Effect of Nitrogen and Nitrapyrin on Stalk Rot, Stalk Diameter, and Yield of Corn

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Accepted for publication 24 October 1977.

ABSTRACT

WHITE, D. G., R. G. HOEFT, and J. T. TOUCHTON. 1978. Effect of nitrogen and nitrapyrin on stalk rot, stalk diameter, and yield of corn. Phytopathology 68: 811-814.

The effects of various rates of spring- and fall-applied anhydrous ammonia, with and without 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin) on stalk rot, stalk diameter, and yield were determined. Stalk rot from natural infection and stalk rot following inoculation with *Diplodia maydis* or *Colletotrichum graminicola* decreased with increasing nitrogen rates. Nitrapyrin reduced stalk rot in some treatments, however, it did not increase yield or stalk diameter. The reduced stalk rot with increasing rates of nitrogen and at lower rates of nitrogen with nitrapyrin may be due to a continuous supply of nitrogen throughout the growing season.

Additional key words: *Diplodia maydis*, *Gibberella zeae*, *Colletotrichum graminicola*, nitrapyin, leaching, denitrification, anhydrous ammonia, *Zea mays*, maize.

Several studies have been made to evaluate the effect of various nutrients on stalk rot of corn. Nitrogen (N) has been reported to both increase (1) as well as decrease stalk rot severity (4, 6). This variable effect of N on stalk rot appears to be influenced by factors such as the stalk rot pathogen, susceptibility of the plant, source of nitrogen, and relative availability of other nutrients (1, 5, 6, 7, 12).

Recently the use of a nitrification inhibitor, 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin) has been reported to reduce stalk rot caused by *Diplodia maydis* (Berk.) Sacc. [syn. *D. zeae* (Schw.) Lev.] and *Gibberella zeae* (Schw.) Petch when mixed with fall-applied anhydrous ammonia (10). In addition to a reduction in stalk rot, nitrapyrin also increased yield, increased stalk diameter, and reduced kernel infection by *F. moniliforme* (10).

Nitrapyrin inhibits *Nitrosomonas* spp. in soil thus reducing the rate of nitrification (conversion of NH_4-N to NO_3-N) (9). This may result in a reduced potential for loss of nitrate-N by leaching or denitrification and an increased uptake of ammonium-N by the plant. Increased uptake of ammonium may affect concentrations of other elements in the plant through cation:cation, cation:anion, and anion:anion interactions.

The purpose of this research was to determine the effects of nitrapyrin applied with various rates of anhydrous ammonia on stalk rot caused by *D. maydis* and *Colletotrichum graminicola* (Ces.) Wils. following artificial inoculation and on stalk rot due to natural infection. Other research on the same plots (8) provided data on nitrogen uptake at physiological maturity, distribution of ammonium and nitrates in the soil, and concentration of elements other than nitrogen in the plant tissue at various growth stages. Relationships between stalk rot and these other parameters were evaluated.

00032-949X/78/000 137$03.00/0

MATERIALS AND METHODS

The experimental site was located at the Agronomy South Farm, Urbana, IL on a Drummer-silty clay loam (Typic Hapliquoll) that had been planted with corn the previous year. Experimental plots (6 × 15 m) were arranged in a randomized complete block design with treatments replicated three times. Anhydrous ammonia was applied in bands 75 cm apart at a depth of 17 cm both with and without nitrapyrin in the fall (17 October 1975) and in the spring (4 April 1976). A four-knife applicator with 44-kg capacity ammonia tanks was used. Nitrapyrin was mixed with ammonia just prior to applications. Nitrogen rates, nitrapyrin, and time of application are listed in Table 1. Potassium (K) as KCl was applied in the fall of 1975 at the rate of 100 kg of K_2O/ha. Soil analysis prior to application of KCl indicated an extractable K level of 300 kg/ha. Eight rows per plot of a single-cross corn hybrid (B73 × Mo17) were planted on 16 April 1976. After a stand was established, the corn was thinned to 54,000 plants/ha.

Diplodia maydis was cultured at room temperature on moistened, sterile oats in 1-liter flasks. After incubation for 5 wk the oat substrate was placed in cheese cloth bags and kneaded in distilled water to release conidia. *Colletotrichum graminicola* was cultured on oatmeal agar plates for 3 wk and conidia were washed with distilled water from the culture surface. The concentrations of conidia suspensions of both organisms were estimated with a hemacytometer and adjusted to 2×10^5 conidia/ml by dilution with distilled water.

Inoculations were made using a 50-ml Vaco Pistol-Grip Rubber Plunger Syringe (Ideal Instruments, Inc., Chicago, IL 60612) fitted with a 2.3-mm diameter (11-gauge) stainless-steel needle. Needles were cut to 7-9 cm in length, hammered shut at the tip, and the tips were ground to a sharp point. Small holes were drilled in both sides of the needles about 1 cm from the tips. These altered needles could be punched into corn stalks without becoming clogged with stalk tissue.

Plants were inoculated 65 days after planting (13 days after the full silk stage) by injecting 2 ml of inoculum into the first elongated internode above the brace roots. Twenty-four plants of the third and the sixth row of each plot were inoculated with *D. maydis* and 12 plants of these rows were inoculated with *C. graminicola*.

