Vol.3, No.4, 582-587 (2012)
doi:10.4236/as.2012.34070

Allelopathic potential of *Artemisia biennis* (biennial wormwood)

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Received 3 March 2012; revised 14 April 2012; accepted 17 May 2012

ABSTRACT

In this study, *Artemisia biennis* was seeded in a greenhouse and raised to an average plant height of 100 cm. Aboveground plant portions were harvested and partitioned into leaves and stems, and dried; while roots were either removed from some soil (soil – roots) or left in soil (soil + roots). Greenhouse studies were conducted to evaluate the allelopathic potential of *A. biennis* leaves, roots, and stems; and soil – roots, and soil + roots on *Solanum melanocerasum* plant height and fresh weight plant^{-1}. When 5 g of root and stem biomass were added to soil, *S. melanocerasum* plant height and fresh weight plant^{-1} was reduced by 75 and 88%, respectively. In contrast, 5 g of leaf biomass caused an increase in *S. melanocerasum* plant height and fresh weight plant^{-1} by 35% and 43%, respectively; whereas, 20 g of leaf biomass depressed both variables by 50% and 65%, also respectively. Plant height was more suppressed when *S. melanocerasum* grew in soil – roots as opposed to soil + roots, whereas fresh weight plant^{-1} was similar between soil treatments. *S. melanocerasum* plant height was reduced by 70 and 55% when grown in soil – roots and soil + roots, respectively. In contrast, *S. melanocerasum* fresh weight plant^{-1} was reduced by 76% in both soil treatments. The reduction in *S. melanocerasum* plant attributes in this study is indicative of the allelopathic potential of *A. biennis*. Furthermore, *A. biennis* allelopathy is differentially expressed among plant parts, primarily in roots. This may explain how *A. biennis* is capable of dominating a habitat once it becomes established. The presence of extractable compounds with herbicidal activity could increase the potential usefulness of *A. biennis*.

Keywords: Allelopathic Potential; *Artemisia biennis*; Biennial Wormwood; Garden Huckleberry; *Solanum melanocerasum*

1. INTRODUCTION

Artemisia biennis is native to northwest U.S. and western Canada [1] and has now spread and become a problem in several crops in the United States, Southern Canada, and Europe [2,3]. Originally classified as a biennial weed of meadows, roadsides, and waste areas [1,4], *A. biennis* infestations became larger in size and more frequent in cultivated fields in the 1990s [3,5]. The successful spread of *A. biennis* has been largely attributed to the production of numerous seeds, estimated to be between 400,000 to one million per plant [4,5], which are very small and easily spread by wind or water. *A. biennis* can form large patches that can adversely affect surrounding vegetation and which is suspected to be via its allelopathic potential.

Allelopathic activity has been characterized among several *Artemisia* species, e.g., *A. annua* (annual wormwood) [6-8], *Artemisia princeps* var. *orientalis* [9], and *A. vulgaris* [10]. However, unlike other *Artemisia* species, *A. biennis* has become an established weed of several cropping systems and is suspected to be expanding its habitat range which warranted its placement on a "Weeds to Watch" list by several scientists of the North Central States of the United States [11].

Upon emergence, *A. biennis* appears to negatively affect surrounding vegetation via stunting which suggests allelopathy may be involved. In preliminary studies, greenhouse soil amended with aboveground *A. biennis* biomass reduced fresh weights of selected crops (*Zea mays*, *Glycine max*, *Helianthus annuus*, and *Triticum aestivum*) by 10% and selected weed species (*Setaria viridis*, *Solanum melanocerasum*, *Amaranthus retroflexus*, and *Avena fatua*) by 18%. Greenhouse soil containing belowground *A. biennis* biomass reduced crop and weed fresh weight on average by 39% and 61%, re-

spectively [12,13]. *S. melanocerasum* was the most sensitive species and greenhouse soil containing *A. biennis* biomass from plants that were 45 cm or taller resulted in reduction in fresh weight by 65 and 90% from aboveground and belowground biomass, respectively.

Seasonal variation in allelopathic potential has been reported for *Artemisia princeps* var. *orientalis* [9] and *Artemisia annua* [8]; and showed that extracts from wheat straw were more inhibitory to *Echinochloa crusgalli* germination and seedling growth than were extracts from hulls and leaves [14]. *A. biennis* allelopathy can be of better utility if variation in phytotoxicity in plant parts is characterized. The objective of this study was to evaluate the allelopathic potential of *A. biennis* leaf, stem, and root biomass partitioned from plants, and soil used to grow *A. biennis* plants.

