

Administration of Diazinon was discontinued on August 7, 1961. There was no apparent excretion of the insecticide in milk even at the extremely high level of intake. Diazinon is apparently altered in some manner in the digestive system of the dairy cow since the insecticide was recovered from the butterfat separated from milk to which it had been added.

A maximum single nontoxic dose of

10 mg. per kg. has been reported by Radeleff (2) for adult cattle. The two cows at the highest level received 8.2 and 10.0 mg. per kg. daily for a period of 7 days with no apparent symptoms of toxicity.

It appears that forage treated with Diazinon at reasonable rates might be ensiled and fed to dairy cows without concern for residue contamination of the milk or toxic effects on the cow.

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SOIL ADSORPTION OF HERBICIDES

The Adsorption of Monuron and Diuron by Hawaiian Sugarcane Soils

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Chemical analysis of monuron and diuron in aqueous solutions shaken with Hawaiian sugarcane soils has been used to study the equilibrium adsorption and desorption of these chemicals. All soils show greater adsorptive capacity and bond strength for diuron than for monuron; both chemicals exhibit normal isothermal adsorption equilibria. Binding energy, measured as the resistance to desorption, was found to be directly related to adsorptive capacity.

TWO PRE-EMERGENCE HERBICIDES, 3-(*p*-chlorophenyl)-1, 1-dimethylurea (monuron) and 3-(3,4-dichlorophenyl)-1, 1-dimethylurea (diuron), have been used extensively in sugarcane in Hawaii since 1951 and 1953, respectively. Total soil residues from rates of 4 to 5 pounds of active chemical per acre decreased to negligible quantities in about 12 months. Soil residues from normal use are essentially zero during the entire second year of the 2-year crop cycle, and no residual buildup has occurred. Effective control of annual weeds may vary from zero to 90 days, with the average around 40 to 50 days.

Considerable variation in weed control and crop injury, not associated with weed species or climatic factors, has occurred over the approximately 75,000 acres treated each year. Analysis of cane tissues (total plant above ground) and soil samples for monuron or diuron has consistently shown that cane injury could be closely related to plant residue levels in young cane, but that the same injury had little or no apparent correlation with total soil residue from a 0- to 6-inch soil profile sample. Thus, a uniform application rate was producing variable results which appeared to be associated with soil factors.

This article describes experiments designed to measure the adsorptive capacity of a variety of Hawaiian sugarcane soils for monuron and diuron. A series of experiments is also described to support the conclusion that the ad-

sorptive bond strength of soils for monuron and diuron is directly related to the adsorptive capacity. Additional work with other soil active herbicides is in progress.

Hawaiian soils, except for small coral areas, are derived from volcanic basalts or andesites. Although the parent material was fairly uniform, wide variations in climate and vegetation, and in the age of successive lava flows, account for the major differences. Sugarcane is grown on four islands at elevations ranging from near sea level to about 2500 feet, in areas of rainfall from 10 to 150 inches per year, and at average air temperatures of from 78° F. at sea level to about 72° F. in the higher regions with little summer-winter variation. In rainfall areas of less than 60 inches per year, cane is irrigated. Soil types (great soil groups) on which sugarcane is grown include mainly Low Humic Latosols, Humic Latosols, Hydrol Humic Latosols, Humic Ferruginous Latosols, Alluvial, Gray Hydromorphic, and Dark Magnesium Clays with smaller amounts of numerous others. In general, soils are acidic (pH 4 to 8), high in iron, titanium, and aluminum oxides, and low in silica. Clays are principally kaolinite or oxides in the Latosols and montmorillonite in the darker gray soils. Weathering has reduced the soils in most areas mainly to clays (6). Carbonaceous residues vary from 0 to 15% with no peat or muck soils.

Experimental Procedure

Adsorption of Monuron and Diuron on Soils. A 50-gram sample of air-dry soil, ground to pass a 2-mm. mesh screen, was mixed with 100 ml. of an aqueous solution containing 6.25 mg. of monuron and 2.5 grams of K₂SO₄ per liter and shaken for 15 minutes at an ambient temperature of 26° ± 2° C. The liquid was decanted through Whatman No. 12 filter paper, and the clear filtrates were analyzed to determine the amount of unadsorbed chemicals by the colorimetric micromethod of Young and Gortner (8).

The procedure used for most of the determinations utilized 12.5 µg. of monuron per gram of soil. Other measurements, especially for the Freundlich plots, were made at levels from 3.9 to 312 µg. of monuron per gram of soil. Values given are averages of two or more determinations; blank determinations were run to correct for chemical residue in the soil.

Diuron adsorption was determined similarly on 50 grams of soil with 100 ml. of an aqueous solution containing 5.76 mg. of diuron and 2.5 grams of K₂SO₄ per liter.

Phosphate determinations for comparison of adsorptivity and fixation were made on 50 grams of soil with 100 ml. of an aqueous solution containing 500 mg. of P from KH₂PO₄ and 2.5 grams of K₂SO₄ per liter.

The K₂SO₄ prevented serious de-

flocculation of the soils, permitted rapid, clear filtration, and did not affect the results. Length of shaking time, up to 4 hours, did not significantly change the amount of chemical adsorbed.