Ratings for stalk-rot reaction were made 28 and 29 days after inoculation. Plants inoculated with *D. maydis* were split lengthwise through the inoculated internode and were rated as follows: 1 = 0-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100% of the inoculated internode discolored, and 5 = discoloration beyond the inoculated internode (2). Plants inoculated with *C. graminicola* were rated on the basis of number of internodes 50% or more discolored.

Diameter of the first elongated internode of fifteen plants in row four were measured at harvest. Percent plants with rotted stalks in the two center rows (rows 4 and 5) was recorded at harvest. Plants were considered to have rotted stalks if the base of the stalk readily collapsed when pressed between the thumb and forefinger. The two center rows were harvested for grain yield when the moisture was approximately 20%. Grain yield was then adjusted to 15.5% moisture.

RESULTS

In general, stalk rot following inoculation with *D. maydis* decreased with increasing N rates (Table 1). There were no differences due to spring or fall application of the same N and nitrapyrin rate. There was a highly significant decrease in stalk rot when 1.12 kg/ha nitrapyrin was added to fall applied 134 kg/ha N. There was no effect due to nitrapyrin at other rates or times of N application.

As with Diplodia, Colletotrichum stalk rot also decreased as N rates increased (Table 1). There was no difference due to spring or fall application of the same nitrapyrin and N rates. The only significant effect due to nitrapyrin at the same N rate and time of application was with spring-applied 67 kg/ha N where stalk rot reaction was increased by the addition of 0.56 kg/ha nitrapyrin.

Stalk rot from natural infection was significantly decreased by the addition of nitrapyrin to spring- and fall-applied 67 kg/ha N (Table 1). As with stalk rot following inoculation, there were no differences due to time of application of the same nitrapyrin and N rate. When low rates of N (67 kg/ha) were compared to high rates of N (268 kg/ha) without nitrapyrin, the percent infected stalks was higher at the lower N rates. Symptoms on rotted plants and isolations from stalks indicated the most prevalent stalk rot pathogens were *G. zeae* and *C. graminicola*.

Stalk diameters increased with increasing N rates (Table 1). No effect was found due to time of application or nitrapyrin.

Grain yields increased with increasing N rates (Table 1). A yield reduction occurred when nitrapyrin was added to fall applied 67 kg/ha N. Yield from the fall 67 kg/ha N rate without nitrapyrin was relatively high, and appears to be abnormally high when compared to spring applications. There was no other effect of nitrapyrin or time of application on yield.

TABLE 1. Stalk rot reactions[a], percent plants with naturally rotted stalks[b], stalk diameters and yield of corn as affected by nitrogen, nitrapyrin, and season of application

Treatment			Stalk rot				
Nitrogen (kg/ha)	Nitrapyrin (kg/ha)	Season of application	*D. maydis* reaction	*C. graminicola* reaction	Natural infection (%)	Stalk diameter (cm)	Yield (q/ha)
0	0	...	4.2	3.5	9.2	2.3	46.6
67	0	Fall	3.9	3.2	13.8	2.5	103.1
67	0	Spring	3.8	3.2	16.9	2.5	88.1
67	0.56	Fall	3.8	4.0	5.0	2.4	78.6
67	0.56	Spring	4.1	4.2	8.3	2.5	91.0
134	0	Fall	3.2	2.4	13.1	2.6	124.3
134	0	Spring	2.7	2.1	11.2	2.6	135.9
134	0.56	Spring	2.3	2.2	8.5	2.6	128.8
134	1.12	Fall	2.5	2.2	8.0	2.7	130.7
134	1.12	Spring	2.6	2.7	9.3	2.7	124.5
268	0	Fall	2.5	2.1	6.2	2.7	138.2
268	0.56	Fall	2.5	2.0	3.6	2.7	138.9
268	0	Spring	2.3	2.0	9.6	2.7	140.9
	LSD[c]	($P = 0.05$)	.49	.94	7.4	.14	14.1
	LSD	($P = 0.01$)	.67	1.27	...	.20	19.1

[a]Reaction following inoculation with *Colletotrichum graminicola* or *Diplodia maydis*. *Diplodia maydis* ratings were based on average rating (scale 1 to 5) of percentage discoloration of the first elongated internode of 48 plants per plot in three replications, and those for *C. graminicola* were based on the average number of internodes discolored 50% or more of 24 plants per plot in three replications.

[b]Percent natural infection based on number of plants with rotted stalks out of 72 plants examined in each of three replications.

[c]Abbreviation LSD = least significant difference.

DISCUSSION

The most interesting aspect of this study is the decrease in stalk rot with increasing nitrogen rates. This trend is in agreement with results from preliminary studies in 1975 with spring applications of various rates of anhydrous ammonia with and without nitrapyrin on corn inoculated with *D. maydis* (11). These findings agree with previous work (6) where ammonium sulfate or ammonium chloride decreased Diplodia stalk rot, but does not agree with much of the earlier work which generally indicates an increase in stalk rot with increasing amounts of nitrogen. Earlier work, however, was done with sources of nitrogen other than anhydrous ammonia.

