

## A Lack of Evidence for an Ecological Role of the Putative Allelochemical ( $\pm$ )-Catechin in Spotted Knapweed Invasion Success

Amy C. Blair · Scott J. Nissen ·  
Galen R. Brunk · Ruth A. Hufbauer

Received: 19 July 2006 / Revised: 23 August 2006 /  
Accepted: 28 August 2006 / Published online: 6 September 2006  
© Springer Science + Business Media, Inc. 2006

**Abstract** Allelopathy is a notoriously difficult mechanism to demonstrate. There has been a recent resurgence of interest in allelopathy because of the work done on the invasive weed spotted knapweed and its putative allelochemical, ( $\pm$ )-catechin. In this study we collected and analyzed soil samples taken from three, long-term knapweed infested sites in Montana, USA during the summer and fall of 2005. We only detected catechin in all the soil cores at one time point (August, 2005) at two of the sites. Field levels from these two sites were nearly three orders of magnitude lower than what has previously been reported to cause reduced growth in a sensitive native species. Fourteen percent of the remaining soil cores contained low but detectable levels ( $<0.11$  ppm) of ( $\pm$ )-catechin. Additional experiments indicated that soil moisture appears to play a significant role in whether or not catechin degrades rapidly or remains in the soil. Adding to previous work, this paper sheds doubt on the importance of this chemical in spotted knapweed invasion success.

**Keywords** Allelopathy · Novel weapons hypothesis · *Centaurea maculosa* · *Centaurea stoebe* · Spotted knapweed · ( $\pm$ )-Catechin · Invasive species

### Introduction

The role of allelopathy in defining plant distribution patterns has fallen in and out of favor with plant ecologists (Harper, 1977; Romeo, 2000). Over the past 5 years there has been a resurgence of interest in this topic, largely through the work on ( $\pm$ )-catechin as a putative allelochemical of *Centaurea stoebe* (previously misnamed *C. maculosa*, spotted knapweed) (Bais et al., 2002; Weir et al., 2003; Perry et al., 2005; Thelen et al., 2005). This work has led to the high profile ‘novel weapons hypothesis’, which states that the success of some invasive plant species may be due to the possession of novel allelochemicals that native species have never encountered (Callaway and Ridenour, 2004).

---

A. C. Blair (✉) · S. J. Nissen · G. R. Brunk · R. A. Hufbauer  
Department of Bioagricultural Sciences and Pest Management, Colorado State University,  
Fort Collins, CO 80523–1177, USA  
e-mail: amyblair@lamar.colostate.edu

Previously, we questioned the importance of ( $\pm$ )-catechin (hereafter referred to as catechin) in spotted knapweed's invasion (Blair et al., 2005). Specifically, we (1) found published techniques to be unrepeatable, (2) found levels of catechin production more than an order of magnitude less than previously reported, (3) showed that a species described as sensitive to catechin (*Festuca idahoensis*) only exhibited slightly reduced growth at concentrations 10 times higher than previously reported to cause 100% mortality, and (4) found no measurable catechin at two spotted knapweed sites in Montana, USA. In regards to this last point, it was noted that the soil samples were collected in the late fall, when plants may be less likely to be actively producing the root exudate. This paper addresses this issue by sampling and analyzing soil from three knapweed-infested sites during the summer and fall.

## Methods and Materials

Spotted knapweed is a self-incompatible Eurasian biennial or perennial that threatens grassland diversity and productivity across western North America (Roché and Roché, 1991). Spotted knapweed is commonly called *C. maculosa* in North America; however, there remains much confusion regarding the nomenclature. There are two cytotypes of the plant, which are treated either as separate species or as subspecies. *Centaurea maculosa* Lam. has been used for both the diploid and tetraploid form. The diploid form has been synonymized with *Centaurea stoebe* L. spp. *stoebe* (Ochsmann, 2000). The tetraploid has been referred to as *C. biebersteinii* DC or *C. stoebe* L. spp. *micranthos* (Gmelin ex Gugler) Hayek. Ochsmann (2000) argues that the *C. stoebe* name takes priority. The *C. stoebe* species group consists of several taxa of uncertain status that are difficult to distinguish morphologically. Herein, we refer to the plant by its common name, spotted knapweed.

