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250

RESEARCH ON DICYANDIAMIDE AS A NITRIFICATION INHIBITOR AND FUTURE OUTLOOK

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ABSTRACT

The action and decomposition of dicyandiamide (DCD), a nitrification inhibitor, is discussed.

DCD is especially efficient when used with animal manure slurries or potato starch waste water. As a consequence, nitrate leaching can be reduced, yields and N uptake increased. DCD-amended mineral N fertilizers applied once can substitute for split N applications, thus reducing labor costs without any loss in crop yield and quality. With wheat and sugar beets, use of a DCD-containing product ("Alzon 22") reduced the requirement of N for maximum yield. New formulations, such as DCD plus a reducing substance, ammonium thiosulfate (ATS), or new inhibitors, such as guanylthiourea (GTU), will receive more attention in the future.

INTRODUCTION

Nitrogen (N) from humus and fertilizers (e.g. urea) is mineralized and nitrified by soil microorganisms. This process is an essential characteristic of fertile soils. The nitrate (NO_3^-) that is produced can be taken up by plants or is leached (especially during the "critical" nongrowing season) or denitrified (under anaerobic soil conditions and higher temperatures, especially during the growing season). It is mainly N from harvest residues, immobilized N that is remineralized and then nitrified, and N from animal manure (especially as manure slurry applied in the fall) that is subject to leaching. Mineral N fertilizers, however, are usually applied during the spring when NO_3^- leaching may be less.

The concept of controlled nitrification is to let natural mineralization take its course to produce ammonium (NH_4^+) and then to temporarily inhibit the first step of nitrification, the conversion of NH_4^+ through use of specific inhibitors or "N stabilizers." In this way, N is "preserved" in the root-penetrated zone as NH_4^+ which is less subject to loss from soil. Naturally occurring nitrification inhibitors have been known for a long time, e.g., root exudates and decomposition products of soil organic matter such as tannins (1,2). The production of synthetic nitrification inhibitors gained importance during the 1960s, especially in the United States, Japan, and West Germany. Most widely known are nitrapyrin (N-serve) and dicyandiamide (Didin or DCD).

A concept of using a nitrification inhibitor with N fertilizers includes the following objectives:

(i) to minimize N losses by NO_3^- leaching (in late fall/winter) and by denitrification (under temporarily water-logged soil conditions and warm temperatures), thus enabling a more efficient utilization of soil and fertilizer N;

(ii) to regulate N supply (amount, form, and application time), e.g., by offering N in the form of NH_4^+ to vegetable and animal feed crops over certain periods in order to reduce their NO_3^- contents and otherwise increase their nutritional value;

(iii) to avoid NO_3^- stress during the first stages of plant development from a phytopathological point of view (diminish susceptibility to fungal infection, etc.).

Thus, nitrification inhibitors fit very well into modern fertilizer use systems and apparently contribute to real progress in fertilizer management (3).

In the last ten years, intensive research has been conducted on the action and potentials of dicyandiamide (DCD) as a nitrification inhibitor, especially in our institute (4).

WHAT IS DCD AND HOW DOES IT WORK?

Characteristics of DCD

DCD is the dimeric form of cyanamide with a relatively high water solubility (23 g/L at 13°C) and contains about 65% N. DCD constitutes about 10% of total N (more recently only 5%) in the well-known fertilizer, calcium cyanamide ("lime nitrogen"), and is responsible for its slow release effect. It originates from cyanamide at high temperatures and alkaline conditions (Fig. 1). DCD has been classified as a "non-toxic substance" (5); the LD_{50} is 10 g/kg body weight, which is about 3 times higher than for NaCl. It inhibits the first stage of nitrification, the oxidation of NH_4^+ to NO_2^- (Fig. 2), its effectiveness being dependent on DCD rate and temperature (Table 1). At 12°C, DCD was found to decompose completely after 12 weeks while at 4°C, 12% remained undecomposed even after 17 weeks.

DCD specifically affects <u>Nitrosomonas</u> <u>europaea</u> (6). Presumably, this effect is due to reaction of the C ≡ N group of

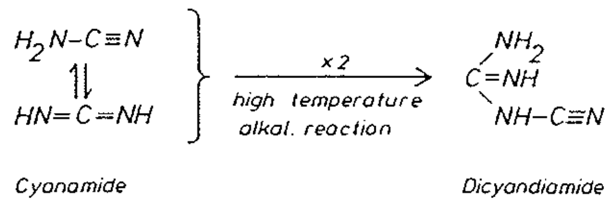

FIG. 1. Formation of Dicyandiamide

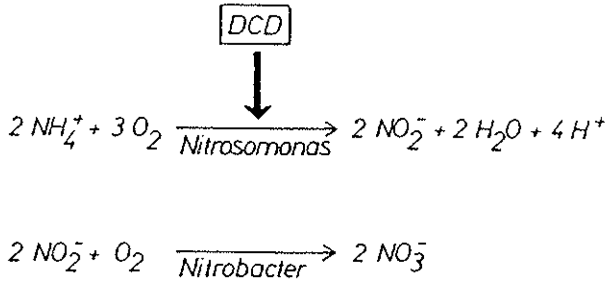

FIG. 2. Nitrification Inhibiting Effect of DCD

Table 1. Decomposition of Dicyandiamide, as Affected by Temperature

	% of added DCD-N remaining in soil			
weeks	0°C	4°C	6°C	12°C
2	88	88	80	76
8	80	73	68	40
12	-	-	56	0
14	62	42	-	-
17	57	12	-	-

