

## Effect of Nitrogen Source and Dicyandiamide on Growth and Water Relations of Cotton

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### ABSTRACT

Nitrification inhibitors such as dicyandiamide (DCD) may improve N efficiency for cotton (*Gossypium hirsutum* L.) grown on sandy Coastal Plain soils. Research has demonstrated that cotton is sensitive to DCD, and field experiments suggest a possible link between cotton response to DCD and rainfall distribution. A greenhouse experiment was conducted to investigate the effect of DCD nod N source on growth and water relations of cotton on a typical Coastal Plain soil. Cotton (Deltapine 90) was grown in pots containing a Norfolk sandy loam (fine-loamy, siliceous, thermic, Typic Paleudults). Nitrogen (50 mg kg<sup>-1</sup>) as NaNO<sub>3</sub> or urea, and DCD (0, 2.5, 5, 10, 15, and 20 mg DCD-N kg<sup>-1</sup>) were applied to the soil at first true leaf. Soil water content, leaf xylem water potential ( $y_1$ ), and stomatal conductance were monitored during a 3-d drying period, commencing at first bloom, following which plants were harvested. Both N source and DCD affected plant growth and water relations, but there were no significant interaction effects. Fertilization with NaNO<sub>3</sub> increased leaf dry weight 9.1% compared with fertilization with urea. Plants fertilized with NaNO<sub>3</sub> depleted soil moisture faster than plants fertilized with urea, resulting in lowered stomatal conductances and more negative  $y_1$  throughout the drying period. Dicyandiamide linearly reduced leaf area and dry weight, and stem dry weight. Dicyandiamide did not affect soil-water depletion,  $y_1$ , or stomatal conductance in the morning. Under more stressful afternoon conditions, DCD, especially at rates  $\geq 10$  mg DCD-N kg<sup>-1</sup>, increased stomatal conductance over the range of available soil water. Dicyandiamide-induced increases in stomatal conductance under conditions of nonlimiting soil water could increase photosynthesis and possibly lint yield. In years when soil water is limiting, however, additional stress from DCD phytotoxicity could result in yield reductions.

*Additional Index Words:* *Gossypium hirsutum* L., nitrification inhibitor, DCD, stomatal conductance, leaf water potential, NH<sub>4</sub><sup>+</sup>-N, NH<sub>3</sub>-N.

NITRIFICATION INHIBITORS may offer an alternative to splitting N applications for improving the efficiency of N applied to cotton grown on sandy soils of the southeastern Coastal Plain. The nitrification inhibiting properties of dicyandiamide (DCD) (H<sub>2</sub>NC[NH]NHCN) have been known since the early 1900s (Cowie, 1918), but only recently have formulations of N fertilizers containing DCD become commercially available. Dicyandiamide is nonvolatile, water soluble (Reider and Michaud, 1980), and chemically and physically stable (SKW Product Studies, 1983). The compound is also soluble and stable in anhydrous NH<sub>3</sub> (Ashworth and Rodgers, 1981). These

properties enable DCD to be effectively formulated with a wide variety of N fertilizers, including urea, NH<sub>4</sub><sup>+</sup>-salts, N solutions, animal manures, and anhydrous NH<sub>3</sub>.

Dicyandiamide has been shown to increase yields of winter wheat (*Triticum aestivum* L.) (Rodgers et al., 1985; Rodgers and Ashworth, 1982) and grain sorghum (*Sorghum bicolor* (L.) Moench] (Touchton and Reeves, 1985); however, research involving DCD applications to cotton has indicated that cotton is sensitive to DCD. The only two reports of DCD applications to cotton have both described pot trials (Reddy, 1964; Reeves and Touchton, 1986). In separate experiments, Reddy (1964) applied 50 or 110 mg N kg<sup>-1</sup> soil as NaNO<sub>3</sub> or (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> in combinations with 0, 3.3, 6.7, and 16.7 mg DCD-N kg<sup>-1</sup> to cotton grown in a Cecil sandy loam (clayey, kaolinitic, thermic, Typic Hapludults). The 16.7 mg DCD-N kg<sup>-1</sup> concentration resulted in visual phytotoxicity symptoms and reduced dry matter yields, regardless of N source. Reeves and Touchton (1986) applied 60 mg N kg<sup>-1</sup> soil in varying ratios of urea/DCD to cotton grown in a Norfolk sandy loam (fine-loamy, siliceous, thermic, Typic Paleudults). Dry weights of shoots and roots were reduced as the proportion of N as DCD was increased. The effect could not entirely be accounted for by lack of availability of DCD-N as dry weights were reduced to below those of a zero-N check when >67% of the N was supplied as DCD.

