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Degradation of the nitrification inhibitor 1-amidino-2-thiourea in soils, and its action in Nitrosomonas pure culture and soil incubation experiments

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Abstract

The degradation of guanylthiourea (GTU) via 3,5-diamino-1,2,4-thiadiazole (TDZ) to dicyandiamide (DCD) was studied in selected soils. All three compounds could be determined by HPLC. GTU decomposed rapidly (within hours-days), the reaction from TDZ to DCD continued more slowly (within days-weeks). Soil type and temperature had an essential effect on the rate of degradation; conspicuous was a more rapid breakdown of GTU in presence of ammonium sulfate (AS) than in combination with urea.

Each compound is a nitrification inhibitor; in *Nitrosomonas* cell suspensions, 0.5 ppm GTU and 10 ppm TDZ achieved an effect comparable to 200 ppm DCD.

The combination of these two effects — degradation in soil and inhibition of nitrification — were studied in soil incubation experiments. The three substances had inhibitory effects also in soil, however at significantly different application rates (20 ppm GTU or TDZ and 30 ppm DCD). Using these concentrations, AS/DCD and urea/GTU showed similar effects.

Urea/GTU retarded nitrification by the factor 1.7 as compared to urea/DCD. AS/GTU had no advantage over AS/DCD which can be explained by the more rapid degradation of GTU in presence of AS.

Urea/GTU apparently presents a promising possibility to utilize N-fertilizers more efficiently.

Introduction

Nitrification inhibitors in agriculture are known to retard selectively the bacterial transformation of NH_4^+-ions into NO_3^- in soil. In contrast to ammonium, nitrate is susceptible to losses by denitrification and leaching. The latter process is of immediate public interest because of its impact on ground- and drinking-water quality. Numerous nitrification inhibitors are known, but few are acceptable for a broad agricultural use due to possible toxic side effects [8, 14]. Some highly effective compounds like N-serve are volatile and thus cannot be combined with solid fertilizers like urea. With increasing importance of urea (U) as a nitrogen source the demand for a suitable nitrification inhibitor is growing. In presence of urea most of the inhibitors show poorer performance than in combination with ammonium-sulfate (AS) [4, 15]. Furthermore, adding nitrification inhibitors to urea [2, 14] may enhance NH_3 volatilization. Intensive research has been done with the nitrification inhibitor dicyandiamide (DCD) [1, 2, 3, 4, 5, 13, 17, 18, 19, 20, 21]. No toxic effects of DCD or its metabolites are known and it can be combined with solid fertilizers. The usual application rate (ca. 15 kg/ha) is considerably high (N-serve ca. 2 kg/ha). The combination U/DCD gives distinctly poorer effects than AS/DCD.

Some workers have attempted to discover related compounds which are more effective than DCD, but with similar advantages. Thiourea was found to

be a promising substance according to experiments in *Nitrosomonas europaea* pure culture media, but failed to perform equally in soil incubation experiments; the required application rate (ca. 25 kg/ha) was too high [6, 20].

1-amidino-2-thiourea or guanylthiourea (GTU), is patented as nitrification inhibitor [12]. In comparative tests 10–20 ppm GTU in soil showed similar effects to 5 ppm N-serve [16]; elsewhere, 10 ppm GTU performed somewhat worse than 10 ppm DCD [4]. The degradation of GTU in soil and plant was studied in China; it was established, that GTU decomposes via 3,5-diamino-1,2,4-thiadiazole (TDZ) to DCD [22] (Fig. 1).

The metabolite TDZ has a chemical structure similar to 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole, a nitrification inhibitor known as 'Dwell'. This is one of the most effective inhibitors, but is not suitable for agricultural use due to its volatility and fungicidal side effects [8, 10, 15, 16].

The goal of the following studies was to examine whether the compounds GTU or TDZ are more effective than DCD in soil and the extent to which their nitrification inhibitory effect is due to the release of DCD.

Material and methods

Inhibitors: TDZ, GTU: SKW Trostberg
DCD: Merck

Soils: characteristics given in Table 1.

