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Chemical Interaction in the Invasiveness of Cogongrass (*Imperata cylindrica* (L.) Beauv.)

Tran Dang Xuan,* Tsuneaki Toyama, Masakazu Fukuta, Tran Dang Khanh, and Shinkichi Tawata*

Faculty of Agriculture, Department of Subtropical Bioscience and Biotechnology, University of the Ryukyus, Okinawa 903-0213, Japan

From gas chromatography−mass spectrometry (GC−MS), numerous plant growth inhibitors were found in the rhizome and root exudates of cogongrass, one of the most problematic weeds in the world. *iso*-Eugenol, *iso*-ferulic acid, linoleic acid, ferulic acid, and vanillin were the major chemicals in the rhizome (88.1−392.2 μg/g of fresh root), while 4-acetyl-2-methoxyphenol was the principle substance (872.6 μg/plant) in the root exudates. In fields, the use of cutting and plowing reduced weed biomass and weed density of cogongrass >70%. However, the alternative invasion of beggar tick might be a problem, because its density and biomass increased 33.3 and 62.5%, respectively. Chemicals from cogongrass showed selective effects against tested invasive species. Of them, 2,4-di-tert-butylphenol was the most potent (78.3−100% of inhibition), followed by *iso*-eugenol and 4-acetyl-2-methoxyphenol. These compounds may play important roles in the invasiveness of cogongrass and might be promising parent constituents of synthesis to develop novel herbicides for control of invasive plants.

KEYWORDS: Cogongrass; inhibition; invasive species; root exudates; rhizome; *Bidens pilosa*; *Leucaena leucocaphala*; *Echinochloa crus-galli*

INTRODUCTION

Cogongrass (*Imperata cylindrica* (L.) Beauv.) has been reported as one of the 10 most troublesome weed species. It is a major impediment to reforestation efforts in southeast Asia and is responsible for thousands of hectares of lost native habitat in the southeastern U.S. (*1*). The weed has been reported to cause harm to the production of 35 crops in 73 countries (*2*−*4*). In Japan, the fast invasiveness of this weed is hindering agricultural practices and increasing the cost of weed control because a strictly limited use of herbicides is permitted, despite glyphosate, sethoxydim, fluazifop, DPA, and tetrapion being effective against the noxious weed (*5*). Yandoc et al. (*6*) studied the use of two parasitic fungi of *I. cylindrica*, including *Bipolaris sacchari* (E. J. Butler) Shoemaker and *Drechslera gigantea* (Heald and Wolf) Ito, to suppress the growth of cogongrass; however, practical application of the research has not been approached.

A few naturally occurring compounds with medicinal properties have been identified in cogongrass, such as imperanene that inhibits platelet aggregation (*7*), graminones A and B with vasodilative activity (*8*), and cylindols A and B, which has 5-lipoxygenase inhibitory activity (*9*), and chromones with glutamate-induced neurotoxicity (*10*). Although cogongrass is a serious weed species worldwide, the chemical component of the weed, especially that of its rhizome, which is demonstrated to be responsible for the strong invasiveness of the weed, has not been well-documented. Inderjit and Dakshini (*11*) reported that several phenolic fractions in the extracts of the foliage, rhizome, and root exudates of cogongrass inhibited the growth of mustard [*Brassica juncea* (L.) Czern and Coss.] and tomato (*Lycopersicon esculentum* Mill.). Koger et al. (*12*) noted that extracts of foliage and root residues of cogongrass inhibited the growth of the selected grass and broadleaf species. However, identification and quantification of compounds responsible for the inhibitory activity were not investigated.

Other than cogongrass, beggar tick (*Bidens pilosa* L.), Leucaena (*Leucaena leucocaphala* L. de Wit), and barnyardgrass (*Echinochloa crus-galli*) are also causing problems to agricultural production and are considered as noxious invasive species (*4*, *13*−*16*). They are among the most common plants found in the vicinity of cogongrass. *B. pilosa* is an annual native to tropical America, belonging to the Asteraceae family, and widely distributed in tropical and subtropical regions in the world (*4*). *L. leucocaphala* is a prevalent invasive species in the tropics. This legume plant produces mimosine, a nonprotein amino acid, which is toxic to other plant species and animals (*14*). *E. crus-galli* is a native of Europe and India. It is now a common weed of most of the agricultural areas of the world (*4*, *15*, *16*).

This study aims to (i) identify and quantify plant growth inhibitors in the rhizome and root exudates of cogongrass, (ii) clarify the chemical interaction of *I. cylindrica* against *B. pilosa*, *L. leucaphala*, and *E. crus-galli*, and their spontaneous competition in fields, and (iii) search for chemicals derived from cogongrass that are potent to control invasive plants.

