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Effects of 2,4-DB on Yield and Pod Development in Peanuts

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ABSTRACT

The herbicide 2,4-DB [4-(2,4-dichlorophenoxy) butyric acid] was not readily absorbed by peanut (*Arachis hypogaea* L.) leaves, was not accumlated in the nut at harvest and was very slowly metabolized to 2, 4-D (2,4-dichlorophenoxyacetic acid). In contrast, pigweed (*Amaranthus retroflexus* L.) readily absorbed the 2,4-DB and rapidly converted it to 2,4-D. The 2,4-D was subsequently translocated to the apical regions of the pigweed and resulted in severely reduced growth or death.

Applications of 0.9 kg/ha of 2,4-DB between maximum pegging and early pod (fruit) enlargement reduced yield and affected quality and pod size. Repeated applications of 0.45 kg/ha of 2,4-DB did not adversely affect the peanut. An analytical procedure sensitive to 0.1 ppm of 2,4-D and 0.2 ppm of 2,4-DB is described for analysis of fresh plant forage and nuts.

Keywords: herbicide, peanuts, pod development, 2,4-DB

Preplant incorporated herbicides such as benefin (N-butyl-N-ethyl-a,a,a-trifluoro-2,6-dinitro-p-toluidine) are commonly used for weed control in peanuts. These herbicides are most effective against small seeded annual grass and broadleaf weeds. Much of the early work on post emergence weed control in peanuts involved applications of 2,4-D, MCPA (2-methyl-4-chlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), all of which controlled young broadleaf weeds but resulted in various degrees of injury to the peanuts (4, 8, 9). Generally, injury increased as rates increased. Rawson (9) reported that applications of 2,4-D or MCPA heavy enough to provide effective weed control could cause serious loss of yield while similar applications of 2,4-DB had little effect on yield. The use of 2,4-DB to control the escape or more resistant broadleaf weeds is now registered for peanuts (11).

Phenoxy herbicides are generally toxic to broadleaved weeds or crops. However, legumes are somewhat tolerant of 2,4-DB because they do not convert the butyric acid side chain into acetic acid as readily as most broadleaf plants (3, 12, 13). Recent research indicated that the tolerance of legumes to 2,4-DB also arises from a combination of poorer spray retention, less effective absorption and translocation, and more rapid degradation of limited amounts of the 2,4-D arising from beta-oxidation of 2,4-DB (5, 6, 14).

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Absorption and metabolism of 2,4-DB have been studied in depth in soybeans, forage legumes, and some weed species (3, 5, 6, 7, 10, 14) but not in peanuts. The objectives of this study were to determine the effect of rate and time of application of 2,4-DB on growth and yield of peanuts; to determine if enlarged pods were produced following application of 2,4-DB; if so, to determine the conditions necessary for enlarged pod development; and to determine residue levels at various times in the crop and a susceptible weed species.

Materials and Methods

Field Experiments

In field tests, seed of Starr variety of Spanish peanut were planted uniformly at the rate of 112 kg/ha in 1.02 m rows. Each plot was two rows wide by 6.1 m long. Postemergence broadcast treatments using the dimethylamine salt of 2,4-DB were applied with a compressed air bicycle sprayer in 187 L of water per ha. Unless otherwise noted, all treatments were replicated four times.

Residue studies at Yoakum, Texas were conducted on field grown peanuts treated two weeks after emergence with 0.45, 0.90 and 1.79 kg/ha of 2,4-DB or 2,4-D to determine relative absorption of the two herbicides and to determine if 2,4-DB is degraded to 2,4-D by the peanut plants under field conditions. Samples were taken at 0, 1, 4 and 7 days after treatment. Four replications of 22 plants were taken from each treatment plus check plots for analysis. Additional plants were treated as above at pegging and analyzed for residue at harvest. Postemergence studies were conducted at Yoakum, Texas to determine the effect of 2,4-D or 2,4-DB on residues in peanuts at harvest. In Test 1, 2,4-DB was applied at 0, 0.56 or 0.84 kg/ha. Samples were taken at harvest for residue analysis from 6.1 m of row. Six replications of forage and nuts were taken from each treatment in Test I and five replications of nuts were taken from each treatment in Test II.

