T H E S I S

THE USE OF CARBON DISULFIDE FOR THE CONTROL OF THE COMMON WILD MORNING GLORY

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Submitted by

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for the Degree of Master of Science

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This is to certify that Ira Hatfield has translated for me assigned passages of technical German bearing upon his graduate Botanical work.

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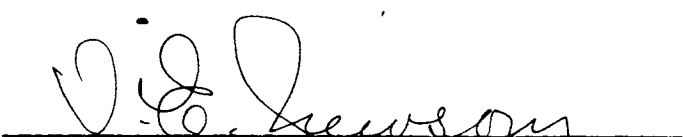

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THE USE OF CARBON DISULFIDE FOR THE CONTROL OF THE COMMON WILD MORNING GLORY

INTRODUCTION

The wild morning glory (Convolvulus arvensis) is one of the most noxious perennial weeds of Colorado, and its eradication is extremely difficult. The extensive use of carbon disulfide as a fumigant and as an insecticide, has suggested the possibility of its use for the control of this weed. Preliminary field tests showed encouraging but variable results, the reasons for which were difficult to explain.

The chemical compound carbon disulfide, was discovered by Lampadius of Freiburg in 1796 (6). In 1856 and 1857, sixty years after this discovery, Doyere (7) exhibited the insecticidal properties of carbon disulfide by demonstrating that small quantities of the substance could be used in grain pits as an effective means of destruction of all weevils and their eggs. He noted not only the killing properties of the chemical in relation to the insects, but he also observed that the quality of the grain was not altered, and that the odor quickly disappeared upon exposure of the grain to free air.

It was not until 1859 that carbon disulfide was used

underground (7). In that year, Thénard applied the insecticide to his Phylloxera-infested vineyard, but because of the large doses used, his vines also were killed. By 1873, however, experimental results were so encouraging that more than 200,000 acres received annual treatment with the chemical.

Since that time many uses have been made of carbon disulfide as an underground poison. Several of the continental European countries performed extensive experimental tests with the chemical, and by 1900 much work has been done relative to the nature and uses of the poison. A considerable number of tests were also made in the United States. Hilgard (11), in 1876, recommended the use of carbon disulfide as a ground squirrel exterminator in California. In 1890, Cook (4) referred to the underground use of the chemical in Kansas and Nebraska for exterminating prairie dogs, and alluded to the practicability of carbon disulfide as a means of destroying ants and cabbage maggots. According to Slingerland (10), Cook was the first to experiment with carbon disulfide on the root maggots.

Webster (12), in 1899, reported the results of experiments with carbon disulfide as an insecticide for lice on the roots of peach trees, but because the mortality of the lice was accompanied by death to the trees, the report was unfavorable to the use of the chemical. Becker (3)

in 1918, wrote that the effect of the treatment of 190 peach trees for the peach tree borer was disastrous. The owner of the orchard wrote,"The trees treated with carbon disulfide are 50 per cent killed and those treated that are not dead I found all the roots near the surface of the ground, are killed and trees are not making any new growth, but have plenty of borers in them." Becker concluded: "Our experience with carbon disulfide indicates that it is too dangerous to recommend for use in controlling borers."

Published records of the underground application of carbon disulfide as a means of control for the common wild morning glory (Convolvulus arvensis), date back to 1919, in which year Mayhew (2), farm advisor of Monterey County, California, began investigations relative to the usefulness of this substance as a weed herbicide. Various later California experiments explain the usefulness of carbon disulfide for the control of small patches of morning glory. Ayers et al. (1), report that carbon disulfide has been used successfully in Idaho for the extermination of small patches of this weed pest.

Due to these successes and the encouraging results obtained in Colorado when this herbicide was used , experiments were planned that would give definite information concerning the effect of the carbon disulfide vapors upon

the roots of the common wild morning glory.* These experiments, as presented in the following thesis, included the development of a chemical indicator that would be usefully sensitive to carbon disulfide gas; the development of a formula and of an apparatus for measuring the liquid to gas volume change of carbon disulfide, and field, laboratory, and microscopic observations concerning the effect of the chemical gas upon the regeneration of the morning glory roots.

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* Unpublished data collected by C. F. Rogers, Deputy State Entomologist, Colorado Agricultural College.