2. MATERIALS AND METHODS

The *A. biennis* seed used in this study was collected from mature plants in soybean fields near Fargo, ND (46˚55'N, 96˚50'W) and Fergus Falls, MN (46˚17'N, 96˚05'W), in the fall of 2000. Mature flower heads were collected and hand crushed to release the seed, and 710- and 300- µm-mesh screens (No. 25 and 50) were used to separate seed from large extraneous plant material. All *A. biennis* seeds collected were stored at 5 C until used. Preliminary studies were conducted in greenhouses at North Dakota State University, Fargo, ND from 2004 to 2005 using the Fargo seedlot to assess allelopathy potential (data not presented). Allelopathic potential was characterized further in greenhouses at Fargo from 2005 to 2006 using the Fargo seedlot (run 1), and at Northwest Missouri State University, Maryville, MO from 2007 to 2008 using the Fergus Falls seedlot (run 2).

A. biennis was seeded in a commercial potting mix in 10-cm-wide by 12-cm-deep plastic pots, which were watered to field capacity. Approximately 150 *A. biennis* seed were spread on the damp potting soil of each pot. Numerous seeds were used because of the small size of *A. biennis* seed and variable germinability. Dry growth medium was lightly sprinkled over the seed to create a planting depth of about 2 mm, and pots were watered again. Transparent plastic was placed over the pots to increase the humidity and temperature for uniform *A. biennis* germination and emergence [5]. This method of incubation resulted in relatively fast and uniform *A. biennis* emergence. A total of 70 pots were established and placed in a greenhouse which was set at a temperature of 25˚C ± 2˚C.

Upon seedling emergence, the transparent plastic was removed and *A. biennis* seedlings were watered when necessary and fertilized weekly with 53 ml of a 3.7 g·L^{-1} solution of 15:30:15 (N_2-P_2O_5-K_2O) fertilizer. At 2 wk after emergence, *A. biennis* seedlings were thinned to one plant per pot. Thinned plants were uniform in height and were grown and later harvested when the average *A. biennis* plant height was 100 cm and plants were starting to initiate floral primordia. Upon harvesting, aboveground *A. biennis* plants were partitioned into leaf and stem biomass, and air-dried in the greenhouse for 10 d. Pots containing soil with *A. biennis* roots were left to dry for 7 d and then root biomass was removed from dry soil. The soil from 50 pots was broken up and sifted with sieves so as to remove all root hairs which were combined with larger roots to form root biomass. Soil from which roots were removed was combined and used in later experiments. The remaining 20 pots containing soil and *A. biennis* roots were combined and used in the soil experiment. *S. melanocerasum* was chosen as the assay species based on preliminary experiments.

After drying, *A. biennis* leaf, root, and stem biomass was ground separately using a grinding mill. The ground up leaf, root, and stem *A. biennis* biomass was apportioned into doses of 0, 5, 10, and 20 g and each portion was mixed with 150 g of a commercial potting mix and placed in a 10-cm wide by 8 cm-deep plastic pot. Each pot was seeded with 25 *S. melanocerasum* seeds, watered, and placed in a greenhouse set at a temperature of 25˚C ± 2˚C and with a 16-h photoperiod under natural sunlight supplemented with high-pressure mercury halide lamps (4.7 µE m^{-2}·s^{-1}). *S. melanocerasum* plants were allowed to grow in soil amended with *A. biennis* biomass for 21 d following emergence whereupon plant height and fresh weight were determined. Plant height was determined by measuring each plant within the pot from the soil surface to the highest extended leaf tip. Fresh weight was determined by harvesting and weighing all aboveground plant parts from each pot. Plant heights and fresh weights from each pot were averaged before statistical analysis.

Soil used to grow *A. biennis* plants was combined and partitioned into two portions. One portion had all roots removed (soil – roots), whereas roots were left intact in the second portion (soil + roots). For each soil, 150 g was placed in a 10-cm wide by 8 cm-deep plastic pot. Each pot was seeded with 25 *S. melanocerasum* seeds, watered, and placed in a greenhouse as previously mentioned. *S. melanocerasum* plants were allowed to grow for 21 d following emergence whereupon plant height and fresh weight were determined as previously outlined. An untreated control in which pots contained 150 g of a commercial potting mix was included.

