Relative Phytotoxicity of Dicyandiamide and Availability of its Nitrogen to Cotton, Corn, and Grain Sorghum[1]

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ABSTRACT

The nitrification inhibitor, dicyandiamide (cyanoguanidine) (DCD), can improve fertilizer N efficiency; however, yield reductions and phytotoxicity from the use of DCD have been reported. A greenhouse experiment was designed to determine the effect of DCD on growth, chlorophyll concentration and nutrient concentration of corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and grain sorghum [*Sorghum bicolor* (L.) Moench.]. Dicyandiamide-N/urea-N combinations of 0:60, 5:55, 10:50, 20:40, 30:30, 40:20, 50:10, and 60:0 mg kg^{-1} soil were applied to pots containing a Norfolk sandy loam (fine-loamy, siliceous, thermic, Typic Paleudults) cropped to each of the three species. Increasing the proportion of N as DCD decreased plant dry weight and leaf chlorophyll concentration and increased stem-leaf N concentrations. Nitrogen recovery decreased curvilinearly from 103% to 4%. from 64% to -6%. and from 72% to 4% for corn, cotton, and sorghum, respectively. with increasing proportion of N as DCD-N. The effects of DCD-N on stem-leaf tissue concentrations of P, K, Ca, Mg, Fe, Mn, Zn, and Cu varied with DCD-N concentration, plant species, and nutrient element. At lower DCD-N concentrations, most nutrient element concentrations were affected by uptake of NH; -N derived from urea; while higher concentrations of DCD-N resulted in increased nutrient element concentrations as a result of reduced plant growth.

Additional Index Words: Zea mays, Gossypium hirsutum, Sorghum bicolor, nitrifiication inhibitor, DCD, nutrient composition, chlorophyll.

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DICYANDIAMIDE (cyanoguanidine) (DCD), $C_2H_4N_4$, is a dimer of cyanamide, and is an effective nitrification inhibitor (Hauck, 1980; Nommik, 1958; Reddy, 1964a; Rodgers and Ashworth, 1982). In addition to its nitrification inhibiting properties, DCD contains 67% N. In soil, DCD undergoes decomposition to ammonium and nitrate (Reider and Michaud, 1980; Amberger and Vilsmeier, 1979), and the nitrogen in DCD is thus eventually plant-available. The decomposition rate increases with organic carbon (Reddy, 1964a), Fe^{3+} hydroxides (Amberger and Vilsmeier, 1979), and temperature (Vilsmeier, 1980). Data from Reddy (1964a) indicated that in the presence of an organic carbon source (sucrose), substantial mineralization of 67 mg kg^{-1} DCD-N took place in a Cecil sandy loam after only 15 d. Reider and Michaud (1980) reported that rapid mineralization, in three soils (DCD-N, to NH; -N and NO_3- -N) began after 28 d and was complete after approximately 70 d.

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Although DCD is an effective nitrification inhibitor and can improve fertilizer N efficiency, yield reductions and phytotoxicity from the use of DCD have been reported. Cowie (1918) reported toxicity symptoms in pot trials with barley (*Hordeum vulgare* L.) from concentrations of nitrogen as DCD (DCD-N) exceeding 18 mg kg^{-1} soil. In field trials, however, no injurious effects were noted from 30 kg ha^{-1} of DCD-N. Nommik (I 958) noted leaf injury symptoms in oats (*Avena sativa* L.) when rates of DCD-N exceeded 28 kg ha^{-1}. Reddy (1964b) reported that 16.7 mg kg^{-1} DCD-N decreased dry matter yields of wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), maize (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and tomato (*Lycopersicon esculentum* Mill.), but Increased yields of Coastal bermuda grass [*Cynodon dactylon* (L.) Pers.]. The decreases in dry weights were, in part, dependent on the interaction between plant species and N source. In a field experiment with wheat (Sommer and Rossig, 1978), DCD in combination with urea increased N uptake, but reduced grain yields by 10%. Maftoun and Sheibany (1979) reported that DCD decreased green and dry tissue weights of pot-grown soybeans [*Glycine max* (L.) Merr.]. The soil concentration of DCD-N needed to reduce fresh and dry weights by 50% was 72 and 58 mg kg^{-1}, respectively. Visual symptoms of DCD phytotoxicity, other than reduced growth, on wheat (Allison et al., 1925; Reddy, 1964b), barley (Cowie, 1918), oats (Nommik, 1958; Reddy, 1964b), and corn, tomato, and cotton (Reddy, 1964b) include leaf tip and margin chlorosis and necrosis. These symptoms suggest that one effect of DCD phytotoxicity may be reduced synthesis or increased degradation of chlorophyll.

