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Soil Biology & Biochemistry 38 (2006) 401–404

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Potential of butyric acid for control of soil-borne fungal pathogens and nematodes affecting strawberries☆

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Received 14 October 2004; received in revised form 24 May 2005; accepted 31 May 2005

Abstract

The effects of butyric acid were evaluated on fungal and nematode endo-parasites of strawberries under controlled laboratory conditions. *Verticillium dahliae*, *Rhizoctonia fragariae*, *R. solani*, *Phytophthora fragariae*, and a *Pythium* sp. were killed after a 2-d incubation in butryic acid-treated sand (0.88 and 8.8 mg g^{-1}). No fungal growth occurred in the presence of vapors from 0.1 and 1 M butyric acid solutions. Gall formation on tomato roots by *Meloidogyne hapla*, and *M. incognita* was reduced by 73–100% relative to controls when egg masses were incubated in butyric acid solution (0.1, 1 M) or treated sand (0.88 and 8.8 mg g^{-1}). Drenching strawberry plants infested with *Pratylenchus penetrans* with butyric acid (0.1 and 1 M) reduced nematode densities by 98–100%. These results suggest that butyric acid warrants further evaluation as an alternative to synthetic soil fumigants for control of nematodes and fungal pathogens in strawberry.

Keywords: Fungicide; Nematicide; Strawberries

Fumigation for control of soil-borne pathogens and weeds in strawberry fields has become routine (Maas, 1998). Researchers and growers are actively seeking alternative strategies to suppress disease and pest incidence. Amendment of soil with crop residues or other types of organic matter can suppress plant-parasitic nematodes (Muller and Gooch, 1982; Rodríguez-Kabana, 1986; McSorley and Frederick, 1999) and fungal pathogens (Lazarovits, 2001). One proposed mode of action involves accumulation of low-molecular-weight organic acids. These are produced by fermentative microorganisms and are readily oxidized to CO_2 and water by microorganisms under aerobic conditions. Organic acids are both phytotoxic (Takijima, 1964) and nematicidal, with butyric acid as the most effective (Johnston, 1959). Browning et al. (2004) demonstrated a 94–100% reduction in population densities of ectoparasitic nematodes affecting turfgrasses when held in sand amended with butyric acid (BA), with exposure to BA vapors resulting in a 96–100% reduction in nematodes. Another organic acid, propionic, is used as a fungicide in grain storage (Luprosil®, BASF, Mount Olive, NJ). The effectiveness of butyric acid in suppressing root pathogenic fungi and endoparasitic nematodes affecting strawberries was evaluated under controlled laboratory conditions.

1. Fungi

Verticillium dahliae Kleb ((24527, American Type Culture Collection, Manassas, VA), *Rhizoctonia fragaria* Husan & McKeen (provided by J. LaMondia, Connecticut Agricultural Experiment Station, Windsor, CT), *Rhizoctonia solani* Kühn (provided by N. Jackson, University of Rhode Island, Kingston, RI), and a *Pythium* sp. (provided by N. Jackson) were cultured on ½ strength potato dextrose agar (Bacto® Difco PDA; Becton Dickinson & Co., Sparks, MD) and incubated at 18–21 °C. *Phytophthora fragariae* Hickman var. *fragariae* (provided by N. Mossier, USDA Horticultural Crops Research Laboratory, Corvallis, OR) was cultured on V8 media (Englander and Roth, 1980) and incubated at 15 °C. Sterile, washed sand (10 g) was placed

☆ Contribution no. 4038 from the Rhode Island Agricultural Experiment Station, Kingston, RI.

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0038-0717/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2005.05.020

in 20-ml glass vials and 1.5 ml butyric acid ((99% purity; Sigma-Aldrich Co., St. Louis, MO) solutions (prepared from a 0.67 M stock) added to achieve final BA concentrations of 0, 0.88 μg, 8.8 μg, 88 μg, 0.88 mg, and 8.8 mg g^{-1} (n = 5). Five plugs (6-mm diam.), removed from the perimeter of fungus cultures, were transferred to each vial, sealed and incubated for 2, 4, and 7 d. To evaluate survival, plugs were retrieved aseptically, transferred to growth media, and growth recorded as + or − after 7 and 14 d. Sclerotia of *R. solani* and *R. fragariae* and microsclerotia of *V. dahliae* were harvested from 2-month-old cultures, and transferred to BA-treated sand and incubated for 2, 4 or 7 days. Following incubation, individual sclerotia and agar blocks containing microsclerotia were transferred to media and subsequent growth monitored.

