Review of the Metabolism and Decomposition of Diquat and Paraquat

H. H. FUNDERBURK, JR., AND G. A. BOZARTH

The absorption, translocation, and distribution patterns for diquat and paraquat are similar. In most cases these herbicides will move in an acropetal direction, but basipetal movement will occur only under certain conditions. Diquat and paraquat are relatively stable compounds in higher plants but both are degraded by soil microorganisms. Degradation of both compounds occurs rather rapidly in the presence of ultraviolet light, and a proposed pathway for the degradation of paraquat is included. Persistence studies indicated that paraquat is more persistent than diquat.

Diquat [6,7-dihydrodipyrido(1,2-*a*:2′,1′-*c*) pyrazidinium salt] and paraquat (1,1′-dimethyl-4,4′-bipyridinium salt) are herbicides that have been used successfully in a number of situations, but information on the fate of these herbicides in the environment in which they are used is limited (Allen, 1960; Amling, 1963; Bailey and White, 1964; Brian, 1964; Calderbank, 1960, 1964; Calderbank *et al.*, 1961; Coats *et al.*, 1966; Colby, 1963; Cronshey, 1961; Funderburk and Lawrence, 1963a, 1963b, 1964; Gunn and Tatham, 1961; Harris and Warren, 1964; van Oorschot, 1964; Zweig *et al.*, 1963). This is especially true in an aquatic environment (Beasley, 1966; Blackburn and Weldon, 1963; Funderburk and Lawrence, 1963b, 1964; Lawrence *et al.*, 1963; Yeo, 1964; van der Zweep, 1964). The main objective of this paper is to summarize the information available on the fate of diquat and paraquat in the environment.

The herbicidal properties of diquat and paraquat were discovered by Brian *et al.* (1956). Some quaternary ammonium compounds have been used as redox indicators under the name of viologens since 1933 (Michaelis and Hill, 1933). Michaelis and Hill (1933) showed that the first step in this reduction involved the addition of one electron to the quaternary salt to form a stable, water-soluble, intensely colored free radical. Both diquat and paraquat respond in a similar manner (Mees, 1960). Homer and Tomlinson (1959, 1960) and Homer *et al.* (1960) studied the redox properties, stereochemistry, and the mode of action of several dipyridyl quaternary salts and found that phytotoxicity and reduction were related. Therefore, they hypothesized that herbicidal activity depends on the ability of the active compounds to form free radicals by the uptake of one electron. These workers found that diquat and paraquat had redox potentials of −349 and −446 mv., respectively.

According to Commoner and Pake (1954), free radicals are most likely to arise in biological systems from oxidation and from irradiation. These workers also reported that the concentration of free radicals produced by radiation increased with increasing doses. Commoner and Pake (1954) further reported that high unpaired-electron content exists in many biological systems. In fact, free radicals are normally formed during photosynthesis (Calvin, 1959a, 1959b).

Mees (1960) reported that light increased the kill obtained with diquat, without being essential for herbicidal action. The light effect was observed only in green tissues. Mees also reported that the herbicide first stimulated and then inhibited respiration.

Absorption and Translocation

To study the metabolism of a herbicide by a living plant, it is usually customary to determine absorption, translocation, and distribution of the chemical (Yamaguchi and Crafts, 1958).

Absorption and translocation data for the dipyridyl herbicides are variable. Funderburk and Lawrence (1963b) reported that there was little movement of the dipyridyls in either the acropetal or basipetal direction in the submersed plant water star grass [*Heteranthera dubia* (Jacq.) Macm.]. These authors (Funderburk and Lawrence, 1964) further showed that, when dipyridyl were applied to nutrient solutions in which alligator weed [*Alternanthera philoxeroides* (Mart.) Griseb.] and bean (*Phaseolus vulgaris*) were growing, there was absorption by the root system and subsequent translocation via the xylem to the shoots.

Apparently, there is less movement when dipyridyls are applied to foliage (Funderburk and Lawrence, 1964). This probably is due to the desiccating effect of these materials on green tissue and subsequent disruption of routes of transport. Baldwin (1963) has shown that considerable foliar-applied diquat can be transported from the point of application if the treatment is followed by a 24-hour dark period.