METHODS

The Development of an Indicator for Detecting the Presence of Given Quantities of Carbon Disulfide

Obvious inaccuracies accompany the use of biological indicators such as aphids, as a means of testing the behavior of carbon disulfide in the soil. As was suggested by Fleming (5), the withdrawal of air from the soil and the making of tests upon it, is also open to serious objections.

Due to the need of an indicator which could be used for determining the rate and amount of carbon disulfide gas in the soil, tests were made with color indicators reactive to carbon disulfide. It was found that thymol blue (thymol-sulphonphthalein) could be advantageously used when mixed in proper proportions. Thymol blue in a 0.05 per cent solution in 95 per cent alcohol proved to be very satisfactory. A smooth surface filter paper was saturated with the liquid and allowed to dry. The paper when dry is a yellow to orange color. When exposed to the carbon disulfide gas, the color changes from the yellow-orange to a deep purple.

Determinations were made pertinent to the range of color change. Various atmospheric concentrations of the carbon disulfide gas were obtained by allowing the required quantity of the liquid carbon disulfide to evaporate into

a given volume. These determinations showed that the thymol blue indicator was not sensitive to carbon disulfide in atmospheric concentrations below 10 per cent, but that it was sensitive to all higher concentrations.

The search for an accurate chemical indicator necessitated a formula which would express the change in volume when a given quantity of liquid becomes a gas. Until this relationship was established, it was impossible to accurately know the atmospheric concentrations to which the roots were subjected. No adequate formula to express this volume change was found in the literature. Although Fleming (5) suggested a formula by which the amount of carbon disulfide contained in a liter of saturated air could be approximately estimated, it was inaccurate for the necessary determinations.

Experiments with carbon disulfide as an agent for killing roots of perennial weeds demanded an expression for the volume of liquid necessary to produce a given concentration of vapor in a known volume, and a formula for the liquid-to-gas volume ratio. The two following methods for the development of the formulae are based on the general gas laws.*

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* The formulae and the apparatus used for this phase of the work, were jointly developed by C. F. Rogers and the author of this thesis. This portion of the thesis is being published separately.

Method I.

For the volume of liquid required to produce a given gas concentration by mass, or by a given volume of pure gas, the following symbols may be used:

M = the molecular weight of the liquid substance.

gmv = the gram-molecular volume (22.38 liters).

C = the concentration of the gas in decimal fraction of total pressure or of volume.

L = the volume of the gas expressed in liters.

D = the density of the liquid of molecular weight M.

When the liquid becomes a pure gas under standard conditions, the term $\frac{M}{gmv}$ expresses the mass per liter of pure gas, and the equation

$$W = \frac{M\,L}{gmv} \qquad (1)$$

gives the mass of gas for the volume of L liters of pure gas.

To find the mass of a gas when it is in concentration C, or C parts of the whole, the equation is

$$W = \frac{C\,M\,L}{gmv} \qquad (2)$$

The mass of the gas is of course equal to that of the liquid which evaporated to produce it, and "W" is therefore the mass of both the gas and the liquid. The volume of the liquid "V_ℓ" is obtained by dividing formula (2) by D.

$$V_\ell = \frac{W}{D} = \frac{C\,M\,L}{gmv\,D} \qquad (3)$$

The real values of this equation will be found up to the

vapor tension of the liquid at the temperature, T_1, of the experiment, and the limiting value of C is $\frac{vP}{P_1}$, where vP is the vapor pressure of the liquid at T_1, and P_1 is the barometric pressure of the experiment.

The values of equation (3) are in terms of standard conditions of temperature and pressure. Under normal conditions of experimental work, the use of the temperature correction factor $\frac{T_1}{T}$, is necessary, in which T_1 is the Absolute temperature of the experiment and T is the standard on the Absolute scale. By Boyle's Law the pressure correction for ordinary conditions is $\frac{P}{P_1}$, in which P is the standard pressure. The vapor tension of a liquid is not affected by barometric pressure, so that the partial pressure of the vapor increases with decrease in barometric pressure, but the factor $\frac{P}{P_1}$ enters into the equation when the liquid has a high vapor tension, and the cencentration is below $\frac{vP}{P_1}$ at T_1. The whole expression then becomes

$$V_l = \frac{C\ M\ L\ T_1\ P}{gmv\ D\ T\ P_1} \qquad (4)$$

If the volume of the liquid and the resulting gas are to be compared, the equation

$$V_r = \frac{V_g}{V_l} \qquad (5)$$

will express the ratio, "V_r" being the volume ratio of change from liquid to gas. The reciprocal equation

$$\frac{1}{V_r} = \frac{V_l}{V_g} \qquad (6)$$

will give the gas-liquid-volume ratio.

