Weed Science 2012 60:212–218

Tabanone, a New Phytotoxic Constituent of Cogongrass (*Imperata cylindrica*)

Antonio L. Cerdeira, Charles L. Cantrell, Franck E. Dayan, John D. Byrd, and Stephen O. Duke*

Cogongrass is a troublesome, invasive weedy species with reported allelopathic properties. The phytotoxicity of different constituents isolated from roots and aerial parts of this species was evaluated on garden lettuce and creeping bentgrass. No significant phytotoxic activity was detected in the methylene chloride, methanol, or water extracts when tested at 1.0 mg ml^{-1}. However, the total essential oil extract of cogongrass aerial parts was active. Bioactivity-guided fractionation of this extract using silica gel column chromatography led to the identification of megastigmatrienone, 4-(2-butenylidene)-3,5,5-trimethyl-2-cyclohexen-1-one (also called *tabanone*), as a mixture of four stereoisomers responsible for most of the activity. Tabanone inhibited growth of frond area of lesser duckweed, root growth of garden onion, and fresh weight gain of garden lettuce with 50% inhibition values of 0.094, 3.6, and 6.5 mM, respectively. The target site of tabanone is not known, but its mode of action results in rapid loss of membrane integrity and subsequent reduction in the rate of photosynthetic electron flow.

Nomenclature: Tabanone, 4-(2-butenylidene)-3,5,5-trimethyl-2-cyclohexen-1-one; cogongrass, *Imperata cylindrica* (L.) Beauv. IMCY; creeping bentgrass, *Agrostis stolonifera* L.; lesser duckweed, *Lemna aequinoctialis* Welw.; garden lettuce, *Lactuca sativa* L.; garden onion, *Allium cepa* L.

Key words: Allelochemical, allelopathy, phytotoxin, essential oil.

Cogongrass is one of the 10 most troublesome and problematic weed species in the world (MacDonald 2004). It is a perennial, rhizomatous grass endemic to tropical and subtropical regions throughout the world and often overtakes areas disturbed by human activities (MacDonald 2004). The plant has become naturalized in the Americas, northern Asia, Europe, and Africa. In the United States, cogongrass thrives in the southeast as a highly invasive species, and has been recognized by the U.S. Department of Agriculture (USDA) as a federal noxious weed. This species is a major impediment to reforestation efforts and is responsible for thousands of hectares of lost native habitat in the southeastern United States (MacDonald 2004). In Mississippi, cogongrass is found in highly variable ecological conditions and in soils varying widely in texture, organic matter content, nutrient status, and pH (4.4 to 8.0) (Bryson et al. 2010). It prefers full sun but will tolerate some shade.

Cogongrass is a medicinal plant in Asia, and the rhizome can be used for this purpose (Chang 2008). Medicinal bioactive compounds produced by this species include cylindol A, an inhibitor of 5-lipoxygenase activity (Matsunaga et al. 1994a); cylindrene, a sesquiterpenoid with inhibitory activity on contractions of vascular smooth-muscle (Matsunaga et al. 1994b); graminone B, a lignan with vasodilative activity (Matsunaga et al. 1994c); and imperanene, a phenolic compound with platelet aggregation inhibitory activity (Matsunaga et al. 1995). Bioactivity-guided fractionation of the methanolic extract of the rhizomes of cogongrass yielded the compounds 5-hydroxy-2-(2-phenylethyl)chromone and 5-hydroxy-2-[2-(2-hydroxyphenyl)ethyl]chromone that showed significant neuroprotective activity against glutamate-induced neurotoxicity in primary cultures of rat cortical cells (Yoon et al. 2006).

The foliage, rhizome, and root residues of cogongrass have allelopathic properties that repress the germination and growth of several weed species (Koger and Bryson 2004; Koger et al. 2004). Water extracts of cogongrass foliage and roots inhibited germination and seedling growth of bermudagrass [*Cynodon dactylon* (L.) Pers.] and Italian ryegrass [*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot] at concentrations as low as 0.5% (wt/vol). Furthermore, incorporation of foliage and rhizome plus root residues in soil at concentrations as low as 0.25% (wt/wt) inhibited seed germination and shoot and root growth, indicating that cogongrass tissue and extracts may contain allelochemicals that contribute to its invasiveness and extreme competitiveness. Additional studies showed that rhizome and root exudates of cogongrass contain numerous plant growth inhibitors with selective effects against tested invasive species at the level of 0.1 mg ml^{-1} (Xuan et al. 2009). The most potent component identified was 2,4-di-tert-butylphenol, followed by iso-eugenol and 4-acetyl-2-methoxyphenol.

The phytotoxicity of cogongrass leaf extracts has also been associated with the presence of *p*-coumaric and *o*-coumaric, gentisic, vanillic, benzoic, and *p*-hydroxybenzoic acids; vanillin; and *p*-hydroxybenzaldehyde (Eussen and Niemann 1981). The activity in cold and hot water extracts of fresh and dry plants parts and natural rain leachates of cogongrass was linked to these compounds, in addition to caffeic, ferulic, chlorogenic, and syringic acids (Hussain and Abidi 1991). The leachates of leaves and roots/rhizomes of cogongrass affected seed germination and seedling characteristics of radish (*Raphanus sativus* L.), Indian mustard [*Brassica juncea* (L.) Czern.], fenugreek (*Trigonella foenum-graecum* L.), and garden tomato [*Solanum lycopersicum* L. var. *cerasiforme* (Dunal) Spooner, J.J. Anderson, & R.K. Jansen] (Inderjit and Dakshini 1991). The root/rhizome leachate was more inhibitory than the leaf leachate. The same study also found that of the 19 fungi detected in soils, *Aspergillus fumigatus*, *Aspergillus niger*, and *Aspergillus candidus* decreased and *Aspergillus flavus* increased in soils with cogongrass.