Each experiment was conducted twice in a completely randomized design and treatments were replicated three times. Data were subjected to ANOVA using PROC GLM in SAS. The assumptions of random, homogeneous residuals with a normal distribution were checked to ensure the validity of ANOVA. The amount of biomass (0,

5, 10, and 20 g) was treated as a nested effect. This was necessary to allow the model to account for differences in the allelopathic potential of equal weight of biomass from different sources (leaves, roots, and stems). In the evaluation of allelopathic potential of *A. biennis* biomass, ANOVA indicated significant treatment effects. Therefore, regression analysis was performed to relate amount of *A. biennis* biomass to number of plants, plant height and weight plant^{-1} of *S. melanocerasum*.

ANOVA indicated that there was no difference between runs in the evaluation of allelopathic potential of soil – roots and soil + roots treatments. Therefore data were combined and reanalyzed and where significant effects were detected treatment means were compared using the Tukey-Kramer HSD test. The level of significance was defined at $P \leq 0.05$.

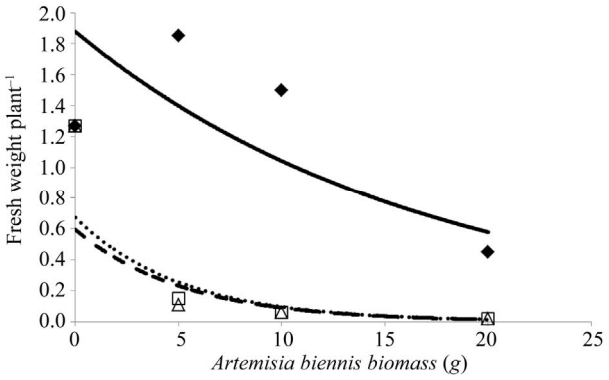
3. RESULTS AND DISCUSSION

Results from ANOVA indicated that the addition of *A. biennis* leaf, root, and stem biomass to soil significantly affected *S. melanocerasum* fresh weight plant^{-1} ($P < 0.0001$) and average plant height ($P < 0.0001$). A significant effect was detected among blocks ($P = 0.0189$) for *S. melanocerasum* fresh weight plant^{-1} but this effect accounted for only 1.6% of the total variation. Therefore, *S. melanocerasum* fresh weight plant^{-1} data were combined and reanalyzed. ANOVA detected significant effects due to soil – roots and soil + roots treatments on *S. melanocerasum* average plant height ($P < 0.0001$) and fresh weight plant^{-1} ($P < 0.0001$).

3.1. Allelopathic Potential of *A. biennis* Leaf, Root, and Stem Biomass

Increasing the amount of *A. biennis* root and stem biomass added to soil from 5 to 20 g resulted in 88% or greater reduction in *S. melanocerasum* fresh weight plant^{-1} compared to the untreated control (**Figure 1**). In contrast, the addition of 5 and 10 g of *A. biennis* leaf biomass to soil resulted in an increase in *S. melanocerasum* fresh weight plant^{-1} of 43 and 15%, respectively, compared to the untreated control. When the amount of leaf biomass added to soil was increased to 20 g, *S. melanocerasum* fresh weight plant^{-1} was reduced by 65%, compared to the untreated control.

Increasing the amount of *A. biennis* root and stem biomass to soil from 5 to 20 g caused 75% or greater reduction in average *S. melanocerasum* plant height compared to the untreated control (**Figure 2**). In contrast, the addition of 5 g of *A. biennis* leaf biomass caused an increase in *S. melanocerasum* plant height of 35%, whereas the addition of 10 and 20 g of leaf biomass to soil caused 2 and 50% reduction in plant height, respectively, compared to the untreated control.



Fihure 1. Influence of amount of *Artemisia biennis* leaf, root, and stem biomass (0, 5, 10, and 20 g) mixed with greenhouse potting mix on *Solanum melanocerasum* fresh weight plant^{-1} 21 days after emergence under controlled environment. *A. biennis* rates were mixed with 150 g of greenhouse potting mix prior to seeding *S. melanocerasum*. Leaf biomass (♦, solid line): $y = 1.8781e^{-0.059x}$ ($R^2 = 0.64$); root biomass (□, dotted line): $y = 0.6732e^{-0.194x}$ ($R^2 = 0.89$); stem biomass (Δ, broken line): $y = 0.5947e^{-0.189x}$ ($R^2 = 0.84$).