Desorption of Monuron and Diuron from Soils. Equilibrium adsorption of monuron and diuron was established using 50 grams of air-dry soil and 100 ml. of aqueous solution containing 6.24 p.p.m. monuron or 5.76 p.p.m. diuron as described above. After 15 minutes shaking, an additional portion of dis-

tilled water containing 2.5 grams of K_2SO_4 per liter was added, and the diluted mixture was shaken again for 15 minutes; the liquid was filtered as before and the filtrates were analyzed. Diluent levels from 100 ml. to 900 ml. in 100-ml. increments were added to give solution ratios of from 50 grams per 200 ml. to 50 grams per 1000 ml.

Direct determination of water-soluble monuron was made by shaking 10 grams of air-dry field soils containing variable amounts of residual monuron with 100

ml. of K_2SO_4 solution; filtration and analysis of the monuron was carried out as before. The equilibrated, desorbed, water-soluble fractions were plotted (as per cent of total residue present in the soil) against monuron adsorption indices (as per cent adsorbed) for the same soils determined separately.

Discussion and Conclusions

This investigation consisted in part of determining the physical adsorption, by

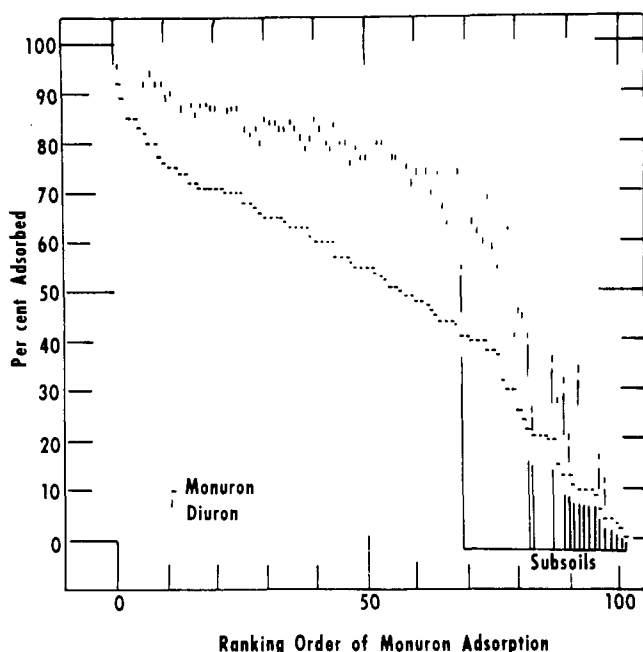


Figure 1. Adsorption of monuron and diuron on Hawaiian sugarcane soils

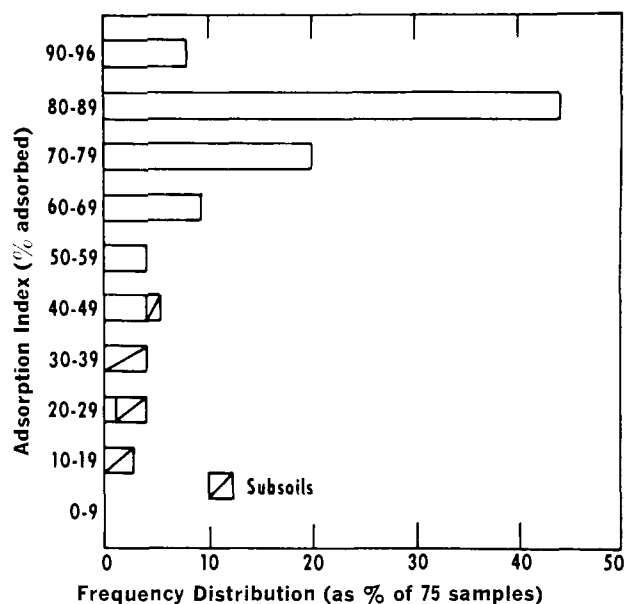


Figure 3. Frequency distribution—diuron

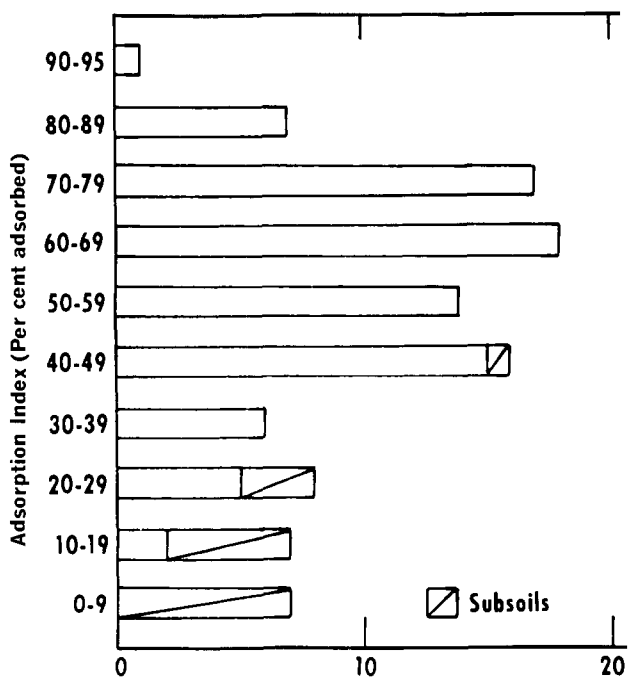


Figure 2. Frequency distribution—monuron

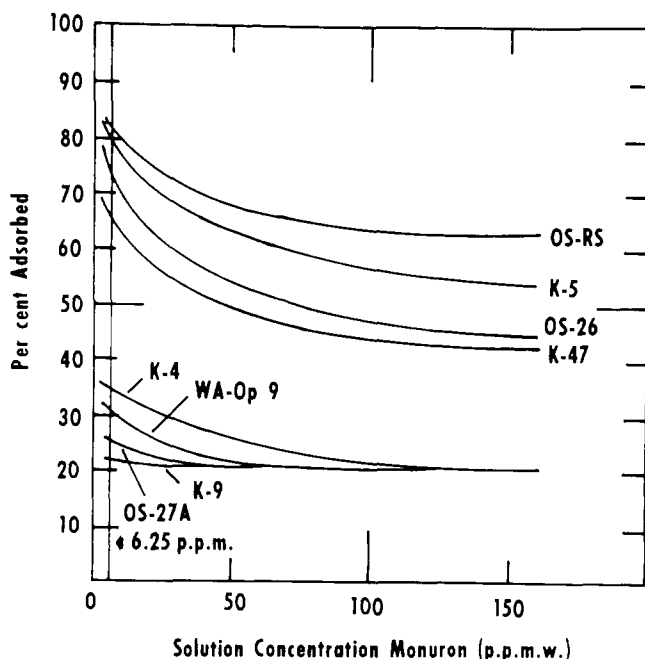


Figure 4. Effect of concentration of monuron on soil adsorption at constant soil weight and solution volume

the method described, of monuron on 101 soil samples and of diuron on 75 of the same samples representing a variety of soil types. Figure 1 presents data obtained with soils ranked in descending order of monuron adsorption from a high value of 92% absorbed to a low of 0%. Adsorption of diuron was always greater numerically than monuron on the same soils, showing a range of from 96 to 12%. Comparison of equilibrium magnitudes for the two chemicals ranked the soils in roughly the same relative order. Figures 2 and 3 present the data as frequency distributions plotted against 10-unit adsorption intervals.

Soil adsorption, for this investigation, has been expressed as per cent monuron or diuron adsorbed on a given weight of soil (50 grams) from an aqueous solution (100 ml.) of a standardized concentration of the respective herbicide. The variability of this per cent adsorption, or adsorption index, with herbicide concentration for a fixed soil-solution ratio is presented in Figure 4 for monuron and in Figure 5 for diuron. Figure 6 indicates the effect on the adsorption index of a variable amount of soil mixed with a constant volume of solution. To the extent shown in these figures, selection of a herbicide concentration and soil-solution ratio was arbitrary, and individual soil measurements are relative and depend on maintaining the same experimental conditions. However, this source of uncertainty can be eliminated by the log x log plots at several concentrations described below.

