SOME PHYSICAL AND BIOCHEMICAL EFFECTS OF FUMIGANTS IN SOILS

by

JEROME J. SIEGEL

A THESIS

Submitted to the School of Graduate Studies of
Michigan State College of Agriculture and
Applied Science in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Soil Science

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Approved L. M. Turk

ABSTRACT Jerome J. Siegel

Some Physical and Biochemical Effects of Fumigants in Soils

The dispersion characteristics of 1-3 dichloropropene and 1-2 dibromoethane were studied using adsorption isotherms and radioactive tracer techniques. It was determined that the compeunds were sorbed by soils and that this sorption was primarily a function of the organic soil colloids. Diffusion of the compounds was greatest in a sandy soil, next greatest in a clay loam, and least in a muck. Optimum diffusion was obtained in a soil at the moisture equivalent. Diffusion in a water saturated soil was limited by the low solubility of the compounds in water, while they were strongly sorbed in an air-dry soil in a small volume around the injection point. The compounds diffused laterally and downward with little penetration into the soil mass above the injection point. Field and greenhouse experiments indicated that Dowfumes N and W-40 were also fixed in soils and under certain climatic conditions, it was possible to attribute certain plant injuries to residual effects of these fumigants in the soils.

The use of 1-3 dichloropropene and 1-2 dibromoethane as soil fumigants accomplished a partial sterilization of the soil and resulted in a reduced activity of the oxidizing organisms. This caused a lowering of the bio-electric potential of the soils. Also, nitrification was inhibited permitting the accumulation of large quantities of ammonium in the soils. Dowfumes N, W-40, and MC-2 retarded nitrification of ammonium which was added to soils with Dowfume N being most effective in this respect.

Soybeans grown in culture solution with ammonium as their source of nitrogen responded favorably to an increase in calcium in the nutrient substrate. The plants were characterized

by an abnormally high protein content and matured earlier than soybeans whose sole source of nitrogen was nitrate. The ammonium decreased the uptake of other cations by the plants. Soybeans growns on soils which had been fumigated with Dowfumes N, W-40, and MC-2 also showed favorable growth responses when the calcium content of the soils was increased. There was a decrease in the mineral content of the plants grown on the fumigated soils which was attributed to the fact that large amounts of ammonium were taken up by these plants.

The growth of soybeans and onions on muck soils which were treated with Dowfume N showed that fumigation could be of benefit at a time when available nitrogen is a limiting factor in plant growth. The plants matured earlier than those on the untreated soils, had a higher protein content, and produced higher yields.

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INTRODUCTION

Various volatile soil fumigants have been employed in recent years in an attempt to control certain pathogens which attack numerous host plants and thus decrease crop production. They have been used with varying degrees of success against organisms such as the bulb nematode, Ditylenchus dipsaci, which infests certain onion fields and, H. schactti, a sugar beet nematode. They have also been used to control weeds in the preparation of seed beds and green-house benches.

Investigations concerning the use of these fumigants have been reported which indicate that they affect plant growth in a manner which cannot be explained by pathogen or weed control. It is obvious that the successful use of any toxic material for the control of organisms will depend upon the ability of this material to disperse through the soil and reach these organisms in their micro-habitats. The subsequent growth of plants in this soil will then depend upon the ability of the soil to give up the fumigant to the atmosphere or the tolerance of the plant to the fumigant if it remains in the soil. It is also obvious that if certain species of the micro-biologic population are eliminated from the soil, the subsequent dynamic balance which is established will have its effect on plant nutrition.

The purpose of this study was to investigate certain of these factors using commercially available fumigants and

their active components.

EXPERIMENTAL SOILS AND MATERIALS

Unless otherwise stated, the following soils were used in this study:

1. Oshtemo loamy sand: pH 5.6, organic matter 0.48%.
2. Brookston clay loam: pH 6.3, organic matter 7.86%.
3. Carlisle muck: a well decomposed organic soil with a pH of 6.5 and 13.6% mineral matter upon ashing.

The soil class was determined by the Bouyoucos hydrometer method (10), the pH by a Beckman pH meter, and the organic matter content by oxidation with 30% hydrogen peroxide (37).

The fumigants used were:

1. Dowfume N: active components are isomers of 1-3 dichloropropene.
2. Dowfume W-40: ethylene dibromide in an inert solvent.
3. Dowfume MC-2: methyl bromide with 2% chloropicrin.
4. 1-3 dichloropropene.
5. 1-2 dibromoethane. (This term is synonomous with ethylene dibromide and the two are used interchangeably in this study.)

PART I

Adsorption and Diffusion of Fumigants in Soils

With the advent of a more widespread use of soil fumigants, a reconsideration of certain aspects of the general problem has become necessary. Among these aspects is the movement of the fumigant vapors through the soil mass and their retention in the soil.

It is a well known fact that both the colloidal inorganic and colloidal organic fractions of the soil are capable of sorbing polar and semi-polar compounds (11,30). Thus, the ability of a fumigant to disperse through the soil will depend upon the chemical nature of the fumigant and the composition of the soil. An experiment was devised to test the degree of adsorption of certain fumigant vapors in selected soils and the ability of the soils to retain the vapors.

The use of radioactive isotopes in tracer work has been widely practiced in recent times. This technique applied to the tracing of fumigants in soils has the advantage, through its unusual sensitivity, of being able to detect very low concentrations of material whether it exists as vapor in the soil air, dissolved in the soil water, or sorbed by the colloids. It was possible to prepare "labeled" samples of 1-3 dichloropropene and 1-2 dibromoethane and thus use a direct approach in tracing the movements of these compounds in soil.

LITERATURE SUMMARY

Most of the literature concerning the dispersion of fumigant vapors through the soil deals with the practical aspects of injection spacing for maximum control of organisms. Taylor (50) set up certain hypotheses for the most efficient distribution of fumigant vapors through the proper spacing of injections. This was based on experimental determinations of dispersion ranges for satisfactory control of specific organisms. With a biological assay method using the larvae of Popilia japanica, Fleming and Baker (18) found that vapors of carbon bisulfide did not diffuse uniformly in all directions but moved laterally and downward from the point of injection forming a cone-shaped region with an apex close to the point of injection. Higgins and Pollard (22) concluded that soil fumigated with carbon bisulfide is characterized by a high concentration of the fumigant in the injection zone and immediately below it. There is a rapid decrease in concentration as the surface is approached. Schmidt (40) in another bio-assay method using the rice weevil, Sitophilus oryza L., reported that the vapors of chloropicrin and D-D mixture (a mixture of 1-2 dichloropropane, and two isomers of 1-3 dichloropropene) move most rapidly in a soil of moderate moisture content. The movement is less rapid in dry soil and least rapid in very wet soil. Retention time of the vapors in the soil is of the same order.

Polyakov (33) reported that chlorine penetrates sand to a considerable depth. However, absorption of the chlorine increases with the increase of organic matter in the soil. Chisholm and Koblitsky (14) found that methyl bromide was strongly sorbed by peat. Clay was less effective and sand still less in sorbing the compound. They also found that dry soil sorbed more than wet. Fuhr and Bransford (20) however, found little to no sorption of methyl bromide in a sandy clay containing 11% water. They reported marked sorption of $COCl_2$, H_2S, HCN, and SO_2 on the same soil.

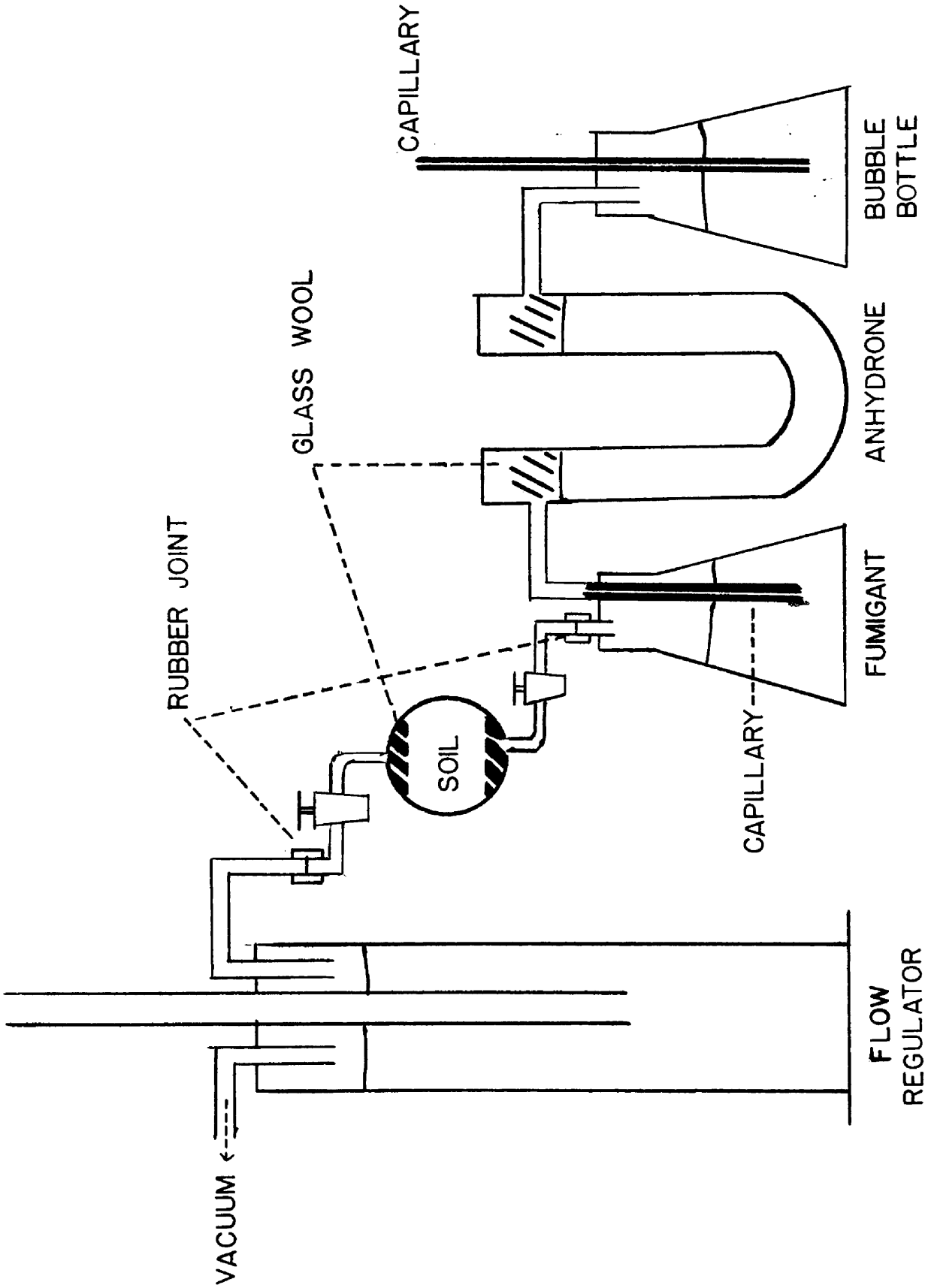
EXPERIMENTAL

Adsorption of Dichloropropene and Ethylene Dibromide on Soils.

Under constant conditions of temperature and pressure the amount of vapor adsorbed by an adsorbent will vary with the nature of the vapor and the nature of the adsorbent.

The illustration in figure 1 depicts the apparatus used in this experiment. The soils used were a Brookston clay loam, a muck, and Wyoming Bentonite (montmorillonitic clay). The soils were air-dry containing 3.2, 12.4, and 9.2 percent moisture respectively. After screening through a 0.5 mm. sieve, the soils were individually placed in the adsorption bulb. 1-3 dichloropropene and 1-2 dibromoethane vapors were then drawn through the bulb at the rate of 50 ml. of vapor per minute. (The rate of flow was determined by

FIGURE 1. APPARATUS FOR THE DETERMINATION OF ADSORPTION & DESORPTION CHARACTERISTICS



inserting a gas burette into the system.) Periodically, the adsorption bulb was removed from the system and weighed on an analytical balance. The quantity of vapor adsorbed per gram of soil (x/m) was then calculated.

The data for the adsorption of the dichloropropene and dibromoethane on the two soils and on the Bentonite are presented in table 1. The data are for a soil temperature of 25° C.

Table 1. The adsorption characteristics of 1-3 dichloropropene and 1-2 dibromoethane on Bentonite, Muck, and Brookston soil.*

Vapor** concentration	Bentonite		Muck		Brookston	
	x/m***	x/m	x/m	x/m	x/m	x/m
	Dichloro-propene	Dibromo-ethane	Dichloro-propene	Dibromo-ethane	Dichloro-propene	Dibromo-ethane
15	.0018	.0021	.0020	.0028	.0025	.0026
40	.0047	.0051	.0058	.0063	.0078	.0083
100	.0109	.0123	.0095	.0103	.0123	.0136
250	.0201	.0214	.0251	.0268	.0170	.0189
700	.0260	.0267	.0398	.0405	.0220	.0241
850	.0281	.0280	.0396	.0406	.0230	.0264
1000	.0319	.0334	.0400	.0405	.0238	.0264
1300	.0334	.0342			.0234	.0274
1900	.0367	.0399			.0235	.0278
2400	.0368	.0398				

* Average of three determinations.
** The quantity of vapor passed through the soil in ml.
*** Weight of vapor in grams adsorbed per gram of soil.

The source of fumigant was then disconnected from the system and air was passed through the soil. The desorption data are presented in table 2.

Table 2. The desorption characteristics of 1-3 dichloropropene and 1-2 dibromoethane on Bentonite, Muck, and Brookston soil.*

Air ** concentration	Bentonite		Muck		Brookston	
	x/m***	x/m	x/m	x/m	x/m	x/m
	Dichloro-propene	Dibromo-ethane	Dichloro-propene	Dibromo-ethane	Dichloro-propene	Dibromo-ethane
0	.0368	.0398	.0400	.0405	.0235	.0278
25	.0276	.0293	.0387	.0310	.0191	.0243
100	.0108	.0185	.0292	.0284	.0155	.0205
500	.0016	.0045	.0290	.0280	.0073	.0115
800	.0003	.0001	.0289	.0280	.0070	.0053
1000					.0072	.0050

* Average of three determinations.
** The quantity of air passed through the soil in ml.
*** Weight of vapor in grams adsorbed per gram of soil.

The adsorption and desorption data are presented graphically in figures 2 and 3.

Both the dichloropropene and the ethylene dibromide were adsorbed by the three soils. The maximum adsorption in both cases occurred on the muck, the next greatest on the Bentonite, and the least on the Brookston. Desorption was complete on the Bentonite. However, the muck and Brookston retained quantities of the compounds which could not be removed by the passage of air. These data are summarized in table 3.

Table 3. The percentages of 1-3 dichloropropene and 1-2 dibromoethane adsorbed and immobilized by Bentonite, Muck, and Brookston soil.

Soil	1-3 dichloropropene		1-2 dibromoethane	
	Maximum adsorption	Immobilized*	Maximum adsorption	Immobilized*
Bentonite	3.7%	0	4.0%	0
Muck	4.0%	62.2%	4.1%	60.5%
Brookston	2.4%	30.1%	2.8%	18.0%

* Percentage of fumigant adsorbed which could not be removed by aeration.

Apparently the great surface area of the Bentonite adsorbs a considerable quantity of the compounds, but probably the bonding forces are weak electrostatic or Van der Waal's forces since the energy required to remove the materials was not great. On the other hand, the muck and

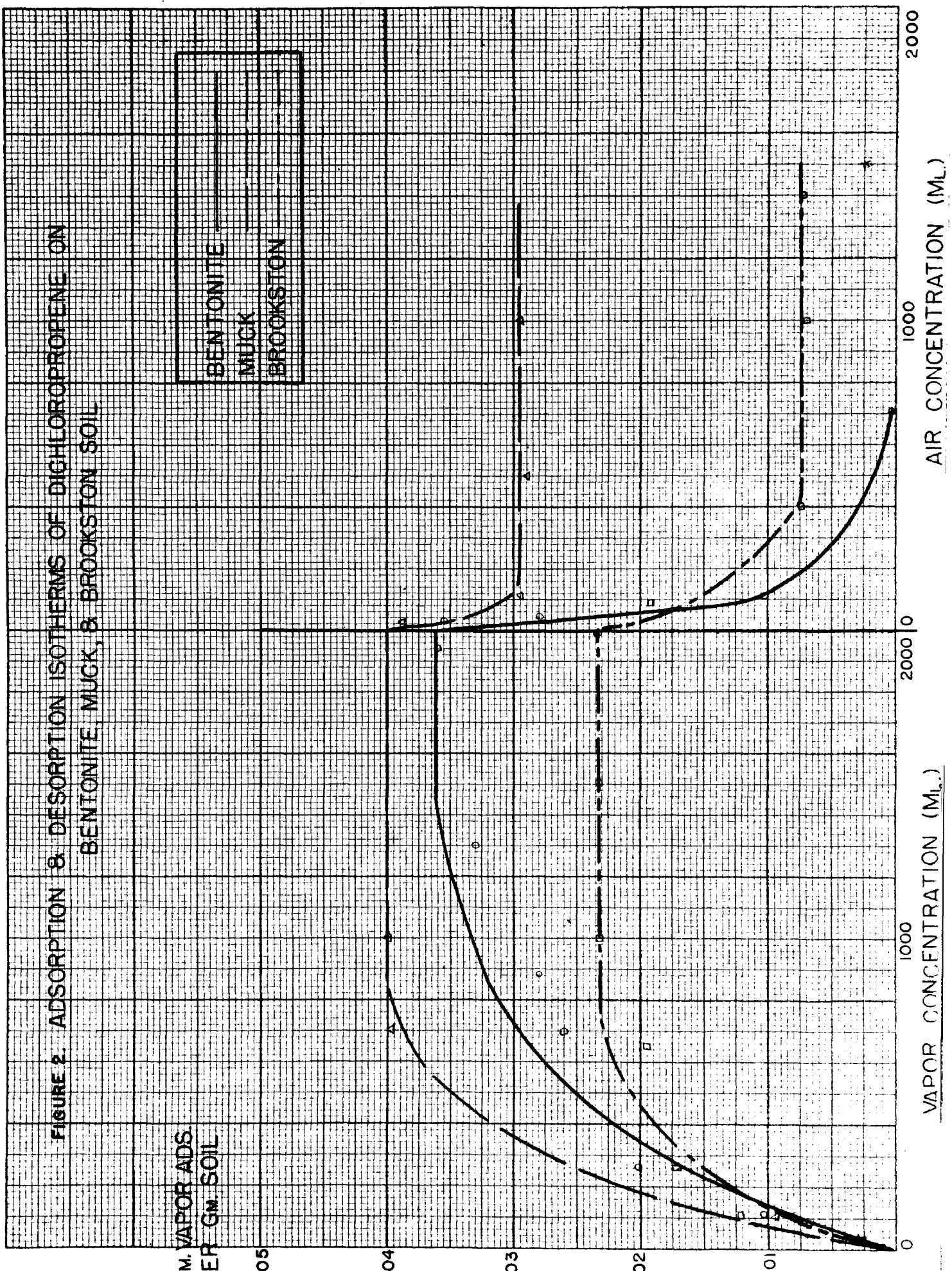


FIGURE 2. ADSORPTION & DESORPTION ISOTHERMS OF DICHLOROPROPENE ON BENTONITE, MUCK, & BROOKSTON SOIL

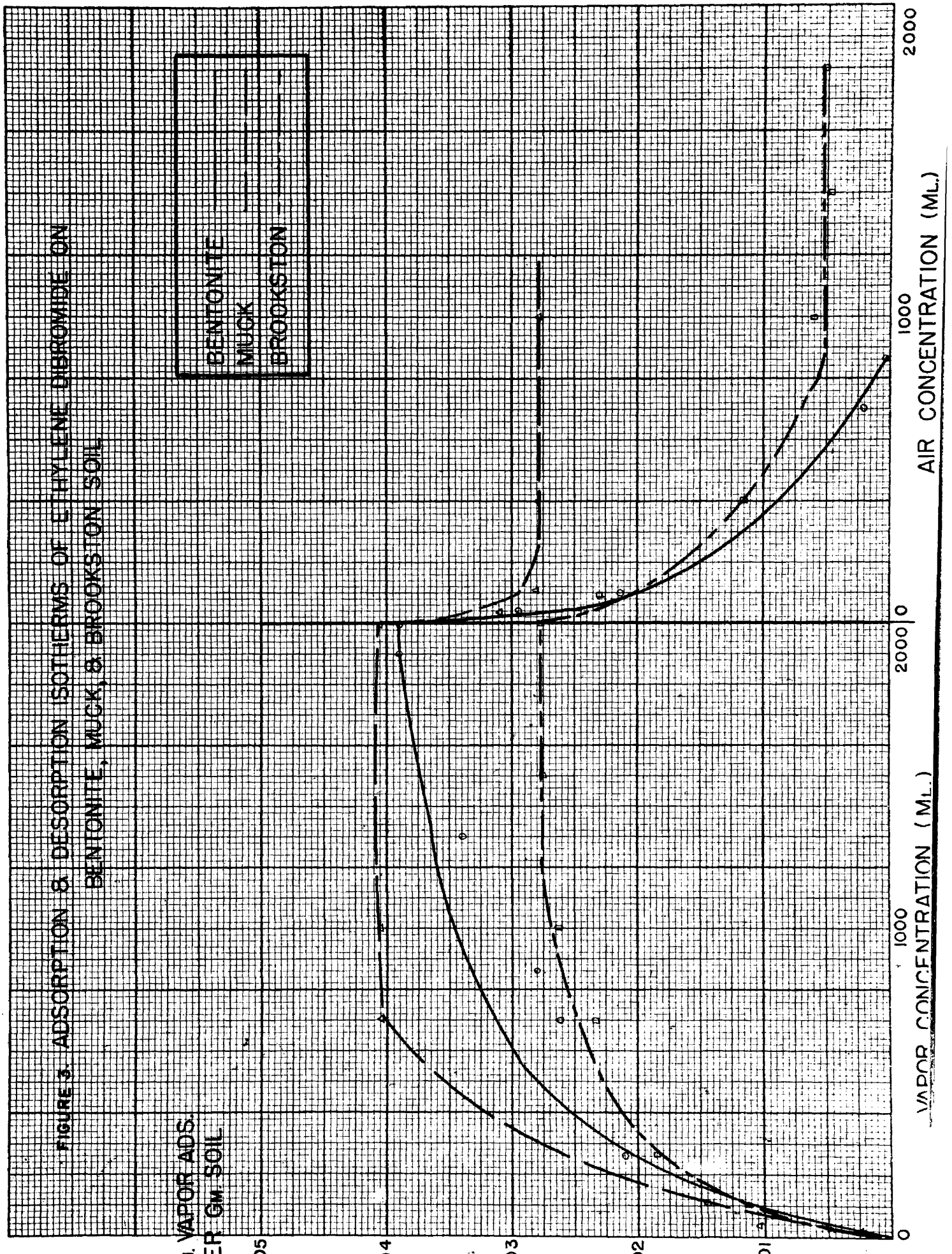


FIGURE 3. ADSORPTION & DESORPTION ISOTHERMS OF ETHYLENE DIBROMIDE ON BENTONITE, MUCK, & BROOKSTON SOIL

Brookston data suggest that the compounds enter into a stronger chemical bonding with the organic colloids, because it was impossible, with the system used, to remove more than a fraction of the amount adsorbed. The amounts of fumigant retained by the soils was related to their organic matter content. The relationship between the soil organic matter and these fumigants is not difficult to comprehend when it is realized that compounds such as resins, alkaloids, phenols, organic acids and their derivatives, (41, 30) exist as dynamically functioning parts of the organic matter.