In the present study, our objective was to reexamine cogongrass for potential allelochemicals, using a systematic bioassay-guided isolation approach that we have successfully used in the past for discovery of phytotoxic phytochemicals (e.g., Castro et al. 2010, Morimoto et al. 2009). We identified a new phytotoxic constituent of the essential oil of the aerial

DOI: 10.1614/WS-D-11-00160.1

* First author: Scientist, Brazilian Department of Agriculture, Agricultural Research Service, EMBRAPA/Environment, Jaguariúna, São Paulo, 13820-000 Brazil; second, third, and fifth authors: Research scientists, United States Department of Agriculture, Agricultural Research Service, Natural Products Utilization Research Unit, University, MS 38677; fourth author: Professor, Plant and Soil Sciences, Mississippi State University, Mississippi State, MS 39762. Corresponding author's E-mail: Stephen.Duke@ars.usda.gov

parts of cogongrass, megastigmatrienone, also known as *tabanone*. Tabanone caused injury via an unknown mechanism of action resulting in loss of plasma membrane integrity.

Materials and Methods

Raw Material and Plant Extraction. Cogongrass raw material was collected in May and June 2010 in Oktibbeha County, MS. A voucher specimen number MISS 79386 has been deposited in the Pullen Herbarium on the University of Mississippi campus. Fresh roots and aerial parts were air-dried at room temperature in a fume hood for 48 h, providing 500 g of both root and shoot material. After grinding in a Wiley Mill grinder (Thomas Scientific, Swedesboro, NJ 08085), 300 g of each plant part was soaked in solvents of increasing polarity. Plant material was soaked sequentially for 24 h in each solvent followed by Buchner funnel filtration, rotary evaporation, and lyophilization. This provided root (CH_2Cl_2 [102.0 mg], 95% ethanol [367.1 mg], and H_2O [183.5 mg]) extractables and aerial part (CH_2Cl_2 [83.0 mg], 95% ethanol [324.3 mg], and H_2O [203.1 mg]) extractables. For production of the essential oil extract, 1 kg of fresh-dried aerial parts of cogongrass was hydrodistilled for 96 h, providing 210.5 mg of essential oils (Figure 1).

Bioassays of Garden Lettuce, Creeping Bentgrass, and Garden onion. Silica gel column chromatographic fractionation of the CH_2Cl_2 extract of the leaves and roots guided by garden lettuce and creeping bentgrass bioassays, according to Dayan et al. (2000), was used to isolate phytotoxic fractions. A filter paper (Whatman No. 1; Whatman Inc., Clifton, NJ 07014) and 5 mg of garden lettuce seeds or 10 mg of creeping bentgrass seeds were placed in each well of a 24-well multiwell plate (Corning Inc., Corning, NY 14831-0001). Test fractions were dissolved in acetone, CH_2Cl_2, or water, depending on the solubility of extractables, and mixed with distilled deionized (DDI) H_2O. The final concentration of acetone was 3%. To each test well, 250 µl of the DDI H_2O mixture was added. Only solvent and DDI H_2O were added to each control well. Plates were covered, sealed with Parafilm, and incubated at 26 C in a Conviron growth chamber set at 173 µmol m^{-2} s^{-1} continuous photosynthetically active radiation. Phytotoxicity was qualitatively evaluated by visually comparing the amount of germination of the seeds in each well with the nontreated controls after 7 d (Dayan et al. 2000). A qualitative estimate of phytotoxicity was evaluated by using a rating scale of 0 to 5, where 0 was no effect, and 5 was no germination of the seeds. Each experiment was repeated, and treatments were triplicated within each repetition. The same methodology was repeated for garden lettuce and garden onion measuring the weight (garden lettuce) and the length of roots (garden onion) to determine the concentration required for 50% inhibition (I_{50}).

Lesser Duckweed Bioassay. The method of Michel et al. (2004) was used to test the phytotoxicity of tabanone on lesser duckweed. Lesser duckweed stocks were grown from a single colony consisting of a mother and two daughter fronds in a beaker on modified Hoagland media containing 1,515 mg L^{-1} KNO_3, 680 mg L^{-1} KH_2PO_4, 492 mg L^{-1} $MgSO_4 \cdot 7H_2O$, 20 mg L^{-1} Na_2CO_3, 1,180 mg L^{-1} $Ca(NO_3)_2 \cdot 4H_2O$, 0.5 mg L^{-1} $MnCl_2$, 0.025 mg L^{-1} $CoCl_2$, 0.025 mg L^{-1} $CuSO_4 \cdot 5H_2O$, and 18.355 mg L^{-1} Fe-EDTA. The medium was adjusted to pH 5.5 with 1 M NaOH and filtered through a 0.2-µm filter (No. 431118, Corning Inc.). Each well of nonpyrogenic polystyrene sterile six-well plates (CoStar 3506, Corning Inc.) was filled with 4,950 µl of Hoagland media, 50 µl of DDI water, and 50 µl of acetone or EtOH containing the appropriate concentration of test compound. The final concentration of acetone or EtOH was 1%. Two, three-frond colonies from 4- to 5-d-old stock cultures were placed in each well. Total frond area per well was recorded by the Scanalyzer (LemnaTec, Würselen 02405 412600, Germany) image analysis system from days 0 to 4 (Michel et al. 2004). Percentage of increase at days 1 to 4 was determined relative to baseline area at day zero.

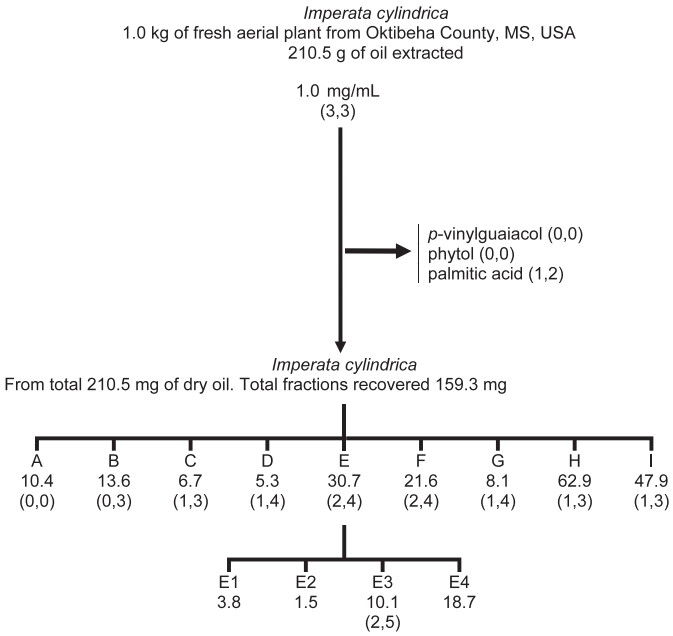

Figure 1. Bioassay guided fractionation of the essential oil of cogongrass, showing the amount recovered and phytotoxicity. Parentheses denotes toxicity at 1.0 mg ml^{-1} to (lettuce, creeping bentgrass) unless noted otherwise. Key: 0, no effect; 5, maximum effect.

