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Crabgrass (*Digitaria sanguinalis*) Allelochemicals That Interfere with Crop Growth and the Soil Microbial Community

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ABSTRACT: Three chemicals, veratric acid, maltol, and (−)-loliolide, were isolated from crabgrass and their structures were identified by spectroscopic analysis. The chemicals were detected in crabgrass root exudates and rhizosphere soils, and their concentrations ranged from 0.16 to 8.10 μg/g. At an approximate concentration determined in crabgrass root exudates, all chemicals significantly inhibited the growth of wheat, maize, and soybean and reduced soil microbial biomass carbon. Phospholipid fatty acid profiling showed that veratric acid, maltol, and (−)-loliolide affected the signature lipid biomarkers of soil bacteria, actinobacteria, and fungi, resulting in changes in soil microbial community structures. There were significant relationships between crop growth and soil microbes under the chemicals' application. Chemical-specific changes in the soil microbial community generated negative feedback on crop growth. The results suggest that veratric acid, maltol, and (−)-loliolide released from crabgrass may act as allelochemicals interfering with crop growth and the soil microbial community.

KEYWORDS: *allelopathy, veratric acid, maltol, (−)-loliolide, root exudates, soil microorganism*

INTRODUCTION

Many weeds interfere with the growth and development of crop plants that are grown in their vicinity through the release of allelochemicals.[1−3] Crabgrass (*Digitaria sanguinalis* (L.) Scop., Poaceae) is a widespread weed in nonirrigated farmlands and usually infests cultivated fields and reduces crop productivity. A heavy infestation of crabgrass results in adverse effects on the growth and yield of crop plants, particularly in wheat, maize, and soybean fields.[4,5] It has long been suspected that an allelopathic mechanism may be an important factor for the interference of crabgrass with the crop plants.[6,7] However, the allelochemicals from the weed involved against crop plants are largely unknown.

Weed−crop allelopathic interactions are mediated by allelochemicals released from plants into the environment, mostly into the soil, and the search for allelochemicals has been pursued extensively.[2,3] However, allelochemicals are still a controversial issue due to inadequate methodology and neglect of soil interactions.[8,9] Collecting plant material, grinding it up, and taking an extract for bioassay is not allelopathy, which involves exudates, not extracts. The presence of the phytotoxic chemicals in the plant extracts does not imply that they can be exuded into the environment to exert an allelopathic effect.[8,10] Furthermore, numerous phytochemicals have been isolated and identified from various plants, following the determination of their phytotoxicity at arbitrary concentrations rather than at actual quantities in soil-grown plants.[2,8] It is not appropriate to call them allelochemicals until they at least have been shown to be present in the soil-grown plants and shown to be bioavailable in soil at sufficient concentrations to affect vegetation either directly or indirectly through effects on soil microbes.[8,10−12] Accordingly, the objectives of this study were to identify and quantify the phytotoxic chemicals from crabgrass extracts, root exudates, and rhizosphere soils and to evaluate their effects on ecologically relevant crops, namely, wheat, maize, and soybean, and on soil microbial biomass carbon and community, with an attempt to further our understanding of allelopathic interference of weed with crop plants mediated by allelochemicals in cropping systems.

MATERIALS AND METHODS

Instruments. High-resolution mass spectrometry experiments were carried out with IonSpec Ultima FTMS and FABMS instruments with a VG-ZAB-HS (VG Instrument Co., Crawley, UK). The NMR spectra were measured with a Bruker ARX-600 NMR spectrometer (Bruker Instrument Co., Karlsruhe, Germany). All chemical shifts were reported as δ values relative to the peak for TMS. Optical rotation was measured with a Perkin-Elmer model-241 MC polarimeter (Perkin-Elmer Co., Waltham, MA, USA).

Plant Materials, Soils, and Chemicals. Three crop plants, namely, wheat (*Triticum aestivum*), maize (*Zea mays*), and soybean (*Glycine max*), were used in this study. These crop plants were selected on the basis of their ecological relevance with the crabgrass (*D. sanguinalis*) in the local cropping systems. Crabgrass plants were collected from the fields at the Shenyang Ecological Experiment Station, Chinese Academy of Sciences (northeastern China, N 41° 31′, E 123° 24′) during their growing season in 2010.

Soils for bioassays and for microbial study were collected from the same experimental field. The soil belongs to the class Hapli-Udic Cambisol (FAO Classification) with a pH of 6.63 ± 0.15. Soil fertility status was as follows: organic matter content of 1.68 ± 0.21%; total N, 1.2 ± 0.07 g/kg; available N, 35.64 ± 5.67 mg/kg; total P, 0.53 ± 0.04 g/kg; available P, 26.13 ± 3.21 mg/kg; total K, 2.20 ± 0.35 g/kg; available K, 37.60 ± 6.51 mg/kg. Rhizosphere soils tightly adhering to the roots of crabgrass plants were collected and used for the quantitation of allelochemicals.[13,14] The crabgrass plants grown in the field

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were carefully uprooted and air-dried, and the roots were separated from the plants. The roots were taken in tubes and vigorously shaken to shatter adhered soils. The soils were stored and used for the quantitation of allelochemicals when required.

Crabgrass plants (10 g) were freeze-dried, powdered, and homogenized with 100 mL of distilled water. The homogenate was filtered, and the filtrates were used as plant extracts. Twenty crabgrass seedlings were grown in a pot with 200 mL of hydroponic solution (KNO_3, 0.61 g/L; $Ca(NO_3)_2$, 0.95 g/L; $(NH_4)_3PO_3$, 0.12 g/L; $MgSO_4$, 0.50 g/L; pH, 6.0). The pot was placed in a sterile environment growth chamber at 24 ± 1 °C with a 12 h photoperiod. The solution in the pot was collected and filtered after 14 days. The filtrate was concentrated in vacuo to yield the root exudates. Other chemicals and organic solvents were purchased from local suppliers (Beijing, China) and were of the highest purity available.

