

DETECTION OF 2,4-D WITH COTTON

(GOSSYPIUM HIRSUTUM)

ROOT BIOASSAY

By

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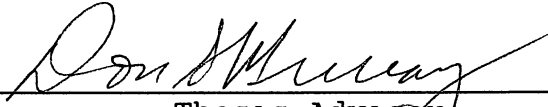
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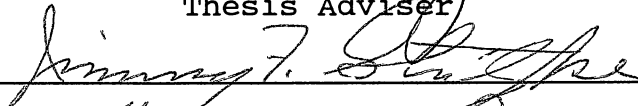
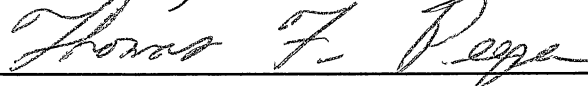

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## INTRODUCTION

This thesis is a manuscript to be submitted for publication in Weed Technology, a Weed Science Society of America publication. Articles in that journal are peer reviewed and must report original experiments repeated over time and/or space.



DETECTION OF 2,4-D WITH COTTON

(GOSSYPIUM HIRSUTUM)

ROOT BIOASSAY

## Detection Of 2,4-D With Cotton

(Gossypium hirsutum)

### Root Bioassay

Abstract. Laboratory bioassay experiments using pregerminated cotton seed were conducted using soil treated with a 2,4-D oil-soluble amine salt in petri dishes to determine the sensitivity range of cotton to 2,4-D and establish suitable units of concentration increase. Field experiments were also conducted near Perkins and Stillwater, Oklahoma, in 1989 to compare the activity of an amine salt formulation of 2,4-D and a low volatile ester formulation of 2,4-D as well as determining the effect of soil type on the detection and persistence of 2,4-D. Soil was sampled to a depth of 8 cm the day of application and 1, 2, and 4 weeks after application. The soil samples were bioassayed, and the 2,4-D concentrations were estimated using a standard curve. Laboratory bioassay experiments using pregerminated cotton seed and soil treated with a 2,4-D amine salt in aluminum pie-pans were conducted to determine if the developed method could be used to detect a 10 parts per billion by weight (ppbw) concentration of 2,4-D as an on-farm type bioassay technique. The initial laboratory bioassay experiments indicated that there was a nonlinear growth inhibition response to 2,4-D in the 0 to 500 ppbw concentration range. An approximately doubling or logarithmically scaled 2,4-D concentration increase

represented the effect of 2,4-D on cotton root growth adequately in the 0 to 400 ppbw concentration range. The field experiments indicated the differences in the soil types and the 2,4-D formulations were not consistently significant factors in the detection of 2,4-D activity or persistence. The most important factors in determining 2,4-D activity were the application rate and the time after application. The laboratory bioassay experiments using 2,4-D treated soil in aluminum pie-pans was a successful method for the detection of 10 ppbw 2,4-D, and may be useful as an on-farm type bioassay technique. Nomenclature: 2,4-D (2,4-dichlorophenoxyacetic acid); cotton, Gossypium hirsutum L. 'GP 3774' and 'Paymaster 145'.

Additional index words. Cotton root bioassay, on-farm bioassay.

#### INTRODUCTION

Conventional tillage systems for cotton production normally use eight or more tillage operations. Generally more than half of these tillage operations are directly or indirectly committed to the control of weeds. The Food Security Act of 1985 mandated that erodible lands in the U.S. have a conservation plan proposed by 1990 and those plans, as approved, must be implemented by 1995. A no-tillage or reduced tillage system, which maintains some crop residue on the soil surface, may permit a producer to comply with erosion control requirements and thus continue cotton

production without crop rotation.

Weed control is one of the major difficulties which limits the success of conservation tillage systems in cotton. Without late-fall or spring tillage, weeds become established and are present prior to planting in the following spring (4, 6, 8, 16). For example horseweed [*Conyza canadensis* (L.) Cronq] which is not commonly present in conventional tillage systems has been reported to be present by many researchers the first year in reduced tillage systems (3, 7, 15, 16). Control of horseweed and other weeds before or at planting is very important to establishing a good crop stand and to the success of a conservation tillage system (3, 5, 12, 16).

The use of 2,4-D can be an effective, economical method of controlling weeds which have emerged prior to planting cotton in conservation tillage systems (1, 6, 8, 9, 10, 16). It has been reported that seedling cotton is much more susceptible to 2,4-D than cotton at later growth stages (11). Thus, unacceptable cotton stand reductions may occur when cotton is planted too soon after a 2,4-D application (1, 6). After a pre-plant 2,4-D application a bioassay may be a useful tool for determining when cotton may be safely planted.

The objectives of this research were to determine: a) a rapid soil-cotton bioassay technique, b) a range of 2,4-D soil concentrations in which cotton is responsive, c) the effect of two 2,4-D formulations and two soil types in the

bioassay standards, d) the effect of the 2,4-D formulations on cotton root growth in field experiments, and e) the effectiveness of an on-farm type bioassay using soil in aluminum pie-pans.

