

Identification of Phytotoxic Substances from Early Growth of Barnyard Grass (*Echinochloa crusgalli*) Root Exudates

Tran Dang Xuan · III Min Chung · Tran Dang Khanh · Shinkichi Tawata

Received: 7 September 2005 / Revised: 28 November 2005 /
Accepted: 26 December 2005 / Published online: 5 May 2006
© Springer Science + Business Media, Inc. 2006

Abstract Barnyard grass is a problematic weed worldwide. It competes with crops and causes reduction in crop yields. In this study, barnyard grass suppressed rice emergence, and the degree of rice inhibition was proportional to the density of barnyard grass. Root exudates of barnyard grass reduced germination and growth of lettuce, rice, and monochoria. Fifteen compounds potentially involved in the phytotoxic activities of barnyard grass were isolated and identified, including phenolics, long-chain fatty acids, lactones, diethyl phthalate, acenaphthene, and derivatives of phthalic acids, benzoic acid, and decane. Quantities of diethyl phthalate, decanoic acid, myristic acid, stearic acid, 7,8-dihydro-5,6-dehydrokavain, and 7,8-dihydrokavain were 2.7, 11.1, 19.6, 35.5, 10.3, and 15.5 µg/ml of barnyard grass root exudates, respectively. The two lactones exhibited the greatest inhibition, followed by the phenolics and the derivatives of phthalic acids. Fatty acids had stronger suppression than diethyl phthalate and ethyl ester-4-ethoxy-benzoic acid. The acenaphthene and decane derivatives were the least phytotoxic. The phytotoxins released by barnyard grass roots showed strong inhibition on growth of broadleaf indicator plants and paddy weeds, but were less effective on barnyard grass itself and rice. Our study revealed that in addition to competition, barnyard grass also interferes with rice and other plants in its surroundings by chemical means.

Keywords Allelopathy · Allelochemicals · Barnyard grass · Exudates · Inhibition · Rice · Weed

T. D. Khanh and I. M. Chung are members of the research team of Friendly Environmental Low Input Natural Herbicide New Material Study of the Konkuk University.

I. M. Chung (✉) · T. D. Khanh
Department of Crop Science, Konkuk University,
Seoul 143-701, Republic of Korea
e-mail: imcim@konkuk.ac.jp

T. D. Xuan · S. Tawata
Department of Bioscience and Biotechnology, Faculty of Agriculture,
University of the Ryukyus, Okinawa 903-0213, Japan

Introduction

Barnyard grass [*Echinochloa crusgalli* (L.) Beauv.], an annual grass, has been reported to cause problems in at least 61 countries and in at least 36 different crops (Holm et al., 1991). It is a major weed in paddy fields as it competes with rice (*Oryza sativa* L.) and causes reduction in rice yield. Competition from 25 barnyard grass plants/m² can cause 50% reduction in rice yield (Chin, 2001). Some weeds exude allelochemicals that suppress crop growth as well as other weed species in fields (Tang and Young, 1982; Qasem and Foy, 2001). However, differentiating between allelopathic effects and resource competition in the field is not easily achieved (Yamamoto et al., 1999). Therefore, the identification of allelochemicals exuding from roots in laboratory experiments, where the competitive factors can be eliminated, is useful to understand the phytotoxic activities of such weeds. Yamamoto et al. (1999) reported that during germination and early growth, barnyard grass inhibited the growth of cockscomb (*Celosia cristata* L. var. *kunze*), timothy (*Phleumpratense* L.), cress (*Lepidium sativum* L.), amaranth (*Amaranthus viridis* L.), rice, lettuce (*Lactuca sativa* L.), and barnyard grass. *p*-Hydroxymandelic acid, an allelochemical exuding from young barnyard grass roots, strongly inhibited the growth of rice at 59.5–178.6 μ M (Yamamoto et al., 1999). Most of the studies on rice–weed interactions focus on the weed-suppressing potential of rice, many of which concentrate on using rice allelopathy to control barnyard grass (Rimando et al., 2001; Chung et al., 2003). Major allelochemicals in rice capable of suppressing barnyard grass have been detected including phenolics, indoles, momilactones, and terpenoides (Khanh et al., 2005). Chung et al. (2003) and Jung et al. (2004) noted that barnyard grass was the least susceptible to rice allelopathy among the paddy weeds. Barnyard grass is in the same family as rice (Graminae), which may be one of the reasons for its superior ability to compete against rice. On the other hand, this noxious weed may also release growth inhibitors that inhibit emergence of rice and other paddy weeds. However, except for *p*-Hydroxymandelic acid, the composition of its root exudates, which may be responsible for such allelopathic activities, has not been investigated. Therefore, we conducted this research to isolate and identify allelochemicals in root exudates of barnyard grass and to examine their bioactivities against several important plant species.