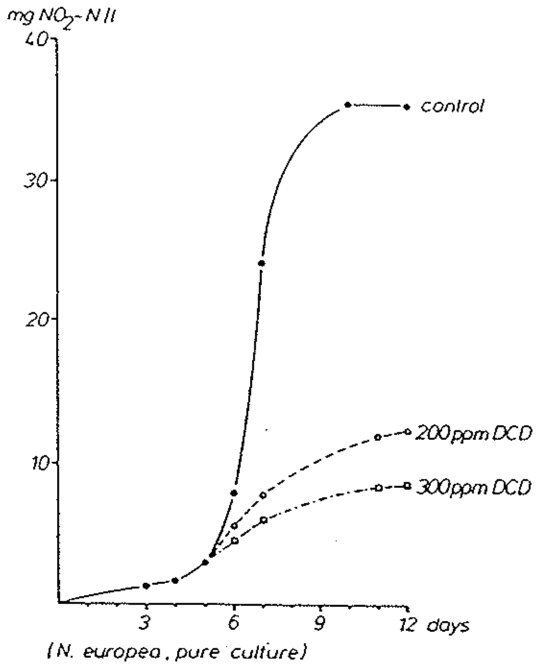

FIG. 3. Effect of Dicyandiamide on Nitrite Production

DCD with sulfhydryl or heavy metal groups of the bacteria's respiratory enzymes. With pure culture of Nitrosomonas europaea, this specific inhibition of NO_2^- formation was observed at concentrations of 200 to 300 mg/L (Fig. 3).

However, the same cultures after being transferred to a DCD-free medium were able to oxidize NH_4^+ up to about 90% of their original capability. This implies that DCD is a bacteriostatic and not a bactericidal chemical.

Other microorganisms, especially the heterotrophs that are mainly responsible for the so-called "biological activity" or biomass production in soil, are not affected by DCD (Fig. 4) (7).

Even after 20 years of continuous fertilization with granulated calcium cyanamide containing 10% DCD-N, no negative

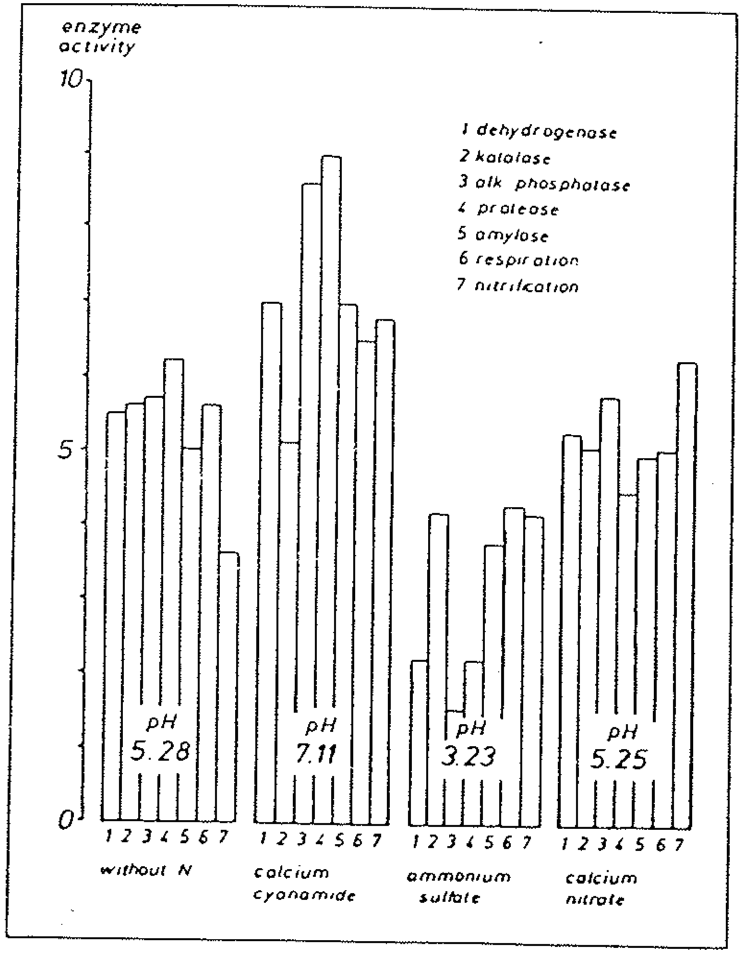

FIG. 4. Enzyme Activity and Fertilizer Use. Field Trials with Different N-Forms (over 50 years) on a Sandy Silty Loam

effect on most soil enzymes and biomass production was observed in soil sampled each fall. On the contrary, the very high enzyme activities that were observed resulted from the increase in soil pH caused by the high lime content of this fertilizer (about 60% CaO). Further, numerous investigations of the activities of different soil enzymes, as well as biomass and CO_2 measurements in different soil types, confirmed these results (8).