#### MATERIALS AND METHODS

Bioassay response range experiments. Bioassay experiments were conducted to determine the 2,4-D concentration range that reduced cotton root growth. The 2,4-D formulation, soil type, and cotton cultivar used in these experiments was; 2,4-D oil-soluble amine salt<sup>1</sup>, Zaneis sandy loam (Udic Argiustoll), and 'GP 3774' cotton, respectively. The physical and chemical characteristics of this soil are detailed in Table 1.

The procedure used was a modification of the soil-petri dish bioassay as described by Parker (13). The soil was air dried and screened through a 2 mm sieve. Ten ml aliquots of 2,4-D solution were used to treat 490 g of soil to achieve known soil concentrations of 2,4-D. The treated soil was mixed in a Liquids-Solids Blender<sup>2</sup> for 2 min. A strip of paper towel 2 to 3 cm wide by 15 cm long was placed in the bottom of each 10 by 1.5 cm petri dish so that a 3 to 4 cm

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<sup>1</sup>2,4-D oil-soluble amine salt (n-oley1-1,3-propylenediamine salt of 2,4-dichlorophenoxyacetic acid; Dacamine®).

<sup>2</sup>Paterson - Kelley Co., Inc. Executive Office and Plant. East Stroudsburg, PA 18301.

portion of the paper towel was protruding from the petri dish. The petri dish lids were pressed on the petri dishes filled with 100 g of soil to spread the soil. The soil was moistened to near field capacity by placing the protruding portion of the paper towel in distilled water. After the soil was moistened a straight line was marked in the soil across the petri dish 2 to 3 cm from the edge to facilitate measurement of root growth. Cotton seeds were pregerminated at  $25 \pm 1$  C in trays lined with paper towel. Four pregerminated cotton seeds with 0.5 to 2.5 cm radicals were placed on the soil surface. The seeds were aligned near one side of the petri dish, and the root tips were placed along the reference line marked in the soil. Seeds with similar radicle lengths were placed in each petri dish, and the dishes were planted by replication in an effort to reduce variation within petri dishes and replications. The petri dishes were then sealed with transparent tape, and the protruding portion of the paper towel was removed to reduce water loss during the incubation period. The petri dishes were inverted at a 45 degree angle to promote root growth along the lid of the petri dishes. After approximately 24 hours at  $28 \pm 1$  C cotton root growth was measured.

In this experiment the cotton radicle length at planting was 0.5 to 2.5 cm with an average length of approximately 1 cm. The 2,4-D concentrations used in this experiment ranged from 0 to 500 ppbw in increments of 50 ppbw. The experiment was conducted in a randomized complete block design with

four replications, one petri dish/replication with four seeds/petri dish for a total of 16 observations/concentration level. The experiment was repeated four times.

The root growth data collected in this experiment and in the following experiments were converted to a percentage of the untreated check and then subjected to an analysis of variance and protected LSD test (0.05 probability level). The experiment runs 1, 3, and 4 were not significantly different. Experiment run 2 was significantly different.

Low concentration experiments. Experiments were conducted to better define the effect of 2,4-D on cotton root growth at low 2,4-D concentrations. The same bioassay procedure that was described previously was used in these experiments. In these experiments the number of concentrations below 100 ppbw was increased, and concentrations of 200 and 400 ppbw were included to use a similar range of concentrations as the previous experiment. The 2,4-D concentrations used were 0, 5, 10, 25, 50, 100, 200, and 400 ppbw. The same 2,4-D formulation, soil type, and cotton cultivar were used in conducting this experiment as in the previous experiment. The cotton radicle length at planting was 0.5 to 2.5 cm with an average length of approximately 1.8 cm. This experiment was conducted in a randomized complete block design and had four replications, one petri dish/replication with four seed/petri dish for a total of 16 observations/concentration level. This experiment was repeated twice.

The two runs of the experiment were pooled together. The mean root growth values for individual petri dishes were regressed against the log of the 2,4-D concentrations using a non-linear iterative regression procedure<sup>3</sup> with an equation for a sigmoidal curve

$$(\hat{y} = \frac{1}{1 + e^{-(m - b(\log x - \log \text{largest } x))}}). \text{ The } R^2 \text{ values reported are}$$

based on the regression of the means.

Field experiments. Field experiments were conducted at two locations using two 2,4-D formulations to evaluate the effect of soil type and formulation differences on cotton root growth. The field experiments were established in north central Oklahoma on a Zaneis sandy loam (Udic Argiustoll), and a Easpur loam (Fluventic Haplustoll). The physical and chemical characteristics of these soils are detailed in Table 1. The experimental design was a randomized complete block with 4 replications with 3.7 by 4.6 m plots. A 2,4-D amine salt<sup>4</sup> and a low volatile ester formulation of 2,4-D<sup>5</sup> were applied at the rates of 0.27, 0.53, 1.07, and 2.13 kg ae ha<sup>-1</sup> on October 10, 1989 with a compressed air tractor sprayer. The treatments were applied

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<sup>3</sup>Marquardt iterative method. 1985. SAS/STAT User's Guide. SAS Institute, Inc., Cary, NC 27511-8000.