Methods and Materials

Plants

Alfalfa (*Medicago sativa* L.), lettuce, sesame (*Sesamum indicum* L.), and rice (cv. Koshihikari) seeds from commercial sources (Wakaba company, Japan) were used. Seeds of barnyard grass, monochoria (*Monochoria vaginalis* Presl var. *plantaginea* Solms-Laub.), and Indian jointvetch (*Aeschynomene indica* L.) were collected from Miyazaki fields, Japan, in 2003. Empty and undeveloped seeds were discarded by floating in tap water. The remaining seeds were air-dried and stored hermetically at -20°C . These were sterilized with 1% sodium hypochlorite for 30 min and rinsed many times with distilled water before use. The germination percentage was randomly checked, and all were greater than 90%.

Effects of Barnyardgrass on Rice Growth

Ten healthy rice seeds were sown evenly in a Petri dish (9 cm diam) lined with filter paper and moistened with 10 ml distilled water. To the Petri dish, 10, 50, and 100 seeds of barnyard grass were added evenly between the rice seeds. Five replicates of all treatments were placed in an incubator (25°C, 4000 lx, with an 8-hr day/16-hr night cycle, humidity: 75%) using a completely random design. After 7 d, the number of germinated rice seeds was counted, and the length of shoots and roots was measured.

Barnyardgrass Exudates

Ten grams of healthy barnyard grass seeds was put in a glass pot with 100 ml of distilled water for 7 d in a growth chamber (25°C, 4000 lx, with an 8-hr day/16-hr night cycle, humidity: 75%). The distilled water was changed every 2 d. The resulting root exudates were filtered and set as the original dose, and a one-half strength solution was also prepared. Effects on emergence of rice, lettuce, and monochoria were examined by using methods similar to those described above against 20 seeds of each indicator plant. In another trial, 100 ml of the barnyard grass exudates was extracted with 70% MeOH in a shaking bath at 40°C for 24 hr. The solution was filtered, evaporated to dryness, dissolved in MeOH, and analyzed by thin-layer chromatography (TLC).

TLC Experiment

Many TLC solvent systems were tested. The combination of chloroform/ethyl acetate/acetic acid (10:3:1) gave the best separation and was selected. TLC plates were coated with 500- μ m layer of silica gel (Merck). The prepared barnyard grass root exudates were applied to the TLC plate (16 \times 20 cm). R_f values of the colored spots detected under the UV light were recorded, and the plate area was scraped and eluted with methanol. After evaporation to dryness, the residue was dissolved in distilled water, adjusted to 50 ppm, and tested for its effects on germination and growth of barnyard grass. Spots that inhibited lettuce emergence were collected, dissolved in acetone, and used for gas chromatography–mass spectrometry (GC-MS) analysis.

GC-MS Analysis

An aliquot of 2 μ l was injected (splitless) into the GC-MS (QP-2010, Shimadzu Co., Japan). The data were obtained on an ID-BPX5 column (30 m \times 0.25 mm ID, 0.25- μ m film thickness, SGE, Australia). The carrier gas was helium, and the GC oven temperature program was as follows: 50°C hold for 5 min, rate of 5°C/min to 280°C, and hold for 5 min. The injector and detector temperatures were set at 220 and 280°C, respectively. The mass range was scanned from 15 to 900 amu. The control of the GC-MS system and the data peak processing were carried out by means of the Shimadzu's GC-MS solution software, version 2.1.

Quantification of Allelochemicals in Exudates of Barnyardgrass

Phytotoxic compounds identified in the exudates were quantified. Pure reference standards were purchased from Wako (Japan). Two lactones were purified in our

laboratory. All compounds were dissolved in acetone and by using similar GC conditions to those described above to quantify the constituents of the barnyard grass root exudates by comparing the retention time and areas between the standard chemicals and the samples. However, some of the identified constituents could not be quantified as they could neither be purchased nor purified in our laboratory.

Effects of Isolated Compounds on Plant Growth

Dilution of pure reference chemicals detected in exudates of barnyard grass at 100 ppm was prepared and examined for their influence on emergence of alfalfa, monochoria, lettuce, Indian jointvetch, sesame, and rice (var. Koshihikari) using the reported bioassay method.

Statistical Analysis

All treatments were arranged in a completely randomized design with at least three replications. Bioassays were replicated five times. Data were analyzed with SAS version 6.12 (SAS Institute, 1997) using analysis of variance and least significant difference at the 0.05 probability level.

