

Growth Retardants Mitigate Verticillium Wilt and Influence Yield of Cotton

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ABSTRACT

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The growth retardants tributyl[(5-chloro-2-thienyl)methyl]phosphonium chloride (TTMP), chlormequat (CCC), and N,N-dimethylpiperidinium chloride (DPC), when applied to the foliage in June or July, slightly mitigated symptom expression of Verticillium wilt of cotton caused by *Verticillium dahliae* and in most cases markedly reduced the internal population of *V. dahliae* propagules in the upper petioles of plants. These chemicals also increased the yield of cotton cultivar SJ-2 by 10–29% on land

infested with *V. dahliae* and on noninfested land. The effective dosages of TTMP were 40–80 g/ha. Increasing the dosage to 160 g/ha had no effect on yield. CCC at 10 and 25 g/ha increased yield, but had no effect at 50 g/ha, and yield was decreased by 75 g/ha. However, yield was increased by DPC most effectively at 25 to 50 g/ha. Plant height was not affected by TTMP but was decreased by increasing dosages of DPC or CCC. The number of bolls set was slightly, but not significantly, greater on treated plants.

Additional key word: cycocel.

Verticillium wilt (caused by *Verticillium dahliae* Kleb.) of cotton can be controlled only partially by using resistant cultivars. Because a high level of resistance is lacking, other methods of control have been sought. Systemic fungicides, such as benomyl, were only partially effective and not economical (10). Results of our research (3,5,12,23,24) on chemical control of Verticillium wilt showed that growth retardant chemicals delayed the onset and slightly mitigated the severity of symptoms, and reduced the internal population of *V. dahliae* propagules in petioles. We report here the results of an extensive study of these nonfungitoxic, but biologically active, compounds in the field to determine their effect on the internal population of *V. dahliae* and on the yield of cotton seed and lint. Some of these results already have been summarized (9,10).

MATERIALS AND METHODS

Chemical application. The growth retardant chemicals tested were chlormequat (CCC) obtained as Cycocel®, (2 chloroethyl)trimethylammonium chloride, N,N-dimethylpiperidinium chloride (BASF 083 OOW) (DPC), and tributyl[(5-chloro-2-thienyl)methyl]phosphonium chloride (CHE 8728) (TTMP). Chlormequat was obtained from American Cyanamid Co., Princeton, NJ 08540, DPC from BASF Corporation, Parsippany, NJ 07054, and TTMP from Mobay Chemical Corporation, Kansas City, MO 64120. Mobay has withdrawn TTMP from commercial field testing. All chemicals were water soluble, and dosages are given as the active ingredient. The wetting agent, Tween-20 (polyoxyethylene sorbitan monolaureate) was added to the aqueous solution (0.1%, v/v) of the chemicals to increase wetting of the leaves. The materials were applied to cotton plants in some experiments at the initial square (flower bud) formation in late June and in others at early flower

formation in early July. Application was made with back pack sprayers (AgriTechnical Associates, Riverside, CA 92521) pressurized with CO₂ (1.4 kg-force/cm²) using a hand-held wand fitted with a Spraying Systems® 8004 fan nozzle. In most experiments, full coverage of both under and upper sides of leaves was obtained with 935 L/ha (100 gal/acre). In one experiment, a lower volume, 187 L/ha (20 gal/acre) of the same formulation was compared to 935 L/ha. Application was made in the morning when wind drift was minimal.

The Wilcox field (a Cajon fine sandy loam near Strathmore, Tulare County, California) had a long history of severe Verticillium wilt. Disease incidence usually reached 75–100% each year. The field was rotated each year with milo. Plots were either one or two rows bordered by guard rows, and 12.2 m (40 ft) in length. Plots were laid out in a randomized block design with six replications. Some experiments were done in field plots (a Panoche clay loam at the West Side Field Station of the University of California at Five Points, CA) in which the incidence of Verticillium wilt was negligible.

In all experiments, plants were grown on raised beds (two rows [96.5 cm apart] per bed) and thinned to ~ nine plants per meter of row; the population was about 98,800 plants/ha. Except where designated otherwise, cotton cultivar SJ-2 was planted; cultivars SJ-3 and SJ-4 were more tolerant to *Verticillium dahliae* than was SJ-2.