Metabolism by Higher Plants. Funderburk and Lawrence (1964) fed C^{14}-labeled diquat and paraquat to alligator weed and C^{14}-labeled paraquat to bean via nutrient solutions. After various periods of time up to 3 weeks, shoots of these plants were homogenized. The homogenates were centrifuged several times to clean up the extract for thin-layer and paper chromatography. In all cases there was only one radioactive spot on the autoradiographs of chromatograms and it corresponded to authentic diquat or paraquat. Results of partition chromatography of the extracts with various organic solvents, as well as CO_2 studies, also indicated that diquat and paraquat were not metabolized by alligator weed and bean.

Botany and Plant Pathology Department, Auburn University Agricultural Experiment Station, Auburn, Ala.

Metabolism by Soil Microorganisms. Bozarth *et al.* (1965, 1966) and Bozarth (1966) determined the effect of 0, 10, 100, 500, 1000, and 10,000 p.p.m. of paraquat on the microflora of a sandy loam soil (Cahaba loamy fine sand). In this investigation, the effect of paraquat on the total number of fungi was determined 4, 8, and 21 days following treatment. This study indicated that there was little effect on the number of fungi except possibly at the highest rate after a 21-day incubation period (Table I).

In a similar experiment, the effect of paraquat on the total number of bacteria and actinomycetes was determined (Table II). At the 4-day incubation period there was very little difference in numbers among all treatments receiving paraquat. After 8 days all treatments of paraquat showed a large increase in numbers of bacteria and actinomycetes, but after 21 days this increase in numbers was apparent only at the 500-, 1000-, and 10,000-p.p.m. treatments.

Paraquat-tolerant microorganisms were isolated from Cahaba loamy fine sand that had been enriched with paraquat, serially diluted, and plated on media selected for the particular groups of microorganisms. From these plates a fungus and a bacterium, which grew rather well on high concentrations of paraquat, were selected for additional studies.

The fungus, *Neocosmospora vasinfecta*, was capable of reducing paraquat to the colored free radical. This indicated that paraquat accepted electrons from some metabolic process that took place in the fungus. Electrochromatography of the medium containing C^{14}-labeled paraquat in which the fungus was cultured indicated that there was no degradation of paraquat.

The bacterial isolate (not identified) also was capable of reducing paraquat to the colored free radical. Extracts from the liquid medium containing 1000 p.p.m. of C^{14}-labeled paraquat in which the bacterial isolate was grown were subjected to electrochromatography after 2, 4, 8, and 16 days. Figure 1 is a picture of autoradiographs of the thin-layer electrophoresis plates that were spotted with filtrates of the bacterium. There was no apparent degradation after 2 days, but after 4 days there were two additional radioactive spots. A large amount of radioactive material usually remained at the origin, which probably was paraquat adsorbed to particles that did not move in this particular system. The 8- and 16-day autoradiographs were similar to the one for the 4-day incubation period. Of the two main spots on the plates, other than paraquat, one moved approximately two thirds the distance of paraquat toward the negative pole and the other spot moved a short distance toward the positive pole, indicating that one of the degradation products had a negative charge. One of the metabolites moved in a manner similar to 1-methyl-4,4′-dipyridinium ion, which has one less positive charge than paraquat (Figure 2).

Figure 3 shows a comparison between a chromatogram from the bacterial filtrate and a chromatogram spotted with methyl- and ring-labeled paraquat. Here again, movement of one of the degradation products was similar to that of 1-methyl-4,4′-dipyridinium ion. The other degradation product moved in a manner similar to

Table I. Effect of Paraquat on the Number of Fungi after Various Incubation Periods

Treatment, P.P.M.	Time, Days		
	4	8	21
	Per Cent		
0	100	100	100
10	91.7[a]	101.6	110.3
100	107.8	94.1	94.5
500	97.2	99.4	110.3
1,000	94.4	111.8	110.3
10,000	94.4	85.2	79.3

[a] Data are expressed as per cent of check.

Table II. Effect of Paraquat on the Number of Bacteria and Actinomycetes after Various Incubation Periods

Treatment, P.P.M.	Time, Days		
	4	8	21
	Per Cent		
0	100	100	100
10	96.9[a]	141.4	103.5
100	100.0	135.7	103.5
500	102.1	145.7	132.8
1,000	99.0	150.0	139.7
10,000	107.3	151.4	151.7

[a] Data are expressed as per cent of check.