The only report of DCD phytotoxicity to corn and cotton limited DCD-N rates to 3.3, 6.7, and 16.7 mg kg^{-1} (Reddy, 1964b). No efforts have been made to quantify DCD's phytotoxicity at rates that could result from banded application of N formulated with DCD. In addition, the relative phytotoxicity of DCD to grain sorghum [*Sorghum bicolor* (L.) Moench.] has not been reported.

The objectives of this study were to determine the availability and the phytotoxicity of DCD-N to crops commonly grown in coastal Plain soils. The effects of DCD-N on plant growth, nutrient uptake, and chlorophyll concentration were used as criteria for determining availability and phytotoxicity of DCD.

MATERIALS AND METHODS

Four to seven seeds of 'Ring Around 1502' corn, 'Deltapine 90' cotton and 'Northrup King Savanna 5' grain sorghum were planted in separate 20-cm diam plastic containers containing 2.7 kg of air-dried Norfolk sandy loam (tine-loamy, siliceous, thermic, Typic Paleudults) which had been sieved

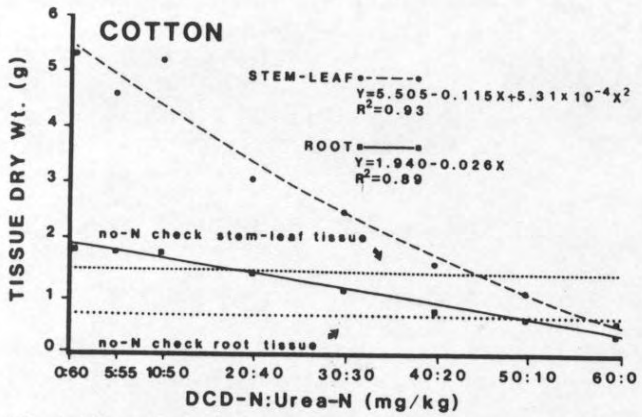

Fig. 1. Effect of DCD-N on dry weight of cotton stem-leaf and root tissue (X = mg kg ' DCD-N).

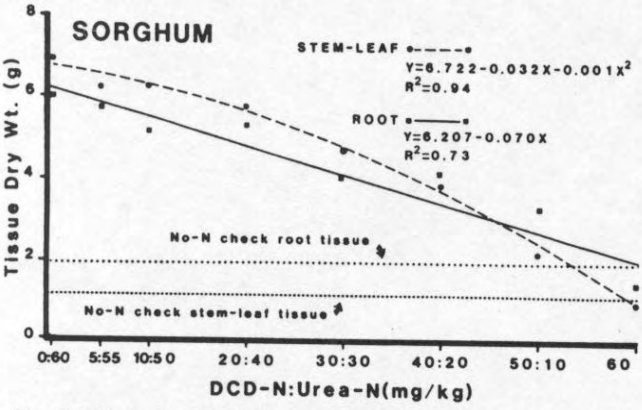

Fig. 3. Effect of DCD-N on dry weight of sorghum stem-leaf and root tissue (X = mg kg ' DCD-N).

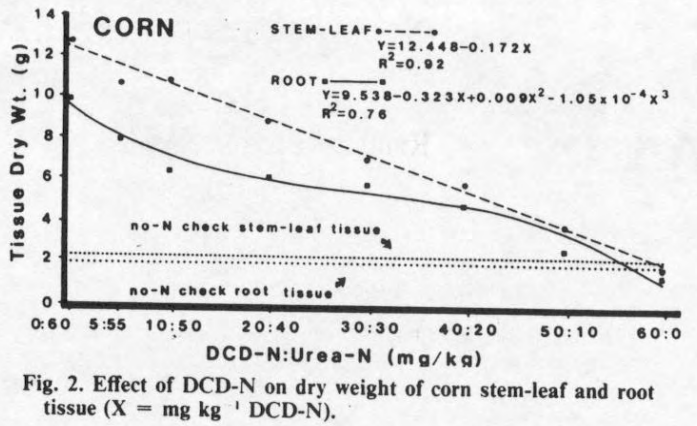

Fig. 2. Effect of DCD-N on dry weight of corn stem-leaf and root tissue (X = mg kg ' DCD-N).

