Anhydrous Ammonia as a Soil Fungicide Against Fusarium and Fungicidal Activity in the Ammonia Retention Zone

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Cooperative Investigations, Soil and Water Conservation Research Division and Crops Research Division, ARS, USDA, and the Experiment Stations of Idaho, Montana, Oregon, Utah, and Washington. Scientific Journal Paper No. 3368, College of Agriculture, Washington State University.

Accepted for publication 20 March 1970.

ABSTRACT

Populations of *Fusarium roseum* f. sp. *cerealis* 'Culmorum', *F. solani* f. sp. *pisi*, and total fusaria (including saprophytes) declined to zero, or nearly so, within the ammonia retention zone in both Palouse silt loam (PSL) and Ritzville silt loam (RSL) in the laboratory following injection with liquid anhydrous ammonia (NH_3). In soil 0-2 cm from the site of injection, the *Fusarium* populations began to drop within 1 day after injection and reached zero by 14 days. In soil 4-5 cm from the site of injection, the respective *Fusarium* populations declined less rapidly, but approached and in some cases reached zero by 49 days after injection. Fusaria outside the NH_3 retention zone were not significantly affected, whereas inside the zone no recovery had occurred by 225 days after injection.

Initially, and particularly in the centermost portions of the retention zone, NH_3 apparently caused most of the destruction of the fusaria. Nitrite (NO_2^-) accumulated with time, and probably constituted an additional source of fungicidal action. In PSL incubated at 24 C, more than 30 to 35 ppm NO_2^- was detected within 4 days in soil 0-2 cm and 2-4 cm from the NH_3 injection site and within 14 days in the 4-5 cm zone. Concentrations exceeding 100 ppm NO_2^- were measured in PSL incubated at 6 C. Additions of 35 ppm NO_2^- (KNO_2) to PSL reduced populations of all fusaria to undetectable numbers within 1 week. High pH in the absence of NH_3 and 100 ppm N as nitrate (NO_3^-) (KNO_3) had no significant effect on the *Fusarium* populations. When soil "sterilized" by gamma-irradiation (9.8 megarads) and later aseptically reinfested with Culmorum was injected with liquid anhydrous NH_3, reductions in populations of Culmorum were less marked by comparison with those in nonsterile field soil. The reduced fungitoxicity of injected NH_3 in sterile soil was attributed largely to the lack of NO_2^- accumulation. Phytopathology 60:1227-1232.

Wheat (*Triticum aestivum* L.) is foremost among crops affected by root diseases that have not been commercially controlled by fungicides. For economic reasons, available soil fumigants and fungicides cannot be used, even with the present-day high wheat production capabilities. Systemic fungicides may offer new possibilities for disease control, but their use for wheat production is still some time away.

Eno et al. (9) injected anhydrous ammonia (NH_3) into a Florida sand and reduced populations of fungi, bacteria, and nematodes. Neal et al. (15) found that NH_3 rapidly killed sclerotia of *Phymatotrichum omnivorum*. McCallan & Setterstrom (13) reported that NH_3 is toxic to many different fungi. Others have reported similar observations (2, 3, 4, 5, 6, 8, 10, 17).

In the Pacific Northwest, anhydrous NH_3 is a primary N source for wheat production. Low cost, ease of application, fungicidal properties, and the need of N for wheat growth suggest the possibility of using anhydrous NH_3 additionally to control soil-borne diseases of wheat. The present study was undertaken to determine the feasibility of using anhydrous NH_3 as a fungicide against *Fusarium* in two Pacific Northwest soils. The results of laboratory studies are presented here. Results of field studies and additional laboratory studies will follow.

MATERIALS AND METHODS.—*The pathogens.*—*Fusarium roseum* (Lk. ex Fr.) emend. Snyd. & Hans. f. sp. *cerealis* (Cke.) Snyd. & Hans. 'Culmorum' was selected as a test organism because it is an important soil-borne pathogen of wheat (7) and exists largely as free-living chlamydospores in soil (7, 14). Presumably, it would be vulnerable to effects of NH_3. *Fusarium solani* (Mart.) Appel & Wr. f. sp. *pisi* (F. R. Jones) Snyd. & Hans. exists in the higher rainfall region where peas are grown in rotation with wheat, and was selected as a second test organism because it also exists in soil as free-living chlamydospores.