The effect of rate and time of application of 2,4-DB on peanut yield and herbicide residues in the nut was investigated in a study at Prairie View, Texas. Peanuts were treated two, four or six weeks after planting using 0.45 of 0.90 kg/ha of 2,4-DB. Repeated applications of 0.45 kg/ha were also made at two plus four weeks and at two plus four plus six weeks after planting. Yields were taken and peanuts were analyzed for herbicide residues.

The effect of application of 2,4-DB on pod development was investigated in two experiments at Prairie View, Texas. In the first experiment, 2,4-DB treatments of 0.45 and 0.90 kg/ha were applied pre-bloom and post-bloom at 30 and 55 days from planting. In the second experiment, the same rates were applied at 3 to 8 day intervals beginning 21 days after planting and continuing until 62 days after planting. The dates of application were selected to expose plants to the herbicide during all stages of development from pre-bloom through early pod development. The stage of development of the peanut plants at each application date was noted and plant response was recorded. Peanuts were harvested at maturity, placed in bags, dried to below 10% moisture, threshed, cleaned, weighed and graded. A volume displacement test was conducted to determine the size of pods from the various treatments to determine if pod size was significantly affected by treatment.

A susceptible weed, pigweed, was treated in College Station, Texas at three growth stages for comparison. Pigweed was treated at heights of 10, 18 and 53 cm. The taller plants were beginning to produce flower heads. A broadcast treatment of 0.56 kg/ha of 2,4-DB was applied as in the peanut tests. Residue samples were taken at 4 and 48 hours after application and at weekly intervals for the next eight weeks. Regrowth on samples not killed by the treatment were also analyzed for residue.

Greenhouse Experiments

Peanuts were grown in 1500 g Sawyer loamy sand in plastic pots 15 cm in diam. Five seed were planted per pot. Peanut seedlings were treated using the laboratory spray table described by Bouse and Bovey (2). All treatments were replicated four times and each experiment repeated twice. Samples were analyzed for both 2,4-DB and its metabolite 2,4-D.

In studies to determine if age of plants at time of spraying affected absorption and metabolism of 2,4-DB, peanuts were treated at a rate of 1.12 kg/ha of 2,4-DB at 7, 10, 12, 14, 20 and 27 days after planting. All peanuts were harvested 35 days after planting. Each plant was weighed and measured prior to extraction.

Low levels of absorption of 2,4-DB in other legumes have been reported (7, 11). Peanuts may be expected to gain some resistance to the herbicide because of this lack of absorption. To maximize penetration of 2,4-DB through the waxy cuticle, a surfactant and higher herbicide rates were employed. Peanut seedlings were treated two weeks after emergence with 2,4-DB at rates of 1.12 or 2.24 kg/ha or at 1.12 kg/ha with 1 or 5% X-77, a surfactant containing a mixture of alkylarylpolyethyleneglycol and free fatty acids in isopropanol. Controls were sprayed with water or water plus surfactant for comparison. Plants were harvested 2 weeks after treatment. Weight and height measurements were taken.

For comparison, pigweed seedlings were grown to a height of 6 cm in flats in a greenhouse and sprayed with 1.12 kg/ha 2,4-DB as in the peanut test except that 10 plants were used for each replication. Samples were taken at 0, 1 and 2 weeks after treatment.

Baur et al. (1) concluded that maximum penetration of herbicides occurred when the treated plant was kept in a moist environment to prevent drying of the treating solution. To test this principle, peanuts were germinated and allowed to grow for seven days after emergence prior to treatment. The first pair of primary leaves was treated with 8 ul of a solution of 2,4-DB in 0.1% X-77 containing 25 ug/ul for a total of 200 ug of 2,4-DB per plant. The treated plants were immediately placed into a 100% relative humidity chamber to maintain the treating solution in a moist condition. After 24 hr, the plants were harvested, separated into treated leaf, upper leaf, stem and root sections and analyzed for 2,4-DB and 2,4-D.