The soils were collected fresh from the field, air dried to about 40% of the water holding capacity (WHC), sieved (2 mm) and stored at 0–4° C. All experiments were run in duplicate unless otherwise specified. Fertilizers and inhibitors were added as solutions and thoroughly mixed with the soil in polyethylene bottles. The samples were adjusted to 60% WHC unless otherwise specified and incubated at the appropriate temperature for up to 24 weeks. The polyethylene bottles allowed gas exchange; evaporation losses during the experiments

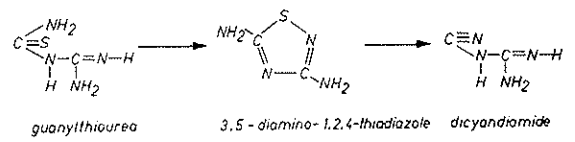

Fig. 1. Decomposition of GTU.

were replaced with distilled water. The extraction of nitrate and inhibitors was carried out by shaking the soil (50–100 g) for one hour with up to 200 ml distilled water, taking in account the remaining soil water. Following filtration, ammonium was determined in the soil water solution with an ammonia specific electrode (Orion 9512), and DCD, TDZ, NO_3^-, and NO_2^- determined in untreated filtrate by HPLC as recommended by Vilsmeier [19]. For HPLC-determination of GTU, a recorded wavelength of 253 nm was used instead of 220 nm. Nitrite in *Nitrosomonas* cell suspensions was determined colorimetically with sulfanilamide and N-(1-naphtol)-ethylenediamine at 546 nm [7, 21].

Degradation experiments

Experiment 1: various soils, air-dried = 50 g dry weight ± 15 mg GTU (6.9 mg GTU-N), 15°C

Experiment 2: soil Dürnast, air-dried = 10 g dry weight ± 15 mg GTU at 4°, 15° and 30°C

Experiment 3: 300 g fresh soil (Dürnast) + 60 mg N as urea or AS including inhibitor-N (3.1 mg GTU-N = 6.6 mg GTU), 50% WHC, incubation in greenhouse at 17°–25°C

Experiments with Nitrosomonas *cell suspensions*

Experiment 4:
Nitrosomonas europaea ('Nm 35', Univ. Hamburg') was cultivated as described by Zacherl [21] in nutrient solution according to Krümmel and Harms [11]. An equivalent of 50×10^{-6} g of bacteria protein was added to 10^{-1} mol/l tris-(hydroxymethyl) aminomethane ($C_4H_{11}NO_3$) buffer at pH 8.0, containing 10^{-4} mol/l $(NH_4)_2SO_4$ and the inhibitors: none, 200 ppm DCD, 10 ppm TDZ, or 0.5 ppm GTU. These tests were run with 6 replicates.

Nitrification experiments with soils

Experiment 5:
soil Dürnast, air-dried = 50 g dry weight + 10 mg urea-N, 4 replicates. The inhibitors were applied as

Table 1. Soil properties.

Soils	pH_{CaCl2}	C	N	Clay	Silt	$CaCO_3$
			%			
Dü Dürnast loess brown earth	6.4	1.11	0.12	20	66	0.1
Sc Schrobenhausen sandy brown earth	6.2	0.84	0.04	6	10	0
Vö Vötting humic calcareous gley	7.5	3.10	0.36	26	18	41.3
Ws Weihenstephan loess brown earth	6.1	0.72	0.09	25	59	0.2
Pt Pettenbrunn gley-like soil	5.7	1.18	0.14	22	66	0
Mi Mintraching	7.3	43.40	0.49	35	16	10.0
o Hohenbachern brown earth from tertiary sand	5.7	1.10	0.12	12	29	0

1.5 mg DCD, 1.0 mg TDZ, or 1.0 mg GTU equivalent to 1.0/0.5/0.5 mg inhibitor-N.