The dilution assay method used for estimating the internal population of *V. dahliae* propagules in plant tissue was modified (13) from that of Saalting (19). A petiole was selected from an upper, fully expanded but not senescent, leaf on at least 20 randomly-selected plants in each replication. The petioles were refrigerated prior to isolation, surface-disinfested by washing 5 min in 0.5% sodium hypochlorite, washed in sterile water, blotted dry, and the ends were cut off and discarded. Small pieces (2.0 g fresh weight) were blended with 200 ml of sterile water in a Sorvall Omnimixer at full speed for three 20-sec periods. A 1:10 dilution was made with sterile distilled water and 1-ml samples were plated on the surface of sodium polypectate agar (15) in petri dishes and

incubated at 21–24 C. After 8–12 days the microsclerotial colonies were counted.

Disease severity was estimated by evaluating about 20–50 plants in each replication for the approximate percentage of leaf area with foliar symptoms of *Verticillium* wilt. Disease severity was based on percentage of leaf area affected: 0 = healthy; 1 = 1–25%; 2 = 26–50%; 3 = 51–75%; and 4 = 76–100% to complete defoliation. Disease incidence was calculated by dividing the number of plants with foliar symptoms by the total number of plants evaluated.

RESULTS

Effect of growth retardant chemicals on *Verticillium* wilt. In general, disease severity was only slightly reduced by the growth retardants. At 40, 80, and 160 g/ha, TTMP applied at initial square formation 21 June and at early flowering 15 July reduced the incidence and severity of the disease only slightly. In a similar experiment, CCC at 10, 25, 50, and 75 g/ha and TTMP at 40 g/ha only slightly reduced either the incidence or severity of disease. In

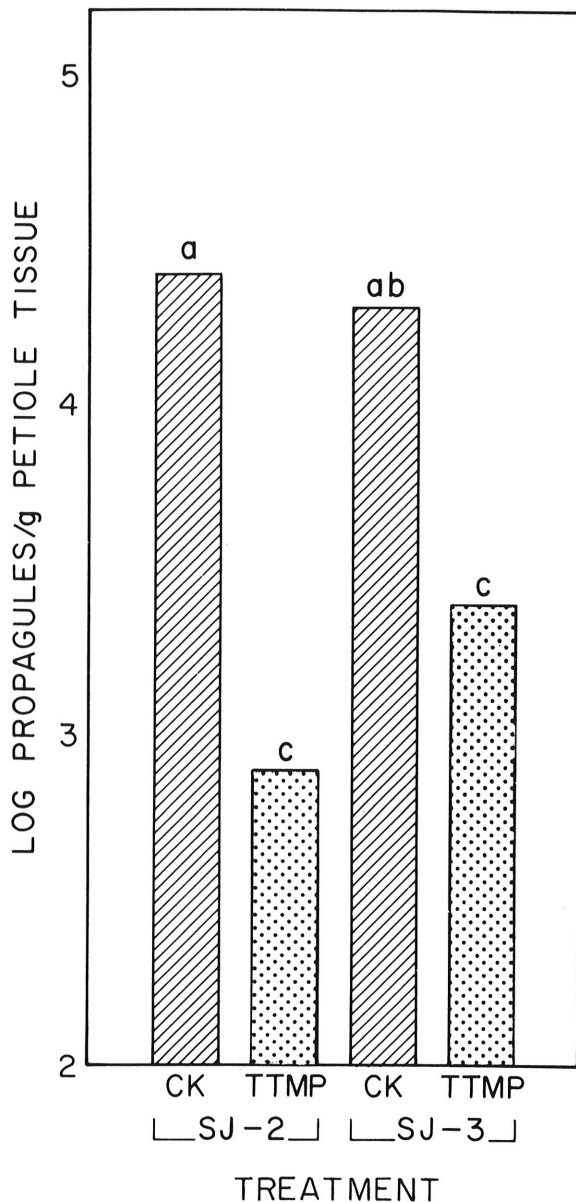


Fig. 1. The effect of tributyl[(5-chloro-2-thienyl)methyl]phosphonium chloride (TTMP) applied (80 g/ha) in mid-June to cotton foliage of two cultivars on the population of *Verticillium dahliae* in petiole tissue on 5 September 1974. Letters a, b, and c indicate different statistical populations, $P = 0.01$.

1975, TTMP (80 g/ha), CCC (25 g/ha), and DPC (25 g/ha) had no effect on incidence of disease (approximately 100%), but the severity was significantly reduced by CCC in all experiments. Both DPC and TTMP reduced the severity in two experiments.

