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Carbon Dioxide as a Potential Water Disinfestant for Phytophthora Disease Risk Mitigation

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Abstract

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The spread of *Phytophthora* spp. through irrigation systems and natural waterways can have a significant impact on plant health and requires mitigation. Pressurized carbon dioxide (CO_2) can inactivate *Phytophthora nicotianae* zoospores but its effectiveness at low pressure and on other species was unknown. This study evaluated the effect of injected CO_2 at 63 to 4,000 ppm in irrigation water on zoospore survival of four *Phytophthora* spp. and infectivity of *P. nicotianae* zoospores. Zoospore survival of *P. nicotianae*, *P. tropicalis*, and *P. pini* was reduced by over 90% at 4,000 ppm and was reduced by 40% at 125 to 2,000 ppm after a 2-h exposure. Survival of *P. megasperma* was less affected by injected CO_2, with a reduction of 37.1% at ≤4,000 ppm. CO_2 treatments at 4,000 ppm for 30 or 120 min of water infested with *P. nicotianae* at zoospore concentrations of 1,000 and 5,000 ml^{-1} reduced disease incidence of annual vinca (*Catharanthus roseus*) by 92 and 75%. Comparable efficacy was observed in the CO_2 treatment at 2,000 ppm. The CO_2 treatments at <2,000 ppm also significantly reduced disease caused by water infested at 1,000 zoospores ml^{-1}. These results indicate that CO_2 may have potential as a safe and effective water disinfestant for *Phytophthora* spp.

The spread of many plant pathogens, including the causal agent of sudden oak death in forestry and horticultural industries, has been associated with infested irrigation water sources (3,11,26). Chlorination is a widely used water treatment because of its effectiveness and relatively low cost at the recommended concentration of 2 ppm (13). However, chlorine gas is extremely hazardous and its effectiveness is influenced by pH, organic and inorganic chemicals in the water, and other factors (13,14,23). Chlorination is most efficacious at a pH of 5 to 6.5, and its efficacy drops with increasing pH (e.g., it can be as low as 20% at pH 8) (13,22). Such low efficacy may be common because pH in many irrigation water sources is high (up to 10.3), particularly during growing seasons (10).

It has been known for more than 100 years that carbon dioxide (CO_2) can exert an inhibitory effect on microbial growth (27). CO_2 has been used in the food industry and elsewhere for inactivation of microorganisms because of its safe, cost-effective, and straightforward application without extensive safety training and equipment. Pressurized CO_2 has been used as a fumigant or injected into juice or milk to kill microorganisms, preventing food spoilage and increasing shelf life (4,7,8,15,19,25). Its use has also been extended to sewage treatment for *Escherichia coli* contamination (20). For plant pathogens, pressurized CO_2 has been effective for inactivating *Phytophthora nicotianae* inocula (1,2). In recent studies, CO_2 was shown to work well at low pressures for inactivation of microorganisms in liquids (1,5,21,24). The treated liquid becomes weakly acidic due to dissolved CO_2 effectively killing pH-sensitive microbes (1,5). CO_2 as an acidifier could improve chlorination efficiency and also, by low pH itself, suppress *Phytophthora* spp., including *P. ramorum*, which are favored by a basic environment (17,18). However, little information is available regarding the effectiveness and cost of CO_2 as a disinfestant or acidifier. This study tested the efficiency of different concentrations of injected CO_2 in irrigation water on zoospore survival and resulting plant infection.

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Materials and Methods

Pond water processing and CO_2 treatments. Water was collected from a nursery irrigation reservoir at a wholesale ornamental nursery located in eastern Virginia on 27 February, 6 April, and 7 June 2012 (Table 1, samples 1, 2, and 3, respectively). For each experiment, 10 liters of water were taken and filtered through membranes with pore sizes of 5.0 and 0.45 μm to remove algae, zoosporic microorganisms and most bacteria in the water, eliminating their interference. Processed water was stored at 4°C and equilibrated to room temperature before pH measurement and use.

Compressed (50 psi) CO_2 was bubbled through 2.5 liters of the filtered water in 3.8-liter plastic containers at a rate of 0.67 liter min^{-1} for 8 min. This resulted in a CO_2 concentration of approximately 4,000 ppm; this estimate was based on the CO_2 mass flow rate with Boyle's law at 25°C and 1 atmosphere of pressure. The bubbled water was then immediately diluted with nonbubbled control water to obtain CO_2 concentrations of 2,000, 1,000, 500, 250, 125, 63, and 0 ppm. The dilutions were made three independent times for each experiment, and 100 ml of each dilution concentration was placed in 167-ml tissue culture containers (Greiner Bio) and used immediately. Duplicates were prepared for pH measurements with a UB10 pH/MV Meter (Denver Instrument) to estimate dissolved CO_2 levels. CO_2 injection reduced the pH of all three irrigation water samples. The pH of all samples injected with CO_2 at 4,000 ppm and their dilutions are listed in Table 1. The dissolved CO_2 levels were estimated with the aquarium CO_2 calculator $CO_2 = 3 \times KH \times 10^{(7\text{-}pH)}$ (http://www.fishfriend.com/aquarium_co2_calculator.html). KH refers to carbonate hardness. It is measured in degrees and converted from $CaCO_3$ ppm (1° KH is equal to $CaCO_3$ at 17.9 ppm). Because $CaCO_3$ in the irrigation water reservoir was 63 ppm, KH of the water samples was 3.5°. The injected CO_2 levels were much higher than those estimated (Table 1) and many of the levels were outside the measurement range of water CO_2 sensors currently on the market. As such, injected CO_2 levels instead of dissolved CO_2 levels were used throughout in this study.

***Phytophthora* spp. and zoospore suspension preparation.** Isolates of *P. megasperma* (42D2), *P. nicotianae* (45H1), *P. pini* (previously *P. citricola*; 43H1), and *P. tropicalis* (7G9) from irrigation water as well as an annual vinca isolate of *P. nicotianae* (3A12) from Virginia were used in this study. Zoospore production followed the method described previously (18). Briefly, agar plugs of