Results and discussion

1. Degradation experiments

a) Degradation of GTU in various soils (experiment 1)

DCD and TDZ could be analyzed simultaneously in an aqueous soil extract. In a second run, TDZ and GTU were determined at 253 nm. GTU can be extracted from soil with water, and is obviously not adsorbed, but sorptive processes apparently occured on the HPLC-column causing a considerable variation of the GTU-values. In case of TDZ the employed method was very efficient, only in one sample a standard deviation of 7% was noticed (Fig. 2, soil Mintraching after 3 d). Since all three compounds, DCD, TDZ, and GTU contain the same number of N-atoms, results are presented as 'mg N' to illustrate the molar reaction. The recovery of GTU as metabolites TDZ and DCD is high, but decreases, e.g. in soil Pettenbrun significantly after 6 days (Fig. 2); at this point, the DCD-concentration has passed its maximum and the metabolites of DCD, guanylurea, guanidine, and urea, appearing by this time [17], cannot be measured with the employed method [7].

GTU disappeared in some samples already after 4 h, or in other soils not later than after 3 d (Fig. 2). The degradation of TDZ to DCD was much slower, with great variations: a maximum DCD-concentration was measured in soil Pettenbrunn after 2 weeks, in soil Schrobenhausen or Vötting only after 10 or 14 weeks resp. (Fig. 3). In particular the two soils with a comparably slow GTU degradation differ widely in pH-value (6.2/7.3), clay (6%/35%), and organic matter content (1.7%/8.8%). None of the analyzed soil properties can explain the longer persistence of GTU in these two soils. The high clay content in soil Pettenbrunn may have been essential for the rapid breakdown of GTU compared to soil Hohenbachern, since all other properties of these two soils are similar.

From the fact that all soils showing a slow reaction from GTU to DCD exhibit pH-values over 6, it might be concluded that a higher pH-value retards decomposition.

Further general conclusions concerning the influence of soil parameters on the rate of GTU-degradation cannot be drawn from this experiment.

b) Effects of temperature and N-form on degradation of GTU

The following experiments were performed with soil Dürnast to obtain results comparable to the numerous experiments concerning the breakdown of DCD in this soil [3, 17, 18].