Phytotoxicity-Guided Fractionation. Column chromatography was performed using a Biotage, Inc. Horizon Pump (Charlottesville, VA 22906) equipped with a Horizon Flash Collector and fixed wavelength (254 nm) detector. Initially, 210.5 mg of extractables from the aerial part hydrodistillation of the roots was separated on a Biotage 40 + M normal-phase chromatography column (40 to 63 mm, 60 Å, 40 by 150 mm) running at 32 ml min^{-1} using a hexane : EtOAc step-gradient beginning with 100 : 0 to 90 : 10 over 1,200 ml; followed by 90 : 10 to 80 : 20 over 600 ml; followed by 80 : 20 to 50 : 50 over 350 ml; and finishing with 50 : 50 to 0 : 100 for 600 ml. The 24-ml fractions were collected and recombined based on thin-layer chromatography (TLC) similarities and ultraviolet chromatogram (280 nm) peak profiles into nine distinct fractions of 159.3 mg total and submitted to bioassay. Those fractions were named A, B, C, D, F, G, H, I, and J, weighing 10.4, 13.6, 6.7, 5.3, 30.7, 21.6, 8.1, 62.9, and 47.9 mg, respectively. Fraction E was further purified using the Biotage 40 + M column (40 to 63 µm, 60 Å, 40 by 150 mm) running at 32 ml min^{-1} using a hexane : EtOAc step-gradient as described before. The 24-ml

https://doi.org/10.1614/WS-D-11-00160.1 Published online by Cambridge University Press

portions were collected and recombined based on TLC similarities into four subfractions. Subfraction E3 (10.1 mg) was further subjected to bioassay (Figure 1).

Chemical Analysis. Chemical standards, essential oils, and fractions were analyzed by gas chromatography–mass spectrometry (GC-MS) on a Varian CP-3800 GC coupled to a Varian Saturn 2000 tandem mass spectrometry (MS/MS; Varian Medical Systems, Palo Alto, CA 94304-1038). The GC was equipped with a DB-5 fused silica capillary column (30 m by 0.25 mm, with film thickness of 0.25 μm), operated using the following conditions: injector temperature, 240 C, column temperature, 60 to 240 C at 3 C min^{-1} then held at 240 C for 5 min; carrier gas, He; injection volume, 1 μl (splitless). The MS mass ranged from 40 to 650 *m/z*, filament delay of 3 min, target total ion chromatography of 20,000, a prescan ionization time of 100 μs, an ion trap temperature of 150 C, a manifold temperature of 60 C, and a transfer line temperature of 170 C.

^{1}H–nuclear magnetic resonance (NMR) and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a Varian Inova 600 MHz spectrometer. All ^{13}C multiplicities were deduced from 90° and 135° DEPT (distortion-less enhancement by polarization) experiments. High-resolution mass spectra were obtained using an Agilent Technologies (Santa Clara, CA 95051-7201) 1100 high-performance liquid chromatography system coupled to a JEOL USA, Inc. (Peabody, MA 01960-3862) AccuTOF (JMS-T100LC). Commercial tabanone was purchased from BOC Sciences (Shirley, NY 11967).

Whole-Plant Phytotoxicity Assays. The postemergence activity of tabanone was tested in the greenhouse on the following grass weed species: barnyardgrass [*Echinochloa crus-galli* (L.) Beauv.] and large crabgrass [*Digitaria sanguinalis* (L.) Scop.]; and broadleaf weed species: redroot pigweed (*Amaranthus retroflexus* L.), velvetleaf (*Abutilon theophrasti* Medik.), field bindweed (*Convolvulus arvensis* L.), and hemp sesbania [*Sesbania herbacea* (P. Mill) McVaugh], as described before (Dayan et al. 2011).

Tabanone was applied on 3-wk-old plants using a Generation III Spray Booth(Devries Manufacturing, Inc., 28981 870th Ave., Hollandale, MN 56045) equipped with a model TeeJet EZ 8002 nozzle (TeeJet Technologies, Springfield, IL 62703) with a conical pattern and an 80° spray angle. The height from nozzle to soil level was 40 cm for all experiments. The spray head was set to move over the plants at 1.5 km h^{-1}, and the apparatus was calibrated to deliver the equivalent of 360 L ha^{-1}.

The PRE activity of tabanone was tested on large crabgrass and velvetleaf in 30 ml glass vials filled with agriculturally relevant Commerce silty clay loam soil, collected in a field that has never been treated with herbicides near the USDA Jamie Whitten Delta States Research Center in Stoneville, MS.

Membrane Integrity Assessment by Electrolyte Leakage Measurement. The effect of tabanone on plant plasma membranes was determined according to the method developed by Duke and Kenyon (1993) modified into a three-step protocol (Dayan and Watson 2011). Garden cucumber (*Cucumis sativus* L.) seedlings ('straight eight') were grown in a growth chamber with a 16/8 h light/dark cycle for 10 d. Twenty-five, 4-mm cotyledon discs were placed on a 2% sucrose–1 mM 2-(*N*-morpholino)ethanesulfonic acid buffer (MES, pH 6.5) containing various concentrations of tabanone in 60 by 15 mm petri plates. Each plate contained 5 ml of buffer. Control tissues were exposed to the same amount of acetone as treated tissues but without the tabanone. The final concentration of acetone in the dishes was 1% (v/v). Plates were incubated in darkness for 16 h before exposure to high light intensity (1,000 μmol m^{-2} s^{-1}) photosynthetically active radiation in an incubator (model E-30-B; Percival Scientific, Boone, IA 50220-8140). Measurements were made using an electrical conductivity meter (model 1056; Amber Science, Eugene, OR 97402-4147) equipped with a model 858 Conductivity Macro Flow cell at the beginning of the dark incubation period, after 16 h (overnight); at which time, the samples were placed in light, and a final measurement was taken after 8 h of light exposure. Each experiment consisted of three replicates. Maximum conductivity was measured by boiling three samples of each treatment for 20 min.