Decomposition of DCD in Soil

The nitrification inhibiting effect of DCD persists on the average for 1 to 3 months, depending on temperature, water content, organic matter, and pH of the soil (9,10). Initial decomposition takes place on surfaces of metal oxides (especially iron oxides and hydroxides) by catalytic addition of water to DCD to form guanylurea (Fig. 5) (11). This compound is transformed mainly by microorganisms through further addition of water, desamination, and decarboxylation to guanidine, and finally, to urea which is quickly degraded by the enzyme, ureas. The end products of DCD degradation, therefore, are CO_2, NH_3, and H_2O (12). Under anaerobic conditions and in soils poor in clay, the breakdown is much slower (13,14). DCD can be regarded to be also a slow release N fertilizer, albeit the amount usually added to soil is relatively small as compared with the amount of fertilizer N with which the DCD is applied. It is important to emphasize that only DCD but none of its metabolites is effective as an inhibitor.

In the wet tropics of Costa Rica where losses of N via leaching are very high, nitrification was found to be very slow in a strongly acid Inceptisol. After 8 weeks at 20° C, 83% of the applied urea-N + DCD was recovered as NH_4^+. In an Andisol with much higher nitrifying activity, nitrification was retarded as long as DCD levels remained high (complete decomposition occurred after 8 weeks) (15,16).

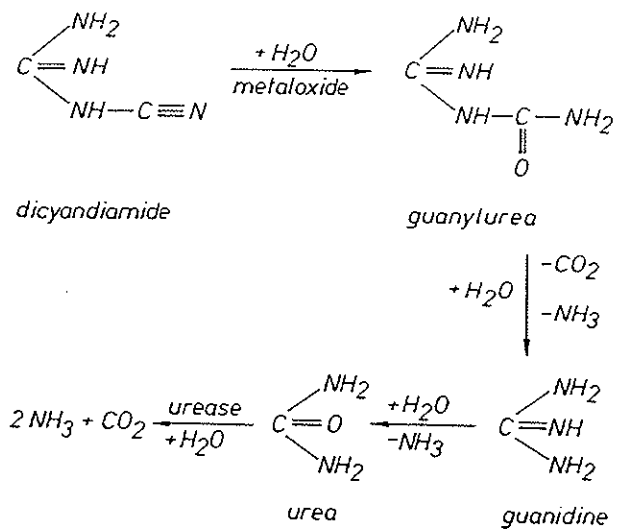

FIG. 5. Decomposition of Dicyandiamide

Physiological Properties of DCD

When used in higher amounts, especially at onset of growth or in nutrient solution--which is, however, a rather unusual fertilizer practice and does not comply with fertilizing recommendations--DCD can be taken up by plants in small quantities, but with little adverse effects (eventually, minor necroses). It is transported in the xylem, exuded at the leaf margins by way of transpiration, and is in this way physiologically more or less ineffective (17,18).

DCD AS AN ADDITIVE TO ANIMAL MANURE SLURRY AND WASTE WATER

Animal Manure Slurry

Slurry accumulates in intensive animal farming in large amounts. It contains on the average 2 kg NH_4^+-N/m³ which is nitrified within 2 to 3 weeks during the fall, 1 to 3 months during winter, or 5 to 6 weeks during early spring, the rate of nitrification depending on soil temperature.

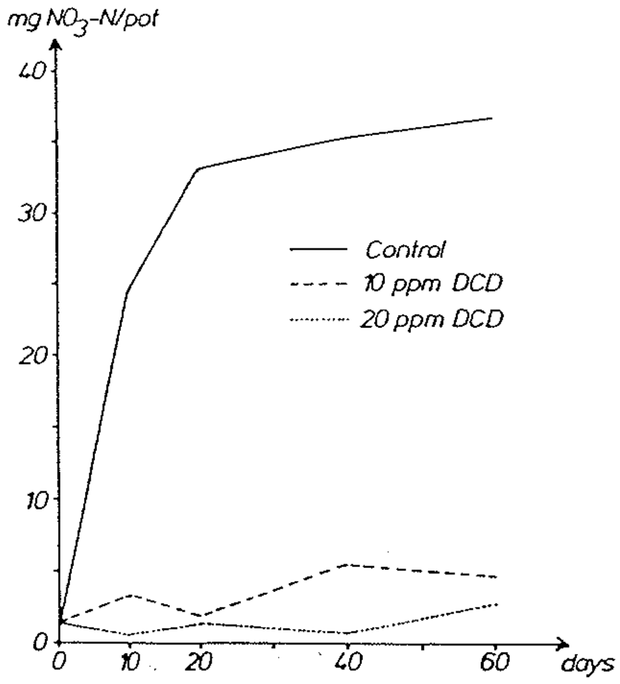

FIG. 6. Inhibition of Nitrification from Cattle Slurry by DCD in Incubation Trials with Soil (silty loam, pH 6.5, 400 g + 20 g slurry, 14°C, 50% of field capacity)

The resultant large quantity of NO_3^- in the soil is subject to leaching and can be quite a problem representing a significant loss of N with resultant decrease in crop yield, as well as possible pollutant of ground water. Leaching takes place under Mideuropean climatic conditions mainly between December and April and can reach 60-100 kg N/ha after slurry application in October/November.

