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Allelopathic Substance Exuded from a Serious Weed, Germinating Barnyard Grass (*Echinochloa crus-galli* L.), Roots

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Abstract. The allelopathy of a serious weed, barnyard grass (*Echinochloa crus-galli* L.), was investigated. Root exudates of young barnyard grass showed allelopathic effects and plant-selective activity and inhibited root elongation of all plants tested. With respect to shoot growth, the exudates did not show inhibition of barnyard grass only. The allelopathic substance was isolated and identified as *p*-hydroxymandelic acid by NMR. *p*-Hydroxymandelic acid strongly inhibited shoot growth and root elongation of all plants tested. The effects of three congeners of *p*-hydroxymandelic acid were tested on rice shoot growth. In the biological activity exhibited in rice, shoot growth was related to the hydroxyl groups.

Key Words. Allelopathic substance—Allelopathy—Barnyard grass (*Echinochloa crus-galli* L.)—Root exudate—Plant growth regulator—*p*-Hydroxymandelic acid

It is well known that barnyard grass is a serious worldwide weed (Rice 1984), and an allelopathic substance that affects other species has been confirmed (Tang and Young 1982), but the compound has not yet been identified. There are many reports about weeds concerning allelopathy, but the isolation and identification of the allelochemical substances are difficult (Chang-Hung and Hui-Jung 1976, Fuerst and Putnam 1983) because the separation of allelopathic effects from other competitive plants, or indirect sources, in the field is not easily done. Recently, allelochemicals from the mucilage of some germinating plant seeds have been found in laboratory experiments. A potent growth-promoting substance was isolated from the exudates of germinating cress (*Lepidium sativum* L.) and *Arabidopsis thaliana* seeds and identified as sodium 2-*O*-rhamnopyranosyl-4-deoxy-α-L-*threo*-hex-4-enopyranosiduronate, named lepidimoide, by spectral analysis (Hasegawa et al. 1992, Kosemura et al. 1993, Yokotani-Tomita et al. 1997). Kushima et al. (1998) reported that vanillic acid exuding from germinating watermelon seeds showed plant-selective activity on shoot growth of five etiolated species of plants tested.

In this study, we focused on a growth-inhibiting substance as an allelochemical in barnyard grass during germination and at the early seedling development state because allelopathy is already recognized at this time (Hasegawa et al. 1992, Kosemura et al. 1993, Yokotani-Tomita et al. 1997). The isolation, identification, and bioactivity of the growth regulator in root exudates were examined.

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Materials and Methods

Plants

Barnyard grass (*Echinochloa crus-galli* L. Beauv. var. *crus-galli*) and cockscomb (*Celosia argentea* L. var. *kunze*), timothy (*Phleum pratense* L.), cress (*Lepidium sativum* L.), amaranth (*Amaranthus viridis* L.), rice (*Oryza sativa* L.), and lettuce (*Lactuca sativa* L.) were sterilized with 1% sodium hypochlorite for 30 min and rinsed with distilled water. They were then sown in a tray (27.5 × 40.0 × 7.0 cm) with two layers of moistened filter paper and incubated at 25°C for 2 days.

Fifteen uniform etiolated barnyard seeds were incubated in a concentric circularity in a Petri dish (9.0 cm) containing 20 mL of 0.5% agar medium. Other uniform germinating seeds were incubated around the barnyard grass when the barnyard grass was incubated for 2, 4, 6, 8, 10 and 12 days. Petri dishes were put into the tray with moistened filter paper and then covered with Saran Wrap to retain humidity and incubated at 25°C. During incubation, they were kept in white fluorescent light (13 μmol m^{-2} s^{-1}) for 16 h every day. After 4 days, shoots and roots were measured. 200,000 Barnyard grass seeds were sterilized with 1% sodium hypochlorite for 30 min, rinsed with distilled water, put on a stainless steel net (1-mm mesh) in two trays (27.5 × 40.0 × 7.0 cm) containing 0.3 liter of distilled water, respectively, and then cultured at 25°C for 2 days. The culture solution was then removed and replaced with distilled water. During incubation, the seeds were kept in white fluorescent light for 16 h every day.