Results

Effects of Barnyardgrass on Rice Growth

Barnyard grass was sown at different densities to examine its effects on rice growth. Germination was not influenced by the presence of barnyard grass, but elongation of rice roots and shoots was suppressed when density of barnyard grass was increased (Table 1). At the rate of 10 seeds per Petri dish, no impact on root length was observed, but shoot length was significantly reduced, relative to the control. When the density of barnyard grass increased to 50 and 100 seeds per Petri dish, inhibition of rice roots was increased by 30–40%. However, shoot length was not significantly affected by the increase in barnyard grass density. This experiment found that barnyard grass had a chemical effect on rice emergence. Barnyard grass density was positively correlated with rice root and shoot length ($r = 0.94$ and 0.81 , respectively; Table 1). The inhibitory effect on rice growth was proportional to the density of barnyard grass.

Table 1 Inhibitory effects of barnyard grass on rice growth

Correlation coefficients of barnyard grass density against rice root and shoot length: $r = 0.94$ and 0.81 , respectively. Means with the same letter in a column are not significantly different at $P = 0.05$.

Barnyard grass density (seeds)	Inhibition (% of control)		
	Germination	Root length	Shoot length
10	0.0a	3.0a	26.2b
50	0.0a	30.0b	31.6b
100	0.0a	39.4c	31.3b

Table 2 Effects of barnyard grass exudates on emergence of lettuce, rice, and monochoria

Concentration	Inhibition (% of control)		
	Germination	Root length	Shoot length
Lettuce			
1/2	15.8a	44.1a	13.7a
1.0	30.6b	94.5b	88.2b
Rice			
1/2	0.0a	33.2a	26.2ab
1.0	5.0a	48.1b	27.5b
Monochoria			
1/2	7.4a	28.6a	28.7a
1.0	25.9b	65.0b	28.6a

Means with the same letter in a column are not significantly different at $P = 0.05$.

Effects of Barnyardgrass Exudates on Lettuce, Rice, and Monochoria

Barnyard grass root exudates were selected to determine potential phytotoxicity toward plants that are commonly found nearby such as rice and monochoria. The root exudates exhibited strong inhibitory effects on emergence of lettuce, rice, and monochoria (Table 2). Germination of lettuce and monochoria was reduced at the two applied doses in comparison with the controls, whereas no effect on rice germination was found. The roots of these indicator plants were more affected than their shoots. At one-half dose, the roots of monochoria and rice were suppressed by a similar level (about 30%), whereas lettuce roots were reduced by about 45% (Table 1). However, shoot length of lettuce was less affected than rice and monochoria at this concentration. At the original full dose, lettuce emergence was suppressed by about 90%. On the other hand, emergence of rice and monochoria was not significantly reduced as compared to the one-half dose, with the exception of monochoria root, which was inhibited by 65%. This experiment reveals that barnyard grass releases toxic compounds during germination that strongly reduce the growth of lettuce, rice, and monochoria.

TLC Experiment

Several spots were observed on TLC plates under UV light and examined for their activities on lettuce germination and growth at 50 ppm. Of these, only five spots suppressed lettuce emergence, with Rf values of 0.24, 0.29, 0.35, 0.42, and 0.72

Table 3 Effects of spots isolated from TLC on emergence of lettuce at 50 ppm

Spots	Rf values	Inhibition (% of control)		
		Germination	Root length	Shoot length
1st	0.24	44.4c	57.5c	33.5b
2nd	0.29	11.1ab	28.6ab	20.0b
3rd	0.35	5.6a	14.6a	2.6a
4th	0.42	22.2b	37.9b	18.0b
5th	0.72	60.6d	77.7d	54.2c

Means with the same letter in a column are not significantly different at $P = 0.05$.

(Table 3). These were selected for analysis. At 50 ppm, the compounds contained in each spot significantly reduced germination and growth of lettuce, as compared to the control, with the exception of the third spot that had no significant effect on lettuce germination. The constituents of the fifth spot exerted the greatest influence on lettuce emergence followed by the first spot (Table 3), whereas the third spot was the least inhibitory.

GC-MS Analysis

Fifteen compounds were detected in the five spots collected by TLC as shown in Table 4. Their chemical structures are described in Fig. 1. The first spot consisted of two phenolics [2-ethyl-phenol and 2,4-bis (1,1-dimethyl)-phenol] and three derivatives of phthalic acids (dimethyl ester-phthalic acid, butyl 8-methylnonyl ester-phthalic acid, and diisooctyl ester-phthalic acid). The second spot contained diethyl phthalate and a derivative of benzoic acid (ethyl ester-4-ethoxy-benzoic acid). Derivatives of decane (2,3,7-trimethyl-decane and 2-methyl-dodecane) and acenaphthene were detected in the third spot. Three long-chain fatty acids were observed in the fourth spot, including decanoic acid, myristic acid, and stearic acid. In the fifth spot, two lactones were identified [7,8-dihydro-5,6-dehydrokavain (DDK) and 7,8-dihydrokavain (DHK)]. All of these 15 constituents have been identified in barnyard grass root exudates for the first time.