The population of *Verticillium dahliae* propagules in petioles from upper fully-expanded leaves was almost always reduced by the growth retardants. In one experiment, the internal population was reduced by TTMP (40 g/ha) in the late season (5 Sept 1974) as shown in Fig. 1. Even though earlier in the season the internal population of *V. dahliae* was reduced slightly by both CCC (10, 25, 50, and 75 g/ha) and TTMP (40 g/ha), the differences were not statistically significant. In another experiment to test several other experimental growth retardant chemicals, nearly all treatments reduced the population of propagules markedly even when the assays were made > 2 mo after application (Table 1).

Effect of growth retardants on yield. The dosage of CCC on cotton was extremely critical. The first dosage response experiment was conducted with CCC on the Wilcox farm, where *Verticillium* wilt was a factor. Yield of cultivar SJ-2 was increased by CCC at 10 and 25 g/ha, but no significant effect was detected at 50 or 75 g/ha (Table 2). The response of the more resistant cultivar SJ-3 generally was similar to that of cultivar SJ-2, but the increase in yield was less. Yields of both cultivars were increased by TTMP (40 g/ha). In a subsequent experiment in 1975, CCC increased yield of SJ-2 at similar dosages.

At the West Side Field Station, where *Verticillium* wilt was not a

TABLE 1. Effects of several plant growth regulators applied by foliar spray (25 June 1974) on cotton plants (cultivar SJ-2) in the field on suppression of *Verticillium dahliae* in petiole tissue

Treatment	g/ha	Propagules/g petiole tissue ^x sampled:			
		15 July	31 July	13 Aug.	5 Sept.
DPC	100	100 ab ^z	50	100 ab	400 c
BAS 83 394 X	100	150 ab	50	0 b	1,050 bc
BAS 0660 W	150	3,550 a	100	100 ab	1,300 bc
BAS 0640 W	150	0 a	50	100 a	1,550 bc
Rohm and Haas 531	445	0 b	200	400 ab	3,400 abc
TTMP	40	220 a	100	100 ab	1,800 ab
No treatment	0	2,350 ab	10,400	500 a	7,400 a

^x Petioles from the uppermost fully expanded leaves were collected randomly, surface disinfested with sodium hypochlorite, blended and diluted in water, and plated on sodium polypectate agar for 10 days.

^y The chemical names are: DPC, N,N-dimethylpiperidinium chloride; BAS 83 394 X, N,N-dimethyl-hexahydro-pyridazinium bromide; BAS 0660 W, N,N-dimethyl-morpholinium chloride; BAS 0640 W, N,N-dimethyl-(β -chloroethyl)hydrazonium chloride (34); Rohm and Haas 531, sodium 1-(*p*-chlorophenyl)-1, 2-dihydro-4,6-dimethyl-2-oxonicotinate; and TTMP, tributyl[(5-chloro-2-thienyl)methyl] phosphonium chloride.

^z Numbers followed by the same letter are not significantly different, $P = 0.05$.

TABLE 2. Effect of chlormequate (CCC) at different dosages and TTMP applied by foliar spray (25 June 1974) on yield of two cultivars of cotton at the Wilcox farm

Chemical ^a	Yield ^b of seed and lint (g/plot—two rows 12.2 m long)				
	Conc. (g/ha)	Cv. SJ-2		Cv. SJ-3	
		Yield (kg/ha)	Prop. of control (%)	Yield (kg/ha)	Prop. of control (%)
CCC	75	3,250 ab	93.7	3,161 a	90.8
CCC	50	3,521 abc	101.5	3,560 abcd	102.2
CCC	25	3,906 cde	112.6	3,753 bcde	107.8
CCC	10	4,048 de	116.7	3,855 cde	110.7
TTMP	40	4,163 d	120.0	3,928 cde	112.8
No treatment	...	3,470 abc	100.0	3,483 ab	100.0

^a Acronyms: CCC is (2-chloroethyl)trimethyl ammonium chloride and TTMP is tributyl[(5-chloro-2-thienyl)methyl]phosphonium chloride.

^b Numbers followed by the same letter are not significantly different, $P = 0.05$.

factor, yield increases were obtained by applying CCC at 10 and 25 g/ha. Higher dosages either were ineffective or reduced yield. Yield was increased significantly by TTMP (40 and 80 g/ha) (Table 3).

