Benzaldehyde as a Soil Fumigant, and an Apparatus for Rapid Fumigant Evaluation

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Abstract. An apparatus was developed for the rapid and facile evaluation of soil fumigants in a controlled manner using small volumes of soil. The apparatus consisted of a manifold to which were attached six canisters containing a loamy sand soil (adjusted to -100 kPa soil water potential). The soil was infested with either conidia of *Fusarium oxysporum* or *Trichoderma harzianum*; sclerotia of *Sclerotinia minor*; ascospores of *Talaromyces flavus*; vermiculite colonized with *Pythium aphanidermatum*; or beet (*Beta vulgaris* L., cv. Detroit Red) seed colonized with *Rhizoctonia solani*. Using nitrogen gas (N_2) as a carrier gas, either N_2 or N_2 plus benzaldehyde was passed continuously through the soil for 24, 48, or 72 hours. At all three exposure times, benzaldehyde + N_2 reduced viability of *R. solani* and *S. minor*, and reduced populations of *P. aphanidermatum* and *T. harzianum*. Populations of *F. oxysporum* were reduced after 48 and 72 hours of exposure to benzaldehyde, whereas populations of *T. flavus* were reduced only after 72 hours of exposure. Fumigation with benzaldehyde + N_2 for 24 hours did not affect soil pH 1 week after exposure, but fumigation for 48 or 72 hours temporarily lowered pH from an average of 6.86 to 5.57 and 5.32, respectively. The biocontrol fungus, *T. flavus*, was less sensitive to benzaldehyde than the pathogens or the biocontrol fungus, *T. harzianum*. Thus, combining *T. flavus* with benzaldehyde to enhance biocontrol may be possible.

Methyl bromide (MB) has played a major role in preplant soil treatments for certain high-value crops like strawberries and tomatoes. Growers fumigate soil with methyl bromide to rid it of various soilborne root pathogens, harmful nematodes, and weeds before planting. About 80% of the methyl bromide produced in the United States is used to ready fields before planting [U.S. Environmental Protection Agency (EPA), 1995].

The projected loss of MB as a soil fumigant in 2005 has underscored the need for reliable, environmentally and economically acceptable alternative methods for soil disinfestation (United Nations: Environmental Program, 1992, 1994). Agriculture has also depended heavily on MB for quarantine treatments for the exportation of fruit and vegetables (EPA, 1995). A variety of alternatives to MB has been proposed, including other chemical fu-

migants (Canullo et al., 1992; Ohret et al., 1995), heat sterilization (Litshitz et al., 1983), irradiation (Luckey, 1980), and solarization (Katan and DeVay, 1991). An additional alternative may be the use of naturally occurring plant volatiles (Wilson et al., 1987, 1997). Benzaldehyde is commonly found in fruit as a flavor

compound and readily decomposes to benzoic acid and water (Do et al., 1969). Benzaldehyde has been reported to have activity as a fungicide (Wilson et al., 1987) in vitro and as a nematocide in soil (Bauske et al., 1994).

Evaluation of the effectiveness of soil fumigants applied to planting beds or greenhouse containers to control pathogens, insects, and weed seeds is difficult. Present methodologies require that the soil be fumigated in situ. This involves the fumigation of large volumes of soil and elaborate evaluation procedures to determine the effectiveness of the fumigant. In the present investigation, we devised an apparatus to more conveniently and rapidly evaluate the effectiveness of fumigants for the control of soilborne pathogens.

We elected to test natural soil fumigants in a system that allowed the continuous flow of the fumigant through a container rather than placing the soil and fumigant together in a closed system. This was to prevent high CO_2 buildup that occurs in a closed system. Carbon dioxide is fungicidal to soilborne pathogens (data not shown) and its activity confounds the effects of potential fumigants when they are tested. Also, continuous flow of the fumigant through the soil provided a uniform concentration and distribution of the test fumigant.

This research was undertaken to 1) develop a system for the rapid and reliable evaluation of soil fumigants, and 2) evaluate the capacity of a naturally occurring product to kill plant pathogenic and biocontrol fungi in soil.

Materials and Methods

Fumigation apparatus. The soil fumigation apparatus (Figs. 1–3) consisted of a manifold (Figs. 1D, 2C) to which multiple gastight, cylinder-shaped canisters (Figs. 1H, 2A), each with a small opening in the end, were attached with a quick-coupler (Fig. 2B). Small pieces of rock wool were placed at the openings at each end of the soil-containing canisters to prevent clogging by soil particles. Pressurized

Fig. 1. Schematic of soil fumigation apparatus: (A) nitrogen tank and regulators; (B) stainless steel gastight container; (C) plastic container filled with charcoal loaded with fumigant; (D) stainless steel manifold to which gas flow regulator and soil-containing canisters are attached; (E) gas flow regulator; (F) quick coupler attachment for easy removal of soil-containing canisters; (G) soil-containing canisters; (H) pathogen- and antagonist-infested soil; and (I) outlet nipple to which flow meter is attached.