Field experiments on the same soil have shown erratic responses to preplant and banded applications of urea containing DCD (unpublished data). Averaged over 3 N rates (67, 101, and 134 kg ha<sup>-1</sup>), urea formulated with 10% of the N as DCD-N reduced seed cotton yield 30 and 10%, respectively, in 2 yr, but increased yield 13% in another year. Weather data indicated that in both years when yield reductions occurred, plants were subjected to drought stress prior to peak bloom. The yield increase occurred in a growing season with a more favorable rainfall distribution.

Erratic plant responses to nitrification inhibitors can be caused by a number of factors and their interactions. These include N rate applied, soil temperature, moisture, texture, pH, organic matter, and biological activity (Keeney, 1980; Slangen and Kerkhoff, 1984). The interaction of nitrification inhibitors and plant water status has not been well-researched. This interaction results from two primary causes, the inhibition of nitrification with resulting increased plant uptake of NH<sub>4</sub><sup>+</sup> ions, and the effect of the nitrification inhibitor, per se, on the plant's physiological processes.

Form of N has been shown to influence plant water relations. Ammonium-N in comparison with NO<sub>3</sub><sup>-</sup>-N inhibited water uptake, decreased leaf xylem water potentials ( $\psi_1$ ) and increased leaf diffusive resistances of tomato (*Lycopersicon esculentum* Mill.) (Quebedeaux

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Published in Soil Sci. Soc. Am. J. 52:281-285 (1988).

and Ozbun, 1973; Pill et al., 1978; Pill and Sparks, 1982) and Ostrich fern [*Matteuccia struthiopteris* (L.) Todaro] (Prange and Ormrod, 1982). The inhibitor nitrapyrin (2-chloro-6-[trichloromethyl] pyridine) has also been shown to reduce  $\psi_1$ , and increase leaf diffusive resistance of tomato plants grown in  $\text{NO}_3\text{-N}$  fertilized medium (Pill, 1981).

Nitrapyrin and DCD both inhibit the cytochrome oxidase involved in  $\text{NH}_3$  oxidation by *Nitrosomonas* (Hauck, 1980), and phytotoxicity symptoms are similar for both inhibitors (Reeves and Touchton, 1986; Rufner et al., 1984). These similarities, as well as inferences from field data, suggest that DCD may affect water relations of cotton.

The objectives of this greenhouse study were to determine the effects of N form and DCD concentrations on plant growth and water relations of cotton grown in a sandy Coastal Plain soil.

## MATERIALS AND METHODS

Ten seeds of the cotton cultivar 'Deltapine Acala 90' were planted in separate 22-cm-diam., 5.45-L, plastic containers containing 6.35 kg (oven-dry weight basis) of Norfolk sandy loam that had been sieved through a 2.5-mm screen. The initial soil pH was 5.8, and Mehlich I (Mehlich, 1953) P, K, Ca, and Mg (Hue and Evans, 1979) averaged 39, 76, 325, and 53 mg  $\text{kg}^{-1}$ , respectively. Organic matter content averaged 10.3 g  $\text{kg}^{-1}$  and cation exchange capacity averaged 3.6 cmol<sub>c</sub>  $\text{kg}^{-1}$ . Initial total N and inorganic N averaged 0.38 g  $\text{kg}^{-1}$  and 4 mg  $\text{kg}^{-1}$ , respectively. Ten days prior to planting, 6.0 g of dolomitic limestone (90% calcium carbonate equivalent, CCE) was mixed with the soil in each pot and each pot was watered to saturation. Pots were fertilized at planting, and weekly thereafter, with 2X Hoagland's solution (Hoagland and Arnon, 1950) minus N to ensure that no mineral deficiencies would confound results. At the first true-leaf stage of development (15 d after emergence), plants were thinned to 3 plants per pot and treatments were applied as aqueous solutions to the soil surface of each pot except the zero-N check pots. Water (0.5 L) was applied to all pots immediately after treatment applications to leach treatments into the soil.