### Knapweed-Infested Field Sites and Sampling Protocol

Three field sites were sampled through time to assess catechin production over the course of a growing season (see Table 1 for site characteristics). The soil around five, randomly selected spotted knapweed plants was sampled from 3 to 5 cm depth using 6 cm diam cores. Three cores were taken adjacent to and directly under the knapweed crown for a total of 6 cores per plant, pooled, and mixed. Bulk soil samples (200 g) were collected from outside the three infested sites to serve as control soils. All samples were frozen within 1 h of collection and shipped on ice to Colorado State University, where they were stored at  $-20^{\circ}\text{C}$  until analysis.

### Quantitation of Catechin in Soil

We extracted and analyzed catechin in the soils using HPLC following protocol of Blair et al. (2005). We used field soils fortified with 5 ppm catechin to assess extraction efficiencies.

### Catechin Persistence in Wet vs. Dry Soil

Because catechin was found above trace levels in August only (see results below), we conducted an additional experiment to evaluate the persistence of catechin in these field soils. Based on previous work (Blair et al., 2005), we hypothesized that catechin may have been able to build up in August because the soils were quite dry, but that it would degrade if

**Table 1** Knapweed infested site and soil characteristics

| Site MT, USA              | Infestation Duration | Soil Type | pH  | % Organic Matter | Sample Dates          | Recovery Efficiency % | Catechin (ppm)         | % Moisture |
|---------------------------|----------------------|-----------|-----|------------------|-----------------------|-----------------------|------------------------|------------|
| Nelson Gulch <sup>a</sup> | 12 y                 | Windham   | 7.9 | 4.2              | 7/5/05 <sup>b</sup>   | 87.4                  | trace (1) <sup>f</sup> | 5.1 ± 0.7  |
| 46°34.373' N              |                      | Sandy     |     |                  | 8/4/05 <sup>c</sup>   | +100.0                | 1.1 ± 0.3              | 2.0 ± 0.05 |
| 112°8.820' W              |                      | Loam      |     |                  | 9/26/05 <sup>d</sup>  | 79.0                  | trace (1) <sup>f</sup> | 11.2 ± 0.4 |
| 4273 m                    |                      |           |     |                  | 10/27/05 <sup>e</sup> | 90.6                  | 0                      | 14.1 ± 1.3 |
| Beck Hill <sup>a</sup>    | 20 y                 | Tanna     | 7.5 | 3.5              | 9/14/05 <sup>a</sup>  | 43.6                  | 0                      | 9.7 ± 0.4  |
| 46°29.472' N              |                      | Sandy     |     |                  |                       |                       |                        |            |
| 112°43.422' W             |                      | Loam      |     |                  | 10/28/05 <sup>e</sup> | 65.6                  | 0                      | 8.2 ± 0.3  |
| 4497 m                    |                      |           |     |                  |                       |                       |                        |            |
| Jens Exit <sup>a</sup>    | >15 y                | Blossberg | 7.2 | 6.1              | 8/5/05 <sup>c</sup>   | +100.0                | 1.3 ± 0.4              | 5.0 ± 0.2  |
| 46°36.359' N              |                      | Sandy     |     |                  | 9/25/05 <sup>d</sup>  | 71.8                  | Trace (2) <sup>f</sup> | 15.1 ± 0.4 |
| 113°00.832' W             |                      | Loam      |     |                  | 10/28/05 <sup>e</sup> | 47.4                  | Trace (2) <sup>f</sup> | 12.5 ± 1.4 |
| 4136 m                    |                      |           |     |                  |                       |                       |                        |            |

<sup>a</sup> Plant species at Nelson and Beck include bluebunch wheatgrass (*Pseudoroegneria spicata*), Idaho fescue (*Festuca idahoensis*), and 5% scattered ponderosa pine (*Pinus ponderosa*). Species at Jen include sandberg bluegrass (*Poa secunda*) and Idaho fescue (*Festuca idahoensis*).