If in equation (4), $T_1 = T$, $P_1 = P$, and C and L become unity, then

$$V_g = \frac{M}{gmv\ D} \qquad (7)$$

The volume ratio, V_r, will be

$$V_r = \frac{V_g}{\frac{M}{gmv\ D}}, \text{ or } \frac{gmv\ D\ V_g}{M} \qquad (8)$$

If V_g becomes equal to unity, the volume ratio will be

$$V_r = \frac{gmv\ D}{M} \qquad (9)$$

Method II.

As stated under equation (3) the values for a volume of gas obtained from a liquid, are limited by the vapor tension of the liquid at the temperature T_1, and the concentration C, therefore, can be expressed as $\frac{vP}{P_1}$. Values for C at T_1, which are greater than $\frac{vP}{P_1}$ are unreal and have no experimental significance.

The part of the gram-molecular volume which the pure gas under standard conditions would occupy is $\frac{vP}{P}$. Expressing this as V_g, the volume of the gas, the equation becomes

$$V_g = \frac{vP\ gmv}{P} \qquad (10)$$

Since the $\frac{vP}{P}$ ratio holds for the mass as well as volume, $\frac{vP\ M}{P}$ is the gram mass of the gas in the gram-molecular volume when reduced to standard conditions. Dividing by D as in (3),

V_ℓ is obtained, but in the form

$$V_\ell = \frac{vP}{P} \frac{M}{D} \qquad (11)$$

By equation (5), and substituting for V_g and V_ℓ,

$$V_r = \frac{V_g}{V_\ell} = \frac{gmv}{M} \frac{D}{M} \text{ which is equation (9)}$$

Another approach to the V_r ratio uses formulae (3) and (10), and modifies (3) with (11). To calculate the limit of equation (3) under standard conditions, let C be equal to $\frac{vP}{P}$, and L be equal to the gram-molecular volume. Then

$$V_\ell = \frac{vP}{P} \frac{M}{D} \frac{gmv}{gmv} = \frac{vP}{P} \frac{M}{D} \qquad (12)$$

which is identical with (11) that was developed without (3).

It must be remembered in these equations that the volumes of V_ℓ are in milliliters, and the volume ratios will be correct only when the gram-molecular volume is in the same units.

These formulae hold for a pure liquid or a know molecular mixture, and may be verified experimentally by the simple apparatus shown in the diagram. A large graduated cylinder G, to which is attached an outlet O, is inverted over water in the vessel A, and is supported by a ring R on the stand S. The outlet O is a glass tube which extends from within 2 cm. of the base of the graduate, bends sharply over the mouth, down to a level above the rim of the vessel A when the graduate is inverted. It is closed by a heavy rubber tube and a screw clamp. The vessel is filled with water