Dispersion of Dichloropropene Through Soils

It is possible to obtain an isotope of chlorine having a radioactive nucleus with a mass number of 36 and an extremely long half-life ($t_{\frac{1}{2}} = 10^6$ years) (3). The disintegration of this isotope proceeds according to the following reaction: $17Cl^{36} \longrightarrow 18A^{36} + B^-$. The maximum energy of the negative beta particle spectrum is 0.64 M.E.V. The assay of this radioactive isotope of chlorine depends on the detection of these beta particles. This can be accomplished with a G-M tube with a minimum window density, i.e. 1.9 mg. per cm^2. Because the emmission energy is low, considerable self-absorption occurs in the sample material. Therefore, the samples must be kept as thin as possible for maximum counts.

Radioactive 1-3 dichloropropene was obtained by placing 3.5 ml. of the compound in contact with 5 microcuries of $_{17}Cl^{36}$ in the form of 1.03N HCl in a closed glass container. The contact was maintained for 24 hours with occasional shaking. A small sample of the dichloropropene was then removed and tested for beta emission. The test indicated that a transfer of some of the radioactive isotopes for the chlorine on the compound had taken place. The emission from the dichloropropene was not due to occlusion of the radioactive HCl as can be seen in table 4.

Table 4. Titration data on HCl after contact with 4 ml. of 1-3 dichloropropene for 24 and 48 hours.

24 hours		48 hours	
1.0090 N HCl	0.1007 N NaOH	1.0090 N HCl	0.1007 N NaOH
ml.	ml.	ml.	ml.
1.00	10.02 10.02	1.00	10.015 10.02
2.00	20.035 20.04	2.00	20.03 20.03

A drop of the radioactive dichloropropene was placed on the center surface of three soils contained in Petri dishes. The soils, at the moisture equivalent, were 1, Oshtemo loamy sand (9.6 percent moisture), 2, Brookston clay loam (31.6 percent moisture), and 3, muck (131 percent moisture). A strip of x-ray film was placed over the dishes and kept in place for 24 hours. Duplicate dishes

were set up and x-ray film was kept over them for 48 hours. Plates 1 and 2 show positive contact prints made from these negatives.

The plates exposed to the Oshtemo soil show no localization of the radioactive dichloropropene. Apparently the diffusion was lateral and downward since there is no evidence of particle impingement on the film except at a few scattered points. Because of the low emission energy, the radiation was for the most part absorbed by the soil. The Brookston soil plates show a strong localization of radiation around the point of application. There is an indication of lateral diffusion at the soil surface. The radiation distribution is even more localized in the muck soil, indicating strong sorption at the point of application. There is little evidence of diffusion outside of that accomplished by "wetting" at the surface. There are indications of slight surface diffusion with time as a comparison of the 24 hour and 48 hour radiographs demonstrates.

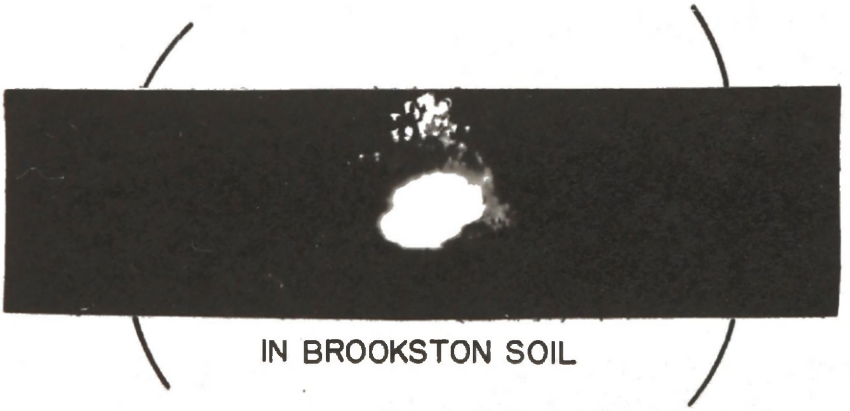
The information gained from these radiographs is purely qualitative, but it supports the evidence gained from the adsorption studies as to the role of organic matter as it affects the dispersion of the fumigants.

The labeled 1-3 dichloropropene was next injected in the Brookston clay loam contained in 3 gallon glazed crocks

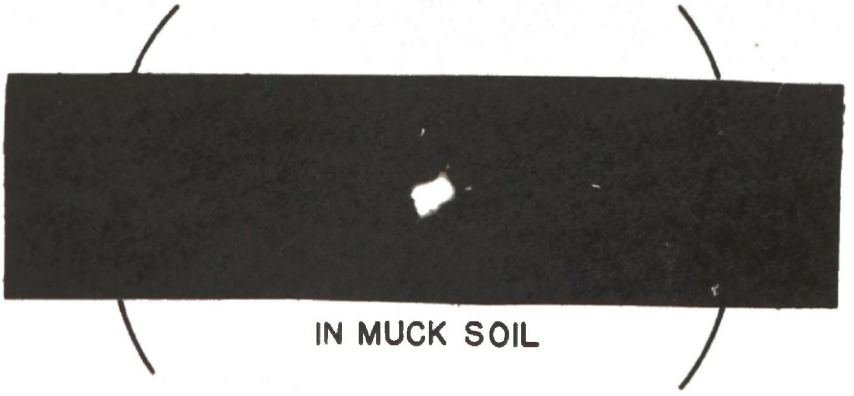
PLATE 1.
RADIOGRAPHS OF RADIOACTIVE DICHLOROPROPENE INJECTED AT THE SURFACE OF SOILS CONTAINED IN PETRI DISHES.

(24 HOUR EXPOSURE FROM THE TIME OF INJECTION.)

IN OSHTEMO SOIL



IN BROOKSTON SOIL

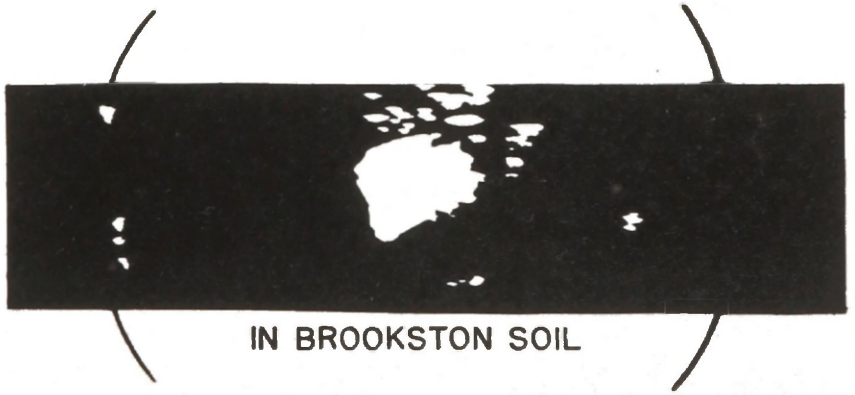


IN MUCK SOIL

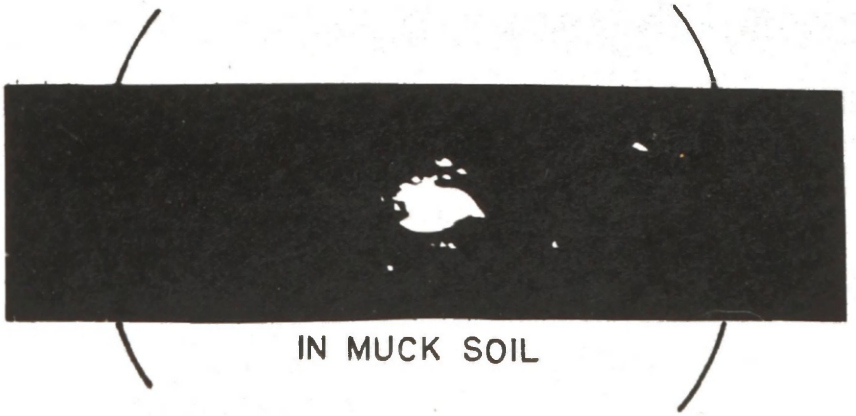
PLATE 2.
RADIOGRAPHS OF RADIOACTIVE DICHLOROPROPENE INJECTED AT THE SURFACE OF SOILS CONTAINED IN PETRI DISHES.

(48 HOUR EXPOSURE FROM THE TIME OF INJECTION.)

IN OSHTEMO SOIL



IN BROOKSTON SOIL



IN MUCK SOIL

with a diameter of 22cm. The Brookston soil was chosen for this study because it had significant quantities of both colloidal organic and mineral matter. The soil in one crock was at the moisture equivalent; in another, the soil was saturated (54.1 percent moisture). The temperature of the soils was 24.7° C. An injection of the dichloropropene of 0.869 gm. equivalent to 0.75 ml. was made in the center of each crock and 6 cm. from the surface of the soil.

The soil was allowed to stand for 8 days and was then sampled with a 2 cm. diameter steel "core sampler" where indicated in figures 4 and 5. The steel edge of the sampler served to run the outside surface of the cores together and minimize loss of any fumigant which might exist as vapor in the soil air. The cores were then divided into 6 cm. lengths and each length was placed in a flask containing 30 ml. of toluene.

Because of the low emission energy of the beta particles involved, it was necessary to devise a system for isolating the labeled dichloropropene or a reaction product from it containing the radioactive chlorine before emission counts could be made. It was found that if a solution of dichloropropene in toluene were refluxed in the presence of fused sodium, (this is possible because the boiling point of toluene is higher than the melting point of sodium), a water extract of the reaction products would

yield sodium chloride with the chlorine coming from the dichloropropene.

The flask containing the soil and toluene was agitated long enough to break up the soil core and the toluene extract was decanted into a boiling flask. Enough additions of toluene and decantations were made to bring the total extract up to 60 ml. Sodium was then added to the boiling flask (0.15 grams was calculated to give a probable excess to that necessary for a complete reaction with the dichloropropene in each sample) and the mixture was refluxed for 2 hours. Sufficient ethyl alcohol was then added to react with the unreacted sodium. The solution was then mixed with several additions of water in a separatory funnel and the water soluble extract was drawn off into a small metal container and evaporated on a hot plate.

Solid sodium chloride along with sodium hydroxide from the reaction of the excess sodium with the ethyl alcohol formed a thin layer at the bottom of the container and emission counts were taken directly from this layer. The thickness of the layer was approximately constant because the same amount of sodium was used in each reaction vessel. Thus, the absorption of radiation could be considered to be constant. The number of emission counts recorded on a scaler from each sample was taken to be proportional to the concentration of dichloropropene in the toluene extracts from the soil cores. The data are tabulated in

table 5.

In order to extract the humus from the soil samples, 50 ml. of a 2 percent solution of sodium hydroxide was added to the soil and the mixture was vigorously agitated. After standing for a few hours, a 10 ml. sample was removed by a pipette from the supernatent suspension and was evaporated to dryness in a metal container. The thickness of this humus layer was approximately equal to that of the salt layer described above. The number of counts recorded from this humus layer was multiplied by a factor of 5 to compensate for the fraction of humus not evaporated and counted. In this instance, the number of emission counts recorded was considered to be proportional to the amount of dichloropropene tied up in or associated with the colloidal organic matter. These data are presented in table 5.

Table 5. Emission counts from radioactive 1-3 dichloropropene injected in Brookston clay loam at 2 moisture levels. *

Soil Sample	At moisture equivalent		At saturation	
	Toluene extract **Counts per minute	NaOH extract Counts per minute	Toluene extract Counts per minute	NaOH extract Counts per minute
0A	28	285	78	252
1A	9	53	5	55
2A	0	0	0	0
3A	0	0	0	0
4A	0	0	0	0
0B	129	630	186	438
1B	58	147	6	192
2B	14	0	6	66
3B	8	0	5	0
4B	T	0	5	0
0C	120	0	38	104
1C	49	0	9	37
2C	6	0	T	0
3C	T (1)	0	0	0
4C	0	0	0	0
1A' (2)	10	56	6	58
1B'	55	140	6	200
1C'	41	0	8	30

* The average of 3 close counts per each sample.
** Calculated on the basis of 1024 counts for each determination less background of 30 counts per minute.
(1) Little, but significant, radiation as determined by long counts.
(2) Symetrical core samples to those of the same code no.

Dispersion patterns incorporating the data in table 5 are presented in figures 4 and 5. It can be seen that the dichloropropene disperses through the soil in two phases; 1, the air-water phase or that part of the dichloropropene which can be extracted with toluene, and 2, the humus phase. Most of the fumigant is associated with the humus and is tied up in a relatively small volume of soil. The fraction traveling in the air-water phase diffuses farther and can be considered to form the boundary limits of the dispersion pattern. The symmetrical core samples taken yield radiation counts of approximately the same magnitude and thus indicates that dispersion of the dichloropropene is horizontally symmetrical.

The dispersion of the dichloropropene in the humus phase of the soil at the moisture equivalent is limited to a small volume around the point of injection. The movement in this phase is downward with a minimum of lateral dispersion. The movement in the air-water phase is also downward with a tendency toward greater lateral dispersion than exists in the humus phase. It seems probable that most of the dichloropropene in the air-water phase surrounding and immediately below the injection point exists in the air pores in equilibrium with a small amount of the compound in the water phase. As the fumigant concentration diminishes with lateral dispersion, it is probable that a greater proportion of it exists in the soil water.

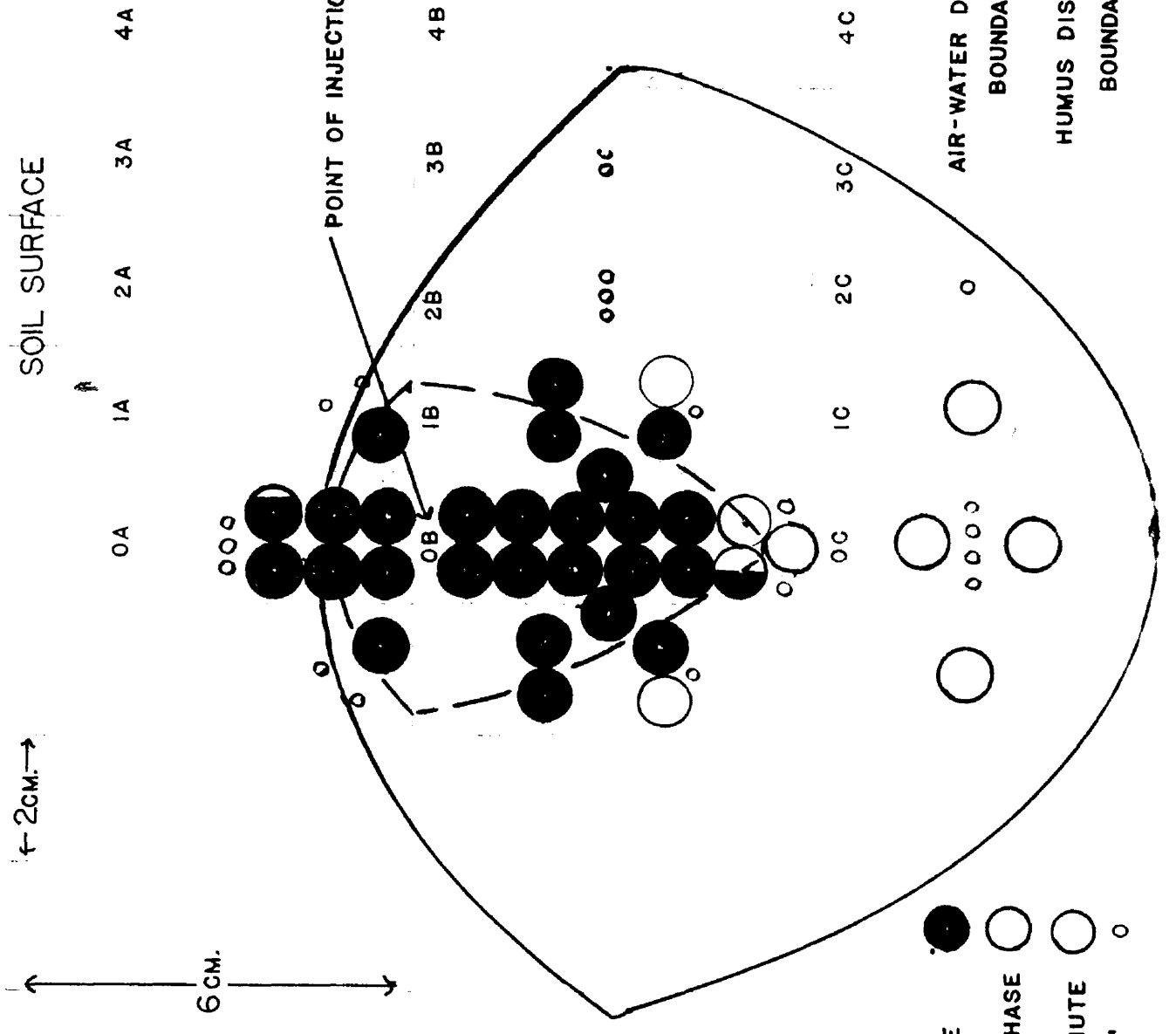


FIGURE 4. DISPERSION PATTERN OF DICHLOROPROPENE IN BROOKSTON AT THE MOISTURE EQUIVALENT 3C INT

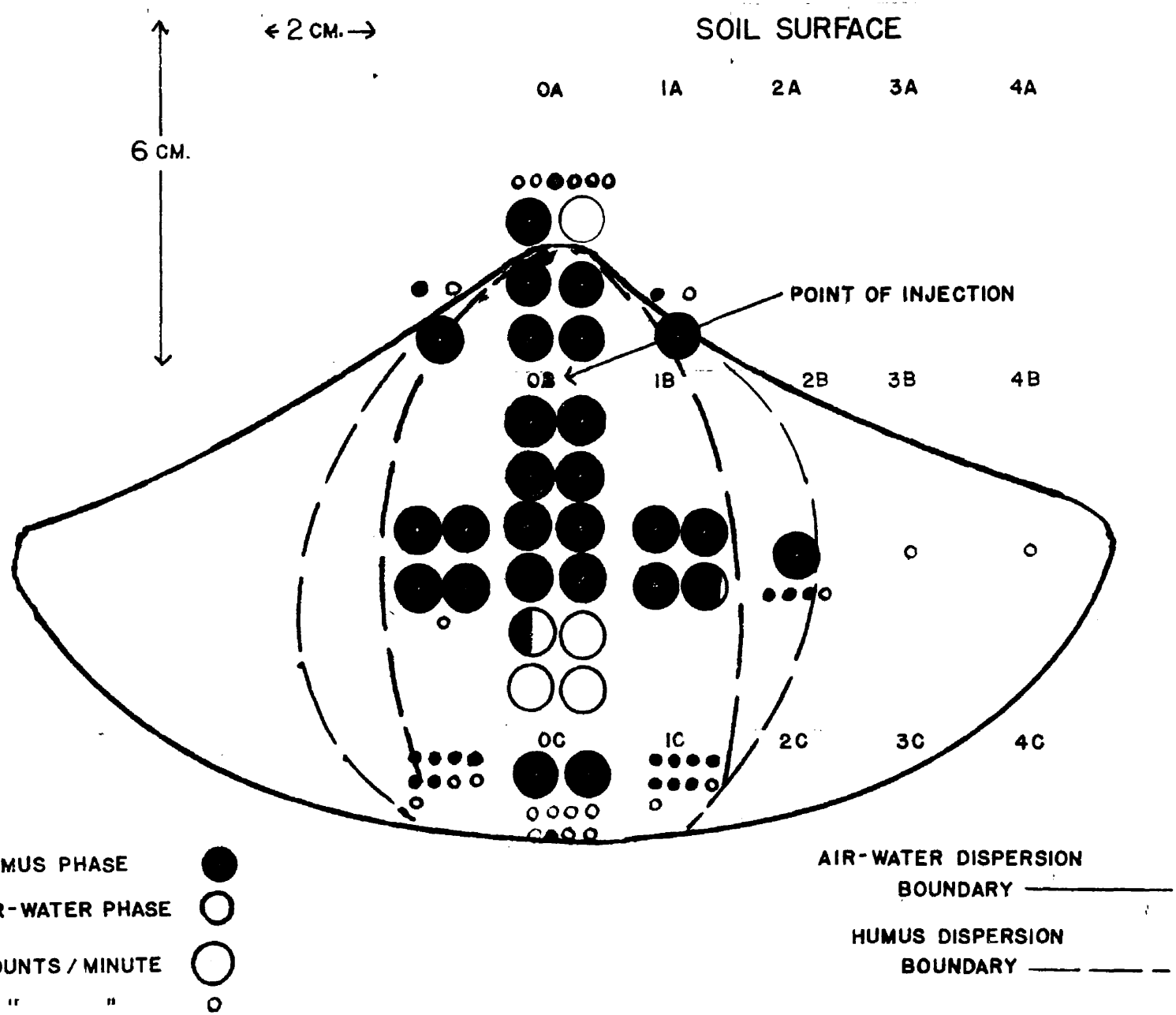


FIGURE 5. DISPERSION PATTERN OF DICHLOROPROPENE IN SATURATED BROOKSTON

There is a greater lateral dispersion of the dichloropropene in the humus phase of the moisture saturated soil. Diffusion downward in the air-water phase is strongly diminished and the small and relatively constant concentration of the fumigant in this phase, lateral to the point of injection suggests that its movement is limited by its low solubility in water.