Although no attempt was made to select soil samples in proportion to the relative abundance of the soil types planted to sugarcane, this has been accomplished to some extent by random

collection of samples from experimental herbicide test areas as well as by making a consistent collection of one soil from each major soil family in each group. The most extensive great soil groups have the greatest number of subgroupings and in consequence the largest number of samplings were taken from these groups. Where subsoils were definitely included, they were designated in Figures 1, 2, and 3 since their adsorption capacity was always low in comparison with surface soils. An abnormally low adsorption for a soil type may have been indicative of admixed top and subsoil. Abnormally high levels of chemicals were fixed in soils which have been mixed with carbon particles from sugar mill stacks. One such soil (a Humic Ferruginous Latosol) adsorbed 74 and 87% monuron and diuron, respectively, in the mill carbon area as compared with 30 and 41% in the same soil without carbon fallout. The carbon is in the form of small flakes due to the use of the by-product cane fiber as the principal source of fuel for the mills.

Within certain limitations, and when adequate sampling was made, soil ad-

sorptive patterns in the topsoils were found to be related to soil type as shown in Table I.

Samplings in the same soil family on different islands showed a fair degree of correspondence. The Kahana family of Low Humic Latosols (Table I) most nearly resembles continental U. S. soils high in silt (6).

Sherburne and Freed (4) have measured the adsorptive capacity of 12 Oregon soils. The results were expressed as micrograms of monuron per gram of soil on a ratio of 50 grams of soil per 125 ml. of solution containing 200 p.p.m. of monuron. Showing data on eight Hawaiian soils nearly in correspondence with their experimental conditions, Table II illustrates relative levels involved.

Typical Hawaiian soils exhibit adsorption properties for monuron more nearly corresponding to the Palouse silt loam (47%) and the muck soil (69%) which Sherburne and Freed consider atypical of Oregon soils. The astonishing fact is that 25% of the Hawaiian soils studied adsorbed more than 69% of the applied monuron under the experimental con-

Table I. Adsorption of Monuron as Related to Soil Types

Type	Range of Adsorption	Monuron Adsorption (%)	
		Topsoil	Subsoil
Dark magnesium clay	High	55-83	...
Gray Hydromorphic	High	55-89	...
Humic Latosol	Intermed.-high	65-72	...
Hydrol Humic Latosol	Intermed.-high	57-71	20-40
Humic Ferruginous Latosol	Intermediate	30-53	10-13
Low Humic Latosols			
Molokai family	Variable-high	40-92	...
Kahana family	Low	30-50	2-11
Hailimaile silty clay	Low	20-21	0-4