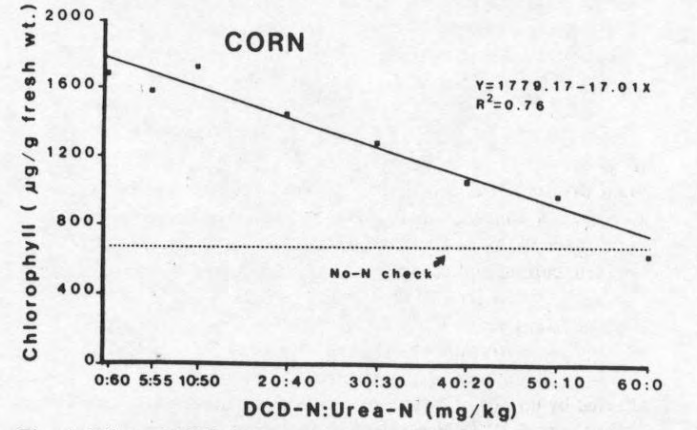

Fig. 4. Effect of DCD-N on total chlorophyll concentration of corn leaves (X = mg kg ' DCD-N).

through a 0.25-cm screen. The initial soil pH was 6.2, and Mehlich I (Mehlich, 1953) P, K, Ca, and Mg (Hue and Evans, 1979) averaged 120, 128, 1053, and 130 kg ha^{-1}, respectively. Organic matter content averaged 13.8 g/kg and cation exchange capacity averaged 4.2 cmol (+) kg^{-1}. Pots were fertilized at planting, and weekly thereafter, with Hoagland's solution (Hoagland and Amon, 1950) minus N to ensure that no mineral deficiencies would confound results from DCD treatments. Seven d after emergence, corn and cotton were thinned to 2 plants per pot, and sorghum was thinned to 4 plants per pot. Eleven d after emergence. N was supplied at 60 mg kg^{-1} as aqueous solutions of DCD-urea combinations to the soil surface of every pot except the 0-N check pots. Dicyandiamide-N/urea-N combinations used as treatments were 0:60, 5:55, 10:50, 20:40, 30:30, 40:20, 50:10, and 60:0 mg kg^{-1} soil.

The experimental design was a split plot in a randomized complete block with live replications. Main plots were plant species and subplots were DCD treatments. Statistical analyses included analysis of variance, and first, second, and third order polynomial regression of growth variables, chlorophyll concentrations, and mineral concentrations on DCD N rates.

Forty-five d after emergence (34 d after treatment initiation), plants were harvested and separated into aboveground (stem-leaf portions and roots. Soil was washed from roots and all tissue was rinsed in deionized water before drying for 72 h at 60°C. Tissue was then weighed and ground to pass a 40-mesh screen. Nitrogen concentrations were determined with a LECO CHN-600[3] carbon-hydrogen-nitrogen analyzer. Apparent N recovery was defined as the difference in N content of all plant tissue (root + stem-leaf tissue) in each N-treated pot and the N content of all plant tissue from O-N control pots. Concentrations of P, K, Ca, Mg, Fe, Mn, Zn, and Cu were determined from wet-ashed samples analyzed with an Inductively Coupled Argon Plasma spectrophotometer (ICAP). The third leaf from each plant in each pot was frozen immediately and stored for 4 d at - 10°C before preparation for chlorophyll determinations. Two subsamples of leaf tissue were taken from leaf midsectons for chlorophyll determinations. The tissue was chopped into ap proximately 0.5-cm diam sections, extracted twice with 80% (v/v) acetone for 12 h at 5°C, and made to an equal volume (50 mL). Absorption at 663 and 645 nm was determined with a spectrophotometer and total chlorophyll (µg g^{-1}) was calculated according to Arnon's procedure (Arnon, 1949).