Residue Analysis

Treated leaves were first washed with two 100 ml aliquots of 0.1% NH_4Oh in distilled water to remove any herbicide on the surface. The sample was then blended in a Waring Blendor with 25 ml of 0.1 N CHL and 100 ml 2-propanol for 10 min at high speed and suction filtered. The filtrate was added to 100 ml 5% $NaHCO_3$ plus sufficient 5% KOH to obtain a pH of 10 and washed twice with 100 ml petroleum ether. This clean-up step effectively removed the oil from nut samples and much of the pigment from forage samples. The procedure worked equally well on peanuts and pigweed samples.

Basic samples were then acidified by dropwise addition of concentrated HCl to pH of 2.0. The water was extracted three times with 75 ml portions of diethyl ether. The ether extracts were combined and evaporated to dryness. Acids of 2,4-DB and 2,4-D were methylated by adding 10 ml BF_3 in methanol solution (12.5% w/v) and heating until BF_3 fumes evolved. After cooling, 8 ml water and 10 ml hexane were added and transferred to a separatory funnel for shaking and separation. The hexane fraction was retained in a glass vial for analysis.

Samples were analyzed by injecting 2 ul of the hexane solution into a Barber-Colemen 5360 gas chromatograph equipped with a radium-226 electron capture detector. The 1.83 m spiral glass column was packed with 10% DC-200 on 100/200 mesh Gas-Chrom Q. Injector, column and detector temperatures were 260, 210 and 230°C, respectively. Prepurified nitrogen at 2.1 kg/cm^2 was used as a carrier gas. Herbicide concentrations within samples were determined by comparing peak heights produced by 2 ug of 2,4-D and 5 ug 2,4-DB in 10 ml hexane. The retention times for 2,4-D and 2,4-DB were 2.5 and 5 minutes and the limits of detection for 25 g samples were 0.1 ppm and 0.2 ppm, respectively.

Results and Discussion

Field Experiments

Within four hours peanut seedlings treated with 0.45, 0.90 or 1.79 kg/ha of 2,4-DB had absorbed 18.0, 20.2 or 17.5%, respectively, of the herbicide applied (Table 1). After treatment with similar rates of 2,4-D, 53.4, 57.4 or 56.4% had entered the treated leaf. The uptake of 2,4-D was significantly higher than that of 2,4-DB. No significant beta-oxidation to 2,4-D within the plant was evident. Peanuts treated with 2,4-DB showed no signs of hormone-like activity while those treated with 2,4-D showed symptoms of stem and leaf curl, a decline in vigor, and in many cases, permanent stunting or death. At harvest, residues in forage samples treated with 2,4-DB at or shortly after pegging were reduced below the range of sensitivity except for the 1.79 kg/ha rate which had 0.60 ppm of 2,4-DB and 0.22 ppm of the metabolite 2,4-D. Residue in forage treated with 2,4-D was 0.56 and 1.34 ppm of 2,4-D when treatment was 0.90 or 1.79 kg/ha, respectively.

Table 1. Absorbed (TL) and unabsorbed (LW) 2,4-DB or 2,4-D in peanut seedlings 0 to 7 days after treatment in the field at Yoakum, Texas.

Treatment (kg/ha)	µg/plant at 0 hr	Percent of herbicide recovered at various days after treatment[a]							
		0 day[b]		1 day		4 days		7 days	
		LW	TL	LW	TL	LW	TL	LW	TL
2,4-DB 0.45	575a	82a	18d	61b	35cd	27b	13cd	1c	3ab
2,4-DB 0.90	1200b	80a	20d	86a	36cd	41a	13cd	1c	2bc
2,4-DB 1.79	2725c	83a	18d	87a	29d	47a	12cd	2bc	1c
2,4-D 0.45	575a	47bc	53b	37cd	56b	8d	21bc	2bc	3ab
2,4-D 0.90	1225b	43c	57b	45c	44c	14cd	25b	2bc	4a
2,4-D 1.79	2650c	44c	56 b	37cd	33d	14cd	25b	4a	4a

[a]Values in columns for the same day followed by the same letter do not differ at the 1% significance level as determined by Duncan's multiple range test. LW=Leaf Wash and TL=Treated Leaf

[b]Leaf wash was completed within four hours after treatment.