Table 4 Substances identified from spots that showed inhibition on emergence of lettuce and their quantities in barnyard grass exudate

Compounds	Retention time (min)	Molecular weight	Amount in exudates of barnyard grass ($\mu\text{g/ml}$)
Spot 1			
2-Ethyl-phenol	19.4	122	–
Dimethyl ester-phthalic acid	27.8	194	–
2,4-bis (1,1-Dimethyl)-phenol	29.4	206	–
Butyl 8-methylnonyl ester-phthalic acid	39.9	223	–
Diisooctyl ester-phthalic acid	50.1	279	–
Spot 2			
Ethyl ester-4-ethoxy-benzoic acid	29.9	194	–
Diethyl phthalate	31.5	222	2.7 ± 0.01
Spot 3			
2,3,7-Trimethyl-decane	22.5	141	–
2-Methyl-dodecane	24.1	169	–
Acenaphthene	26.1	153	–
Spot 4			
Decanoic acid	30.7	200	11.1 ± 0.03
Myristic acid	35.5	228	19.6 ± 0.02
Stearic acid	43.9	284	35.5 ± 0.06
Spot 5			
7,8-Dihydro-5,6-dehydrokavain	33.4	230	10.3 ± 0.05
7,8-Dihydrokavain	43.2	232	15.5 ± 0.03

–: Measurement was not conducted.

\pm : Standard errors ($N = 5$).

Only 6 of the 15 detected compounds were quantified, as the remaining chemicals were uncommon and could neither be purchased nor successfully purified. Diethyl phthalate accounted for 2.7 $\mu\text{g}/\text{ml}$ of the root exudates (Table 4). The quantities of decanoic acid, myristic acid, and stearic acid were 11.1, 19.6, and 35.5 $\mu\text{g}/\text{ml}$, respectively. The amounts of lactones were 10.3 and 15.5 $\mu\text{g}/\text{ml}$ for DDK and DHK, respectively.