The soils.—A Palouse silt loam (PSL) containing a natural infestation of approximately 3,700 Culmorum and 1,000 *F. solani* f. sp. *pisi* propagules/g was collected from a field near Pullman, Whitman County, Washington. A Ritzville silt loam (RSL) containing an estimated 12,000 Culmorum propagules/g but no *F. solani* f. sp. *pisi* was collected from near Ritzville, Adams County, Washington. Both soils were collected from the surface-10 cm of the respective fields, air-dried, passed through a 2-cm screen, and stored at −6 C until used. Some chemical and physical properties of the two soils are given in Table 1.

Injection of liquid anhydrous NH_3 and sampling of the NH_3 retention zone.—Each soil was wetted to approximately −2 bars water potential and then packed into 1-gal cans that were previously split in half longitudinally, taped back together, and lined with polyethylene bags. Liquid anhydrous NH_3 was then

TABLE 1. Some physical and chemical properties of Palouse and Ritzville silt loams

Property	Palouse	Ritzville
pH of saturated paste	6.0	6.3
pH of 1:10 soil: 2 N KCl supernatant	4.9	5.2
Organic carbon (%)	1.58	0.92
Total N (%)	0.119	0.105
Cation exchange capacity at pH 7 (meq/100 g)	17.4	14.3
Water content at 1/3 bar matric water potential (%)	25.5	23.2
Sand (%)	21.4	27.6
Silt (%)	54.0	58.4
Clay (%)	24.6	14.0

injected into each soil-filled can with a dispenser similar in principle and design to that of Papendick & Parr (16). The NH_3 was dispensed through a small diam tube, the aperture of which was placed 10 cm below the soil surface. Upon release, the NH_3 vaporized rapidly and moved radially outward from the injection site. The rate of application (730 mg N/can) was sufficient to form a spherical NH_3 retention zone about 5 cm in radius. Following injection, the polyethylene bags were closed to minimize evaporative water loss.

One-half the cans containing PSL were incubated at 24 C, the other half at 6 C. These temp were arbitrarily selected to provide information on effectiveness of NH_3 as a fungicide in both warm and cold soils. All cans containing the RSL were incubated at 24 C. Three cans of PSL at each incubation temp were taken for sampling at 1, 4, 14, 28, 49, and 77 days after injection of anhydrous NH_3. PSL at 24 C was also sampled at 225 days after injection. RSL was sampled at 1, 7, 28, 49, and 77 days after injection. At each sampling date, the cans of soil were split into hemicylinders by pressing a piece of sheet metal between the pre-split can halves. The NH_3 retention zone was thereby divided into equal hemispheres, each being exposed on the newly opened surface of each hemicylinder. Sampling consisted of removing successive subhemispherical regions of soil with boundaries 0-2, 2-4, and 4-5 cm from the NH_3 injection point. Samples were taken by rotating a wire ring to loosen soil around the NH_3 infection point. The smallest ring was used first, followed by use of successively larger rings. The soil loosened by each successive ring (Fig. 1) was put in a glass flask, stoppered, and retained for analysis. Soil from outside the NH_3 retention zone was taken for analysis from all treatments at each sampling date to serve as controls.

Measurement of soil pH and forms of N in the retention zone.—Samples from the hemispheres of the retention zone were placed in centrifuge tubes and suspended in 2 N KCl at a soil:solution ratio of 1:10. The suspensions were shaken mechanically for 5 min and then centrifuged at 2,000 rpm for 5 min. Equilibrium extracts were analyzed for $NH_4^+ + NH_3$, NO_3^-, and NO_2^-, using the steam distillation method of Bremner (1). Periodic confirmation of the NO_2^- analysis was made using a modified Griess-Ilosvay method (1). The soil pH was measured in the supernatant extract