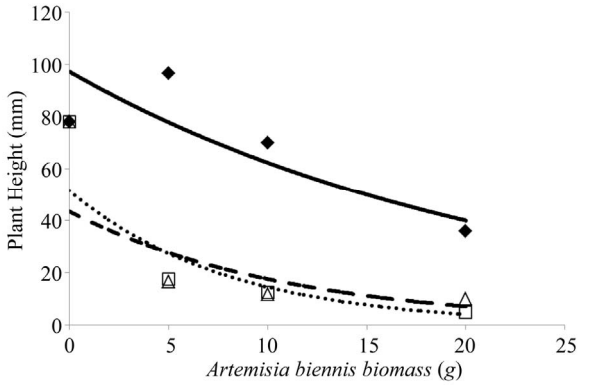


Figure 2. Influence of amount of *Artemisia biennis* leaf, root, and stem biomass (0, 5, 10, and 20 g) that was mixed with greenhouse potting mix on *Solanum melanocerasum* average plant height 21 days after emergence under controlled environment. *A. biennis* rates were mixed with 150 g of greenhouse potting mix prior to seeding *S. melanocerasum*. Leaf biomass (♦, solid line): $y = 97.276e^{-0.044x}$ ($R^2 = 0.78$); root biomass (□, dotted line): $y = 51.57e^{-0.126x}$ ($R^2 = 0.89$); stem biomass (Δ, broken line): $y = 43.482e^{-0.09x}$ ($R^2 = 0.67$).

In this study *A. biennis* allelopathy was demonstrated and we showed that phytotoxic effects vary among plant parts with effects most evident on *S. melanocerasum* average plant height and fresh weight plant^{-1} (**Figures 1** and **2**). Several related species within the *Artemisia* genus are allelopathic. For example, extracts from *A. annua* (annual wormwood) leaves have been shown to inhibit growth of *A. retroflexus* and *Chenopodium album* (common lambsquarters) [15]. Our study indicated that *A. biennis* leaf biomass was inhibitory to *S. melanocerasum* though more plant material was required to illicit the

response compared to root and stem biomass. Monoterpenoids are secondary plant metabolites recognized for their role against plant-directed pathogens, herbivores, or competing plant species [6,7]. Differences in concentration of monoterpenoids in *A. feddei* and *A. scoparia* leaves and stems have been shown to vary by season [16]. Investigation of the seasonal variation in allelopathic potential of *A. princeps* on the germination and seedling growth of *Lactuca sativa* and *Achyranthes japonica* [17]. The highest phytotoxic effect was from extracts collected in July from *A. princeps* plants and least from extracts collected in April and October. In our preliminary study, we found *A. biennis* allelopathy to be highly expressed when plants were 45 cm or taller (data not presented) and that the allelopathic potential is differentially expressed among plant parts, hence supporting the work by other researchers [16,17].

3.2. Allelopathic Potential of Soil Used to Grow *A. biennis*

Soil that was used to grow *A. biennis* plants for the leaf, root, and stem biomass study and from which all root material was removed resulted in a 55% reduction in average height of *S. melanocerasum* plants 21 d after emergence (**Figure 3(a)**). The soil plus roots treatment resulted in a 70% reduction in *S. melanocerasum* plant height compared to the untreated control. *S. melanocerasum* fresh weight plant^{-1} was similar among the soil treatments and was reduced by 76% compared to the untreated control (**Figure 3(b)**).

A. biennis allelopathy appears more evident in the soil and perhaps is an indication of root exudation as the primary release of phytotoxins. When roots were removed from soil that was used to grow *A. biennis* plants and placed in new soil prior to seeding *S. melanocerasum*, reduction in weight plant^{-1} was 88% or greater and was 75% greater for plant height (**Figures 1** and **2**). Despite detecting a slight difference between the soil – roots and soil + roots treatments (**Figure 3**), potential *A. biennis* allelopathy was demonstrated and appears to be primarily soil-related.

A. annua produces several biologically active compounds, including artemisinin, which is a sesquiterpene lactone known to be phytotoxic *in vitro* [6,15]. The importance of artemisinin production in plants extends well beyond allelopathy. Artemisinin is a very effective antimalarial drug due to its action against the *Plasmodium* spp. parasites which cause malaria [18].