Isolation and Identification of Allelopathic Substances

The root exudates (2–12 days) were concentrated to dryness *in vacuo* at 35°C. The concentrate (13.8 g) was dissolved in water and separated into two fractions, one with a M_r above 5×10^3 and the other below 5×10^3 by molecular exclusion chromatography (Mol cut, Millipore Corp.). The active fraction (below 5×10^3) was split into three fractions, acidic, basic, and neutral, using cation and anion exchange cartridges (Sep-Pak CM and QMA, Waters). The acidic fraction (279.5 mg) showed the highest biological activity. The active fraction was then further purified by HPLC (Tosoh, TSK gel SCX (H^+), ϕ 7.8 × 300 mm). The fraction with a retention time of 16–20 min was concentrated to dryness at 35°C. This fraction was purified by HPLC again under the same conditions, and the fraction with a retention time of 18.1 min was dried, yielding 1.0 mg.

Spectrometric Analysis

The substance isolated in this study was analyzed by ^1H and ^{13}C NMR.

Bioassay

The seeds of cockscomb, timothy, cress, amaranth, lettuce, and rice were placed on filter paper moistened with 500 μL of test solution containing barnyard exudate solution from roots in a 3.3-cm Petri dish. The Petri dishes were kept in the light for 16 h and the dark for 8 h for 4 days, and their shoot or root lengths were then measured.

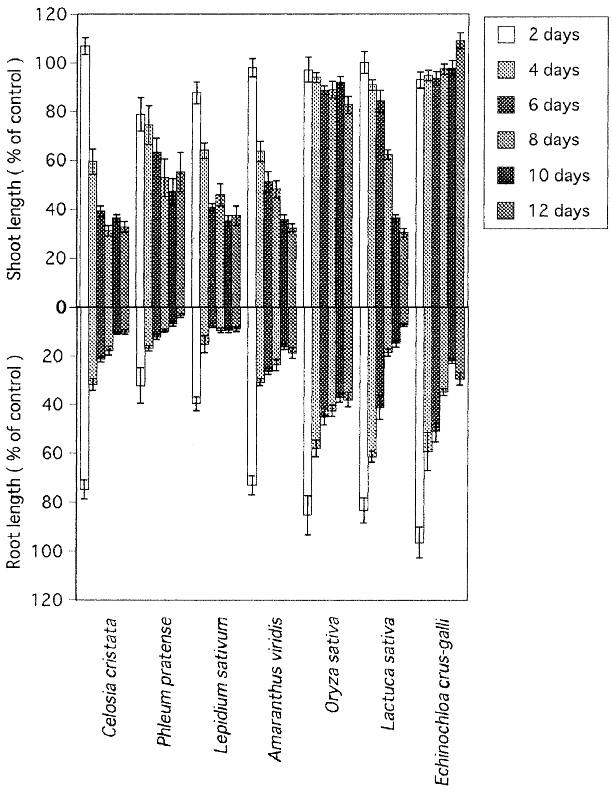


Fig. 1. Effects of exudate from young barnyard grass roots on the shoot growth and root elongation of seven species of plants. Bars indicate average ± S.E.

Results and Discussion

Fig. 1 shows the effects of barnyard grass seeds on the shoot growth and root elongation of cockscomb, timothy, cress, amaranth, lettuce, rice, and barnyard grass. When they were incubated together with barnyard grass seeds, the shoot growth and root elongation of all plants tested, except the shoot growth of barnyard grass, were inhibited. This allelopathic influence increased gradually during incubation (Fig. 1). These results indicated that a plant-selective allelopathic substance was exuded from germinating barnyard grass seed when the seeds were 2–12 days old.