Peanut yields were not significantly affected by the 2,4-DB or 2,4-D treatments in Test I and Test II at Yoakum, Texas. In Test II, 2,4-D versus 2,4-DB, a canopy of weeds prevented most of the spray from reaching the peanut plants. This resulted in a lack of typical growth response by peanuts from the 2,4-D treatment. Residue levels in both nuts and forage for Test I and in nuts for Test II were below detectable limits.

Applications of 2,4-DB at rates of 0.45 or 0.90 kg/ha at two, four or six weeks after emergence and repeated applications of 0.45 kg/ha at two and four or two, four and six weeks after emergence did not significantly affect the yield of peanuts. No residue of 2,4-DB or the metabolite 2,4-D was detected in harvested peanuts at any rate or time of application.

Enlarged pods were observed at harvest and

Table 2. Effect of 2,4-DB upon peanut yield and quality. Prairie View Experiment Station, Texas.[a]

Rate (kg/ha)	Application Days After Planting	Yield (kg/ha)	Quality Components Percentage[b]			
			SMK	OK	TK	Hulls
Check	None	2616a	71.0a	4.5b	76.3a	23.7b
0.45	30	2533a	70.0a	4.6b	76.5a	23.5b
0.45	55	2607a	70.2a	3.0c	75.0a	25.0b
0.45+0.45	30+55	2576a	69.8a	3.8bc	75.3a	24.7b
0.90	30	2445a	69.8a	4.8b	75.6a	24.4b
0.90	55	2638a	64.4b	5.7a	72.1b	27.9a

[a]Values within columns followed by the same letter do not differ at the 1% significance level as determined by Duncan's multiple range test.

[b]SMK=sound mature kernels, OK=other kernels, and TK=total kernals.

Table 3. Effect of 2,4-DB upon peanut yield and pod size. Prairie View Experiment Station, Texas.[a]

Rate (kg/ha)	Application days after planting	Yield (kg/ha)	Pod Volume (cc)	Pod Density (g/cc)
0.45	21	1818a	145.9e	0.60ab
0.90	21	2042a	153.8cde	0.61a
0.45	28	1964a	149.7de	0.60ab
0.90	28	2028a	151.4de	0.59ab
0.45	33	1978a	49.1de	0.60ab
0.90	33	1873a	149.0de	0.58ab
0.45	36	1923a	156.0cd	0.59ab
0.90	36	1936a	167.5b	0.57bc
0.45	40	1932a	154.1cd	0.61a
0.90	40	1873a	155.6cd	0.59ab
0.45	44	1914a	155.5cd	0.57bc
0.90	44	1579a	174.3ab	0.53c
0.45	48	1850a	167.5b	0.57bc
0.90	48	1850a	178.8a	0.53c
0.45	54	1864a	159.4c	0.59ab
0.90	54	1877a	169.4b	0.56c
0.45	62	2079a	155.0cd	0.60ab
0.90	62	1873a	150.9de	0.59ab
Check	None	2019a	150.0de	0.60ab

[a]Values within columns followed by the same letter do not differ at the 1% significance level as determined by Duncan's multiple range test.

significantly lower percentages of sound mature kernels (SMK) and total kernels (TK) and higher percentages of other kernels (OK) were produced in plots receiving 0.90 kg/ha of 2,4-DB at the post bloom state 55 days after planting (Table 2). A significantly higher percentage of hull and decreased value per ton also resulted from this treatment.

