Bull. Environ. Contam. Toxicol. (2001) 66:801–807

DOI: 10.1007/s00128-001-0079-x

Bulletin of Environmental Contamination and Toxicology

Compounds with Selective Toxicity Toward the Musty-Odor Cyanobacterium *Oscillatoria perornata*

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Received: 26 September 2000/Accepted: 17 March 2001

Our efforts continue in the discovery of compounds that may be useful as new selective algicides to help prevent musty "off-flavor" in farm-raised channel catfish (*Ictalurus punctatus*). The filamentous cyanobacterium (blue-green alga) *Oscillatoria perornata* is common in catfish aquaculture ponds in west Mississippi (van der Ploeg et al. 1992) and produces the musty-odor compound 2-methylisoborneol (MIB) (Martin et al. 1991). In catfish, MIB is mainly absorbed across the gills (Boyd and Tucker 1998) with rapid accumulation in the adipose tissue occurring within hours (Martin et al. 1988; Johnsen 1989; Johnsen and Lloyd 1992). Depuration (removal) of MIB from catfish is slow and can take days or weeks depending on several factors including water temperature, rate of catfish metabolism, and the concentration of MIB in the catfish flesh (Johnsen and Lloyd 1992; Johnsen et al. 1996). Catfish deemed by processors to be off-flavor must be held by the producer until they become "on-flavor." Such delays in harvesting can result in financial losses to the producer from additional feed costs, forfeiture of income from foregone sales, interruption of cash flows, and potential losses of held catfish to disease, water quality deterioration, and bird depredation (Engle et al. 1995; Tucker 2000). In 1998, off-flavor problems in farm-raised catfish may have cost producers as much as $60 million (Tucker 2000). *O. perornata* has been attributed as being the major cause of musty off-flavor in pond-cultured catfish in the Mississippi Delta (van der Ploeg et al. 1992; van der Ploeg and Tucker 1993).

Current pond-management practices to alleviate musty off-flavor problems include application of chemicals to catfish ponds to kill or prevent the growth of *O. perornata*, thereby eliminating the MIB-producing microorganism. Copper-based products, such as copper sulfate and chelated-copper algicides, and diuron are the only compounds (herbicides) under current United States Environmental Protection Agency (USEPA) approval for use as algicides in catfish production ponds. Diuron is currently under restricted-use label registration whereby, catfish farmers must follow strict guidelines on application regimes. Unfortunately, copper-based algicides and diuron have broad-spectrum toxicity towards non-target organisms (e.g., catfish) and are not very selectively toxic towards noxious species of cyanobacteria compared to other types of desirable phytoplankton (e.g., green algae). Green algae are preferable to cyanobacteria in catfish aquaculture

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ponds for several reasons: 1) they have not been linked with musty off-flavor problems in farm-raised catfish; 2) they are better oxygenators of the water than cyanobacteria; 3) they are a better base for aquatic food chains than cyanobacteria; and 4) they are not commonly associated with toxin production (Paerl and Tucker 1995). Without careful application of copper-based products and diuron, the entire phytoplankton community can be poisoned and possibly killed resulting in low dissolved oxygen levels and increased levels of ammonia (Boyd 1973). Such conditions can hamper catfish health and ultimately result in the death of catfish. The discovery of new environmentally safe, selective algicides for use in catfish production ponds would benefit catfish aquaculture.

Previous studies have identified several synthetic (herbicides) and natural compounds that are selectively toxic towards *O. perornata*. Unfortunately, the herbicides identified by Schrader et al. (1998) as having a high degree of toxic selectivity toward *O. perornata* are not suitable for further testing (e.g., pond testing) due to such considerations as high application rates required, high economic cost of the compound, and potential toxicity to fish and mammals (e.g., diquat and paraquat have high mammalian toxicity). A large number of natural compounds remain unscreened to determine their potential as new selective algicides. In this study, previously untested herbicides and natural compounds were screened to discover potential new lead compounds to be used as selective algicides for controlling *O. perornata* in catfish production ponds.

MATERIALS AND METHODS

Algal cultures used in the screening were *Oscillatoria perornata* (previously identified as *Oscillatoria* cf. *chalybea*), isolated by van der Ploeg et al. (1995), and *Selenastrum capricornutum* (provided by Dr. J.C. Greene, USEPA, Corvallis, OR), a representative species for green algae that is used by USEPA as a test organism in toxicity studies. Unialgal cultures were grown and maintained under the same conditions as those used by Schrader et al. (1997).

