

Inhibitory Effect of Nitrapyrin on Three Genera of Ammonia-Oxidizing Nitrifiers

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Five strains of *Nitrosomonas* and one each of *Nitrospira* and *Nitrosolobus* were examined for sensitivity to the nitrification inhibitor nitrapyrin. Considerable variation in sensitivity was observed, with some strains about five times more resistant than others. Sensitivity to nitrapyrin varied more with strain than with genus.

Nitrification of ammonia or ammonia-yielding fertilizers by the nitrifying bacteria results in oxidized forms of nitrogen susceptible to loss from the soil by leaching, denitrification, and volatilization. Inhibition of the activities of the nitrifiers thus has the potential to conserve fertilizer nitrogen added to agricultural soils.

Nitrapyrin [2-chloro-6-(trichloromethyl)pyridine] is the most widely used inhibitor of nitrification. It inhibits the first step of nitrification, the oxidation of ammonia to nitrite (4, 5). The inhibitory characteristics of nitrapyrin have been demonstrated in pure culture only with the ammonia oxidizer *Nitrosomonas europaea*. The objective of this study was to examine the sensitivity of other strains of *Nitrosomonas* and other genera of ammonia oxidizers to nitrapyrin.

Seven strains of ammonia oxidizers were tested. Five were from the genus *Nitrosomonas*, and one each were from the genera *Nitrospira* and *Nitrosolobus*. The *Nitrosomonas* strains were selected on the basis of apparent differences in morphology or growth responses. Four of the strains of *Nitrosomonas* (*N. europaea* ATCC 17198, Tara 7/15, EK, and SI), the *Nitrospira* (Sp-1) strain, and the *Nitrosolobus* (Fargo) isolate were described previously (2). The remaining strain of *Nitrosomonas*, D41, was isolated from an intertidal sediment by using a marine salts medium (1).

Concentrated solutions of nitrapyrin (Dow Chemical Co., technical grade 93.7%, lot 37) were prepared in ethanol for initial experiments and in dimethyl sulfoxide (DMSO) for later experiments. A 0.4% solution of nitrapyrin in ethanol was used to prepare stock solutions of 20, 5, and 1 μg of nitrapyrin per ml in ethanol-water (ethanol 0.5%). These stock solutions were filter sterilized. Addition to the nitrifier medium resulted in final concentrations of 0.2, 0.05, and 0.01 μg of nitrapyrin per ml and 0.005% ethanol.

With DMSO as the solvent, stock concentrations contained 2,500, 250, 50, and 12.5 $\mu\text{g}/\text{ml}$. These solutions were filter sterilized and diluted 250:1 when added to the nitrifier medium, giving concentrations of 10, 1.0, 0.2, and 0.05 μg of nitrapyrin per ml and 0.4% DMSO.

The freshwater and saltwater (strain D41 only) media in these studies were described elsewhere (1, 2). Flasks containing 200 ml of medium were inoculated with 0.5 ml of exponentially growing culture. Growth was allowed to proceed until about 0.05 mM nitrite had been formed. Samples of 10 ml were then transferred to culture tubes. Triplicate culture tubes received either 0.1 ml of nitrapyrin in 0.5% ethanol or 0.4 ml of nitrapyrin in DMSO. *Nitrosomonas* strains Tara 7/15, SI, and EK, *Nitrosolobus* strain Fargo, and *Nitrospira* strain Sp-1 received nitrapyrin in 0.5% ethanol. The later studies (*Nitrosomonas* strains *N. europaea*, SI, and D41) received nitrapyrin in DMSO. Controls received either 0.1 ml of 0.5% ethanol or 0.4 ml of DMSO. Nitrite as measured by the method of Bremner (3) was used as an index of growth. Incubation temperatures were maintained between 23 and 25°C.

Figure 1 shows the production of nitrite for three of the initial five incubations. A maximum nitrapyrin concentration of 0.2 $\mu\text{g}/\text{ml}$ was chosen because it was the concentration at which Campbell and Aleem (4) observed complete inhibition of growth for their strain of *N. europaea*. Inhibition as complete and as rapid as that observed by Campbell and Aleem (4) was seen here at 0.2 $\mu\text{g}/\text{ml}$ for only one strain of ammonium oxidizer, Tara 7/15 (Fig. 1a). Although not as immediate, inhibition appeared to be almost complete at 0.2 $\mu\text{g}/\text{ml}$ with the *Nitrospira* isolate (Sp-1, Fig. 1b). *Nitrosolobus* strain Fargo (Fig. 1c) continued to produce nitrite at a decreased but exponentially increasing rate with nitrapyrin present at 0.2 $\mu\text{g}/\text{ml}$. Responses of the two remaining