The effects of growth retardants at squaring time (mid-June) and at flowering time (early July) were compared at different dosages. There was no effect due to time of application. At the lower dosages of 10 and 25 g/ha, CCC and DPC increased the yield significantly, but a dosage of 75 g/ha of DPC decreased yield (Table 4). In a similar experiment, CCC at 10 g/ha, DPC at 25 g/ha, and TTMP at 80 g/ha significantly increased yield from 8 to 11%.

Increasing the dosage of TTMP applied at first squaring (mid-June), at early flowering (early July), or at both times did not affect yield in another experiment. The yield of cotton seed and lint increased from 2,644 kg/ha to 2,970 kg/ha by TTMP (112% of control). Neither dosages nor time of application had any significant effect on yield.

In 1976, CCC and DPC were compared on cultivar SJ-2, which is moderately susceptible to Verticillium wilt and on cultivar SJ-4, which is moderately resistant. A yield increase due to optimal dosages of chemicals occurred on SJ-2 but not on SJ-4. The amount of water (187 L/ha or 935 L/ha) in which the chemicals were applied did not affect the yield. Since the differences detected for SJ-4 were not significant, only the data for SJ-2 compared to the control for SJ-4 are shown in Fig. 2.

Effect of CCC and DPC on plant height and boll set. Dosages of TTMP at 40, 80, and 160 g/ha did not affect plant height in field experiments in 1973, 1974, and 1975. Both CCC and DPC consistently reduced the height linearly with increased dosage (Fig.

3, and Tables 3 and 4).

Boll set tended to increase with optimum dosage of growth retardants, but the differences were not statistically significant.

DISCUSSION

The research reported here was initiated to gain more information on the effect of nonfungitoxic growth-regulating chemicals on the internal population of *V. dahliae* in infected cotton plants and on the yield of cotton in infested fields. Horsfall and Dimond (14) included chemicals that acted on the metabolism of the host in their definition of a chemotherapeutant. Davis and Dimond (6) found that 2,4-D ([2,4-dichlorophenoxy]acetic acid) effectively reduced vascular discoloration caused in tomato plants by *Fusarium oxysporum* f. sp. *lycopersici*. Sinha and Wood (21) reported that CCC delayed and mitigated the symptoms of *Verticillium albo-atrum* (dark mycelial type) on tomato. Buchenauer reported that CCC delayed and mitigated symptoms of *Fusarium wilt* and *Verticillium wilt* of tomato (2). Rationale and experimental data on the effects of growth regulators on partial control of vascular wilt and other diseases have been reviewed extensively by Bockmann (1) and by Erwin (9,10).

The reason for delayed onset of *Verticillium wilt* symptoms in the presence of nonfungitoxic growth regulators is not yet understood. In cotton, Buchenauer and Erwin (4) showed by thin-layer chromatography that treatment with the growth retardant N,N-dimethylpiperidinium iodide (DPI) increased the concentration of the extractible phytoalexin, hemigossypol (25,26). The compound

TABLE 3. Effect of several growth regulators applied by foliar spraying at initial squaring (budding) time 24 June 1975 on yield of cotton at the West Side Station (San Joaquin Valley, California) where the incidence of *Verticillium wilt* was less than 5%

Treatment ^m	Dosage (g/ha)	Yield of seed and lint ⁿ			Prop. of control (%)	Plant height 7 Aug (cm)
		(kg/ha)	P = 0.05	P = 0.01		
CCC	10	6,587	a	w	117	102.1
DPC	25	6,329	ab	w	112	93.1
TTMP	80	6,283	ab	wx	112	123.6
TTMP	40	6,132	ab	wx	109	118.7
CCC	25	6,022	bc	wx	107	97.1
Control (water)	0	5,630	c	y	100	119.1
Control (0.1% Tween 20)	0	5,630	c	y	100	118.3
CCC	50	4,610	d	yz	82	89.4
CCC	75	4,302	d	yz	76	85.4

^m Acronyms: CCC is (2-chloroethyl)trimethyl ammonium chloride; DPC is N,N-dimethyl piperidinium chloride; and TTMP is tributyl[(5-chloro-2-thienyl)methyl]phosphonium chloride.

ⁿ Values followed by the same letter do not differ significantly; a-d P = 0.05; w-z P = 0.01.