DCD added to animal slurry at a rate of 10-20 ppm blocks nitrification for about 2 months at 14° C (Fig. 6). In a field study where polyethylene flasks filled with soil, animal slurry, and DCD at a rate of 20 kg/ha were buried in the soil, the following results were obtained (Fig. 7):

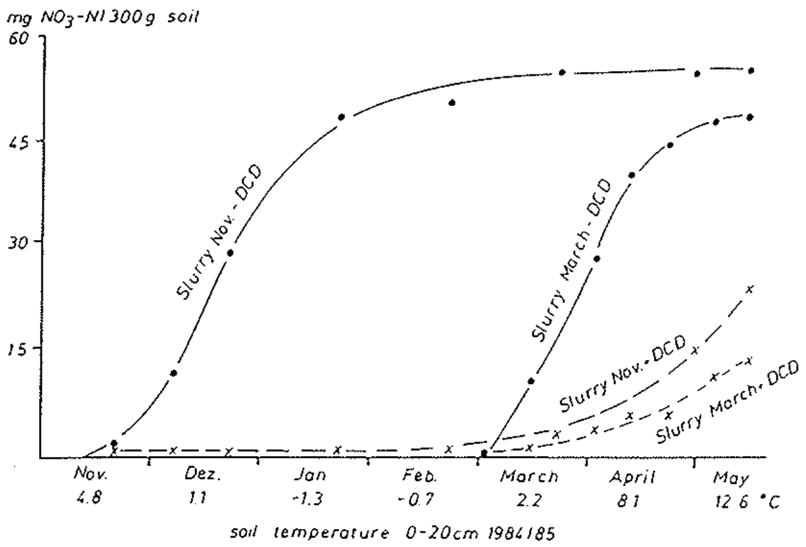

FIG. 7. Nitrification of Cattle Slurry (at out-door temperatures November - May.)

Eighty percent of the NH_4^+-N of animal slurry applied in November without DCD was nitrified by January even at soil temperatures of 1° C; however, with DCD, nitrification occurred not earlier than April. "March slurry" without DCD was nitrified within a few weeks; with DCD, nitrification did not begin until May in response to increasing soil temperature (19).

Therefore, adding DCD to 50 m^3/ha animal manure slurry reduced NO_3^- leaching considerably and NH_4^+-N was "preserved" for use of the following crop.

In a field trial with silage maize, DCD addition to animal slurry increased yields by 23 to 45% and N uptake by 10 to 27%, depending on weather and time of application (Table 2) (20).

Table 2. Slurry Application to Silage Maize.

slurry application (kg NH_4^+-N/ha)	silage maize yields dt dry m./ha	removal kg N/ha
1982/83		
November (145)	169	63
+ "Didin"	303	111
February (140)	268	88
+ "Didin"	286	95
1983/84		
October (182)	84	81
+ "Didin"	108	109
April (108)	92	79
+ "Didin"	110	93

30 kg "Didin"/ha
Soil: brown earth-loess (Weihenstephan), pH 6.3

The beneficial effect of DCD even in combination with "spring slurry" (when little leaching occurs), might also be the result of a decrease in N loss via denitrification.

In lysimeter experiments (Table 3), slurry applied in March as compared with "October slurry" resulted in somewhat higher beet and sugar yields and markedly decreased N leaching (21). "August slurry" with rape as a green manure gave similar yields of beets but lower sugar yields and minimal NO_3^- leaching. This implies that N derived from slurry that was taken up by the green manure crop was not yet fully available to the succeeding crop during early growth.

DCD addition to slurry usually resulted in slightly higher beet and sugar yields; the October application resulted in higher N uptake and considerably less leaching of N.

Table 3. Slurry Application to Sugar Beets: Effect of Different Application Times on Beet and Sugar Yields, N Uptake and N Leaching; Results of a Lysimeter Experiment at "Weihenstephan" (800 mm Annual Rainfall; Loess-Brown Earth)

slurry application (100 kg NH_4^+-N/ha)	beet yields (fresh) dt/ha	sugar yields (corrected) dt/ha	removal by beets + leaves kg N/ha	leaching kg N/ha
August, rape as green manure	713	142	164	50
October				
without "Didin"	695	142	165	116
with "Didin"	715	147	187	91
March				
without "Didin"	711	150	166	68
with "Didin"	728	149	166	75
L.S.D.$_{5\%}$	36	9	22	18

(25 kg "Didin"/ha)

Potato Starch Waste Water

Another possibility for DCD use is its addition to waste water from potato starch production. This waste contains about 0.6 kg N/m^3 as organic N compounds (e.g., amino acids, amides) and NH_4^+. Amounts customarily used for sprinkle irrigation are very high (300 to 400 kg N/ha). The organic N compounds are degraded microbially within 2 or 3 weeks during autumn.

