decomposition (Inderjit and Duke, 2003). Though only aqueous tissue extracts were used in this study, the results reveal the potential of inhibition effect of the invasive plant *B. pilosa* var. *radiata* on the congeneric species *B. bippinata*. However, it has been found that both hydrophobic and hydrophilic root exudates of *B. pilosa* inhibited seedling growth of some crop species (Stevens and Tang, 1985). Accordingly, further studies need to be conducted to examine the effect of hydrophobic component of tissue extracts and to further verify the alleochemical effect of this species on the same species and other species in field. In addition, the concentration of tissue extracts used in this study was referenced the study of *Lolium rigidum* (a plant with a productivity of 425 kg ha^{-1}) by San Emeterio et al. (2004). A survey on the residue of *B. pilosa* var. *radiata* produced in field and therefore the actual concentration of leachate released into the soil is necessary to evaluate the dosage effect of tissue extracts on its neighbours. In addition, microbes and other compositions in soil may change the way an allelochemical works (El-Khatib et al., 2004; Vivanco et al., 2004). Environment conditions, for example, the precipitation and nutrient level, may also play a role in allelopathy by amplifying of reducing the effect (Kobayashi, 2004; San Emeterio et al., 2004; Cipollini et al., 2008). How these factors interact with allelochemicals released by *B. pilosa* var. *radiata* deserve further investigation.

In conclusion, *A. conyzoides*, *B. bipinnata* and *B. pilosa* var. *radiata* showed differential response to aqueous tissue extracts of *B. pilosa* var. *radiata*. The aqueous tissue extracts inhibited seedling growth of *B. pilosa* var. *radiata* while enhanced that of *A. conyzoides*. Root and leaf extracts significantly inhibited seed germination of *B. bipinnata*. Similar taxa would have similar ecological niche. Resuls of this study revealed that the effect of aqueous tissue extracts on seed germination and seedling development was more deterious to *B. pilosa* and *B. bipinnata* than to *A. conyzoides*. The pattern implys a high ability of *B. pilosa* to compete against similar taxa. A high allelopathic inhibiton of related neighbours in early stages of development would reduce the threat that they become competitiors later on. In addition, the inhibition effect of *B. pilosa* on *B. radiatioa* may partially explain the overwhelming dominance of *B. pilosa* var. *radiata* over *B. bipinnata* when they are growing sympatrically.

ACKNOWLEDGEMENTS

We thank Mr. Pao-Yuan Chen and his family for allowing us to get access to the orchard farm and for their hospitality. This study was supported by a grant from the National Science Council (NSC 94-2621-B-002-010) of Republic of China.

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比較入侵植物大花咸豐草的組織水溶液萃取液對其共域植物的影響

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(收稿日期：2009 年 4 月 8 日；接受日期：2009 年 6 月 16 日)

摘要：大花咸豐草 (*Bidens pilosa* L. var. *radiate*) 是臺灣平地常見雜草，目前被列為臺灣二十種入侵性嚴重的植物之一。本文研究大花咸豐草根、莖、葉水溶液萃取液對同種，以及共域的鬼針草和白花霍香薊 (*Ageraturem conyzoides*) 種子發芽及幼苗生長(以胚根和胚莖的總長度計算) 的影響；目的在瞭解大花咸豐草組織萃取液是否會抑制其共域植物的表現，以及其影響的程度是否有所不同。結果發現：根和葉水溶液萃取液顯著降低鬼針草的種子發芽率，大花咸豐草的種子發芽率也明顯受根水溶液萃取液抑制；然而白花霍香薊的種子發芽率卻完全未受這三種組織水溶液萃取液的影響。莖、葉水溶液萃取液顯著抑制大花咸豐草胚根的長度，導致大花咸豐草在這兩種處理下，其幼苗的生長顯著降低；雖然葉水溶液萃取液促進鬼針草胚莖長度，但幼苗整體的生長並沒明顯受三種組織水溶液萃取液的影響；三種組織水溶液萃取液均顯著促進白花霍香薊的胚根和胚莖的長度，因此三種組織水溶液萃取液顯著增加白花霍香薊幼苗生長。這些結果顯示，大花咸豐草水溶液萃取液對其共域植物的確有不同程度影響；又當大花咸豐草和鬼針共域時，鬼針族群數量減少，可能和其種子發芽率受到大花咸豐草抑制有關。

關鍵詞：白花霍香薊、組織水溶液萃取液、大花咸豐草、鬼針草、入侵植物。