TABLE 4. Effect of several growth regulators applied at initial squaring (budding) (S) time, 24 June 1975, and at flowering (F) time, 18 July, on yield of cotton at the Wilcox farm

Treatment ^m	Growth stage	Dosage (g/ha)	Yield of seed and lint ⁿ			Prop. of control (%)	Height 26 Aug (cm)
			(kg/ha)	P = 0.05	P = 0.01		
TTMP	S	80	3,782	a	x	108	115.8
DPC	S	25	3,769	a	xy	108	104.3
DPC	F	10	3,743	ab	xy	107	124.1
DPC	F	25	3,734	ab	xy	107	121.2
DPC	S	10	3,718	ab	xy	106	121.1
CCC	F	25	3,698	ab	xy	106	109.6
DPC	F	50	3,628	abc	xy	104	116.2
Control		0	3,492	abc	xy	100	130.5
DPC	F	75	3,401	bc	xyz	97	112.5
Control (0.1% Tween 20)		0	3,389	bc	xyz	97	121.3
DPC	S	50	3,304	cd	yz	94	101.2
DPC	S	75	3,030	d	z	87	77.8

^m Acronyms: CCC is (2-chloroethyl)trimethyl ammonium chloride; DPC is N,N-dimethyl piperidinium chloride; and TTMP is tributyl[(5-chloro-2-thienyl)methyl]phosphonium chloride.

ⁿ Values followed by the same letter do not differ statistically; a-d P = 0.05; and x-z P = 0.01.