<sup>4</sup>2,4-D amine salt (diethanolamine salt of 2,4-dichlorophenoxyacetic acid; Weedar 64-A®).

<sup>5</sup>2,4-D low volatile ester (2,4-dichlorophenoxyacetic acid butoxyethyl ester; Weedone LV4®).



to tilled soil surfaces, free of vegetation.

Following herbicide application, two soil samples were taken from each plot, each sample contained 15 to 25 soil cores 2.5 cm in diameter and 8 cm deep. Samples were collected from each plot within an hour of application and at 1, 2, and 4 weeks after application. The soil samples were stored at -5 C until they were used in the cotton bioassay. The field samples were removed from the freezer to thaw 1 to 2 days prior to the start of the bioassay procedure. Each sample was then mixed thoroughly by hand and the larger soil aggregates in the sample were crushed to be a size suitable for use in the petri dishes. One hundred g of soil from each field sample was weighed into a petri dish. The field samples collected at the time of application and 4 weeks after application were near field capacity, therefore these samples were not moistened. The percent soil moisture was determined in both soils for each sampling date by drying soil at 105 C for 24 hrs. Standards were included for both 2,4-D formulations and soil types using 'Paymaster 145' cotton. Concentrations of 2,4-D used in the standards were 0, 3, 6, 13, 25, 50, 100, 200, 400, and 800 ppbw. This process was repeated three times with the soil samples collected on the day of application, 1 week, and 2 and 4 weeks after application.

The bioassay experiments were conducted in a randomized complete block design, and the bioassay replications corresponded to the field plot replications. The field

experiments at both locations had four replications with two samples for two petri dishes/replication and four seeds/petri dish for a total of 32 observations/treatment. The standards for both soil types and 2,4-D formulations had four replications from each 500 g lot of treated soil with one petri dish/replication for a total of 16 observations/standard concentration level/soil type/2,4-D formulation.

Protected LSD values were calculated in the field experiments to compare means for different sampling times within levels of formulation and rate. Similarly, LSD values were calculated to compare means for 2,4-D formulations and rates within a single sampling time.

The three runs of the standards were pooled together. Soil types and 2,4-D formulations in the pooled data set were not significantly different; therefore, the data were further pooled over soils and formulations to have 192 observations/concentration level. The pooled data set was regressed using the same procedure used in the Low concentration experiments to calculate a line from the standards to estimate the 2,4-D concentrations in the field samples. The  $R^2$  values reported are based on the regression of the means by 2,4-D concentration. Concentrations of 2,4-D were estimated for the field samples using the line regressed from the standards pooled by concentration. The standard error of the mean was used to predict the upper and lower values for the observed 2,4-D concentrations.

On-farm type bioassay experiments. Experiments were conducted to determine the effectiveness of a soil bioassay procedure using pregerminated cotton seed and 2,4-D treated soil in aluminum pie-pans. The 2,4-D concentrations used were 0 and 10 ppbw. The 2,4-D formulation, soil type, and cotton cultivar used in these experiments was; 2,4-D amine salt, Zaneis sandy loam, and 'Paymaster 145' cotton, respectively. The soil was air dried and screened through a 2 mm sieve. Fifteen ml aliquots of 2,4-D solution were used to treat a 985 g lot of soil to achieve known 2,4-D soil concentrations. The treated soil was mixed in a Liquids-Solids Blender for 2 min. Eighty ml of distilled water was added to each lot of soil to moisten the soil to near field capacity. The soil and water was hand mixed in plastic bags. Twenty five pregerminated cotton seed were placed in the bottom of 20 cm diameter aluminum pie-pans. Seeds with similar root lengths were placed in the pie-pans of the same replication, and the average initial root length was estimated for each replication. In the first experiment replications 1, 2, 3, and 4 had initial root lengths of approximately 1.3, 1.0, 0.8, and 0.5 cm, respectively. In the second experiment replications 1, 2, 3, and 4 had initial root lengths of approximately 2.5, 1.8, 1.8, and 1.5 cm, respectively. Then the soil was placed over the seed in the pie-pans and packed gently. The pie-pans were covered with aluminum foil to reduce moisture loss during the incubation period. The temperature during the first

experiment was  $23 \pm 1$  C, and during the second experiment the temperature was  $24 \pm 1$  C. The experiments had four replications with 25 seed/replication. In the first experiment root growth was measured 24 hours after planting, and in the second experiment root growth was measured 48 hours after planting to determine if cotton root growth inhibition was more pronounced with the longer incubation period.

Initial root lengths of each replication were subtracted in the on-farm type bioassay experiments, and the data were converted to a percentage of the untreated check in each experiment. The experiments were not significantly different, therefore they were pooled. LSD values were then calculated for the means of the pooled data set.