Isolation and Identification of Allelochemicals. A total of 20 kg of air-dried crabgrass plants was soaked with 70% aqueous MeOH at a temperature of 25 °C, extracted for 24 h, and filtered. The filtrate was concentrated in vacuo, and the concentrated extracts were successively partitioned three times with petroleum ether (PE), CH_2Cl_2, and EtOAc. Each of the extracts was subsequently concentrated, and the residues were used in the bioassay with wheat. The active CH_2Cl_2 extract was subjected to silica gel CC (5 cm × 80 cm) by eluting stepwise with a mixture of 500 mL PE/CH_2Cl_2/MeOH (10:0:0, 8:2:0, 6:4:0, 4:6:0 2:8:0, 0:10:0, 0:9:1, 0:8:2, 0:7:3, 0:6:4, 0:5:5, and 0:0:10, v/v/v). Resulting fractions were screened using a bioassay-guided approach.[15] Finally, three fractions with phytotoxic activity were obtained. The first fraction eluted with PE/CH_2Cl_2/MeOH (6:4:0, v/v/v) was further purified by silica CC (2.5 cm × 40 cm) with 200 mL of *n*-hexane/acetone (10:1, 8:2, 6:4, v/v), and the *n*-hexane/acetone (8:2) eluate was collected and concentrated. The concentrate was diluted with MeOH and centrifuged. The yellow precipitate was further purified by TLC on 5 cm ×10 cm silica gel plates developed with CH_2Cl_2 to give veratric acid (23 mg). The second fraction eluted with PE/CH_2Cl_2/MeOH (4:6:0, v/v/v) was purified by Sephadex LH-20 (20−150 μm, 1 cm × 25 cm) with MeOH, resulting in a green solid. The solid was washed with *n*-hexane to remove the green color and recrystallized to yield maltol (13 mg). The third fraction eluted with PE/CH_2Cl_2/MeOH (0:10:0, v/v/v) was further purified by ODS (YMC 120A 50 μm, 1 cm × 25 cm) with H_2O containing increasing amounts of MeOH to obtain (−)-loliolide (15 mg).

*Data for veratric acid (3,4-dimethoxybenzoic acid, **1**):* white amorphous powder; mp 198−200 °C; ESI-MS ($C_9H_{10}O_4$) *m*/*z* 182.9 ($[M + H]^+$), 204.9 ($[M + Na]^+$); ^{1}H NMR (600 MHz, $CDCl_3$) *δ* 3.88 (3H, s, H-3OMe), 3.94 (3H, s, H-4OMe), 6.93 (1H, d, *J* = 6.9 Hz, H-5), 7.55 (1H, brs, H-2), 7.63 (1H, brd, *J* = 6.9 Hz, H-6).

*Data for maltol (3-hydroxy-2-methyl-4-pyrone, **2**):* colorless solid; mp 159−161 °C; molecular formula $C_6H_6O_3$ determined by accurate mass spectrometry (HR-ESI, *m*/*z* 127.0392 $[M + H]^+$); ^{1}H NMR (600 MHz, $CDCl_3$) *δ* 2.37 (3H, s, CH_3), 6.42 (1H, d, *J* = 5.4 Hz, pyran-H), 7.71(1H, d, *J* = 5.4 Hz, pyran-H); ^{13}C NMR (150 MHz, $CDCl_3$) *δ* 14.3 (C-7), 113.1 (C-6), 143.2 (C-3), 149.1 (C-2), 154.2 (C-4), 173.0 (C-5).

*Data for (−)-loliolide (5,6,7,7a-tetrahydro-6α-hydroxy-4,4,7a-trimethylbenzofuran-2(4H)-one, **3**):* white crystal; mp 146−148 °C; molecular formula $C_{11}H_{16}O_3$ determined by accurate mass spectrometry (HR-ESI, *m*/*z* 197.1181 $[M + H]^+$); $[\alpha]_D^{20}$ = −93.1° (*c* 0.006, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) *δ* 1.25 (3H, s, H-9), 1.36 (3H, s, H-11), 1.45 (3H, s, H-10), 1.50 (dd, *J* = 14.5, 3.7 Hz, H-2*β*), 1.74 (dd, *J* = 13.8, 4.0 Hz, H-4*β*), 1.98 (dt, *J* = 14.5, 2.6 Hz, H-2*α*), 2.35 (1H, brs, OH-3), 2.46 (1H, dt, *J* = 13.8, 2.6 Hz, H-4*α*), 4.30 (1H, m, *J* = 3.3 Hz, H-3), 5.66 (1H, s, H-7); ^{13}C NMR (150 MHz, $CDCl_3$) *δ* 26.4 (C-10), 26.9 (C-11), 30.6 (C-9), 36.0 (C-1), 45.6 (C-4), 47.2 (C-2), 66.6 (C-3), 87.1 (C-5), 112.7 (C-7), 172.3 (C-8), 183.1 (C-6).