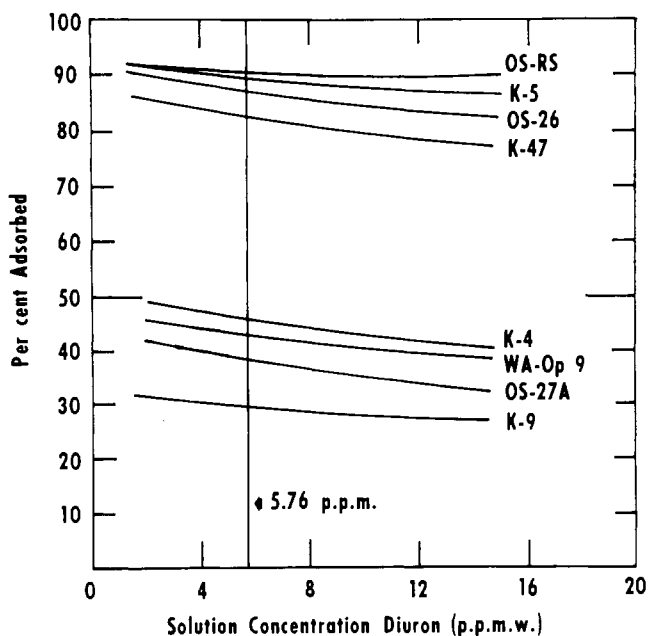


Figure 5. Effect of diuron concentration on soil adsorption at constant soil weight and solution volume

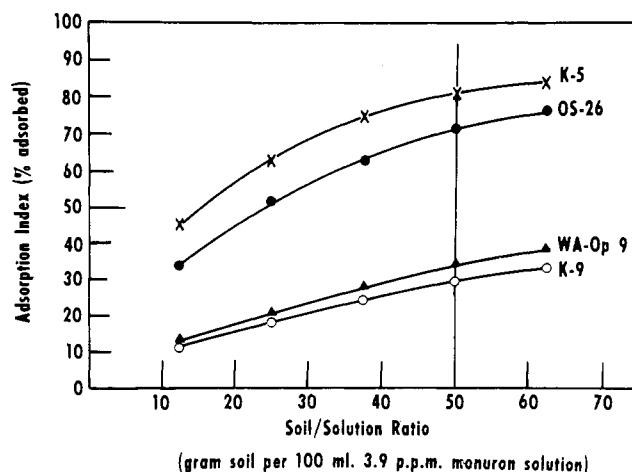


Figure 6. Effect of soil weight to solution volume ratio on soil adsorption at constant monuron concentration

ditions, and yet organic matter content was rarely if ever over 15%. All but seven soils (all subsoils) of the 101 in this study adsorbed over 9% of the applied monuron.

Diuron adsorption was greater than 60% for all but 15 of 75 soils, and of those 15, nine were known to be subsoils.

Preparatory work for the study established that soil pH, presence or absence of soluble sulfate or phosphate anions, soluble monovalent cation (K^+), and inorganic acids and bases had little or no effect on the observed results. Soil adsorption could be increased by adding carbons and could be decreased by surface active agents, implying molecular adsorptive forces of the Van der Waals type.

Other investigators (2) have used the empirical isothermal Freundlich relationship: $x/m = KC^n$. A plot of the chemical concentration fixed on the soil (x/m) against the equilibrium concentration in the soil solution (C) over a sufficient range of concentrations plotted on log x log paper resulted in a series of straight line plots for different soils. Extrapolation of the lines to a unit equilibrium solution concentration (i.e., $C = 1$) gave a corresponding x/m value equal to K , which was designated as the amount of herbicide adsorbed on the soil in equilibrium with 1 p.p.m. in the soil solution. The slope of the lines (n) could be calculated from the data or by inspection of the plot. Figures 7 and 8 show such plots based on six observations at different concentrations of eight Hawaiian soils, four of high and four of

relatively low adsorption. The data are summarized in Table III.

The lines were nearly parallel with slopes averaging about 0.8 to 0.9. A commercial wood charcoal and an activated carbon (Darco G-60, Atlas Powder Co.) gave, with monuron, parallel lines of slope 0.34 and K values (at $C = 1$) of 312 and 5.15×10^4 , respectively (Figure 9). Since the slopes of the log x log plots vary greatly for herbicide chemicals other than those reported here, as well as for the carbons, the K values alone are insufficient to characterize adsorption behavior of a herbicide on a group of soils; the K value and the line slope (n) for a given

chemical would, however, be sufficient to give a characteristic plot adequate for most practical concentration ranges. The merit of the isothermal adsorption method is that the results are not influenced by soil-herbicide-solution ratios.

Investigation of the phosphate fixation of 52 soil samples showed no correlation whatever with monuron adsorption. Hawaiian soils show rather high levels of phosphate fixation: 17 samples gave 90 to 99% equilibrium fixation (from KH_2PO_4), 13 samples 80 to 89%. Both influence (7) and the lack of influence (7) of soil phosphorous on diuron toxicity to plants have been noted by Upchurch in different investigations. More re-

Table II. Comparison of Monuron Adsorption on Some Oregon and Hawaiian Soils

	Sherburne and Freed (4)	Present Work
Soil-solution ratio	50 grams/125 ml.	50 grams/100 ml.
Monuron in solution	200 p.p.m.	156 p.p.m.
Monuron, $\mu\text{g./gram soil}$	500	312
Monuron absorption data	$\mu\text{g./G. (\%)}$	$\mu\text{g./G. (\%)}$
Oregon sand soil-sandy loams	21-24 (4-5)	
Oregon silt and clay loams	21-44 (4-9)	
Oregon Aiken clay loam	90 (18)	
Oregon Palouse silt loam	237 (47)	
Oregon muck soil	3453 ^a (69)	
Hawaiian mixed soil		200 (64)
Hawaiian Gray Hydromorphic, K-5		169 (54)
Hawaiian Alluvials, OS-26, K-47		133, 140 (43, 45)
Hawaiian Regosol, K-4		64 (20)
Hawaiian Low Humic Latosols, WA-Op 9, K-9, OS-27A		64-68 (21-22)

^a 83.3% organic; monuron at 5000 $\mu\text{g./gram}$, 5 grams of soil/125 ml.