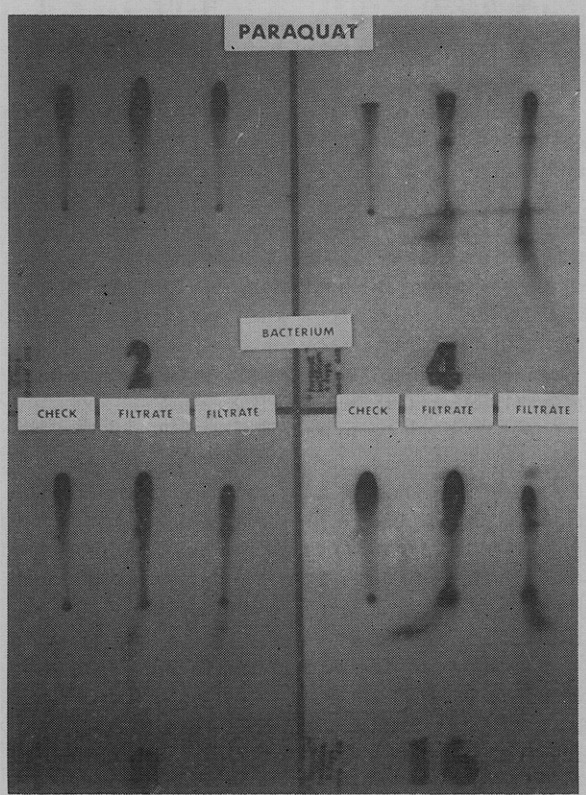

Figure 1. Autoradiographs of thin-layer electrophoresis plates

Spotted with filtrates from cultures of bacterial isolate that contained radioactive paraquat, after 2, 4, 8, and 16 days' incubation. Check = C^{14}-methyl-labeled paraquat; filtrate = filtrate from bacterial cultures; filtrate + = a combination of check and filtrate (top = cathode; bottom = anode)

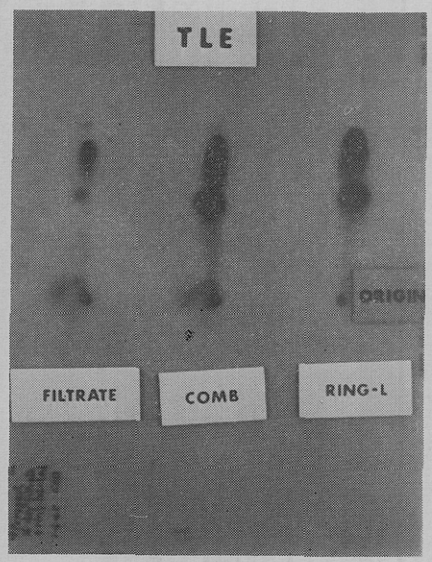

Figure 2. Autoradiograph of a thin-layer electrophoresis plate

Spotted with the following: Filtrate = filtrate from the 16-day-old bacterial culture containing radioactive paraquat; ring-L = ring-labeled paraquat containing 1-methyl-4,4′-dipyridinium ion as a contaminant; comb = combination of the two

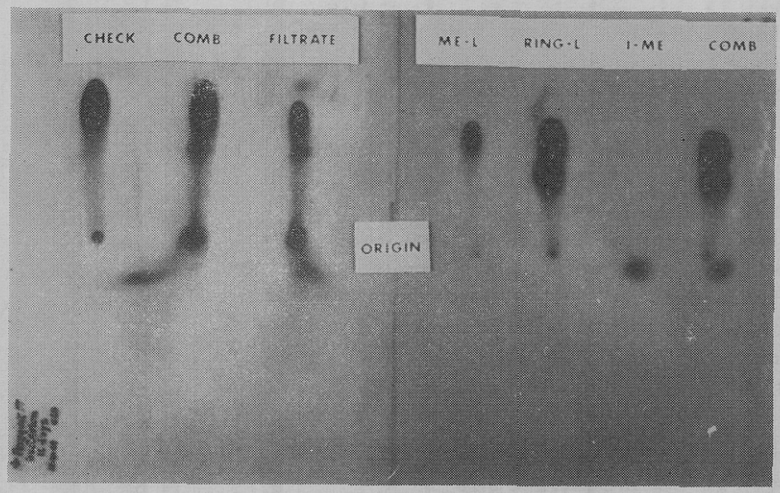

Figure 3. Autoradiographs of thin-layer electrophoresis plates

(Top = cathode; bottom = anode) spotted with the following: Left: check = filtrate from uninoculated culture medium containing methyl-labeled paraquat; comb = combination of check and filtrate; filtrate = filtrate from 16-day-old bacterial culture containing methyl-labeled paraquat. *Right:* Me-L = methyl-labeled paraquat; ring-L = ring-labeled paraquat containing 1-methyl-4,4′-dipyridinium ion; 1-ME = 1-methyl-4-carboxypyridinium ion; comb = combination of the three