*To whom correspondence should be addressed: Faculty of Agriculture, Department of Subtropical Bioscience and Biotechnology, University of the Ryukyus, Okinawa 903-0213, Japan. Telephone/Fax: 81-98-895-8744. E-mail: g056003@agr.u-ryukyu.ac.jp, b986097@agr.u-ryukyu.ac.jp.

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MATERIALS AND METHODS

Solvents and Reference Chemicals. Acetone, methanol, ethyl acetate, margaric acid, hydrocinnamic acid, 4-acetyl-2-methoxyphenol, 2,4-di-tert-butylphenol, pantolactone, *trans-p*-coumaric acid, stearic acid, benzoic acid, syringic aldehyde, linoleic acid, coumaran, *iso*-eugenol, *p*-vinylguaicol, ferulic acid, *iso*-ferulic acid, *iso*-vanillic acid, palmitic acid, vanillin, cinnamic acid, myristic acid, pentadecanoic acid, and syringic acid were of analytical grade and purchased from Wako Pure Chemical Industries, Japan.

Cogongrass Rhizomes. Fresh rhizomes of cogongrass growing around the Farm Center of the University of the Ryukyus in June 2006 were collected, sealed in plastic bags, and immediately transferred to the laboratory. The rhizomes were cleaned with tap water and then distilled water, dried at 60 °C for 48 h, well ground by a Wiley mixer, and kept in the dark at 5 °C for extraction.

Root Exudates. Similar plants of cogongrass including the rhizome (20 cm of length) were planted in plastic boxes (diameter, 15 cm; height, 12 cm; average weight of the rhizome per plant, 3 g) filled with 500 mL of 0.5% potato dextrose agar (PDA). The pots were transferred to a growth chamber (25 °C, 4000 lux, with an 8/16 h day/night cycle, humidity at 75%). After 7 days, the plants were removed and the agar was centrifuged at 10000 rpm for 20 min. The supernatant was separated and kept in the dark at 5 °C for further experiments.

Extraction Protocol. An amount of 10 g of rhizome was extracted with 100 mL of 70% MeOH, shaken in a water bath (25 °C) for 1 day, and filtered. The supernatant was evaporated until dryness to examine the influence of the extract on the emergence of barnyardgrass in a bioassay. Subsequently, MeOH was evaporated and hydrolyzed with 4 N NaOH (1:1, v/v) for 4 h at 50 °C. After pH was adjusted to 1.5, the aqueous phase was extracted 3 times with each of 100 mL of ethyl acetate. The ethyl acetate fraction was evaporated to dryness and dissolved in acetone for gas chromatography−mass spectrometry (GC−MS) analysis. Furthermore, 1 L of the root exudates was extracted with 5 L of 70% MeOH and applied to a similar extraction protocol of the rhizome as mentioned above to obtain samples for bioassays and GC−MS analysis.

Tested Plants. Four species, including barnyardgrass (*E. crus-galli*), beggar tick (*B. pilosa*), Leucaena (*L. leucocaphala*), and cogongrass (*I. cylindrica*) were used. Seeds of *E. crus-galli* were collected in the field of Miyazaki Prefecture, Japan, in 2006. The seeds of the other species were harvested in the fields of the Agricultural Farm, University of the Ryukyus, Japan, in 2007. For *E. crus-galli*, *B. pilosa*, and *L. leucocaphala*, empty and undeveloped seeds were discarded after floating in tap water. The remaining seeds were then air-dried and hermetically stored at −25 °C. These seeds were sterilized with 1% sodium hypochlorite for 30 min and well rinsed several times with distilled water before used. The harvested spikelets of *I. cylindrica* was kept at −25 °C for 1 month, then spread on the surface of a 0.5% potato dextrose agar (PDA) box, and kept in the dark at 25 °C, humidity at 70%, at 2−3 days for germination stimulation. The germination percentages of these invasive species were randomly checked and showed over 90%.

Bioassays. The extracts of the rhizome and root exudates were dissolved in distilled water and diluted to different doses at 10−1000 ppm to examine the effects against the growth of barnyardgrass. An aliquot of 10 mL of each solvent was put in a Petri dish (9 cm in diameter), lined with filter paper, and sowed with 10 seeds of barnyardgrass. The plates were transferred to an incubator (25 °C) placed in the dark. Treatments with distilled water only were used as the controls. After 5 days, shoot and root lengths of barnyardgrass were measured. The influence of the extracts at different concentrations against the growth of barnyardgrass was calculated and expressed to the inhibitory concentration (IC_{50}, μg/mL), which indicates the doses that inhibit 50% growth of the indicator plant. Therefore, a smaller value of IC_{50} shows a stronger inhibitory activity.