Dispersion of Ethylene Dibromide Through Soils

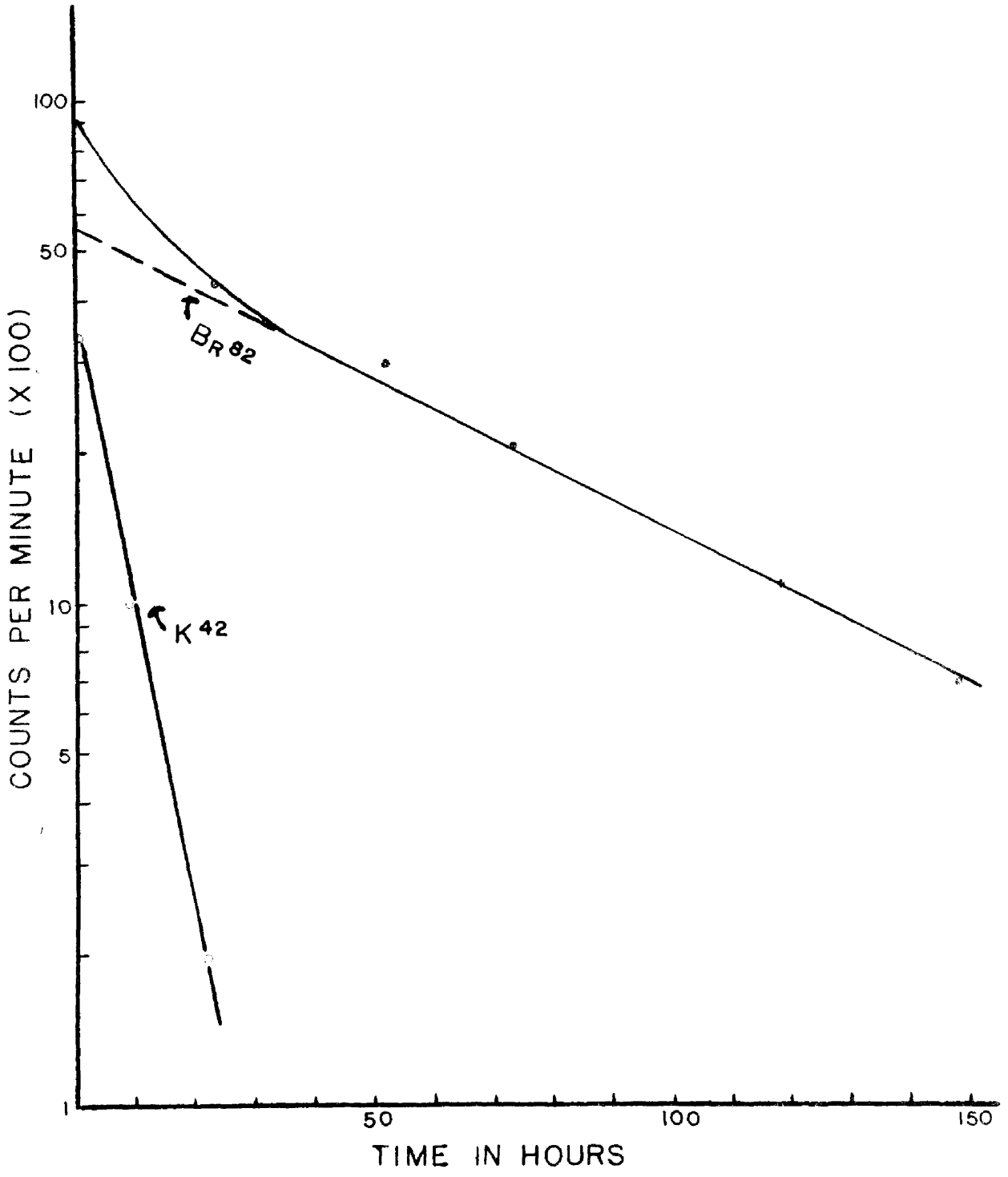
Radioactive bromine can be obtained as KBr with the bromine having a mass number of 82. This isotope of bromine has a half life of 34 hours and disintegrates to yield negative beta particles with a maximum energy of 0.465 M.E.V. Associated with each beta disintegration are three gamma rays in cascade with energy levels of 0.547, 0.787, and 1.35 M.E.V. (36). This high energy gamma emission allows for a different tracer technique than was used with the dichloropropene since it is possible for the gamma radiation to penetrate a relatively large mass of soil.

A quantity of radioactive KBr equal to approximately 40 millicuries of energy was dissolved in 2 ml. of water and this was placed in contact with 4 ml. of 1-2 dibromoethane in a small glass reaction vessel. After standing for 24 hours, a small amount of the dibromoethane was removed and tested for particle emission. The fact that some emission was detected indicated that an exchange of

some of the active bromide ions from the KBr solution had exchanged with some of the bromide on the dibromoethane. Approximately 0.75 ml. of the labeled compound was injected into the Brookston clay loam which was contained in 3/16 in. lead boxes. The soil in one of the boxes was air dry and in the other, at the moisture equivalent. The boxes were 26 cm. square and 16 cm. deep. One side and the cover of each box had 6mm. holes drilled through in strategic places. The injection of the compounds was made in the center surface of the soils and 3cm. deep. Periodic emission counts were made by placing a G-M tube hooked into a scaler against the sides and covers of the boxes. The tube was shielded with a $1\frac{1}{2}$ in. lead casing and was attached to a columnating device consisting of a 6 in. steel tube containing a series of lead discs with 6 mm. perforations in the center. Thus, the columnator could be placed directly against the openings in the lead boxes and comparable counts made repeatedly from the same place.

In order to ascertain the rate of decay of the Br^{82}, the columnated G-M tube was focused on the reaction vessel containing the remainder of the dibromoethane and radioactive KBr. Periodic emission counts were made from a standardized position. The decay curve is presented in figure 6. Because the potassium in the KBr is also radioactive ($t_{\frac{1}{2}}$ = 12.4 hours), the counts recorded on the scaler

FIGURE 6. COMPOSITE DECAY CURVE FOR THE TWO RADIOACTIVE ISOTOPES IN K^{42} Br^{82}.



included particle emission from the potassium as well as the bromine. However, since the half-life of the potassium is shorter than that of the bromine, after a time the counts recorded were due primarily to the bromine. When plotting the decay against time using a logarithmic scale, it can be seen that the decay curve becomes a straight line. This straight line portion of the curve is due to the emission from the bromine and if it is extrapolated to time = 0, the decay for bromine alone is ascertained. Using this extrapolated value, it was possible to compare the emission counts recorded from the boxes containing the soils at any given time by adjusting the magnitude of these counts by a factor which made them comparable to those at the time of the first reading. The data recorded in tables 6 and 7 give the adjusted counts recorded from the soils through time.

Table 6. Emission counts from radioactive 1-2 dibromoethane injected in Brookston soils at two moisture levels: lateral dispersion from vertical axis through point of injections. *

Distance from axis through injection point. (cm.)	Counts per minute corrected to time of first reading.**							
	Air dry soil				Soil at moisture equivalent			
	Time after injection (hours).							
	3	24	48	72	3	24	48	72
	2 cm. below soil surface							
0	387	371	369	360	356	312	347	349
2	111	90	82	87	184	162	157	155
6	94	84	77	82	89	75	84	82
10	90	85	73	77	92	84	79	85
	4 cm. below soil surface							
0	790	705	701	709	612	542	553	561
2	145	183	181	173	292	333	268	263
6	94	100	103	97	114	101	127	121
10	90	82	85	88	93	85	78	86
	8 cm. below soil surface							
0	124	200	212	221	338	362	311	302
2	118	138	142	150	287	314	327	319
6	89	90	85	75	193	204	218	207
10	92	87	84	80	90	85	84	82

* Average of three close counts for each determination.

** All counts recorded below 95 per minute are considered to be part of a variable background and are not considered to be indicative of the presence of the compound.

Table 7. Emission counts from radioactive 1-2 dibromoethane injected in Brookston soils at two moisture levels: vertical dispersion along the axis of injection.*

Distance along axis through injection point. (cm.)	Counts per minute corrected to time of first reading.**							
	Air dry soil				Soil at moisture equivalent			
	Time after injection (hours).							
	3	24	48	72	3	24	48	72
4	790	705	701	709	612	542	553	561
6	586	488	458	449	515	469	480	484
8	124	200	212	221	338	362	311	302
10	89	122	125	128	92	175	208	217
14	94	85	87	77	85	127	134	125

* See table 6.
** See table 6.

That the volume occupied by the compounds in either soil consisted approximately of a symmetrical solid of revolution with a vertical axis running through the point of injection and perpendicular to the soil surface can be seen from the data in table 8.

Table 8. Emission counts from radioactive 1-2 dibromo-ethane injected in Brookston soils at two moisture levels: counts taken from the soil surfaces.*

Distance from vertical axis running through injection point.	Counts per minute recorded 24 hours from the time of injection and corrected to the three hour readings.**	
	Air dry soil	Soil at moisture equivalent
0	1492	1114
2 cm. 90°	327	374
2 cm. 180°	348	361
2 cm. 270°	338	348
2 cm. 360°	318	359
6 cm. 90°	86	179
6 cm. 180°	86	191
6 cm. 270°	90	187
6 cm. 360°	82	187

* See table 6.
** See table 6.

The rates of diffusion of the ethylene dibromide at the two moisture levels are presented in figures 7 and 8. It can be seen that the movement of the compound is more rapid in the vertical than in the horizontal direction. Also, the concentration of the compound shows a greater localization along the vertical axis through the injection point. The greatest amount of dispersion takes place within

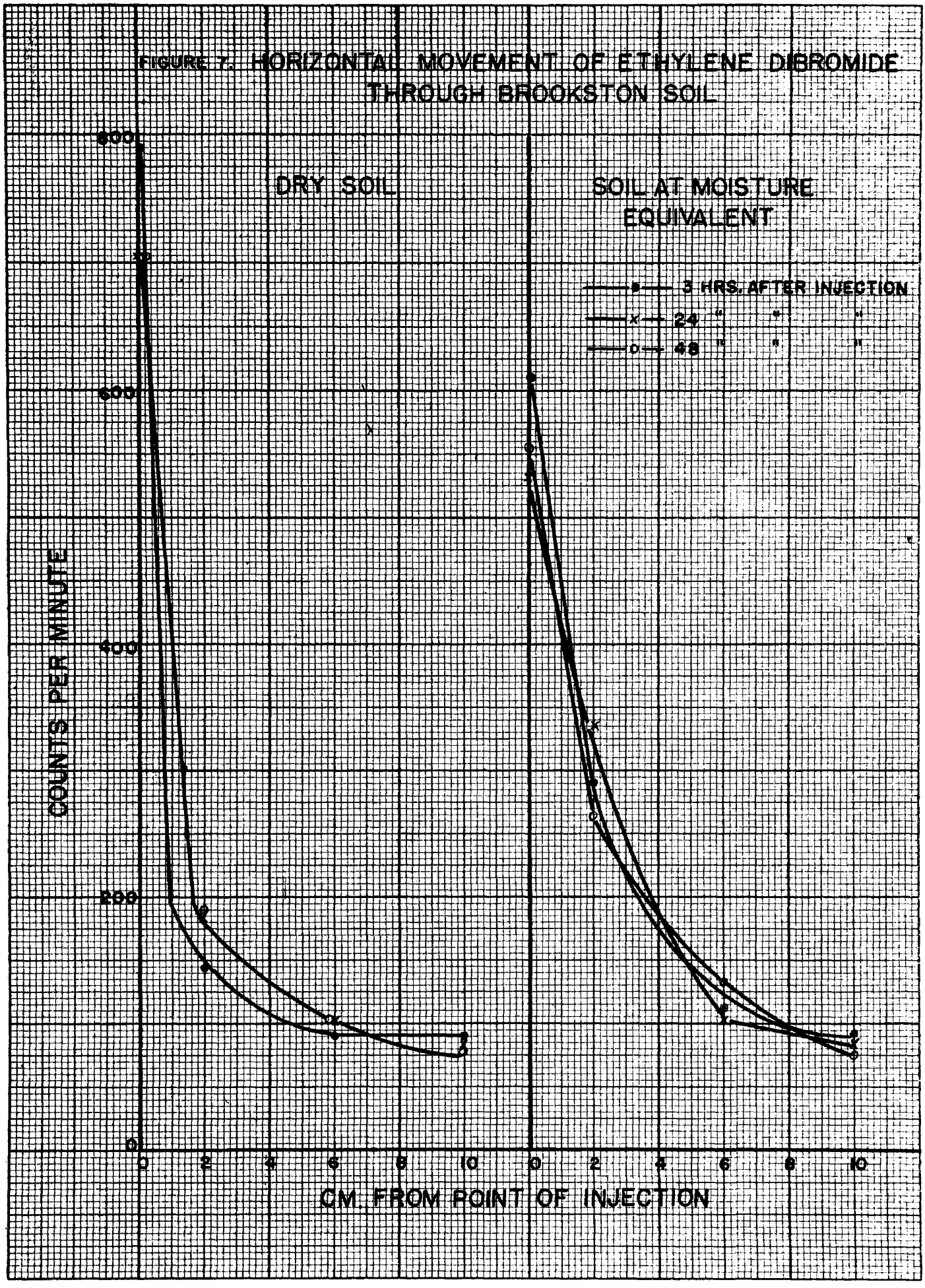


FIGURE 7. HORIZONTAL MOVEMENT OF ETHYLENE DIBROMIDE THROUGH BROOKSTON SOIL
DRY SOIL
SOIL AT MOISTURE EQUIVALENT
3 HRS. AFTER INJECTION
24 " " "
48 " " "
COUNTS PER MINUTE
800
600
400
200
0
0 2 4 6 8 10
0 2 4 6 8 10
CM. FROM POINT OF INJECTION

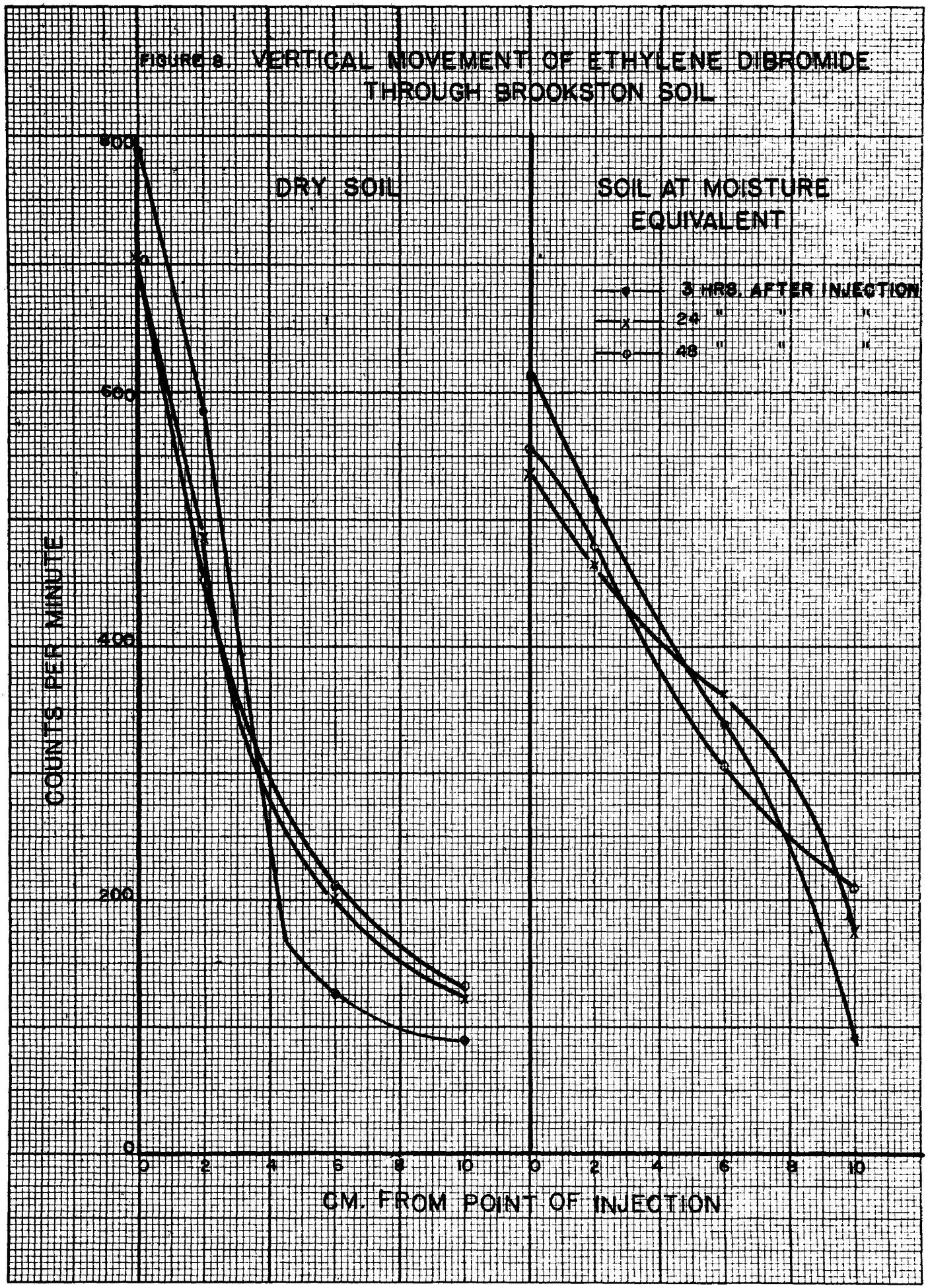


FIGURE 8. VERTICAL MOVEMENT OF ETHYLENE DIBROMIDE THROUGH BROOKSTON SOIL

the first three hours after injection and the dispersion limit was for all practical purposes, reached after 24 hours. It is interesting to note that at the 48 hour determination, there appeared to be a movement of the fumigant against a concentration gradient in the immediate vicinity of the injection point. This can be seen in the soil having the greater amount of water. Since it is improbable that movement against a concentration gradient took place, it seems more logical that the reaction with or adsorption of the compound by the organic matter gave it new characteristics, and a solution effect with the water could cause this movement in localized areas. It is also possible that hydrolysis of the ethylene dibromide in areas of high concentration could result in the formation of bromides which would diffuse through the water phase.

The stabilized dispersion patterns of the ethylene dibromide in the Brookston soil at the two moisture levels are given in figures 9 and 10. It can be seen that the compound disperses through a greater volume of soil when the soil is at the moisture equivalent than it does when the soil is air-dry. It is also evident that the area of greatest concentration around the point of injection is more diffuse in the wetter soil. In neither case was there any significant penetration of the compound into the soil mass above the point of injection other than that caused by a surface effect due to wetting.