Fungi were also exposed to BA vapors. A watch glass, placed in the bottom of a sterile plastic desiccator (polypropylene body, polycarbonate top; vol. = 7 l; one per BA concentration), received 35 ml of a 0, 0.1 mM, 1.0 mM, 10 mM, 0.1 M, or 1 M BA solution. Six petri dish bottoms (10-cm-diam.), filled with media and seeded with a 6-mm-diam. fungus plug, were arranged on a 2-tier rack in each desiccator. Cultures were incubated in sealed desiccators until colony growth in the control reached the edge of the dishes, at which time all colony diameters were measured. Sclerotia of *R. solani* and *R. fragariae*, and microsclerotia of *V. dahliae* were also exposed to BA in the vapor phase for 7 days. Following incubation, sclerotia were transferred to growth media and subsequent growth evaluated. All data was subject to one-way analysis of variance and means compared with using the least significant difference test ($P < 0.05$).

Incubation in BA-treated sand had a consistent effect on fungus survival in all species tested. Concentrations of 8.8 and 0.88 mg BA g^{-1} were lethal after 2 d in every instance, except in the case of *R. solani*, whereas incubation in 0.088 mg BA g^{-1} had no effect on fungus survival. Exposure of *R. solani* to 0.88 mg BA g^{-1} killed 44% of the plugs, a significant decrease relative to the controls ($P < 0.01$). The vapor phase was similarly detrimental to fungi. Growth of all fungi was suppressed by vapors of 1 M BA solution (Table 1). *Rhizoctonia* spp. and *Pythium* grew in vapors from 0.1 M BA although at a slower rate than controls. Radial growth rates of *Pythium*, *R. fragariae*, and *V. dahliae* were greater in vapors of 0.1 and 1 mM BA than in their absence. No growth resulted from sclerotia of *R. solani*, *R. fragariae*, or *V. dahliae* following two days in BA-amended sand (0.88 and 8.8 mg g^{-1}) or 1 week in BA vapors (0.1 M and 1 M), whereas 100% growth occurred in the controls.

Soil-borne fungal pathogens can severely reduce nutrient and water uptake by strawberry plants as evidenced by wilting, and reduced yield and runner production. Exposure to high concentrations of BA, whether in solution or vapor phase, not only suppressed mycelial growth but also killed the over-wintering survival structures. The toxicity of BA to fungi likely involves the undissociated form of the acid, which can diffuse rapidly across the cell membrane and cause acidification of the cytoplasm (Rothstein, 1965). Fungi are capable of utilizing organic acids, including BA, as a carbon source (Perlman, 1965). *Candida* spp. added to a 10% swine manure slurry consumed odor-causing fatty acids present in the waste, including 100% of the butyric acid (Kim et al., 2004), while volatile fatty acids present in a 10% swine manure slurry killed microsclerotia of *V. dahliae* (Tenuta et al., 2001), indicating differing levels of tolerance among species.

2. Nematodes

The importance of developmental stage or association with host tissue to the susceptibility of endoparasitic nematodes to the adverse effects of BA was examined.

Sensitivity of juvenile root knot nematodes, *Meloidogyne hapla* Chitwood and *M. incognita* (Koford & White) Chitwood (both provided by G. Abawi, Cornell University, Ithaca, NY), to BA was evaluated by immersing approximately 300 juveniles ((24-h-old) in BA diluted with Ringer's solution [(g l^{-1}) NaCl, 8; $CaCl_2$, 0.2; KCl, 0.2;

Table 1
Mean ± SEM ($n = 5$) radial growth (mm day^{-1}) of different fungal isolates in the presence of butyric acid vapors

Isolate	Concentration of butyric acid solution					
	0	0.1 mM	1 mM	10 mM	0.1 M	1 M
P. fragariae 95–02	1.9a ± 0.1	0.9b ± 0.1	0.3c ± 0.1	0.2cd ± 0.1	0d ± 0.10	0d ± 0
P. fragariae 95–15	1.5a ± 0.0	1.5a ± 0.0	1.4a ± 0.0	0.9b ± 0.1	0c ± 0	0c ± 0
P. fragariae 95–02	1.7a ± 0.1	1.9a ± 0.1	0.5b ± 0.1	0.2c ± 0.1	0c ± 0	0c ± 0
Pythium sp.	16.6c ± 0.3	26.3a ± 0	23.2b ± 0.5	13.9d ± 0.7	3.1e ± 0.5	0f ± 0
R. fragariae-AGA	11.5b ± 0.5	15.8a ± 0	15.3a ± 0.3	15.8a ± 0	2.8c ± 0	0d ± 0
R. fragariae-AGG	12.4b ± 0.2	15.8a ± 0	15.8a ± 0	15.8a ± 0	1.8c ± 0.2	0d ± 0
R. fragariae-AGI	14.6a ± 0.3	15.8a ± 0	15.8a ± 0	15.8a ± 0	7.6b ± 0.8	0c ± 0
R. solani	19.6a ± 0.1	19.4a ± 0.38	18.9a ± 0.30	15.1b ± 1.20	0.8c ± 0.3	0c ± 0
V. dahliae	1.9c ± 0.03	2.7a ± 0.05	1.2d ± 0.06	2.3b ± 0.04	0e ± 0	0e ± 0

Values in the same row followed by the same letter are not significantly different ($P < 0.01$).