PHYTOTOXINS FROM ROOT EXUDATES OF BARNYARDGRASS

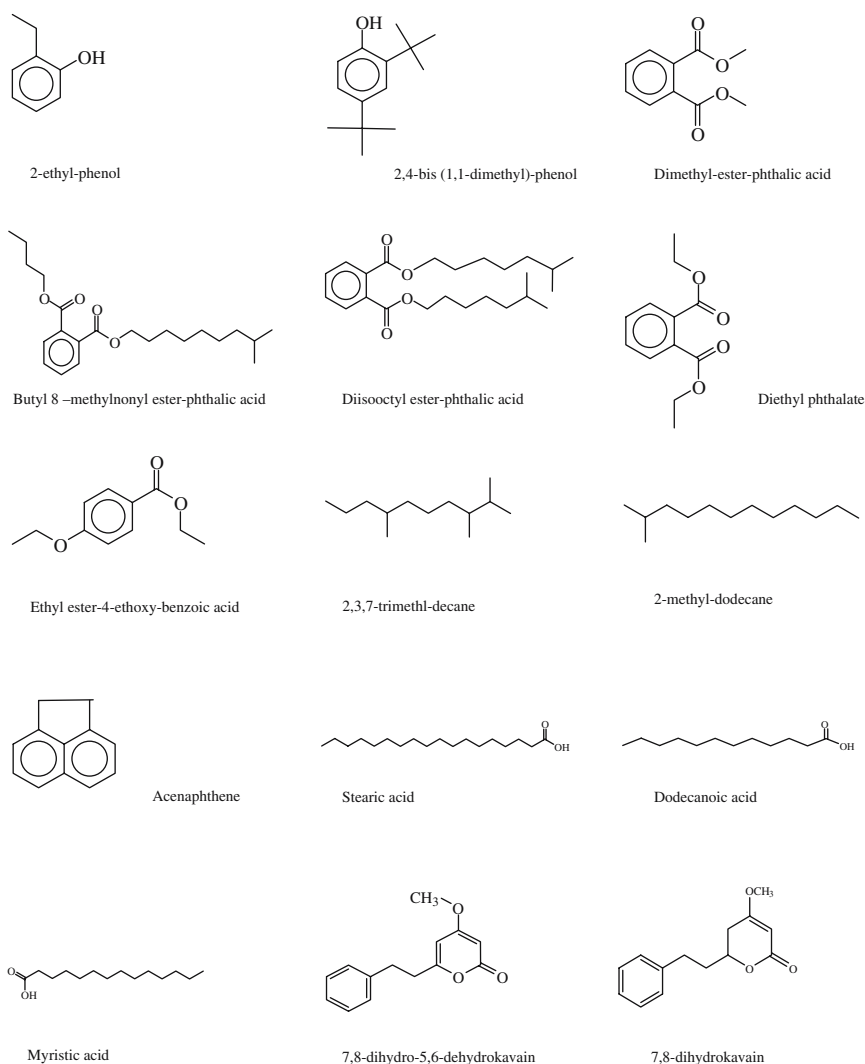


Fig. 1 Chemical structure of chemicals involved in inhibitory activities of barnyard grass root exudates

Effects of Chemicals in Root Exudates of Barnyardgrass on Plant Emergence

The effects of the two lactones on lettuce were the most detrimental, followed by the two phenolics and derivatives of phthalic acids (Tables 3 and 4). Fatty acids gave greater suppression than the derivative of benzoic acid (ethyl ester-4-ethoxy-benzoic acid; Tables 3 and 4). Acenaphthene and derivatives of decane exhibited the least effects among identified substances (Tables 3 and 4).

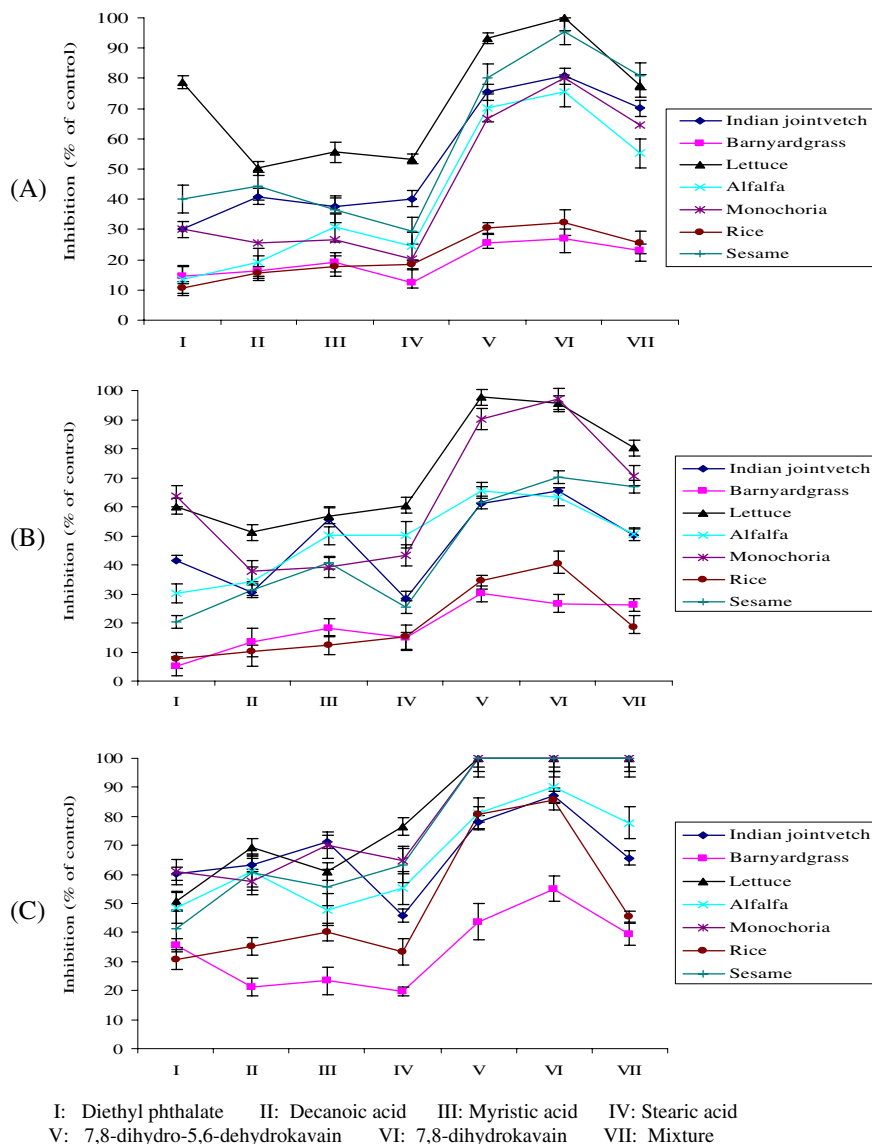


Fig. 2 Effects of six individual compounds isolated from barnyard grass root exudates on emergence of seven plants [(A) germination, (B) shoot length, and (C) root length] at 100 ppm. Vertical bars represent standard errors