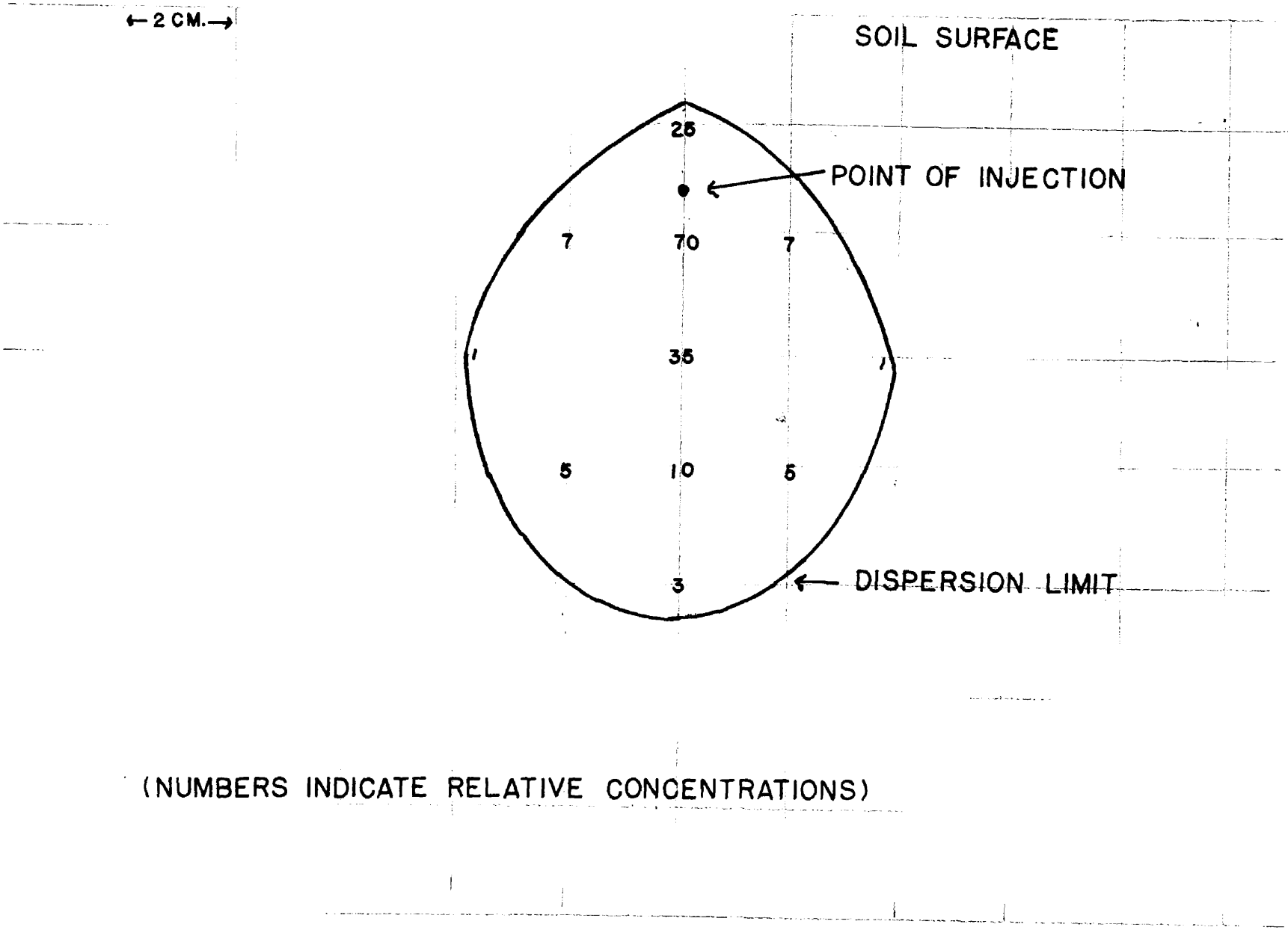


FIGURE 9. STABILIZED DISPERSION PATTERN OF ETHYLENE DIBROMIDE IN AIR DRY BROOKSTON SOIL

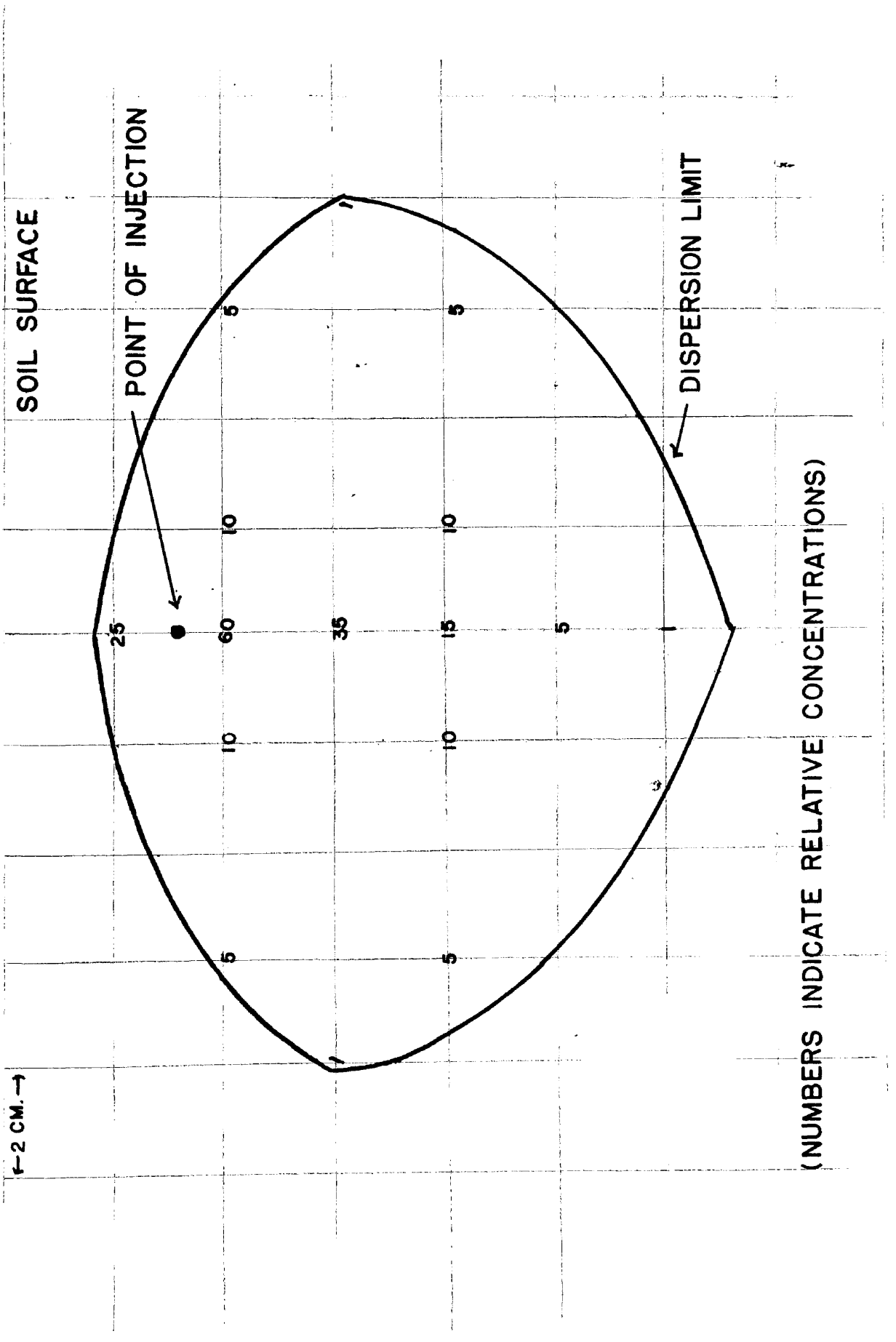


FIGURE 10. STABILIZED DISPERSION PATTERN OF ETHYLENE DIBROMIDE IN BROOKSTON SOIL AT THE MOISTURE EQUIVALENT POINT

Field and Greenhouse Observations

Certain qualitative data on the spread and retention of certain fumigants in soils was gained from the following observations.

It is not uncommon to notice the odor of Dowfume N or Dowfume W-40 in muck soils for periods of one year or longer after fumigation has taken place. This is usually true if the interceding time is characterized by a great deal of rainfall. Thus, the fumigant is believed to be carried to the surface by a rise of the water table. However, consideration must also be given to the fact that if the period following fumigation is wet and cool, and if a certain quantity of the fumigant is fixed in the soil and localized around the point of injection, then biological "detoxication" of the fumigant will be retarded and quantities will remain in the soil per se for long periods of time.

A field of muck soil in Chandler's Marsh near St. Johns, Michigan was fumigated in the fall of 1949 with Dowfume N at the rate of 40 gallons per acre. The field was planted to carrots in 1950, and abnormal branching of the roots was noticed. Plate 3 shows some typical carrots pulled from this field. That this branching was not due to root knot nematode damage can be seen by the fact that the roots do not have the knotted appearance typical of this kind of damage. It seems more probable that the carrot roots grew down into the soil until they reached a

Plate 3. Typical branched carrots pulled from a field of muck soil fumigated with Dowfume N.

concentration of a toxic substance which damaged the meristems which in turn stimulated lateral branching and growth from the crowns. In this case, the fumigant was injected approximately three inches below the surface which was also equal to the extent of vertical growth of the carrot roots. Spot checks made of the stand showed that approximately 30 percent of the carrots were branched in this manner. Those carrots which were not branched were also not greater than three inches in length for the most part. They were, however, grouped so closely together that lateral branching was probably impeded.

One-gallon crocks were filled with Oshtemo loamy sand and Brookston clay loam and soy beans were planted

in them. After the seeds germinated the plants were thinned to three per pot which approximately formed an equilateral triangle of about five inches on each side. When the plants were about nine inches tall, 1 ml of dichloropropene or ethylene dibromide was injected into the soil half way between two plants and one inch below the surface of the soil. The soils were at the moisture equivalent at the time. The dosages used were far greater than the amount normally recommended for field use. Plates 4 and 5 show the results of the use of these compounds in the two soils.

Plate 4. Crock 1 contained Brookston clay loam. Ethylene dibromide was injected between the two plants on the right. Crock 2 contained Oshtemo loamy sand. Ethylene dibromide was injected between the two plants on the left.

Plate 5. Left - Oshtemo loamy sand. Dichloropropene was injected between the plants on the lower right. Right - Brookston clay loam. Dichloropropene was injected between the plants on the left.

All the plants wilted after injection of either compound in the Oshtemo soil. The wilting took place within 12 hours after the injections were made. The plants never recovered from the wilting and died from what appeared to be an inability to take up water through the root systems. Examination of the roots showed that the surfaces were highly corroded. This corrosion was probably due to direct contact with the fumigants. In the Brookston soil, there was only a slight wilting of the two plants between which the fumigant was injected. This was true upon the use of either of the compounds. The third plant in each crock was unaffected and continued normal growth. After a few days, the visible symptoms of wilt on the affected plants disappeared and all plants continued to grow. Examination of the roots of these plants showed only

small areas of corrosion and this only on those roots which were adjacent to the point of injection of the compounds. Though the roots of each of the three plants in each pot were dispersed more or less through the entire soil mass, the effective area for absorption of water could be considered to be unaffected in those plants not adjacent to the point of injection.

It is believed that the above observations support the hypothesis that large quantities of the fumigant are fixed in certain soils and that this fixation is primarily a function of the amount of colloidal organic matter present. As was previously shown, a colloidal inorganic soil fraction of large surface area would probably adsorb certain quantities of the fumigants, but the bonding energy is not strong enough to give the concentration gradients exhibited in these cases.

DISCUSSION

The results of these experiments indicate that the extent of dispersion of dichloropropene and of ethylene dibromide are approximately equal in identical soils at the same moisture level. There is little, if any, penetration of the fumigants into the soil mass above the point of injection if the soils are allowed to remain undisturbed after treatment. The dispersion is lateral and downward from the point of injection with the greatest

concentration of the fumigant localized around a vertical axis through the point of injection. Probably because of its greater weight per unit volume, the ethylene dibromide shows a greater concentration at the lower depths of penetration than does the dichloropropene.

There is evidence as indicated by the data that large proportions of the compounds are concentrated in a relatively small volume of soil around the point of injection, and that this concentration is primarily due to an association of the compounds with the colloidal organic matter present in the soil. This association may be due to strong chemical adsorption or to mutual solution. Thus, it was shown by the use of radioautographs that dispersion of the dichloropropene was greatest in the sandy soil containing very small amounts of organic matter; next greatest in the clay loam containing relatively large amounts of organic matter; and least in the muck which contained extremely large amounts of organic matter. There is evidence to believe that the same relationships hold for the ethylene dibromide. It was shown that a colloidal mineral soil constituent does not play a great role in fixation of the compounds in the soil.

In consideration of the moisture content of the soils at the time of fumigation, the soils at the optimum moisture level (moisture equivalent) allowed for the greatest vertical penetration of the vapors. There was

also an indication of lateral dispersion but a very great concentration gradient existed around the point of injection. With the soil containing enough water to saturate it, vertical movement of the dichloropropene was decreased, but lateral dispersion was more extensive and the concentration gradients were not so severe. It seems probable that dispersion of the organic fraction is more extensive under these soil conditions. Also, the unassociated fraction of the applied compound has more water in which to dissolve and movement of the compound in the water phase is limited by its low solubility in water. There is evidence to believe that the ethylene dibromide behaves in the same manner. Dispersion was least extensive in all directions in the air-dry soils.

Further evidence which showed the effects of certain soils as they pertain to fumigant dispersion was gained from observations made on soybeans which were grown in the Oshtemo and Brookston soils and later fumigated with Dowfumes N and W-40. The plants growing in the sandy soil all died in a very short time after treatment with either fumigant. The roots were strongly corroded and it appeared as though the plants died from an inability to take up water. Treatment in the clay loam soils did not materially affect the plants. It was apparent that large quantities of the fumigants were fixed within the immediate vicinity of the point of injection and thus did not reach the plant roots and damage them. Further observations made on carrot

roots growing in a muck soil nine months after the soil was treated with Dowfume N showed damage to the roots which was attributed to a residual effect of the fumigant in the soil.

There were indications that dispersion of the different compounds and fumigants used was almost complete after three hours and that a stabilized equilibrium was reached within 24 hours. It also became apparent that the compounds traveled through two phases; the humus phase and the air-water phase in which an equilibrium exists between the compound vapor and solution forms. The effectiveness of these fumigants will thus depend upon the relative concentration in these two phases and the severity of their action in the different phases.

PART II

Effect of Fumigation on Soil Biochemistry

Though soil fumigants are used to control specific plant pathogens, attention must also be paid to the effects that these treatments have on the microbial population as a whole. One finds many instances in the literature of reported effects on crop growth different from that which could be expected from control of the pathogens. The purpose of this portion of the study was to study certain changes which occurred in the biochemistry of the soil as a result of fumigation.

LITERATURE SUMMARY

Early research with chemical disinfectants has established the fact that their use as fumigants accomplishes only a partial sterilization of the soil (15). Wakeman and Starkey (57) confirmed this and showed that partial sterilization results in an increase in the bacterial population and in ammonium accumulation. Work by Matthews (26) indicated that fumigation with certain compounds increased the bacterial population while other compounds did not affect it. In general, the majority of the early workers agreed that the beneficial results obtained from soil fumigation was due to increased bacterial activity which made increased amounts of nutrients available

for plant growth (16, 38, 39).

More recently, Stark and Smith (47) reported that low dosages of chloropicrin had little effect on nitrate formation, but as the dosage was increased, nitrification was inhibited. In no case was ammonification inhibited, but the total amount of nitrogen made available for plant growth was materially increased only where high dosages of the chloropicrin were used. Bogopolskii and Bershova (9) reported an increase in the yields of tomatoes, oats, and potatoes after fumigation with chlorine and phenol. The activity of the ammonifying and nitrogen fixing bacteria was increased, while that of the nitrifying bacteria was decreased. Similar results were reported by Smith and Wenzel (46) using benzene hexachloride as a fumigant.

As a result of the increased microorganism activity and the elimination of certain species, other related effects have been noted in soils after fumigation. Beames and Butterfield (7) reported that the oxygen content in non-sealed pots was decreased by 80% after fumigation with methyl bromide. Sherman and Fujimoto (43) showed that the exchangeable iron in the soil was increased after treatment with chloropicrin and D-D mixture and Timonin (52), while working on the manganese deficiency disease of oats, reported that plants grown on "manganese deficient" soil were free from manganese deficiency symptoms after the soil

was treated with chloropicrin or formaldehyde. The treatments greatly reduced or completely eradicated the bacteria capable of oxidizing manganese.

EXPERIMENTAL

Carbon Dioxide Production and Redox Potential after Fumigation

One method for measuring changes in the metabolic activities of soil microorganisms is to measure carbon dioxide production in the soil (56). Also, in view of the fact that the bio-electric or redox potential is the sum total of the oxidizing and reducing tendencies in the soil, it was believed that investigation as to the nature of this potential would offer a valuable insight on the state of the soil system after fumigation. Redox potential in and of itself is probably not too important since it varies so greatly from soil to soil, but changes in the potential may be related to the state of the soil after treatment and help to explain certain of the phenomena observed after treatment. Volk (55) has developed a laboratory method for the determination of redox potential where measurements are made on a soil sample immersed in nitrogen saturated water. Bueher, (12), however has noted that the bubling of nitrogen through soil suspensions results in a decrease in the redox potential whether or not the soils were puddled or sterilized. Quispel (35) has suggested that accurate measurements of the redox

potential of the soil system can only be made in place and has developed a method to accomplish this.

An experiment was devised to simultaneously measure the carbon dioxide production and redox potential in soils after fumigation. The apparatus used is diagrammed in figure 11. Oshtemo loamy sand, Brookston clay loam, and Carlisle muck were placed in the spherical glass containers and saturated with distilled water. The quantities of soil necessary to bring the soil surface to one-half inch of the opening at the top of the containers was 500, 450, and 175 grams respectively. The soils were then brought to approximately the moisture equivalent by draining off the excess moisture with a 40 cm. column of water attached to the small opening at the bottom of the glass container. The cork containing the platinized platinum electrode, the KCl in agar bridge, and the intake and outlet tubes were placed in the opening of the container and saturated with paraffin to insure an air-tight seal. Carbon dioxide free, moisture saturated air was then passed over the soil and bubbled through a standard barium hydroxide solution. The rate of air flow was maintained at approximately 40 ml. per minute. Periodic titrations of the barium hydroxide with standard sulfuric acid indicated the quantities of carbon dioxide evolved from the surface of the soil through given periods of time.

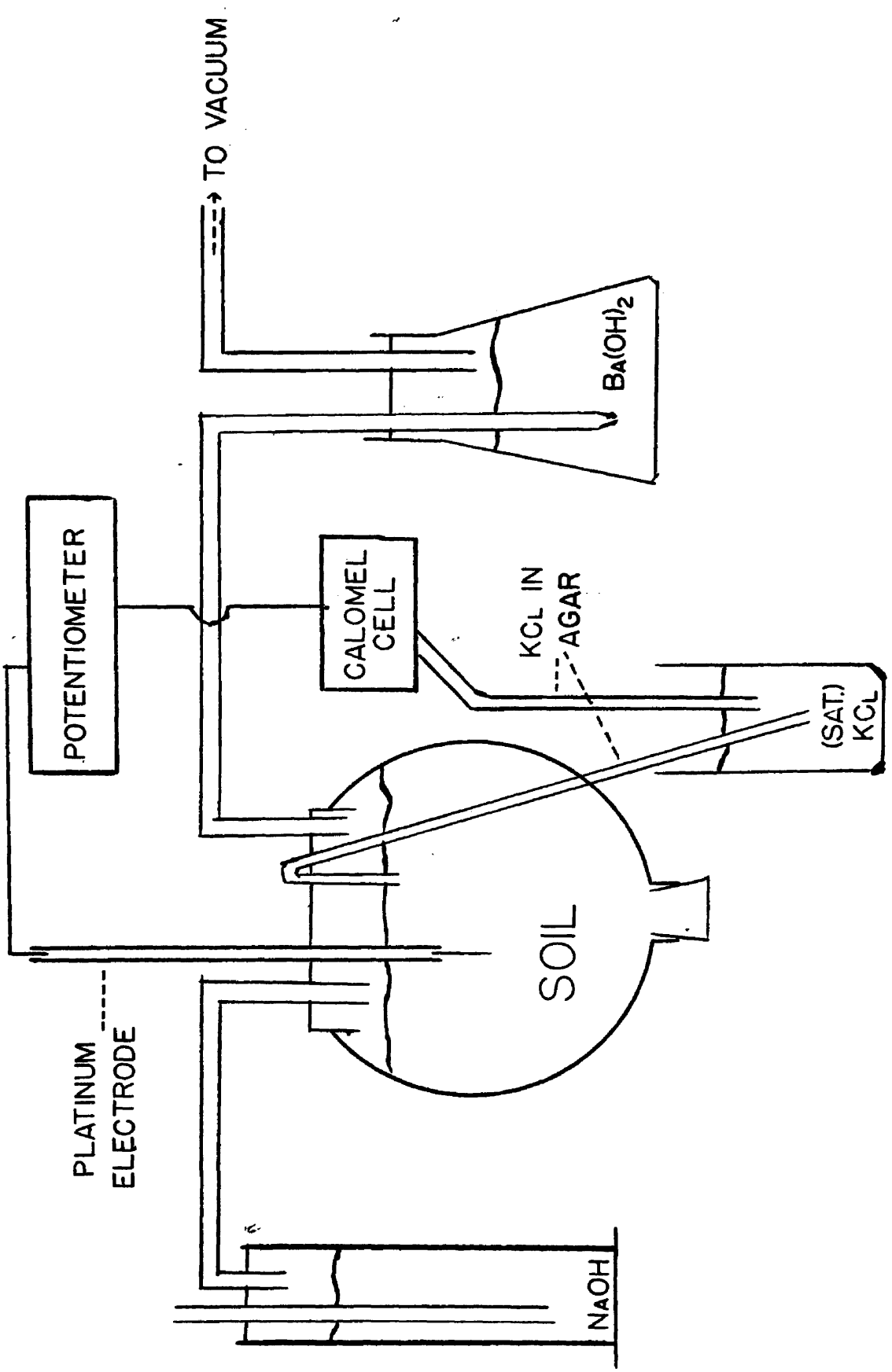


FIGURE II. APPARATUS FOR THE DETERMINATION OF REDOX POTENTIAL AND CARBON DIOXIDE PRODUCTION

Simultaneous with the measurement of carbon dioxide evolution, the redox potential was measured by attaching the platinum electrode to a potentiometer and completing the circuit through a calomel cell connected through a saturated KCl solution to the KCl in agar bridge. In this system the redox potential of the soil could be measured in place and variations in the potential through time plotted as a continuous function. The soil was considered to function as a half cell being compared to a standard calomel cell in order to obtain a measurement of the overall state of its oxidation-reduction tendency.

After the electrodes in the soils had come to a state of equilibrium as determined by constant voltage readings, the soils were fumigated with 0.50 ml of 1-3 dichloropropene and 1-2 dibromoethane. The compounds were introduced into the center of the soil mass by pushing a hypodermic needle attached to a syringe through the cork stopper and slowly ejecting them. The hole in the stopper was then resealed with paraffin. The carbon dioxide which evolved from the soil and reacted with the barium hydroxide was subsequently evaluated for two hour periods at various intervals after fumigation and a comparison was made with these quantities of carbon dioxide and that evolved from untreated soils. Table 9 presents these data.

Table 9. A comparison of carbon dioxide production in Oshtemo, muck, and Brookston soils after treatment with 1-3 dichloropropene and 1-2 dibromoethane.

Soil	Time after treatment (hours)	Mg. CO_2 evolved per 2 hr. period			% CO_2 produced compared with untreated soils	
		Treatments				
		Dichloro-propene	Dibromo-ethane	Untreated	Dichloro-propene	Dibromo-ethane
Oshtemo	3	1.1	3.2	4.8	24	68
	15	0.9	1.0	4.5	19	23
	28	3.1	7.6	3.8	81	200
	40	5.1	6.5	3.1	163	211
	168	4.9	6.8	2.3	212	297
	240	3.1	6.9	4.0	76	173
	400	3.0	3.3	3.6	82	93
	740	3.0	7.2	4.1	73	175
	1000	4.2	6.7	3.8	110	176
Muck	3	4.4	7.6	10.8	41	70
	15	1.8	4.6	11.0	16	42
	28	10.6	17.2	10.6	100	162
	40	13.2	22.4	10.4	127	215
	168	12.8	29.2	8.3	154	352
	240	11.2	40.5	8.9	112	455
	400	11.4	37.4	11.4	100	314
	740	10.7	19.9	7.6	141	263
	1000	8.3	12.7	7.2	115	176

Table 9. (continued)

Soil	Time after treatment (hours)	Mg. CO_2 evolved per 2 hr. period			% CO_2 produced compared with untreated soils	
		Treatments				
		Dichloro-propene	Dibromo-ethane	Untreated	Dichloro-propene	Dibromo-ethane
Brookston	3	3.5	4.0	9.2	38	43
	15	7.6	16.0	10.5	72	152
	28	16.8	30.5	10.7	157	285
	40	18.5	30.0	9.0	206	333
	168	9.4	26.8	6.8	138	395
	240	9.1	28.2	7.9	115	357
	400	8.5	13.4	5.5	154	244
	740	11.4	25.4	7.2	158	354
	1000	5.8	14.4	5.5	106	262

As could be expected there were variations in the amounts of carbon dioxide released from both the treated and untreated soils from time to time. It was felt however, that a comparison between the magnitudes of carbon dioxide produced by the treated soils and that produced by the untreated soils for a given period of time (the quantity produced by the untreated soil was taken to be 100 percent for the time interval under consideration) gave a valid index of the state of microbial activity in the soil. Of course, these variations do not take into account the micro-variations which exist through smaller increments of time.

The data on the redox potential measurements made simultaneously with the carbon dioxide production measurements are presented in table 10. It was possible to obtain potentials of remarkably close magnitudes for the soils within each type so that the variations within each type are comparable. It can be seen that there is a tendency which is especially noticeable in the untreated soils for the potential to rise with time. This was probably due to increased aeration caused by a slight drying of the soils. The rise in potential is most pronounced in the sandy Oshtemo soil and least in the muck.

Table 10. Redox potentials of Oshtemo, Muck, and Brookston soils after treatment with 1-3 dichloropropene and 1-2 dibromoethane.