[3]Mention of a trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the U. S. Dep. of Agric., and does not imply its approval to the exclusion of other products that may also be suitable.

RESULTS AND DISCUSSION

Visual Phytotoxicity Symptoms

Three d after treatment application, foliar toxicity symptoms appeared on grain sorghum treated with all rates of DCD-N. Symptoms were initially manifested as leaf tip necrosis, which proceeded marginally downward. Minor leaf tip was evident in corn treated with 20 mg kg^{-1} DCD-N and marginal necrosis developed on corn plants treated with >30 mg kg^{-1} DCD-N. Five d after treatment application, cotton treated with $\geq$ 40 mg kg^{-1} DCD-N developed mottled foliar chlorosis. Fourteen d after treatment application, cotton treated with $\geq$ 10 mg kg^{-1} DCD-N developed mottled foliar chlorosis and marginal necrosis. Symptoms were

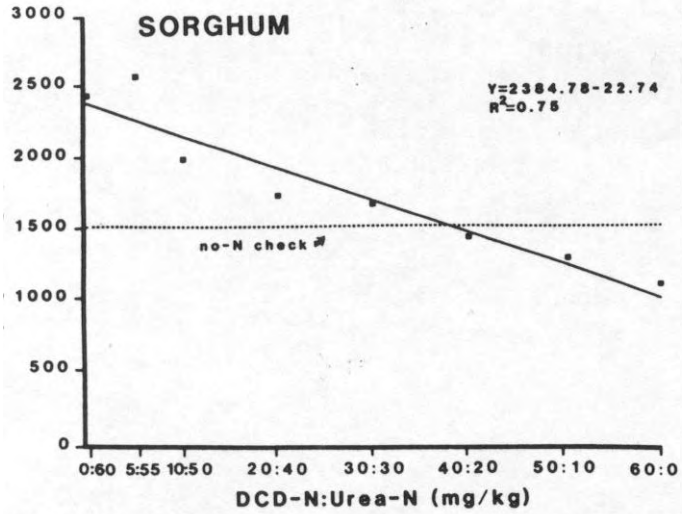

Fig. 5. Effect of DCD-N on total chlorophyll concentration of sorghum leaves (X = mg kg $^{-1}$ DCD-N).

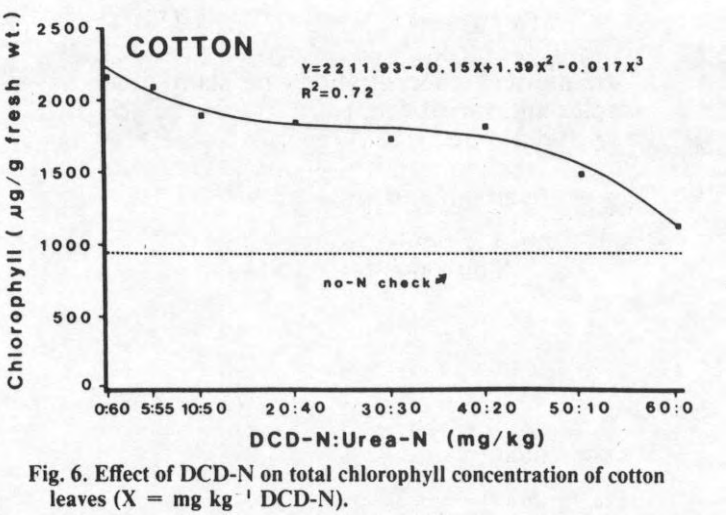

Fig. 6. Effect of DCD-N on total chlorophyll concentration of cotton leaves (X = mg kg $^{-1}$ DCD-N).

more severe in sorghum and cotton plants than in corn plants treated with equivalent amounts of DCD-N. For all species, the severity of symptoms intensified with DCDN rate.

The 60 mg kg^{-1} N rate was inadequate and visible N deficiency symptoms developed in corn and, to a lesser extent, sorghum. The N deficiency symptoms were directly related to plant size, and thus were commensurate with amount of N supplied as urea. DCD toxicity symptoms were distinct from N deficiency symptoms and, unlike N deficiency symptoms, developed quickly after treatment applications.