By adding DCD, NO_3^- leaching between cropping seasons was reduced by 40 to 70%. Yields of a subsequent crop (2 cuts of rye grass) were increased two- to three-fold (Table 4) (22).

DCD-AMENDED INORGANIC FERTILIZERS

In an incubation trial (at 14° C) with ^{15}N-labelled ammonium sulfate, ammonium sulfate-nitrate, or urea, DCD inhibited nitrification considerably for 63 days (Table 5) (23).

On light soils under high rainfall during the growing season, use of DCD-amended N fertilizers, e.g., ammonium sulfate ("Alzon 22") or urea ("Alzon 47") with 10% of the total N as DCD-N, made it possible to supply N as NH_4^+ to crops for an extended time period and to reduce the NO_3^- and oxalate contents of vegetables and feed crops. Single application of these products in lieu of split applications can be advantageous (especially on very light sandy or rendzina soils) from a labor-saving and economic point of view; fertilizer N may be conserved or used by crops more efficiently (Table 6) (23).

In intensive cereal and sugar production, multiple split applications of N are used. Thereby, temporary over-supply of nitrate, prolific leaf growth at tillering, and high crop susceptibility to diseases is avoided.

Equivalent wheat yields were obtained for a single application of "Alzon" fertilizer and the conventional triple-split application of unamended fertilizer N (Table 7) (24).

Table 4. Effect of "Didin" with Potato Starch Waste Water in Pot Trials with Rye Grass.

waste water application (≈ 384 mg NH_4^+-N/pot)	leaching (during winter) mg N/pot	removal mg N/pot
August		
without "Didin"	128	89
with "Didin"	79	231
November		
without "Didin"	143	104
with "Didin"	42	255
L.S.D.$_{5\%}$	15	12

(120 mg "Didin"/pot)
Soil: Sandy Loam, pH 5.9

Table 5. Turnover of ^{15}N-Ammonium Sulfate (AS) and Ammonium Sulfate-Nitrate (ASN) Combined with Dicyandiamide (DCD) (in % of Added N After 63 Days)

DCD application	AS		ASN	
	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N
- DCD	1	89	1	92
+ DCD	75	10	55	33

Treatment: 100 g Soil (Silty Loam), pH 6.5
20 mg ^{15}N as AS or ASN $\pm$ 2 mg DCD-N
14°C, 50% of Field Capacity

Table 6. Potato and Potato Starch Yields, as Affected by Ammonium Sulfate-Nitrate (ASN) with and without Dicyandiamide (DCD)

fertilizer added		fresh matter dt/ha	starch dt/ha
without N		215	41
200 N	ASN	325	55
200 N	ASN/DCD	354	58
240 N	ASN	326	55
240 N	ASN/DCD	347	56

ASN in 4 applications; ASN/DCD in 3 applications.
Rendzina soil (east of Munich).

A high initial N dressing as Alzon increased population density while high applications of NO_3^- in the spring results in excessive tillering (24).

In experiments with sugar beets, N supply could also be optimized with "Alzon 22." Maximum yield was obtained with a single application of 100 kg N/ha as compared with 200 kg N/ha as calcium ammonium nitrate (Fig. 8). Wet spring weather resulted in markedly higher N losses following calcium ammonium nitrate application than with "Alzon," as measured by soil NO_3^- contents in June (25).

PRESENT AND FUTURE DEVELOPMENTS

In our experiments, we observed a temperature-dependent decomposition (10), as well as a slower degradation of DCD, under reducing conditions (24).

Because the first step of breakdown takes place on surfaces of metal oxides, the idea arose to combine DCD with reducing

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Table 7. Fertilizer Systems for Winter Wheat (cv. Kronjuwel)

fertilizer application (kg N/ha)			yield parameter			yield
early vegetation	late tillering	late dressing	spikes/ m^3	weight (g)/ 1000 seeds	kernels/ spike	(86% dr. m) dt/ha
1983						
1 65 CAN	30	50 CAN	638	39.8	27	68.0
2 145 AS/DCD	-	-	723	40.0	24	68.0
1984						
1 65 CAN	30 CAN	50 CAN	633	41.8	29	77.2
2 145 AS/DCD	-	-	702	39.6	28	77.3
					L.S.D.5%	4.0

CAN = Calcium ammonium nitrate
AS = Ammonium sulfate
Soil: Brown Earth-Loess, pH 6.4, Nmin 75 kg/ha