Time after treatment (hours)	Redox potential in positive millivolts.								
	Oshtemo			Muck			Brookston		
	* 1.	2.	3.	1.	2.	3.	1.	2.	3.
0	548	550	553	489	475	476	460	466	468
1	474	522	555	298	203	480	315	316	462
3	435	506	553	294	190	481	313	317	466
15	435	507	557	295	185	485	326	305	470
28	436	505	555	301	188	478	330	310	475
40	405	500	555	295	190	480	338	305	475
88	400	473	560	297	193	482	341	303	478
168	402	473	559	297	193	480	343	304	481
240	392	481	563	296	187	478	344	304	482
400	390	470	567	297	175	480	338	302	480

Table 10. (continued)

Time after treatment (hours)	Redox potential in positive millivolts.								
	Oshtemo			Muck			Brookston		
	* 1.	2.	3.	1.	2.	3.	1.	2.	3.
538	390	475	570	304	175	482	342	302	485
740	392	469	581	310	181	485	342	311	490
884	395	470	586	312	183	491	346	313	495
1000	400	476	592	312	183	490	350	318	497

* The following treatments were used in each soil:

1. 0.5 ml of 1-3 dichloropropene
2. 0.5 ml of 1-2 dibromoethane
3. check, 0.5 ml of distilled water

Both the redox potential and carbon dioxide production data are graphed for each soil and treatment in figures 12 through 17. In all cases where the soil was fumigated, there is an initial depression of the carbon dioxide produced followed by a rise above that of the untreated soils after approximately 40 hours. The rise is somewhat earlier in the dibromoethane treated soils than in those treated with dichloropropene. This might possibly be explained by a more rapid hydrolysis of the former forming ethylene glycol which is available as a source of energy for the microorganisms. The eventual rise in the carbon dioxide production is also greater in those soils treated with the ethylene dibromide than in those treated with the dichloropropene. The magnitude of the rise is greatest in the muck soil followed by the Brookston and then the Oshtemo. It can be seen that the high carbon dioxide production was sustained in the fumigated muck and Brookston soils throughout the length of this experiment (1000 hours). The Oshtemo soil returned to its "normal" dynamic equilibrium after approximately 400 hours.

The redox potential of the soils dropped within an hour after fumigation. That this drop was not due to the addition of a liquid to the soil can be seen from the fact that the untreated soils did not show a drop in potential with the addition of a quantity of water equivalent to that of the fumigant. There appeared to be no

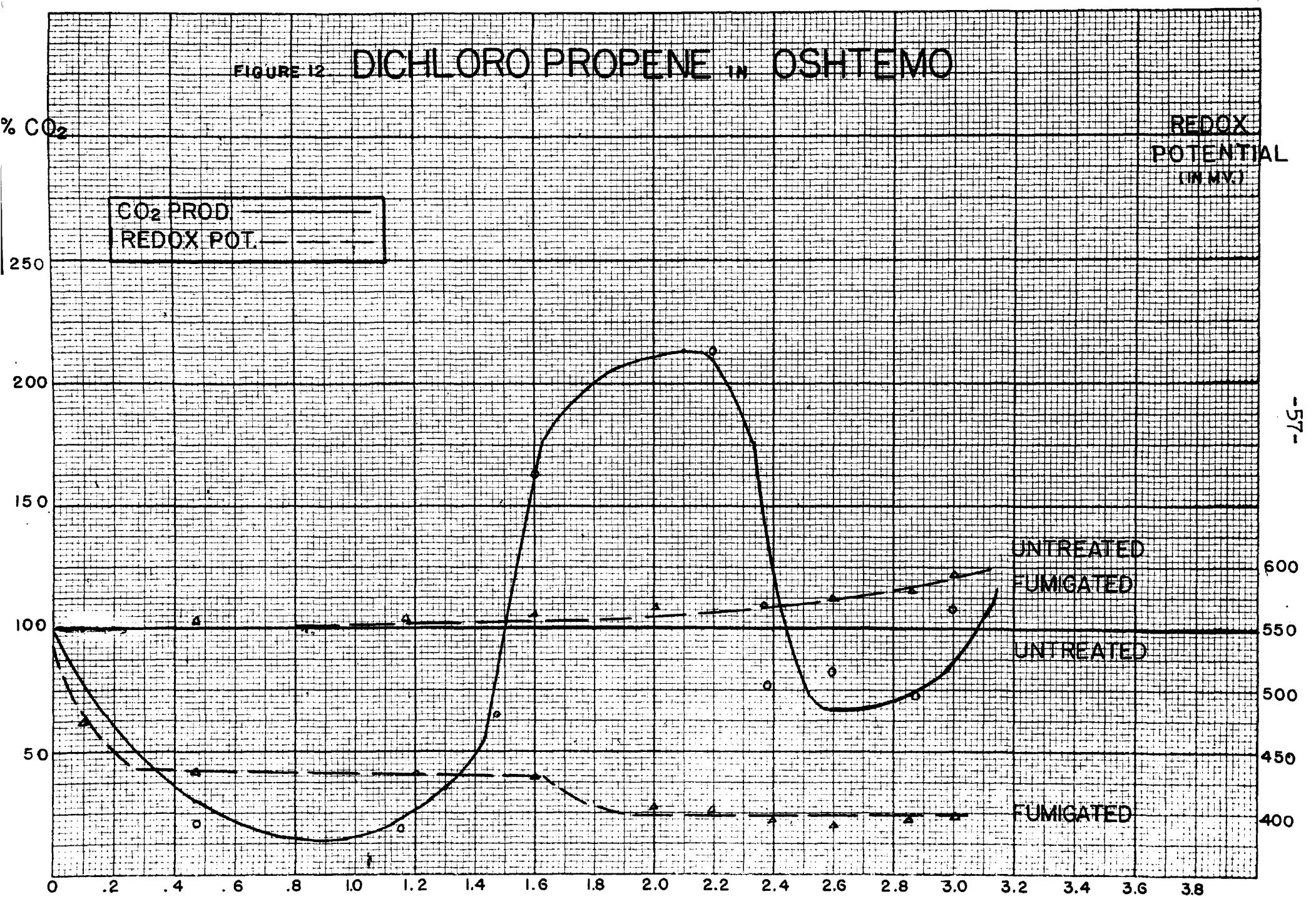


FIGURE 12. DICHLORO PROPENE IN OSHTEMO
% CO_2
REDOX POTENTIAL (IN MV.)
CO_2 PROD.
REDOX POT.
250
200
150
100
50
600
550
500
450
400
UNTREATED
FUMIGATED
UNTREATED
FUMIGATED
0
.2
.4
.6
.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8

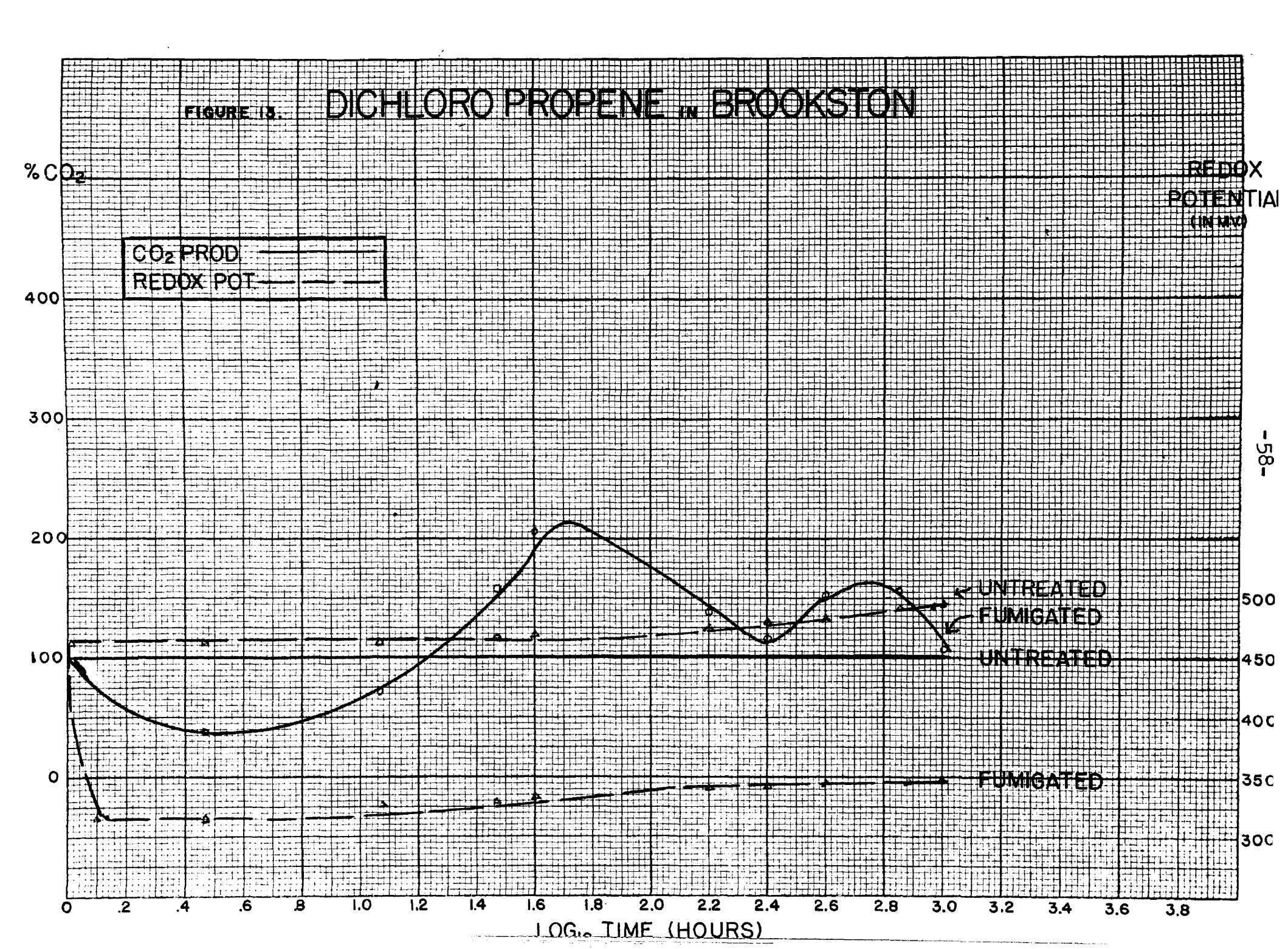


FIGURE 13. DICHLORO PROPENE IN BROOKSTON
% CO_2
REDOX POTENTIAL (IN MV)
CO_2 PROD.
REDOX POT.
400
300
200
100
0
500
450
400
350
300
UNTREATED
FUMIGATED
UNTREATED
FUMIGATED
0 .2 .4 .6 .8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 3.6 3.8
LOG10 TIME (HOURS)

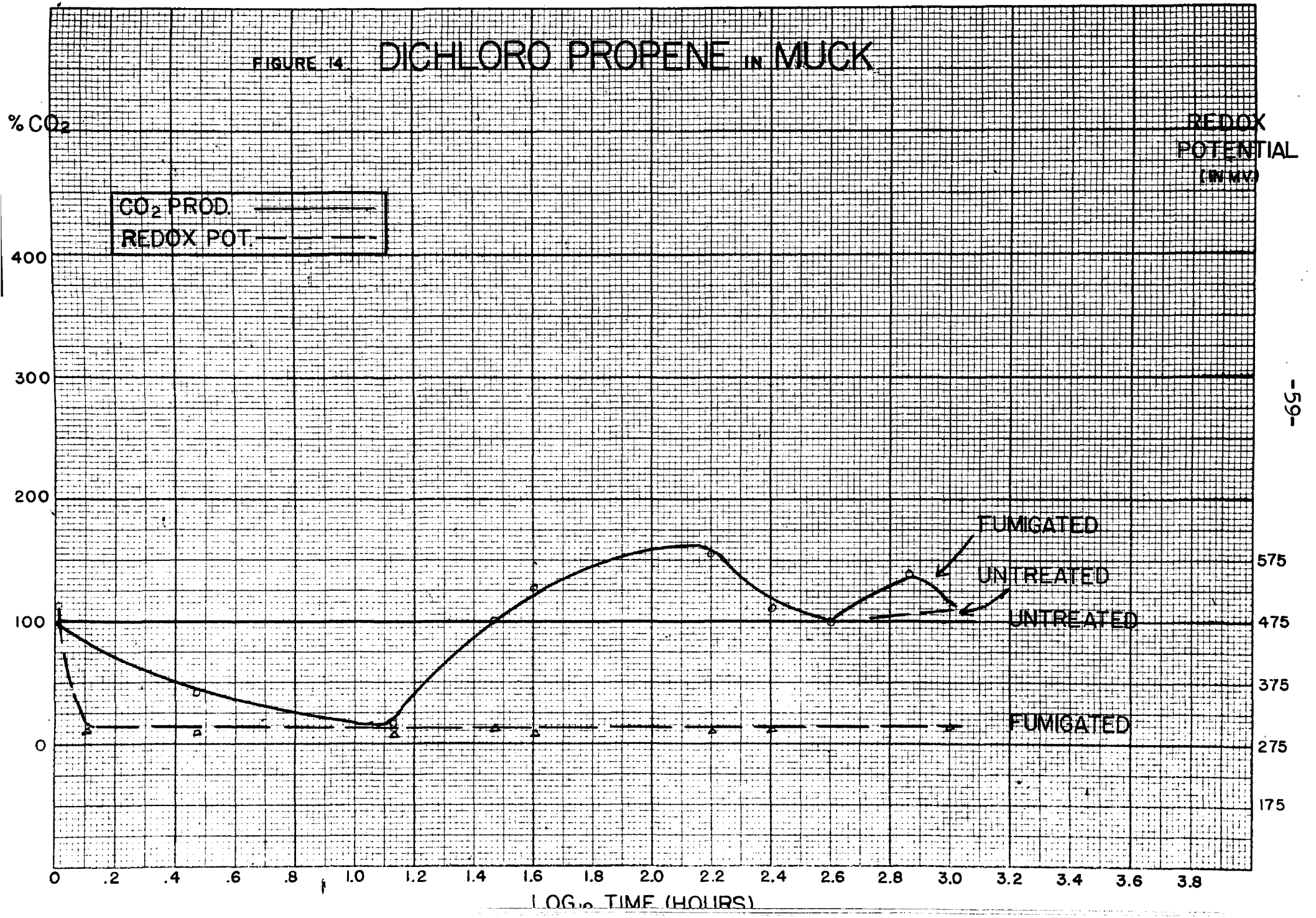


FIGURE 14. DICHLORO PROPENE IN MUCK
% CO_2
REDOX POTENTIAL (IN MV.)
CO_2 PROD.
REDOX POT.
400
300
200
100
0
575
475
375
275
175
FUMIGATED
UNTREATED
UNTREATED
FUMIGATED
0 .2 .4 .6 .8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 3.6 3.8
LOG$_{10}$ TIME (HOURS)

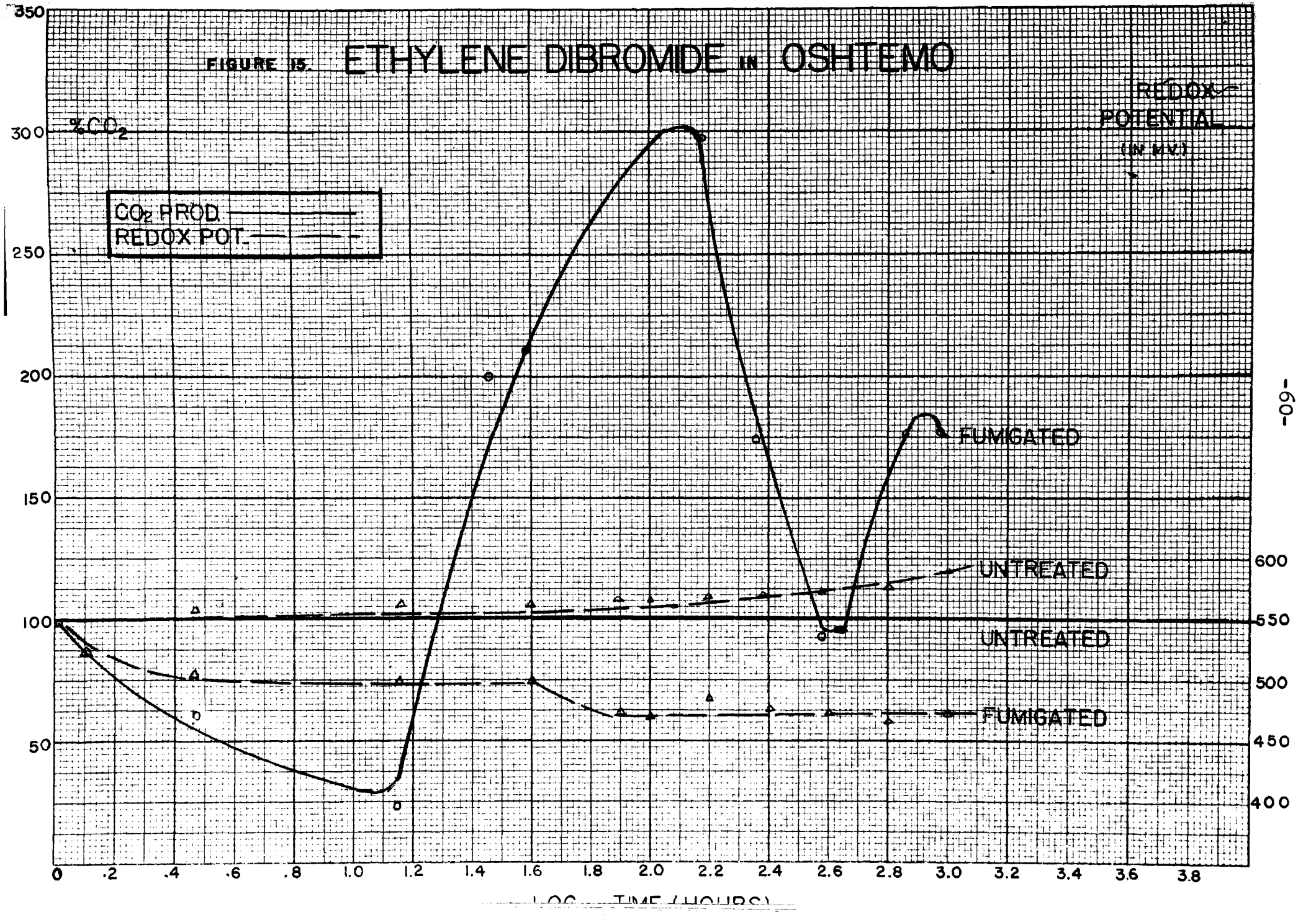


FIGURE 15. ETHYLENE DIBROMIDE IN OSHTEMO
%CO2
REDOX POTENTIAL (IN MV.)
CO2 PROD.
REDOX POT.
FUMIGATED
UNTREATED
UNTREATED
FUMIGATED
350
300
250
200
150
100
50
600
550
500
450
400
0
.2
.4
.6
.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
LOG TIME (HOURS)

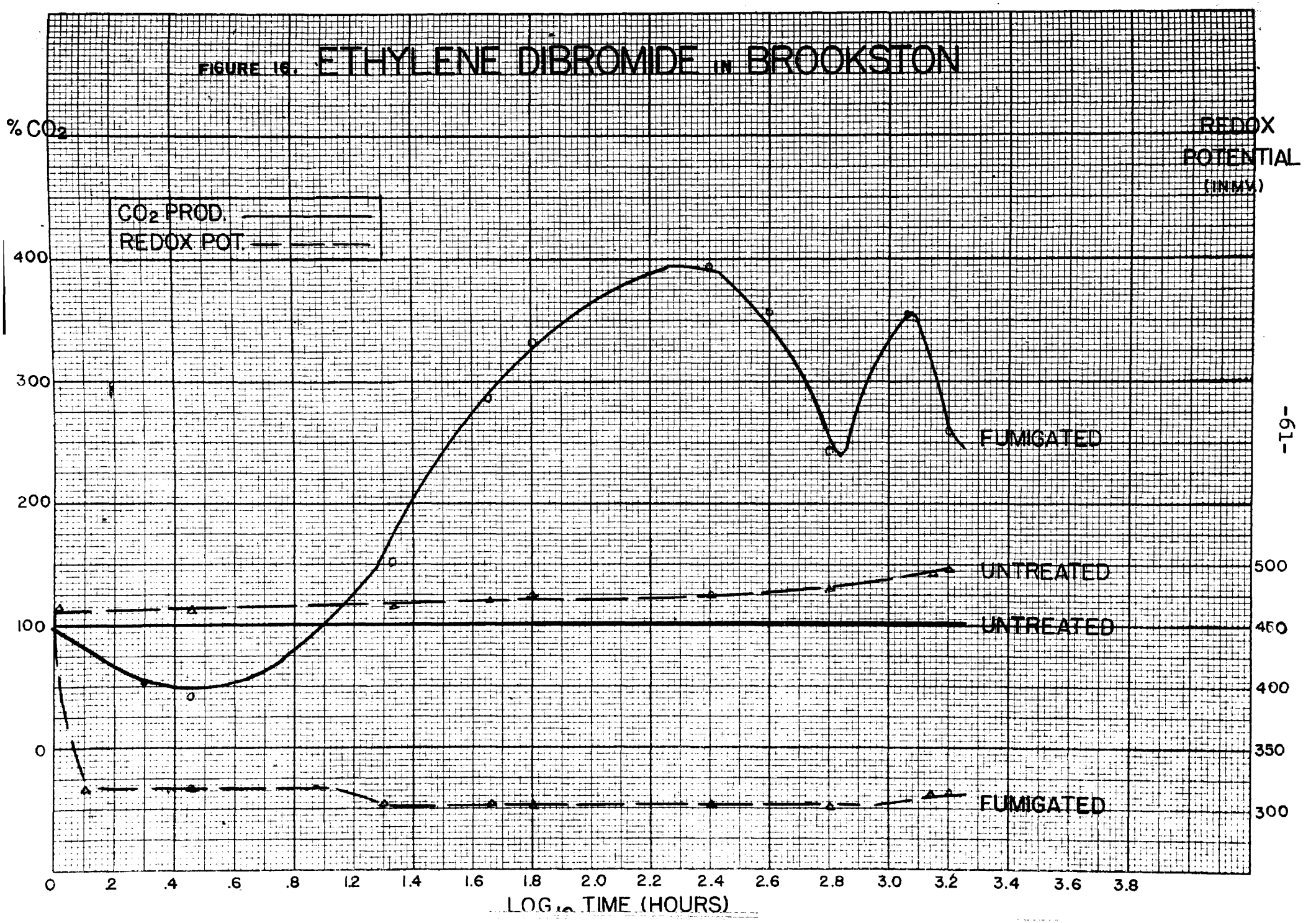


FIGURE 16. ETHYLENE DIBROMIDE IN BROOKSTON
% CO_2
REDOX POTENTIAL (IN MV.)
CO_2 PROD.
REDOX POT.
400
300
200
100
0
FUMIGATED
UNTREATED
UNTREATED
FUMIGATED
500
450
400
350
300
0
.2
.4
.6
.8
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
LOG$_{10}$ TIME (HOURS)