#### RESULTS AND DISCUSSION

Bioassay response range. The response of cotton roots to 2,4-D was similar three of four times the experiment was repeated (Table 2). For unknown reasons experiment run two was different from the other runs. The 2,4-D concentration factor was highly significant in each run of the experiment. Root growth was significantly reduced by 50 ppbw in the experiments. Also the 150 ppbw 2,4-D concentration reduced root growth significantly from the 50 ppbw concentration. However, the procedure was less effective distinguishing between 200 and 500 ppbw of 2,4-D. The different magnitude of response per unit of concentration increase suggested a

nonlinear growth inhibition response to the concentration of 2,4-D in the 0 to 500 ppbw range. This nonlinear root growth response is in agreement with results previously reported (2, 14).

Low concentration experiments. Response of cotton roots to 2,4-D was similar both times the experiment was conducted (Table 3). In both runs of the experiment the effect of the 10 ppbw concentration was significantly different from the untreated check. An increased root growth inhibition for a given 2,4-D concentration was observed in this experiment compared to the previous experiments. The different sensitivity levels in the two experiments may possibly be due to seeds with longer initial radicle lengths being used in the Low concentration experiments. However, experiments were not conducted to investigate the effect of initial cotton root length on the sensitivity of cotton to 2,4-D.

Cotton root growth response to the 2,4-D concentration in the soil is shown in Figure 1. Root growth is reported as a percentage of the untreated check. The 2,4-D concentration factor was highly significant. This indicates that the change in 2,4-D concentration was responsible for the differences observed in the treatments. The use of an approximately doubling or logarithmically scaled 2,4-D rate increase represents the effect of 2,4-D concentration on cotton root growth well in this set of experiments. The curve fitted to the means had an  $R^2$  squared value greater than 0.99.

Within the low concentration experiments the concentration range of 0 to 400 ppbw was used to show the effect of 2,4-D on cotton root growth in the Zaneis sandy loam soil adequately. It was concluded from these experiments that a doubling or logarithmically scaled concentration rate increase would be more appropriate than a concentration increase of 50 ppbw increments for the standards in the field experiments.

Field experiments. The three times the standards were repeated the changes in 2,4-D concentration had similar effects on cotton root growth (Table 4). The 2,4-D concentration was highly significant in the standards with a probability value of less than 0.001. When the runs were pooled, the effect of the 3 ppbw soil 2,4-D concentration was significantly different from the untreated check.

Using the regression line calculated from the standards, the 2,4-D concentration in the field samples was estimated. The regression line of the standards had a  $R^2$  value of 0.99 (Figure 2). Because the line used to estimate herbicide concentration is based on the log of 2,4-D concentration, the accuracy of the estimated values decreases with increasing 2,4-D concentrations.

The analysis of variance of the field sample data indicated that the application rates of both formulations of 2,4-D were significant in both soil types at all sampling times (Table 5 and Table 6). There was also a decrease in cotton root growth inhibition of both 2,4-D formulations in

both soil types as time after application increased. The 2,4-D formulations were significantly different 1, and 2 weeks after application. The soil types were significantly different 0, and 4 weeks after application. The effect of the different 2,4-D formulations and the different soil types was not consistent across sampling times and is thought to be confounded with the differences in location.

The biological activity estimated in ppbw of the 2,4-D in the Zaneis sandy loam and the Easpur loam decreased as time after application increased (Table 7 and Table 8). The 0.53 kg ae ha<sup>-1</sup> and higher rates of both 2,4-D formulations were still active 4 weeks after application. There was also a response to the initial application rate of 2,4-D 4 weeks after application. The persistence of 2,4-D activity is attributed to the cool temperatures of the fall months (Figure 3 and Figure 4). However, this experiment was done primarily to test the usefulness of this technique in the field and not necessarily to measure 2,4-D persistence.

In the Zaneis sandy loam, the low rate of 2,4-D amine and ester decreased from an initial concentration of 80 and 102 ppbw to 1 and 0 ppbw 4 weeks after application respectively. The high rate of 2,4-D amine and ester decreased from 640 and >800 ppbw initially to 150 and 68 ppbw 4 weeks after application respectively.

In the Easpur loam, the low rate of 2,4-D amine and ester decreased from an initial concentration of 36 and 30 ppbw to 0 and 16 ppbw 4 weeks after application respectively. The

high rate of 2,4-D amine and ester decreased from 178 and 252 ppbw to 179 and 201 ppbw 4 weeks after application respectively.

The bioassay of the field samples indicated that both 2,4-D formulations increased in root growth inhibition from the day of application to 2 weeks after application in the Easpur loam; the increase was less in the Zanies sandy loam. This may be attributed to the rainfall event which occurred 4 days prior to the 2,4-D application (Table 9). The different moisture levels affected the amount of soil placed in the petri dishes (Table 10). The different amounts of soil thus affected the concentration of 2,4-D in each sample. This procedural effect may have caused the increase in the 2,4-D root growth inhibition from the day of application to 2 weeks after application which was observed in both soil types.