An allelopathic substance, which showed inhibitory activity for shoot growth and root elongation of cockscomb, timothy, cress, amaranth, lettuce, rice, and barnyard grass seedlings, was isolated from the exudates of 2- to 12-day-old grown barnyard grass roots (Fig. 2). NMR spectra of isolated substance was absolutely coincident with of authentic *p*-hydroxymandelic acid (Fig. 3). The ^1H NMR data δ (400 MHz, D_2O) were: 7.19 (2H, d), 6.77 (2H, d), 5.07 (1H, s), ^{13}C NMR data were: δ(400 MHz, D_2O), 177.4 (COOH), 156.9 (C_4), 131.0 (C_3, C_5), 129.7 (C_2, C_6), 116.6 (C_1), 173.3 (*a*).

The biological activity of *p*-hydroxymandelic acid on barnyard grass growth was not greater than that on the growth of the other plants tested. Phenolic compounds have been reported widely as highly potent inhibiting substances on seed germination and shoot growth or seedling root elongation (Kushima et al. 1998, Rice 1984, Yamada et al. 1995). The effects of the phenolic compounds, *p*-hydroxymandelic acid, 3,4,-dihydroxyphenylacetic acid, and mandelic acid, were tested on rice shoot growth (Fig. 4 and Table 1). *p*-Hydroxymandelic acid gave high inhibition at concentrations higher than 30 mg/liter, and 3,4,-dihydroxyphenylacetic acid had weak inhibitory activity, but mandelic acid did not show equivalent inhibitory effects at the concentrations used. Therefore, at least the OH at the *p* position is needed to induce inhibition, and the *p*-OH position of mandelic acid may play some important roles in inhibitory activity.

The effects of the exudate from young barnyard grass roots on rice shoot growth did not give as strong an inhibition as was shown on shoot growth of other plants, except in the case of barnyard grass (Fig. 1). *p*-Hydroxymandelic acid significantly inhibited rice shoot and root growth. On the other hand, we also confirmed the presence of a growth-promoting substance, a lepidimoide-like substance, during the purification process (data not shown). Yamada et al. (1995) reported that lepidimoide is widespread in exudates from many plant

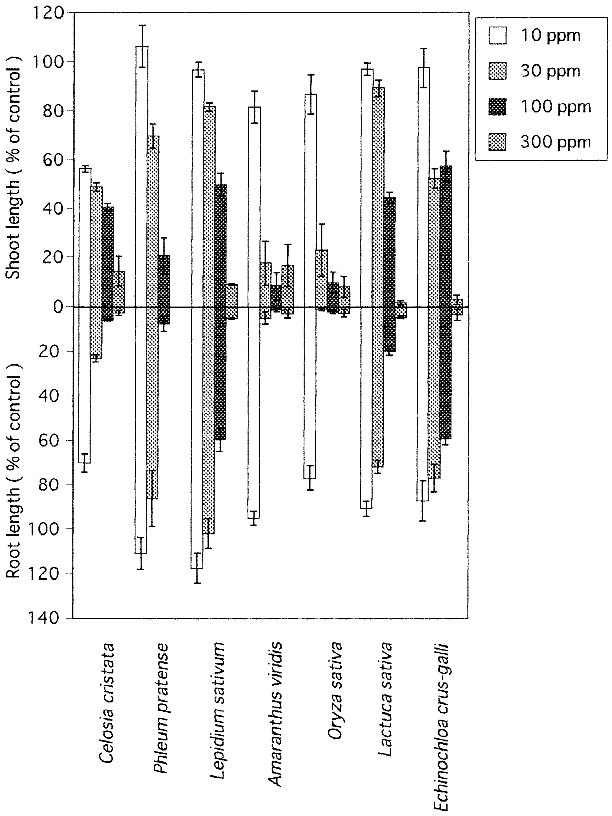


Fig. 2. Effects of the isolated substance on the shoot growth and root elongation of seven species of plants. Bars indicate average ± S.E.