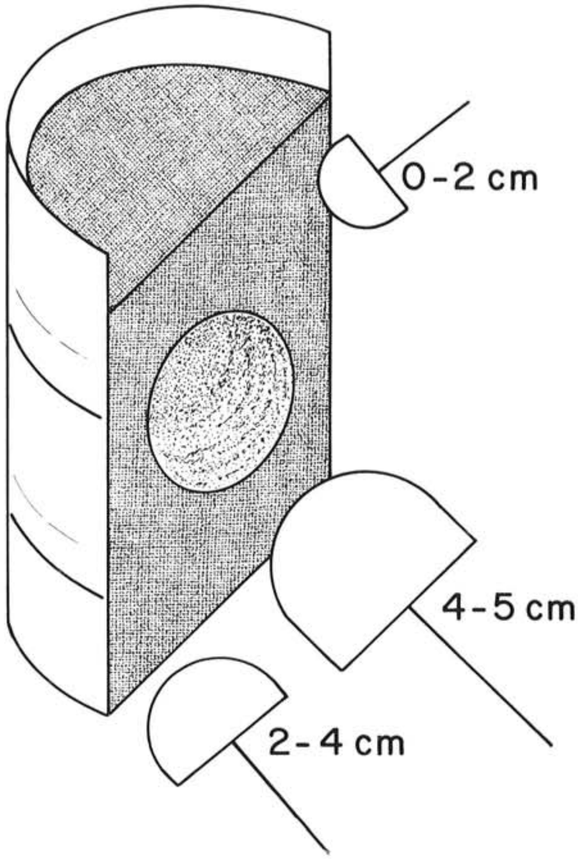

Fig. 1. Diagram of a divided cylinder of soil containing a hemispherical NH_3 retention zone and the wire rings (2, 4, and 5 cm radii) used to remove concentric layers (0-2, 2-4, and 4-5 cm radii) of the NH_3 retention zone.

of a 1:10 soil:2 N KCl suspension rather than as the commonly reported pH of a saturated soil paste. The pH values reported in this paper are roughly one unit lower than the pH values measured in pastes.

Estimates of the Fusarium population.—Two 1-g samples of soil were taken from each sample of the retention zone soil, suspended in 0.1% water agar (1:1,000 dilution), and dispensed on plates of the peptone-PCNB-agar medium of Nash & Snyder (14), 1 ml/plate. Three plates were prepared from each of the two diluted samples, making a total of six plates/subhemispherical sample and 18 plates (three replications)/time and location within the retention zone. Plates were incubated for 4 to 5 days in diffuse light before counts were made on the numbers of colonies of Culmorum, *F. solani* f. sp. *pisi,* and total fusaria.

RESULTS.—*Fusarium populations in the NH_3 retention zone.*—Within 1 day following injection of liquid anhydrous NH_3 into soil, the population of total fusaria had decreased appreciably in all regions of the retention zone of both soils (Table 2). The rate of decline was greatest in the central region at the higher temp (24 C). The fungicidal effect appeared to progress outward from the central region into the 2-4 and finally the 4-5 cm regions of the retention zone. No

TABLE 2. Populations of *Fusarium roseum* f. sp. *cerealis* 'Culmorum', *F. solani* f. sp. *pisi*, and total fusaria in Palouse and Ritzville silt loams at different times following injection of liquid anhydrous ammonia into the soils

Incubation temp	Days of incu-bation	*F. roseum* f. sp. *cerealis* 'Culmorum' 0-2 cm[a]	2-4 cm	4-5 cm	check[b]	*F. solani* f. sp. *pisi* 0-2 cm	2-4 cm	4-5 cm	check	Total fusaria 0-2 cm	2-4 cm	4-5 cm	check
						Palouse silt loam							
6 C	1	1.00[c]	1.48	1.48	3.50	0.27	0.41	1.01	1.02	5.10	7.74	9.21	12.53
	7	0.13	0.47	2.88	3.43	0	0.47	0.87	1.03	0.20	1.60	11.55	12.52
	14	0.07	0.68	2.03	3.42	0.07	0.13	0.88	1.21	0.48	2.65	10.37	13.28
	28	0	0.33	1.73	3.40	0	0.20	0.47	1.10	0	1.93	7.80	12.60
	49	0	0	0	3.33	0	0	0	1.61	0	0	0	11.10
	77	0	0	0	3.41	0	0	0	1.52	0	0	0	12.30
24 C	1	1.22	1.22	1.90	3.75	0.27	0.67	0.68	1.02	5.54	6.03	9.57	12.52
	7	0	0.82	2.51	3.76	0	0.14	1.16	1.02	0.48	4.82	12.43	12.53
	14	0	0	0.07	3.92	0	0.07	0.07	0.85	0	0.54	1.27	13.30
	28	0	0	0.07	3.70	0	0	0	1.10	0	0.13	0.87	12.60
	49	0	0	0.06	3.57	0	0	0	1.38	0	0.06	0.85	11.10
	77	0	0	0	3.60	0	0	0	1.22	0	0	0.54	12.00
	225	0	0	0	3.16	0	0	0	1.58	0	0	0.68	10.83
						Ritzville silt loam							
24 C	1	0	1.43	6.07	11.55				0	0.13	4.97	17.19	30.22
	7	0.13	0.53	3.38	10.74				0	0.20	2.77	13.74	28.65
	28	0	0	0.94	14.43				0	0.13	0.06	3.38	32.56
	49	0	0	0.92	12.52				0	0	0	2.92	30.81

[a] Radial distance from the NH_3 injection site.