Twenty-five d after treatment application, cotton plants treated with DCD-N wilted during midday, even though pots were well watered. Wilting occurred even in plants treated with 5 mg kg^{-1} DCD-N, which did not exhibit any foliar toxicity symptoms.

Effect of DCD on Plant Growth

Increasing the proportion of N as DCD-N decreased dry weights of stem-leaf and root tissue of all three species. Cotton (Fig. 1) was more adversely affected by DCD-N than corn (Fig. 2) or sorghum (Fig. 3). Cotton stem-leaf and root dry weights were reduced to that of the no-N check at DCD-N concentrations of approximately 45 and 48 mg kg^{-1} soil, respectively (Fig. 1). Stem-leaf and root dry weights of corn and sorghum did not decrease to the level of the O-N check until all N was supplied as DCD-N.

Effect of DCD on Chlorophyll Concentrations

Total chlorophyll concentration of corn leaves decreased as the proportion of N as DCD-N increased (Fig. 4). Dicyandiamide-induced reductions in chlorophyll concentrations of corn leaves may have been masked by N deficiency symptoms exhibited by the larger plants in treatments where large proportions of N were supplied as urea.

Dicyandiamide-N concentrations greater than approximately 34 mg kg^{-1} decreased sorghum leaf chlorophyll concentrations to less than the O-N check (Fig. 5). The sensitivity of sorghum leaf chlorophyll concentrations to DCD reflected this species' exhibition of visible toxicity symptoms. Foliar symptoms developed more quickly and were more severe on sorghum than on corn.

Chlorophyll concentration of cotton leaves decreased curvilinearly with increasing DCD-N concentration; however, chlorophyll concentration in the O-N check was less than that of any treatment with N supplied as DCD-N (Fig. 6). The severity of foliar symptoms in cotton was not accurately reflected in chlorophyll determinations. By the end of the experimental period, cotton in treatments with 50 or 60 mg kg^{-1} DCD- N had partially defoliated. The leaf sampling procedure was thus not as accurate for cotton as for corn and sorghum.

Both DCD and nitrapyrin (2-chloro-6-[trichloromethyl] pyridine) inhibit the cytochrome oxidase involved in ammonia oxidation by *Nitrosomonas* (Hawk, 1980). The toxicity symptoms observed in this experiment and reported by others (Reddy, 1964b; Nommik, 1958) are similar to those reported for nitrapyrin toxicity (Rufner et al., 1984). Rufner et al. (1984) reported that nitrapyrin reduced the number of chloroplasts per cell and altered the structural integrity of chloroplasts in radish (*Raphanus sativus* L. cv. Cherry Belle). Given the similarities in visual toxicity symptoms and mode of action of nitrapyrin and DCD, it may be that DCD affects chloroplasts in a manner similar to that observed with nitrapyrin.

Availability of DCD-N

Generally, the concentration of N in stem-leaf tissue of all three species increased with the proportion of N supplied as DCD-N (Table 1). The increase in N concentrations of stem-leaf tissue was caused by reduced plant growth resulting from increased concentrations of DCD-N. However, apparent N recovery decreased curvilinearly from 103% to 4%, from 64 to -6%, and from 72 to 4% for corn, cotton, and sorghum, respectively, with increasing proportion of DCD-N. The rate of decomposition of DCD to ammonium is dependent on such factors as soil type (Reddy, 1964a; Reider and Michaud, 1980), temperature (Vilsmeier, 1980), and organic carbon (Reddy, 1964a). Data in Table 1 indicate that under our experimental conditions, DCD-N was unavailable for plant uptake within the 34-day experimental period.