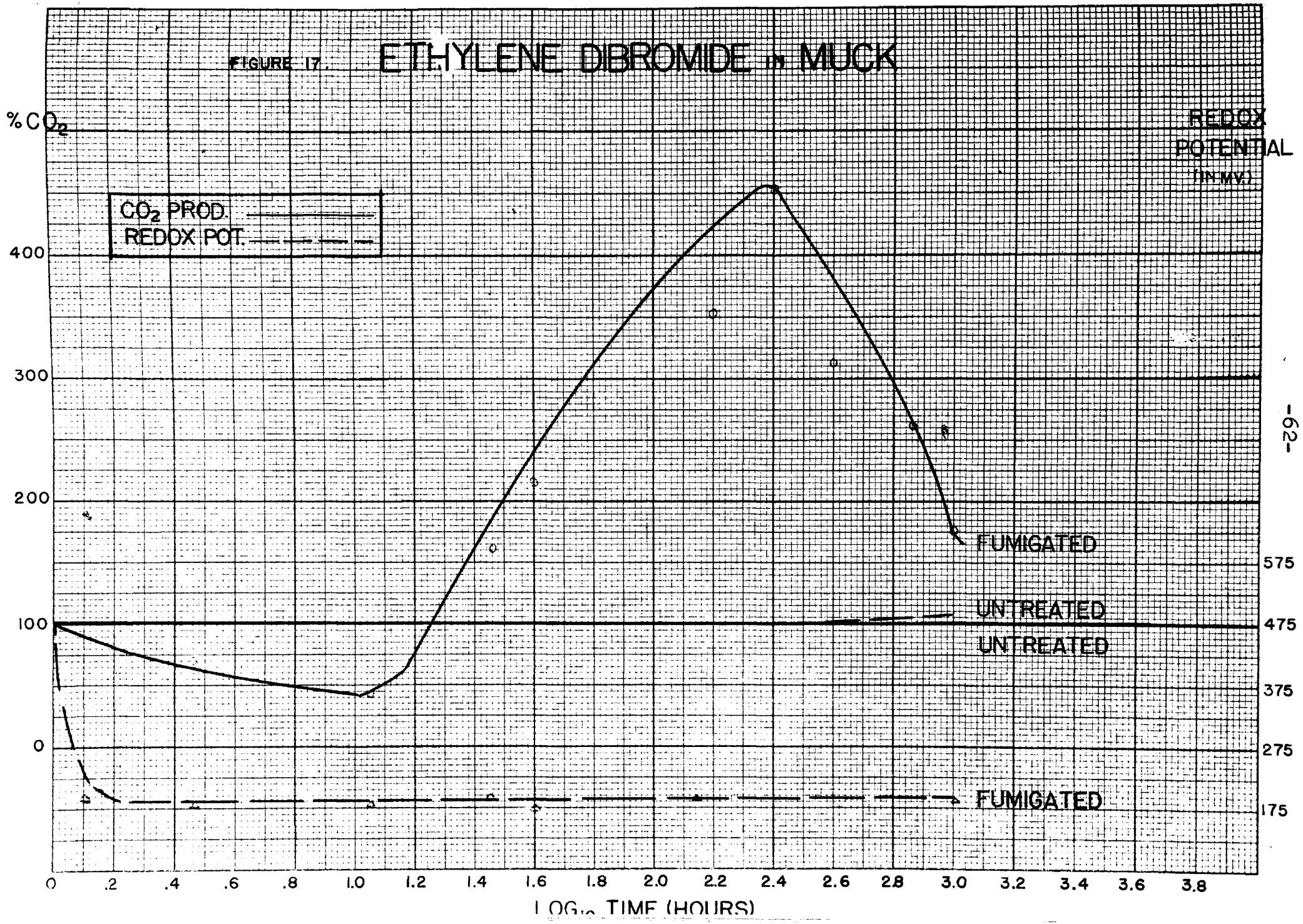


FIGURE 17. ETHYLENE DIBROMIDE IN MUCK
% CO_2
REDOX POTENTIAL (IN MV.)
CO_2 PROD.
REDOX POT.
400
300
200
100
0
575
475
375
275
175
FUMIGATED
UNTREATED
UNTREATED
FUMIGATED
0 .2 .4 .6 .8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8 3.0 3.2 3.4 3.6 3.8
LOG10 TIME (HOURS)

significant difference in the potential drop between fumigants, but the drop was greater in the muck than in the soils containing less organic matter. The drop in potential leveled off followed by a second drop when the carbon dioxide production rose to a maximum. This second drop was accentuated in the Oshtemo soil.

Ammonification and Nitrification in Soils Fumigated With Dichloropropene and Ethylene Dibromide

Because the data on carbon dioxide production and redox potential indicated that there was an increase in microbial activity and that the oxidation-reduction state of the soil was altered after treatment with these two compounds, an attempt was made to determine the effect of these fumigants on the quantities of available nitrogen in the soil and the forms in which this nitrogen existed.

Investigations on the nitrogen nutrition of plants (25) have shown that there are three important factors which must be considered with respect to the nitrogen supplying power of soils. They are, 1, the total amount of nitrogen which can become available to a plant through a period of time, 2, the form of this available nitrogen, and 3, the rate of mineralization of the organic nitrogen or the regeneration of the nitrogen after the supply has been reduced to a minimum because of plant feeding or leaching. In order to study the effect of fumigation on the regeneration factor as well as the others in the

Oshtemo, Brookston, and muck soils, the soils were first leached with distilled water to remove the nitrate nitrogen. All the ammonium nitrogen was also removed except for a small amount which was adsorbed by the colloids. The leaching was accomplished in 500 ml. spherical glass containers with an opening at the bottom. The soils were then immediately brought to the moisture equivalent and fumigated with 0.5 ml. of 1-3 dichloropropene and 1-2 dibromoethane by injection of the compounds into the soil mass 1 cm. below the soil surface. The soils were kept at the moisture equivalent throughout the study by periodically adding enough distilled water to bring the soil and container up to weight.

The soil was then periodically sampled and tested for ammonium and nitrate nitrogen. The testing methods used were those described by Peech and English (32), that is, the use of brucine for the determination of nitrate nitrogen and a modification of Nessler's reagent for ammonium. The determinations were compared with standards in a photoelectric colorimeter. A modification of the sampling and extracting procedure suggested by the above authors was used. Instead of extracting the soil in an air dry condition with sodium acetate extracting solution, it was extracted in the moist state immediately after sampling. It was felt that this would give a truer picture of the state of the ammonium and nitrate in the

soil at the time of sampling because the aeration and time necessary for drying was bound to alter the nitrogen status. Approximately 5 gm. of soil was removed from the container and placed in an Erlenmeyer flask containing 50 ml. of the extracting solution (10% sodium acetate buffered at pH 4.8 with acetic acid). After shaking the flask for two minutes, the contents was filtered through Whatman #40 filter paper. The determinations were made on the extract and the soil remaining on the filter paper was dried in an oven and its dry weight determined. The amounts of ammonium and nitrate were then calculated on the basis of the oven dry weight of the soil.

Table 11 gives the data on the amounts of ammonium and nitrate nitrogen present in the soils after 2 days, 7 days, and then weekly intervals from the time of fumigation. Periodic tests for nitrite nitrogen using the sulfanilic acid-naphthylamine method (19) showed no significant accumulation of this form in any of the soils. The greatest amount recorded was 2 p.p.m. and this only at one week after fumigation in the muck soil. Despite the fact that the brucine test for nitrates included nitrites, its use seemed to be warranted because of its rapidity and accuracy and the fact that nitrite concentrations were very low. It is apparent from the data that treatment with dichloropropene and ethylene dibromide retarded nitrification in all three soils. This resulted

Table 11. Nitrogen regeneration data on leached Oshtemo, Brookston, and muck soils after fumigation with 1-3 dichloropropene and 1-2 dibromoethane. *

Time after fumigation	TREATMENT								
	Untreated			Dichloropropene			Dibromoethane		
	Mg. N per kg. dry soil								
	Total	NH_4	NO_3	Total	NH_4	NO_3	Total	NH_4	NO_3
	OSHTEMO								
0	1.0	1.0	0.0	1.0	1.0	0.0	1.0	1.0	0.0
2 days	6.8	2.0	4.8	17.1	17.1	0.0	9.7	9.7	0.0
1 week	13.1	2.0	11.1	28.3	28.3	0.0	18.3	18.3	0.0
2 "	18.4	2.0	16.4	28.6	27.6	1.0	24.5	24.5	0.0
3 "	17.4	1.0	16.4	30.1	29.1	1.0	21.2	19.4	1.8
4 "	15.3	1.0	14.3	18.1	16.3	1.8	19.3	18.3	1.0
5 "	17.4	1.0	16.4	22.3	21.3	1.0	22.6	21.6	1.0
6 "	23.9	2.3	21.6	18.1	17.1	1.0	18.1	17.1	1.0
7 "	19.4	1.0	18.4	23.1	21.3	1.8	18.1	17.1	1.0
8 "	17.4	1.0	16.4	20.1	18.3	1.8	20.3	18.5	1.8

Table 11. (continued)

Time after fumigation	TREATMENT								
	Untreated			Dichloropropene			Dibromoethane		
	Mg. N per kg. dry soil								
	Total	NH_4	NO_3	Total	NH_4	NO_3	Total	NH_4	NO_3
	BROOKSTON								
0	3.3	2.3	1.0	3.0	2.0	1.0	3.0	2.0	1.0
2 days	5.8	4.0	1.8	32.1	29.1	3.0	23.4	21.6	1.8
1 week	16.4	9.2	7.2	70.3	67.0	3.3	45.2	43.4	1.8
2 "	36.1	8.5	27.6	65.0	60.8	4.2	54.8	51.8	3.0
3 "	48.2	9.0	39.2	72.0	66.5	5.5	57.4	53.2	4.2
4 "	50.3	5.1	45.2	78.1	73.0	5.1	53.7	48.6	5.1
5 "	49.3	7.2	42.1	72.1	67.0	5.1	57.3	51.8	5.5
6 "	48.7	7.2	41.5	65.0	60.8	4.2	58.2	49.4	8.8
7 "	51.7	8.0	43.7	62.3	51.8	10.5	54.5	43.4	11.1
8 "	49.7	7.6	42.1	63.7	53.2	10.5	51.1	40.6	10.5

Table 11. (continued)

Time after fumigation	TREATMENT								
	Untreated			Dichloropropene			Dibromoethane		
	Mg. N per kg. dry soil								
	Total	NH_4	NO_3	Total	NH_4	NO_3	Total	NH_4	NO_3
	MUCK								
0	15.3	12.3	3.0	13.8	10.8	3.0	14.6	11.6	3.0
2 days	31.0	26.2	4.8	53.3	50.2	3.3	46.6	43.6	3.0
1 week	75.9	45.4	30.5	135.8	128.6	7.2	70.0	67.0	3.0
2 "	100.3	55.1	45.2	142.5	131.4	11.1	104.0	99.2	4.8
3 "	121.6	67.0	54.6	132.5	120.4	11.1	120.4	114.1	6.3
4 "	125.7	65.3	60.4	137.7	121.3	16.4	119.4	108.3	11.1
5 "	139.5	59.1	80.4	139.4	131.4	8.0	115.4	96.1	18.3
6 "	139.8	48.8	91.0	130.3	119.2	11.1	126.8	110.4	16.4
7 "	145.3	53.1	92.2	149.0	141.0	8.0	108.3	89.5	18.8
8 "	127.1	41.0	86.1	142.8	132.3	10.5	117.4	105.1	12.3

* Average of two determinations.

in large accumulations of ammonium nitrogen for at least eight weeks after treatment. There was little evidence to indicate that this condition would not prevail for extended lengths of time beyond the eight weeks.

The nitrogen regeneration graphs developed from these data and presented in figures 18, 19, and 20, more clearly demonstrate some of the other relationships. It can be seen that there was a steady rise in the available nitrogen present in the soil after leaching until a maximum was reached. From then on, there was a fluctuation around this maximum with time. This was to be expected and is a function of the dynamic nature of the soil organisms. The height of the curves or the maximum nitrogen made available in any given soil is a partial function of the quantity and nature of the organic matter present. Thus the muck soil regenerated more available nitrogen than the Brookston soil and the latter more than the Oshtemo. However, within the Brookston and Oshtemo soils, fumigation raised the total amounts of available nitrogen somewhat above that present in the untreated soils. The dichloropropene was more effective in accomplishing this than was the ethylene dibromide. This difference in the total available nitrogen was not as apparent in the muck soil. Perhaps of greater significance than the total amount of available nitrogen in the soil as affected by fumigation was the rate of regeneration of

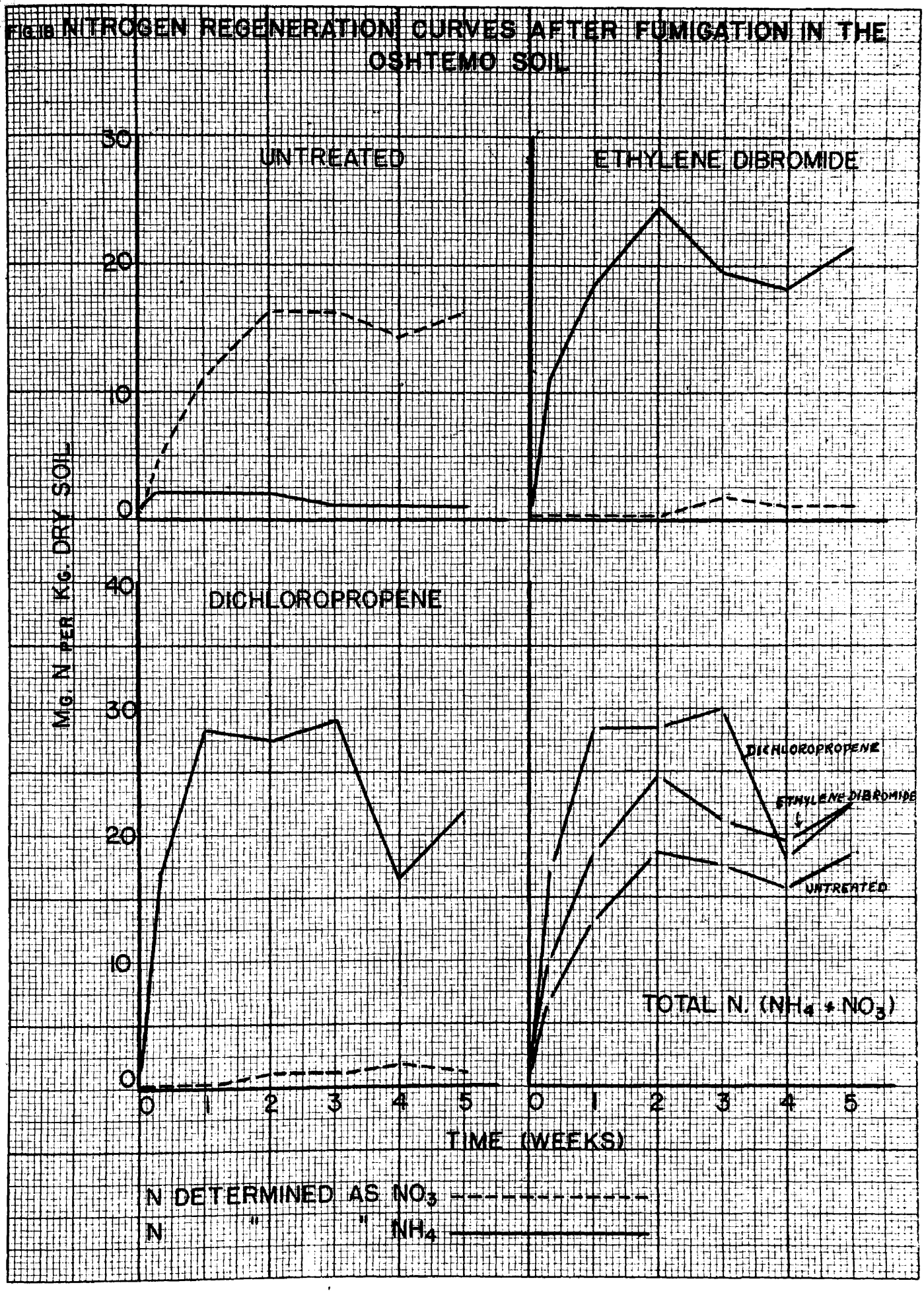


FIG 18 NITROGEN REGENERATION CURVES AFTER FUMIGATION IN THE OSHTEMO SOIL
UNTREATED
ETHYLENE DIBROMIDE
DICHLOROPROPENE
MG. N PER KG. DRY SOIL
0
10
20
30
40
0 1 2 3 4 5
TIME (WEEKS)
DICHLOROPROPENE
ETHYLENE DIBROMIDE
UNTREATED
TOTAL N. (NH_4 + NO_3)
N DETERMINED AS NO_3
N " " NH_4

FIG. 19 NITROGEN REGENERATION CURVES AFTER FUMIGATION IN THE BROOKSTON SOIL

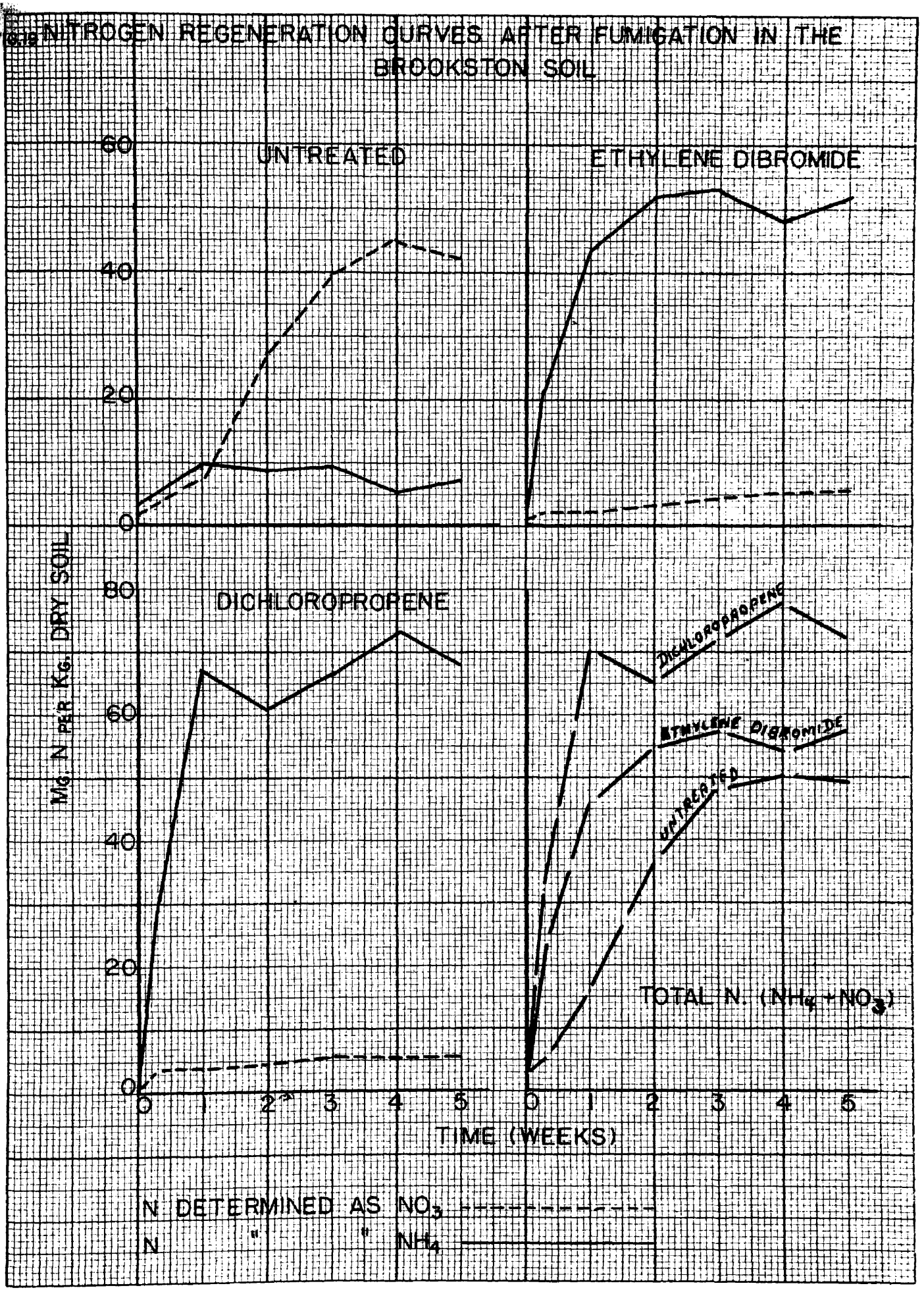
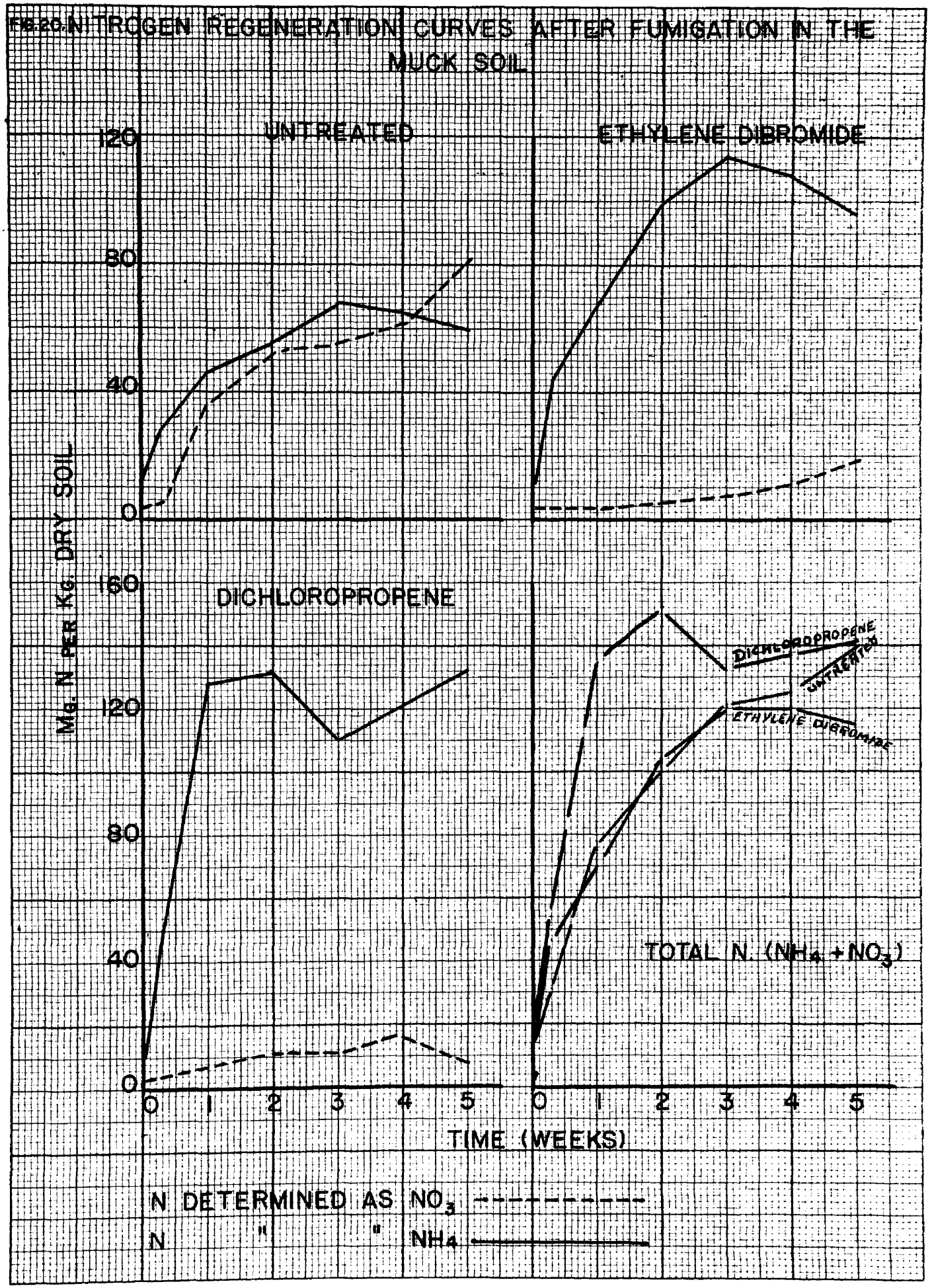


FIG. 20. NITROGEN REGENERATION CURVES AFTER FUMIGATION IN THE MUCK SOIL



this nitrogen. It can be seen from the regeneration curves that slope of the line is much steeper in the curves representing the fumigated soils than in those representing the untreated soils. This rate is especially emphasized in the soils treated with dichloropropene. This accelerated rate of regeneration would probably be of benefit to plant growth if the seeding took place when environmental conditions were not favorable for nitrate production. It is also conceivable that the accelerated rate of regeneration could maintain the supply of readily available nitrogen to the plant as the plant roots take it up from the soil.