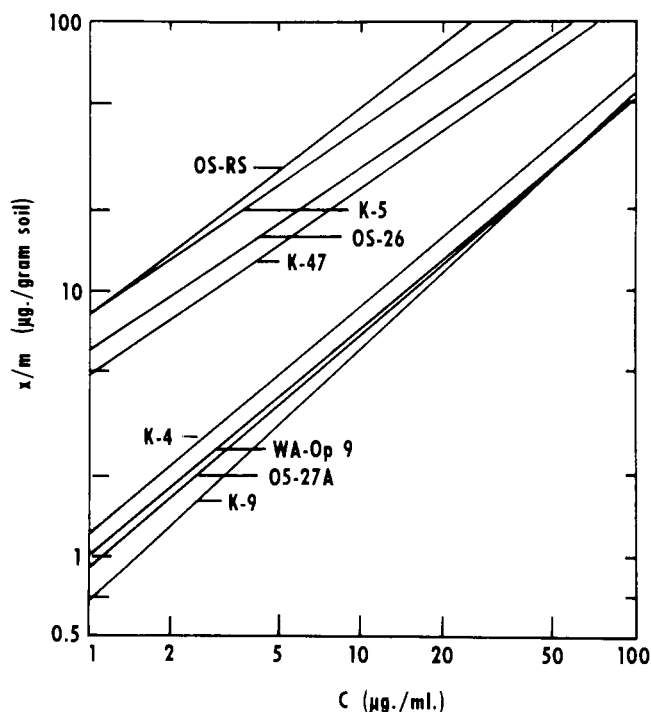


Figure 7. Isothermal equilibrium adsorption plots of monuron on eight Hawaiian soils

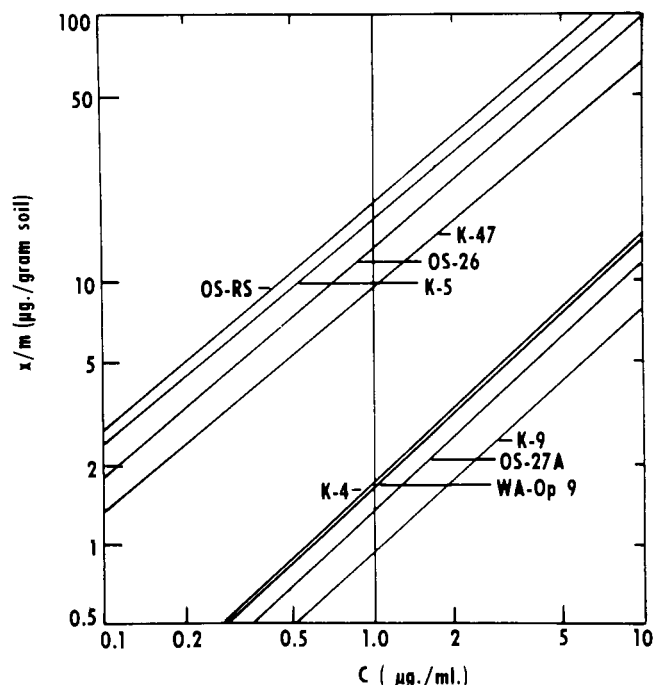


Figure 8. Isothermal equilibrium adsorption plots of diuron on eight Hawaiian soils

cently (3), inhibition by monuron and diuron of catalytic cofactors in photosynthetic phosphorylation under aerobic conditions has been shown to be independent of the presence or absence of inorganic phosphate. Fixation of phosphate in all subsoils was very high (80 to 99%) and the available P was generally low. Monuron and diuron injury to sugarcane grown on soils low in herbicide adsorption capacity has often been associated with nutrient deficiencies.

Adsorption of *p*-chloroaniline, the hydrolytic degradation product from monuron, showed about the same relative order and degree as monuron in a group of eight soils.

Desorption of monuron or diuron from a field soil to which the chemical has been applied was studied as an aid in determining the phytotoxic level of the soil solution in contrast to total soil residue. As a corollary, a method was sought to determine how much adsorbed chemical residue could be leached from soil under given conditions and how strong the adsorptive bondings of different soils were. Soil residues of monuron may be appreciable eight to 12 months after application, yet effective weed control may be maintained only as

long as the soil solution in contact with weed seedlings exceeds and maintains the required toxicity level. This period of time is of vital importance, not only from economics of use, but from the appearance, maximum level, and disappearance of residues in sugarcane. The analysis of Hawaiian soils for residual total monuron is completely unrelated to the phytotoxicity of those soils either for weeds or for sugarcane. For this reason, a method was needed to determine soil solution concentrations that might be expected from a given application, and data were needed on the desorptive tendency of the chemical when water was added to soil as a measure of soil solution availability.