The six quantified compounds were tested individually for their influence on germination and growth of alfalfa, barnyard grass, Indian jointvetch, lettuce, monochoria, rice, and sesame at 100 ppm [405.5, 500.0, 438.5, 352.1, 434.8, and 431.0 μM for diethyl phthalate, decanoic acid, myristic acid, stearic acid, DDK, and DHK, respectively] (Fig. 2). In general, diethyl phthalate showed greater inhibition on growth of indicator plants than the fatty acids. The two lactones, however, inhibited indicator plant growth far more than the other compounds. The growth of all tested plants, except for barnyard grass and rice, was strongly inhibited by the quantified compounds.

The phytotoxins released by barnyard grass root exudates were least effective against rice and barnyard grass itself as compared to other broadleaf species (Fig. 2). Germination and shoot growth of barnyard grass and rice were not much affected; however, the growth of rice root was significantly suppressed by DDK and DHK and the mixture (60–70% of inhibition). The inhibitory effect of the two lactones on root elongation of barnyard grass was markedly lower than the effect on rice.

Discussion

Certain weeds are known to release allelochemicals into the environment and suppress both crops and other weeds in their vicinity (Bais et al., 2003). Therefore, the invasiveness of certain weeds may also be dependent on their allelopathic activities including the influence of exuded phytotoxins against neighboring plants. Numerous allelochemicals from weeds have been identified and reported to exhibit strong phytotoxic activity. However, most of them are from upland weeds (Qasem and Foy, 2001). Allelochemicals detected in upland weeds belong to several chemical classes, including phenolics, sesquiterpenes, flavonoids, terpenes, alkaloids, and their derivatives (Qasem and Foy, 2001). Little information on allelochemicals from weeds in rice paddies has been documented, although weeds cause serious interferences in agricultural production. Much of current research is concentrating on the biological control of rice paddy weeds in attempts to minimize the use of synthetic herbicides.

Major research efforts have focused on evaluating the ability of certain crop species to suppress barnyard grass infestation and on enhancing the allelopathic potential of these crop species, especially in rice (Chung et al., 2003). The incorporation of phytotoxic plant material into soil to suppress paddy weeds such as barnyard grass has also been investigated (Xuan et al., 2004), as well as the search for allelochemicals to control barnyard grass (Jung et al., 2004). In contrast, the allelopathic potential of this harmful weed species has not received much interest. Xuan et al. (2001, 2002, 2003, 2004) selected plants with strong weed-suppressing ability and applied them to soil 2 d after transplanting at 1–2 tons/ha. Weed biomass was controlled up to 80%, and rice yield increased by 20%. However, barnyard grass appeared to be the most difficult of the paddy weeds to control. The cause of this phenomenon is not known, although it is possible that chemical interference may have a role.

Yamamoto et al. (1999) reported that barnyard grass exhibited allelopathic potential. Root exudates of young barnyard grass suppressed root elongation of the seven plant species tested (cockscorn, timothy, cress, amaranth, rice, lettuce, and barnyard grass). A phenolic acid (*p*-Hydroxymandelic acid) was isolated and

identified in the root exudates that inhibited rice growth at concentrations of 59.5–178.6 μM . This compound has been shown to be involved in the phytotoxic activity of barnyard grass against rice (Yamamoto et al., 1999). However, to date, no further information about allelochemicals released by barnyard grass has been reported.