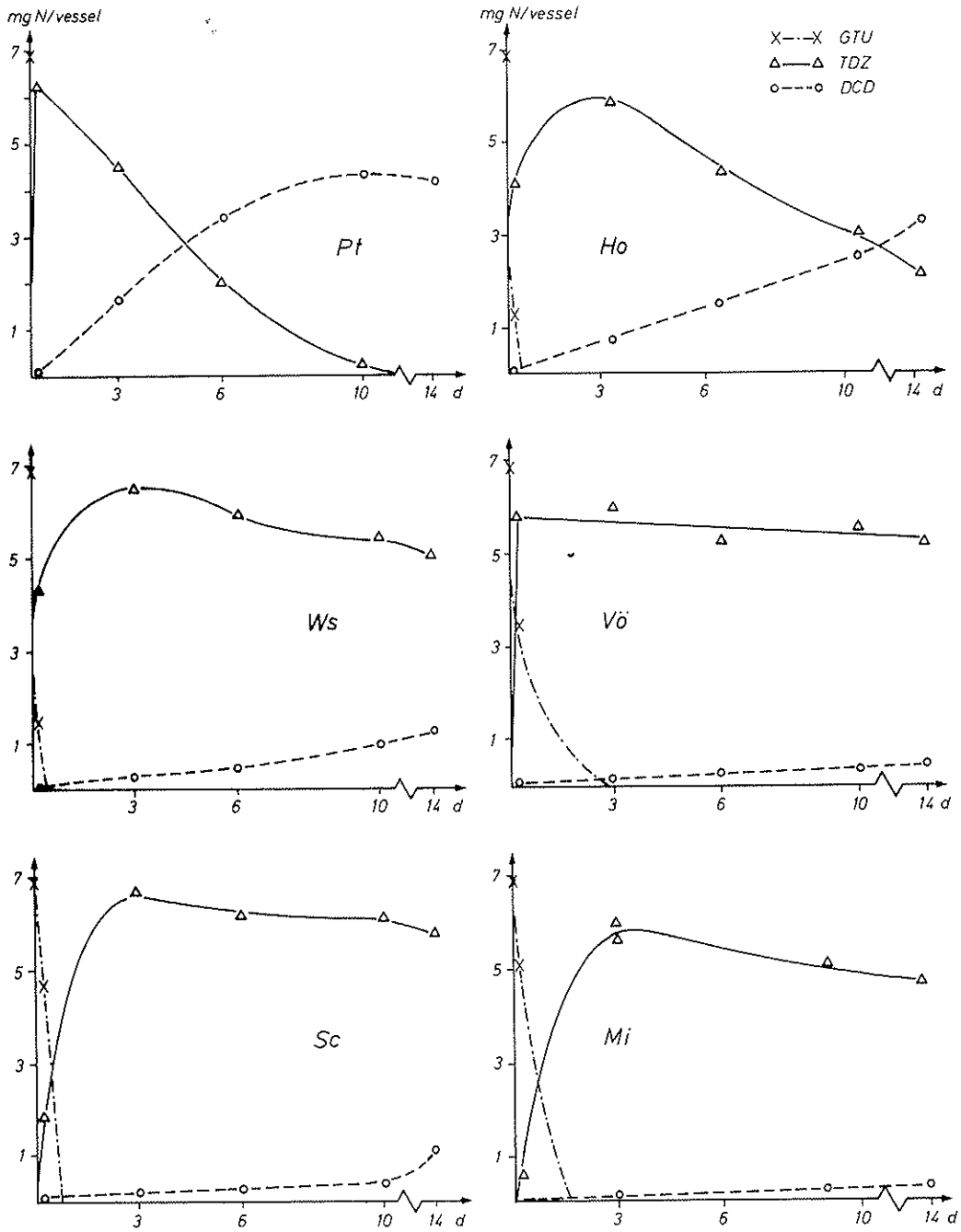

Fig. 2. Degradation of GTU in soils. Pt = Pettenbrunn; Ho = Hohenbachern; Ws = Weihenstephan; Sc = Schrobenhausen; Vö = Vötting; Mi = Mintraching. (50 g soil dry m. + 15 mg GTU = 6.9 mg N/vessel, 15°C).

Influence of temperature (experiment 2). Effects of temperature on the reaction GTU → DCD were remarkable (Fig. 4); a higher temperature resulted in an accelerated release of DCD from GTU. As the rate of breakdown of DCD increases in the same way [7, 17], the maximum DCD concentration observed at 30° and 15°C was smaller than at 4°C.

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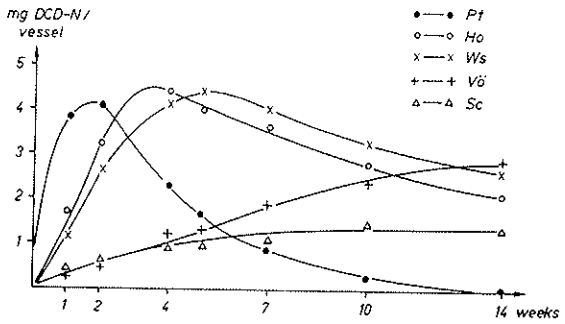

Fig. 3. Degradation of GTU to DCD in soils. Pt = Pettenbrunn; Ho = Hohenbachern; Ws = Weihenstephan; Sc = Schrobenhausen; Vö = Vötting. (50 g soil dry m. + 15 mg GTU = 6.9 mg N/vessel, 15°C).

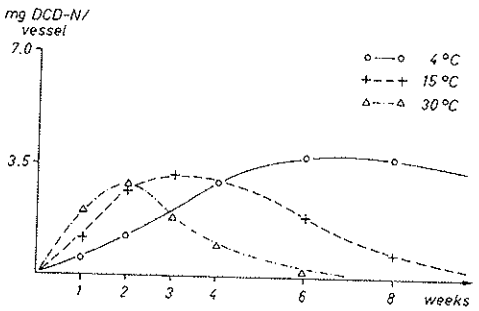

Fig. 4. Degradation of GTU to DCD in soil Dürnast at various temperatures. (100 g soil dry m. + 15 mg GTU = 6.9 mg N/ vessel, 15°C).