Quantitation of Allelochemicals. The quantitation of veratric acid, maltol, and (−)-loliolide in crabgrass root exudates and rhizosphere soils was performed by a liquid extraction/solid-phase extraction followed by HPLC. The rhizosphere soils were extracted with 50% aqueous MeOH, agitated for 12 h at 24 °C, and then centrifuged at 1000*g* for 10 min. The root exudates and soil extracts were evaporated

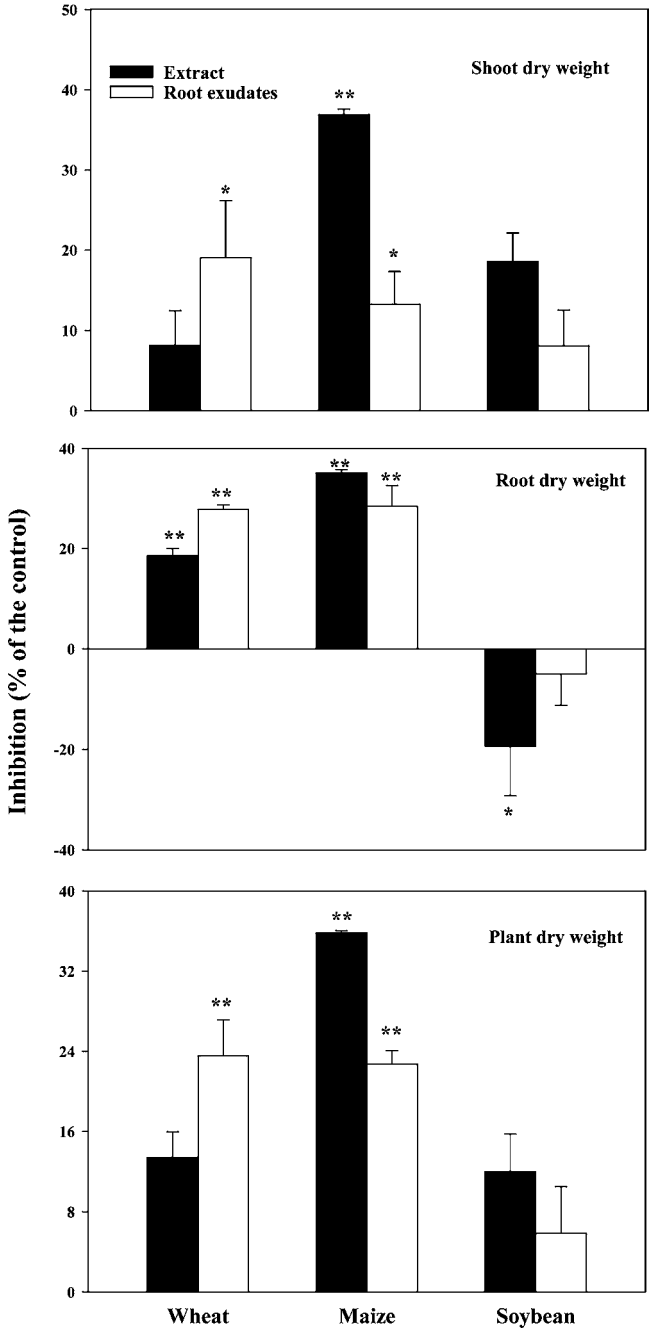

Figure 1. Effect of the extracts and root exudates of crabgrass on the growth of three crop plants. The significance of the difference between treatment and control is represented by ∗ (0.05) or ∗∗ (0.01) with Student's *t* test.

to dryness individually with N_2. The residues were dissolved in 50% aqueous MeOH and loaded onto reversed phase C_{18} Sep-Pak cartridges (Waters Co., Milford, MA, USA) before being analyzed by HPLC. The quantitative analysis was carried out with an Agilent 1100 HPLC (Agilent Co., Palo Alto, CA, USA) instrument equipped with a C_{18} reversed-phase column (Agilent Zorbax SB-C_{18}, 4.6 mm × 250 mm, 5 μm) with a diode array detector. The flow rate was 0.8 mL/min at a column temperature of 40 °C, and the injection volume was 5 μL. For maltol, the mobile phase was a mixture of MeOH and 0.5% aqueous AcOH (3:7, v/v) and detected at 275 nm. The retention time was 7.2 min. For veratric acid and (−)-loliolide, the mobile phase was a mixture of MeCN and H_2O (2:8, v/v) and detected at 210 nm. Their retention times were 4.5 min for veratric acid and 18.1 min for (−)-loliolide. The quantitation was achieved by regression analysis of

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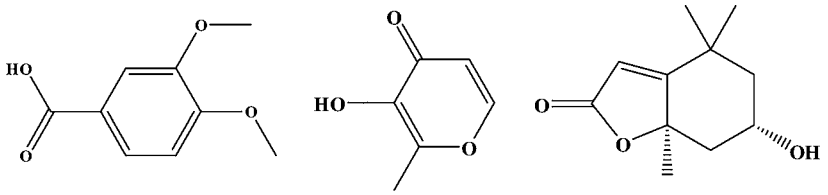

veratric acid (**1**) maltol (**2**) (-)-loliolide (**3**)

Figure 2. Structures of three allelochemicals from crabgrass.

the peak areas against standard concentrations. The mean recoveries of known amounts of the chemicals added into soil were 88.3% (veratric acid), 79.3% (maltol), and 77.5% [(−)-loliolide]. These mean recoveries were used to correct the concentrations determined in the rhizosphere soils.

Bioassays. Inhibitory activity of crabgrass plant extracts, root exudates, and the identified chemicals on the growth of three crop plants (wheat, maize, and soybean) were evaluated using the pot-culture method.[3] Fifteen pregerminated seeds were sown on each 5 cm × 5 cm pot containing 100 g of soil collected from the experimental site. After emergence, the seedlings were thinned to 10 plants per pot, and then the extracts, root exudates, and identified chemicals were added to each of the treated pots, respectively. The extracts and root exudates were diluted with distilled water to prepare a concentration of 100 μg/g. Veratric acid, maltol, and (−)-loliolide were each added to the soil at a concentration of 8, 1, and 3 μg/g soil, respectively. These concentrations were an approximate quantity as determined in crabgrass root exudates (Figure 3). The control pots received water only. All pots were placed in an environmental chamber with a temperature of 25 °C and 65−90% relative humidity maintained. Pots were watered and randomized once a day. After 14 days, the seedlings were each harvested and dried for at least 48 h at 80 °C, and their dry weights were recorded.