Equilibrium adsorption of monuron in solution placed in contact with soil depends on the ratio of soil to solution (Figure 6) and on the concentration of monuron in the solution (Figure 4). Equilibrium desorptions of monuron and diuron were measured in two ways. First, the chemicals were adsorbed in the usual way at 50 grams of soil per 100 ml. of solution, utilizing 12.5 μg . of monuron per gram of soil or 11.5 μg . of diuron per gram of soil. After equilibration, additional K_2SO_4 solution was added in

increments from 100 to 900 ml. and the diluted mixtures were again shaken, filtered, and analyzed for soluble chemical. Results are given in Figure 10 for a single soil of mixed origin, adsorption index 75 by the standard method. A maximum of 46% of the adsorbed fraction of monuron (35% of the diuron) could be released from this relatively high adsorptive soil by ten-fold dilution with water. However, 5.05 μg . of monuron and 6.43 μg . of diuron per gram of soil remained fixed.

The Freundlich equation sorption and desorption plot (Figure 11) at numerous concentration levels of chemical with this soil and the foregoing discussion indicate that the adsorptive process may not be a simple equilibrium but a combination of two or more phenomena which may be indicative of different types of binding or the availability or energy of the binding sites.

A second desorptive method consisted of examining field soil samples to which monuron had been applied. A desorptive soil-solution ratio of 10 grams per 100 ml. of K_2SO_4 solution was selected and was held constant for all soils. The equilibrium solution concentration (calculated as per cent of the total monuron residue) was plotted against the adsorptive indices of the soils for monuron. The scatter diagram of 43 soils (Figure 12) clearly showed that ease of removal of adsorbed monuron was directly related to soil adsorptivity.

Sund (5) has reported on the adsorptive capacity of seven continental U. S. soils for another herbicide, 3-amino-1, 2, 4-triazole (amitrole). His zero days after treatment corresponds well with the desorptive method in this study inasmuch as he adsorbed the chemicals at 20% of the soil moisture-holding capacity, then diluted the samples with further water for analysis. If his muck soil is

Table III. Freundlich Isothermal Adsorption Constants at Unit Soil Solution Concentration for Eight Hawaiian Soils

Soil ^a and Type	Adsorption Index (%)		K Values		n	
	Monuron	Diuron	Monuron	Diuron	Monuron	Diuron
OSRS, Mixed	75	90	8.0	19.5	0.80	0.85
K-5, Gray Hydromorphic	76	89	8.0	17	0.70	0.85
OS-26, Alluvial	71	87	6.0	13.5	0.70	0.85
K-47, Alluvial	61	81	4.8	9.6	0.70	0.84
K-4, Regosol	26	46	1.2	1.7	0.86	0.94
WA-Op 9, Low Humic Latosol	24	45	1.0	1.65	0.80	0.94
OS-27A, Low Humic Latosol	22	41	0.9	1.35	0.88	0.94
K-9, Low Humic Latosol	21	26	0.7	0.94	0.94	0.92

^a Symbols identify source of individual soils.

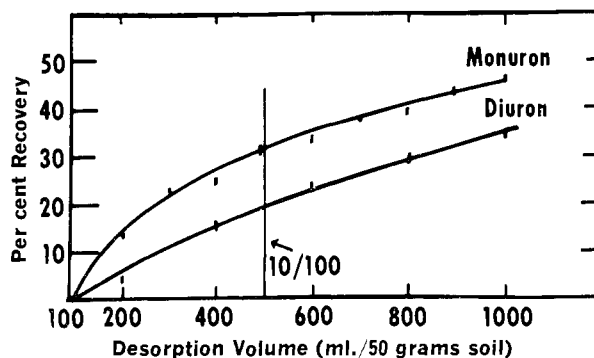
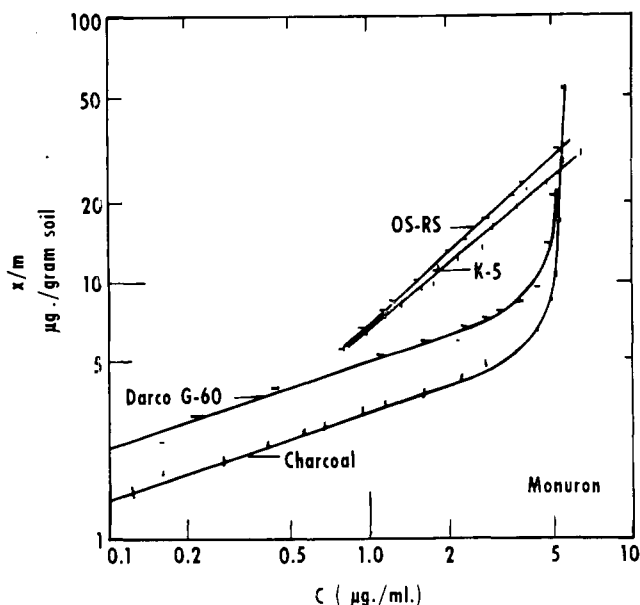


Figure 10. Equilibrium desorption of monuron and diuron (Soil: OS-RS at 75% monuron and 90% diuron)

Figure 9. Isothermal adsorption plots of various carbons and soils

(Ordinates: Darco G-60 $x/m \times 10^4$, Charcoal $x/m \times 10^2$, Soils x/m)