GC−MS Analysis. An 1 μL aliquot of each sample was injected into GC−MS (QP-2010, Shimadzu Co., Kyoto, Japan). The data were obtained on a DB-5MS column, 30 m length, 0.25 mm inner diameter, and 0.25 μm thickness (Agilent Technologies, J&W Scientific Products, Folsom, CA). The carrier gas was helium, and the GC oven temperature program was 100 °C hold for 3 min, rate of 7 °C/min to 280 °C, and hold for 5 min. The injector and detector temperatures were set at 250 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 Shimadzu's GC−MS solution software, version 2.4.

Chemical Identification and Quantification. Chemicals in the rhizome and root exudates were identified and quantified by comparing the retention times, peak areas, and mass spectra between the standard chemicals and the samples with the Shimadzu GC−MS solution software as mentioned above and from the literature. Four dilutions of 0.05, 0.1, 0.5, and 1.0 mg/mL were used to obtain standard curves of the reference chemicals ($r > 0.99$) for quantification. However, some of the identified constituents could not be quantified because they could neither be purchased nor purified in our laboratory. Contents of quantified compounds in the rhizome and root exudates were expressed as μg/g of fresh weight and μg/plant, respectively.

Effects of Identified Chemicals on the Growth of Invasive Species. The influence of the purchased compounds as mentioned above against the growth of *B. pilosa*, *L. leucocaphala*, *I. cylindrica*, and *E. crus-galli* was examined. The reference chemicals at 0.1 mg/mL diluted in distilled water were prepared and tested for their effects on the elongation of the shoot and root of the four plants growing in the Petri dish using the aforementioned bioassay method. The inhibitory activity of each reference chemical is expressed as the average of the suppressive magnitude on elongation of the shoot and root of the tested species. This experiment was repeated twice.

Spontaneous Competition of Invasive Species in Fields. In 2006−2007, strongly infested cogongrass areas in the Agricultural Farm, University of the Ryukyus, Japan, were selected for field experiment. It was divided into different plots of 3×4 m^2. Plants in the plot were removed by sickle, and the plot was afterward well-plowed by hoes. Plots without plant cutting and plowing were used as the controls. Each treatment was replicated 3 times in a completely randomized design. Water was provided daily. After 3 months, plant number, plant height, and fresh and dry biomass of *I. cylindrica* and other invasive species in all treatments were investigated.

Statistical Analysis. All treatments were arranged in a completely randomized design with at least three replicates. Data were analyzed using SAS version 6.12 (*17*), applying two-way analysis of variance (ANOVA) using the standard error (SE) of the difference to separate treatment means ($p = 0.05$).

RESULTS

Inhibitory Effects of Rhizome and Root Exudates. The inhibition of extracts of the rhizome and root exudates on root growth was 2−3-fold higher than that of the shoot of barnyardgrass (**Figure 1**). However, the effects differed between the shoot and root, of which the root exudates were more inhibitive on shoot growth than that of the rhizome extract. In contrast, the rhizome exhibited a significantly stronger reduction on root growth (IC_{50} = 293.8 μg/mL) than that of the root exudates (IC_{50} = 362.4 μg/mL). It appears that the rhizome and root exudates of cogongrass may contain growth inhibitors that were suppressive upon the emergence of barnyardgrass.

Detection of Chemicals from Rhizome and Root Exudates. From the use of GC−MS, a total of 36 compounds were identified in the rhizome and root exudates of barnyardgrass. Of which, 27 substances were found in the rhizome, and the root exudates had 24 constituents (**Table 1**). Of these, 12, 9, 10, and 2 compounds were phenolic acids, phenols, long-chain fatty acids, and lactones, respectively. The other compounds were clionasterol, coumaran, and methyltartronic acid.