[b] Nonammoniated check soil.

[c] Each number represents thousands of propagules/g of oven-dry soil and is an average of counts made with 18 dilution plates.

significant changes in population of any of the fusaria occurred outside the NH_3 retention zone. Within the retention zone, on the other hand, *Fusarium* populations were still low or undetectable 225 days after injection, at which time the experiment was terminated.

The effect of soil pH and forms and concn of N on the Fusarium population.—Nitrogen concn were highest in the center of the retention zone and decreased with distance away from the injection site (Table 3). Initially, most of the N existed as $NH_4^+ + NH_3$ but,

TABLE 3. Concentration of different forms of N and the pH in the retention zone of Palouse and Ritzville silt loams at different times following injection of liquid anhydrous ammonia into the soils

Incubation Temp	Days	0-2 cm[a] $NH_4^+ + NH_3$	NO_3^-	NO_2^-	pH	2-4 cm $NH_4^+ + NH_3$	NO_3^-	NO_2^-	pH	4-5 cm $NH_4^+ + NH_3$	NO_3^-	NO_2^-	pH
C		*ppm N*				*ppm N*				*ppm N*			
						Palouse silt loam							
6	1	1,878[b]	0	25	8.5	1,314	0	30	8.0	496	0	0	6.5
	4	1,286	6	66	7.9	785	3	28	7.1	423	10	3	6.5
	7	1,094	4	113	7.5	858	13	151	6.8	433	20	75	5.9
	14	1,436	10	44	7.7	1,118	12	37	6.8	822	20	65	6.0
	28	1,249	15	61	7.6	988	20	105	7.1	520	28	138	6.0
	49	949	28	185	7.3	660	27	161	6.1	391	34	88	5.6
	77	677	150		6.7	474	126		6.4	304	100		5.9
24	1	1,726	0	0	8.0	1,257	0	0	7.7	629	0	0	6.7
	4	1,387	19	75	7.7	1,461	32	68	7.4	760	25	26	6.7
	7	1,615	26		8.0	1,420	31		7.8	975	24		7.2
	14	1,016	32	38	6.6	792	30	32	6.0	503	36	82	5.8
	28	695	71	65	6.1	485	100	36	5.5	295	105	31	5.4
	49	535	165	30	6.0	321	173	28	5.4	195	157	14	5.2
	77	439	218		6.3	239	184		5.0	138	173		5.0
						Ritzville silt loam							
24	1	899	0	0	8.9	794	0	0	8.7	627	0	18	7.8
	7	1,017	0	0	7.9	856	0	0	7.6	597	0	0	7.2
	28	721	125	63	7.0	505	138	20	6.2	323	124	34	5.6
	49	466	157	33	6.3	377	159	26	5.7	266	155	22	5.2
	77	307	162	0	5.5	312	218	0	5.4	198	203	0	4.9

[a] Radial distance from the NH_3 injection site.

[b] All concn are expressed as averages of three replicates of actual concn minus the concn in nonammoniated check soil.

with time, these decreased and concn of NO_3^- increased. Nitrite was detected on the first sampling date in PSL incubated at 6 C and slightly later in PSL and RSL incubated at 24 C. The pH of the retention zone, as with $NH_4^+ + NH_3$ concn, was highest in the center of the retention zone at the beginning of the incubation period and decreased with time and as the concn of $NH_4^+ + NH_3$ decreased.

Salts containing NH_4^+, NO_2^-, and NO_3^- were mixed with individual samples of PSL in amounts sufficient to approximate the concn found for each in the NH_3 retention zone during the period of highest accumulation following injection of anhydrous NH_3. Specific salts and rates applied were: NH_4Cl at 1,500 ppm N; KNO_3 at 100 ppm N; and KNO_2 at 0, 1, 10, 25, 35, and 50 ppm N. In addition, we adjusted soil pH to 9.0 with KOH to determine whether the increased pH detected in the retention zone was responsible for changes in the *Fusarium* population. Potassium and chloride ions were nontoxic to the test organisms at these application rates. The treated soils were incubated for 3 weeks (1 week where NO_2^- was added) before estimates of the *Fusarium* population were made.