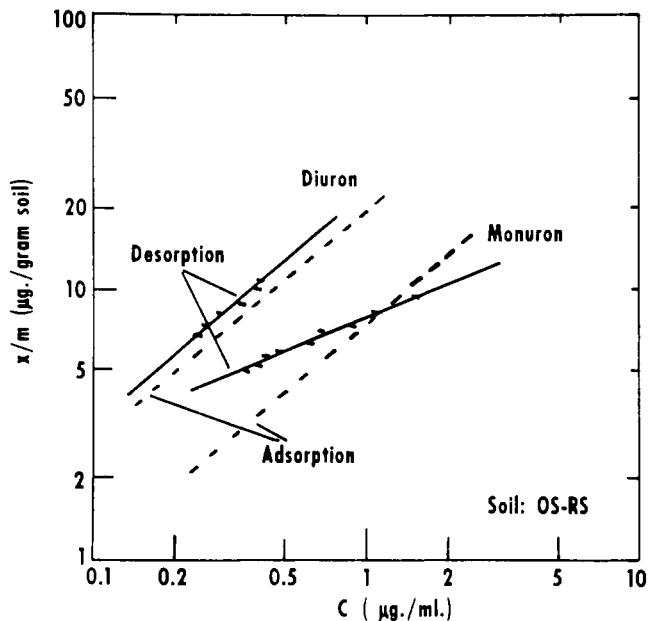


Figure 11. Isothermal equilibrium adsorption and desorption of monuron and diuron on a single soil

deleted from consideration, only a Raub loam (Indiana) showed characteristics in the range of Hawaiian soils: 67% of the amitrole remained fixed under his conditions. Sund's five other soils showed: Duke's sand (Long Island), 7%; Maury loam (Indiana), 21%; Yolo sandy loam (California), 11%; Cecil sandy loam (North Carolina), 37%; and Croton silty loam (New Jersey), 26%. Note was made of the correlation of his figures with base exchange and the tendency of amitrole to form metal complexes with di- and trivalent cations.

From the data presented, field performance of monuron and diuron is viewed as a dynamic complex involving four principal factors: the intrinsic phytotoxicity of the chemical, the soil adsorptive characteristics, microcrystalline regions of undissolved monuron or diuron, and the supply of water. These chemicals applied as wettable powder suspensions to soil act as slowly dissolving reservoir supplies whose rate of solution will depend on the chemical and on the availability of water. Upon solution, a dynamic equilibrium between soil and chemical in soil solution will be established wherein various concentration factors depend on soil adsorptivity, water-soil ratio, and concentration of chemical in solution. Solution of the herbicide will proceed as long as water is continuously available or until the soil solution in contact with the solid reservoir source becomes saturated. When water is removed as evaporation occurs, the soil solution will become saturated, and the herbicide may precipitate as redistributed crystallites to form secondary reservoir sources.

Further wetting of the soils will again

result in an equilibrium (desorption plus additional solution from the undissolved reservoir), the status of which will depend again on the soil adsorptivity and the various concentration and soil-solution ratios. Vertical movement of the herbicide will result from: low soil adsorptivity and large amounts of water under conditions of good drainage. Under most conditions in this study, vertical distribution of the herbicide does not occur to any extent. Leaching has occurred in exposed subsoils and probably in small areas of porous, well drained lava cinder, the latter being a mechanical washing of the herbicide particles.

Weed control, in such a scheme, will occur as long as: The level of herbicide in the soil solution is sufficient to exceed and maintain the minimum intrinsic phytotoxicity level for the chemical and weed population in question; a reservoir supply of undissolved herbicide and (periodic) water is maintained to supply the soil-solution deficit brought about by adsorption and depletion. This depletion occurs by plant root absorption (weed and crop), photo- and hydrolytic decomposition, evaporation, leaching, and microbial decomposition.

The value of such work as this lies in part in predicting field results. For example, experience indicates that as a first approximation, the micrograms of residual chemical per gram of soil may be taken as the amount in pounds per acre contained in an acre half-foot of