Among the 36 identified compounds, 15 compounds were detected in both rhizome and root exudates, including benzoic acid, coumaran, hydrocinnamic acid, vanillin, 4-acetyl-2-methoxyphenol, vanillic acid, *iso*-vanillic acid, syringic aldehyde, myristic acid, ferulic acid, pentadecanoic acid, *iso*-ferulic acid, linoleic acid, stearic acid, and palmitic acid (**Table 1**). However,

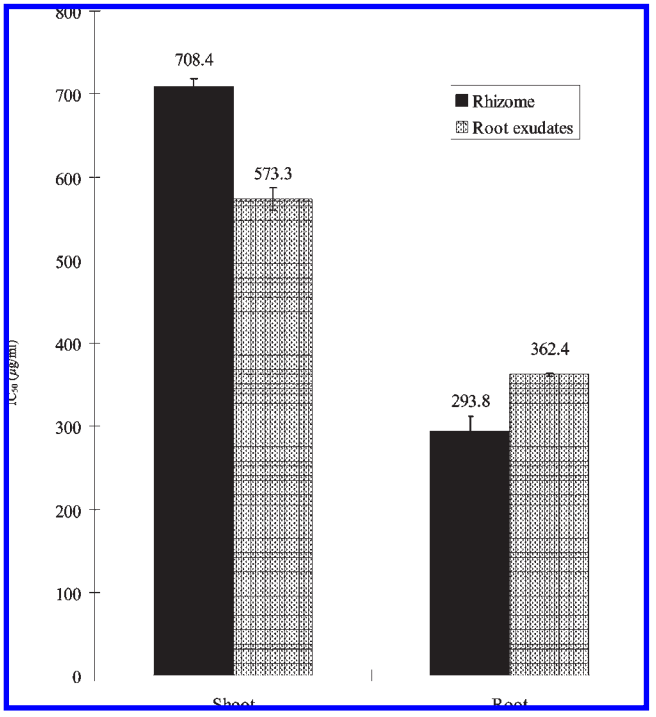

Figure 1. Inhibition (IC_{50}) of rhizome and root exudate extracts of cogongrass on the growth of the shoot and root of barnyardgrass. Bar values are means ± SE (n = 3).

there were 12 and 9 substances found only in the rhizome and root exudates, respectively.

Quantification of Phytotoxic Substances. There were 18 compounds in the rhizome of cogongrass quantified, as shown in **Figure 2**. *iso*-Eugenol, *iso*-ferulic acid, linoleic acid, and ferulic acid were the major constituents in the rhizome (392.2, 288.9, 252.5, and 217.3 μg/g of fresh root, respectively). Three compounds were >50 μg/g, including *p*-vinylguaicol, vanillin, and *trans*-*p*-coumaric acid. Contents of other detected compounds in the rhizome were in 19.7−43.8 μg/g.

Quantified substances in the root exudates were benzoic acid, coumaran, vanillin, 4-acetyl-2-methoxyphenol, syringic aldehyde, ferulic acid, linoleic acid, stearic acid, and palmitic acid (**Figure 2**). Of which, 4-acetyl-2-methoxyphenol was the principal constituent (872.6 μg/plant), whereas quantities of the other compounds were 78.8−220.7 μg/plant. Although *p*-vinylguiaicol, cinnamic acid, pentadecanoic acid, and syringic acid were detected (**Table 1**), their peak areas were too small (data not shown) that the quantification could not be carried out.

Inhibitory Effects on the Emergence of Invasive Species. Among identified substances, 20 compounds were examined for their influence against the growth of *B. pilosa*, *L. leucocaphala*, *E. crus-galli*, and *I. cylindrica* itself (**Table 2**). Of them, 9, 4, and 5 constituents were phenolic acids, phenols, and long-chain fatty acids, respectively, while the other compounds were coumaran and pantolactone. In general, the inhibitory magnitude on *B. pilosa* was the maximum, followed by *L. leucaena* and *E. crus-galli*. Many compounds were not inhibitive against *I. cylindrica* because they strongly stimulated the weed growth (**Table 2**). The phenolic acids showed 50−80% inhibition on *B. pilosa* growth, but they were less suppressive against *L. leucocaphala* (18.0−68.0% of inhibition). The emergence of *E. crus-galli* was in lower reduction than *L. leucocaphala* (9.8−74.7%), except that ferulic acid and vanillin slightly stimulated its growth.

Table 1. Chemicals Identified in Rhizome and Root Exudates of Cogongrass[a]