Photosynthetic Efficiency Measurement by Chlorophyll Fluorescence. Photosynthetic electron rate was measured as described before (Dayan et al. 2009). Three cucumber cotyledon discs were placed on their adaxial side on the surface of a bathing medium with 0 to 10 mM tabanone as described above and incubated in darkness. Control samples received an equivalent volume of solvent. After 1.5 h, the plates were transferred to red light for 30 min before measurements were made. Photosynthetic quantum yield (Y) and electron transport rate were measured using a pulse-modulated fluorometer (model OS5-FL; Opti-Science, Tyngsboro, MA 03051). The instrument was set on kinetic mode and adjusted so that the initial instantaneous fluorescence signal (Ft) value in the control samples was approximately 210. Photosynthetic electron transport rate was determined by the following light treatment: each cycle consisted of a 0.8 s pulse of saturating light generated with a laser diode actinic source to saturate photosystem II (PSII), followed by a 1-s far-red light pulse used to reoxidize PSII, and a 20-s delay to allow PSII to regain steady-state conditions. A total of eight cycles were run for each sample. The samples were returned to darkness for an additional 14 h and were then illuminated for a final 8 h. Chlorophyll fluorescence measurements were repeated at those times.

Presence of Tabanone in Soil under Cogongrass. Soil was harvested from two separate locations where cogongrass was growing. The first soil investigated was obtained at the time of collection of cogongrass in Oktibbeha County, MS, which was the source of the plant material for this study (additional details above). The second soil was harvested in August 2011 from 22-L pots where cogongrass was grown in the greenhouse for 1 yr. In both instances, 600 g of soil was collected under the growing plants by lifting the soil/plant mass and gently shaking the unit while loose soil was collected below. The loose soil appeared to be clean of root material, and the depth of collection varied from 5 to 30 cm . The soil samples were divided into equal parts and extracted with either methanol (125 ml) or methylene chloride (125 ml) under stirring for 1 h. The solvent was concentrated into 1 ml by rotary evaporation under vacuum, and the entire contents placed into a 2-ml GC-MS vial for analysis. Soil extracts were analyzed by GC-MS as described above and compared directly with pure tabanone.

The recovery efficiency of tabanone from soil was determined with a spiking experiment. A portion of the soil from the above experiment was divided into four equal, 10-g parts and labeled A to D. To parts A and B, 5 ml of a tabanone solution (2 mg ml^{-1}) in methanol was added, providing 10 mg of tabanone in 10 g of soil. To the controls, parts C and D, 5 ml of methanol was added. The four parts were allowed to air dry in a fume hood for 48 h. After drying, a 2-h extraction was performed on all parts under stirring. Parts A and C were extracted with methylene chloride (50 ml), and B and D were extracted with methanol (50 ml). Afterward, each part was vacuum filtered and concentrated by rotary evaporation to approximately 5 ml. The filtrates were then brought to volume in a 10 ml volumetric flask in methanol and were directly analyzed by GC-MS as described above. External standard tabanone solutions were prepared at 1.0, 0.1, and 0.01 mg ml^{-1} and analyzed in triplicate, as described above, providing a linear range of response factors. The average response factor method was used to determine the amount of tabanone present in all four soil samples, and the subsequent percentage was calculated based on the possible theoretical total.

Statistical Analysis. Data from dose–response and time–course experiments were analyzed with the dose–response curve module (Ritz and Streibig 2005) using R Version 2.2.1 (R-Development-Core-Team, 2009). Analysis of the means was performed using the SAS statistical software program (Version 10; SAS Institute Inc., Cary, NC 27513-2414).

Results and Discussion

Bioactivity of Organic, Aqueous Extracts, and Essential Oil of Cogongrass. Aerial and root tissues of cogongrass were extracted sequentially with methylene chloride, methanol, and water. None of these extracts were phytotoxic to garden lettuce and creeping bentgrass when tested at 1.0 mg ml^{-1}. On the other hand, the oil obtained from the steam distillation of 1.0 kg of plant material was injurious to garden lettuce and creeping bentgrass at 1.0 mg ml^{-1} (Figure 1).

Identification of Active Constituents in Cogongrass Essential Oil. Preliminary GC-MS, Kovat number identification, and TLC analysis of the essential oil (Figure 2) revealed the presence of three well-known compounds (Figure 3), namely, phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol), palmitic acid (hexadecanoic acid), and vinyl phenol or *p*-vinylguaiacol (2-methoxy-4-vinylphenol). These compounds were not significantly phytotoxic against garden lettuce and creeping bentgrass in the 24-well microassays (Figure 1). Because no activity was detected on these major constituents, purification was further conducted using normal-phase column chromatography with a hexane/ethyl acetate linear gradient to produce fractions A to I (Figure 1).

Fraction E contained active constituents, so that sample was further fractionated by Biotage column separation, and the four subfractions were collected as shown in Figure 1. The NMR analysis of the most active subfraction (E3) identified its constituent as tabanone, which consists of a mixture of four stereoisomers of megastigmatrienone (Aasen et al. 1972) (Figure 3). Commercial tabanone had a GC profile similar to that of the E3 fraction (Figure 4) with a similar ratio of the four stereoisomers of megastigmatrienone in addition to identical MS data for each of the isomers. Therefore, commercial tabanone was used for the subsequent experiments.