Soil Microbial Analysis. A series of 150 mL vials were filled with 100 g of soils collected from the experimental site. The vials were treated with the identified chemicals in the crabgrass at concentrations similar to those in the root exudates [8 μg/g soil of veratric acid, 1 μg/g soil of maltol, and 3 μg/g soil of (−)-loliolide], respectively. The vials were airtight with lids and then incubated in a growth chamber at a temperature of 28 °C. The vials were taken out from the chamber randomly after different incubation periods (1, 3, 6, or 9 days). The soil samples were divided into two subsamples and used for the microbial biomass carbon (MBC) and phospholipid fatty acid (PLFA) analyses as described below.

MBC was determined using the chloroform fumigation−extraction method with minor modifications.[16] Every sample of the soil incubated in vials was divided into two portions, each consisting of 10 g of dry soil. One portion was fumigated with ethanol-free chloroform for 24 h in the dark at 25 °C, and the other portion was not fumigated (control). Both fumigated and unfumigated soils were extracted with 0.5 M K_2SO_4 solution for 30 min. The supernatant was filtered, and the filtrate was frozen. The amount of extractable carbon was determined with K_2CrO_4 oxidation method. MBC was calculated by MBC = E_c/K_c, where E_c = (C extracted from fumigated soil) − (C extracted from unfumigated soil) and K_c with a value of 0.38 is a calibration.[16]

PLFA analysis was conducted according to the method previously developed in the authors' laboratory.[13,14] Briefly, triplicate 4 g (dry weight) subsamples of freeze-dried soil were extracted with mixture of $CHCl_3$/MeOH/citrate buffer (1:2:0.8, v/v/v) and the phospholipids were separated from other lipids on silica gel-filled solid-phase extraction cartridges (0.50 g of Si; Supelco Inc., Bellefonte, PA, USA). The samples were then subjected to mild alkaline methanolysis, and the resulting fatty acid methyl esters (FAMEs) were separated before being quantified and identified by GC-MS. Identification of FAMEs was based on retention time comparisons to FAME controls (Supelco Inc.). Quantitation was carried out by calibration against standard solutions of nonadecanoate methyl ester (C19:0), which were also used as the internal standard.

A total of 22 PLFAs were identified in the soil samples. Among them, the fatty acids present in proportions >0.5% were used in the analysis. The fungal biomass was assessed by quantifying 18:3ω6, 18:2ω9,12c, and 18:1ω9c. The actinobacterial biomass was indicated by the presence of the biomarker 10Me18:0. The sum of 14:0, i15:0, a15:0, 15:0, i16:0, 2-OH 16:0, 16:1ω7c, i17:0, cy17:0, 17:0, cy19:0, and 20:0 was used to assess bacterial biomass. Among them, i15:0, a15:0, i16:0, and i17:0 were considered to be representative of Gram (+) bacteria, and 16:1ω7c, cy17:0, 2-OH 16:0 and cy19:0 were considered to be representative of Gram (−) bacteria. cy17:0 and cy19:0 were biomarkers of cyclopropyl phospholipid fatty acids. The ratios of saturated/unsaturated were reflected by (14:0, i15:0, a15:0, 15:0, 2-OH 14:0, 3-OH 14:0, i16:0, 16:0, 2-OH 16:0, i17:0, cy17:0, 18:0, 10Me 18:0, cy19:0, 20:0)/(16:1ω7c, 16:1ω9c, 18:3ω6, 18:2ω9,12c, 18:1ω9c, 18:1ω9t).

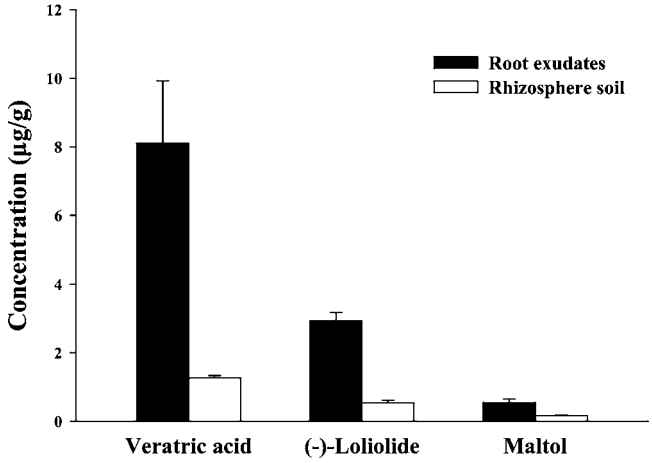

Figure 3. Concentrations of veratric acid, maltol, and (−)-loliolide in crabgrass root exudates and rhizosphere soils.

Data Analysis. Data were presented as means ± standard error (SE) from three independent experiments with three replications for each determination. Analysis of variance (ANOVA) and multiple comparisons were carried out with the SPSS10.0 program. The correlation analysis used bivariate correlation coefficients carried out with the SPSS16.0 program. Discriminant analysis was performed with the STATISTICA software package, version 6.0 (Statsoft Inc., Tulsa, OK, USA). Data used in the discriminant analysis plots were transformed using sample unit totals to represent relative abundance of each PLFA (mole percent of total PLFA).