number	substances	RT	MW	rhizome	root exudates
1	methyltartronic acid	4.15	134	+	−
2	pantolactone	4.78	130	+	−
3	benzoic acid	5.78	122	+	+
4	coumaran	8.35	120	+	+
5	4-phenyl-2-butanone	8.77	120	−	+
6	pelargonic acid	9.23	158	−	+
7	*p*-vinylguaiacol	10.32	150	+	−
8	hydrocinnamic acid	24.84	150	+	+
9	capric acid	11.29	172	−	+
10	vanillin	12.01	152	+	+
11	4-hydroxy-2-methoxybenzadehyde	12.04	152	−	+
12	undecanoic acid	12.23	186	−	+
13	cinnamic acid	13.05	148	−	+
14	4-acetyl-2-methoxyphenol	13.74	166	+	+
15	2,6-di-tert-butyl-4-hydroxytoluene	14.05	220	+	−
16	2,4-di-tert-butylphenol	14.05	206	+	−
17	*iso*-eugenol	14.15	164	+	−
18	lauric acid	15.13	200	−	+
19	vanillic acid	15.29	168	+	+
20	acetoveratrone	16.02	180	+	−
21	*iso*-vanillic acid	16.51	168	+	+
22	3′,5′-dimethoxyacetophenone	16.55	180	+	−
23	syringic aldehyde	16.83	182	+	+
24	1-(4-hydroxy-3,5-dimethoxyphenyl)-ethanone	18.05	196	−	+
25	myristic acid	18.61	228	+	+
26	syringic acid	19.89	198	−	+
27	ferulic acid	20.38	194	+	+
28	*trans*-*p*-coumaric acid	20.62	164	+	−
29	3-hydroxy-4,5-dimethoxybenzoic acid	20.84	198	+	−
30	pentadecanoic acid	20.19	242	+	+
31	*iso*-ferulic acid	20.85	194	+	+
32	margaric acid	23.12	270	+	−
33	linoleic acid	24.13	280	+	+
34	stearic acid	24.52	284	+	+
35	palmitic acid	24.63	256	+	+
36	clionasterol	29.22	414	+	−

[a] +, detected; −, not detected. Abbreviations: RT, retention time; MW, molecular weight.

Among tested phenols, 2,4-di-tert-butylphenol was the most inhibitive against all tested species (78.3−100.0%). *iso*-Eugenol obtained 44.2−99.3% reduction, when 4-acetyl-2-methoxyphenol showed 6.7−56.2% of suppression. The chemical structures of these compounds are shown in **Figure 3**. Syringal aldehyde exhibited higher inhibition than 4-acetyl-2-methoxyphenol (59.4−76.1%); however, in contrast, it promoted growth of cogongrass by 55.6% (**Table 2**). The long-chain fatty acid, coumaran, and pantolactone exerted lower effects than the phenolics and phenols. These compounds retarded growth of *B. pilosa* by 27.5−68.7%; however, many among them stimulated the emergence of *E. crus-galli* and *I. cylindrica*. Growth of *L. leucocephala* was stunted but was in much lower inhibition than *B. pilosa* (1.1−29.8%).

Spontaneous Competition of Invasive Species in Fields. In the fields that were strongly infested by cogongrass, the emergence of *B. pilosa*, *L. leucocephala*, and *E. crus-galli* was found. However, at 3 months after cutting and plowing, the fields were alternatively invaded by *B. pilosa*, whereas *L. leucocephala* and *E. crus-galli* were not found (**Table 3**). The fresh and dry weight and plant number of cogongrass were controlled by about 70%, whereas the weed biomass and weed density of *B. pilosa* were increased by 62.5 and 33.3%, respectively. It appears that there was a strong competition between *I. cylindrica* and *B. pilosa* in cogongrass