The experimental design was a factorial arrangement of N source X DCD rates in a randomized complete block with five replications. Nitrogen sources were urea and  $\text{NaNO}_3$ . Nitrogen rate (apart from DCD-N) was 50 mg  $\text{kg}^{-1}$  soil. Dicyandiamide rates were 0, 2.5, 5.0, 10.0, 15.0, and 20.0 mg DCD-N  $\text{kg}^{-1}$  soil (DCD contains 67% N). A duplicate of the design was arranged on an adjacent greenhouse bench. Gypsum blocks (Soil Moisture Equipment Corp., Santa Barbara, CA), calibrated for gravimetric soil-water content, were placed in each pot of one set of the experiment. Pots were watered as needed, based on gypsum block resistance readings, so that plants were not water stressed prior to the water

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relations measurement period. At first bloom, (45 d after treatment application, 60 d after emergence), at 1700 h CST, pots were watered to saturation (0.31 kg  $\text{H}_2\text{O kg}^{-1}$  dry soil). Stomatal conductance, leaf water potential (leaf xylem pressure potential,  $\psi_1$ ), and soil-water content were measured from 0830 to 1000 and from 1400 to 1530 h CST during a 3-d drying period starting 61 d after emergence. Stomatal conductances (abaxial + adaxial conductances in parallel) of the youngest fully expanded leaves were measured with a LI-1600 steady-state porometer (LI-COR, Inc., Lincoln, NE.) from the set of pots with gypsum blocks. Because of the destructive nature of  $\psi_1$  measurements, uppermost fully expanded leaves were excised from the duplicate set of pots without gypsum blocks, placed immediately in small plastic bags, and transferred to a pressure chamber (Soil Moisture Equipment Co., Santa Barbara, CA) for determination of  $\psi_1$ . Technical problems prevented  $\psi_1$  measurements during the afternoon of the first day of the drying period.

Sixty-seven days after emergence, plants were harvested and separated into leaves, squares and blooms, stems, and roots. Roots were washed free of soil, blotted dry, and weighed. Leaf area was determined on a LI-COR LI-3100 area meter. All plant organs were then dried for 72 h at 60 °C and weighed.

Statistical analyses included analysis of variance and regression analysis using the General Linear Models (GLM) procedure of SAS Inst. (Freund and Littell, 1981). Fisher's protected least significant difference (LSD,  $P \leq 0.05$ ) was used to separate means among N sources. There were no N source X DCD interaction effects on any variable; therefore, only main effects are presented throughout the paper.

## RESULTS AND DISCUSSION

### Plant Growth

#### Nitrogen Source Effects

Nitrogen applied as  $\text{NaNO}_3$  increased total dry weight of cotton plants compared with fertilization with urea (Table I). This increase was primarily the result of an increase in leaf tissue. The number of fruiting structures (squares and blooms) per plant was also increased by fertilization with  $\text{NaNO}_3$  as compared with fertilization with urea. Nitrogen recovery was less ( $P \leq 0.006$ ) for plants fertilized with urea rather than  $\text{NaNO}_3$  (95.3 vs. 102.5%). Although precautions were taken to minimize urea hydrolysis and  $\text{NH}_3$  volatilization (soil pH in zero-N check pots averaged 6.6 at the end of the experiment and all pots were watered to incorporate treatments into the soil), it is possible that  $\text{NH}_3$  volatilization reduced the efficiency of urea.

#### Dicyandiamide Effects

Plant dry weight decreased linearly as DCD rate increased (Fig. 1). The decrease was due to reductions in both stem and leaf dry weights (Fig. 2). Leaf area

Table 1. Effect of N source on growth of cotton in the greenhouse 67 d after emergence.

| N source        | Dry wt. |           |           |        | Root fresh wt. | Leaf area       | Squares + blooms        |
|-----------------|---------|-----------|-----------|--------|----------------|-----------------|-------------------------|
|                 | Total   | Roots     | Stems     | Leaves |                |                 |                         |
|                 |         |           | g         |        |                | cm <sup>2</sup> | no. plant <sup>-1</sup> |
| $\text{NaNO}_3$ | 41.63   | 10.56     | 15.19     | 13.76  | 70.30          | 1681.3          | 2.15                    |
| Urea            | 39.25   | 10.19     | 14.65     | 12.61  | 69.56          | 1592.3          | 1.80                    |
| LSD (0.05)      | 1.65    | 1.19 (NS) | 0.71 (NS) | 0.69   | 5.11 (NS)      | 92.0 (NS)       | 0.26                    |
| Zero-N control  | 10.36   | 3.12      | 3.24      | 4.01   | 23.6           | 432.0           | 0                       |