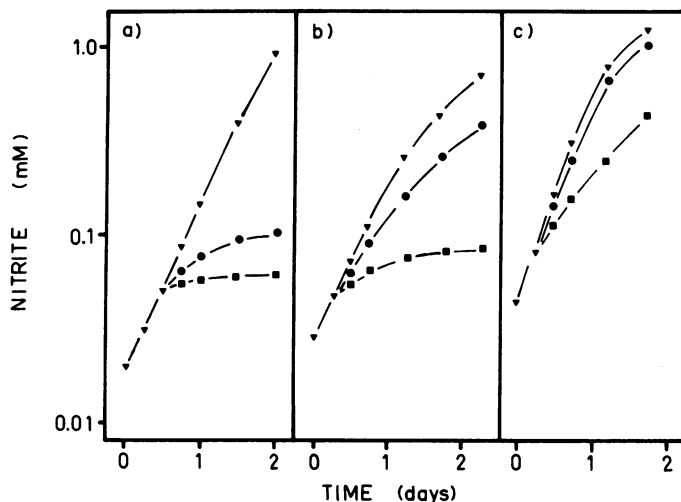


FIG. 1. Ammonia oxidation by *Nitrosomonas* strain Tara 7/15 (a), *Nitrosospira* strain Sp-1 (b), and *Nitrosolobus* strain Fargo (c) as inhibited by nitrapyrin: control (∇); 0.05 $\mu\text{g/ml}$ (\bullet); 0.2 $\mu\text{g/ml}$ (\blacksquare).

strains, both *Nitrosomonas*, resembled that of *Nitrosolobus*.

The results for two of the strains incubated with higher concentrations of nitrapyrin are given in Fig. 2. At 1 μg of nitrapyrin per ml, the SI strain was completely inhibited after a short lag. The response of *N. europaea* (not shown) was almost identical to the response of the SI strain, whereas the D41 strain (Fig. 2b) appeared to be slightly less sensitive.

Campbell and Aleem (4) reported that 1 $\mu\text{g/ml}$ was required for complete and immediate inhibition of ammonium oxidation, and that 0.2 μg of nitrapyrin per ml was required for effective control of growth of their strain of *N. europaea*. We found that complete and immediate inhibition could require as much as 10 μg of nitrapyrin per ml for some strains of ammonium oxidizers, and that 0.2 $\mu\text{g/ml}$ was ineffective in stopping growth for five of the seven cultures tested. Variation in sensitivity does not seem to be associated with generic differences, since strains of the genus *Nitrosomonas* included the most sensitive (Tara 7/15) and the least sensitive (D41). The range of sensitivity among strains seems to be about a factor of five.

From an agronomic point of view, variation in sensitivity might be very important. Goring (5) found that effective inhibition of nitrification required between 0.05 and 20 $\mu\text{g/g}$ depending on soil type. He attributed the differences to variations in pH and organic matter content. It appears that strain sensitivity may also be a factor. It seems likely that soils which show good response at low-level applications of nitrapyrin may have very sensitive strains present. Contin-

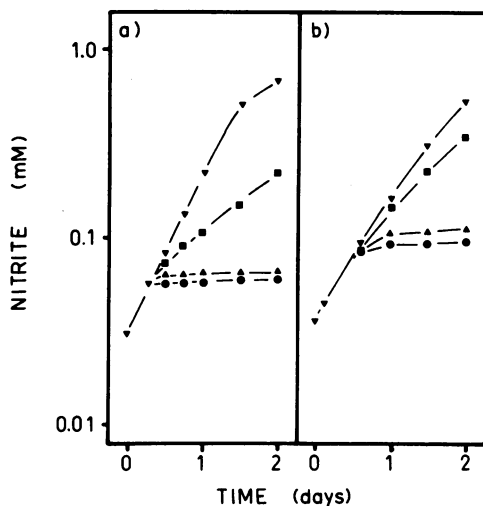


FIG. 2. Ammonia oxidation by *Nitrosomonas* strain SI (a) and *Nitrosomonas* strain D41 (b) as inhibited by nitrapyrin: control (∇); 0.2 $\mu\text{g/ml}$ (\blacksquare); 1.0 $\mu\text{g/ml}$ (\blacktriangle); and 10 $\mu\text{g/ml}$ (\bullet).

ued application of nitrapyrin, however, might select for less sensitive strains, leading to a need for progressively higher application rates.

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