Influence of the N-form (ammonium sulfate or urea, experiment 3). In this experiment, GTU was applied at a concentration which had been determined in incubation experiments as an effective rate (20 ppm GTU) (Fig. 5).

The decomposition of GTU to DCD was quite

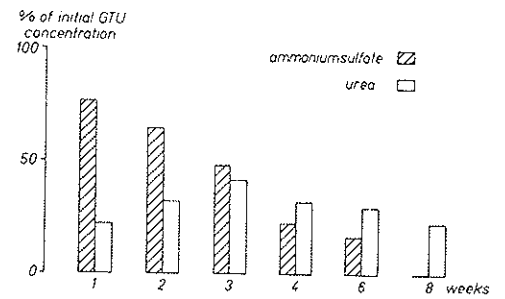

Fig. 5. Degradation of GTU in soil Dürnast in presence of ammonium sulfate or urea. (300 g fresh soil + 60 mg N as urea or ammonium sulfate including 6.6 mg GTU = 3.1 mg N/vessel, greenhouse 17–25°C).

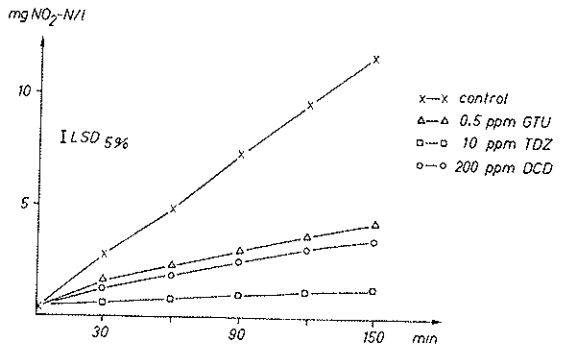

Fig. 6. Effect of GTU, TDZ, and DCD on NO_2 production in Nitrosomonas europea cell suspensions.

different in presence of urea as compared to the presence of ammonium sulfate. In combination with urea, GTU was decomposed at a much slower rate than in the case of AS addition. The maximum DCD concentration in the U/GTU treatment was reached only after 3 weeks, whereas in combination with AS ca. 60% of the GTU applied were already converted to DCD after 1 week. After 4 weeks, DCD concentrations in the AS/GTU treatment were significantly lower than in the case of U/GTU.

Influence of soil moisture on degradation of GTU. Soil moisture in a range of 50 to 100% WHC had no detectable effect on the degradation of GTU to DCD, but DCD is decomposed slowlier with increasing moisture [3, 7]. Dry or flooded conditions, however, essentially retarded decomposition of GTU and TDZ in soils (values not shown) [7].

2. Nitrification inhibiting effects of GTU, TDZ, and DCD in Nitrosomonas *cell suspensions (experiment 4)*

The degradation experiments in soils showed that GTU is converted rapidly via TDZ to DCD. This raised the question whether the nitrification inhibitory effect is caused by GTU itself, or its metabolites TDZ and DCD. The metabolites of DCD do not give such effects [17]. In order to reduce the observation time and consequently the influence of degradation to a minimum, highly active cell suspensions of *Nitrosomonas europaea* were used.

After a series of preliminary experiments, similar nitrification inhibitory effects, as measured by NO_2-production, were observed with 0.5 ppm

GTU, 10 ppm TDZ, and 200 ppm DCD (Fig. 6). GTU was thus much more effective than its metabolites TDZ and DCD. TDZ has not been described as a nitrification inhibitor before; due to the analogy of its chemical structure to the known inhibitor 'Dwell' (3-ethoxy-5-trichloromethyl-1,2,4-thiadiazole) such an effect was to be expected.