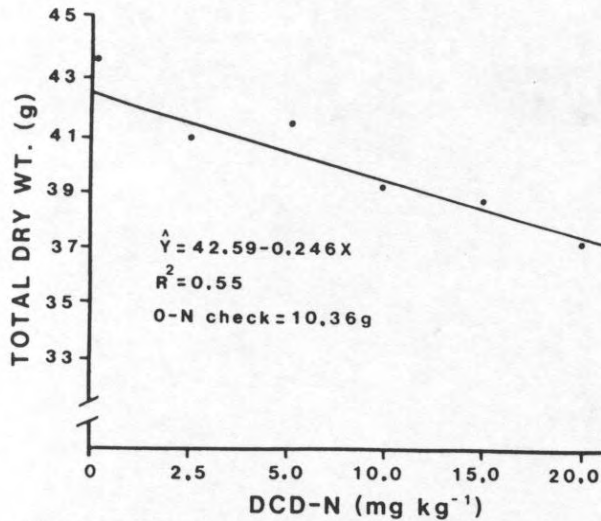


Fig. 1. Effect of DCD on total dry weight of greenhouse grown cotton harvested 67 d after emergence. The  $R^2$  is calculated from individual data points; model significant at the 0.01 level.

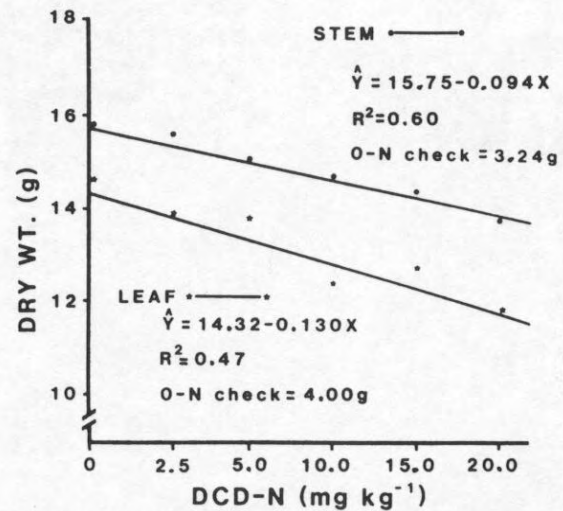


Fig. 2. Effect of DCD on dry weight of stem and leaf tissue of greenhouse grown cotton harvested 67 d after emergence. The  $R^2$  values are calculated from individual data points; both models significant at 0.01 level.

was reduced similarly to leaf dry weight (data not shown). Dicyandiamide reduced root fresh weight (Fig. 3) but did not affect root dry weight (data not shown). Maftoun and Sheibany (1979) postulated that DCD-induced growth suppression was the result of reduced lateral root formation and main root elongation accompanied by reduced water and nutrient absorption. Amberger and Vilsmeier (1983) in pot trials with oats (*Avena sativa* L.) and spring wheat (*T. aestivum* L.) reported that water-soluble DCD was taken up by plants and located mainly in leaves and straw. They did not detect DCD in roots. Our results, here and previously (Reeves and Touchton, 1986), in conjunction with those of Amberger and Vilsmeier (1983), would suggest that the primary site of phytotoxicity of DCD lies in green tissue, and not in root tissue.

Table 2. Effect of N source on soil-water depletion and water relations of greenhouse grown cotton plants during a 3-d drying period commencing at first bloom.

