Effects of Fungicides on Nitrogen Transformations in Soilless Potting Media

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Abstract. Fungicides were applied at label rates to two commercial soilless potting media in which *Iris siberica* L. crowns had been potted and were subsequently grown under greenhouse conditions. Effects of fungicides on urea hydrolysis were inconsistent and generally insignificant. Ammonium oxidation was inhibited to varying degrees by Truban, Benlate, Banol, and Subdue. In a subsequent experiment, the same fungicides were added to cropped samples of the same media in vitro, followed 12 hours later by a solution containing urea and ammonium. Urea hydrolysis was essentially unaffected by fungicide treatments. Subsequent oxidation of ammonium was inhibited by Truban and Banol only in one medium. Transient accumulation of nitrite was inhibited by Truban but stimulated by Benlate in both media. When added to pure cultures of *Nitrosomonas europaea* and *Nitrobacter agilis*, Truban completely inhibited oxidation of ammonium and nitrite. Benlate partially inhibited oxidation of ammonium and nitrite, while Subdue and Banol partially inhibited oxidation of ammonium but not nitrite. Chemical names used: [Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] (benomyl); *N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)alanine methyl ester (metalaxyl); [2-chloro-6-(trichloromethyl)pyridine] (nitrapyrin); 5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (ethazol); Propyl[3-(dimethylamino)propyl]carbamate monohydrochloride (propamocarb).

Soluble fertilizers used for production of crops in soilless media often contain high proportions of urea or ammonium nitrogen. Thus, hydrolysis of urea and oxidation of ammonium in these media influence N nutrition of plants (Elliott, 1986, 1988a, 1988b; Niemiera and Wright, 1986a, 1986b; Vetanovetz and Peterson, 1987a, 1987b; Wright, 1987). Accumulation of NH_4^+ can result from hydrolysis of organic N sources, such as urea, occurring more rapidly than oxidation of NH_4^+ to NO_2^- . Accumulation of NO_2^- results from oxidation of NH_4^+ to NO_2^- occurring more rapidly than oxidation of NO_2^- to NO_3^- . Transient accumulation of NH_4^+ or NO_2^- is, therefore, the result of differences in activity of NH_4^+ -oxidizing and NO_2^- -oxidizing microbes. This difference could be the result of differences in population sizes, species, or serotype, or environmental factors such as pH, oxygen, or substrate concentration. Urea hydrolysis tends to increase medium pH (Vetanovetz and Peterson, 1987b), which favors increased activity of NH_4^+ oxidizers but may suppress NO_2^- oxidizers (Bunt, 1988).

Urea hydrolysis and nitrification rates are generally low in uncropped media and in peat, but increase with time (Elliott, 1986; Vetanovetz and Peterson, 1987a), presumably associated with increased microbial populations rather than plant activity, since increases occur in media in the absence of plants (H.J.L. and G.C.E., unpublished; Vetanovetz and Peterson, 1987a). Urea hydrolysis in soil is catalyzed both by living organisms and exocellular urease (Bremner and Mulvaney, 1978).

Fungicides applied to control pathogens may also affect urea

hydrolysis and nitrification (Dhanaraj, 1988; Narayana Rao, 1988). Ethazol and metalaxyl are patented as soil nitrification inhibitors for field-grown crops (Adams, 1987; Rideout and Jones, 1987). In a pine bark-peat-sand mixture, ethazol was an effective inhibitor of nitrification, but metalaxyl suppressed nitrification only slightly (Stratton and Good, 1987). Benomyl has been reported to stimulate accumulation of NO_2^- plus NO_3^- in soils (Mazur and Hughes, 1975; van Faassen, 1974). Van Faassen (1974) reported that 20 ppm benomyl in liquid medium inhibited oxidation of nitrite, but 200 ppm inhibited oxidation of both ammonium and nitrite. Propamocarb may also affect nitrification in soil (Foster and McQueen, 1977; Simon-Sylvestre, 1985).

Although nitrification is generally associated with chemoautotrophic bacteria such as *Nitrosomonas* and *Nitrobacter*, acid-tolerant heterotrophic microbes, especially fungi, may be significant contributors to nitrification in acid soils (Lang and Jagnow, 1986). Since peat- or bark-based media tend to be acidic, acid tolerance would be required for activity, and fungicides might thus be particularly inhibitory to heterotrophic nitrification.

The objective of this research was to test the effects of soil-drench fungicides on urea hydrolysis and nitrification in soilless potting media.