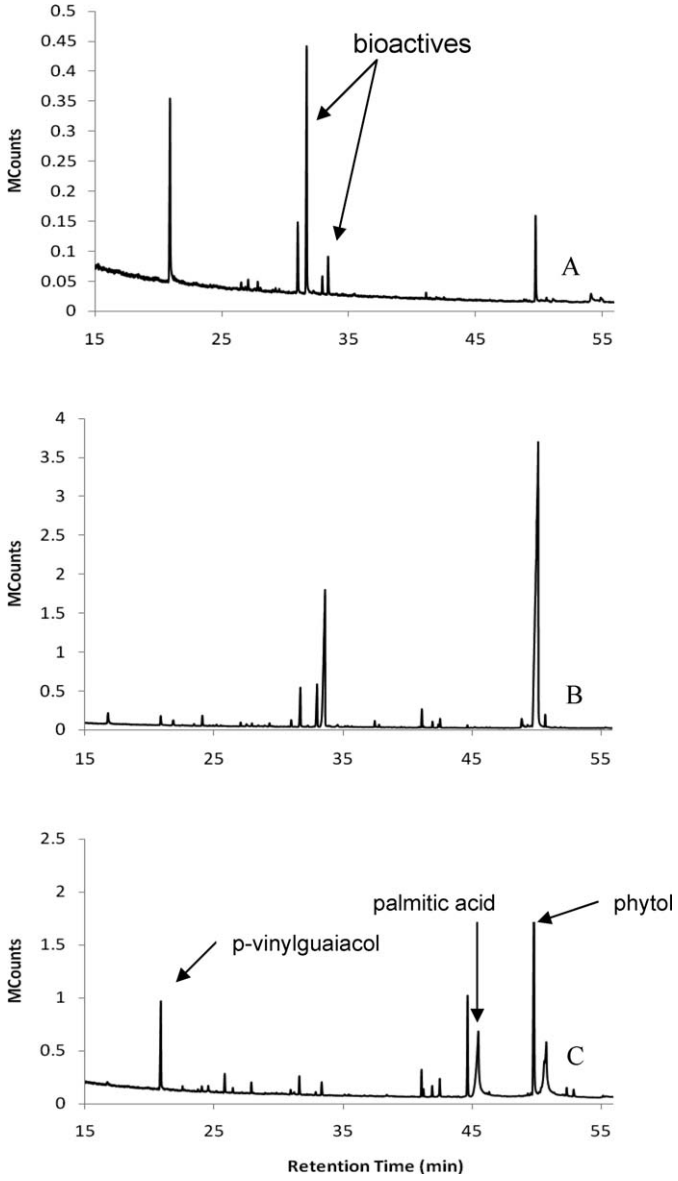

Figure 2. Gas chromatography–mass spectrometry (GC-MS) analysis of (A) fraction E, (B) fraction F, and (C) the essential oil extract from cogongrass.

Phytotoxicity of Tabanone. Tabanone inhibited the growth of the aquatic plant lesser duckweed, with an I_{50} value of 94 μM (Figure 5). This level of potency against *Lemna* species is similar to that of the commercial herbicides EPTC, 2,4-D, and dicamba (Michel et al. 2004). Tabanone was much less active on both garden lettuce (Figure 6) and garden onion (Figure 7), with I_{50} values of 6.5 and 3.6 mM, respectively.

Tabanone had no POST activity on the selected grass and broadleaf weeds tested (data not shown, supplemental figure S1) and was mildly active as a germination inhibitor when amended to soil at the rate of 0.1% (w/w) (Table 1). At 1% (w/w) in the soil, germination was completely inhibited.

Effect of Tabanone on Membrane Stability and Photosynthesis. Tabanone caused electrolyte leakage from garden

https://doi.org/10.1614/WS-D-11-00160.1 Published online by Cambridge University Press

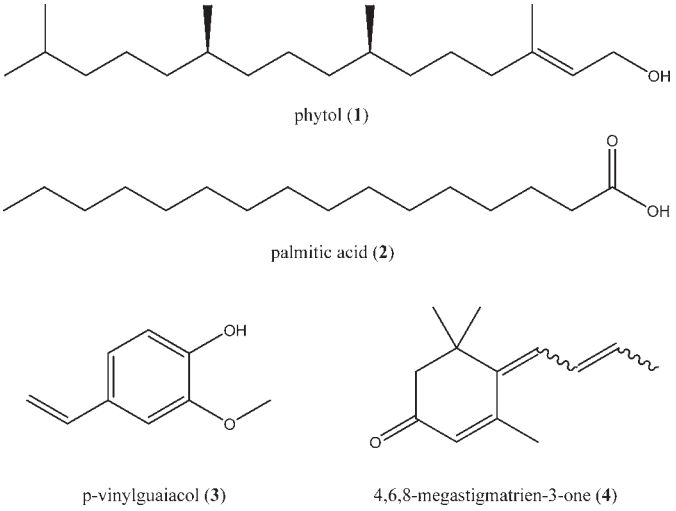

Figure 3. Chemical structure of major compounds and isomers identified the essential oil of cogongrass: (A) 3,7,11,15-tetramethyl-2-hexadecen-1-ol; (B) hexadecanoic acid; (C) 2-methoxy-4-vinylphenol; (D) 4,6,8-megastigmatrien-3-one (tabanone).

cucumber cotyledons after 16 h of dark incubation (Figure 8). Electrolyte leakage continued during the subsequent 8 h of light exposure. The level of electrolyte leakage followed a dose-dependent pattern, with I_{50} values of 3.5 ± 0.3 and 2.1 ± 0.2 mM after 16 h dark incubation and 8 h light exposure, respectively. The photosynthetic transport rate of garden cucumber cotyledon discs was affected by similar high doses of tabanone (Figure 9).