Only NO_2^- accumulation appeared to significantly account for the decline in populations of *Fusarium* (Fig. 2, 3). Exposure of the fusaria in soil to 100 ppm N as NO_3^- and to a pH of 9.0 for 3 weeks caused slight reductions in the populations of total fusaria and *F. solani* f. sp. *pisi*, but not of Culmorum. Treatment of soil with 1,500 ppm N as NH_4^+ reduced the populations of Culmorum, *F. solani* f. sp. *pisi*, and total fusaria (Fig. 2), but to a much smaller degree than that which occurred in the NH_3 retention zone (Table 2) for an equivalent 3-week incubation period. On the other hand, the *Fusarium* population was undetectable when soil was treated with 35 ppm NO_2^- and incubated for 1 week (Fig. 3). Concn of this magnitude were measured in the NH_3 retention zone (Table 3).

Experiments with irradiated soil.—The relationship between NO_2^- accumulation and the decline of *Fusarium* populations in the NH_3 retention zone was studied further using soil sterilized by gamma irradiation to prevent nitrification. Three lots of soil (3 kg each) were sealed in polyethylene bags and exposed to cesium-137. The irradiation dosage was between 7.8 and 9.8 megarads, with an average dosage of 8.83 megarads. This soil was then aseptically reinfested with Culmorum by adding a water suspension of conidia of the fungus to the soil. Incubation was for 3 weeks, during which time the conidia converted into chlamydospores. Sealed bags of the reinfested soil were placed in 1-gal cans and aseptically injected with anhydrous NH_3 at a rate of 730 mg NH_3-N/can. Two weeks after injection, the soil was sampled as described for nonsterile soil, with the exception that a region 5-6 cm from the injection site was sampled in addition to samples 0-2, 2-4, 4-5 cm from the NH_3 injection site, and a distant nonammoniated region.

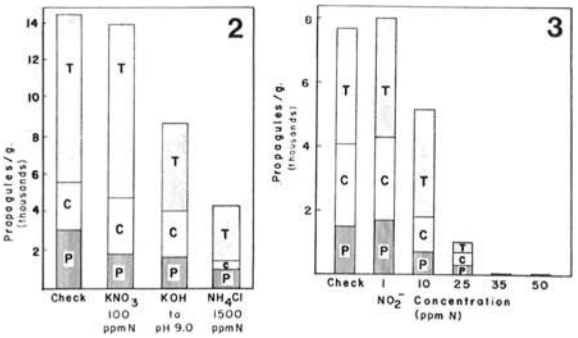

Fig. 2-3. 2) Influence of 100 ppm NO_3^-, 1,500 ppm NH_4^+, and pH 9.0 on populations of *Fusarium roseum* f. sp. *cerealis* 'Culmorum' (C), *F. solani* f. sp. *pisi* (P), and total fusaria (T) in Palouse silt loam following 3 weeks of incubation. **3)** Influence of NO_2^- on the populations of *Fusarium roseum* f. sp. *cerealis* 'Culmorum' (C), *F. solani* f. sp. *pisi* (P), and total fusaria (T) in Palouse silt loam following 1 week of incubation.

The Culmorum population decreased in irradiated soil following injection of anhydrous NH_3 (Table 4) but not as much as in nonsterile soil (Table 2). Populations were markedly reduced in the centermost portion of the retention zone by 14 days after injection, but not in the peripheral regions of the zone. This is in contrast to nonsterile soil where, after 14 days at the same temp (24 C), the Culmorum populations were undetectable in all regions of the retention zone. Accumulation of NO_2^- and NO_3^- did not occur in the irradiated soil (Table 4).

Discussion.—NH_3 injected as a liquid into soil was effective as a fungicide against *Fusarium*. Populations of Culmorum, *F. solani* f. sp. *pisi*, and total fusaria, estimated by dilution plate-counts, were markedly less in the NH_3 retention zone than in untreated soil outside the retention zone.