RESULTS AND DISCUSSION

The extracts and root exudates of crabgrass reduced the dry weight of wheat, maize, and soybean with an exception of soybean root dry weight (Figure 1), indicating that both extracts and root exudates contained the phytotoxic chemicals. Three phytotoxic chemicals were obtained from the crabgrass extracts (Figure 2). Veratric acid and maltol were each identified by comparison of their HR-MS and NMR data with the literature.[17,18] The ^{1}H NMR spectrum of the third chemical indicated the presence of three methyl and two methylene proton signals in the high field, a hydroxy proton signal, a methine proton signal of carbon

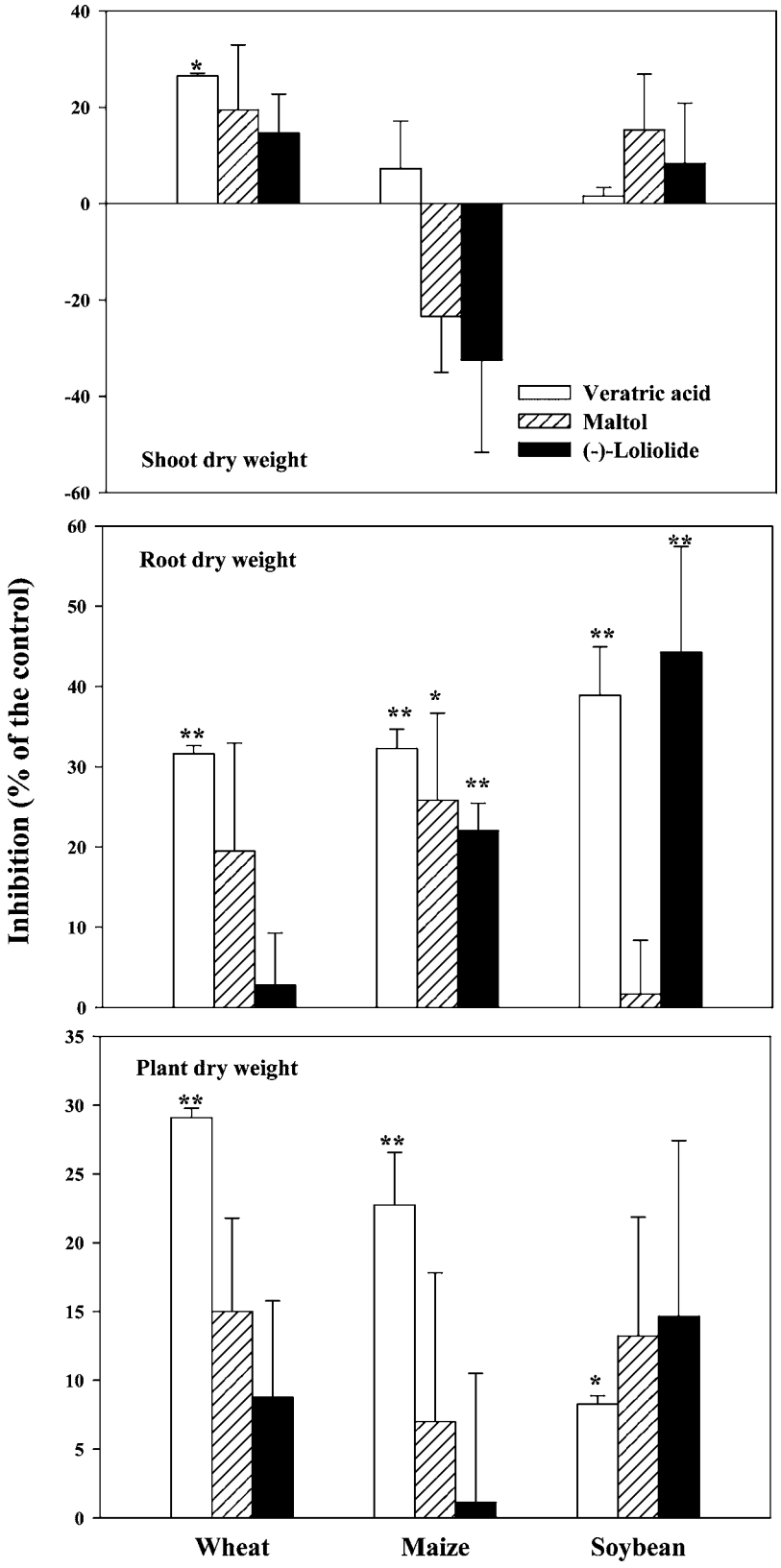

Figure 4. Effect of veratric acid, maltol, and (−)-loliolide on the growth of three crop plants. The significance of the difference between treatment and control is represented by ∗ (0.05) or ∗∗ (0.01) with Student's *t* test.

connecting with oxygen, and an olefin proton signal. Its ^{13}C NMR were given 11 carbon signals including a carbonyl carbon and two unsaturated ethylenic carbons. The presence of those three low-field carbons suggested that the chemical structure was α,β-substituted quaternary carbon on the unsaturated lactone. Furthermore, δ_c 172.3 was a quaternary carbon from hydrogen and carbon spectral information; it was β-position full substituted on the unsaturated lactone. The carbon spectra also

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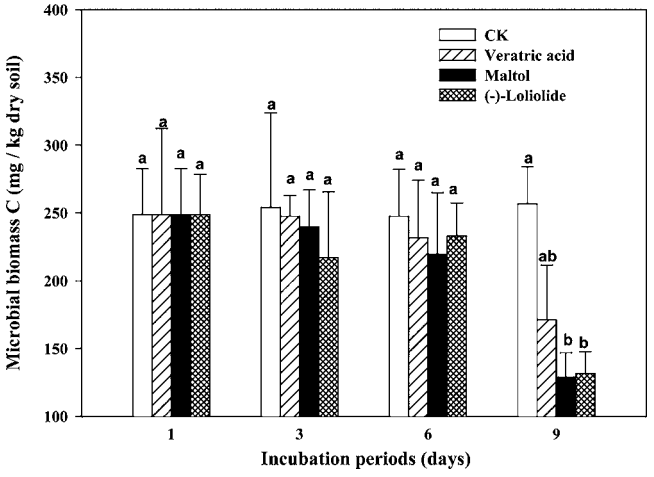

Figure 5. Effect of veratric acid, maltol, and (−)-loliolide on soil microbial biomass carbon at different incubation periods. Columns with different letters indicate significant differences between veratric acid, maltol, (−)-loliolide, and control (CK), at $P < 0.05$, analysis of variance (ANOVA), followed by Tukey's honestly significant difference test.

showed one carbon connecting with oxygen, one oxygenated tertiary carbon, and a series of the specific carbons to sesquiterpene structure. From analysis of its NMR and optical rotation, and comparison with literature data for loliolide and isololiolide,[19−21] this phtotoxic chemical was (−)-loliolide (Figure 2).

