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

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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 butyric 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

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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 ($P < 0.01$).

$NaHCO_3$, 0.2] to concentrations of 0, 0.1 mM, 1 mM, 10 mM, 0.1 M, or 1 M. (n = 5). After 4 h, nematodes were rinsed and transferred to Ringer's solution for a 24-h recovery period and viability assessed. Galls with obvious egg masses were then harvested in 1–2 cm root segments from Rutger's tomato plants and two were transferred to each of 10 replicate vials containing a BA solution (0, 0.1 or 1 M) for 24 h, or sand amended with BA (0, 0.88 and 8.8 mg g^{-1}) for 48 h. Galls were transferred to 4-week-old tomato seedlings potted in a pasteurized sand/soil mix (3:1). Plants were harvested and their roots examined for nematode galls after 6 weeks.

In addition, strawberry roots infested with lesion nematodes (*Pratylenchus penetrans (Cobb)* Filipjev & Schuurmans Stekhoven (provided by J. LaMondia)), a migratory endoparasite, were drenched with BA solutions. Uniformly-sized rooted runners of *Fragaria* x *ananassa* Duchesne 'Honeyoye' strawberry were potted in a pasteurized sand/soil mix in 6-cm pots (160 ml) and infested with approximately 1200 lesion nematodes. One month later, 10 replicate pots were drenched with 50 ml of a 0, 0.1 or 1 M BA solution. After a week, lesion nematodes were extracted from growing media with Baermann trays, and collected from roots for 14 d using a modified Seinhorst mist apparatus (Barker et al., 1985).

Immersion of juvenile root knot nematodes in a 10 mM solution for 4 h resulted in 76% mortality, whereas 0.1 M and 1 M killed 100% of juveniles (Fig. 1). When egg masses were incubated in 0.1 and 1 M BA solutions or in sand amended with 0.88 and 8.8 mg BA g^{-1}, subsequent gall formation by *M. hapla* and *M. incognita* was reduced relative to the controls (Fig. 2). A drench of 0.1 M and 1 M BA (which eventually killed the strawberry plants) reduced numbers of lesion nematodes significantly relative to untreated controls (Fig. 3), indicating that strawberry roots offered little protection from BA. This is a critical factor in control, since many pests and disease-causing organisms survive in soil in association with host tissue. Nematodes stunt plants, reduce yield, and increase disease incidence through wounding. Both root knot and lesion nematodes possess a wide host range, including many weed species, such that control through cultural practices is difficult.

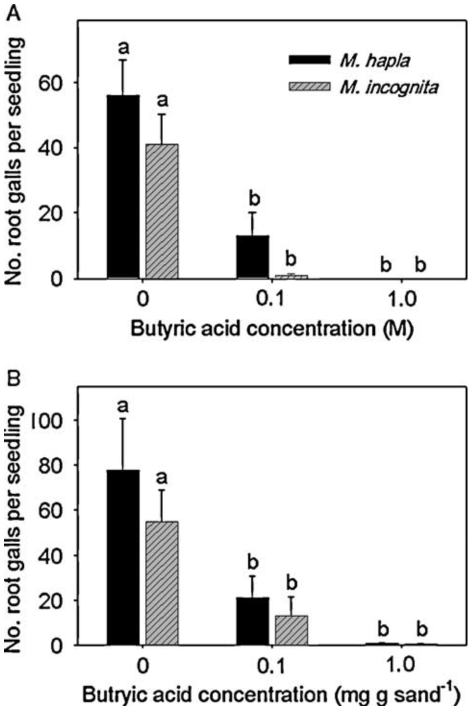

Fig. 2. Number of galls formed on tomato roots (mean ± SEM; $n = 10$) subsequent to (A) immersion of egg masses of *M. hapla* and *M. incognita* in butyric acid solutions of different concentrations for 24 h, or (B) a 2-d incubation in sand amended with different concentrations of butyric acid. Bars designated with the same letter are not significantly different ($P < 0.01$).