Although NH_3 per se apparently is toxic to chlamydospores of *Fusarium*, our results suggest that in the NH_3 retention zone, NH_3 was not the only factor responsible for reduction in the *Fusarium* population. Nitrite accumulated in the peripheral regions as well as elsewhere in the retention zone in both soils, particularly at the lower temp, and may account for slow decline of the populations of *Fusarium* in peripheral regions (4-5 cm). The *Fusarium* population did not reach zero until 7 or 11 weeks after injection. Exposure to 35 ppm N as NO_2^- for 1 week was highly toxic to members of the genus *Fusarium*, including Culmorum and *F. solani* f. sp. *pisi*. Nitrite and nitrate were not detected in soil sterilized by gamma irradiation before reinfestation with Culmorum and injection with NH_3, and here reductions in the numbers of Culmorum propagules occurred primarily in the centermost region of the retention zone. Presumably, NH_3 is fungicidal in the central region, but because of adsorption and reaction with soil, concn of NH_3 progressively diminished at increasing distances away from the injection site and, hence, the extent of its toxic action was progressively decreased. With time, however, and particularly in cold or poorly drained soils, NO_2^- may accumulate and serve as an additional source of toxicity.

The observation that 35 ppm N as NO_2^- inhibits

TABLE 4. Forms and concn of N, the pH, and the population of *Fusarium roseum* f. sp. *cerealis* 'Culmorum' in the retention zone 2 weeks after injection of liquid anhydrous NH_3 into irradiated-reinfested Palouse silt loam incubated at 24 C

Sample region[a] (cm)	$NH_4^+ + NH_3$	NO_2^-	NO_3^-	pH	Culmorum Propagules/g	Culmorum % Detectability
	ppm N					
0-2	1,240[b]	0	0	7.64	3.6[d]	4
2-4	950	0	0	7.47	14.8	16
4-5	756	0	0	6.96	44.4	51
5-6	355	0	0	6.14	78.3	87
Check[c]	0	0	0	6.26	89.4	100

[a] Radial distance from the NH_3 injection site.
[b] All concn are expressed as average of three replicates of actual concn minus the nonammoniated check soil concn.
[c] Nonammoniated check soil with the ppm N considered as base zero.
[d] Each number represents thousands of propagules/g of oven-dry soil and is an average of counts made on 18 dilution plates.

Fusarium in PSL is similar to that of Zentmyer & Bingham (18) for *Phytophthora cinnamomi*. Thirty ppm N as NO_2^- inhibited growth of *P. cinnamomi* on cornmeal agar at pH 6.5 and also parasitism of avocado seedlings growing in liquid culture at pH 6.5.

Henis & Chet (11) showed that elevated pH due to NH_3 could account for the fungicidal effects of NH_3 against sclerotia of *Sclerotium rolfsii*. This explanation seems inadequate to explain our results with *Fusarium*, since pH 9.0 (higher than any pH detected in the soils used for this study) did not cause a significant reduction in the population of the test pathogens. On the other hand, a high pH may favor the accumulation of nitrite and in this way contribute to reductions in populations of *Fusarium*.

Temperature changes in the retention zone resulting from exothermic and endothermic reactions of liquid anhydrous NH_3 with soil following injection likewise seem inadequate to account for the reduction in population of *Fusarium*. Khasawneh & Parr (12) reported temp increases of 11 C in the retention zone immediately following injection of liquid anhydrous NH_3 in soil initially at room temp. In a similar trial, we measured temp as high as 44 C in the proximity of the injection point in dry soil initially at room temp. However, the dissipation of heat was very rapid and the high temp persisted for less than 1 min. A temp of 40 C for 2.5 hr is harmful to Culmorum (Smiley, *unpublished data*), but exposure at this temp for only a few min probably has little effect on the fungus. On the other hand, Cochrane (4) states that certain fungicides are more effective if the temp used for toxicity tests are near the thermal-death point of the fungus. A possible interaction between NH_3 and high temp at the time of injection could be a factor in the population reductions recorded in our study.

Thus, it appears that anhydrous NH_3 used as a fertilizer has potential as a soil fungicide. The degree of effectiveness, however, may depend on how thoroughly the soil can be treated with the chemical and whether the rates needed are phytotoxic, or whether treated soil can be rendered safe for a crop by the time of planting. Since an accumulation of NO_2^- may account for at least part of the decline in the *Fusarium* population in the retention zone, the effectiveness of NH_3 may also depend on existence of field conditions conducive to NO_2^- accumulation. Studies are now underway to investigate the potential of NH_3 as a fungicide under field conditions.

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