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Fig. 2. Soil fumigation apparatus: (A) three manifolds in a hood with six soil-containing canisters attached to a fumigant container and nitrogen tank; (B) soil canister detached from the quick coupler attached to the manifold; (C) three soil canisters with flow regulators between the manifold and the quick couplers; and (D) digital flowmeter attached to the nipple at the end of the soil-containing canister.

Fig. 3. Stainless steel can in which fumigants are placed: (A) benzaldehyde being added to the charcoal in the inner container; and (B) can showing the inlet from the nitrogen tank and outlet to the manifold.

gas flowed from the manifold through each canister and the flow-rate was regulated with an adjustable gas-flow valve (Fig. 2C) between the manifold and the soil canister. A stainless steel gas pressure tank (Fig. 3 A and B) was attached to a container that held the volatiles to be tested as soil fumigants. The container was also attached to the manifold through a gastight hose (Fig. 1). Gas pressure was released from the pressure tank, which pressurized the container with the volatiles to be tested. The volatile was then propelled through the manifold with N_2 gas, where it was distributed throughout the canisters containing the soil (Fig. 1H). To avoid microbial toxicity that occurs under anaerobic conditions and high CO_2 buildup, the fumigant flowed continuously through the canister rather than being retained in an airtight compartment. This system allowed uniform retention of the candidate fumigant in the soil within the canister for a defined period and the treatment of multiple replicates at the same time. Uniform fumigation of each canister was obtained by maintaining the same flow rate through each canister. Once soil samples were fumigated, the canisters were opened and soils destructively assayed for fungal activity after 24, 48, and 72 h.

Fumigant application. The fumigant benzaldehyde was impregnated onto a carbon support (Fig. 1C) by means of the incipient wetness method (i.e., the liquid fumigant was added to the carbon up to the point of saturation). One hundred milliliters of liquid benzaldehyde was added to 280 g of activated charcoal. The benzaldehyde-impregnated carbon was deposited in a 1-L aluminum container. This container (Fig. 1B) was equipped with a gas inlet, which reached almost to the bottom, and a gas outlet orifice on the top. Nitrogen gas (N_2) from a pressurized tank was connected to the fumigant container and manifold holding the soil-containing canisters. As N_2 flowed thorough the aluminum container, the fumigant, which was desorbed from the carbon support, was transported through the canisters containing soil (Fig. 1G). Each canister contained 100 g of soil and was subjected to a volumetric flow rate of $50 \text{ mL} \cdot \text{min}^{-1}$ at ambient temperature (Fig. 2D).

For control treatments, soil was exposed to N_2 alone. Preliminary experiments indicated that exposure of the fungi to N_2 alone was equivalent to exposure to air.

To determine the amount of fumigant applied to the soil during the fumigation process, the weight of the fumigant container plus fumigant was recorded initially and again after 1-d intervals. This was then converted to milligrams of volatile per grams of soil, yielding values of 0.263, 0.109, and 0.470 for 24, 48, and 72 h, respectively.

Soil infestation. Four pathogens were selected to represent unrelated taxonomic groups. They were *Fusarium oxysporum*, *Pythium aphanidermatum*, *Rhizoctonia solani*, and *Sclerotinia minor*. In addition, the effect of benzaldehyde was evaluated on two biocontrol fungi, *Trichoderma harzianum* and *Talaromyces flavus*.

Soil used in all experiments was Galestown gravelly loamy sand (pH 5.8, and 77.8% sand, 12.6% silt, 9.6% clay, 0.6% organic matter, cation exchange capacity 4.0) collected in Beltsville, Md. Soil was infested with fungi the day it was collected, passed through a 0.5-cm-pore-size screen, and sent by overnight mail to Kearneysville, W. Va., for fumigation.

All fungi used were from the collection of the Biocontrol of Plant Diseases Laboratory, U.S. Dept. of Agriculture, Agricultural Research Service, Beltsville, Md. *F. oxysporum* FGOR3 (Larkin et al., 1996) is an orange-colored mutant derived from *F. oxysporum* and is pathogenic on watermelon (Larkin et al., 1993). It is comparable to the wild-type *F. oxysporum* in growth, sporulation, and pathogenicity.