Materials and Methods

Fungicides and development of nitrification activity during cropping (Expt. 1). *Iris siberica* were planted in four replicate 1.5-dm³ pots each of Metro-Mix 220 (MM220) and Metro-Mix 350 (MM350) (W.R. Grace, Fogelsville, Pa.). Exact composition of these media is proprietary, but MM220 contains sphagnum peat, vermiculite, and perlite, while MM350 contains sphagnum peat, vermiculite, and processed bark ash. Uncropped MM220 pH was 6.7, and uncropped MM350 pH was 6.6, measured in a saturated slurry. The fungicides Banol, Benlate, Subdue, and Truban were applied according to label directions (Table 1) the next day. Samples were obtained from each pot with a core sampler the following day, and after 2, 4, and 9 weeks. All samples were air-dried at room temperature under a downdraft hood with HEPA-filtered air, thoroughly mixed and screened through a 2-mm mesh sieve before assays. Am-

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Table 1. Quantity of active ingredient of fungicides applied.

Fungicide	Active ingredient	Experiment		
		1	2	3
		mg/pot	mg/tube	
Banal	Propamocarb	320	2.7	16
Benlate	Benomyl	180	1.5	9
Subdue	Metaxyl	6	0.05	0.3
Truban	Ethazol	30	0.30	1.5

monium oxidation rates were determined by measuring NO_2^- accumulation in the presence of chlorate (Elliott, 1988a). Urea hydrolysis was determined by measuring disappearance of urea in samples incubated 4 hr at 37C with 5 mmol urea/cm³ medium in 50 mM Tris buffer, pH 8.0 (Elliott, 1988a). Data were statistically analyzed using SAS General Linear Model (GLM) (SAS, 1985), with treatments, media, and sample dates as class variables.

Fungicides in potting media in vitro (Expt. 2). Fungicide solutions were formulated at label concentrations and added at 2.5 ml/tube to 15-cm³ samples of medium (Table 1) in 50-cm³ polyethylene screwcap conical bottom centrifuge tubes. An untreated control was included. Samples of cropped media were composites pooled from pots in which iris plants had been grown. Cropped MM220 pH was 6.7, and cropped MM350 pH was 5.6, measured in a saturated slurry. Five milliliters of solution containing 7.5 mM urea and 0.75 mM $(\text{NH}_4)_2\text{SO}_4$ was added 12 hr later. Soluble N compounds were extracted from samples after 0 to 7 days of incubation at 25C by adding 15 ml 1.0 M KCl containing 5 ppm phenylmercuric acetate, shaking 1 hr on a reciprocating platform shaker at 180 excursions/min, centrifuging 5 min at 2400 × g, and filtering through medium retention, qualitative grade filter paper (VWR 613; VWR, San Francisco, Calif.). Four replicate tubes were extracted for each medium, treatment, and length of incubation. Urea, NH_4^+ and NO_2^- were assayed calorimetrically (Bremner, 1982; Keeney and Nelson, 1982). Data were analyzed using the SAS GLM procedure (SAS, 1985) with media and fungicides as class variables and length of incubation as a continuous variable.

Fungicides in pure cultures (Expt. 3). Fungicide solutions were added to tubes containing 40 ml autoclaved selective media for *Nitrosomonas europaea* (ATCC strain 19718) and *Nitrobacter agilis* (ATCC strain 14123) at concentrations similar to those in the aqueous phase of the media in Expt. 2 (Table 1). There were five replicates per treatment. Additional treatments included 10 ppm nitrapyrin (N-Serve), an inhibitor of ammonium oxidation, and 10 mM NaClO_3 , an inhibitor of nitrite oxidation, to media for *Nitrosomonas* and *Nitrobacter*, respectively. Treated culture media were inoculated with pure cultures of the two microorganisms, mixed thoroughly, and incubated on a reciprocating shaker held in darkness at 25C. Aliquots of culture media were withdrawn at intervals and assayed calorimetrically for NH_4^+ or NO_2^- . Banol interfered with the calorimetric determination of NH_4^+ in culture media (but not soilless media extracts), so appearance of NO_2^- was used as a measure of NH_4^+ oxidation in pure cultures..

Results

Fungicides and development of nitrification activity during cropping Expt. 1). Fungicide applications at the start of cropping did not significantly affect the development of urea hydrolytic activity in either medium (data not shown). Truban decreased NH_4^+ oxidation, expressed as nitrite accumulation, in both media

(Fig. 1), but the effect was more persistent in MM220 than in MM350. Banol and Benlate inhibited NH_4^+ oxidation in MM220 at 0 and 4 weeks after application, respectively. Banol and Subdue inhibited NH_4^+ oxidation in MM350 up to 2 weeks after application.