Plant Tissue Nutrient Element Concentrations

The effects of increasing proportion of N as DCD-N on nutrient concentrations in plant tissue were complex and varied with plant species (Table 2). Sahrawat and Keeney (1984) reported that a number of studies have demonstrated increased P uptake resulting from retardation of nitritication. Reduction in rhizosphere pH resulting from plant NH_4^+ -N uptake (Smiley, 1974) would facilitate P uptake. The increased P concentration of corn, cotton, and sorghum stem-leaf tissue at concentrations exceeding 20, 10, and 10 mg DCD-N kg^{-1} soil, respectively, probably resulted from reductions in growth caused by DCD (Fig. 1, 2, 3) rather than increased NH_4^+-N uptake. Nitrogen recovery data indicated that little DCD-N was decomposed to plant available $NH4^+$ -N during the course of the experiment (Table 1).

Retardation of nitrification and increased uptake of NH_4^+-N will decrease Ca and Mg uptake (Nielsen and Cunningham, 1964; Mathers et al., 1982). The depression of Ca and Mg concentrations at concentrations of DCD-N <30 mg kg^{-1} soil is most likely due to increased uptake of NH; -N derived from urea, while the increase in Ca and Mg concentrations with DCD-N treatments exceeding 30 mg kg^{-1} is due to the reduced growth of plants caused by DCD.

The increased K concentration of corn stem-leaf tissue is best explained by reductions in plant growth (Fig. 2). The decrease in K concentration of cotton stem-leaf tissue with DCD-N concentrations >40 mg kg^{-1} soil cannot be attributed to reductions in plant growth, Thus, DCD per se may have an effect on K uptake.

The general trend for concentrations of Fe, Zn, Mn, and Cu to increase with DCD-N rate is most likely due to the interaction of reduced plant growth from

Table 1. Effect of DCD-N concentration on stem-leaf tissue N concentrations and N recovery.

Treatment DCD-N/ Urea-N	Tissue N concentration			N recovery‡		
	Corn	Cotton	Sorghum	Corn	Cotton	Sorghum
mg kg^{-1}	g kg^{-1} dry wt			%		
0:60	9.8	19.1	13.2	103	64	72
5:55	10.0	20.1	16.1	82	58	75
10:50	10.0	17.4	14.5	81	57	72
20:40	10.3	22.9	13.3	71	42	60
30:30	11.2	24.2	15.2	57	35	50
40:20	10.9	25.7	15.8	43	16	42
50:10	12.4	27.0	17.0	23	9	26
60:0	16.1	23.0	19.8	4	−6	4
No-N check	8.8	13.0	11.0			
Regression model†	C*	C*	L**	L**	L**	Q**
R^2	0.73	0.63	0.26	0.88	0.96	0.88

*,** Significant at the 5 and 1% probability levels, respectively.
† L = linear, Q = quadratic, C = cubic fitted regression model; R^2 values are for regressions calculated from individual data points.
‡ N recovery = N content of treated plants (root + stem-leaf tissue) − N content of O-N check plants (root + stem-leaf tissue).

Table 2. Effect of DCD-N on nutrient element concentrations of corn, cotton, and sorghum stem-leaf tissue.