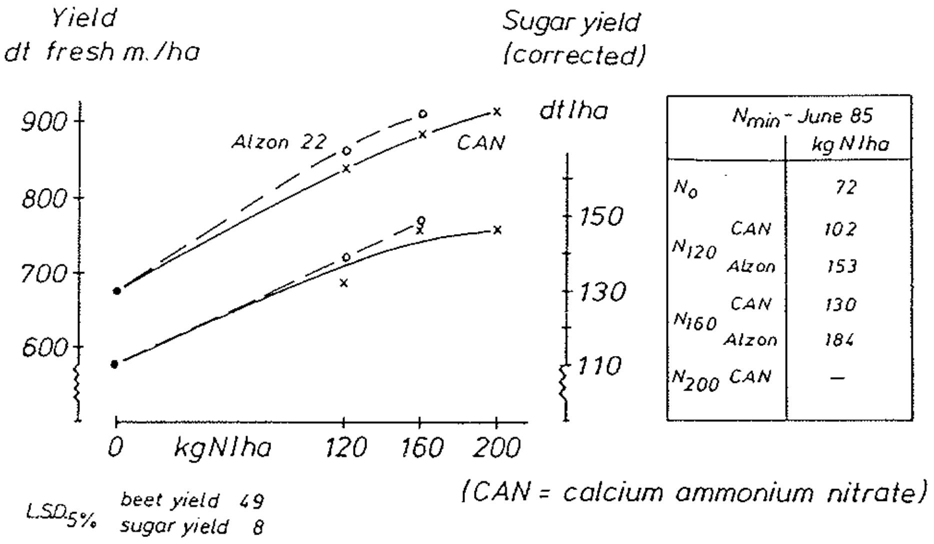

FIG. 8. Effect of "Alzon" on Yield of Sugar Beets (Brown Earth - Loess, Weihenstephan, 1985.)

substances, for example, ammonium thiosulfate (ATS), in order to inactivate iron oxides and prolong the nitrification inhibiting effects.

ATS is a reducing substance with 19% N; according to Goose, et.al (26), it also may act as a nitrification and urease inhibitor before it is degraded.

In one study (8), about 65% of added DCD was decomposed after 1 day under the conditions of the experiment, as compared with only 31% of DCD with ATS and 52% of DCD as "Didin 380," a formulation containing ATS (Table 8).

In addition, to reduce the amount of chemical additive needed, we looked for chemicals with more potent nitrification inhibiting effects. Guanylthiourea (GTU) proved to be a promising chemical, both because it is less phytotoxic than DCD and because of its urease inhibiting effect.

Table 8. Influence of Ammonium Thiosulfate (ATS) on the Decomposition of Dicyandiamide

treatments	DCD-N (% of added) after 24 hours of shaking
1. 1 mg DCD-N	35
2. 1 mg DCD-N + 1 mg ATS-N	48
3. 1 mg DCD-N as "Didin 380" (= 38% Didin + 38% ATS + water)	69

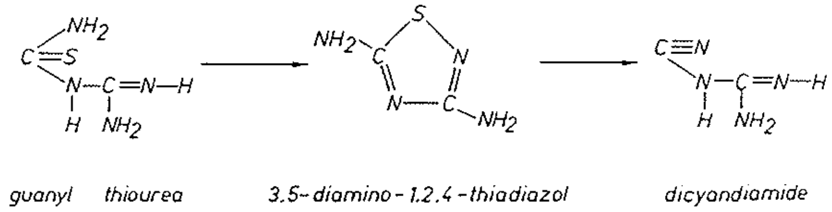

FIG. 9. Reaction of Guanylthiourea

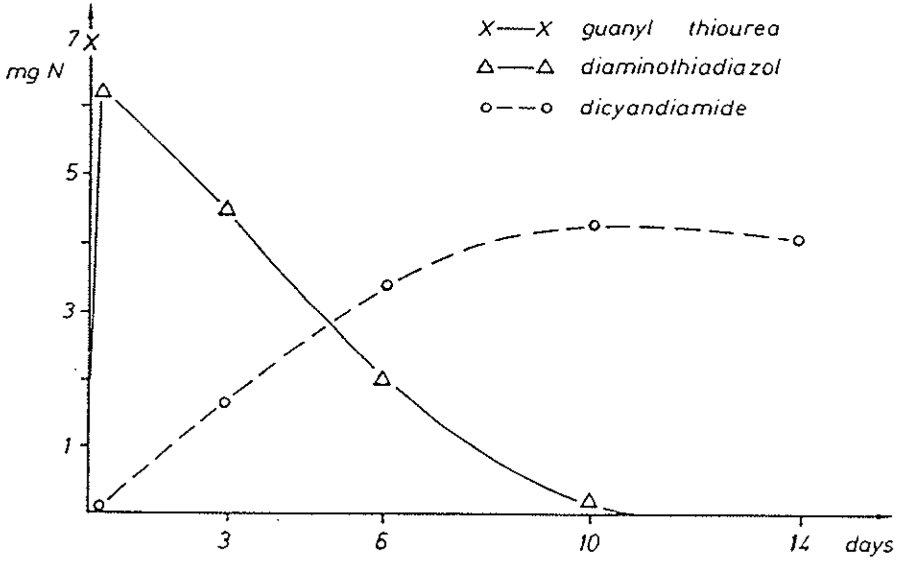

FIG: 10. Degradation of Guanylthiourea (6.9 mg N) in Pettenbrunn Soil (gley - like soil, pH_{CaCl_2} 5.7, 22 % clay, 2.4 % org. matter)