The rapid microplate bioassay of Schrader et al. (1997) was used to screen the compounds listed in Table 1. Technical grade compounds and solvents were used in this study, and compensations for purity were applied when preparing stock solutions. The procedures of Schrader et al. (1997) in preparing stock solutions were used. Reusable fused silica, 96-well microplates (Hellma Cells, Inc., Forest Hills, New York) were used with compounds dissolved in hexane. Microplates were placed in a Percival Scientific model I-36LL growth chamber (Percival Scientific, Inc., Boone, IA) at 28-30°C and illuminated by overhead fluorescent lamps (20 W) at a light intensity of 18-24 $\mu E/m^2/sec$. Optical densities of each well were measured at 24-h intervals for 5 d at 650 nm using a Packard model SpectraCount microplate photometer (Packard Instrument Company, Meriden, CT). Mean values of the optical density measurements for each concentration and controls were graphed, and graphs were used to determine compound LCIC (Lowest-Complete-Inhibition Concentration). LCIC values were used to calculate differential sensitivity (DS) values (LCIC *S. capricornutum*/LCIC *O. perornata*).

Table 1. Compounds[a] screened.

Compound	Solvent	Manufacturer
Abscisic acid	Ethanol	Sigma Chemical Co.
Acifluorfen-methyl	Ethanol	Rohm and Haas
Alloxan monohydrate	H_2O	Aldrich Chemical Co.
Anthranilate	Methanol	Sigma Chemical Co.
Benzofluorfen	Methanol	Rohm and Haas
Benzoic acid	Ethanol	Mallinckrodt Inc.
3-Carene	Hexane	Aldrich Chemical Co.
trans-Caryophyllene	Ethanol	Fluka Chemical Corp.
Chrysophanic acid	Ethanol	Aldrich Chemical Co.
1,4-Cineole	Ethanol	Fluka Chemical Corp.
Cutrine-Plus	H_2O	Applied Biochemists, Inc.
β-Cyano-L-alanine	H_2O	Sigma Chemical Co.
Diayangambin	Methanol	A. Rimando[b]
Ellagic acid	Ethanol	Sigma Chemical Co.
Epiyangambin	Methanol	A. Rimando[b]
Fluorodifen	Ethanol	Chem Service, Inc.
Gallic acid	Ethanol	Eastman Kodak Co.
Glufosinate	H_2O	Riedel-de Haën
α-Humulene	Ethanol	Sigma Chemical Co.
Lactofen	Ethanol	PPG Industries, Inc.
S-Limonene	Ethanol	Aldrich Chemical Co.
L-Lysine	H_2O	Aldrich Chemical Co.
Methyl anthranilate	Methanol	Aldrich Chemical Co.
Methylene blue	Ethanol	Aldrich Chemical Co.
Myrcene	Ethanol	Aldrich Chemical Co.
Nitrofen	Methanol	Rohm and Haas
Nitrofluorfen	Methanol	Rohm and Haas
Oxadiazon	Ethanol	Rhône-Poulenc
Oxyfluorfen	Methanol	Rohm and Haas
Primaquine	Ethanol	Aldrich Chemical Co.
Prometryn	Methanol	Chem Service, Inc.
Protocatechuic acid	Ethanol	Sigma Chemical Co.
Quinone A-7[c]	Ethanol	Chem Service, Inc.
Shikimic acid	H_2O	Sigma Chemical Co.
Streptonigrin	Methanol	J. Hajdu[d]
Sulcotrione	Ethanol	Zeneca Agrochemicals
Vulgarone B	Ethanol	K. Meepagala[e]
Zopfiellin	Methanol	Nissan Chem. Ind., Ltd.
Zosteric acid	H_2O	PhycoGen, Inc.

[a]Compounds in italics are herbicides. [b]A. Rimando, USDA, ARS, NPURU, University, MS. [c]2-(3-Chloro-3-methylbutyl)-3-hydroxyl-1,4-naphthoquinone. [d]J. Hajdu, Dept. of Chemistry, California State University, Northridge, CA. [e]K. Meepagala, USDA, ARS, NPURU, University, MS.

RESULTS AND DISCUSSION

An ideal selective algicide for controlling *O. perornata* in catfish aquaculture ponds is one that includes the following characteristics: 1) environmentally-safe (low persistence); 2) non-toxic to non-target organisms (i.e., catfish) at recommended application rate; 3) inexpensive for the catfish producer in terms of application rate; and 4) has a high degree (i.e., at least one order of magnitude) of toxic selectivity towards *O. perornata* compared to non-target phytoplankton (e.g., green algae). In our study, chrysophanic acid, β-cyano-L-alanine, diayangambin, ellagic acid, gallic acid, glufosinate, α-humulene, L-lysine, primaquine, quinone A-7 [2-(3-Chloro-3-methylbutyl)-3-hydroxy-1,4-napthoquinone], streptonigrin, and zosteric acid are selectively toxic towards *O. perornata* (Table 2). Chrysophanic acid is greater than one order of magnitude more toxic to *O. perornata* than towards *S. capricornutum* (DS = 2). Of the compounds screened in this study, acifluorfen-methyl is the most toxic towards *O. perornata* (LCIC = 0.1 μM) but is not selectively toxic (*S. capricornutum* LCIC = 0.1 μM). Among the selectively toxic compounds, quinone A-7 is the most toxic towards *O. perornata* (LCIC = 1 μM), followed by chrysophanic acid, β-cyano-L-alanine, and streptonigrin (LCIC = 10 μM for each).