This method of cotton root bioassay was useful in detecting a wide range of 2,4-D concentrations. The bioassay was also sensitive to a 2,4-D concentration of 3 ppbw. In the standards, the different 2,4-D formulations were not significantly different from one another, and the soil types were not different from one another. In the field samples, the bioassay technique was an effective tool in determining the activity of 2,4-D remaining in the soil. On-farm type bioassay experiments. The results of these experiments indicate that 10 ppbw concentration of 2,4-D reduced cotton root growth 16 and 13 percent (Table 11).



There was no significant difference in replications within experiments, and there was no significant difference between the 24 hr and 48 hr experiments. Therefore, the data were pooled resulting in a 15 percent root growth reduction for the pooled data. The longer incubation period did not increase the effect of 2,4-D; therefore, there is no apparent advantage to the longer incubation period. This simple method of determining if small amounts of 2,4-D is still active in soil after an earlier application may provide a farmer with valuable information on the presence and activity of 2,4-D in the soil. However, additional field research is needed to determine if the detectable concentration of 2,4-D is correlated with cotton stand reductions, reduced early season growth, and/or yield reductions.

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Table 1. Physical and chemical characteristics of soils.

Soil	pH	Sand	Silt	Clay	Organic
					matter <sup>1</sup>
		————— % —————			
Easpur loam	6.1	48	30	22	0.7
Zaneis sandy loam	5.4	58	24	18	0.5

<sup>1</sup>Determined by Oklahoma State University soil testing laboratory.

Table 2. Cotton root growth 1 day after seeding into Zaneis sandy loam treated with 0 to 500 ppbw of 2,4-D in 50 ppbw increments.

2,4-D concentration  (ppbw)	Experiment run			
	1	2	3	4
	————— % of untreated <sup>1</sup> —————			
0	100	100	100	100
50	69	41	71	51
100	55	17	55	48
150	47	13	50	44
200	32	22	38	41
250	32	11	34	32
300	36	11	28	29
350	27	7	30	26
400	33	8	27	21
450	28	7	27	25
500	32	9	20	19
LSD (0.05)	11	12	13	13

<sup>1</sup>Mean growth of untreated for runs 1, 2, 3, and 4 was; 41, 31, 35, and 26 mm.

Table 3. Cotton root growth 1 day after seeding into Zaneis sandy loam treated with 0 to 400 ppbw of 2,4-D.

2,4-D concentration  (ppbw)	Experiment run		Runs
	1	2	combined
	————— % of untreated <sup>1</sup> —————		
0	100	100	100
5	90	90	90
10	59	75	67
25	40	58	49
50	28	40	34
100	14	26	20
200	8	11	9
400	6	6	6
LSD (0.05)	16	15	11

<sup>1</sup>Mean growth of untreated for run 1 and 2 was 31 mm.

Table 4. Cotton root growth 1 day after seeding averaged across soil types and 2,4-D formulations within each run. Runs combined were averaged over soil types, 2,4-D formulations, and runs.

2,4-D concentration  (ppbw)	Experiment run			Runs combined
	1	2	3	
	————— % of untreated <sup>1</sup> —————			
0	100	100	100	100
3	94	91	84	89
6	92	89	84	88
13	88	86	72	82
25	78	67	55	66
50	48	51	35	45
100	30	33	19	27
200	16	17	11	15
400	9	7	6	7
800	6	7	4	5
LSD (0.05)	12	10	9	6

<sup>1</sup>Mean growth of untreated in Zanies soil for run 1, 2, and 3 was 29, 28, and 36 mm. Mean growth of untreated in Easpur soil for run 1, 2, and 3 was 30, 26, and 32 mm.



Table 5. Cotton root growth 1 day after seeding into Zaneis sandy loam at 0 to 4 weeks after application.

2,4-D form. <sup>1</sup>	Rate applied (kg ae ha <sup>-1</sup> )	Weeks after application				LSD(0.05)
		0	1	2	4	
		— % of untreated <sup>2</sup> —				
Amine	0.27	33	44	31	98	13
Ester	0.27	27	42	43	100	15
Amine	0.53	19	17	10	80	15
Ester	0.53	12	32	16	89	13
Amine	1.07	7	6	4	53	11
Ester	1.07	4	15	17	50	11
Amine	2.13	5	4	4	20	7
Ester	2.13	2	14	6	37	9
LSD(0.05)		7	9	9	18	

<sup>1</sup>Formulation.

<sup>2</sup>Mean growth of untreated for 0, 1, 2, and 4 weeks after application was 37, 33, 40, and 29 mm.

Table 6. Cotton root growth 1 day after seeding into Easpur loam at 0 to 4 weeks after application.