that of 1-methyl-4-carboxypyridinium ion. Both of these compounds, the degradation product and the 1-methyl-4-carboxypyridinium ion, had a similar type erratic movement on the thin-layer plates. The erratic movement of 1-methyl-4-carboxypyridinium ion can possibly be explained on the basis of its existing as a zwitterion. The pH of the solvent used in these investigations was approximately 4.5, which was probably near the isoelectric point for this compound. Thus, any small change in pH would influence movement of 1-methyl-4-carboxypyridinium ion, toward either the anode or cathode.

These studies suggest that a possible pathway of degradation by the bacterial isolate consists of first demethylating the parent molecule, followed by ring cleavage of one of the heterocyclic rings to form eventually the carboxylated 1-methylpyridinium ion (Figure 4). Other intermediate compounds probably are formed during the degradation of paraquat, but none of these has been reported at the present time. The fate of the radioactive methyl group, which apparently is cleaved from paraquat, has not been accounted for.

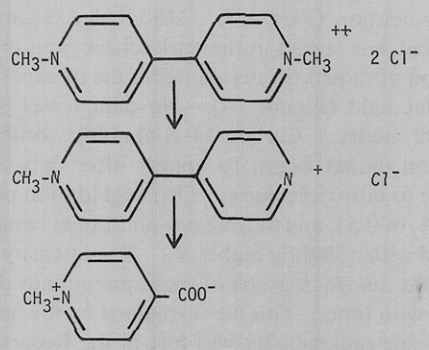

Figure 4. Proposed pathway of paraquat degradation by a bacterial isolate

Fate of Diquat and Paraquat in an Aquatic Environment. Beasley *et al.* (1965) reported that the dipyridyls are very persistent in the bottom mud of pools and ponds. In pools to which diquat and paraquat were applied at the rate of 0.3 pound per surface acre in 1962, 1.2 to 7.9 p.p.m. of paraquat and a trace to 1.7 p.p.m. of diquat were found in 1966. The data from this study and many others led Beasley (1966) to conclude that paraquat was somewhat more persistent than diquat in the hydrosoils.

Beasley (1966) also treated fish in several ways with C^{14}-labeled diquat and subsequently sacrificed the fish to determine the location and form of the radioactive material. He found that radioactivity in most organs increased with time, which suggested that the herbicide (or labeled atom) was distributed throughout the fish by the blood; this might be anticipated because of the complete water solubility of the herbicide. In all phases of the investigations, maximum radioactivity was detected in the digestive tracts, and autoradiographs of electrochromatograms of plasma from treated fish revealed that only one radioactive compound was present. The relative movement of this compound was the same as for authentic diquat. From these studies, one must conclude that diquat was relatively stable in fish.

Beasley also analyzed tissues and organs of channel catfish collected from pools which received single applications of unlabeled 1-p.p.m. diquat or paraquat, two applications of 1-p.p.m. diquat, or paraquat applied at 3-month intervals. No diquat or paraquat residue was detected in organs or tissues of fish collected 5 months after a single application or 2 months after the second treatment. Fish from pools treated or retreated with diquat contained 0 to a trace of diquat in the contents of

the digestive tract, and fish from pools treated or retreated with paraquat contained in excess of 2 and 6.9 p.p.m. of paraquat, respectively, in the digestive tract contents.

Photodecomposition of Diquat and Paraquat. This problem has been studied by Slade (1965, 1966) and by Funderburk *et al.* (1966a, 1966b). Slade showed that ultraviolet light from a mercury vapor lamp as well as sunlight was capable of degrading paraquat. He identified two degradation products as 1-methyl-4-carboxypyridinium ion and methylamine hydrochloride, and proposed the degradation pathway shown in Figure 5. In addition to paraquat, Funderburk *et al.* (1966a) studied the photochemical degradation of diquat. The data in Figure 6 show that diquat is degraded rapidly; the same is true for paraquat (Funderburk *et al.*, 1966a). Approximately 50% of the C^{14} from both compounds was lost after 48 hours and more than 75% after 96 hours. This phase of the investigation measured the effect of ultraviolet light on the dipyridyls in the dry form, and apparently both chemicals were degraded to volatile compounds.