Although quinone A-7 is the primary phytotoxic compound of the group of compounds screened due to its high degree of toxicity towards *O. perornata* (LCIC = 1 μM), additional studies determining its impact on catfish and the environment are needed. Some of the other compounds screened in this study have previously been identified as having phytotoxic properties such as β-cyano-L-alanine (Yoshikawa et al. 2000), L-lysine (Kaya and Sano 1996), and methyl anthranilate (Dorr et al. 1998). In addition, Cutrine-Plus® is a commercially available chelated copper algicide. Methyl anthranilate and Cutrine-Plus® do not have high degree of toxic selectivity towards *O. perornata* (based on LCIC results). β-Cyano-L-alanine was previously reported to be anticyanobacterial with growth inhibition of *Oscillatoria amphibia* NIES-361 at 3.1 μg/mL or 27.2 μM (Yoshikawa et al. 2000). In our study, β-cyano-L-alanine completely killed *O. perornata* at 1.14 μg/mL or 10 μM. Yoshikawa et al. (2000) report that β-cyano-L-alanine is non-toxic towards many environmental organisms such as bacteria, yeast and eukaryotic microalgae. Unfortunately, the toxicity requirements of β-cyano-L-alanine for eliminating *O. perornata* (LCIC = 10 μM) are not economically favorable for its use in catfish production ponds. Chrysophanic acid and streptonigrin are also in this category due to LCIC results (10 μM). L-Lysine, although environmentally benign, is not practical for use as a selective algicide due to the high application rates required to kill *O. perornata* (LCIC = 1000 μM).

The compounds 3-carene, trans-caryophyllene, α-humulene, myrcene, and limonene are major volatile constituents of anticyanobacterial crude extracts of tarbush (*Flourensia cernua*) leaves (*Tellez et al. in press*). Unfortunately, none of these individual compounds are very toxic towards *O. perornata*. It is possible

Table 2. Lowest-Complete-Inhibition Concentration of compounds screened.

	Test Organism		
	Oscillatoria perornata	*Selenastrum capricornutum*	
Compound	LCIC (μM)	LCIC (μM)	DS[a]
Abscisic acid	>1000	1000	0
Acifluorfen-methyl	0.1	0.1	0
Alloxan monohydrate	1000	10	0
Anthranilate	1000	1000	0
Benzofluorfen	1	0.1	0
Benzoic acid	>1000	1000	0
3-Carene	>100	>100	0
trans-Caryophyllene	>1000	>1000	0
Chrysophanic acid	10	>100	2
1,4-Cineole	>1000	>1000	0
Cutrine-Plus	10	10	0
β-Cyano-L-alanine	10	100	1
Diayangambin	100	>100	1
Ellagic acid	100	>100	1
Epiyangambin	>100	100	0
Fluorodifen	100	0.1	0
Gallic acid	100	1000	1
Glufosinate	1000	10,000	1
α-Humulene	100	>100	1
Lactofen	>100	1	0
S-Limonene	>100	>100	0
L-Lysine	1000	10,000	1
Methyl anthranilate	1000	1000	0
Methylene blue	1	1	0
Myrcene	>100	>100	0
Nitrofen	100	100	0
Nitrofluorfen	10	1	0
Oxadiazon	100	1	0
Oxyfluorfen	10	0.1	0
Primaquine	100	1000	1
Prometryn	10	1	0
Protocatechuic acid	1000	1000	0
Quinone A-7[b]	1	10	1
Shikimic acid	1000	1000	0
Streptonigrin	10	100	1
Sulcotrione	1000	1000	0
Vulgarone B	1000	1000	0
Zopfiellin	1000	1000	0
Zosteric acid	10,000	100,000	1

[a]DS=Differential sensitivity (as order of magnitude); LCIC of *S. capricornutum*/LCIC of *O. perornata*. [b]2-(3-Chloro-3-methylbutyl)-3-hydroxy-1,4-napthoquinone.

that minor constituents in the active extracts of tarbush may be responsible for the anticyanobacterial properties.