Increased interest in allelopathy over the past several years has been because some allelopathic plants have helped enhance crop protection by providing a basis for herbicide development. For example, the herbicide mesotrione was derived from the structurally similar phytotoxin leptospermone released by the allelopathic species

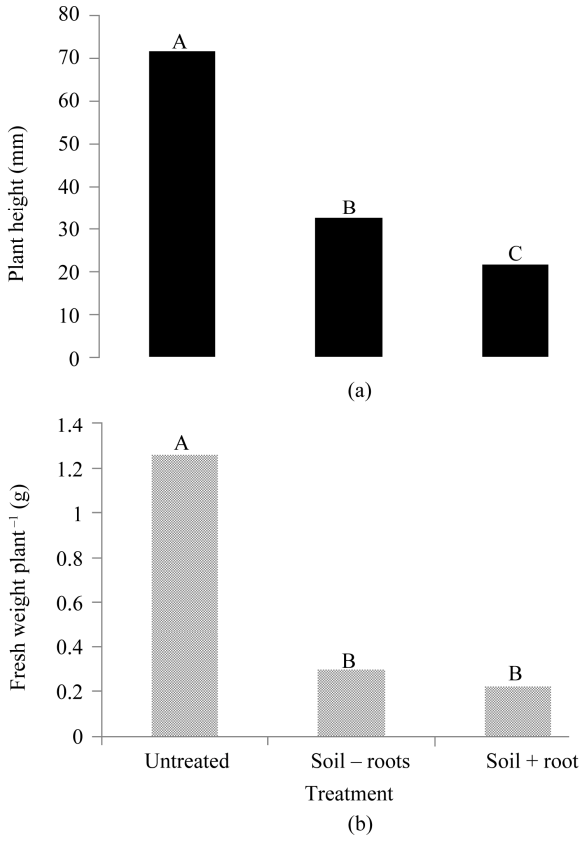


Figure 3. Influence of soil used to grow *Artemisia biennis* plants on (a) average plant height and (b) fresh weight plant^{-1} of *Solanum melanocerasum* plants 21 days after seedling emergence under controlled environment. The "soil – roots" treatment had most of the root material removed whereas no roots were removed from the "soil + roots" treatment. Bars with the same letters are not significantly different (P = 0.05).

Calistemon citinus [19]. Allelochemicals often exhibit selectivity similar to synthetic herbicides [20] and can offer the potential for the discovery of new molecular target sites for herbicides [21]. Utilization of this *A. biennis* trait involves isolating and characterizing plant compounds that are believed to be allelochemicals. [6] isolated artemisinin from *A. annua* plants and demonstrated its phytotoxic effect on several weedy species. However, [15] reported that soil amended with pure artemisinin was not as phytotoxic to *A. retroflexus* as soil amended with *A. annua* leaf extract, suggesting that the inhibitory effects of *A. annua* could not be attributed solely to artemisinin.

Successful extraction of essential oils from aboveground *A. biennis* plant portions has identified compounds that are phytotoxic to some microbes, such as the yeast *Cryptococcus neoformans*, and fungi *Fonseceaea pedresoi* and *Aspergillus niger* [22]. The presence of extractable compounds with herbicidal activity could increase the potential usefulness of *A. biennis*.

4. CONCLUSION

Our research findings indicate that the allelochemicals with herbicidal activity are located mostly in the stem and root biomass compared to leaf biomass (**Figures 1-3**). Increased invasiveness of *A. biennis* is aided partially by the production of allelochemicals, which improves its ability to compete for resources. The most likely scenario is one where *A. biennis* plant exudates (allelochemicals) alter the surrounding soil so as to give it a competitive advantage, which is similar to the allelopathic effect of *Pluchea lanceolata* [23]. Consequently, as *A. biennis* plants grow they produce increasing amounts of phytotoxins that prevent other susceptible species from becoming established. Characterizing *A. biennis* allelopathy to better understand the herbicidal activity of allelochemicals may provide new tools that can be utilized for weed management. However, once biologically-active allelochemicals have been isolated and characterized, they may not mimic their behavior in the soil [15]. Isolated allelopathic compounds from *A. biennis* have been found to have antimicrobial properties [22] which, together with its phytotoxic effects on plants, justify further investigation.

5. ACKNOWLEDGEMENTS

The authors thank S. Hobbie and D. Yazali (Maryville, MO), and S. Mark (Fargo, ND). This material is based upon work supported in part by Northwest Missouri State University Faculty Research Funds and by the Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture (under Agreement No. 2004-34361-14427).

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