<sup>b</sup> plants at bolting stage;

<sup>c</sup> plants at flowering stage;

<sup>d</sup> plants senescing;

<sup>e</sup> basal regrowth (≈2 cm).

<sup>f</sup> less than 0.1 ppm catechin; the number in parentheses refers to the number of cores with trace catechin out of 5.

the soils were moist. We tested this hypothesis using soil from the cores with the highest naturally occurring catechin concentrations from the Nelson and Jens August samples and soils from control areas of these sites fortified at 5 ppm catechin. We moistened samples of 5 g of soil ( $N = 3$  per sample) with 1 ml water and allowed them to sit at room temperature in the dark for 24 h. As positive controls, un-moistened soils with natural or fortified catechin ( $N = 3$  per sample) were subject to the same environment. Catechin was quantitated as above. The percent soil moisture from each field site through time was determined by drying approximately 5 g overnight at 105°C and comparing mass before and after.

## Results

### Quantitation of Catechin in Soil

Recovery efficiencies across the three sites through time averaged  $80.3 \pm 9.4\%$  (mean  $\pm$  SE). The detection limit of our method was 0.02 ppm, well below previously reported levels of catechin found in the field, and much lower than what has been suggested to be biologically active. Catechin was found in all soil cores at the two sites sampled in August (Nelson and Jens) (Table 1). Only 14% of the other soil cores had detectable trace levels of catechin ( $<0.11$  ppm) (Table 1).

### Catechin Persistence in Wet vs. Dry Soil

The naturally occurring catechin was not detectable in the Nelson and Jens soil samples 24 h after they were moistened. Similarly, only 22% and 6% of the catechin was recovered from the moistened fortified Nelson and Jens control soils, respectively. However, the naturally occurring catechin from the same Nelson and Jens cores remained detectable in the un-moistened controls (Nelson =  $1.83 \pm 0.21$  ppm and Jens =  $2.9 \pm 0.5$  ppm). Fortified control soils behaved similarly; 81.4% and 74.8% of the catechin was recovered from the un-moistened Nelson and Jens control soils, respectively.

These data support our hypothesis that the amount of detectable catechin found in these soils through time can be explained by field soil moisture levels. The two sites that had detectable catechin in August 2005 were among the three with the lowest soil moisture levels of any samples (Table 1).

## Discussion

We found catechin in field soils at levels three orders of magnitude lower than have previously shown to be toxic (Blair et al., 2005). Additionally, based on the research herein and our previous work, we believe that it is unlikely that catechin will persist in the field at levels necessary to influence plant distributions. Blair et al. (2005) found catechin to be highly unstable at neutral and basic pH in both water and soil, and even in acidic soils if they were moist. In this study, we found that catechin degraded rapidly in moistened soils from the low or trace amounts naturally present to undetectable ( $<0.02$  ppm). We hypothesize that our inability to find catechin even when the soil moisture was relatively low (i.e., Nelson 7-5-2005, soil moisture  $\approx 5\%$ ) suggests that sporadic precipitation may prevent a build-up of the compound in the soil via the chemical process of degradation and/or enhanced microbial activity.