2,4-D form. <sup>1</sup>	Rate applied  (kg ae ha <sup>-1</sup> )	Weeks after application				LSD(0.05)
		0	1	2	4	
		———— % of untreated <sup>2</sup> ————				
Amine	0.27	55	25	33	101	17
Ester	0.27	60	46	22	75	19
Amine	0.53	30	14	12	74	14
Ester	0.53	41	29	39	51	NSD <sup>3</sup>
Amine	1.07	18	9	7	40	8
Ester	1.07	23	12	5	28	11
Amine	2.13	17	6	3	17	6
Ester	2.13	12	6	3	15	7
LSD(0.05)		12	7	13	19	

<sup>1</sup>Formulation.

<sup>2</sup>Mean growth of untreated 0, 1, 2, and 4 weeks after application was 25, 25, 28, and 23 mm.

<sup>3</sup>No significant difference.

**Table 7.** Observed 2,4-D concentrations in the Zaneis sandy loam at 0 to 4 weeks after application using cotton root growth percentages and the standard curve.

2,4-D formulation	Rate applied  (kg ae ha <sup>-1</sup> )	Weeks after application			
		0	1	2	4
		observed ppbw			
Amine	0.27	80 ± 12 <sup>1</sup>	54 ± 8	88 ± 8	1 ± 4
Ester	0.27	102 ± 11	58 ± 9	55 ± 6	0 ± 3
Amine	0.53	156 ± 19	176 ± 27	302 ± 43	12 ± 4
Ester	0.53	259 ± 26	83 ± 17	195 ± 24	6 ± 4
Amine	1.07	456 ± 71	499 ± 27	666 ± 85	38 ± 7
Ester	1.07	680 ± 104	202 ± 35	178 ± 35	42 ± 7
Amine	2.13	640 ± 115	744 ± 91	780 ± 128	150 ± 23
Ester	2.13	>800	219 ± 46	499 ± 122	68 ± 14

<sup>1</sup>One standard error of the mean.

**Table 8.** Observed 2,4-D concentrations in the Easpur loam at 0 to 4 weeks after application using cotton root growth percentages and the standard curve.

2,4-D formulation	Rate applied	Weeks after application							
		0		1		2		4	
	(kg ae ha <sup>-1</sup> )	observed ppbw							
Amine	0.27	36 ± 4 <sup>1</sup>	113 ± 13	81 ± 16	0 ± 4				
Ester	0.27	30 ± 5	49 ± 6	131 ± 18	16 ± 5				
Amine	0.53	92 ± 9	213 ± 22	263 ± 42	17 ± 5				
Ester	0.53	60 ± 10	97 ± 21	65 ± 16	42 ± 8				
Amine	1.07	164 ± 21	350 ± 38	415 ± 91	62 ± 8				
Ester	1.07	124 ± 15	263 ± 32	581 ± 118	98 ± 14				
Amine	2.13	178 ± 21	507 ± 83	>800	179 ± 30				
Ester	2.13	252 ± 36	507 ± 83	>800	201 ± 35				

<sup>1</sup>One standard error of the mean

Table 9. Precipitation received at the Perkins and Stillwater, Oklahoma locations during the experiment.

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Date	Perkins	Stillwater
10/06/89	3.0	2.4
10/28/89	0.1	Trace
10/29/89	0.1	0.8
10/30/89	3.8	4.0
11/02/89	0.1	Trace

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Table 10. Percent soil moisture determined on a weight basis.

Weeks after application	Zaneis sandy loam	%	Easpur loam
0	11.7		11.7
1	7.4		7.8
2	7.1		7.1
4	8.8		12.3

Table 11. On-farm type bioassay cotton root growth in the Zaneis sandy loam treated with 0 and 10 ppbw of 2,4-D in aluminum pie-pans.

2,4-D concentration	Experiment run		Runs combined
	1	2	
(ppbw)	———— % of untreated <sup>1</sup> ————		
0	100	100	100
10	84	87	85
LSD (0.05)	14	11	11

<sup>1</sup>Mean growth of untreated for run 1 and 2 was 17 and 48 mm.