Peanuts treated with 0.45 and 0.90 kg/ha of 2,4-DB

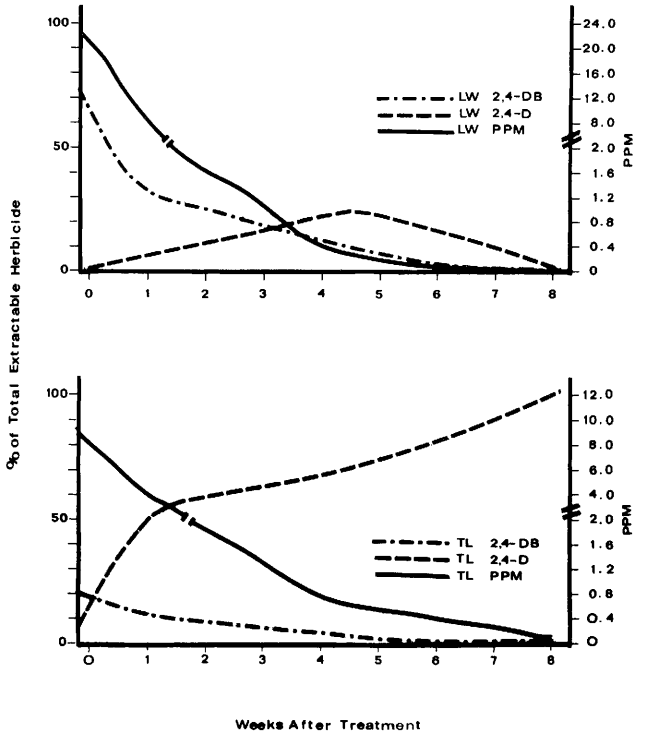

Fig. 1. The occurrence of 2,4-D in leaf wash (LW) and treated leaf (TL) samples of pigweed at weekly intervals 0-8 weeks after application of 0.56 kg/ha of 2,4-DB. PPM data include 2,4-D plus 2,4-DB.

at 40 to 54 days from planting exhibited rolled leaves, especially at the higher rates. This effect occurred from one to two days after application and persisted from three to five days after which most of the leaf roll disappeared. There was also a 24 to 48 hr loss of turgidity in treated plants. Enlarged pods occurred in plots treated with 0.90 kg/ha of 2,4-DB at 40, 44, 48 and 54 days from planting. The greatest reduction in yield (395.4 kg/ha) resulted from the 0.9 kg treatment at 44 days from planting. The lowest grades were obtained from plots treated with 0.9 kg rate applied at 44 and 48 days (Table 3).

The stage of development at which the peanut appears to be most susceptible to 2,4-DB was between the period of maximum pegging and early pod development. The activity of 2,4-DB was increased by high relative humidity following application of the herbicide. Due to the potential yield reduction, and the undersirable change in pod size and density, we believe that each application of 2,4-DB to peanuts should not exceed the 0.45 kg/ha rate, especially during stages from pegging to early pod development under conitions of high relative humidity.

In comparison, pigweed was severely twisted within 16 hours after treatment with 0.56 kg/ha of 2,4-DB. Although no 2,4-D was present in the treating solution, 2,4-D was detected in treated leaf samples within four hours after treatment (Figure 1). Growth of pigweed essentially ceased within two days. Those plants not dead two weeks after

treatment showed a marked swelling along the stem which, when examined in cross section, appeared to be caused by the uncontrolled initiation of lateral roots from the xylem tube tissue to the stem. These plants also became excessively turgid and brittle at ground level and were easily broken off when touched. Plants treated after flower initiation produced a much reduced inflorescence and therefore fewer seed than an untreated plant.

There was a rapid drop in total herbicide during the first week partially due to abscission of many of the larger treated leaves. As the total amount of herbicide decreased, the relative amount of 2,4-D in treated leaf samples steadily increased. By the end of the eighth week all detectable herbicide was 2,4-D. Similarly, all herbicide detected in regrowth samples proved to be 2,4-D. Residues in these samples ranged from 0.04 to 0.18 ppm.

Greenhouse Experiments

Age of peanut seedlings at time of spraying did not significantly affect penetration of 2,4-DB into the treated leaf or metabolism to 2,4-D in peanut seedlings. There were no significant differences in weight or height due to the herbicide treatment. There were no typical auxin type responses of the peanuts to the herbicide. Analysis indicated that less than 10% of the herbicide available on the plant surface entered into the treated leaf.

Increasing rate of application and adding surfactant did not lead to the production of a detectable amount of 2,4-D in the peanut tissue (Table 4). No significant differences between check and treated plants were found for height and weight. Necrotic spots appeared on the foliage of peanuts treated with 2,4-DB plus surfactant, but surfactant alone did not cause these damaged spots. Concurrently, the leaf wash data on these samples (Table 4) showed a highly significant loss in herbicide indicating that these necrotic areas tended to speed up dissipation of the 2,4-DB from the plant surface and increased penetration of 2,4-DB into surrounding living tissues. No 2,4-D was detected in any peanut sample and no hormone-like effects were noted.