When the effect of ultraviolet light on the dipyridyls was studied in aqueous solutions, there was no loss of radioactivity from the solutions, but there was considerable degradation (Figure 7). No change occurred in diquat that was stored in the dark (check) and the concentration of diquat decreased with time of exposure to ultraviolet light (Figure 7*A*). No diquat was present after 192 hours. Carbon-14–containing compounds other than diquat began to appear after only 8-hour exposure to ultraviolet light. One degradation product had an R_f of 0.53, and at least one additional compound appeared with a slightly higher R_f. The intensity of the spot on the autoradiograph of these compounds did not increase with time. This was explained by the fact that considerable radioactivity was lost in the freeze-drying process, and less radioactivity was spotted on the chromatogram for increasing times of exposure to ultraviolet light (Fundberburk *et al.*, 1966a).

The degradation products from paraquat are shown in Figure 7*B*. In this solvent system, the R_f for paraquat

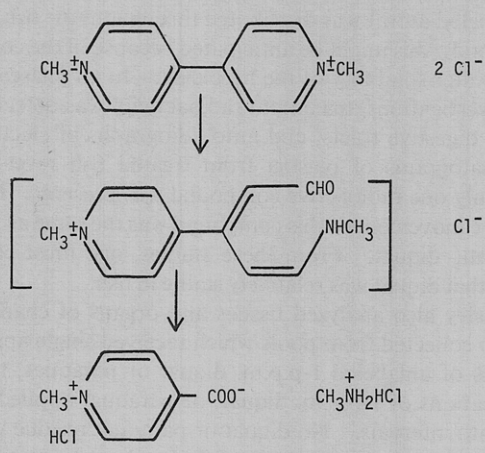

Figure 5. Proposed pathway of paraquat degradation by ultraviolet light

Initial publication (Slade, 1965)

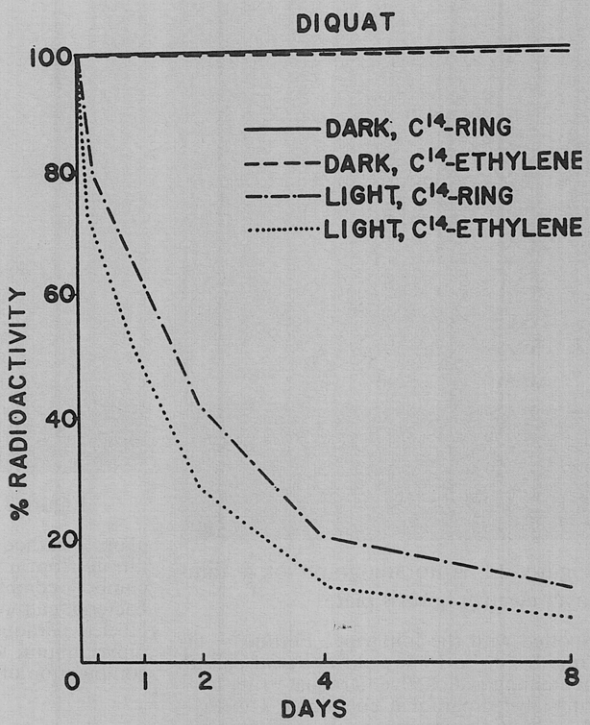

Figure 6. Percentage radioactivity remaining in aluminum planchets

Contains C^{14}-ring– and C^{14}-ethylene–labeled diquat after various periods of exposure to ultraviolet light. Initial publication (Funderburk *et al.*, 1966)

