



Evaluation of a copper hydroxide-based algicide to eliminate propagules of *Phytophthora* spp. in naturally-infested streams in South Carolina, USA: a preliminary report.[†]

Inga M. Meadows^{1,*}, Glenn C. Colburn² and Steven N. Jeffers¹

¹ Department of Entomology, Soils, and Plant Sciences, 114 Long Hall, Clemson University, Clemson, SC 29634, USA

² Department of Plant Industry, 511 Westinghouse Road, Pendleton, SC 29670, USA

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*corresponding author: ingam@clemson.edu

Abstract

Many algicides are registered for use in potable water, irrigation ponds, and swimming pools. Because oomycetes, including species of *Phytophthora* de Bary, are closely related to brown algae, algicides also may prove to be effective at eliminating propagules of *Phytophthora* spp. from water. In laboratory studies, we have shown that two copper-based algicides were lethal to zoospores, sporangia, and chlamydozoospores of several species of *Phytophthora*. These algicides also were lethal to propagules of species of *Phytophthora* naturally occurring in six streams in the northwest region of South Carolina, USA. Recently, we have investigated the effects of season and temperature on the efficacy of an algicide containing copper hydroxide (Cu[OH]₂) as the active ingredient on natural populations of *Phytophthora* spp. in two streams. Water samples of 10 L were collected monthly in February, April, June, August, and November 2009 and were maintained at 5, 10, or 22 °C during treatment. Propagules usually were not detected at 2 h after treatment and never were detected at 4 h after treatment. The copper hydroxide algicide was effective in all five months and over a range of temperatures; therefore, it may provide an effective management strategy for species of *Phytophthora* present in some waterways.

Keywords: algicide; chemical control; copper hydroxide; disease management; *Phytophthora* species.

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Introduction

Copper is toxic to species of *Phytophthora* de Bary and has been used to manage plant diseases caused by fungi and oomycetes for over a century (Erwin & Ribeiro, 1996; Ordish & Mitchell, 1967). Copper-based algicides are registered for use in pools, freshwater lakes, drinking water, irrigation water, water at fish hatcheries, and other water systems. Because species of *Phytophthora* are closely related to brown

algae (Dick, 2001) and can be dispersed naturally in water (Erwin & Ribeiro, 1996; Hwang et al., 2009), these algicides also may be useful for eliminating *Phytophthora* spp. present in waterways. In laboratory studies, we have shown that two algicides with copper hydroxide (Cu[OH]₂) and copper carbonate (CuCO₃) as active ingredients were lethal to zoospores of seven species of *Phytophthora* (*P. cactorum* [Lebert and Cohn] Schröeter, *P. citricola* Sawada, *P. citrophthora* [R.E. Smith and E.H. Smith] Leonian, *P. cryptogea*

Pethybridge and Lafferty, *P. nicotianae* Breda de Haan [syn. *P. parasitica* Dastur], *P. palmivora* [Butler] Butler, *P. ramorum* Werres, De Cock, and Man in't Veld) at 1 h after treatment. Sporangia of three of these species (*P. cactorum*, *P. nicotianae*, *P. ramorum*) were eliminated at 4 h after treatment, and chlamydo spores of *P. ramorum* were eliminated at 8 h after treatment (Colburn & Jeffers, 2008, 2010). In 15-L samples of water collected from six naturally-infested streams in South Carolina (SC), the two copper-based algicides were lethal to propagules of *Phytophthora* spp. 1 h after treatment whereas another algicide with hydrogen dioxide as the active ingredient was less effective (Colburn & Jeffers, 2010). Our objectives for this study were to determine the efficacy of a copper hydroxide-based algicide on propagules of *Phytophthora* spp. in naturally-infested suburban waterways at selected months throughout the year and to evaluate algicide efficacy at various temperatures.