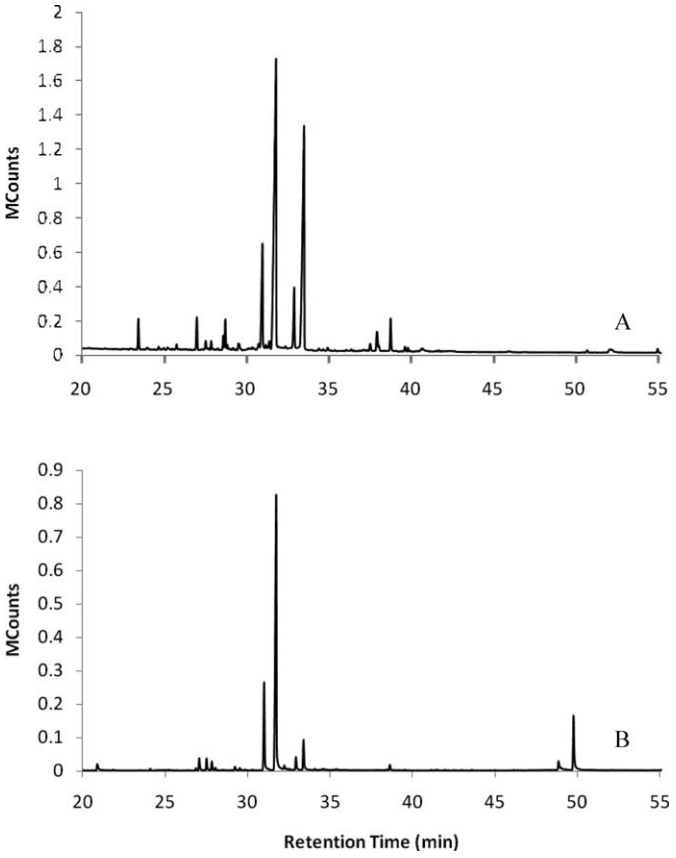

Figure 4. Total ion gas chromatography spectrum of (A) commercial tabanone, with (B) E3 fraction of cogongrass.

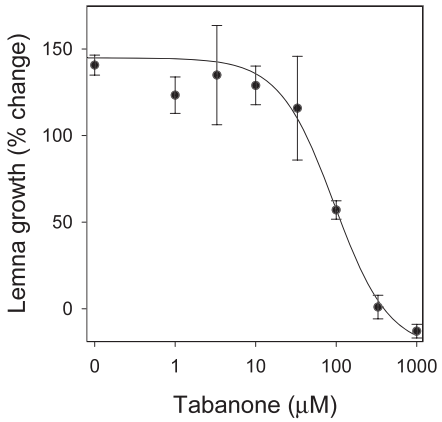

Figure 5. Dose–growth response curve of effects of tabanone on lesser duckweed growth (percentage of change after treatment) 7 d after treatment. Error bars are ± 1 SE; 50% inhibition (I_{50}) was 94 ± 27 µM.

Analysis of Soil Samples for the Presence of Tabanone. Extraction of two soil samples with either methylene chloride or methanol, one collected in an area in Mississippi, where a dense infestation of cogongrass was present, and the other collected in the soils used to grow cogongrass in the greenhouse, failed to detect any tabanone when extracting with either methylene chloride or methanol. To confirm that tabanone can be recovered from soil using our method, 10 mg of tabanone was dried onto 10 g of soil that had been used in the greenhouse study. The tabanone was subsequently extracted using the same technique, and a recovery of 20.4 ± 1.6% was obtained with methylene chloride and 31.6 ± 10.1% with methanol used as the extraction solvents.

Allelopathy has been implicated in the invasiveness of cogongrass (Eussen and Niemann 1981; Hussain and Abidi 1991), and a number of investigations have reported the identification of several organic acids as phytotoxins produced by this noxious plant species. However, these phenolic acids (e.g., benzoic, caffeic, chlorogenic, coumaric, ferulic, gentisic, *p*-hydroxybenzoic, syringic, and vanillic acids) are only mildly phytotoxic and ubiquitous to most plant species, whether allelopathic or not, making them unlikely to be major contributors to allelopathy unless produced at relatively high concentrations (Dayan and Duke 2009). Blum (1996) postulated that, although these compounds are too weak to act as allelochemicals alone, they may contribute some activity

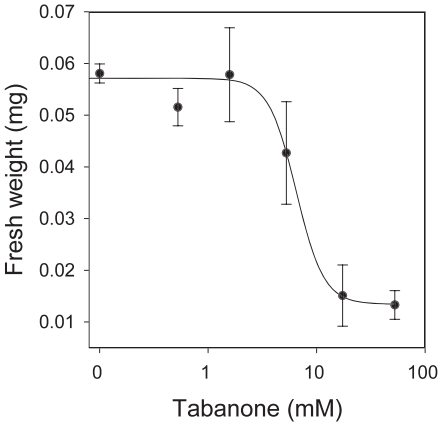

Figure 6. Dose–growth response curve of effects of tabanone on garden lettuce growth 7 d after treatment. Error bars are ± 1 SE. I_{50} was 6.5 ± 1.7 mM.

https://doi.org/10.1614/WS-D-11-00160.1 Published online by Cambridge University Press

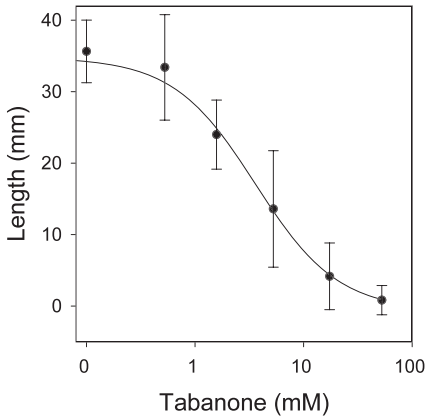

Figure 7. Dose–growth response curve of effects of tabanone on *Allium cepa* root growth 7 d after treatment. Error bars are ± 1 SE; 50% inhibition (I_{50}) was 3.6 ± 1.1 mM.

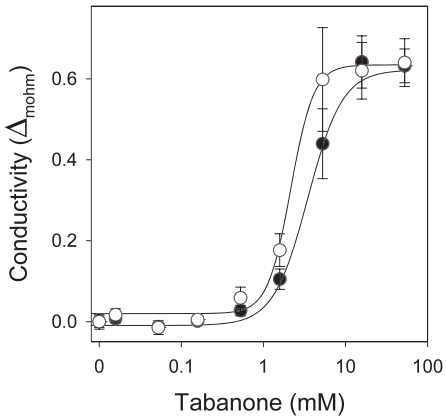

Figure 8. Effect of tabanone on electrolyte leakage of garden cucumber cotyledon discs. In the dark, 50% inhibition (I_{50}) = 3.49 ± 0.27 mM. In the light. I_{50} = 2.15 ± 0.20 mM. Filled circles represent the electrolyte leakage after 16 h dark incubation, whereas white circles represent the leakage after an additional 8 h exposure to light.

when acting in concert with each other or other secondary metabolites. Indeed, mixtures of phenolic acids extend the bioavailability of each acid in soils (Tharayil et al. 2008). Nonetheless, cogongrass also produces other interesting secondary metabolites with known bioactivity, such as chromone analogues with neuroprotective activity (Yoon et al. 2006). Therefore, the objective of this work was to identify other secondary metabolites that may impart the allelopathic potential of cogongrass.