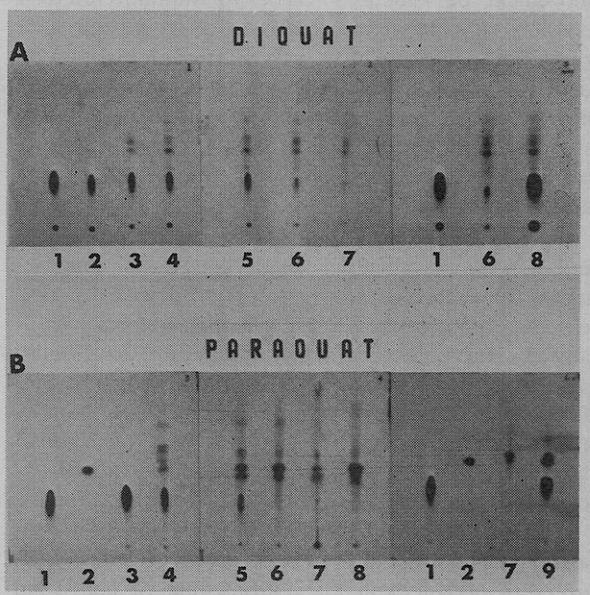

Figure 7. Autoradiographs of thin-layer chromatograms

Spotted with aliquots from solutions containing radioactive diquat or paraquat that had been exposed to ultraviolet light. *A*, 1 = diquat; 2 = check; 3, 4, 5, 6, 7 = diquat solutions exposed to ultraviolet light for 8, 24, 48, 96, and 192 hours, respectively; and 8 = cochromatography of 1 and 6. *B*, 1 = paraquat; 2 = 1-methyl-4-carboxypyridinium chloride; 3 = check; 4, 5, 6, 7, 8 = paraquat solutions exposed to ultraviolet light for 8, 24, 48, 96, and 192 hours, respectively; and 9 = cochromatography of 1, 2, and 7. Initial publication (Funderburk *et al.*, 1966)

Table III. Mean Dry Weight of Wheat Grown in Diquat- and Paraquat-Treated Kaolonite and Bentonite[a]

Adsorbent	Treatment, Mg./G.	Mean Dry Weight	
		Diquat	Paraquat
Kaolonite	0.0	92.4	110.0
	1.	56.7[b]	58.6[c]
	3.5	0[c]	5.8[c]
Bentonite	0.0	122.7	130.8
	50.0	125.3	110.7
	110.0	0[c]	0[c]

[a] From Coats *et al.* (1964), by courtesy of the publishers, Blackwell Scientific Publications, Oxford, England.
[b] Significantly different from the 0-mg. treatment of that adsorbent at the 5% level.
[c] Significantly different at the 1% level as determined by Dunnett's *t*-test.

was 0.34 and the R_f for 1-methyl-4-carboxypyridinium chloride was 0.44. The concentration of paraquat decreased with increased exposure to ultraviolet light, and very little paraquat was present after 48 hours. The first degradation products appeared after 24 hours and the concentration of these compounds increased slightly with time of exposure to ultraviolet light. One of the degradation products had an R_f of 0.44 and cochromatographed with 1-methyl-4-carboxypyridinium chloride. There were at least two additional degradation products with slightly higher R_f values (Funderburk *et al.*, 1966a).

Availability of Diquat and Paraquat to a Biological System. Several workers (Coats *et al.*, 1964, 1965, 1966; Taylor and Amling, 1963) have shown that the dipyridyls are bound tightly to various soil fractions. Taylor and Amling (1963) and Taylor (1964) showed by x-ray analysis that paraquat was adsorbed in the interlayer spaces of bentonite clay. This has been confirmed recently by Weber and Scott (1966). Coats *et al.* (1966), as well as Weber and Scott, reported that the dipyridyls were available to higher plants when adsorbed onto kaolinite. When adsorbed onto bentonite, they were unavailable even when applied at exceedingly high rates (Table III). If this phenomenon holds true for other biological systems—e.g., fungi and bacteria—these molecules might persist for a relatively long period of time when they are adsorbed onto bentonite. This area must receive much additional study if we are to understand the fate of the dipyridyls in soil fully.