3. Nitrification inhibiting effects of GTU, TDZ, and DCD with urea in soils (experiment 5)

Incubation experiments with soils were carried out to test whether GTU is able to express its inhibitory effect in soil, despite rapid degradation, or whether inhibition is caused by its metabolites. Preliminary experiments revealed that in order to obtain similar effects of GTU and DCD, the ratio GTU : DCD (1 : 400 in *Nitrosomonas* cell suspensions) had to be changed to 1 : 1.5 in soil.

In the Dürnast soil, 30 ppm DCD in combination with urea showed a significantly inferior effect to 20 ppm TDZ or GTU. In nearly all samples TDZ performed better than GTU (Fig. 7). Since GTU does not appear in considerable concentrations but decomposes rapidly to TDZ and DCD (Fig. 2), TDZ must be the compound responsible for the inhibiting effect in soil.

In general, all three compounds also showed nitrification inhibiting effects in soil; the degradation processes, however, strongly reduced the advantages of GTU and TDZ over DCD found in *Nitrosomonas* pure culture.

4. Nitrification inhibiting effects of DCD and GTU with urea or ammonium sulfate in soils (experiment 6)

Besides soil type, temperature and form of N greatly influenced the degradation of GTU; whether these processes affect the inhibitory action of GTU in soil was also examined (Fig. 8). The time interval in which 50% of the applied nitrogen are nitrified was chosen as an index for presentation of the obtained data.

At 4°C, urea alone was nitrified more rapidly than ammonium sulfate alone. When inhibitors were added, 24 weeks were insufficient for 50% nitrification in all cases. At temperatures over 8°C,

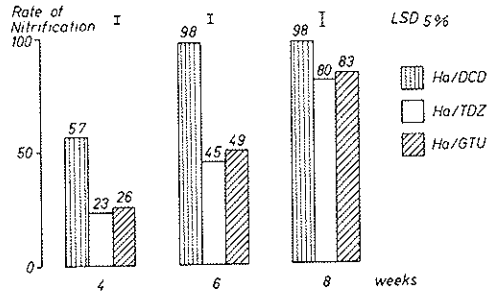

Fig. 7. Effect of GTU, TDZ, and DCD on nitrification of urea in soil Dürnast. (50 g soil dry m. + 10 mg N as urea + 1.5 mg DCD/1 mg TDZ/1 mg GTU).

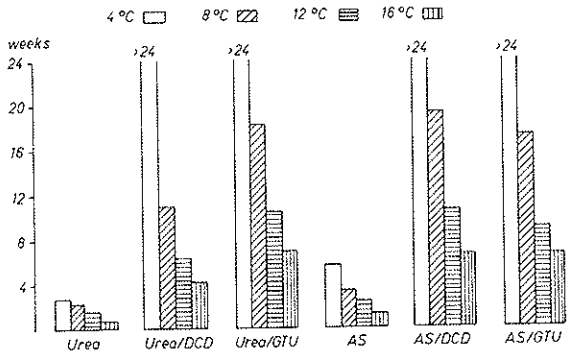

Fig. 8. Time for 50% nitrification of urea or ammonium sulfate in combination with inhibitors at various temperatures, soil Dürnast. (50 g soil dry m. + 10 mg N as urea or ammonium sulfate + 1.5 mg DCD/1 mg GTU).

DCD was more effective in combination with AS than with urea. When GTU was used, the difference between the N-forms was not so pronounced, but the nitrification of urea was more clearly reduced than of AS. At the low temperature, and combined with AS, DCD (30 ppm) was a more effective inhibitor than GTU (20 ppm). In combination with urea, however, 20 ppm GTU showed a distinctly better effect than 30 ppm DCD and gave the same result as AS/DCD.

The combination of urea/GTU as 10% of fertilizer, equivalent to 10 kg GTU at an application rate of 100 kg N/ha, offers a promising possibility for more efficient N fertilization. The degradation processes of GTU are known and, like DCD, it has the advantage over other nitrification inhibitors of being non-volatile thus facilitating combinations with solid fertilizers.

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