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Communication

# First Report of Benzoxazinoid Compounds in Woolly Cupgrass (*Eriochloa villosa* Thunb. Kunth), an Invasive Plant

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**Abstract:** Woolly cupgrass (*Eriochloa villosa* Thunb. Knuth) is a common weed which is native to East Asia. It also was introduced into North America and Europe. The problems related to controlling this annual weed are due to its germination characteristics, low susceptibility to certain herbicides and considerable competitiveness against other plant species. Several genera of the *Panicoideae* subfamily contain benzoxazinoids, therefore we hypothesized that woolly cupgrass also produces these chemicals. To test this hypothesis, the benzoxazinoid content of plants at the flowering stage was investigated. Our results demonstrate the presence of benzoxaziniods in woolly cupgrass and this represents the first report of these compounds in this species within the *Poaceae* family and the *Panicoideae* subfamily. Benzoxazinoids likely account for the rapid spread and ecological success of woolly cupgrass in its invasive range.

**Keywords:** benzoxazinoids; *Eriochloa villosa*; wolly cupgrass; new occurrence

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## 1. Introduction

Woolly cupgrass (*Eriochloa villosa* Thunb. Kunth) is a common weed which is native to East Asia. In the middle of the 20th century, it was introduced into North America, specifically the United States, especially as a weed in corn fields. Soon it spread, and appeared in the southern part of Canada by the start of the 21st century. At the same time, it also was introduced into Europe (Romania, Ukraine, Hungary) [1]. In the last few years, this problematic weed plant has been found in other Western and Eastern European countries [2]. In Hungary, there are numerous regions where this invasive plant is found [3]. The problems in controlling this annual weed are due to its germination characteristics; seeds emerge from deeper soil depths continuously during the growing season [1]. In addition, woolly cupgrass is less susceptible to certain herbicides that are often used against other annual grasses that infest corn fields [4].

The success of a plant species can originate from its ability to suppress other plants. For this purpose, plants produce and exudate—especially through their roots—chemicals with allelopathic properties. Plants from Poaceae use benzoxazinoids against their competitors and pests; thus, benzoxazinoids are key defence chemicals, and help these grasses suppress other plant species. Benzoxazinoids can be classified into three groups: first, lactams, such as 2-hydroxy-1,4-benzoxazin-3-one HBOA; 2-hydroxy-7-methoxy-1,4-benzoxazin-3-one HMBOA; second, hydroxamic acids, such as 2,4-dihydroxy-1,4-benzoxazin-3-one DIBOA; 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one DIMBOA; and third, benzoxazolines, such as benzoxazoline-2-one BOA; 6-methoxy-benzoxazoline-2-one MBOA) [5]. Woolly cupgrass is a member of the *Panicoideae* subfamily. Several genera of this subfamily contain benzoxazinoids, including *Coix*, *Echinochloa*, *Tripsacum* and *Zea* [6]. For example, 10-day-old *Echinochloa* plants contained DIBOA in the glycosidic form (194–267 $\mu g\ g^{-1}$ fresh weight) [7].

Five-week-old *Echinochloa crus-galli* plants in turn contained DIMBOA and DIBOA in the form of glucosides (8.7–18.85 and 4.05–7.5 μg $g^{-1}$ fresh weight, respectively) [8], although HMBOA has not been reported in *E. crus-galli*. Corn and *Coix* spp., however, contain HMBOA, and in considerable amounts [9]. To date, no data have been reported concerning the benzoxazinoid content of wooly cupgrass, which was the goal of this study.

## 2. Materials and Methods

Seeds of woolly cupgrass were collected in 2020 from field-grown plants in Hungary, in the Hajdúság region, near to the city of Debrecen. Ten seeds were sown on 20 May 2021, in 14 cm diameter pots filled with standard potting soil. After germination, three seedlings were randomly selected for use in this study. We had four independent pots. The plants were grown in an open-sided greenhouse, and water was applied at 65% of field water capacity every two days. Sample collection was conducted at the initiation of flowering (Figure 1).

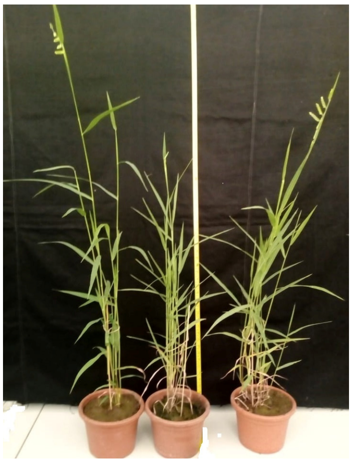

**Figure 1.** Woolly cupgrass plants at the stage of flowering.

Plants were 69-days (about 10 weeks)-old at the time of sampling. The flag leaf, the upper-most leaf, and the youngest parts of the root were collected from four individual plants. Four plants, each grown in different pots, were used to take measurements. The fresh-weight measurements of the harvested tissues were recorded, and these tissues were stored at −80 °C until sample preparation.