Veratric acid is derived from lignin and widely distributes in many kinds of plants and their growing soils.[22−24] Veratric acid has been described as having many bioactivities such as allelopathic action[25] and anti-inflammatory and antifungal activities.[23,24] Maltol is found in various beans and other plant species.[18,26,27] Maltol is usually used as a food additive and a bidentate metal ligand for administered drugs,[26−28] but its allelochemical function is largely unknown. (−)-Loliolide occurs in many plant families and marine alga.[19−21,29] (−)-Loliolide has relatively broad-spectrum bioactivity including antimicrobial,[30] antialgal,[31] and antifeedant and herbicidal activities.[32] All three chemicals have been identified in many plants. However, to the best of our knowledge, they have never been reported in crabgrass.

Substantially different from the phytochemicals in plant tissues, the action of allelochemicals requires their presence in the vicinity of the target plants.[8,10] Although crabgrass extracts contained the phytotoxic chemicals veratric acid, maltol, and (−)-loliolide, this did not mean that crabgrass could release them through the living roots into the surroundings at sufficient concentrations to interfere with crop plants grown in its vicinity. To address this, the quantities of veratric acid, maltol, and (−)-loliolide in crabgrass root exudates and rhizosphere soils were examined. Subsequently, all three phytotoxic chemicals were found in both root exudates and rhizosphere soils, but their concentrations were much greater in the root exudates than in the rhizosphere soils (Figure 3). Soil microbes degrade allelochemicals,[13] resulting in a great reduction of the quantities of veratric acid, maltol, and (−)-loliolide in the rhizosphere soils. Regardless of the root exudates and the rhizosphere soils, veratric acid always had the highest concentrations, followed by (−)-loliolide and maltol. Veratric acid reached a concentration of 8.10 μg/g dry weight in the root exudates, whereas maltol had the lowest concentration of 0.16 μg/g dry soil in the rhizosphere soils (Figure 3). At an approximate concentration as was determined in crabgrass root exudates, all chemicals inhibited the growth of wheat, maize, and soybean,

Table 1. Soil PLFA Concentrations (Nanomoles per Gram Dry Soil) and Selected Microbial Community Characteristics in the Soil Incubated with Veratric Acid (VA), Maltol (M), and (−)-Loliolide (L)[a]