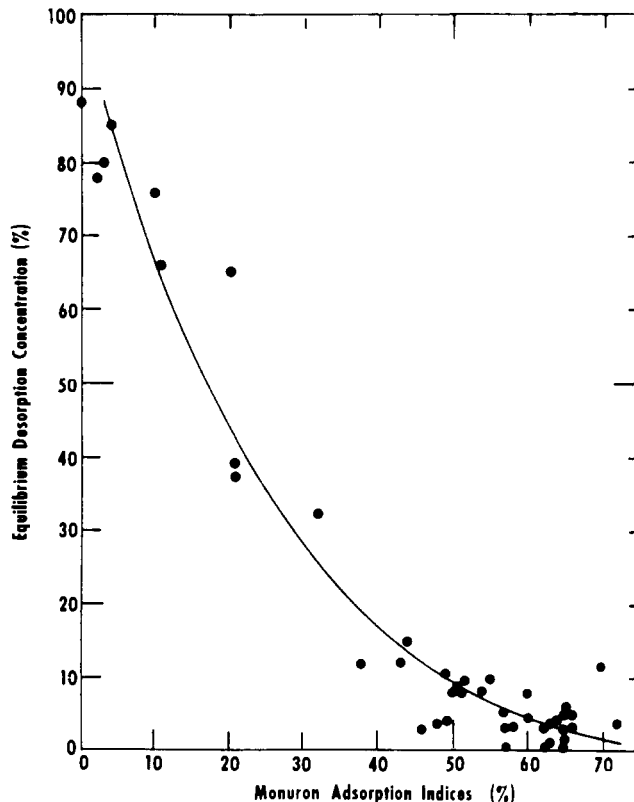


Figure 12. Relationship of adsorption to desorption for 43 Hawaiian soils

soil if the application has been uniform. Assuming arbitrarily that a soil solution concentration of 2 ($C = 2$) p.p.m. monuron is desirable under the experimental equilibrium conditions, a highly adsorptive soil, such as K-5 (Figure 7), will fix 13 µg. per gram to maintain this equilibrium concentration in solution. This then requires 15 p.p.m. total residue in the soil ($x/m = 13$ where $C = 2$ for this soil) or, as stated above, about 15 pounds of active monuron per acre to secure and maintain adequate control of weeds in this soil. The intrinsic phytotoxicities of monuron and diuron are both such that less than 2 p.p.m. is needed in the soil solution for most small weeds; also, the soil-solution ratios are rarely those of the equilibrium method. Nevertheless, this 15-pound amount is consistent with experience for maintaining weed control in this soil. A low adsorptive soil such as OS-27A ($x/m = 1.6$ at $C = 2$) would be expected to show adequate weed control at 2 to 3 pounds of monuron, again consistent with experience.

Since the predictive success of the above method depends on the intrinsic phytotoxicity of the chemical in question on a variety of weed species, efforts are being made to obtain such figures by solution culture bioassay. In the field, diuron and monuron result in about an equal time length of weed control at comparable rates, but diuron has shown less tendency toward leaching to cane root depths.

In a low adsorptive soil, with an index tentatively below 30% in this scheme, weed control without crop injury can best be accomplished by adjusting application rates of monuron or diuron downward consistent with the adsorption levels and ease of desorption. In highly adsorptive soils, again tentatively over 70% ($K = 9.8$ at $C = 2$ for monuron), these chemicals are not active enough to be used. For these areas, new chemicals are needed which must have one or more of the following properties: greater intrinsic toxicity to be effective at lower concentrations, greater solubility in water but not to the point where the reservoir source life is too limited, and lower soil adsorption. These three factors are considered mutually exclusive and capable of

achievement. Other alternatives, such as lowering the adsorptive capacity of some soils by subsoil mixing or other means and increasing the solubility of the herbicides by cosolvents, are being considered.

Acknowledgment

The authors are indebted to Virgil Freed, Oregon State University, for the initial suggestion that led to the procedure adopted.

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FUNGICIDE DETERMINATION

Field Estimation of Stop Mold B Concentrations

A method for the determination of Stop Mold B concentration in wash tanks depends on its reaction with the Folin-Ciocalteu reagent. Standard and unknown solutions of Stop Mold B are reacted with the reagent and the colors formed compared. This method is simple and rapid and it is most useful in the concentration range of 0.4 to 0.8%.

STOP MOLD B (active ingredient sodium *o*-phenylphenate, 34%) is used for the control of storage rot on apples and pears in the Pacific Northwest (5, 6). The fruits are washed in a solution of the fungicide, rinsed with water, packed, and cold-stored. The maintenance of a definite concentration of 0.6% of Stop Mold B in the washing tank during the treatment is important for effective control. Lower concentrations result in poor disease control and higher concentrations are wasteful and could result in excessive residues. A titrimetric method has been used, but is too slow and complicated for packing house use (7). A method was desired that would be simple and rapid, but give at least semiquantitative results.

Unsuccessful tests for phenols included the use of Millon's reagent (3), ferric chloride (3), and 4-aminoantipyrine (4). The only successful test for Stop Mold B was with the Folin-Ciocalteu reagent, which is used for phosphatase determination in whole milk (2). With this reagent, a simple and reliable test was developed which can be performed by packing house personnel.

Materials and Methods

Preparation of Reagents. The Folin-Ciocalteu reagent is prepared as follows (7):

Add 100 grams of sodium tungstate, $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$, and 25 grams of sodium molybdate, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, to 700 ml. of water in a 1500-ml. Florence flask. Add 50 ml. of 85% phosphoric acid and 100 ml. of concentrated hydrochloric acid. Connect to a reflux condenser and reflux gently for 8 hours. Then add 150 grams of lithium sulfate, Li_2SO_4 , 50 ml. of water, and a few drops of liquid bromine. Boil without a condenser for 15 minutes to remove excess bromine. Cool, dilute to 1000 ml., and filter. The finished reagent should have no greenish tint. The reagent should be well protected against dust.

Prepare the test solution by mixing 900 ml. of methanol, 230 ml. of 0.1N hydrochloric acid, and 18.3 ml. of Folin-Ciocalteu reagent. Test this solution against known amounts of Stop Mold B as described below and if the color range is not satisfactory, adjust the amount of acid. The finished reagent is yellow. A green color indicates the presence of reduced materials, which may mean that the reagent will not act

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properly. Increasing the acid content will shift the range of the test to higher concentrations of Stop Mold B; lowering the acid content will provide a range for lower concentrations.

Preparation of Standard Solutions. Prepare concentrations of 0.4, 0.6, and 0.8% by diluting a 34% concentrate of the fungicide. Pint bottles with screw-cap lids are convenient for the storage of standards and the volumes can be measured with a syringe or graduated cylinder.

Test Procedure. Using a 5-ml. syringe carefully transfer a 5-ml. portion of each standard, starting with the weakest, to clean test tubes. Rinse the syringe with the unknown wash solution and transfer a 5-ml. portion into the fourth test tube. Then using a second syringe add exactly 3 ml. of the Stop Mold B test solution to each test tube. Shake the tubes and compare the colors. Estimate the concentration of the unknown wash solution by matching its color with one of the standards. Make the comparison against a white background, using the same light source each day.

The colors produced by the standards should be approximately as follows: 0.4% yellow, 0.6% gray, and 0.8% blue.