Sample preparation and HPLC analyses were carried out according to Lyons et al. [10]. DIMBOA and DIBOA standards were isolated from etiolated corn and rye seedlings using the method developed by Hartenstein et al. [11]. The HMBOA standard was synthetized and provided by Pierre Mateo (Universität Bern, Institute of Plant Sciences). The DIMBOA and DIBOA standards were checked and identified by their melting points and by $^{1}H$ NMR spectra recorded for $CD_3OD$ solutions. For this, we used a Bruker 360 (360 MHz) spectrometer. Chemical shifts were referenced to $CH_3OH$ (δ 3.31 ppm). The melting points of DIMBOA and DIBOA were 168.0–169.0 °C and 163.5–165.0 °C, respectively. A reverse-phase C18 column (Lichrospher RP18-5; 250 × 4.0 mm) was used for HPLC (Jasco, UV/VIS, Japan, Tokyo) measurements. The volume of samples was 20 μL. At the beginning and end of every set of measurements, solutions of pure DIMBOA, DIBOA and HMBOA were used as standards. Standard solutions were prepared from the crystalline form of the three chemicals. Four measurements were conducted for each sample. Statistical analyses were performed using SPSS version 23.0. The data collected for individuals within each group were not normally distributed; therefore, nonparametric tests were used for analyzing these groups.

## 3. Results and Discussion

Our results demonstrate the presence of benzoxazinoid compounds in different tissues of woolly cupgrass (Table 1), and this represents the first report of these compounds in this species.

**Table 1.** Benzoxazinoid content of woolly cupgrass in different parts (mg $g^{-1}$ fresh weight); (Root, Flag leaf, Last fully expanded leaf) $n$ = 4; ±SE.

| Organ/Benzoxazinoid | DIMBOA | HMBOA |
| --- | --- | --- |
| Root | $0.0525 \pm 0.0201^{a}$ | $0.0700 \pm 0.0178^{a}$ |
| Flag leaf | $0.0900 \pm 0.0302^{a}$ | $1.0125 \pm 0.02723^{b}$ |
| Last fully expanded upper leaf | $0.1175 \pm 0.0256^{a}$ | $0.7225 \pm 0.0517^{b}$ |

Note: Different small letters indicate significantly different groups in the columns at $p \leq 0.05$ level.

Our results indicate that there is no significant difference between the DIMBOA and HMBOA content of roots, while leaves contain a higher amount of HMBOA (0.8675 ± 0.1359 mg $g^{-1}$; $n$ = 8; ±SE) compared to DIMBOA (0.1037 ± 0.0191 mg $g^{-1}$; $n$ = 8; ±SE). There were no differences in the DIMBOA content of the plant parts we examined, but the HMBOA content was higher in leaves compared to roots. These compounds are present in intact plant tissues in the glycosidic form [6]. However, as a consequence of our sample preparation method, glycosidase enzymes released aglycones, which we measured.

Compared to previously published results [7,8] the concentrations we measured are similar to the concentrations reported in the shoots of 10-day-old *Echinocloa crus-galli* plants. Young tissues of older plants may contain benzoxazinoids in as high a concentration as in young plants [12]. The shoot and root tissues we analyzed were the youngest tissues of the plants we examined. In contrast, Pethő [8] reported benzoxazinoids at lower concentrations in five-week-old seedlings of barnyard grass compared to the concentration reported here. However, Petho [8] analyzed the entire plant, not just the youngest tissues that we analyzed in this study.

As has been previously reported, the distribution of various benzoxazinoid compounds vary among different species [13], so it is reasonable to suggest that these compounds vary among the taxa in a plant subfamily (*Panicoideae*).

Woolly cupgrass is an invasive plant with high competitive ability and a high capacity to spread rapidly. The presence of benzoxazinoid compounds in the tissues of woolly cupgrass may be one reason for this species' success, which would allow these plants to better tolerate biotic and abiotic stress factors. Moreover, benzoxazinoid exudates into the rhizosphere [12] of woolly cupgrass, which would have allelopathic effects and regulate the germination and growth of potential competitors.

## 4. Conclusions

In this study, we report the first occurrence of benzoxazinoids in woolly cupgrass (*Eriochloa villosa*). The benefits of our findings are complex. Benzoxazinoid production of weedy plant species means they can protect themselves from different pathogens and pests. Plants that produce benzoxazinoid compounds can also use them to outcompete other plant species, and this phenomenon is referred to as allelopathy. Benzoxazinoid compounds can decrease the growth and germination of other plant species. Moreover, benzoxazinoids facilitate mineral nutrient uptake as these compounds form complexes with different metal ions in the soil; as a result, these compounds contribute to plant growth and development. Such benefits likely account for the rapid spread and ecological success of woolly cupgrass in its invasive range.

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