	incubation time																	
	1 day				3 days				6 days				9 days					
PLFA parameter	CK	VA	M	L	CK	VA	L	CK	VA	M	L	CK	VA	M	L	CK	VA	M	L
total PLFA	10.3 ± 2.7a	14.0 ± 0.6a	15.8 ± 0.8b	18.0 ± 0.8b	19.1 ± 1.0a	23.1 ± 0.9b	21.4 ± 1.0a	22.2 ± 0.5a	30.6 ± 3.3a	29.5 ± 0.4a	30.4 ± 3.5a	32.5 ± 0.2a	23.0 ± 1.0a	27.2 ± 0.7a	30.1 ± 4.2a	29.2 ± 0.4a		
fungi	0.7 ± 0.1a	3.3 ± 0.1b	2.1 ± 0.2ab	4.5 ± 0.8b	4.4 ± 0.3a	5.1 ± 0.6a	4.7 ± 0.3a	5.6 ± 0.4a	3.8 ± 0.2a	5.8 ± 0.1b	4.0 ± 0.5ab	4.0 ± 0.3ab	5.1 ± 0.5a	3.2 ± 0.3b	3.7 ± 0.4b	3.7 ± 0.3ab		
actinobacteria	0.2 ± 0.0a	0.4 ± 0.0a	0.7 ± 0.1b	0.8 ± 0.2b	1.5 ± 0.3a	0.3 ± 0.0b	0.2 ± 0.0b	0.3 ± 0.0b	0.9 ± 0.1a	1.2 ± 0.0c	0.2 ± 0.1b	0.2 ± 0.0b	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a	0.2 ± 0.0a		
bacteria	6.1 ± 2.2a	4.9 ± 0.1a	6.5 ± 0.4a	5.6 ± 0.1a	5.5 ± 0.3a	8.4 ± 0.2b	8.0 ± 0.4b	8.2 ± 0.5b	12.7 ± 1.8a	9.5 ± 0.2a	12.5 ± 2.0a	14.3 ± 0.3b	8.5 ± 0.2a	11.5 ± 0.4ab	13.0 ± 2.0b	12.9 ± 0.5b		
Gram (+)	1.5 ± 0.3a	2.6 ± 0.4b	3.0 ± 0.3b	3.0 ± 0.1b	3.1 ± 0.2a	5.3 ± 0.5b	5.1 ± 0.3b	5.2 ± 0.4b	5.5 ± 0.8a	6.5 ± 0.2a	5.7 ± 0.9a	5.9 ± 0.1a	3.6 ± 0.0a	4.6 ± 0.3a	5.9 ± 0.7b	5.1 ± 0.2b		
Gram (−)	0.5 ± 0.1a	1.0 ± 0.1b	1.3 ± 0.0c	1.1 ± 0.0c	1.4 ± 0.2a	1.4 ± 0.1a	1.4 ± 0.1a	1.5 ± 0.1a	1.4 ± 0.2a	1.6 ± 0.0a	1.5 ± 0.2a	1.6 ± 0.0a	1.1 ± 0.0a	1.3 ± 0.1a	1.6 ± 0.2b	1.4 ± 0.0a		
cyclopropyl	0.2 ± 0.0a	0.5 ± 0.1b	0.7 ± 0.0c	0.6 ± 0.0bc	0.7 ± 0.1a	0.8 ± 0.0a	0.8 ± 0.0a	0.8 ± 0.1a	0.8 ± 0.1a	0.9 ± 0.0a	0.8 ± 0.1a	0.9 ± 0.0a	0.6 ± 0.0a	0.8 ± 0.1a	0.9 ± 0.1b	0.8 ± 0.0b		
saturated/ unsaturated	3.2 ± 0.4a	0.5 ± 0.1b	2.6 ± 0.0a	1.6 ± 0.3b	1.3 ± 0.1a	1.4 ± 0.1a	1.4 ± 0.0a	1.4 ± 0.2a	2.7 ± 0.1ab	2.2 ± 0.1b	2.6 ± 0.2ab	2.8 ± 0.1a	1.9 ± 0.1a	2.9 ± 0.3b	2.7 ± 0.1b	2.8 ± 0.2b		
Cy17:0/16:1ω7c	0.7 ± 0.1a	0.7 ± 0.1a	0.7 ± 0.0a	0.7 ± 0.1a	0.6 ± 0.1a	0.7 ± 0.1a	0.7 ± 0.0a	0.7 ± 0.0a	0.7 ± 0.0a	0.7 ± 0.0a	0.7 ± 0.0a	0.7 ± 0.0a	0.8 ± 0.0a	0.8 ± 0.0a	0.8 ± 0.1a	0.8 ± 0.1a		
(i15:0+a15:0)/ 16:0	1.2 ± 0.6a	0.9 ± 0.1a	0.8 ± 0.1a	0.7 ± 0.1a	0.7 ± 0.0a	1.4 ± 0.1b	1.5 ± 0.0b	1.6 ± 0.2b	1.7 ± 0.0a	0.7 ± 0.0a	0.7 ± 0.1a	0.7 ± 0.0a	0.6 ± 0.1a	0.6 ± 0.1a	0.6 ± 0.0a	0.6 ± 0.0a		
18:1ω9c/ 18:1ω9t	0.4 ± 0.0a	0.4 ± 0.0a	1.9 ± 0.2b	1.6 ± 0.3b	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	1.8 ± 0.6b	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0a	0.1 ± 0.0b	0.1 ± 0.0ab	0.1 ± 0.0a		

[a]Different letters indicate significant differences between VA, M, L, and control (CK) at $P < 0.05$, analysis of variance (ANOVA), followed by Tukey's honestly significant difference tests.

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but their inhibition varied with crop species and organs. Reduction of root dry weight was observed in wheat, maize, and soybean. In particular, (−)-loliolide had a great inhibitory effect on soybean roots even at a low concentration of 3 μg/g. However, maltol and (−)-loliolide stimulated rather than inhibited the growth of maize shoots (Figure 4). The results showed that veratric acid, maltol, and (−)-loliolide exuded from crabgrass could act as allelochemicals to interfere with the growth of wheat, maize, and soybean. However, there were different sensitivities in the crop species and their organs in exposure to the allelochemicals.

Similarly, veratric acid, maltol, and (−)-loliolide reduced the soil microbial biomass carbon. The reduction was observed as the chemicals' incubation periods increased, and significant reduction occurred after 9 days (Figure 5). PLFA profiling showed that the signature lipid biomarkers of bacteria, actinobacteria, and fungi were affected by veratric acid, maltol, and (−)-loliolide. A comparison of PLFA patterns of the soils with veratric acid, maltol, and (−)-loliolide showed that differences in PLFA profiles between incubation periods were significant (Table 1). Changes in actinobacteria and total PLFA occurred during early incubation peroids (1−6 days), whereas changes in fungi and bacteria were observed during late incubation peroids (3−9 days). Furthermore, Gram (+) bacteria were significantly induced at the first 3 days, but no significant changes occurred in Gram (−) bacteria during the whole incubation peroid, indicating that Gram (+) bacteria were more sensitive to veratric acid, maltol, and (−)-loliolide than Gram (−) bacteria. In addition, cyclopropyl phospholipid fatty acids, saturated/unsaturated, (i15:0+a15:0)/16:0, and 18:1ω9c/18:1ω9t were changed by veratric acid, maltol, and (−)-loliolide (Table 1). (i15:0+a15:0)/16:0 may reflect the ratio of bacteria to microorganisms,[33] whereas cyclopropyl fatty acids, saturated/unsaturated, and 18:1ω9c/18:1ω9t are the stress indicators. In particular, 18:1ω9c/18:1ω9t may indicate the degree of nutrient deficiency or environmental stress for microorganisms.[34] These parameters indicated that soil microbial communities were influenced by the allelochemicals exuded from crabgrass. The forward stepwise discriminant analysis of PLFAs showed different soil microbial community structures. Compared with the control, the soil treated with veratric acid was the most different from the soil microbial community structure, followed by (−)-loliolide and maltol (Figure 6). The first discriminant function (DF1) accounted for 77.9% of the variance (eigenvalue = 44.561), and the second discriminant function (DF2) accounted for 18.6% of the variance (eigenvalue = 2.848). DF1 was weighted most heavily by the variables of a15:0, 2OH 16:0, and cy19:0 (5.571, −4.491, and −6.789). The other three PLFAs (3OH 14:0, 18:2ω6,9c, and 18:0) also contributed to this function. DF2 appeared to be marked mostly by variables 17:0, 16:1ω7c, 16:1ω9c (2.850, 2.364, and −3.176) and to a lesser extent by a15:0, 2OH 16:0, and cy19:0 (Figure 6).