The effect of nitrapyrin was not consistent in all treatments or methods for evaluation of stalk rot. In general, nitrapyrin decreased stalk rot except in the case of Collectotrichum stalk rot for which there was an increase in stalk rot when nitrapyrin was applied with 67 kg/ha nitrogen.

The effect of nitrapyrin in reducing stalk rot, increasing yield, and increasing stalk diameter was not as great as previously reported (10) when nitrapyrin was added to fall-applied N under conditions conducive to nitrogen loss.

Soil samples taken in the ammonium retention zone (located between the corn rows) indicated that nitrification of fall-applied anhydrous ammonia was complete by 27 April with the exception of the 134 and 267 kg/ha N rates applied with nitrapyrin which were nitrified by 10 June. Nitrification of spring-applied anhydrous ammonia was complete by 28 July with the exception of 268 kg/ha and 134 kg/ha N rate with nitrapyrin (8). After nitrification, the nitrogen would be in a form subject to denitrification or leaching. Since soil samples were not taken from outside the ammonium retention zone, it is not known how much nitrogen loss actually occurred in the plots. Indications are, however, that substantial nitrogen loss did not occur. The lack of a nitrapyrin effect was possibly due to the lack of leaching or denitrification.

It has been suggested that a stalk rot pathogen may be unable to colonize a plant that is absorbing ammonium from the soil to satisfy a portion of the plant's nitrogen requirement (3, 10). Our data do not tend to support this hypothesis since there were no differences between spring or fall application. More of the fall applied nitrogen would be in the nitrate form compared to spring applied nitrogen. Additionally, the ammonium retention zone was located between the corn rows at a depth of 17 cm and was only 10 cm in diameter (8). Presumably, only a small portion of the total plant root system would be located in this zone and have ammonium available for uptake.

Another study (12) indicated that stalk rot was reduced by higher chloride levels in the plant. High chloride levels may delay maturity resulting in less stalk rot. Another possible chloride effect may be due to a decrease in the uptake of nitrate anions and increased uptake of ammonia (3, 12).

Percent chloride, as determined by potentiometric titration, in small plants (fifth-leaf stage) and in stover (physiological maturity) tended to decrease with increasing rates of applied nitrogen (8). No differences were found between season of application and nitrapyrin. Highly significant positive correlations were found between chloride concentrations in small plants and Diplodia stalk rot ratings ($r = 0.53$) and Colletotrichum stalk rot ratings ($r = 0.41$). There was no significant correlation between chloride levels in stover and stalk rot ratings or between stalk rot due to natural infection and chloride levels.

A number of workers have demonstrated that the development of stalk rot is correlated with cell senescence. Additionally, most data would indicate that any factor which tends to delay senescence in corn stalks reduces the severity of rot (1). Therefore, another possible explanation for the general trend of reduced stalk rot with increased N is that a plant with adequate N supply throughout the growing season is more resistant than a plant that has an adequate supply early in the season and is deficient late in the season. This would imply a need for a balanced and continuous N supply throughout the growing season to maintain living cells in the pith for a longer time. Since leaching or denitrification was not a problem in these experiments, plants growing at the higher N rates would have had a greater supply of N throughout the growing season, whereas, plants growing at the lower N rates would be deficient earlier in the growing season and would senesce at a more rapid rate.

It is interesting that the effect of nitrapyrin was more noticeable with stalk rot resulting from natural infection. This is possibly due to the fact that inoculation does not exactly duplicate natural infection because the resistance or susceptibility of the root system is bypassed. This is important since stalk rot fungi usually invade through the roots (1). Nitrapyrin with the N source did reduce the rate of nitrification and the N remained in the ammonium retention zone for a much longer period of time (8). This would allow for a slow release of the nitrate-N to the surrounding soil and possibly to the main root zone. A continuous supply of N may have slowed senescence of roots and therefore colonization by rot fungi. These conclusions are supported by data on total N uptake of plants (8). Fall- and spring-applied nitrapyrin with 67 kg/ha N decreased total N uptake by 27 and 22%, respectively, when compared to fall- and spring-applied N without nitrapyrin. It was also at the 67 kg/ha N rate where the significant effect of nitrapyrin on natural stalk rot occurred. The reduced nitrogen uptake with nitrapyrin may indicate that N remaining in the ammonium retention zone was away from the main root system and positionally unavailable for uptake. However, slow leaching of nitrate-N from the retention zone would allow for a steady source of N in the nitrapyrin treated plots.

The effects of N on soil microorganisms should not be ignored. The presence of nitrate-N around the roots may have reduced the amount of root rot by directly influencing root rot pathogens or by stimulation of organisms antagonistic to root rot pathogens. A reduction in root rot would result in a slower rate of senescence, thus less stalk rot.

Reduction in stalk rot by a steady supply of N can only be suggested by the data presented here and there is a definite need for more investigation into this aspect of the development of stalk rot. As previously reported, nitrapyrin would be most useful where denitrification and leaching of nitrified ammonia is likely to occur (10).

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