| N source                                       | Days after emergence and (time of day)† |            |            |            |         |         |
|--|---|------------|------------|------------|---------|---------|
|  | 61 (am)                                 | 61 (pm)    | 62 (am)    | 62 (pm)    | 63 (am) | 63 (pm) |
| Gravimetric water content, kg kg <sup>-1</sup> |   |            |            |            |         |         |
| NaNO <sub>3</sub>                              | 0.310                                   | 0.310      | 0.259      | 0.196      | 0.097   | 0.081   |
| Urea   | 0.310                                   | 0.310      | 0.283      | 0.219      | 0.113   | 0.098   |
| LSD (0.05)                                     | 0.003 (NS)                              | 0.003 (NS) | 0.018      | 0.026 (NS) | 0.012   | 0.012   |
| Zero-N control                                 | 0.310                                   | 0.310      | 0.285      | 0.282      | 0.263   | 0.236   |
| Stomatal conductance, cm s <sup>-1</sup>       |   |            |            |            |         |         |
| NaNO <sub>3</sub>                              | 1.353                                   | 0.674      | 1.285      | 0.570      | 0.499   | 0.086   |
| Urea   | 1.394                                   | 0.768      | 1.324      | 0.624      | 0.692   | 0.123   |
| LSD (0.05)                                     | 0.127 (NS)                              | 0.070      | 0.148 (NS) | 0.103 (NS) | 0.155   | 0.037   |
| Zero-N control                                 | 0.743                                   | 0.442      | 0.669      | 0.404      | 0.538   | 0.359   |
| Leaf water potential, -MPa                     |   |            |            |            |         |         |
| NaNO <sub>3</sub>                              | 0.994                                   | --‡        | 0.767      | 1.342      | 1.223   | 1.583   |
| Urea   | 0.939                                   | --         | 0.747      | 1.249      | 0.960   | 1.456   |
| LSD (0.05)                                     | 0.073 (NS)                              | --         | 0.036 (NS) | 0.085      | 0.183   | 0.121   |
| Zero-N control                                 | 1.05                                    | --         | 0.625      | 1.175      | 0.813   | 1.055   |

† Pots watered to saturation 60 d after emergence.

‡ Data not taken because of technical problems.

## Plant Water Relations

### Nitrogen Source Effects

The larger plants resulting from NaNO<sub>3</sub> fertilization depleted soil water faster than plants fertilized with urea (Table 2). This resulted in decreased stomatal conductances throughout the imposed drying period (Table 2). Similarly, plants fertilized with NaNO<sub>3</sub> maintained lower (more negative)  $\psi_1$  than plants fertilized with urea (Table 2). Stomatal conductances of zero-N control plants remained lower than N-fertilized plants until soil water became limiting (morning of Day 3 of drying period, 63 d after emergence). This behavior is similar to that reported for bean plants (*Phaseolus vulgaris* L.) by Shimshi (1970). He reported that transpiration rates of N-deficient plants were lower than those of N-supplied plants when soil moisture remained high, but as soil moisture approached the wilting range, transpiration rates of N-deficient plants

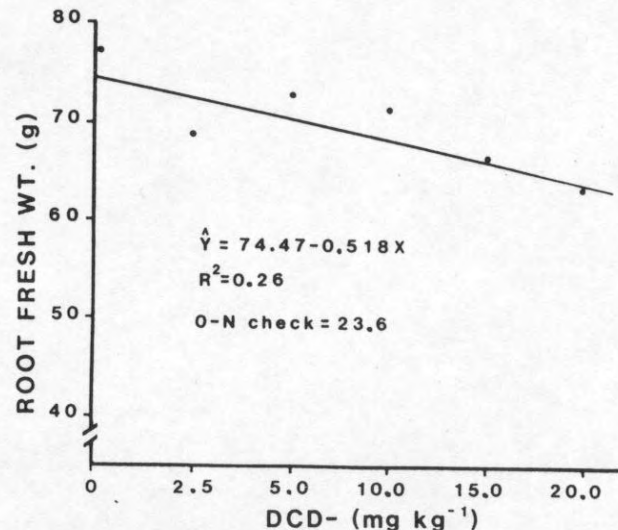


Fig. 3. Effect of DCD on root fresh weight of greenhouse grown cotton harvested 67 d after emergence. The  $R^2$  is calculated from individual data points; model significant at the 0.01 level.

became higher than those of N-supplied plants. Research indicates that  $\text{NH}_4\text{-N}$ , compared with  $\text{NO}_3\text{-N}$ , decreases  $\psi_1$  (Quebedeaux and Ozburn, 1973; Pill et al., 1978; Pill and Sparks, 1982; Prange and Ormrod, 1982). Nitrogen deficiency decreased  $\psi_1$  in greenhouse grown cotton (Radin and Parker, 1979). These findings by other researchers, as well as trends in water depletion (Table 2), indicate that differences in  $\psi_1$  among N sources or between N-fertilized plants and N-deficient plants are reflections of soil-water availability during the drying period and not the result of  $\text{NH}_4$  ion uptake.