Inoculum of *R. solani* isolate Rs23A was prepared on sterile beet seed as previously described by Papavizas and Lewis (1986). To produce inoculum of *Pythium aphanidermatum*, 4 L of vermiculite (grade 3; Schundler, Metuchen, N.J.) were mixed with 1.3 L V-8 vegetable juice (Campbell Soup Co., Camden, N.J.), 6.5 g CaCO₃, and 70 g potato dextrose broth (Difco, Detroit). This mixture and flasks were autoclaved for 1 h each day on two consecutive days. Flasks were inoculated with three 0.7 cm-diameter disks of *P. aphanidermatum* from a 3-week-old water agar culture and incubated under constant fluorescent light under ambient conditions. Eighteen 250-g portions of soil (water content of 4.6%) were mixed with 10 g each of the infested vermiculite mixture. The vermiculite mixture contained $\approx 1.7 \times 10^3$ propagules/g.

Talaromyces flavus isolate Tf-1 was maintained on potato dextrose agar (PDA) (Difco). After 4 to 8 weeks of incubation in the dark at 27 °C, cleistothecia were scraped from the agar and suspended in sterile distilled water (SDW), then disrupted in a blender for 2 min. The resulting suspension was adjusted to 10^6 ascospores/mL. Each of eighteen 250-g portions of soil were mixed thoroughly with 10 mL of this suspension.

Fusarium oxysporum FGOR3 was maintained on PDA in the dark at 25 °C. After 3 weeks of incubation at 25 °C, conidia were collected and blended using the same procedure as for ascospores of *T. flavus* to achieve a suspension of 10^6 conidia/mL. Eighteen 250-g portions of soil were each mixed thoroughly with 10 mL of this suspension.

Sclerotia of *S. minor* isolate 5s-135L were produced on PDA at 25 °C. After 3 weeks, sclerotia were collected by wet sieving through a screen (0.18-mm pore size). Eighteen 250-g portions of soil were each infested with 6.25 g of sclerotia.

Biomass of *T. harzianum* isolate Th-87 was produced as previously described (Papavizas et al., 1984) and stored at 5 °C for up to 6 months before use. Prior to use, *T. harzianum* was plated onto a medium (TME) that is semiselective for *Trichoderma* spp. (Papavizas and Lumsden, 1982) to determine viable colony forming units (CFU). Eighteen 255-g portions of soil were infested with biomass, resulting in 10^6 CFU/g soil (≈ 10 g).

Enumeration of fungal populations. Populations of *F. oxysporum* were determined by dilution plating onto Komada's agar medium (KA) (Komada, 1975). Similarly, populations of *T. flavus* and *T. harzianum* were determined on media that were semiselective for *T. flavus* (TFA) (Marois et al., 1984), or TME, respectively. Additional soil from each replicate was weighed, dried overnight at 100 °C, then reweighed to express colony counts as CFU/g dry weight of soil. Soil infested with *F. oxysporum* or *T. harzianum* was incubated under constant fluorescent light at 25 °C, whereas plates from soil infested with *T. flavus* were incubated in the dark at 27 °C. Colonies of *F. oxysporum*, *T. flavus*, and *T. harzianum* were counted after 6, 10, and 7 d, respectively.

Beet seed infested with *R. solani* were recovered by wet sieving (2-mm pore size), washed 10 min under running tap water, and plated onto water agar (WA). Plates were examined after 24 h incubation in the dark under ambient conditions, and the number of beet seed from which *R. solani* had grown was recorded.

Soil containing *P. aphanidermatum* was serially diluted and plated onto corn meal agar plus antibiotics (CA) (Harris and Sim-

mers, 1968). The number of CFU was recorded after 24 h of incubation under ambient conditions.

Sclerotia of *S. minor* were recovered by wet sieving through nylon mesh (0.18-mm pore size) as previously described (Adams, 1979). Sclerotia were plated onto WA amended with 0.0332 mg/L rose bengal (Fisher, Pittsburgh). After 5 and 10 d incubation in the dark at 25 °C, plates were examined, and hyphal growth was recorded.

Soil pH. For some experiments, after soil was recovered from the canisters and subsamples removed for enumeration of fungi, the remaining soil was again adjusted to -100 kPa and soil pH was determined weekly for each replicate. Ten grams of soil was placed in 25 mL distilled water and mixed for 30 s. The soil suspension was kept under ambient conditions for 1 h, then mixed for 30 s, and the pH was recorded weekly for 4 weeks.

