AQUEOUS TISSUE EXTRACTS OF Conyza canadensis INHIBIT THE GERMINATION AND SHOOT GROWTH OF THREE NATIVE HERBS WITH NO AUTOTOXIC EFFECTS

ABSTRACT - Conyza canadensis is a widespread weed species forming dense populations in most regions of China. Petri dish bioassays with aqueous extracts of the aboveground parts and roots of C. canadensis at three concentrations (0.05, 0.1, and 0.2 g mL⁻¹) were undertaken to investigate the autotoxic effects of C. canadensis, and the possible effects on three dominant native weed species, Plantago asiatica, Digitaria sanguinalis and Youngia japonica. The results showed that seed germination and the shoot length of three native species were significantly inhibited by aqueous extracts of C. canadensis at almost all concentrations that generally increased with increasing extract concentration. However, the seed germination and shoot length of C. canadensis itself was not significantly affected by the same extracts at all concentrations. These results suggested that the potential allelopathic compounds produced by the tissue of C. canadensis may contribute to its invasive success in invading southern China.

Keywords: invasive alien weed, allelopathy, petri dish bioassay.

INTRODUCTION

Conyza canadensis (Asteraceae) is an annual invasive alien and unwanted weed native to North America (Weaver, 2001). It is a versatile and highly adaptive invader that is found in many parts of the world (Dauer et al., 2007; Zaplata, 2011). It was first recorded in Yantai, Shandong province of China in 1862 (Xie et al., 2001), and is now...
widely distributed throughout the 28 provinces and listed as one of the most noxious, invasive plants in China (Weber et al., 2008). This plant generally grows 0.5-1 m tall, and often forms dense populations in most habitats, such as fallow fields, orchard, pastures, roadsides, etc. (Main et al., 2006). It is considered an important agricultural weed because it can reduce agricultural yields by 90% at high densities (100-200 plants m⁻²) (Shields et al., 2006). Seed production is robust with an estimated 100,000 wind-dispersed seeds produced per plant when densities reached 200 plants m⁻² and 200,000 seeds when plant densities were low (10 plants m⁻²) (Bhowmik & Bekech, 1993). On invasion, it establishes quickly in the alien environment, resulting in changes in the structure and function of the native ecosystem.

Allelopathy is a process whereby plants provide themselves with a competitive advantage by putting phytotoxins into the near environment. It is known to play an important role in affecting the community structure, including plant dominance and succession (Lambers et al., 1998). Recently, numerous studies have demonstrated the allelopathic effects of invasive plants on native species in the invaded ranges and provided compelling evidence for allelopathy as an important component of the competitive success of some invaders (Bais et al., 2003; Hierro & Gallaway, 2003; Prati & Bossdorf, 2004; Dorning & Cipollini, 2006; Cipollini et al., 2008; Inderjit et al., 2008; Thorpe et al., 2009; Chapla & Campos, 2010; Pisula & Meiners, 2010). Although the noxious C. canadensis poses a very serious threat to the diversity or abundance of native plant species and agricultural productivity in China, the mechanisms of the invasion of C. canadensis are still poorly understood. A recent study has reported that a total of 42 compounds, including phenol, ketone, acid, alcohol, aldehyde, ester, terpene, etc., were identified from an extract of C. canadensis (Zhang, 2010). Among these, phenol, ketone, and acid were the main components, which might show the allelopathic effects (Zhang, 2010). Shaukat et al. (2003) reported that the aqueous extract of C. canadensis at different concentrations (25, 50, 75, and 100% stock solution) inhibited the germination, root, and shoot growth of six crop species. Presumably, the dominance of C. canadensis in its invaded habitats might be related to chemical interference or allelopathy, which gives it an additional advantage over neighboring native plants.

Because of difficulties in separating the allelopathic effects from resource competition, it is extremely difficult to demonstrate allelopathy in the field (Bais et al., 2003). Instead, extensive research has successfully shown the potential for allelopathy in invasive plants using tissue extracts (Dorning & Cipollini, 2006; Yang et al., 2007; Hussain et al., 2011). A similar approach was applied to study the effects of C. canadensis on seed germination and the shoot growth of three native species and C. canadensis itself under laboratory conditions by using aqueous extracts at different concentrations from the aboveground parts and roots of C. canadensis. We hypothesized that the presence of the extracts of C. canadensis would reduce seed germination and the shoot length of these native species. In contrast, the seeds and seedlings of C. canadensis can explore their adaptive benefit for its own extracts and, therefore, they should be resistant to inhibitory allelopathic effects.

**MATERIALS AND METHODS**

**Sample collection**

Three native herb species, *Plantago asiatica* (Plantaginaceae), *Digitaria sanguinalis* (Poaceae), *Youngia japonica* (Asteraceae), and *C. canadensis* itself were chosen as target species. These native species were often the dominant weed species in most field sites such as roadsides, agricultural fields, disturbed grasslands, and abandoned fields prior to C. canadensis invasion in southern China. From June to October 2010, the seeds of the four target species were collected from the abandoned fields and roadsides in the suburbs of Nanning city, southern China. All the collected seeds were visually examined and all damaged seeds were discarded. After air drying, these seeds were stored in plastic bottles in the dark at 4 °C until further use.

Field grown *C. canadensis* whole plants were collected in situ in Mar 2011. The fresh
Aqueous tissue extracts of *C. canadensis* inhibit the germination of non-native species

Plants were immediately taken to the laboratory, and separated into aboveground (leaf and stem) and belowground (root) components. Each component was first cleaned to remove the dirt, air dried, and ground into powder using a mechanical grinder. The powder was instantly passed through a 100-mesh sieve and kept for use.