The Effect of Fumigation on the Nitrification of Ammonium Sulfate added to Soils

Much of the nitrogen added to the soil as fertilizer is in the form of ammonium sulfate, and it is apparent that fumigation will have its effect on the nitrification of this ammonium and thus affect the form of nitrogen available to plants. An experiment was devised to study the effect of Dowfumes N, W-40, and MC-2 on nitrification of ammonium sulfate added to soils.

Quantities of air dry Oshtemo, Brookston, and muck soils were thoroughly mixed in a rotating drum with enough ammonium sulfate to bring the soils up to approximately 300 mg. of nitrogen determined as ammonium per kg. of dry soil. A quantity of calcium carbonate was added to the

soils to neutralize the effect of the added ammonium sulfate. The soils were then brought to the moisture equivalent and placed in one-gallon glazed pots. The ammonium nitrogen was determined on the moist soil as previously described. The soils were then fumigated with two levels of Dowfume N (equivalent to 40 and 80 gallons per acre), Dowfume W-40 (25 and 50 gallons per acre), and Dowfume MC-2 (1 and 2 pounds per 100 square feet). Duplicate pots were set up. The actual dosages used were 0.8 and 1.6 ml. of N and MC-2, and 0.5 and 1.0 ml. of W-40. The N and W-40 were injected 2 inches under the surface of the soil from a burette while the MC-2 was applied with a "jiffy applicator" under a tar paper cover which was kept sealed for 48 hours. The temperature of the soils at the time of fumigation was 27° C.

After one week, the soils were removed from the pots and sifted through a one-cm. mesh screen. Every effort was made to prevent reinoculation of the soils during this process. The soils were returned to their individual pots. Samples were then taken at 30, 90, and 180 days and analyzed for content of ammonium nitrogen. The soils were maintained at the moisture equivalent with periodic additions of distilled water. The results of the ammonium nitrogen determinations are recorded in table 12.

It can be seen that all the treatments retarded nitrification. More of the added ammonium was recovered

Table 12. The nitrification of ammonium sulfate added to Oshtemo, Brookston, and muck soils, and fumigated with Dowfume N, Dowfume W-40, and Dowfume MC-2. *

Time after fumigation (days)	Mg. N determined as NH_4 per kg. of dry soil. Treatment						
	Untreated	Dowfume N 0.8 ml.	Dowfume N 1.6 ml.	Dowfume W-40 0.5 ml.	Dowfume W-40 1.0 ml.	Dowfume MC-2 0.8 ml.	Dowfume MC-2 1.6 ml.
				Oshtemo soil			
0	310	310	310	310	310	310	310
30	114	300	292	282	304	310	304
90	73	256	295	204	286	290	300
180	51	150	290	110	202	253	304
				Brookston soil			
0	352	352	352	352	352	352	352
30	156	312	324	321	340	336	364
90	84	208	308	207	328	302	346
180	72	142	287	143	195	220	312
				Muck soil			
0	340	340	340	340	340	340	340
30	284	333	364	330	332	382	379
90	111	242	346	214	337	361	358
180	108	183	258	118	211	213	326

* Average of two determinations of samples from each of the duplicate treatments.

from the treated soils than from the untreated soils. This relationship held for the entire incubation period of six months. The efficiency with which the different fumigants retarded nitrification was greater at the higher dosages. Also, Dowfume N and Dowfume MC-2 were more effective in retarding nitrification than was Dowfume W-40. This might be correlated with the ability of the different fumigants to disperse through the soil, though an attempt was made to overcome this difference by screening and stirring the soils after fumigation. The general relationship between the kind of fumigant and rate of application and their effect on nitrification is shown in figure 21. These relationships are plotted for the Oshtemo soil, but it can be seen from the data in table 12 that except for the differences in intensity, they hold for the Brookston and muck soils.

The ammonium recovery was almost 100 percent of the amount of ammonium added six months after fumigation with the higher levels of Dowfumes N and MC-2. It was noted that there was an increase in ammonium in the soils containing great quantities of organic matter after treatment with the above fumigants. This was probably due to the increased ammonification coupled with decreased nitrification after fumigation. In every soil used and with every treatment, it was possible to recover more of the added ammonium six months after fumigation than was

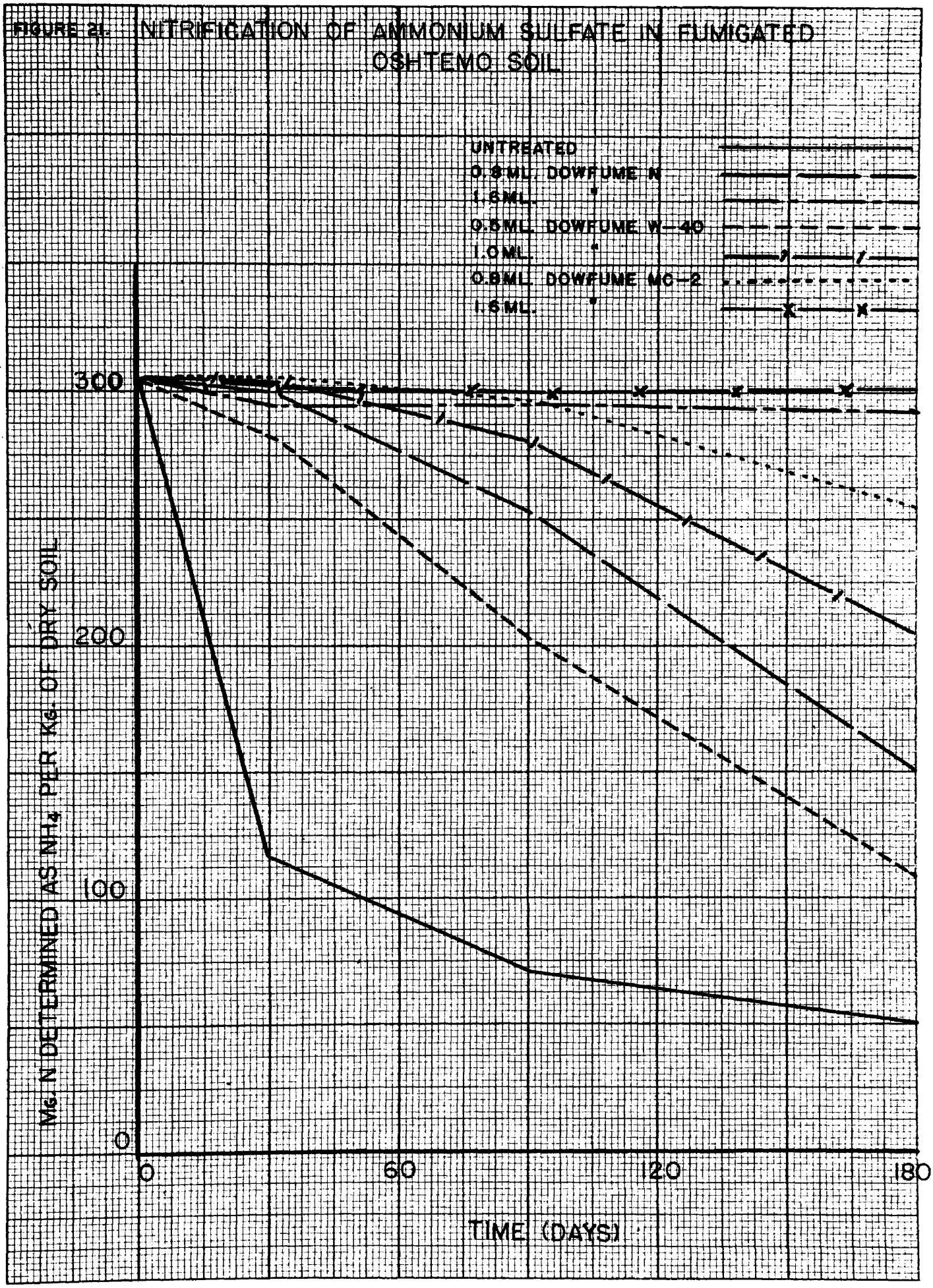


FIGURE 21. NITRIFICATION OF AMMONIUM SULFATE IN FUMIGATED OSHTEMO SOIL

present in the untreated soils.

Field Experiments and Observations

Two 800' x 12' strips of muck soil on the Michigan State College Muck Experimental Farm were fumigated on April 14, 1949. One strip was fumigated with Dowfume N at the rate of 40 gallons per acre and the other with Dowfume W-40 at the rate of 25 gallons per acre. A 12' untreated strip was kept between the two treated strips. Alternate six foot sections the length of the plots were planted to lettuce and Henry spring wheat. The crops were harvested and no significant differences in yield were recorded between those grown on the treated and untreated plots.

On Nov. 4, 1949, 400' sections of the same plots were refumigated with the same fumigants at the same rate of application. No nitrogen was included in the fertilization program and Henry spring wheat and Flambeau soybeans were planted in June, 1950 in alternate six foot strips running the length of the plots. During the growing season, periodic samples were taken from the treated and untreated plots and ammonium and nitrate determinations were made on them. The plots treated with Dowfume N had a slightly higher ammonium content than did the Dowfume N and untreated plots. Observation of the soybeans during their growth period showed that the plants on the Dowfume N

treated soil were making better growth and were greener than the plants on the Dowfume W-40 treated and untreated soils. The differences between the plants on the Dowfume N treated and untreated plots can be seen in plates 6 and 7.

The soybeans were allowed to dry on the vine and the bean yields obtained are reported in table 13.

Table 13. Yield of soybeans on muck soil fumigated with Dowfume N and Dowfume W-40.

Treatment	Date of treatment	
	April, 1949	November, 1949
untreated	16.2 bu. / acre	16.4 bu. / acre
Dowfume N	21.1 bu. / acre	27.4 bu. / acre
Dowfume W-40	15.9 bu. / acre	18.1 bu. / acre

The soil treated with Dowfume N on November, 1949 yielded 68 percent higher than the untreated soil. A possible explanation of this remarkable increase in yield may be deduced from the fact that all crops grown on the muck farm that year showed a tremendous response to additions of nitrogen to the soil. The weather conditions that year were wet and cool far into the growing season which would retard mineralization of the soil organic nitrogen.

Plate 6. Soybeans on right on muck soil treated with Dowfume N. Plants on left on untreated soil.

Plate 7. Soybean plants on left removed from Dowfume N treated plots. Plants on right removed from untreated plots.

Similar responses were obtained for onions planted on muck soil which was fumigated in November, 1950. Fumigation was at the same rate as mentioned above, and the plots were 12' X 200' with an untreated plot separating the fumigated areas. Periodic sampling of the soil gave indications of a higher ammonium content in the Dowfume N treated soil than in the Dowfume W-40 and untreated soils. The ultimate yield of onions is presented in table 14.

Table 14. Yield of onions on muck soil fumigated with Dowfume N and Dowfume W-40.

Treatment	Yield of onions
Untreated	282 bu. / acre
Dowfume N	327 bu. / acre
Dowfume W-40	290 bu. / acre

The Dowfume N treated area yielded 16 percent more onions than did the others. No nitrogen was added to the soil in the fertilization program, but a response attributed to a greater quantity of nitrogen available to the plants can be seen in the greener color of the onions on the Dowfume N treated plot (plate 8).

These soils were not fumigated to control any pathogens. There was no history of crop reverses on these soils due to an infestation of organisms and

Plate 8. Eight rows of onions on the right (two sections) on muck soil fumigated with Dowfume N. Center two sections untreated and onions on the left fumigated with Dowfume W-40.

examination of the plant roots did not give any indications that growth restriction on any of the plots was due to nematodes or other organisms. Though the level of available nitrogen in the Dowfume N treated soils was not materially higher than in the other soils, analysis of the plant sap showed significantly higher concentrations of ammonium in those plants grown in the Dowfume N treated areas. It is well known that the relative level of available nitrogen in the soil is not too indicative of crop response since the plants are constantly taking this nitrogen up from the soil.

Other factors affecting the growth of plants after fumigation can also be attributed to the ammonium and nitrate relationships in the soil after treatment. In order to control an infestation of nematodes in his muck soil, an onion grower in Gun Swamp near Plainwell, Michigan fumigated his soil with Dowfume N at the rate of 40 gallons per acre. The soils were fumigated in the fall of the year and were planted to onions the following spring. Narrow strips of soil were left untreated as a control. Observations made on the onions from the time of planting on through the growing season showed that the onions on the fumigated soils were making better growth and that they had a deep green color. Plate 9 shows this onion as it appeared the first part of August. Note that the

Plate 9. Onion field fumigated with Dowfume N. Light streak through the center of the picture is an untreated area.

onions on the untreated soil are a lighter green color than those on the fumigated soil. The ammonium nitrogen concentration in the fumigated soil averaged 34 p.p.m. while that in the untreated soil averaged 6 p.p.m. at the time this picture was taken. The nitrate nitrogen content of both the treated and untreated soils averaged 58 p.p.m. Examination of the roots of the plants in the untreated areas did not show an extensive nematode infestation so the differences in growth were attributed to the difference in ammonium nitrogen content of the soils.

Towards the middle of August, the onions in the untreated areas began to surpass those in the fumigated areas in vegetative growth. Color differences, though still noticeable, were less distinct. Plate 10 shows typical onions taken from the treated and untreated soils at that time. Protein analysis on composite samples taken from these plants showed that those grown on the fumigated soil had 27.3 percent protein on a dry weight basis compared with 19.2 percent for those grown on the untreated soil. The final yield of onions on the treated plots was materially lower than on the untreated plots though there was no difference between the two in the thickness of stand. The onions on the treated soil matured earlier than the others and did not put on normal bulb growth. This was attributed to the large amount of

Plate 10. The onions on the right are from muck soil fumigated with Dowfume N. Those on the left were taken from untreated soil.

ammonium available to the plants throughout the growing season.

DISCUSSION

It has long been considered that the quantities of carbon dioxide produced in a soil during a given period of time is an index of the activity of micro-organisms in the soil. In the soils treated with dichloropropene and ethylene dibromide the carbon dioxide produced by the soil organisms was increased after a short initial period of depression. This would indicate that the compounds are toxic to selected species of organisms, and those which are not affected increase their activity because of the decreased competition and antagonism of the other species. It seems highly probable that it is the oxidizing organisms which are most affected by these fumigants. One indication of this is the fact that the bio-electric potential in the soils was lowered after the soils were treated. It could thus be considered that the soils were in a "more reduced" state after fumigation with these compounds. The length of time which the abnormally high carbon dioxide production persisted in the soil was a function of the quantity of organic matter present in the soil but the reduced bio-electric potential persisted after the carbon dioxide production began to approximate that of the untreated soil. A lag in the return of the potential to normal is always to be expected, but in this case, there is evidence to indicate

that though the microbial activity does not indefinitely maintain its induced maximum, the new biologic equilibrium persists for an indefinite length of time. Evidence to substantiate this fact exists in the great quantities of the reduced form of nitrogen (ammonium) which accumulated and persisted in the soils. The reports of various investigators showing that iron and manganese have become more available to plants after soil fumigation with various compounds could also be explained as a result of the lowering of the redox potential in the soil since it is known that both these elements are more available to plants in their reduced forms (23, 24).

In all cases where the soil was fumigated with either dichloropropene or ethylene dibromide, subsequent mineralization of the soil organic matter produced quantities of ammonium nitrogen which were not oxidized to the nitrate form. This has important implications as to the subsequent growth of plants in these treated soils. The total amount of available nitrogen was also increased though perhaps not significantly so. However, in all cases where the soils were treated the rate of regeneration of the available nitrogen was materially increased. It is felt that this fact is of great importance with respect to plant growth. In the temperate climate regions, many of the annual crops are planted in the soil at a time when the available nitrogen in

relatively high (the total amount depends upon the environmental conditions and the amount of organic matter present in the soil). This, for the most part, is due to the relatively active mineralization of the organic matter during the warm days of early spring when there is little or no vegetation to take up the nitrogen. However, when the seedlings start growing, they rapidly remove this available nitrogen and continued growth depends upon the ability of the soil to release more available nitrogen. If the rate of mineralization is too slow, more nitrogen must be added to the soil in the form of chemical fertilizer to carry the plant through. With this in mind, it can be seen that if the rate of mineralization of the organic matter could be increased, there would be an ultimate benefit to crop growth. Dichloropropene was most successful in increasing the rate of nitrogen release in the soils used. Ethylene dibromide was less successful but better than the untreated soils except in the case of the muck soil where the great quantities of organic materials present seemed to negate the advantage. An increased rate of nitrogen release would also be beneficial at times when a cold, wet spring retarded microorganism activity and the available nitrogen was leached out of the soils during winter fallow. These phenomena were demonstrated with the increased growth of soybeans and onions on the

fumigated muck soils of the Michigan State College Experimental Farm during 1950.

The use of Dowfume N, W-40, and MC-2 in soils effectively decreased the rate of nitrification of ammonium sulfate added to soils. The high levels of Dowfume N and Dowfume MC-2 (1.6 ml. per gallon crock) practically eliminated nitrification for at least six months. Lower doses of these two fumigants and high and low levels of Dowfume W-40 inhibited nitrification for lesser periods of time. This inhibition becomes important when it is realized that a large portion of the mineral nitrogen now added to soils in the form of chemical fertilizers is added in the ammonium form. In normal untreated soils, it is to be expected that most of the added ammonium will be converted to nitrates within a relatively short period of time. In a soil fumigated with these compounds, the added ammonium will remain in its original form for much longer periods of time. Since the compounds were all effective to a certain degree in inhibiting nitrification, it could be concluded that probably the differential effectiveness between compounds and also between dosages was due to the varying abilities of the different compounds to disperse through the soil and saturate it with the vapors. It is to be expected that the oxidizing organisms which are not reached by the fumigant will eventually begin to

multiphy and increase their effectiveness. However, the differential effectiveness could also possibly be attributed to a difference in the severity of action or toxic properties of the different fumigants.

PART III

Calcium and Nitrogen Relations with Respect to Plant Growth

It was shown that fumigation with the compounds used in this study retarded nitrification and favored the accumulation of ammonium in the soil. The question now arises as to how plant growth would be affected with its primary source of nitrogen in this reduced form.

LITERATURE SUMMARY

Tam, Tam and Clark (48, 49) grew pineapple plants on soils fumigated with D-D mixture, chloropicrin and other disinfectants. With ammonium as the chief source of nitrogen, the plants were characterized as having high N, were dark green, fast growing, and succulent. In connection with this, it is interesting to note that ammonium requires no reduction before it can be utilized in protein synthesis while the nitrate ion must first undergo reduction. However, an adequate supply of carbohydrate in the plant is essential for protein synthesis when ammonium is the source of nitrogen. Carbohydrate does not appear to be as limiting a factor when the nitrogen is supplied in the form of nitrate (44, 53, 54). Though it had long been considered that only high pH values allowed for adequate ammonium assimilation, Arnon and Johnson (6) among others have shown that the hydrogen

ion concentration has no effect on ammonium or nitrate assimilation provided that there is no deficiency of other essential elements.

Concerning ionic relationships in plants, Bear (8) considers that total cation and total anion uptake are each equal to a constant, and he suggests that substitution of ammonium for nitrate may lower the intake of mineral cations and increase that of mineral anions. Arnon (5) in a study on the mineral composition of barley when supplied with ammonium or nitrate as nitrogen sources found that the ammonium-supplied plants had a lower base content but were higher in phosphate than the plants whose nitrogen source was nitrate. Soybeans were grown by Hamner (21) in a culture containing large amounts of phosphorus and little nitrate. The plants showed typical phosphorus toxicity symptoms, but this condition could be alleviated with small additions of nitrate to the culture. These and many other studies including one by Sideris and Young (45) using pineapple plants indicate that cation and anion balance are most important in plant nutrition. They suggest a need for high levels of potassium and other cations when the source of nitrogen to the plant is in the form of ammonium. Prianischnikov (34) showed that high levels of calcium supplied to cotton plants whose source of nitrogen was ammonium allowed the plants to grow extremely well while

lower levels of calcium retarded growth. An interesting effect of ammonium nutrition was cited by Schropp and Arenz (42) who noticed that in certain instances, ammonium was actually excreted from the plant roots unless the level of potassium was sufficiently high.