In this study, barnyard grass suppressed rice emergence, and the degree of rice inhibition was proportional to the density of barnyard grass. Root exudates of recently germinated barnyard grass exerted strong inhibition on germination and growth of lettuce, rice, and monochoria. Monochoria is also a noxious weed and is distributed widely in paddy fields and wet areas in the subtropics and tropics (Holm et al., 1991). Therefore, investigation of the chemical interactions among barnyard grass, rice, and monochoria, in addition to other weeds, is needed. As crop–weed interactions are better understood, the biological elimination of noxious paddy weeds may be more feasible. Lettuce is a common indicator plant used in bioassays related to allelopathy because of its high sensitivity to chemicals at low concentration (Xuan et al., 2004). Diethyl phthalate and phthalic acid derivatives are commercially important chemicals used predominantly as plasticizers in high-molecular-weight polymers, which are toxic to humans, animals, microorganisms, algae, aquatic invertebrates, and fish (Staples et al., 1997; Jonsson and Baun, 2003; Sung et al., 2003; Chen and Sung, 2005; Hu et al., 2005), as well as plants (Herring and Bering, 1988; Saarma et al., 2003). We, at first, thought the presence of diethyl phthalate and derivatives of phthalic acid might be caused by contaminants from either the sample preparation or analytical instruments. Therefore, the existence of these compounds in the root exudates of barnyard grass was examined carefully. Control experiments and samples of root exudates were repeated several times and were analyzed on two different GC-MS. Results from these experiments unequivocally confirmed that these compounds are not derived from contamination because of plasticizers used during extraction and sample preparation or due of solvent impurities. Recent reports show that diethyl phthalate is either bacterium or plant derived. Keire et al. (2001) reported that *Helicobacter pylori* secreted diethyl phthalate as a chemotactic factor. It was the first example of a phthalate ester that is produced by a bacterium. Elzaawely et al. (2006) reported that *Rumex janonicus* Hoult., a perennial herb widely distributed in the subtropics, produces diethyl phthalate. We suggest that diethyl phthalate and derivatives of phthalic acid may be derived from plants and act as a new class of plant phytotoxins. However, this needs confirming in other plant species, and the mechanism of producing these compounds by plants should be examined before concluding that these substances are natural products.

In this research, the most common phenolics found in many reported allelopathic plants were not detected in root exudates of barnyard grass. Three phenolics [2-ethyl-phenol, 2,4-bis (1,1-dimethyl)-phenol, and ethyl ester-4-ethoxy-benzoic acid] and derivatives of phthalic acids did show strong inhibition when isolated from TLC as a mixture. The amounts of these phenolics have not been quantified, but in gas chromatograms, they appeared to have lower peak areas than other identified substances (data not shown). Effects of these phenolics on plant growth need to be determined individually and compared to those of other common phenolic acids.

The two lactones were the most phytotoxic against the growth of seven plants at 431 and 434.8 μM for DDK and DHK, respectively. It is likely that lactones released by barnyard grass roots may play a major role in the allelopathic activities of the

weed. A mixture of these compounds was more inhibitory than diethyl phthalate and fatty acids, but it was lower than the effects of the two lactones individually. In this study, however, all detected phytotoxins in barnyard grass root exudates were present in low concentrations (Table 4), suggesting perhaps that in field conditions, they might not accumulate to the phytotoxic levels needed to affect rice and other weeds (Fig. 2). It is possible that phytotoxins other than those identified here may exist, and they may have allelopathic activity that accounts for the invasiveness of this harmful weed. They might be released by barnyard grass roots at stages other than the early growth stage.

This paper reveals that barnyard grass has allelopathic potential. It releases numerous phytotoxins into the environment during germination and early growth. These compounds could suppress the growth of rice and other plants, depending on the density of the grass in paddy fields. It appears that the phytotoxins that inhibit the growth of rice and other weeds have less influence on barnyard grass growth (Fig. 2). It is possible that phytotoxins released by monocots inhibit the growth of dicots more than the growth of monocots themselves and vice versa. The exudation of these phytotoxins into the environment may contribute to the success of barnyard grass as an invasive species. Both the release of these toxic compounds by barnyard grass at different growing stages and their interactions under natural conditions against crops and other weed species should be further studied.

Acknowledgements The authors thank Japan Society for the Promotion of Science (JSPS) for providing Dr. Tran Dang Xuan a Postdoctoral Fellowship (P04461). They also thank Dr. Alexa Seal for her useful comments on the manuscript.

References

- BAIS, H. P., VEPACHEDU, R., GILROY, S., CALLAWAY, R. M., and VIVANCO. 2003. Allelopathy and exotic plants: from genes to invasion. *Science* 301:1377–1380.
- CHEN, W. L., and SUNG, H. H. 2005. The toxic effects of phthalate esters on immune responses of giant freshwater prawn (*Macrobrachium rosenbergii*) via oral treatment. *Aquat. Toxicol.* 74:160–171.
- CHIN, D. V. 2001. Biological management of barnyardgrass, red sprangletop and weedy rice. *Weed Biol. Manag.* 1:37–41.
- CHUNG, I. M., KIM, K. H., AHN, J. K., LEE, S. B., KIM, S. H., and HAHN, S. J. 2003. Comparison of allelopathic potential of rice leaves, straw and hull extracts on barnyardgrass. *Agron. J.* 95: 1063–1070.
- ELZAAWELY, A. A., XUAN, T. D., and TAWATA, S. 2006. Changes in essential oil, kava pyrones and total phenolics of *Alpinia zerumbet* (Pers.) B. L. Burtt. & R. M. Sm. Leaves exposed to copper sulphate. *Environ. Exp. Bot.* (in press).
- HERRING, H. and BRING, C. L. 1988. Effects of phthalate esters on plant seedlings and reversal by a soil microorganism. *Bull. Environ. Contam. Toxicol.* 40:626–632.
- HOLM, G. L., PLUCKNETT, D. L., PANCHO, J. V., and HERBER, J. P. 1991. The world's worst weeds—Distribution and ecology. Krieger Publishing Company, Malabar, FL, USA. pp. 32, 341, p 609.
- HU, X. Y., WEN, B., ZHANG, S. Z., and SHAN, X. Q. 2005. Bioavailability of phthalate congeners to earthworms (*Eisenia fetida*) in artificially contaminated soils. *Ecotoxicol. Environ. Saf.* 62: 26–31.
- JONSSON, S., and BAUN, A. 2003. Toxicity of mono- and diesters of *o*-phthalic esters to a crustacean, a green alga, and a bacterium. *Environ. Toxicol. Chem.* 22:3037–3043.
- JUNG, W. S., KIM, K. H., AHN, J. K., HAHN, S. J., and CHUNG, I. M. 2004. Allelopathic potential of rice (*Oryza sativa* L.) residues against *Echinochloa crus-galli*. *Crop Prot.* 23:211–218.