Effects of fungicides in potting media in vitro (Expt. 2). Urea hydrolysis was not significantly affected by fungicide treatments in either medium, urea decreasing from ≈ 2.5 to ≈ 0 mol·m⁻³ within ≈ 5 days in both media. Ammonium accumulated to levels in excess of 3 mol·m⁻³ as urea was hydrolyzed (Fig. 2). In MM220, NH_4^+ levels declined significantly at the end of the incubation in the control, Benlate, and Subdue treatments, but not in the Truban or Banol treatments. Truban completely inhibited NO_2^- accumulation, while Benlate markedly increased it, in both media (Fig. 3). In MM220, Banol inhibited NO_2^- accumulation during the final 48 hr of the incubation.

Fungicides in pure cultures (Expt. 3). Ammonium oxidation (NO_2^- formation) by *Nitrosomonas* was completely inhibited by N-serve and Truban, and partly inhibited by Banol, Benlate, and Subdue (Fig. 4). Nitrite oxidation by *Nitrobacter* was completely inhibited by chlorate and Truban (Fig. 5). Benlate partially inhibited NO_2^- oxidation in one trial (Fig. 5a), but in a replicate trial, in which initial rate of oxidation was slower, Benlate completely inhibited NO_2^- oxidation (Fig. 5).

Discussion

The effects of fungicides on the development of NH_4^+ -oxidizing activity, NH_4^+ and NO_2^- accumulation in soilless potting media in vitro, and on the activity of pure cultures of *Nitrosomonas* and *Nitrobacter* are indicative of the complex interrela-

Fig. 1. Effect of fungicides on the development of NH_4^+ oxidation activity (NO_2^- accumulation in the presence of NaClO_3) in soilless potting media under greenhouse conditions. Mean separation within a group by Waller-Duncan k ratio *t* test, *k* = 100. NSD, no significant differences.

Fig. 2. Effect of fungicides on NH_4^+ accumulation in soilless potting media in vitro. Error bars, shown if greater than dimension of symbols, indicate standard deviation, $n = 4$.

Fig. 3. Effect of fungicides on NO_2^- accumulation in soilless potting media in vitro. Error bars, shown if greater than dimension of symbols, indicate standard deviation, $n = 4$.

Fig. 4. Effect of fungicides on NH_4^+ oxidation (NO_2^- accumulation) in pure cultures of *Nitrosomonas europaea*. Standard deviations, $n = 5$, less than dimensions of symbols.

Fig. 5. Effect of fungicides on NO_2^- oxidation in pure cultures of *Nitrobacter agilis*. A and B represent independent trials. Error bars, shown if greater than dimension of symbols, indicate standard deviation, $n = 5$.

tions of N transformations. For example, transient accumulation of NO_2^- in media treated with Benlate (Fig. 3) evidently involves partial inhibition of both NH_4^+ and NO_2^- oxidation by the bacteria responsible for each step (Figs. 4 and 5). However, the degree of inhibition apparently is related to the activity (rate of oxidation) of the microbes involved (Fig. 5).

Effects of fungicides on the activity of microbes in media in situ are likely to be different from effects in pure cultures or in vitro, since microbes in situ might escape suppression because of nonuniform distribution of active ingredient in pots or inactivation of active ingredient by media components. Some NH_4^+ -oxidizing activity was maintained in media treated with Truban under greenhouse conditions (Fig. 1), although Truban completely inhibited activity in pure cultures and in vitro. In addition, the effects of fungicides in vitro could be modified in situ by the activity of plants. Uptake of N could rapidly change concentration of soluble N fractions in the medium, and alterations of substrate : product ratios and concentrations could affect the kinetics of oxidation.

Fungicide applications may have substantial effects on N transformations in soilless potting media, which could in turn be significant for plant growth. In particular, suppression of ammonium oxidation could be harmful for plants susceptible to ammonium toxicity (Haynes and Gob, 1978). Nitrite accumulation also might lead to toxicity (Birch and Eagle, 1969). Inhibition of vitrification by fungicides has been implicated in incidence of fungicide phytotoxicity (Powell, 1988). However, we are not aware of any direct evidence for either ammonium or nitrite toxicity occurring as a result of fungicide applications. Furthermore, it must be borne in mind that the effects we observed are both transient and highly variable. Thus, any potentially deleterious results of suppressed vitrification might occur only under unusual circumstances.

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