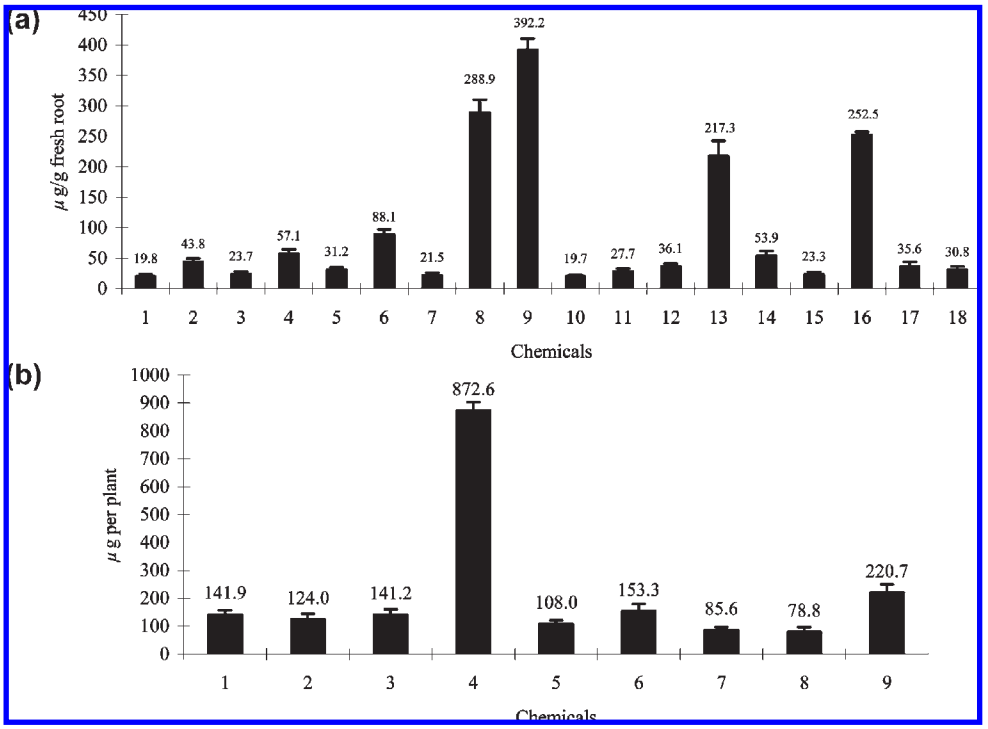

Figure 2. Quantities of several chemicals in (a) rhizome and (b) root exudates of cogongrass. (a) 1, pantolactone; 2, benzoic acid; 3, coumaran; 4, *p*-vinylguiaicol; 5, hydrocinnamic acid; 6, vanillin; 7, 4-acetyl-2-methoxyphenol 8, *iso*-ferulic acid; 9, *iso*-eugenol; 10, 2,4-di-tert-butylphenol 11, *iso*-vanillic acid; 12, syringic aldehyde; 13, ferulic acid; 14, *trans-p*-coumaric acid; 15, margaric acid; 16, linoleic acid; 17, stearic acid; 18, palmitic acid. (b) 1, benzoic acid; 2, coumaran; 3, vanillin; 4, 4-acetyl-2-methoxyphenol; 5, syringic aldehyde; 6, ferulic acid; 7, linoleic acid; 8, stearic acid; 9, palmitic acid. Bar values are means ± SE (n = 3).

Table 2. Effects of Chemicals Identified in Cogongrass on the Growth of Several Invasive Species[a]

chemicals	*B. pilosa*	*I. cylindrica*	*L. leucocaphala*	*E. crus-galli*
		Phenolic Acids		
cinnamic acid	75.4 ± 4.3 b	−14.6 ± 2.3 g	49.4 ± 11.1 c	50.7 ± 2.7 e
iso-vanillic acid	68.9 ± 8.9 bc	−37.1 ± 6.8 i	18.0 ± 1.4 fg	9.8 ± 1.5 h
iso-ferulic acid	81.6 ± 4.5 a	−53.9 ± 7.3 kl	28.7 ± 1.8 de	56.9 ± 9.5 de
ferulic acid	48.8 ± 2.4 e	−65.4 ± 7.6 l	21.2 ± 0.7 f	−6.5 ± 0.8 k
vanillin	56.3 ± 5.9 d	−81.9 ± 26.2 m	29.7 ± 3.7 de	−2.0 ± 0.4 j
hydrocinnamic acid	83.9 ± 7.1 a	−55.6 ± 0.0 k	68.0 ± 3.6 ab	59.4 ± 3.7 d
benzoic acid	73.1 ± 4.1 b	−36.6 ± 4.1 i	62.3 ± 7.3 bc	51.2 ± 0.0 e
syringic acid	69.6 ± 6.5 c	−49.2 ± 5.5 k	17.3 ± 0.9 g	74.7 ± 0.0 c
trans-p-coumaric acid	81.6 ± 5.0 a	−6.4 ± 0.8 f	34.0 ± 3.6 cd	41.4 ± 5.6 f
		Phenols		
iso-eugenol	76.3 ± 5.7 b	70.4 ± 0.0 b	44.2 ± 6.2 c	99.3 ± 0.0 a
syringal aldehyde	76.1 ± 3.0 b	−55.6 ± 4.7 kl	68.0 ± 0.6 ab	59.4 ± 1.9 d
4-acetyl-2-methoxyphenol	56.2 ± 7.5 de	50.2 ± 8.3 c	35.4 ± 3.4 cd	6.7 ± 0.6 i
2,4-di-tert-butylphenol	85.8 ± 3.5 a	100.0 ± 0.0 a	78.3 ± 11.9 a	94.4 ± 0.0 b
		Long-Chain Fatty Acids		
stearic acid	−21.1 ± 8.8 h	14.6 ± 8.0 e	25.7 ± 1.1 de	17.8 ± 3.4 g
myristic acid	68.7 ± 3.4 c	−51.5 ± 2.2	25.9 ± 2.7 de	−43.4 ± 9.4 no
palmitic acid	43.5 ± 4.4 f	23.9 ± 5.4 d	29.8 ± 2.1 de	−58.8 ± 12.7 o
margaric acid	27.5 ± 3.9 g	−17.1 ± 0.3 h	26.5 ± 3.3 de	−12.4 ± 0.3 l
pentadecanoic acid	58.8 ± 1.7 d	33.1 ± 17.2 d	19.4 ± 1.4 f	−14.3 ± 4.5 lm
		Others		
coumaran	63.6 ± 8.9 cd	−43.0 ± 4.4 ij	21.0 ± 2.1 f	−48.8 ± 7.6 no
pantolactone	43.5 ± 5.6 f	−4.6 ± 3.9 f	1.1 ± 0.1 h	−16.9 ± 3.2 m