Materials and Methods

Two suburban streams were selected for this study that were known to be naturally infested with species of *Phytophthora*: the Reedy River in Greenville, SC and an un-named stream (referred to here as Ashley Creek) in Clemson, SC. Water was collected from both streams in each of five months throughout the year in 2009: February; April; June; August; and November. Each month, 18 10-L samples of stream water were collected, and each sample was placed into a 15-L plastic container. Water samples were collected in 1-L aliquots from the surface of the stream in an area where there was unobstructed flow. Containers were transported to the laboratory, and six containers were placed in each of three rooms that were maintained at different temperatures: 5, 10, and 22 °C. Water samples were allowed to equilibrate to the appropriate room temperature before treatment with algicide. Three containers at each temperature were not treated and three were treated with the algicide K-Tea™ (active ingredient = copper hydroxide; SePRO Corporation, Carmel, IN, USA) at the highest rate listed on the label (1 ppm copper). Three 200-mL aliquots were collected from each container at each of three times — just before water was treated (0 h) and at 2 and 4 h after treatment. The temperature of the water in each bucket was recorded prior to collecting aliquots, and each aliquot was filtered through a polycarbonate membrane filter (47 mm in diameter with 3-µm pores; Sterlitech Corp., Kent, WA, USA). Filters were inverted onto plates of PARPH-V8 agar (Ferguson & Jeffers, 1999) — a medium selective for species of *Phytophthora*. Plates were held in the dark at 20 °C for 7 d; filters were removed at 2–3 d, and colonies of *Phytophthora* spp. were counted. Plates were examined regularly over the next 4–5 d for any additional colonies. Densities of *Phytophthora* spp. — i.e. numbers of colony-forming units (CFU) per

200-mL of stream water — were compared in analyses of variance, and mean densities were separated by Fisher's protected least significant difference with $\alpha = 0.05$ (SAS Version 9.2, SAS Institute, Cary, NC, USA). Representative isolates of *Phytophthora* spp. collected during this study were stored on corn meal agar at 15 °C in the dark and have been included in a permanent collection maintained by S. N. Jeffers at Clemson University, Clemson, SC, USA.

Results and Discussion

Based on visible differences in morphological features, it was evident that several different species of *Phytophthora* were present in the two streams; however, the representative isolates collected during this study have not been identified yet. *Phytophthora gonapodyides* (Petersen) Buisman was recovered most frequently, but other species of *Phytophthora* also were recovered. Identification of these isolates based on morphological and molecular characters is currently underway.

Species of *Phytophthora* were detected in all non-treated samples from both streams in all five months. In general, the mean propagule density in non-treated water samples was greater ($P < 0.001$) in the Reedy River (20.5 ± 0.4 CFU) than in Ashley Creek (14.0 ± 0.3 CFU). The Reedy River drains a larger watershed than Ashley Creek, which may explain the greater densities observed in most months.

Except for the water collected from Ashley Creek in August, propagules of *Phytophthora* spp. were not detected in any of the algicide-treated water samples from either stream at 2 h after treatment in all five months — regardless of temperature. In water collected from Ashley Creek in August, a total of 4 CFU were detected at 2 h after treatment: 1 CFU was detected in each set of water samples held at 10 °C and 22 °C and 2 CFU were detected in the water samples held at 5 °C. *Phytophthora* spp. were not detected in any treated water sample at 4 h after treatment. In previous laboratory studies with this algicide (Colburn & Jeffers, 2008, 2010), zoospores were not detected at 1 h after treatment, sporangia were not detected at 4 h after treatment, and chlamydo spores were not detected at 8 h after treatment. Because propagules usually were not detected in water samples at 2 h after treatment, we suspect that the predominant propagules present in the two streams were zoospores. However, it is possible that sporangia also were present in these streams — particularly in Ashley Creek in August.

In this study, a copper hydroxide algicide effectively eliminated propagules of *Phytophthora* spp. in naturally-infested water at each of the three temperatures used (5, 10, and 22 °C). These temperatures cover much of the natural range that occurs in waterways in South

Carolina throughout most of the year (Meadows & Jeffers, unpublished). The highest rate of application recommended on the algicide label was used in this study to insure that the algicide had the greatest potential to be effective; however, lower rates also may be effective but may be influenced more by temperature. Currently, we are conducting trials with different rates of several algicides over a range of temperatures throughout the year.

The mean densities of *Phytophthora* spp. detected each month (i.e. for both streams combined) varied ($P < 0.001$) although there was no distinct seasonal pattern. The highest mean densities in the non-treated water samples occurred in June (21.0 ± 0.2 CFU) and November (20.6 ± 0.9 CFU), and these densities were significantly greater than the densities in water samples from the other three months. In addition, the densities in samples from April (16.7 ± 0.6 CFU), February (14.9 ± 0.4 CFU), and August (12.9 ± 0.6 CFU) all differed significantly. Perhaps this variation is due to local weather conditions prior to and during sample collection rather than to specific seasonal differences.

Conclusion

The copper hydroxide algicide used in this study was effective in all five months and over a range of temperatures; therefore, this algicide may provide an effective management strategy for the species of *Phytophthora* present in some waterways.

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