Experimental design and statistical analysis. Eighteen soil-containing canisters were fumigated during each experiment. Soil samples from each canister of the fumigator were considered one replicate. Means of population counts of soil pathogens and antagonists were compared using the analysis of variance procedure in the Statistical Analysis System

Fig. 4. Effect of benzaldehyde + nitrogen gas on: (A) the ability of *R. solani* to grow from colonized beet seed; and (B) sclerotial germination of *S. minor*. Vertical bars represent standard errors of the means.

Fig. 5. Effects of benzaldehyde + nitrogen gas on populations of the pathogens (A) *F. oxysporum*, and (B) *P. aphanidermatum* and on the biocontrol fungi (C) *T. flavus* and (D) *T. harzianum* in soil. Control treatments received the carrier gas (nitrogen) only. Vertical bars represent standard errors of the means.

(SAS, Cary, N.C.). All experiments were repeated twice. Since there were no significant differences between the two runs of each experiment, runs were combined and the data presented are for the average of the two runs of each experiment.

Results

Benzaldehyde + N₂ significantly reduced the viability of *R. solani* and *S. minor* at all three exposure times (Fig. 4). After only 24, 48, or 72 h exposure to benzaldehyde + N₂, *R. solani* grew from 29%, 35%, and 7.5% of beet seed, respectively, compared to growth from 99% of the beet seed in the control treatment. All sclerotia of *S. minor* exposed to benzaldehyde + N₂ for 72 h failed to germinate whereas 95% of the sclerotia in the control treatment germinated. The 24- and 48-h exposure duration to benzaldehyde + N₂ resulted in some sclerotia germination, but the percentages were

much less than those for the controls. Benzaldehyde + N₂ significantly reduced populations of *F. oxysporum* at the 48 and 72 h exposure times (79% and 81%, respectively), but not at the 24-h exposure time (Fig. 5). Populations of *P. aphanidermatum* were reduced by 88% after 24 h exposure to benzaldehyde + N₂. Benzaldehyde + N₂ did not reduce populations of *T. flavus* after 24- or 48-h exposures, but reduced populations by 58% after 72 h of exposure. Benzaldehyde + N₂ significantly reduced populations of *T. harzianum* by 68% after 24 h exposure, and *T. harzianum* was killed after 48 h exposure to benzaldehyde.

Although fumigation with benzaldehyde + N₂ for 24 h did not affect soil pH 1 week after exposure, fumigation for 48 or 72 h significantly ($P \leq 0.05$) lowered soil pH from an average of 6.86 to 5.57 and 5.32, respectively (Data not shown). Two weeks after fumigation there were no differences among treatments with respect to soil pH.

Discussion

Any "out of bed" screening procedure for soil fumigants cannot fully emulate conditions in the field. When fumigants are tested in completely enclosed gastight containers, the buildup of CO₂ and decrease in O₂, through microbial respiration, produces conditions that are antimicrobial, which confounds the results from the fumigants being tested. Nitrogen gas was selected as a carrier for the fumigants because of its nonreactivity with the fumigant and the soil. When we fumigated with N₂ alone, the effect on soil pathogens was the same as fumigation with air.

Benzaldehyde + N₂ was an effective fumigant against the major soil pathogens *F. oxysporum*, *R. solani*, *P. aphanidermatum*, and *S. minor*. Benzaldehyde is a desirable alternative to MB since it is inexpensive, biodegrades easily, and its eventual breakdown products (CO₂ and H₂O) are harmless to humans and the environment.

The differential effect of benzaldehyde + N₂ on soil pathogens and antagonists may be fortuitous and could provide a way of promoting biological control through fumigation. By selecting natural fumigants that inhibit pathogens and promote antagonists, a favorable environment could be created in the soil for antagonist development and subsequent disease suppression. Canullo et al. (1992) found that repeated treatments of soil with furfuraldehyde significantly increased populations of *Trichoderma* sp. and bacteria.

Although fumigation with benzaldehyde + N₂ significantly lowered soil pH values initially, within 2 weeks the pH returned to the control level. Thus, the changes in soil pH are temporary and should not interfere with agricultural production requirements.

Some fungitoxic plant volatiles may provide alternatives to MB for the fumigation of fruit, as several have been shown to have fungicidal activity (Wilson et al., 1987, 1997). Exploration has only begun of the rich reservoir of natural fungicides produced by plants as alternatives to MB. Combinations of such compounds could yield synergistic fungicidal effects. The screening apparatus that we have described should accelerate exploration of natural volatiles as soil fumigants.

The banning of methyl bromide as a pre-plant treatment for horticultural crops in the year 2005 will cause major problems in the culture of strawberries, tomatoes, and other vegetables. Among the efforts to find effective alternatives to methyl bromide for soil fumigation, the use of natural plant-derived volatiles is promising.

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