Traditional sequential extraction of cogongrass foliage and root with methylene chloride, methanol, and water did not yield any active compounds when the extracts were tested at as high as 1.0 mg ml^{-1}. However, hydrodistillation of a foliage sample yielded an essential oil with a higher level of activity (Figure 1). Bioassay-directed fractionation led to the identification of tabanone. *Tabanone* is a new term loosely used to refer to a mixture of stereoisomers of 4-(2-butenylidene)-3,5,5-trimethyl-2-cyclohexen-1-one. Original isolation work on these stereoisomers was done on Greek tobacco (*Nicotiana paniculata* L.) in the 1960s and 1970s (Aasen et. al 1972, and reference therein), and that mixture appears to play a significant part in the aroma chemistry of tobacco products.

Tabanone was most active on duckweed species but was much less effective against garden lettuce and garden onion. This difference may be due to its lipophilic physicochemical properties. Indeed, tabanone has a calculated (ACD Labs, Toronto, Ontario, Canada M6C 1T4) log *P* value of 3.588. Its low water solubility prevented it from dissolving in the duckweed growth medium and instead formed a film on the surface. Therefore, the actual concentration on the surface of the medium was probably much higher than the intended final concentration, resulting in an artificially higher level of activity. However, in soil, one would expect such a lipophilic compound to adhere to soil particles, producing microenvironments of high concentration, similar to that of highly lipophilic, soil-applied herbicides (e.g., dinitroanilines).

Tabanone's mechanism of action is not known, but a preliminary experiment revealed the ability of this lipophilic molecule to destabilize membrane bilayers in the absence of light. A number of herbicides cause similar loss of membrane integrity by targeting related lipid biosynthesis or respiratory electron transport (Dayan and Watson 2011). Other herbicides need light to cause this type of electrolyte leakage, and yet, other compounds, including many natural products, cause indirect membrane destabilization resulting in cellular leakage (Dayan and Watson 2011).

Tabanone's effect on photosynthesis does not appear to result from direct inhibition of photosynthetic electron flow. Instead, the reduction in electron transport may be due to the general destabilization of membranes, although the effects on photosynthesis occurred at the same high doses as those on cellular leakage (Figures 8 and 9).

No tabanone could be detected in soil samples collected under cogongrass plants. This could be due to no tabanone

Table 1. PRE activity of tabanone on large crabgrass and velvetleaf germination.

Tabanone	Crabgrass	Velvetleaf
% (wt/wt)[a]	(% germination)[b]	
0	62 ± 13 a	84 ± 5 a
0.1	58 ± 13 a	58 ± 18 b
1	0 b	0 c

[a] Tabanone was dissolved in acetone and mixed to soil. Acetone was allowed to evaporate for 3 d before the soil was used in the germination experiment.

[b] Means of five replications followed by standard deviations; numbers in columns followed by the same letters are not statistically different according to Duncan's Multiple Range Test at $\alpha = 0.05$.

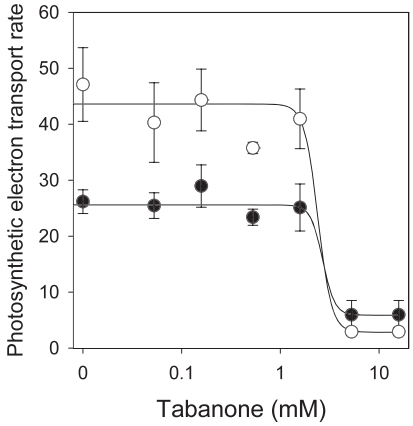

Figure 9. Effect of tabanone on photosynthetic electron transport in garden cucumber cotyledon discs. In the dark, 50% inhibition (I_{50}) = 2.68 ± 2.2 mM. In the light, I_{50} = 2.40 ± 1.6 mM. Filled circles represent the photosynthetic electron rate after 16 h dark incubation, whereas white circles represent the rate after an additional 8 h exposure to light.

https://doi.org/10.1614/WS-D-11-00160.1 Published online by Cambridge University Press

being present or to the inability of our methods to efficiently extract it from soil. As a result, spiking studies were performed to determine whether it was possible to extract tabanone from soil. From the results, it is possible to recover tabanone from spiked soil; however, the recovery was low using this simple, single extraction procedure. Had tabanone been present in the soil at concentrations as high as 0.1% (wt/wt), it should have been detected using this method. Stronger binding to soil components after long-term presence in soil, microbial degradation, and volatility could play a role in our inability to detect it in the soil samples.

Nevertheless, the mild phytotoxicity of tabanone and its apparent lack of accumulation in the soil of growing cogongrass make it unlikely that the compound imparts allelopathic properties to this invasive plant species. Our procedures differed from those of previous investigators probing allelopathy in this species. Others either used plant residues in soil (Koger and Bryson 2004; Koger et al. 2004), root and rhizome exudates (Xuan et al. 2009), or leachates of plant tissues (Hussain and Abidi 1991; Inderjit and Dakshini 1991). Our methods were those of classical bioassay-guided isolation of bioactive compounds that we have used previously to discover phytotoxic compounds from plants and other organisms (Cantrell et al. 2007; Castro et al. 2010; Morimoto et al. 2009). These differences in methods probably account for the different results that we obtained compared with the earlier researchers. The allelochemical or allelochemicals responsible for the apparent allelopathy of cogongrass are still unclear. Future research might focus on combinations of the phytotoxins thus far discovered in realistic bioassays involving plants in soil.

Acknowledgments

The authors thank Solomon Green, III, Robert Johnson, Maria Leticia Zaccaro, and Amber Reichley for technical assistance at the National Center for Natural Products Research, USDA-ARS.

Literature Cited

Aasen, A. J., B. Kimland, S. Almqvist, and C. R. Enzell. 1972. Tobacco chemistry, 15: new tobacco constituents—the structures of five isomeric megastigmatrienones. Acta Chem. Scand. 26:2573–2576.

Blum, U. 1996. Allelopathic interactions involving phenolic acids. J. Nematol. 28:259–267.