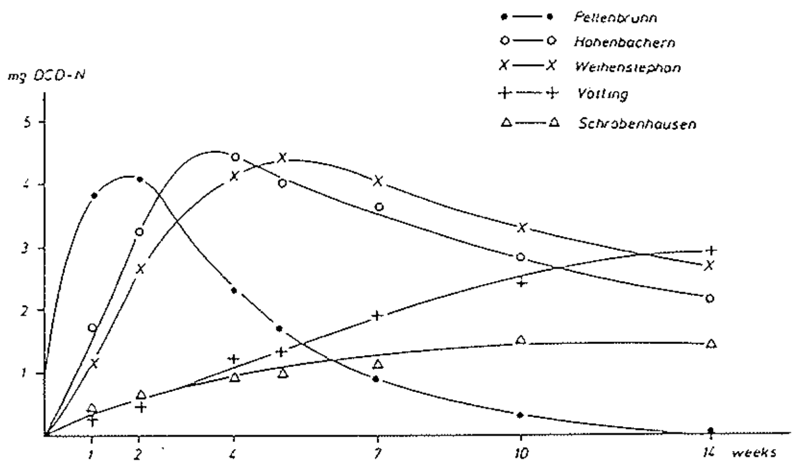

FIG. 11. Transformation of Guanylthiourea (GTU) to Dicyandiamide in Different Soils (6.9 mg GTU added.)

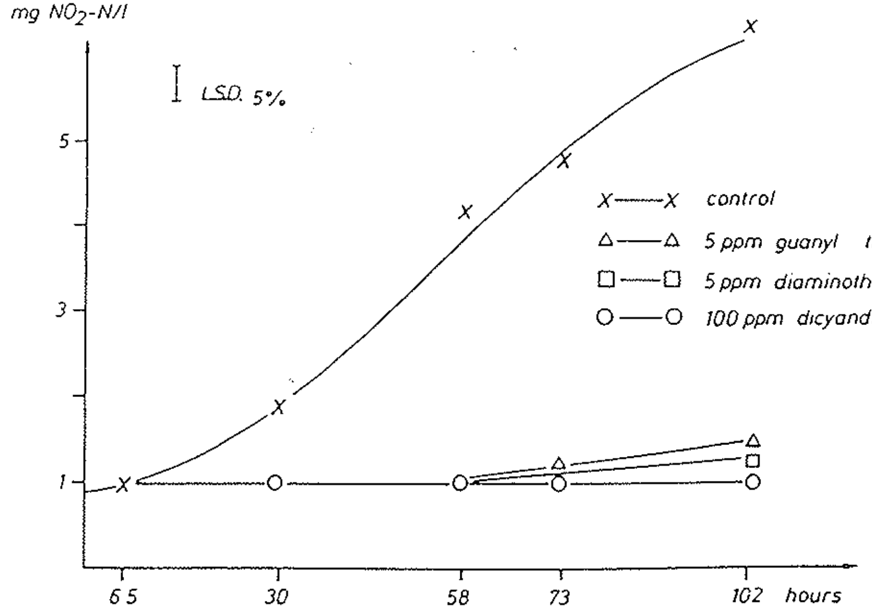

FIG. 12. Growth of Nitrosomanas europaea as Influenced by Guanylthiourea (GTU), 3.5-Diamino-1.2.4-thiadiazol (DTD), and Dicyandiamide (DCD).

GTU is oxidized in the presence of metal oxides to diaminothiadiazol (DTD) and then to DCD (Fig. 9) (27). The formation of DTD takes place in soil relatively quickly (within one day), as evidenced by progressively decreasing amounts of DTD and increasing amounts of DCD (Fig. 10).

The decomposition of GTU to DCD in soils with different clay contents reveals the relatively long persistence of DCD (Fig. 11).

However, GTU (characterized by very low stability) and DTD (with a somewhat longer persistence), show that even at very low concentrations a much stronger inhibiting effect on Nitrosomonas europaea in pure cultures than DCD alone (Fig. 12).

Consequently, GTU or formulations of GTU and DCD may be more efficient in inhibiting nitrification than DCD alone, and smaller amounts of these inhibiting chemicals may be needed.

CONCLUSIONS

The use of nitrification inhibitors is a beneficial technology for efficient management of N fertilizers. Increased efficiencies may still be possible in the near future. The goal is to improve N fertilizer efficiency and decrease NO_3^- losses so as to minimize the economic and environmental risks that are inherent in agricultural production.

REFERENCES

1. Olson, R. K., and W. A. Reiners. 1983. Nitrification in subalpine balsam fir soils: tests for inhibitory factors. Soil Biol. Biochem. 15:413-418.

2. Neal, J. L. 1969. Inhibition of nitrifying bacteria by grass and forb root extracts. Can. J. Microbiolo. 15:633-635.

3. Hauck, R. D. 1980. Mode of action of nitrification inhibitors. p. 19-32. In J. J. Meisinger et al. (ed.) Nitrification inhibitors--potentials and limitations. ASA Spec. Pub. 38. Am. Soc. of Agron., Madison, WI.

4. Amberger, A. 1986. 1986. Potential of nitrification inhibitors. In Modern N-fertilizer management. Z. Pflanzenern. u. Bodenkde. 149:469-484.

5. TNO. 1977. Determination of the acute oral toxicity of dicyandiamide EH in rat. Centraal Instituut voor Voedingsonderzoek, Holland.