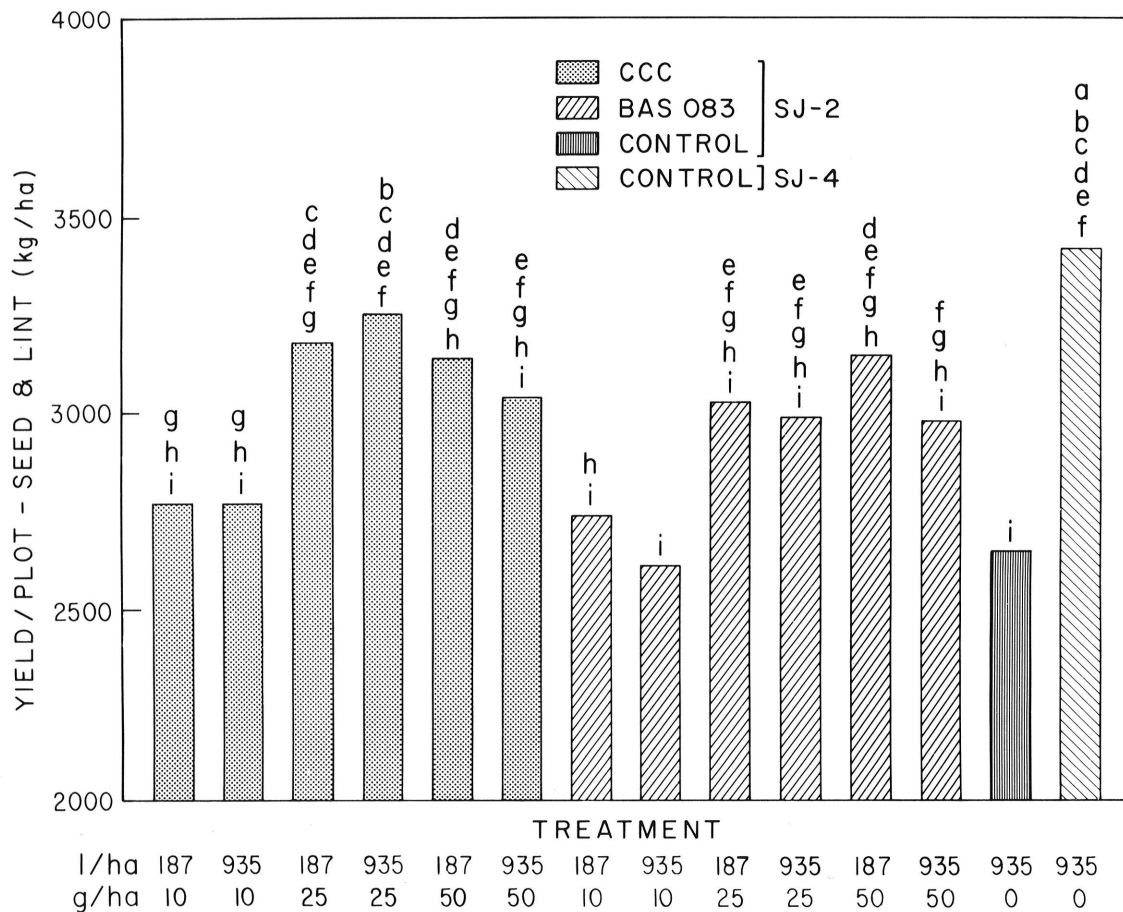


Fig. 2. Effect of application of CCC (chlormequat) and DPC (N,N-dimethylpiperidinium chloride) (BAS 083) applied at early square formation (mid-June) on yield of cotton seed and lint on land infested with *Verticillium dahliae*. The volume of solution used to spray the cotton is given in liters per hectare. The dosage of each chemical is given in grams per hectare. The data for the effect of the growth retardants on cv. SJ-4 was omitted because differences in yield due to chemicals were not significant. Bars with the same letter do not differ significantly ($P = 0.05$).

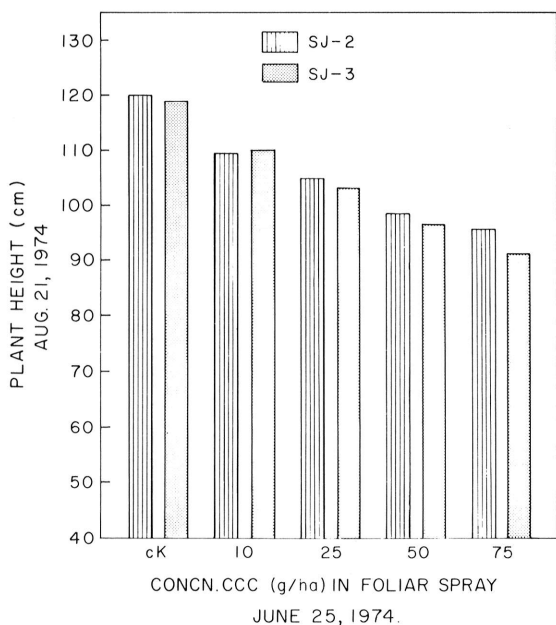


Fig. 3. Reduction of height of two cultivars of cotton plants by application of chlormequat (CCC) at different dosages.

N,N-dimethylpiperidinium chloride (DPC) tested here is an active growth retardant similar to DPI (16,27). Sinha and Wood (21) indicated that tyloses formed more profusely in the xylem of tomato plants treated with CCC. In plants treated with CCC, symptoms of *Verticillium* wilt were delayed. Tyloses probably impeded the translocation of the propagules of *V. albo-atrum* in the xylem tissue.

The side effects of disease control with plant growth regulators have been too serious for the control to be practicable. Dimond (7) commented that this approach to increasing host resistance to fungi may not prove useful because the effect of growth regulators may be too short-lived and the side effects too undesirable. In our early field work with CCC (9), dosages of 200–300 g/ha yield was reduced despite a slight mitigation of symptoms as predicted by Dimond (7). However, in results reported here, the lower dosages of 10 and 25 g/ha actually increased the yield of cotton, slightly reduced the severity of symptoms, and (perhaps of greater phytopathological significance), markedly reduced the internal population of *V. dahliae* propagules in upper petioles. Also TTMP, DPC, and several other growth retardants similarly reduced the internal population of propagules in upper petioles.

The propagule-reducing effect of growth retardants resembled that of resistant cultivars (11). The effect lasted 2–3 mo after foliar treatment. This was contrary to Dimond's prediction that growth regulator effects might be short-lived (7). It was tempting to conclude that growth retardants increased yield because *Verticillium dahliae* had been suppressed in xylem tissue (Fig. 1).

However, yield also was increased by growth retardants in a field in which *Verticillium* wilt was not an important factor (Table 3). Other physiologic effects of growth retardants (eg, those described by Rudolph [18] and Stutte [22] on soybean) also may be responsible for yield increase.

Both CCC and DPC reduce plant height. Since CCC reduces plant height by shortening of internodes (8), the treated plant should have as much photosynthetic area as a nontreated plant and reduction of plant height and lodging might be an advantage.

The growth retardants tested in California might become practical if eventually registered for use because of the extremely small quantities of chemicals required. Because the concentration requirement for CCC and DPC was highly critical, accurate dosage might be a problem under practical agricultural conditions. In India, Singh (20) obtained yield increases with CCC at 10 and 40 g/ha, but not with 320 g/ha. Marani et al (17) decreased yield with 100 g/ha of CCC, but at 50 g/ha, there was no effect on yield. We obtained similar results.

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