Bryson, C. T., L. J. Krutz, G. N. Ervin, K. N. Reddy, and J. D. Byrd. 2010. Ecotype variability and edaphic characteristics for cogongrass (*Imperata cylindrica*) populations in Mississippi. Invasive Plant Sci. 3:199–207.

Cantrell, C. L., S. O. Duke, F. R. Fronczek, W. L. A. Osbrink, L. K. Mamonov, J. I. Vassilyev, D. E. Wedge, and F. E. Dayan. 2007. Phytotoxic eremophilanes from *Ligularia macrophylla*. J. Agric. Food Chem. 55:10656–10663.

Castro, A., C. L. Cantrell, A. L. Hale, and S. O. Duke. 2010. Phytotoxic activity of flavonoids from *Dicranostyles ampla*. Nat. Prod. Commun. 5:1233–1237.

Chang, I.-F. 2008. Ecotypic variation of a medicinal plant *Imperata cylindrica* populations in Taiwan: mass spectrometry-based proteomic evidence. J. Med. Plants Res. 2:71–76.

Dayan, F. E. and S. O. Duke. 2009. Biological activity of allelochemicals. Pages 361–384 *in* A. E. Osbourn and V. Lanzotti, eds. Plant-Derived Natural Products: Synthesis, Function, and Application. New York, NY: Springer.

Dayan, F. E., J. L. Howell, and J. D. Weidenhamer. 2009. Dynamic root exudation of sorgoleone and its in planta mechanism of action, J. Exp. Bot. 60:2107–2117.

Dayan, F. E., J. L. Howell, J. M. Marais, D. Ferreira, and M. E. Koivunen. 2011. Manuka oil, a natural herbicide with preemergence activity. Weed Sci. 59:464–469.

Dayan, F. E., J. G. Romagni, and S. O. Duke. 2000. Investigating the mode of action of natural phytotoxins. J. Chem. Ecol. 26:2079–2094.

Dayan, F. E. and S. B. Watson. 2011. Plant cell membrane integrity as a marker for light-dependent and light-independent mechanisms of action. Pestic. Biochem. Physiol. 101:182–190.

Duke, S. O. and W. H. Kenyon. 1993. Peroxidizing activity determined by cellular leakage, in target assays for modern herbicides and related compounds. Pages 61–66 *in* P. Böger and G. Sandmann, eds. Target Assays for Modern Herbicides and Related Compounds. Boca Raton, FL: Lewis.

Eussen, J. H. H. and G. J. Niemann. 1981. Growth inhibiting substances from leaves of *Imperata cylindrica* (L) Beauv. Z. Pflanzenphysiol. 102:263–266.

Hussain, F. and N. Abidi. 1991. Allelopathy exhibited by *Imperata cylindrica* (L) Beauv. Pak. J. Bot. 23:15–25.

Inderjit and K. M. M. Dakshini. 1991. Investigations on some aspects of chemical ecology of cogongrass, *Imperata cylindrica* (L.) Beauv. J. Chem. Ecol. 17:343–352.

Koger, C. H. and C. T. Bryson. 2004. Effect of cogongrass (*Imperata cylindrica*) extracts on germination and seedling growth of selected grass and broadleaf species. Weed Technol. 18:236–242.

Koger, C. H., C. T. Bryson, and J. D. Byrd. 2004. Response of selected grass and broadleaf species to cogongrass (*Imperata cylindrica*) residues. Weed Technol. 18:353–357.

MacDonald, G. E. 2004. Cogongrass (*Imperata cylindrica*)—biology, ecology, and management. Crit. Rev. Plant Sci. 23:367–380.

Matsunaga, K., M. Ikeda, M. Shibuya, and Y. Ohizumi. 1994a. Cylindol-*a*, a novel biphenyl ether with 5-lipoxygenase inhibitory activity, and a related compound from *Imperata cylindrica*. J. Nat. Prod. 57:1290–1293.

Matsunaga, K., M. Shibuya, and Y. Ohizumi. 1994b. Cylindrene, a novel sesquiterpenoid from *Imperata cylindrica* with inhibitory activity on contractions of vascular smooth-muscle. J. Nat. Prod. 57:1183–1184.

Matsunaga, K., M. Shibuya, and Y. Ohizumi. 1994c. Graminone-B, a novel lignan with vasodilative activity from *Imperata cylindrica*. J. Nat. Prod. 57:1734–1736.

Matsunaga, K., M. Shibuya, and Y. Ohizumi. 1995. Imperanene, a novel phenolic compound with platelet aggregation inhibitory activity from *Imperata cylindrica*. J. Nat. Prod. 58:138–139.

Michel, A., R. D. Johnson, S. O. Duke, and B. E. Scheffler. 2004. Dose-response relationships between herbicides with different modes of action and growth of *Lemna paucicostata*: an improved ecotoxicological method. Environ. Toxicol. Chem. 23:1074–1079.

Morimoto, M., C. L. Cantrell, L. Libous-Bailey, and S. O. Duke. 2009. Phytotoxicity of constituents of glandular trichomes and the leaf surface of camphorweed, *Heterotheca subaxillaris*. Phytochemistry 70:69–74.

R-Development-Core-Team. 2009. R: A language and environment for statistical computing. 2.9.0 ed. Vienna, Austria: R Foundation for Statistical Computing.

Ritz, C. and J. C. Streibig. 2005. Bioassay analysis using R. J. Stat. Softw. 12: issue 5.

Tharayil, N., P. C. Bhowmik, and B. Xing. 2008. Bioavailability of allelochemicals as affected by companion compounds in soil matrices. J. Agric. Food Chem. 56:3706–3713.

Xuan, T. D., T. Toyama, M. Fukuta, T. D. Khanh, and S. Tawata. 2009. Chemical interaction in the invasiveness of cogongrass (*Imperata cylindrica* (L.) Beauv.). J Agric. Food Chem. 57:9448–9453.

Yoon, J. S., M. K. Lee, S. H. Sung, and Y. C. Kim. 2006. Neuroprotective 2-(2-phenylethyl) chromones of *Imperata cylindrica*. J. Nat. Prod. 69:290–291.

Received September 30, 2011, and approved December 5, 2011.