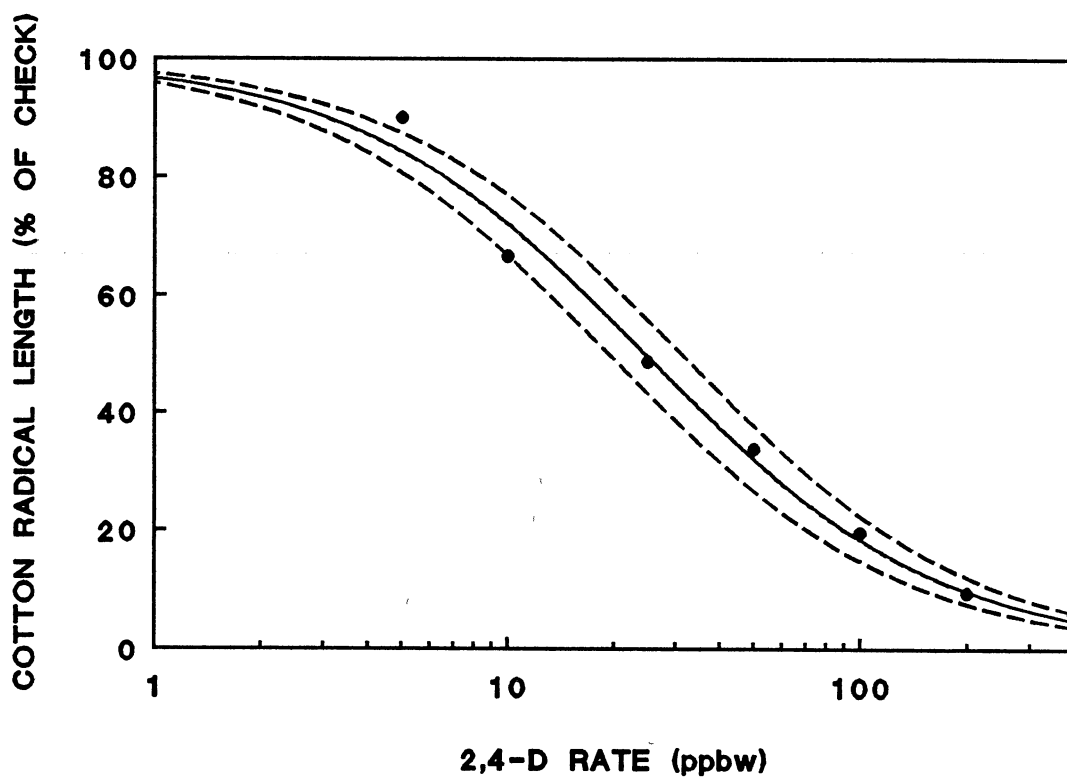


Figure 1. Effect of 2,4-D concentration in the Zaneis sandy loam on cotton root growth. The regression equation is:

$$\hat{y} = \frac{1}{1 + e^{-(0.223 - 2.43(\log x - 1.30))}}$$
 ( $R^2 = 0.99$ ). The dotted lines are an approximation of the 95% confidence interval on the mean.



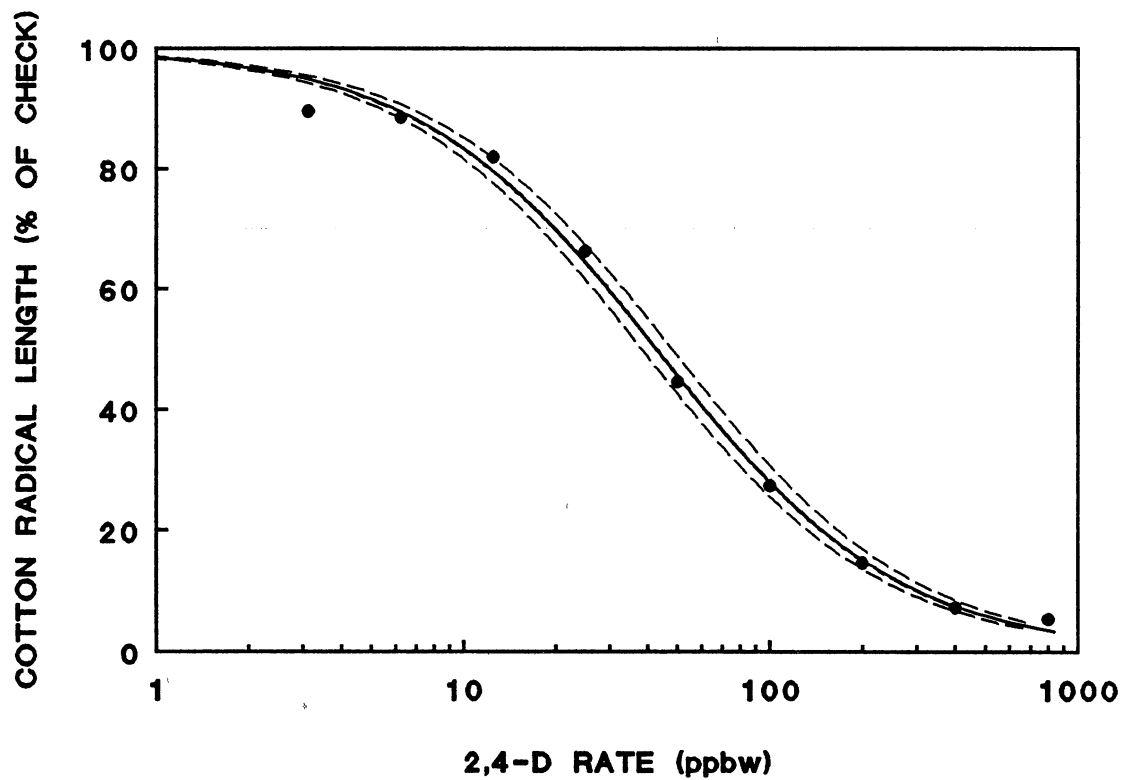


Figure 2. Effect of 2,4-D concentration on cotton root growth averaged across soils, 2,4-D formulations, and runs.