**Extraction procedure**

Twenty grams of each ground component were extracted by soaking in 100 mL of distilled water at 25 °C for 36 h in a shaker. The aqueous extracts were then filtered twice through Whatman No. 1 filter paper (particle retention of 11 μm), and 0.2 g mL⁻¹ aqueous extract was obtained. Using this extract as stock solution, 0.1 g mL⁻¹ and 0.05 g mL⁻¹ extracts were prepared by dilution. The three concentrations (0.05, 0.1, and 0.2 g mL⁻¹) of the aqueous extracts of aboveground parts and the roots of *C. canadensis* were tested for allelopathic potential by a seed germination bioassay.

**Petri dish bioassay**

In March 2011, the seeds of the four species were sterilized in NaOCl (5% v/v) and washed thrice with distilled water before sowing. Twenty seeds of each species were placed on two layers of filter paper in glass Petri dishes (9 cm diameter). Five mL of each extract solution were added to the Petri dishes, while the control treatment (CK) received 5 mL of distilled water. The Petri dishes and filter paper were used as a sterile medium. Three replicates of each treatment were incubated in an illuminated cultivation cabinet setting at 25 °C/20 °C day/night temperature with 70% relative humidity and a 12/12 h light/dark regime. An additional 1 mL of each solution was added every 48 h thereafter. The germination was assessed after every 24 h by counting the number of germinated seeds for up to one week. Seeds were considered germinated when radicles could be observed by the naked eye. After one week, all Petri dishes were transferred to a cold chamber at 4 °C in order to stop seedling growth and then the shoot length was recorded with a measuring tape.

**Statistical analysis**

Germination bioassays were analyzed in a randomized design with three replications. The collected data were statistically analyzed by using a one-way ANOVA, and the LSD test was used to determine the differences between the treatment means at the 5% probability level. Statistical analysis was conducted using SPSS 11.5 software (SPSS Inc., Chicago, Illinois, USA).

**RESULTS**

The seed germination of *C. canadensis*, *P. asiatica*, *D. sanguinalis*, and *Y. japonica* reached an average of 70.9%, 90.2%, 84.3%, and 60.2%, respectively, in the control treatment by the end of the experiment (Figure 1A and B). Both the aqueous extracts of aboveground parts and the roots of *C. canadensis* had no significant effect on its own germination at all three concentrations (0.05, 0.1, and 0.2 g mL⁻¹) compared with the control. In contrary to *C. canadensis*, extracts at almost all concentrations significantly decreased the germination percentage of other species and the percent inhibition increased with increasing concentration of extracts, so that the lowest germination percentage was observed at 0.2 g mL⁻¹ extracts. Furthermore, the aqueous extract of aboveground parts of *C. canadensis* showed stronger inhibition than root extract. For example, the final germination of *P. asiatica*, *D. sanguinalis*, and *Y. japonica* seeds receiving 0.2 g mL⁻¹ of aqueous extract of aboveground parts, was only 20.2%, 14.5%, and 6.2%, respectively (Figure 1A), while 31.5%, 32.2%, and 22.1%, respectively, at the same concentrations of root extracts (Figure 1B).

The final shoot length of *C. canadensis* was not significantly affected by its own extract at all concentrations (Figure 2A and B). However, the shoot length of three native species was significant inhibited by both aqueous extracts of aboveground parts and roots of *C. canadensis* at most concentrations. The shoot length generally reduced with an increasing concentration of extracts for each of the native species. The extract of aboveground parts showed stronger inhibitory effects than root extract on shoot growth. Extract of aboveground parts at a lowest concentration (0.05 g mL⁻¹)
significantly reduced the shoot length of *P. asiatica* and *Y. japonica* (Figure 2A), while there was no significant reduction from root extracts at the lowest concentration (Figure 2B).

**DISCUSSION**

Shoot extracts of *C. canadensis* and volatile compounds from the inflorescence of invasive *Conyza albida*, a closely related species, exerted significant inhibitory effects on the germination and growth of several crop species (Economou et al., 2002; Shaukat et al., 2003). In the present experiment, the aqueous extracts of aboveground parts and the roots of *C. canadensis* greatly inhibited seed germination and the shoot growth of three dominant native herb species, *P. asiatica, D. sanguinalis*, and *Y. japonica*, under laboratory conditions, even at a low concentration (0.05 g mL). Allelopathic compounds that have phytotoxic or at least fitness-reducing effects may be novel for plant competitors without a co-evolutionary history (Callaway & Aschehoug, 2000; Hierro & Callaway, 2003; Callaway & Ridenour, 2004). These non-coevolved competitors may be susceptible to novel phytochemicals in the

**Figure 1** - Effects of different aqueous extract concentrations of aboveground parts (A) and roots (B) of *C. canadensis* on the seed germination of four target species. Each column represents the mean value ± standard error from three replicates. CC, *Conyza canadensis*; PA, *Plantago asiatica*; DS, *Digitaria sanguinalis*; YJ, *Youngia japonica*.

**Figure 2** - Effects of different aqueous extract concentrations of aboveground parts (A) and roots (B) of *C. canadensis* on the shoot length of four target species. Each column represents the mean value ± standard error from three replicates. CC, *Conyza canadensis*; PA, *Plantago asiatica*; DS, *Digitaria sanguinalis*; YJ, *Youngia japonica*. 
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Effect might contribute to its invasive success in southern China. Although our experiment was a preliminary laboratory study it still provides encouraging results and a basis for future research.

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