In contrast, analysis of pigweed seedlings treated with 1.12 kg/ha of 2,4-DB indicated that 2,4-DB was readily absorbed through the leaf surface and subsequently degraded to 2,4-D in the plant tissue (Table 4). Plants were twisted within hours of treatment, particularly in the apical region and all of the treated pigweeds were dead within two weeks. Auxin symptoms noted prior to death included severe stem twisting, leaf epinasty and stem swelling.

Peanut seedlings treated and held at 100% relative humidity for 24 hours absorbed and translocated small quantities of 2,4-DB. However, about 80% of the 2,4-DB still remained on the leaf surface, while

Table 4. Absorption of 2,4-DB as affected by application rates or surfactant and its conversion to 2,4-D in peanut and pigweed plants grown in the greenhouse.[a]

Plant Species	Rate 2,4-DB (kg/ha)	Surfactant X-77 (%)	0 Hour[b] PPM 2,4-DB LW	0 Hour[b] PPM 2,4-DB TL	2 Weeks PPM 2,4-DB LW	2 Weeks PPM 2,4-DB TL	2 Weeks PPM 2,4-D LW	2 Weeks PPM 2,4-D TL
Peanut	1.12	0	45.5b	0.9c	19.6d	0.4c	0 a	0 a
	1.12	1	40.7b	2.6c	1.0d	1.0b	0 a	0 a
	1.12	5	33.0b	22.9a	1.6d	2.8a	0 a	0 a
	2.24	0	93.9a	0.6c	32.2a	1.2b	0 a	0 a
	0	0	0 c	0 c	0 d	0 c	0 a	0 a
Pigweed	1.12	0	57.7b	8.3b	9.8c	3.0a	0.6a	4.7b
	0	0	0 c	0 c	0 d	0 c	0 a	0 a

[a]Values within columns followed by the same letter do not differ at the 1% significance level as determined by Duncan's multiple range test. LW=Leaf wash and TL=Treated leaf.

[b]There was no detectible 2,4-D in 0 hour samples.

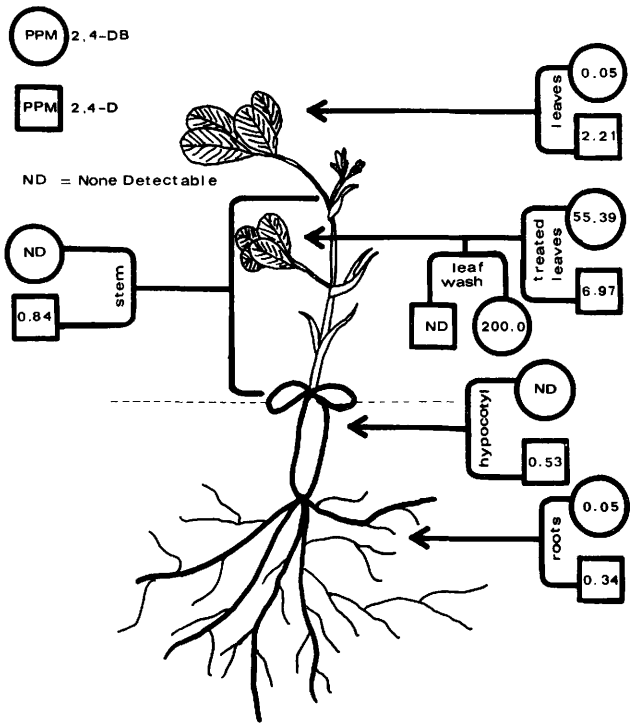

Fig. 2. The distribution of 2,4-DB and it's metabolite 2,4-D in peanut seedlings 24 hours after application to the primary leaf followed by incubation in a 100% relative humidity chamber.

the 2,4-D, apparently formed via beta-oxidation within the treated leaf, proved to be the more mobile product (Figure 2). Visual effects were limited to a few slightly curled leaf petioles.

Treatments of peanuts and pigweed support the theory that 2,4-DB is both non-toxic and immobile but may be converted to 2,4-D through beta-oxidation and cause the herbicidal effects noted. Low absorption appears to be an important part of the mechanism of selectivity in peanuts. These studies indicate that environmental conditions, such as high relative humidity, may increase the possibility of peanut injury due to increased absorbtion of the herbicide.

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