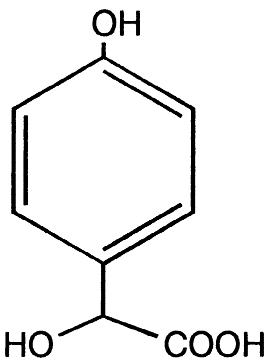
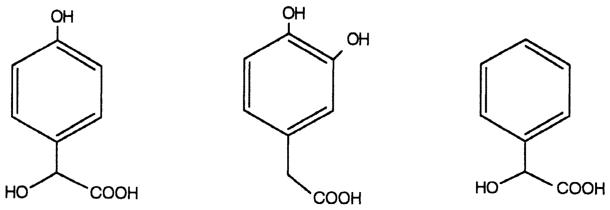


Fig. 3. Structure of the allelopathic substance, *p*-hydroxymandelic acid, exuded from young barnyard grass roots.

species and is exuded from seeds within 2 or 3 days after germination in all plants tested. Therefore, this implies that *p*-hydroxymandelic acid does not exhibit an inhibitory effect during the seed germination period because of the presence of a growth-promoting substance such as a lepidimoide.

These results suggest that the growth-inhibiting substance *p*-hydroxymandelic acid, together with other growth-inhibiting substance, and a growth-promoting



1 2 3

Fig. 4. Tested compounds for structure-activity relationship of phenolic compounds related to *p*-hydroxymandelic acid on the shoot growth of rice. *1, p*-hydroxymandelic acid, *2,* 3,4-dihydroxyphenylacetic acid, *3,* mandelic acid.

Table 1. Inhibitory activity of phenolic acid compounds for the growth of rice shoots and amaranth shoots.

Compounds	I_{40} rice bioassay (ppm)[a]
1	30–100
2	300
3	>300

[a] I_{40} represents the concentration (ppm) of sample which causes 40% inhibition of the growth of rice shoots.

substance, exuded from geminating barnyard grass roots, play some important roles in selective allelopathy.

References

Chang-Hung C, Hui-Jung L (1976) Autointoxication mechanism of *Oryza sativa.* I. Phytotoxic effects of decomposing rice residues in soil. J Chem Ecol 2:353–367

Fuerst EP, Putnam AR (1983) Separating the competitive and allelopathic components of interference: Theoretical principles. J Chem Ecol 9:937–944

Hasegawa K, Mizutani J, Kosemura S, Yamamura S (1992) Isolation and identification of lepidimoide, a new allelopathic substance from mucilage of germinated cress seeds. Plant Physiol 100: 1059–1061

Rice EL (1984) Allelopathy. 2nd ed. Academic Press, New York pp. 301–332

Tang C, Young C (1982) Collection and identification of allelopathic compounds from the undisturbed root system of bigalta limpograss (*Hamarthria altissima*). Plant Physiol 69:155–160

Kosemura S, Yamamura S, Kakuta H, Mizutani J, Hasegawa K (1993) Synthesis and absolute configuration of lepidimoide, a high potent allelopathic substance from mucillage of germinated cress seeds. Tetrahedron Lett 34:2653–2656

Kushima M, Kakuta H, Kosemura S, Yamamura S, Yamada K, Yokotani-Tomita K, Hasegawa K (1998) An allelopathic substance exuded from germinating watermelon seeds. Plant Growth Reg 25:1–4

Yamada K, Anai T, Hasegawa K (1995) Lepidimoide, an allelopathic substance in the exudates from germinated seeds. Phytochemistry 39:1031–1032

Yokotani-Tomita K, Goto N, Kosemura S, Yamamura S, Hasegawa K (1997) Growth-promoting allelopathic substance exuded from germinating *Arabidopsis thaliana* seeds. Phytochemistry 47: 1–2