Literature Cited

Allen, H. P., *Proc. Brit. Weed Control Conf., 5th Brighton* **4,** 625–9 (1960).
Amling, H. J., Turner, J. L., Taylor, T. D., *Proc. Southern Weed Conf.* **16,** 164 (1963).
Bailey, G. W., White, J. L., J. AGR. FOOD CHEM. **12,** 324–32 (1964).
Baldwin, B. C., *Nature* **198,** 872–3 (1963).
Beasley, P. G., Ph.D. dissertation, Auburn University, Auburn, Ala., 1966.
Beasley, P. G., Lawrence, J. M., Funderburk, H. H., Jr., *Proc. Southern Weed Conf.* **18,** 581 (1965).
Blackburn, R. D., Weldon, L. W., *Proc. Southern Weed Conf.* **16,** 365 (1963).
Bozarth, G. A., M.S. thesis, Auburn University, Auburn, Ala. 1966.
Bozarth, G. A., Funderburk, H. H., Jr., Curl, E. A., *Weed Soc. Am. Abstracts* **55** (1966).
Bozarth, G. A., Funderburk, H. H., Jr., Curl, E. A., Davis, D. E., *Proc. Southern Weed Conf.* **18,** 615 (1965).
Brian, R. C., *Weed Res.* **4,** 105–17 (1964).
Brian, R. C., Homer, R. F., Stubbs, J., Jones, R. L., *Nature* **181,** 446–7 (1956).
Calderbank, A., *Agr. Vet. Chem.* **1,** 197 (1960).
Calderbank, A., *Proc. Brit. Weed Control Conf., 7th Brighton* **1,** 312–20 (1964).
Calderbank, A., Morgan, C. B., Yuen, S. H., *Analyst* **86,** 569–79 (1961).
Calvin, M., *Rev. Mod. Phys.* **31,** 147–56 (1959a).
Calvin, M., *Rev. Mod. Phys.* **31,** 157–61 (1959b).
Coats, G. E., Funderburk, H. H., Jr., Lawrence, J. M., Davis, D. E., D. E., *Proc. Southern Weed Conf.* **17,** 308–20 (1964).
Coats, G. E., Funderburk, H. H., Jr., Lawrence, J. M., Davis, D. E., *Proc. Southern Weed Conf.* **18,** 614 (1965).
Coats, G. E., Funderburk, H. H., Jr., Lawrence, J. M., Davis, D. E., *Weed Res.* **6,** 58–66 (1966).
Colby, S. R., Warren, G. F., *Science* **141,** 362 (1963).
Commoner, B. J. T., Pake, G. E., *Nature* **174,** 689 (1954).
Cronshey, J. F. H., *Weed Res.* **1,** 68–77 (1961).
Funderburk, H. H., Jr., Lawrence, J. M., *Nature* **199,** 1011–12 (1963a).
Funderburk, H. H., Jr., Lawrence, J. M., *Weed Res.* **3,** 304–11 (1963b).
Funderburk, H. H., Jr., Lawrence, J. M., *Weeds* **12,** 259–64 (1964).
Funderburk, H. H., Jr., Negi, N. S., Lawrence, J. M., *Weeds* **14,** 240–3 (1966a).
Funderburk, H. H., Jr., Negi, N. S., Lawrence, J. M., *Weed Soc. Am. Abstracts* **41** (1966b).
Gunn, J. S., Tatham, P. B., *Nature* **189,** 808–9 (1961).
Harris, C. I., Warren, G. F., *Weeds* **12,** 120–6 (1964).
Homer, R. F., Mees, G. C., Tomlinson, T. E., *J. Sci. Food Agr.* **6,** 309–15 (1960).
Homer, R. F., Tomlinson, T. E., *J. Am. Chem. Soc.* **504,** 2498–503 (1960).
Homer, R. F., Tomlinson, T. E., *Nature* **184,** 2012–13 (1959).
Lawrence, J. M., Beasley, P. G., Funderburk, H. H., Jr., *Proc. Southern Weed Conf.* **16,** 366–7 (1963).
Mees, G. C., *Ann. Appl. Biol.* **48,** 601–12 (1960).
Michaelis, L., Hill, E. S., *J. Gen. Physiol.* **16,** 859 (1933).
Slade, P., *Nature* **207,** 515–16 (1965).
Slade, P., *Weed Res.* **6,** 158–67 (1966).
Taylor, T. D., M.S. thesis, Auburn University, Auburn, Ala., 1964.
Taylor, T. D., Amling, J. H., *Proc. Southern Weed Conf.*, **16,** 405 (1963).
van der Zweep, W., *Weed Soc. Am. Abstracts* **1964,** pp. 107–8.
van Oorschot, J. L. P., *Proc. Brit. Weed Control Conf., 7th Brighton* **1,** 321–4 (1964).
Weber, J. B., Scott, D. C., *Science* **152,** 1400–02 (1966).
Yamaguchi, S., Crafts, A. S., *Hilgardia* **28,** 161–91 (1958).
Yeo, R. R., *Weed Soc. Am. Abstracts* **107** (1964).
Zweig, G., Tamas, I., Greenberg, E., *Biochim. Biophys. Acta* **66,** 196–205 (1963).

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