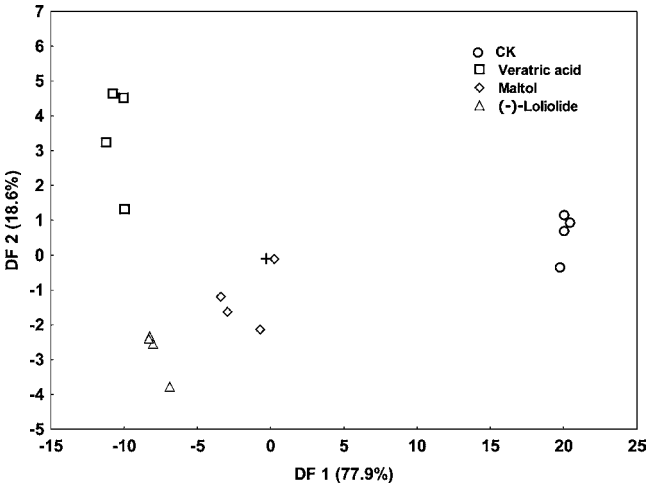

Figure 6. Plots of discriminant analysis of microbial community structure of control and the soil incubated with veratric acid, maltol, and (−)-loliolide. DF indicates a forward stepwise discriminant analysis, and (+) indicates the (0, 0) points.

Soil microbes are mostly heterotrophic organisms depending on the exogenous supply of carbon substrate for growth and development. Root metabolites increase soil organic matter, which favors microbe development and thus increases soil microbial biomass and population.[35] However, allelochemicals, such as veratric acid, maltol, and (−)-loliolide root-exuded from crabgrass in this study, not only provide plant-derived carbon for soil microorganism consumption but also restrict or direct the development of certain soil microbial species.[36] Any effects on microbial species are likely to change the soil microbial community structure and, subsequently, plant growth. Pearson correlation analysis indicated that there were relationships between crop growth and soil microbial community under veratric acid, maltol, and (−)-loliolide application, but the correlation depended on soil

Table 2. Pearson Correlation of Crop Biomass and Soil Microbial Parameters under Veratric Acid, Maltol, and (−)-Loliolide Application[a]

	dry weight								
	wheat			maize			soybean		
microbial parameter	shoot	root	plant	shoot	root	plant	shoot	root	plant
MBC	0.252	−0.264	−0.038	−0.861	0.354	−0.298	0.464	0.604	0.805
total PLFA	**−0.930***	−0.723	−0.856	0.129	**−0.950****	−0.698	−0.227	−0.804	−0.719
fungi	−0.484	−0.166	−0.322	0.490	−0.560	−0.128	−0.121	**−0.924***	0.700
actinobacteria	**0.923***	0.525	0.737	−0.420	**0.957****	0.504	0.603	0.580	0.914*
bacteria	**−0.993***	−0.592	−0.781	0.338	**−0.966****	−0.568	−0.470	−0.693	−0.867
Gram (+)	**−0.955****	−0.613	−0.804	0.320	**−0.981****	−0.592	−0.581	−0.622	−0.865
Gram (−)	−0.838	−0.485	−0.674	0.409	−0.888	−0.455	−0.378	−0.816	−0.863
cyclopropyl	**−0.899***	−0.563	−0.748	0.352	**−0.939***	−0.536	−0.410	−0.765	−0.859
saturated/unsaturated	**−0.988****	−0.704	−0.872	0.200	**−0.997****	−0.688	−0.500	−0.539	−0.796
(i15:0+a15:0)/16:0	0.154	0.704	−0.616	**0.980****	0.058	−0.356	−0.661	−0.019	**0.965****
(18:1ω9c)/ (18:1ω9t)	−0.647	0.935*	−0.112	0.701	0.562	0.131	−0.161	−0.088	−0.790

[a]Boldface indicates significant difference by * (0.05) or ** (0.01).

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microbial parameters, crop species, and organs (Table 2). Significant results (mostly negative) occurred in wheat shoot dry weights and maize root dry weights. There were significantly positive relationships between actinobacteria and wheat shoot and maize root. Similar significantly positive correlations occurred between (i15:0+a15:0)/16:0 and maize shoot and soybean plant. Total PLFA, bacteria, Gram (+) bacteria, cyclopropyl fatty acids, and saturated/unsaturated had significantly negative correlations with wheat shoot or maize root. In addition, there was a significantly negative correlation between fungi and soybean root (Table 2). The results indicated that crabgrass-specific changes in soil microbial communities could generate negative feedback on crop growth through the exudation of allelochemicals.

Allelopathy occurs if the allelochemicals are not only produced in plants but also released by the living plants into their surroundings at phytotoxic concentrations.[10] However, a series of interactions between allelochemicals and soil abiotic and biotic factors may occur when allelochemicals are released through the soil.[11−13] In particular, soil microbial interactions radically alter the environment and give a much better indication of real effects.[12−14] The data generated in this study showed that the concentrations of veratric acid, maltol, and (−)-loliolide root-exuded from crabgrass through the soil were sufficient to elicit the inhibition in crop growth and soil microbial biomass, inducing changes in microbial community structure. Therefore, allelopathic interference of crabgrass with crop plants may be achieved through the release of allelochemicals, veratric acid, maltol, and (−)-loliolide, with direct phytotoxicity and indirect changes in soil microbial community structure.

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