The regression equation is:  $\hat{y} = \frac{1}{1 + e^{-(0.465 - 2.57(\log x - 1.45))}}$

( $R^2 = 0.99$ ). The dotted lines are an approximation of the 95% confidence interval on the mean.

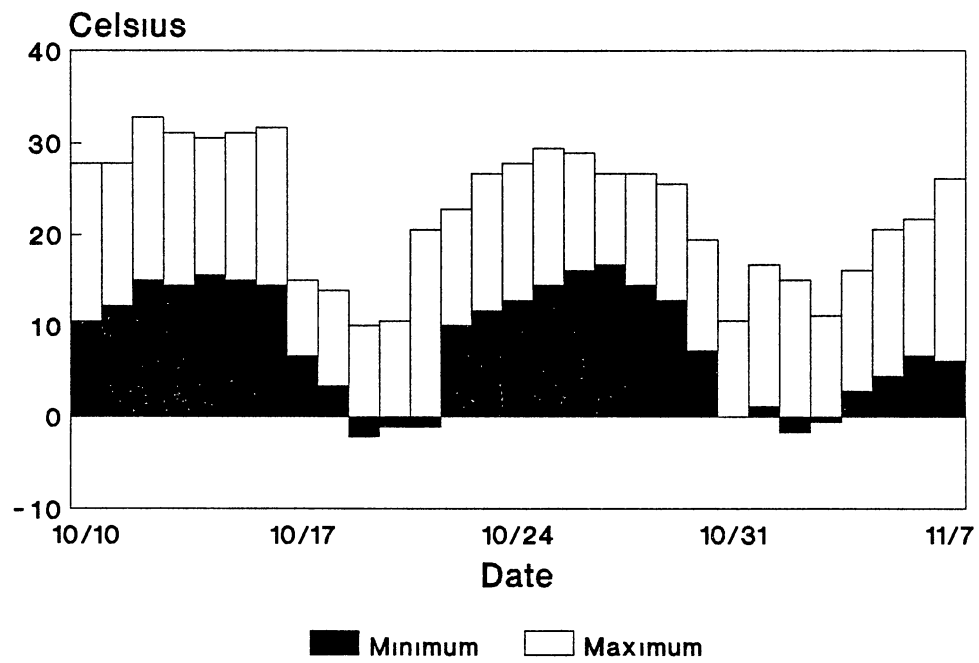


Figure 3. Minimum and maximum temperatures at the Perkins, Oklahoma location during the experiment.

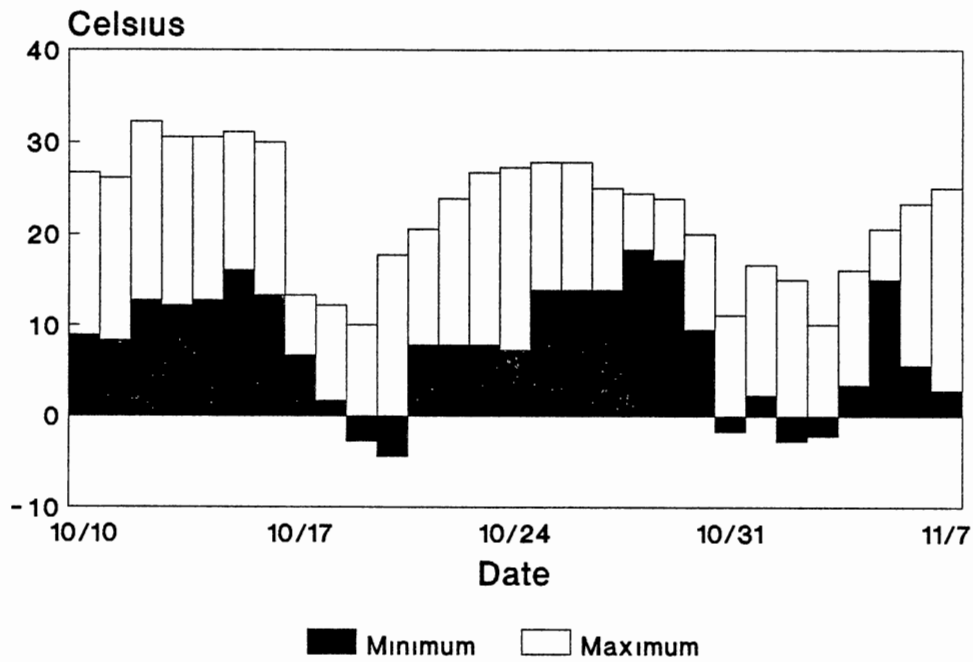


Figure 4. Minimum and maximum temperatures at the Stillwater, Oklahoma location during the experiment.

VITA

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(GOSSYPIUM HIRSUTUM) RESPONSE

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