[a] The means are the average of inhibition on the growth of root and shoot length ± SE (n = 3). Values with (−) are the stimulatory magnitude against controls. Columns with same letters are not significantly different (p = 0.05). Tested dose = 0.1 mg/mL.

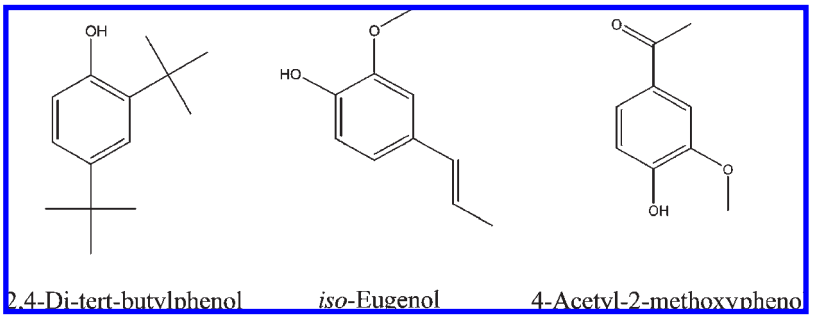

Figure 3. Chemical structures of growth inhibitors were potent against invasive species.

Table 3. Competition between *I. cylindrica* and Other Invasive Species in Fields[a]

plant emergence	*I. cylindrica*	*B. pilosa*	*L. leucocephala*	*E. crus-galli*
fresh weight	68.1 ± 5.5 a	−44.8 ± 6.1 b	*	*
dry weight	70.8 ± 3.1 a	−62.5 ± 6.8 a	*	*
plant height	10.7 ± 2.6 b	−44.8 ± 1.9 b	*	*
plant number	74.2 ± 8.2 a	−33.3 ± 2.3 c	*	*

[a] Means are the inhibitive percentage of cutting and plowing plots as compared to the respective controls (without cutting and plowing) ± SE (n = 3). Values with (−) indicates stimulation against the controls. (*) No emergence was observed after cutting and plowing. Columns with the same letters are not significantly different (p = 0.05).

fields. The cutting and plowing were effective to control cogongrass, but the alternative invasion of *B. pilosa* may be a problem when the emergence of *I. cylindrica* is reduced.

DISCUSSION

The competition with crops and allelopathic activity of cogongrass was reported in some previous reports (*3*, *11*, *18*−*20*); however, this is the first to report about the competition of the weed with other invasive species in fields. A number of plant growth inhibitors detected in the rhizome and root exudates of *I. cylindrica* indicate that they may be involved in the strong invasiveness of the harmful weed.

This study showed that cogongrass contains a rich chemical diversity, of which 27 and 24 compounds were found in the rhizome and root exudates, respectively (**Table 1**). Many among them have been known as plant growth inhibitors, such as benzoic acid, vanillin, hydrocinnamic acid, cinnamic acid, vanillic acid, myristic acid, syringic acid, ferulic acid, *trans-p*-coumaric acid, pentadecanoic acid, margaric acid, syringic aldehyde, linoleic acid, stearic acid, and palmitic acid. However, from this study, 2,4-di-tert-butylphenol, pantolactone, *iso*-eugenol, *iso*-ferulic, and *iso*-vanillic acid also exerted strong inhibition against tested invasive species. In the rhizome, concentrations of *iso*-ferulic acid, *iso*-eugenol, ferulic acid, and linoleic acid were 3−20-fold higher than that of the other compounds. Similarly, the quantity of 4-acetyl-2-methoxyphenol was 2−10-fold greater than other quantified constituents of root exudates (**Figure 2**).