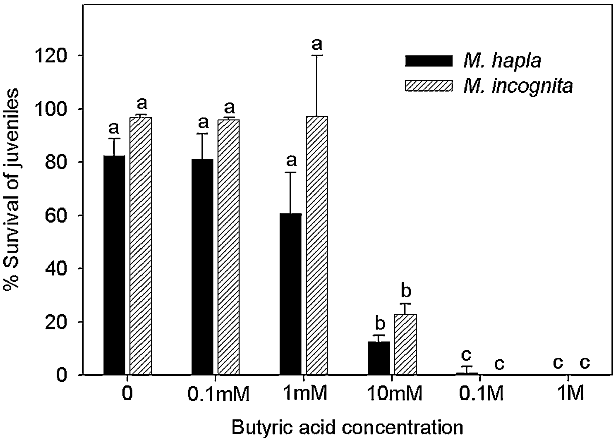

Fig. 1. Survival of free-living juveniles of *Meloidogyne hapla* and *M. incognita* (mean ± SEM; $n = 5$) following a 4-h immersion in butyric acid solutions of different concentration. Bars designated with the same letter are not significantly different ($P < 0.01$).

The data show suppression of fungi and endoparasitic nematodes affecting strawberries through exposure to butyric acid, a natural product of anaerobic fermentation. These results indicate that butyric acid has potential as a pre-plant treatment in strawberry cultivation. In addition, reports of phytotoxicity of organic acids in rice paddies (Takijima, 1964) suggest that butyric acid may also have

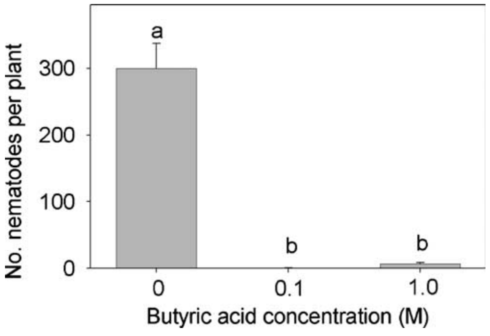

Fig. 3. Number of *P. penetrans* (mean ± SEM; $n = 10$) emerging from strawberry roots over a 2-week period following a 7-d exposure to soil amended with different concentrations of butyric acid. Bars designated with the same letter are not significantly different ($P < 0.01$).

some activity against weeds, another important consideration in strawberry production. To be a viable alternative for the management of strawberry plant pathogens, butyric acid will need to (1) persist long enough to kill pathogens yet dissipate quickly enough to avoid undesirable effects on strawberry plants; (2) have low toxicity towards non-target species; and (3) have costs that are comparable to existing treatments. Persistence of butyric acid in soil will likely be limited through losses via volatilization and leaching. Microbial consumption, suggested by increased growth rates of several fungi in the presence of butyric acid, may also be an important mechanism to diminish persistence. In laboratory experiments using sand-soil mixtures, McElderry et al. (2005) found that exposure of *Tylenchorhynchus* sp. to butyric acid at 8.8 and 88 μg BA g^{-1} soil killed 100% of the nematodes. In addition, BA suppressed soil respiration at concentrations that killed nematodes, but enhanced respiration at lower concentrations, suggesting that microbial utilization of BA as a C substrate depends on the concentration of this organic acid in soil (McElderry et al., 2005). Takijima (1964) reported low levels of soil adsorption for BA, as well as a half-life of 4 days in soil when applied in concentrations found in rice paddy soil.

Butyric acid has a low toxicity to mammals, exhibits slight acute toxicity to aquatic species, and does not have mutagenic potential (Celanese, 2001). In contrast, soil fumigants currently in use are known for their persistence and high toxicity to humans, animals and other species. The pre-plant fumigant options currently available for control of nematodes and fungal pathogens have a high acute and chronic toxicity to a wide variety of non-target species (Ferris, 2001). As such, they pose a more serious health and environmental risk than would butyric acid. The activity of butyric acid as a nematicide and herbicide was investigated previously in rice paddy soil (Johnston, 1959). However, the widespread availability of synthetic pesticides at the time curtailed further interest in butyric acid as an agent for control of plant parasitic nematodes. The results of the present study argue for a field-scale evaluation of butyric acid as an alternative to current treatments, given the likelihood that synthetic fumigants for control of nematodes, weeds and fungi will be unavailable in the near future.

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