#### Dicyandiamide Effects

Dicyandiamide did not affect depletion of soil water or  $\psi_1$  during the dry-down period. Stomatal conductance during the morning was not affected by DCD, but under more stressful afternoon conditions, DCD affected measurements. This effect is demonstrated by regression of stomatal conductance on soil-water content (Fig. 4). Dicyandiamide, at all concentrations, increased stomatal conductance as soil water increased from  $0.12 \text{ kg kg}^{-1}$  to saturation. Soil bulk density at the conclusion of the experiment averaged  $1.52 \text{ Mg m}^{-3}$ . For this soil, at this bulk density,  $0.12 \text{ kg kg}^{-1}$  corresponds to a soil-water tension of 85 kPa. Dicyandiamide concentrations  $\geq 10 \text{ mg kg}^{-1}$  increased responsiveness of stomata to decreasing soil-water content. The effect of DCD on stomatal conductance was not a reflection of soil-water depletion, as was the lowered stomatal conductances of plants fertilized with  $\text{NaNO}_3$ , nor was it due to increased N uptake from mineralization of DCD-N. Nitrogen recovery data indicated no difference in N uptake among DCD rates (data not shown).

#### CONCLUSIONS

Both N source and DCD affected plant growth and water relations, but there were no N source X DCD interaction effects on plant growth or water relations. Decreases in stomatal conductances of  $\text{NaNO}_3$ -fertilized plants compared with urea-fertilized plants were a result of increased plant growth and consequent greater soil-water demand.

Dicyandiamide linearly reduced cotton growth. However, the effect was not substantial until DCD-N rate was between 5 and  $10 \text{ mg kg}^{-1}$  soil. A broadcast application of  $112 \text{ kg N ha}^{-1}$  (a normal rate for cotton on coarse-textured soils) formulated with 10% of the N as DCD-N would result in a concentration of  $5 \text{ mg DCD-N kg}^{-1}$  soil. Higher concentrations in the root zone of cotton from banded applications or from higher rates of N formulated with DCD might adversely affect cotton growth.

Dicyandiamide-induced increases in stomatal conductance over the range of available soil water may offer one explanation for erratic responses of cotton to DCD in field experiments. Nitrogen recovery data (data not shown) indicated these increases cannot be attributed to increased N uptake. Likewise, the lack of any N source X DCD interaction suggests that effects of DCD on this physiological process is the result of the compound itself and not increased plant uptake of  $\text{NH}_4\text{-N}$  as a result of inhibition of nitrification. The

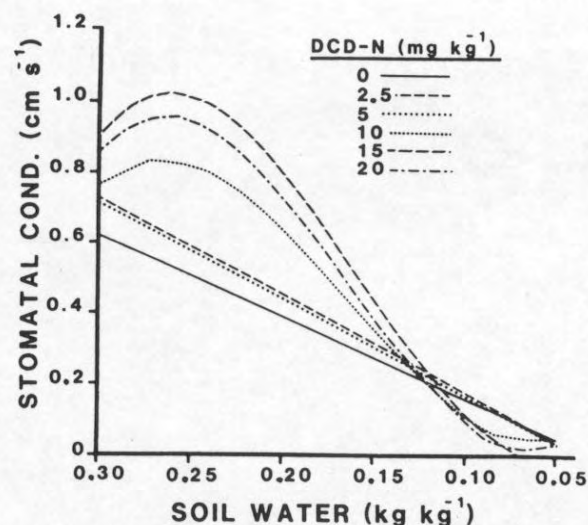


Fig. 4. Relationship between soil water and stomatal conductance of greenhouse cotton plants as influenced by DCD. The  $R^2 = 0.75, 0.87, 0.79, 0.81, 0.83,$  and  $0.86$  for 0, 2.5, 5, 10, 15, and 20 mg DCD-N  $\text{kg}^{-1}$ , respectively (calculated over individual data points). All models are significant at the 0.05 level or greater.

DCD-induced increases in stomatal conductance under conditions of nonlimiting soil water could increase photosynthesis and possibly lint yield. In years when soil water is limiting, however, additional stress from DCD phytotoxicity could result in yield reductions.

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