- KEIRE, D. A., ANTON, P., FAULL, K. F., RUTH, E., WALSH, J., CHEW, P., QUISIMORO, D., TERRITO, M., and REEVE, Jr. J. R. 2001. Diethyl phthalate, a chemotactic factor secreted by *Helicobacter pylori*. *J. Biol. Chem.* 276:48847–48853.
- KHANH, T. D., CHUNG, I. M., XUAN, T. D., and TAWATA, S. 2005. The exploitation of crop allelopathy in sustainable agricultural production. *J. Agron. Crop Sci.* 191:172–184.
- QASEM, J. R., and FOY, C. L. 2001. Weed allelopathy, its ecological impacts and future prospects: a review. *J. Crop Prod.* 4:43–119.
- RIMANDO, A. M., OLOFSDOTTER, M., DAYAN, F. E., and DUKE, S. O. 2001. Searching for rice allelochemicals: an example of bioassay-guided isolation. *Agron. J.* 93:6–20.
- SAARMA, K., TARKKA, M. T., ITAVAARA, M., and FAGERSTEDT, K. 2003. Heat shock protein synthesis is induced by diethyl phthalate but not by di(2-ethylhexyl) phthalate in radish (*Raphanus sativus*). *J. Plant Physiol.* 160:1001–1010.
- SAS Institute, 1997: SAS/STAT User's Guide, ver. 6.12. SAS Institute, Cary, NC.
- STAPLES, C. A., ASAMS, W. J., PARKERTON, T. F., GORSUCH, J. W., BIDDINGER, G. R., and REINERT, K. H. 1997. Aquatic toxicity of eighteen phthalate esters. *Environ. Toxicol. Chem.* 16:875–891.
- SUNG, H. H., KAO, W. Y., and SU, Y. J. 2003. Effects and toxicity of phthalate esters to hemocytes of giant freshwater prawn (*Macrobrachium rosenbergii*). *Aquat. Toxicol.* 64:25–37.
- TANG, C. S., and YOUNG, C. C. 1982. Collection and identification of allelopathic compounds from the undisturbed root system of bigalita limpograss (*Hemarthria altissima*). *Plant Physiol.* 69: 155–160.
- XUAN, T. D., TSUZUKI, E., UEMATSU, H., and Terao, H. 2001. Weed control with alfalfa pellets in transplanting rice. *Weed Biol. Manag.* 1:231–235.
- XUAN, T. D., TSUZUKI, E., UEMATSU, H., and TERA0, H. 2002. Effects of alfalfa (*Medicago sativa* L.) pellets on weed control in rice. *Allelopathy J.* 9:195–203.
- XUAN, T. D., TSUZUKI, E., TERA0, H., MATSUO, M., KHANH, T. D., MURAYAMA, S., and HONG, N. H. 2003. Alfalfa, rice by-products, and their incorporations for weed control in rice. *Weed Biol. Manag.* 3:137–144.
- XUAN, T. D., TSUZUKI, E., TAWATA, S., and KHANH, T. D. 2004. Methods to determine allelopathic potential of crop plants for weed control. *Allelopathy J.* 13:149–169.
- YAMAMOTO, T., YOKOTANI-TOMITA, K., KOSEMURA, S., YAMAMURA, S., YAMADA, K., and HASEGAWA, K. 1999. Allelopathic substance exuded from a serious weed, germinating barnyardgrass (*Echinochloa crus-galli* L.) roots. *J. Plant Growth Regul.* 18:65–67.