6. Zacherl, B., and A. Amberger. 1985. Hemmung der Ammoniumoxidation durch Nitrosomonas europaea mit verschiedenen Nitrifikationshemmstoffen. VDLUFA-Schriftenreihe 11:55-63.

7. Bosch, M., and A. Amberger. 1983. Einfluß langjahriger Dungung mit verschiedenen N-Formen auf pH-Wert, Humusfraktionen, biologische Aktivitat und Stickstoffdynamik einer Ackerbraunerde. Z. Pflanzernern. u. Bodenkde. 146:714-724.

8. Stichlmair, M. 1984. Einfluß des Nitrifikationshemmstoffs Dicyandiamid auf Bodenenzyme und ausgewahlte Bodenmikroorganismen. Diplomarbeit TU Munchen-Weihenstephan.

9. Amberger, A., and K. Vilsmeier. 1979. Dicyandiamidabbau in Quarzsand und Boden. Z. Pflanzernern. u. Bodenkde. 142:778-785.

10. Vilsmeier, K. 1981. Modellversuche zur nitrifikationshemmenden Wirkung von dicyandiamid ("Didin"). Bayer. Landw. Jb. 58:853-857.

11. Vilsmeier, K. 1980. Dicyandiamidabbau im Boden in Abhangigkeit von der Temperatur. Z. Pflanzenern. u. Bodenkde. 143:113-118.

12. Vilsmeier, K. 1981. Action and degradation of dicyandiamide in soils. p. 18-24. In Hauck, R. D., and H. Behnke (ed). Proc. of the Techn. Workshop on Dicyandiamide. Tennessee Valley Authority, Muscle Shoals, AL Dec. 4-5.

13. Vilsmeier, K. 1984. Unsatz von ^{15}N-Dicyandiamid im Boden. VDLUFA-Schriftenreihe, 11:64-73.

14. Amberger, A., and K. Vilsmeier. 1988. Untersuchungen zur Auswaschung von Dicyandiamid und dessen Abbau in uberstauten Boden. Wasser and Abwasserforschung (submitted for publication).

15. Bornemisza, E. 1984. Nitrogen loss reduction with a nitrification inhibitor in the wet tropics of Costa Rica. In Fight against hunger through improved plant nutrition. 9th World Fertilizer Congress, Proceeding Budapest, Hungary Vol. 2, 185-187.

16. Vilsmeier, K., E. Bornemisza, and A. Amberger. 1987. Urea, ammonium sulfate, and dicyandiamide transformations in Costa Rican soils. Fert. Res. 12:255-261.

17. Amberger, A. and K. Vilsmeier. 1983. Stickstoffbilanz von ^{15}N-Harnstoff bzw. ^{15}N-Ammonsulfatsalpeter mit Dicyandiamid in Gefaßversuchen zu Grunhafer und Sommerweizen. Z. Pflanzernern. u. Bodenkde. 146:438-448.

18. Vilsmeier, K. 1984. Stickstoffbilanz von ^{15}N-Ammoniumsulfat mit Dicyandiamid und ^{15}N-Dicyandiamidaufnahme durch Hafer und Weizen. VDLUFA-Schriftenreihe 11:97-107.

19. Vilsmeier, K., and A. Amberger. 1987. Zur nitrifikationshemmenden Wirkung von Dicyandiamid zu Gulle in der Zeit zwischen Spatherbst und Fruhjahr. Z. Pflanzernern. u. Bodenkde. 150:47-50.

20. Amberger, A. Neuere Ergebnisse uber den Einsatz von Dicyandiamid ("Didin") als Nitrifikationshemmstoff in Deutschland. Vortrag Kopenhagen 10.9.84.

21. Gutser, R. and A. Amberger. 1984. Nitratauswaschung nach Gulledungung mit Didinzusatz. Landw. Forsch., Kongreß, 137-145.

22. Amberger, A. and R. Gutser. 1984. N-Wirkung von "Kartoffelfruchtwasser" mit Didinzusatz. VDLUFA - Schriftenreihe 11:305-315.

23. Amberger, A. 1981. Einsatz und Ausnutzung von N-Dungemitteln. Bayer. Landw. Jb. 58:80-88.

24. Mokry, M. and A. Amberger. 1984. Einfluß von Nitrat-, Ammonium- und Mischernahrung mit Dicyandiamid auf Ertragsbildung und Mineralstoffgehalte von Sommerweizen. VDLUFA-Schriftenreihe 11:118-125.

25. Amberger, A. and R. Gutser. 1987. Wirkung und Einsatz von Nitrifikationshemmstoffen in Kombination mit Mineraldungern. Bayer. Landw. Jb. 64:717-726.

26. Goos, R. J., R. P. Voss, and T. E. Fairlie. 1986. Ammonium thiosulfate as a possible nitrification and urease inhibitor. Sulfur in Agric. 10:2-5.

27. Germann-Bauer, M. 1987. Wirkung und Abbau des Nitrifikationshemmstoffes Guanylthioharnstoff. Dissertation TU. Munchen-Weihenstephan.,