Our finding of low or undetectable levels of catechin from field-collected soils are in sharp contrast to the recent reports of catechin found in the field at levels ranging from ~1,550 to 3,300 ppm on average, with a single high value reported at 7,100 ppm (also from Montana, USA) (Bais et al., 2003; Perry et al., 2005; Thelen et al., 2005). It is plausible that differing sites will have varying amounts of catechin, as soil characteristics and microbes will greatly influence the stability and persistence of this compound. However, we are not confident in the accuracy of the remarkably high concentrations reported in these papers. They used a methanol extraction technique that we found to yield between 0 and 17% extraction efficiency with 6 different soil types fortified with 10 ppm catechin (Blair et al., 2005). If extraction efficiency is similar for higher levels of catechin and was on the high end of this range for their soils (e.g., 20%), then their estimates would increase 5-fold to approximately 7,750 to 16,500 ppm, or 7.8 to 16.5 mg of catechin per gram of soil (Bais et al., 2003; Perry et al., 2005; Thelen et al., 2005). Given that our data here suggest catechin is not likely to build up in the soil due to precipitation, this seems like a physiologically unreasonable amount for a plant to produce, and still have carbohydrates remaining for growth and maintenance.

While catechin may be toxic to plants at high concentrations, this study adds to the case that it is unlikely to be a strong force in the successful invasion of spotted knapweed. We predict that rainfall will never allow the chemical to attain toxic levels. However, this research does not rule out the possibility that spotted knapweed is allelopathic, and we suggest that future energies be directed at assaying additional putative allelochemicals.

**Acknowledgment** We would like to thank Celestine Duncan for collecting the soil samples.

## References

- BAIS, H. P., WALKER, T. S., STERMITZ, F. R., HUFBAUER, R. A., and VIVANCO, J. M. 2002. Enantiomeric-dependent phytotoxic and antimicrobial activity of ( $\pm$ )- catechin. A rhizosecreted racemic mixture from spotted knapweed (*Centaurea maculosa*). *Plant Physiol.* 128:1173–1179.
- BAIS, H. P., VEPACHEDU, R., GILROY, S., CALLAWAY, R. M., and VIVANCO, J. M. 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science* 301:1377–1380.
- BLAIR, A. C., HANSON, B. D., BRUNK, G. R., MARRS, R. A., WESTRA, P., NISSEN, S. J., and HUFBAUER, R. A. 2005. New techniques and findings in the study of a candidate allelochemical implicated in invasion success. *Ecol. Lett.* 8:1039–1047.
- CALLAWAY, R. M. and RIDENOUR, W. M. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. *Front Ecol. Environ.* 2:436–443.
- HARPER, J. L. 1977. *Population Biology of Plants*. Academic, London.
- OCHSMANN, J. 2000. Morphologische und molekularsystematische untersuchungen an der *Centaurea stoebe* L.-Gruppe (Asteraceae–Cardueae) in Europa. *Diss. Bot.* 324:242.
- PERRY, L. G., THELEN, G. C., RIDENOUR, W. M., WEIR, T. L., CALLAWAY, R. M., PASCHKE, M. W., and VIVANCO, J. M. 2005. Dual role for an allelochemical: ( $\pm$ )-catechin from *Centaurea maculosa* root exudates regulates conspecific seedling establishment. *J. Ecol.* 93:1126–1135.
- ROCHÉ, B. F. and ROCHÉ, C. T. 1991. Identification, introduction, distribution, and economics of *Centaurea* species, pp. 274–291, in L. F. James, J. O. Evans, M. H. Ralphs, and R. D. Child (eds.). *Noxious Range Weeds*. Westview, San Francisco, California.
- ROMEO, J. T. 2000. Raising the beam: moving beyond phytotoxicity. *J. Chem. Ecol.* 26:2011–2014.
- THELEN, G. C., VIVANCO, J. M., NEWINGHAM, B., GOOD, W., BAIS, H. P., LANDRES, P., CAESAR, A., and CALLAWAY, R. M. 2005. Insect herbivory stimulates allelopathic exudation by an invasive plant and the suppression of natives. *Ecol. Lett.* 8:209–217.
- WEIR, T. L., BAIS, H. P., and VIVANCO, J. M. 2003. Intraspecific and interspecific interactions mediated by a phytotoxin, (–)-catechin, secreted by the roots of *Centaurea maculosa* (spotted knapweed). *J. Chem. Ecol.* 29:2397–2412.