An excellent summary of the various aspects of the nitrogen nutrition of green plants is presented by Nightingale (29) where additional nitrogen relationships such as are affected by oxygen supplied to the plant roots and photoperiodic effects are elaborated. An important related factor was brought out by Burstrom (13) who concluded that in wheat leaves, nitrate reduction depends upon the photosynthetic process while ammonium can be elaborated in darkness.

The effects of carbohydrate content of plants on ammonium and nitrate assimilation and elaboration has already been mentioned. From this and other data, Nightingale (29) concluded that ammonium nutrition results in a rapid depletion of the carbohydrate reserves of a plant if environmental conditions are not favorable for carbohydrate accumulation. An additional consideration, however, is the fact that ammonium assimilation is more rapid than that of nitrate if an adequate supply of oxygen is present and sufficient quantities of other cations are available to the plants. The considerations might explain why Afanaseva (1) found that sterilization

of soils with formalin decreased the time of wheat ripening by four to five days.

EXPERIMENTAL

Growth of Soybeans in Sterile Sand Culture Supplied with Different Sources of Nitrogen and Varying Levels of Calcium

In order to determine the effect of different forms of nitrogen on the growth of soybeans under conditions where there would be no microbial oxidation or reduction of the forms supplied, the following experiment was devised.

Sterile nutrient solutions were added to liter Erlenmeyer flasks containing quartz sand which had been autoclaved at 15 pounds pressure for two 1½ hour periods with a 24 hour interval between treatments. The nutrient solutions contained salts recommended by Ellis and Swaney (17) for the soilless growth of plants. Soybeans were germinated by the vertical paper towel method under sterile conditions and when the plumules began to emerge from between the cotyledons, the seedlings were transferred to the Erlenmeyer flasks and kept in place with a cotton plug. The cultures were aerated periodically by forcing air through the solution through a glass tube which was kept in place in the flask and extended to the bottom. Distilled water was also added through these

Table 15. Sterile nutrient solutions in which soybeans were grown.

Solution	Form of N	ppm N*	ppm Ca	ppm K	ppm Mg	pH	O.P.**
1	Nitrate	120	50	100	100	4.6	1.2
2	Nitrate	120	100	100	100	4.6	1.3
3	Nitrate	120	150	100	100	4.6	1.35
4	Ammonium	120	50	100	100	4.5	1.25
5	Ammonium	120	100	100	100	4.5	1.3
6	Ammonium	120	150	100	100	4.5	1.35
7	Amino acids***	120	50	100	100	3.8	1.50
8	Amino acids	120	100	100	100	3.8	1.55
9	Amino acids	120	150	100	100	3.8	1.60

* Determined as the proportion of N by weight in the ion or compound.
** Osmotic pressure in atmospheres.
*** 50% glycine and 50% aspartic acid by weight.

tubes in order to keep the solution levels constant.

The nutrient solutions as indicated in table 15 were so made up that the variables were the form of nitrogen and the levels of calcium. Among the other cations, the levels of potassium and magnesium were kept constant. Phosphorus was also present in the solutions and the remainder of the anions were primarily sulfates and chlorides. Equal amounts of micro elements were added to each solution in the form of ferric sulfate, manganous chloride, boric acid, and copper sulfate.

The flasks containing the soybeans were then placed in a green house. There were three replications of each nutrient solution. Plates 11 through 14 show pictures of the soybeans after 1, 2, 3, and 4 weeks of growth. It can be seen that after one week's growth, there was no visible difference between the plants receiving the ammonium and those receiving the nitrate. The plants growing in the amino acid solutions were definitely retarded in growth and had a deeper green color than the others. There was no indication of differences due to the different levels of available calcium. After two week's growth, the plants on the nitrate substrate began to grow more rapidly than those on ammonium and chlorosis was beginning to show up in the leaves of the ammonium-supplied plants at the lowest level of calcium. After three weeks, the nitrate supplied plants

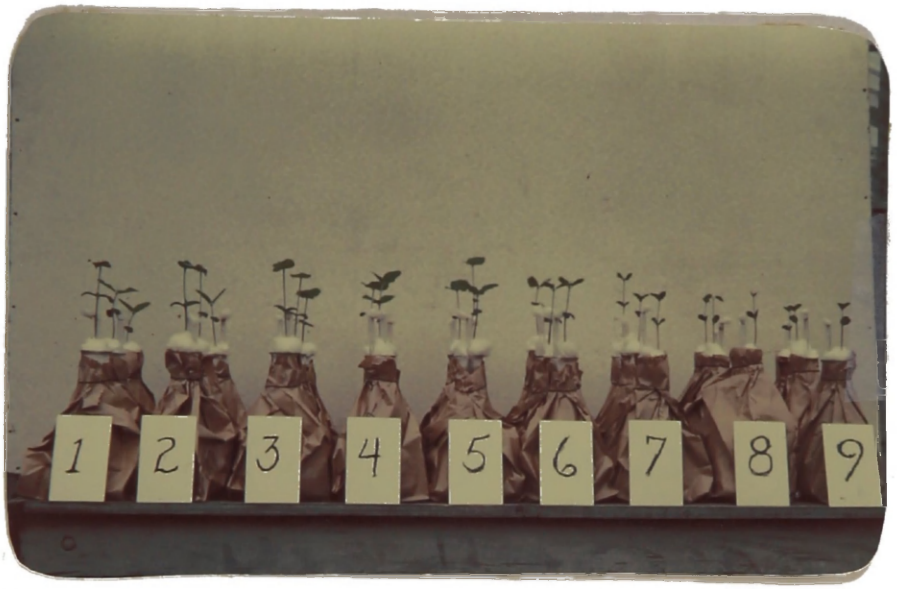


Plate 11. Soybeans in sand culture one week after transplanting. 1. nitrate and 50 ppm Ca, 2. nitrate and 100 ppm Ca, 3. nitrate and 150 ppm Ca, 4. ammonium and 50 ppm Ca, 5. ammonium and 100 ppm Ca, 6. ammonium and 150 ppm Ca, 7. amino acids and 50 ppm Ca, 8. amino acids and 100 ppm Ca, 9. amino acids and 150 ppm Ca.

Plate 12. Soybeans in sand culture two weeks after transplanting. Same legend as plate 11.

Plate 13. Soybeans in sand culture three weeks after transplanting. Same legend as plate 11.

Plate 14. Soybeans in sand culture four weeks after transplanting. Same legend as plate 11.

continued a vigorous growth pattern while those on the ammonium substrate had put on no new vegetative growth since the previous week. They also showed severe chlorosis at the lower levels of calcium and incipient chlorosis at the highest level. The amino acid supplied plants, however, began to put on some vegetative growth and were of a deeper green color than the other plants.

At this time, solution samples were withdrawn from the cultures and tested for the presence of nitrate and ammonium. There was no change in the nitrogen status of those solutions which were made up of ammonium or nitrate but the amino acid solutions indicated the presence of approximately 20 p.p.m. nitrogen in the form of ammonium. Since there was no evidence of microbial activity in these solutions, the presence of ammonium was attributed to acid hydrolysis of the amino acids which yielded the ammonium. Thus, the plants in the amino acid cultures could be considered to be on "low level" ammonium nutrition.

After the fourth week of growth, the nitrate supplied plants were lush and vigorous while there was complete necrosis in the ammonium supplied plants. The only green tissue visible on the latter was on those supplied with the highest level of calcium. The amino acid-supplied plants (low level ammonium) were growing well, had a deep green color, and had begun to blossom and set pods.

After four weeks, the plants were cut and the tissue

was analyzed for protein content. Portions of the tissue were ashed and the ash analyzed for calcium, potassium, and magnesium. The protein analyses were run using a modification of the Kjeldahl process and calcium and magnesium were determined by the methods accepted by the A. O. A. C. (4). The potassium determinations were made with the use of a flame photometer. The results of these analyses are presented in table 16.

It is interesting to note that in the nitrate supplied plants the calcium content increased with an increase in calcium in the substrate. The increase in calcium was accompanied by a decrease of the potassium and magnesium. The higher levels of calcium in the nutrient solution also produced more vegetative growth. There was little difference in the protein content of the plants at the different calcium levels.

The ammonium supplied plants had a very high protein content which was almost double that of those supplied with nitrate. However, plant growth was at a minimum. This could be attributed to the fact that great quantities of carbohydrate were necessary for the synthesis of protein under the given conditions of ammonium nutrition. The percentages of protein decreased slightly as the calcium in the substrate was increased. In these instances just as when the plants were supplied with nitrate, an increase in the calcium content resulted in a decrease in the potassium and magnesium content. However, at the lowest levels of

Table 16. Composition of Soybeans Grown in Sterile Sand Culture with Different Forms of Nitrogen and Three Levels of Calcium*

Solution	Dry wt. of tops (grams)	% Protein	Cations in plant ash (grams)					
			Ca	% Ca	K	% K	Mg	% Mg
1. Nitrate: 50 p.p.m. Ca	1.167	16.78	.00493	.42	.00423	.37	.00360	.31
2. Nitrate: 100 p.p.m. Ca	1.348	17.74	.00585	.44	.00257	.19	.00301	.22
3. Nitrate: 150 p.p.m. Ca	1.484	17.10	.01099	.74	.00225	.15	.00198	.13
4. Ammonium: 50 p.p.m. Ca	.109	34.1	.00030	.29	.00049	.45	.00045	.41
5. Ammonium: 100 p.p.m. Ca	.103	27.9	.00038	.37	.00046	.44	.00040	.39
6. Ammonium: 150 p.p.m. Ca	.167	24.9	.00071	.43	.00040	.24	.00062	.37
7. Amino acids: 50 p.p.m. Ca	.727	19.46	.00353	.48	.00125	.13	.00115	.12

Table 16. (continued)

Solution	Dry wt. of tops (grams)	% Protein	Cations in plant ash (grams)					
			Ca	% Ca	K	% K	Mg	% Mg
8. Amino acids: 100 p.p.m. Ca	.535	19.92	.00240	.45	.00101	.19	.00072	.13
9. Amino acids: 150 p.p.m. Ca	.615	19.70	.00341	.56	.00088	.14	.00080	.14

* Composite Analysis of the Three Replications.

calcium in the nutrient solution, there was no more calcium in the plant ash than was present in the seed. It can be concluded that at the low level of calcium which was supplied to the plants and with ammonium as the source of nitrogen, little or no calcium was taken up by the roots. Observations made during the growth of the plants and the fact that there was some calcium taken up by the plants at the highest level of supply (150 p.p.m.) would tend to indicate that the plants would show better growth if their source of nutrients was higher in calcium content.

The plants which were supplied with amino acids (low level ammonium) contained approximately 18 percent more protein than did those supplied with nitrate and matured earlier. However, they did not put on as much vegetative growth as the latter. The relationship between the amount of calcium supplied and that determined in the plant ash was not as precise as in the previous cases but, this could be attributed to the fact that the amount of ammonium available to the plants was an unknown quantity and undoubtedly varied from flask to flask. The cation relationships in the plant ash however, was the same as in the nitrate and ammonium-supplied plants.

Recalculation of the mineral content of the plants on the basis of the milliequivalents of each cation determined in the analyses brings out some interesting relationships. (table 17)

Table 17. Cation contents of soybeans grown in sterile sand cultures with different forms of nitrogen and three levels of calcium with the data presented in terms of milliequivalents per 100 grams of dry matter.

Solution	Milliequivalents			
	Ca	K	Mg	Total
1. Nitrate and 50 ppm Ca	214	95	250	559
2. " " 100 " "	285	48	185	518
3. " " 150 " "	370	38	108	516
4. Ammonium and 50 ppm Ca	138	110	339	587
5. " " 100 " "	185	107	320	612
6. " " 150 " "	220	60	312	592
7. Amino acids and 50 ppm Ca	248	43	132	423
8. " " " 100 " "	280	41	112	433
9. " " " 150 " "	293	36	109	438

The uptake of calcium, potassium, and magnesium by the nitrate supplied plants was approximately equal to a constant with the potassium and magnesium showing proportional decreases as the calcium increased. The cation constant of the ammonium supplied plants was higher than that of the nitrate supplied plants. This could probably be attributed to the fact that the plants did not put on much vegetative growth and the carbohydrate content was materially decreased. Where the nitrogen source was amino acids (low level ammonium), the cation constant was lower than in the nitrate supplied plants. This would tend to indicate that

ammonium enters into the relationship to the extent of decreasing the uptake of the other cations and would signify that greater quantities of available mineral cations are necessary for the "normal" growth of soybeans when their source of nitrogen is ammonium.

The Relationship of Calcium to Soybean Growth on Fumigated Soils

It was apparent that ammonium affected the mineral uptake of plants and that higher levels of calcium aided plant growth, thus, an experiment was set up to determine the effect of a high level of calcium on the growth of soybeans in fumigated soils.

A soil was composited consisting of 50 percent clay loam and 50 percent by volume of an acid peat (pH 5.6). The soil was placed in one gallon crocks and a quantity of ammonium sulfate equal to 100 p.p.m. N on a dry weight soil basis was added to each pot. In addition to this, 300 p.p.m. of calcium in the form of calcium chloride was added to every second crock and the soil was thoroughly mixed. The soils were then brought up to the optimum moisture and four replicates at the high and four at the low calcium levels were treated with Dowfumes N, W-40, and MC-2 at the rate of 1.6, 1.0, and 1.6 ml. per gallon crock respectively. Analyses for exchangeable mineral contents of the soils indicated that they all contained approximately 37 p.p.m. potassium and 49 p.p.m. magnesium. The soils to which no

calcium had been added contained approximately 74 p.p.m. calcium and those which received the supplementary calcium contained approximately 375 p.p.m. The soils were allowed to stand for one month after the treatment and were then screened and returned to the crocks. Analyses for available nitrogen showed that in the treated soils, the nitrogen was predominately in the form of ammonium, while in the untreated soils it was predominately in the form of nitrate. The pH of all the soils was 5.8.

Soybeans were planted in the soils and after germination, they were thinned to 3 plants per crock. It was obvious from observations made on the plants during the growth period that those on the fumigated soils with the high level of calcium were making better growth than those plants on the fumigated soils with low calcium or on the untreated soils with either high or low calcium. The growth of the plants on the soils high in calcium and treated with Dowfume N was especially pronounced (plate 15). However, the growth of the plants on the treated, low calcium soils seemed to be less in comparison with those on the untreated soils. Some of the plants on the low calcium, treated soils began to blossom at the end of six weeks growth. There was no evidence of blossoming on the other plants. At this point, the plants were harvested and analyzed for protein and mineral content. The results of these analyses are given in table 18.

Table 18. Analyses of Soybeans Grown on Fumigated Soils at Different Calcium Levels.*

Treatment	Dry wt.** (grams)	% Protein	Ca. Mg/gm.	Ca. Me.***	K Mg/gm.	K Me.	Mg. Mg/gm.	Mg. Me.	Total Me.
Untreated	3.27	16.3	1.10	550	.49	122	.36	300	97.2
Untreated - 300 ppm Ca	3.61	16.5	1.20	600	.44	112	.30	250	96.2
1.6 ml. Dowfume N	2.74	20.7	.79	395	.42	105	.28	236	73.6
1.6 ml. Dowfume N - 300 ppm Ca	4.93	17.9	.91	455	.34	85	.23	190	73.0
1.0 ml. Dowfume W-40	2.64	21.3	.85	425	.50	125	.34	283	83.3
1.0 ml. Dowfume W-40 - 300 ppm Ca	3.84	17.6	1.01	505	.36	90	.30	250	84.5
1.6 ml. Dowfume MC-2	2.59	21.8	.82	410	.41	103	.31	258	77.1
1.6 ml. Dowfume MC-2 - 300 ppm Ca	4.41	18.1	.96	480	.39	98	.27	225	80.3

* Average of 4 replications
** Average wt. of 3 plants per crock
*** Me. calculated on the basis of 100 grams of dry plant tissue

Plate 15. Soybeans grown on soil fumigated with Dowfume N. 1. Treated, low calcium soil. 2. Untreated, high calcium soil. 3. Treated, high calcium soil.

It is obvious that the plants on the treated soils contained more protein than did those on the untreated soils. However, the dry weights of these plants were significantly lower when the calcium level in the soil was low. (See analysis of variance in table 19 for difference in weight necessary for significance.) Yet, when the calcium level in the soil was high, the plants on the treated soils put on more growth in addition to containing a greater percentage of protein than those on the untreated soils. This was especially true where the soil was fumigated with Dowfume N. Those plants grown on the soils fumigated with Dowfume W-40 did not have a

Table 19. Analysis of variance of dry weight of soybean plants grown on fumigated soils with low and high calcium levels.

Source	D.F.	S.S	M.S.	F
Total	31	22.81		
Replications	3	.06	.02	.22
Treatments	7	20.80	2.97	33.00 *
Error	21	1.95		

* Significant beyond the 1% level. Application of the "t test" indicated that the differences between the averages of the dry weight of the plants necessary for significance at the 1% level was 0.31 grams.

significantly greater amount of dry matter than did those on the untreated soils when a comparison is made between them on the high calcium level. The responses of the plants grown on the soils treated with Dowfume MC-2 are similar to those of plants grown on the Dowfume N treated soils, though not of the same magnitude.

The calcium uptake by the plants was partially governed by the amount of calcium available in the soil. However, the uptake was less when the primary source of nitrogen available to the plants was in the form of ammonium than when it was in the form of nitrate. An increase in the uptake of calcium was associated with a decrease in the potassium and magnesium content of the

plants. It can be seen that the mineral cation constants in the plants were lower when the primary source of nitrogen was ammonium. It is also obvious however, that an increase in calcium available to the plants growing in the fumigated soils materially aided in the growth responses. This increase in growth due to an increase in the calcium level of the soil was not as pronounced when the primary source of nitrogen available to the plant was in the form of nitrate.

DISCUSSION

It was shown that when the sole source of nitrogen available to plants is in a reduced form, the plants will respond favorably to an increase in calcium in the substrate. Though the protein content of the plants supplied with these forms of nitrogen was higher, total growth was low due to an inability of the plants to synthesize enough carbohydrate which seems to be necessary for elaboration of the ammonium. These plants characteristically matured earlier than plants whose sole source of nitrogen was in the form of nitrate. The mineral cation uptake constant when expressed in milliequivalents per unit of dry weight of tissue was less when the source of nitrogen was ammonium than when it was nitrate. This indicates that ammonium enters into the cation balance relationships and reduces the uptake of other cations by

the plants.

When soybeans were grown on soils which were fumigated with Dowfumes N, W-40, and MC-2, the growth responses brought about by high levels of calcium in the soil were excellent. There were indications that the fumigated soils were much higher in ammonium content than were the untreated soils and conversely lower in nitrate though both forms were present in all soils. It proved futile to try to test the soils for ammonium and nitrate content during the growth period of the plants because of the difficulty of accurate sampling and the fact that ammonium is very rapidly absorbed by the plant roots. However, the responses of the soybean plants growing on the treated soils indicated that their primary source of nitrogen was in a reduced form. The protein content of these plants was higher than that of those growing on the untreated soils and there was a tendency toward earlier maturation. The mineral cation uptake constant was also lower than in the case of the untreated soils. A high calcium content in the soil seemed to reduce the amount of carbohydrate necessary for elaboration of the ammonium because the plants had a much greater dry weight under these soil conditions. At any rate, where there was a large supply of carbohydrate present in the plant due to favorable environmental conditions and a large supply of available calcium in the soil, the growth responses of the

soybean plant to soil fumigation were better than those of the plants grown on the untreated soil regardless of the calcium level. The plants grown on the low calcium, fumigated soils did not put on as much vegetative growth as those grown on the untreated soils.

It appears that a high level of calcium in the soil is of benefit to the growth of soybeans after soil fumigation and probably, increases in the other mineral cations would also be of value.

GENERAL SUMMARY

It was determined that the dispersion of dichloropropene and ethylene dibromide through soils was approximately the same with the dispersion limits being reached within 24 hours from the time of injection of the compounds. The dispersion is lateral and downward from the point of injection with little, if any, penetration of the compounds into the soil mass above the point of injection. The extent of dispersion was greatest in the sandy soil, next greatest in the clay loam, and least in the muck. The amount of moisture exerted considerable influence on the degree of dispersion of the compounds with "optimum" dispersion taking place when the soil was at the moisture equivalent. Outward diffusion from the point of injection was minimized when the soil was air-dry and a water saturated soil cut down on the depth of penetration of the compounds. Quantities of both the dichloropropene and the ethylene dibromide were fixed by the soils and it was shown that this fixation was a function of the amount of colloidal organic matter present. There were indications that the compounds dispersed through the soil in two phases: 1, the air-water phase and 2, the humus phase. It was shown that Dowfumes N and W-40 are also fixed in soils and that it is possible to attribute certain plant injuries to a residual effect of the fumigants in the soils.

The use of dichloropropene and ethylene dibromide as soil fumigants accomplished a partial sterilization of the soil and resulted in a reduced activity of the oxidizing organisms. An indication of this was the fact that the bio-electric potentials of the soils were lowered after treatment with these compounds. Also, the effect of fumigation was to inhibit nitrification and permit the accumulation of large quantities of ammonium in the soils. The rate of ammonification was increased after fumigation, though the total amount of available nitrogen was not materially greater than in the untreated soils. Dowfumes N, W-40, and MC-2 retarded nitrification of ammonium which was added to soils with Dowfume N being most effective in this respect. Thus, plants growing on fumigated soils receive a great part of their nitrogen in the form of ammonium, and their subsequent growth is to a large degree dependent upon their ability to utilize this form of nitrogen.

With ammonium as the source of nitrogen available to soybeans, the plants responded favorably to an increase in calcium in the nutrient substrate. The plants were characterized by an abnormally high protein content and matured earlier than soybean plants whose sole source of nitrogen was nitrate. The calcium, potassium, and magnesium content of the ammonium fed plants was less than those on the nitrate substrate. Soybeans grown on soils

which had been fumigated with Dowfumes N, W-40, and MC-2 also showed favorable growth responses when the calcium content of the soil was increased, and the mineral cation content was lower than that of the plants grown on the untreated soils. The decrease in the mineral content of the plants was attributed to the fact that large amounts of ammonium taken up by the plants on the fumigated soils decreased the uptake of other cations. There were indications that an increase in the cation contents of fumigated soils (especially calcium) would be of benefit to plant growth.

The growth of soybeans and onions on muck soils which were treated with Dowfume N showed that fumigation could be of benefit at a time when available nitrogen is a limiting factor in plant growth. The plants matured earlier than those on the untreated soils, had a higher protein content, and produced higher yields.

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