Species	Treatment DCD-N/Urea-N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
	mg kg^{-1}	g kg^{-1} dry wt				mg kg^{-1} dry wt			
Corn	0:60	1.39	17.31	4.27	3.25	144.7	47.7	25.8	14.1
	5:55	1.54	18.51	4.26	2.78	177.0	48.4	29.5	12.6
	10:50	1.53	19.38	4.41	2.69	144.7	48.7	29.3	13.0
	20:40	1.54	19.56	4.03	2.50	157.4	45.4	25.0	12.4
	30:30	1.84	24.15	4.28	2.40	138.0	55.2	26.8	13.3
	40:20	2.00	28.89	3.78	2.03	178.5	54.2	27.8	14.1
	50:10	2.55	33.29	3.63	2.28	246.0	61.8	33.2	12.6
	60:0	4.40	37.37	4.94	3.42	337.8	76.1	47.6	16.7
	0-N check	3.82	36.60	4.31	2.87	278.8	74.3	41.7	14.3
	Regression model‡	C**	Q**	C*	C*	Q*	Q**	C*	C*
	R^2	0.97	0.92	0.49	0.59	0.77	0.75	0.69	0.68
Cotton	0:60	2.15	16.53	15.34	3.89	154.8	52.7	33.0	12.8
	5:55	2.65	18.55	14.79	3.93	110.0	52.7	34.4	12.0
	10:50	2.04	15.12	15.51	3.78	176.1	52.9	38.9	15.2
	20:40	3.28	20.67	14.28	3.58	170.9	53.7	37.2	13.1
	30:30	4.18	22.53	15.94	3.63	252.1	59.6	45.1	18.5
	40:20	4.49	23.49	14.69	3.58	162.5	56.3	46.1	12.4
	50:10	5.19	22.97	16.93	3.81	228.6	63.0	43.9	14.9
	60:0	4.36	18.79	15.30	4.05	212.5	45.1	33.6	13.7
	0-N check	3.31	11.60	18.79	4.13	245.3	59.3	47.0	12.7
	Regression model‡	C**	C**	NS	Q*	L*	C*	Q**	NS
	R^2	0.79	0.76	--	0.53	0.62	0.49	0.66	--
Sorghum	0:60	1.58	17.64	6.63	2.92	258.3	56.1	27.3	16.2
	5:55	1.80	18.97	5.96	2.77	233.6	58.5	28.6	18.1
	10:50	1.72	18.26	5.93	2.83	289.6	69.7	27.7	14.8
	20:40	1.81	18.53	5.47	2.54	273.2	59.1	26.6	14.3
	30:30	2.07	18.23	5.27	2.31	306.5	60.0	32.3	15.6
	40:20	2.18	19.11	5.77	2.57	267.3	66.1	37.1	20.9
	50:10	2.68	19.62	6.37	2.72	403.1	80.3	31.8	18.4
	60:0	3.65	17.42	7.32	3.09	207.8	96.7	43.5	21.5
	0-N check	3.01	18.06	7.79	3.03	332.6	92.8	34.2	29.3
	Regression model‡	C**	NS	Q**	Q**	L†	C†	L**	L*
	R^2	0.94	--	0.67	0.66	0.53	0.89	0.41	0.43

*,**,† Significant at 5, 1, and 10% probability levels, respectively.
‡ L = linear, Q = quadratic, C = cubic fitted regression model; R^2 values are for regressions calculated from individual data points.

DCD and uptake of NH; -N derived from urea. Retardation of nitrification has been shown to increase concentrations of plant tissue Fe (Touchton et al., 1979) and Zn (Warren et al., 1980).

CONCLUSIONS

The nitrogen in DCD was unavailable for plant uptake; however, the phytotoxic effects of DCD-N cannot fully be attributed to reduced N availability as Allison et al. (1925) postulated. Reddy (1964b) reported that increasing rates of DCD decreased dry weights of maize, oats, and wheat even when supplied with 110 mg kg^{-1} N as $NaNO_3$. In our experiment, cotton growth was reduced to below a O-N check with DCD even when IO or 20 mg kg^{-1} N from urea were available for uptake. The N deficiency that developed in corn, and to a lesser extent in sorghum, probably masked this response in these species. In addition, the distinct and rapid appearance of visible phytotoxicity symptoms in all species, and the reduction of leaf chlorophyll concentration to below that in the O-N check in sorghum treated with >30 mg DCD-N kg^{-1}, suggest that the phytotoxicity of DCD was due to the compound itself as well as to accompanying N deficiency. Corn was less adversely affected by DCD than cotton or sorghum.

The effects of DCD-N on stem-leaf tissue concentrations of P, K, Ca, Mg, Fe, Mn, Zn, and Cu varied with DCD-N concentration, plant species, and nutrient element. At lower DCD-N concentrations, nutrient element concentrations were likely affected by uptake of NH_4^+-N derived from urea, while higher concentrations of DCD-N resulted in increased nutrient element concentrations as a result of reduced plant growth.

Commercial N fertilizers formulated with DCD contain between 5 and 15% DCD-N. Dicyandiamide concentrations in soil resulting from broadcast application of DCD-containing N fertilizers present minimal risks from accompanying toxicity. However, banded applications of these fertilizers might result in toxic DCD concentrations in the root zone of sensitive crops. In these situations, DCD depresson of plant growth and chlorophyll concentrations could reduce photosynthetic capacity, with a potential for yield reductions that may prejudice any increased N efficiency derived from the use of DCD-containing N fertilizer.

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