O. perornata is less sensitive to the protoporphyrinogen oxidase (Protox) inhibiting herbicides screened (acifluorfen-methyl, benzofluorfen, fluorodifen, lactofen, nitrofen, nitrofluorfen, oxadiazon, and oxyfluorfen) than *S. capricornutum* (Table 2). Studies are currently underway in our laboratory to determine the reasons for the reduced sensitivity of *O. perornata* towards Protox inhibitors. Protoporphyrinogen oxidase present in the bacteria *Escherichia coli* and *Bacillus subtilis* are reportedly less sensitive (i.e., more tolerant) to Protox inhibitors than many eukaryotic systems (Dayan and Duke 1997).

Of the compounds screened in this study, quinone A-7 appears to be the lead compound for further studies to determine its usefulness in catfish aquaculture to help prevent musty off-flavor episodes caused by *O. perornata*. Additional studies should include determination of half-life (persistence) of quinone A-7 in catfish pond waters, its toxicity towards non-target organisms (e.g., LC50 for channel catfish), its metabolism and residue accumulation in catfish, and potential accumulation in catfish pond sediments. In addition, efficacy testing of quinone A-7 using limnocorrals placed in catfish ponds (*Schrader et al. in press*) will need to be performed.

REFERENCES

Boyd CE (1973) Summer algal communities and primary productivity in fish ponds. Hydrobiologia 41:357-390

Boyd CE, Tucker CS (1998) Pond aquaculture water quality management. Kluwer Academic Publishers, Boston

Dayan FE, Duke SO (1997) Phytotoxicity of protoporphyrinogen oxidase inhibitors: phenomenology, mode of action and mechanisms of resistance. In: Roe RM, Burton JD, Kuhr RJ (eds) Herbicide Activity: Toxicology, Biochemistry and Molecular Biology, Amsterdam, p 11.

Dorr B, Clark L, Glahn JF, Mezine I (1998) Evaluation of a methyl anthranilate-based bird repellent: toxicity to channel catfish *Ictalurus punctatus* and effect on great blue heron *Ardea herodias* feeding behavior. J World Aquacult Soc 29:451-462

Engle CR, Pounds GL, van der Ploeg M (1995) The cost of off-flavor. J World Aquacult Soc 26:297-306

Johnsen PB (1989) Factors influencing the flavor quality of farm-raised catfish. Food Technol 43:94-97

Johnsen PB, Lloyd SW (1992) Influence of fat content on uptake and depuration of the off-flavor 2-methylisoborneol by channel catfish (*Ictalurus punctatus*). Can J Fish Aquat Sci 49:2406-2411

Johnsen PB, Lloyd SW, Vinyard BT, Dionigi CP (1996) Effects of temperature on the uptake and depuration of 2-methylisoborneol (MIB) in channel catfish *Ictalurus punctatus*. J World Aquacult Soc 27:15-20

Kaya K, Sano T (1996) Algicidal compounds in yeast extract as a component of

microbial culture media. Phycologia 35:117-119
Martin JF, Bennett LW, Graham WH (1988) Off-flavor in channel catfish (*Ictalurus punctatus*) due to 2-methylisoborneol and its dehydration products. Water Sci Technol 20:99-105
Martin JF, Izaguirre G, Waterstrat P (1991) A planktonic *Oscillatoria* species from Mississippi catfish ponds that produces the off-flavor compound 2-methylisoborneol. Water Res 25:1447-1451
Paerl HW, Tucker CS (1995) Ecology of blue-green algae in aquaculture ponds. J World Aquacult Soc 26:109-131
Schrader KK, de Regt MQ, Tucker CS, Duke SO (1997) A rapid bioassay for selective algicides. Weed Technol 11:767-774
Schrader KK, de Regt MQ, Tidwell PD, Tucker CS, Duke SO (1998) Compounds with selective toxicity towards the off-flavor metabolite-producing cyanobacterium *Oscillatoria* cf. *chalybea*. Aquaculture 163:85-99
Tucker CS (2000) Off-flavor problems in aquaculture. Rev Fish Sci 8:45-88
van der Ploeg M, Tucker CS, Boyd CE (1992) Geosmin and 2-methylisoborneol production by cyanobacteria in fish ponds in the southeastern United States. Water Sci Technol 25:283-290
van der Ploeg M, Tucker CS (1993) Seasonal trends in flavor quality of channel catfish, *Ictalurus punctatus*, from commercial ponds in Mississippi. J Appl Aquacult 3:121-140
van der Ploeg M, Dennis ME, de Regt MQ (1995) Biology of *Oscillatoria* cf. *chalybea*, a 2-methylisoborneol producing blue-green alga of Mississippi catfish ponds. Water Sci Technol 31:173-180
Yoshikawa K, Adachi K, Nishijima M, Takadera T, Tamaki S, Harada K-I, Mochida K, Sano H (2000) β-Cyanoalanine production by marine bacteria on cyanide-free medium and its specific inhibitory activity toward cyanobacteria. Appl Environ Microbiol 66:718-722