Of the 36 identified compounds, there were 15 compounds found in both the rhizome and root exudates and 12 and 9 constituents were detected only in the rhizome and root exudates, respectively (**Table 1**). The relation between chemicals in the rhizome of a plant and compounds released by root exudates is still questionable (*14*−*16*, *21*−*24*). Organic substances in the soil are often bound to soil particles and may be rapidly degraded by many environmental and soil factors, such as temperature, light, and the minerals, pH, and microbes of the soil. For instance, mimosine, a toxic nonprotein amino acid in *L. leucocephala* and *Mimosa* spp., is easily degraded into 3,4-DHP by the temperature and pH of the soil and by either mimosine-degrading enzymes derived from the Leucaena itself or soil bacteria (*14*). There were 9 compounds found only in the root exudates of cogongrass, but it is unclear whether they are the secondary products of the chemicals in the rhizome that might be degraded during exudation. *iso*-Ferulic acid, *iso*-eugenol, ferulic acid, linoleic, and 4-acetyl-2-methoxyphenol may play a crucial role, but the other chemicals should also be involved in the strong invasion of cogongrass.

We were unable to estimate the actual concentrations of compounds found in the root exudates in cogongrass-infested soil. The field survey data of this study during 2006−2007 noted that the average density of cogongrass was 1.6 plant/cm^2. Except for 4-acetyl-2-methoxyphenol, the other 8 compounds quantified in the root exudates were in 78.8−220.7 μg/plant, indicating that, theoretically, cogongrass may release 126.1−353.1 μg/cm^2 soil. The concentration of 0.1 mg/mL might not be possibly accumulated in natural fields; however, the dose was chosen because it was close to the minimum of theoretical doses that these compounds might be released in the root exudates. On the other hand, the dose was useful to screen the inhibitory activity of these tested 20 substances against the 4 invasive species. Common growth inhibitors found in cogongrass may not be a concern, but the derivatives of these compounds attached with different functional groups may obtain novel modes of action, which is potent for the development of new herbicides.

Many previous studies used the mixture of tested compounds to compare the activity of each chemical. The inhibition on a plant growth may be attributed to a complex of compounds released/decomposed from the donor plant than by a single substance. However, the mixture of the 20 tested compounds was not used by this study because (i) only 20 of 36 identified compounds were examined because the other substances were neither successfully isolated in our laboratory nor purchased and (ii) the quantities and exuded doses differed among these chemicals and it was difficult to adjust their concentrations in the mixture to fit with their actual spontaneous components in nature. According to our previous reports, a mixture commonly showed a moderate magnitude compared to the activity of individual tested compounds (*20*−*24*).

The use of cutting and plowing was effective to control cogongrass by 70% as well as *L. leucocaphala* and *E. crus-galli* by 100% (**Table 3**). However, the alternative invasion of *B. pilosa* might be a serious problem. A thick density of cogongrass with various plant growth inhibitors exuded into soil might prevent the invasion of *B. pilosa* from a cogongrass field. It is proposed that the use of plants of *I. cylindrica* after cutting as a cover crop in fields may be efficacious to reduce the invasive strength of *B. pilosa*. Compounds identified were graminones A and B (*8*), cylindols A and B (*9*), imperanene (*7*), and chromones (*10*). The plant growth inhibitory activity of these compounds was

unknown, but they are difficult to play a role in the invasion of cogongrass because of their low contents (graminones A and B, 7.0 and 3.5 μg/g, respectively; cylindols A and B, 3.0 and 2.5 μg/g, respectively; imperanene, 4.0 μg/g; chromones, 0.1−1.2 μg/g). Repeated analysis of GC−MS of this study showed no trace of these compounds detected, which may be due to different extracting protocols applied, because they used either hexane or chloroform for extraction and isolation.

In the bioassays, the inhibitory level on the growth of *B. pilosa* was the maximum, followed by *L. leucocaphala*. The emergence of *E. crus-galli* was less inhibitive, whereas many compounds stimulated the growth of *I. cylindrica* (**Table 2**). It was similar to our previous reports that chemicals or extracts of a plant were less suppressive on the growth of itself than on the other plants. For instance, mimosine was not inhibitive against *L. leucocaphala* and *Mimosa* spp. because they are the mimosine producers (*14*). The extract of barnyardgrass showed lower inhibition on the growth of itself than other broadleaf indicator plants and paddy weeds (*15*). However, the compound 2,4-di-tert-butylphenol completely controlled the emergence of cogongrass and showed 78.3−94.4% inhibition against *B. pilosa*, *L. leucocephala*, and *E. crus-galli* (**Table 2**). *iso*-Eugenol and 4-acetyl-2-methoxyphenol also reduced the growth of all tested invasive species, although they were in lower magnitudes than 2,4-di-tert-butylphenol.

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