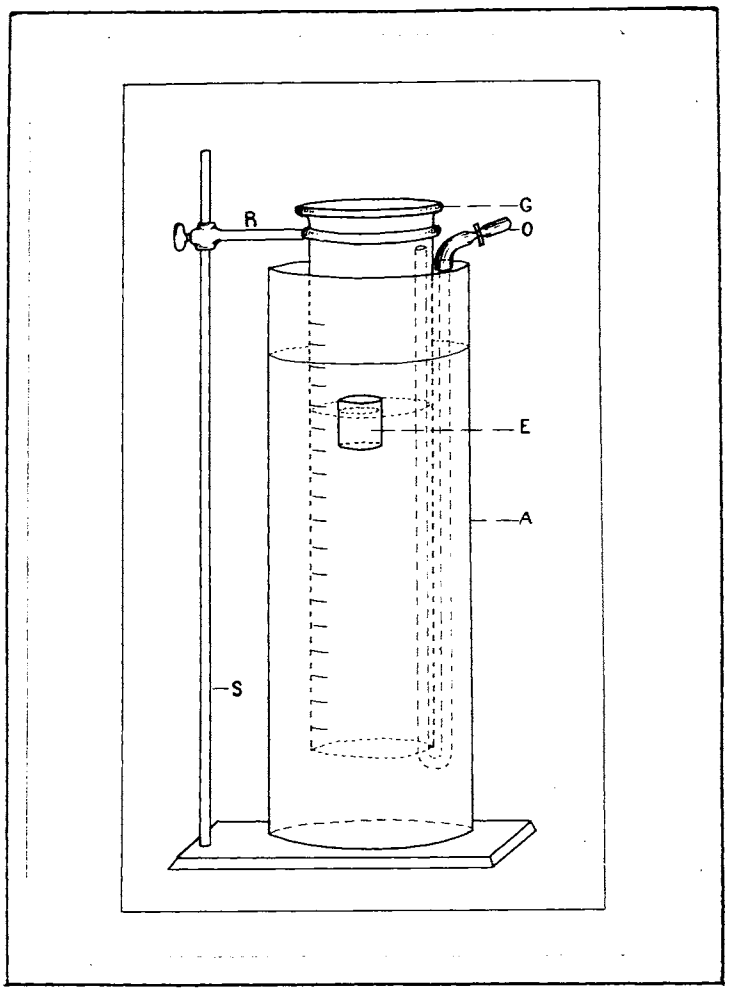

Diagram of apparatus used for measuring the liquid-to-gas volume change.

to a level well above the mouth of the graduate. The evaporator E, is a low-form weighing bottle as nearly filled with the liquid as will permit easy handling.

When the apparatus is to be assembled, the graduate is inverted over the evaporator E, and the excess air is removed by opening the screw clamp and exhausting with a tube run from the outlet O to an aspirator. The water levels should be equalized when the initial and final readings are made, but during the evaporation of the liquid the level within the graduate is unimportant.

Then let

L_1 = reading of the water level in the graduate at beginning of the test.

L_2 = reading of graduate at saturation.

L_3 = a reading of graduate between L_1 and L_2.

vP = partial vapor pressure of liquid at T_1.

t = partial vapor pressure of water at T_1.

A = partial pressure of air at T_1 and L_2.

The sum of the partial pressures of the gases at L_2 may be expressed by the equations

$$A + vP + t = P_1 \qquad (13)$$

and

$$A = P_1 - (vP + t) \qquad (14)$$

To get the fractions of each of these values in terms of atmospheres (P_1) divide (13) and (14) by P_1. Then

$$\frac{A + vP + t}{P_1} = 1 \qquad (15)$$

and

$$\frac{A}{P_1} = 1 - \frac{vP + t}{P_1} \qquad (16)$$

At the beginning of the experiment $A = P_1$. When P_1, vP, t, and L_1 are known, the value of L_2 can be calculated from L_1. By equation (13) the partial pressures of the gases in L_2 at P_1 are $A + vP + t$, and by (16) the partial pressure of the L_1 volume of air in L_2 is $\frac{A}{P}$, or $1 - \frac{vP + t}{P_1}$ Therefore

$$L_1 = L_2 \left(1 - \frac{vP + t}{P_1} \right) \qquad (17)$$

and

$$L_2 = \frac{L_1}{1 - \frac{vP + t}{P_1}} \qquad (18)$$

Prediction of L_2 is useful when it is desired to know the volume to be reached at saturation. This point is essential because during the saturation of air with the vapors of the liquid, water condenses within the weighing bottle and introduces an error. By substituting in equation (3) at its limits

$$V_l = \frac{vP\ M\ L_2}{P_1 g m v\ D} \qquad (19)$$

In order to calculate V_l before the limit of C has been reached, the value of L_2 must be figured and the equation

$$L_2 - L_1 = B \qquad (20)$$

gives the volume difference between the original and the

limiting volumes. The reading L_3 lies between L_1 and L_2 and the difference

$$L_3 - L_1 = H \qquad (21)$$

which gives the volume increase for partial saturation due to evaporation of the liquid. Then

$$\frac{H}{B} = F \ , \qquad (22)$$

the fraction of saturation of L_1 . It becomes a factor of the limit of C. Therefore C is expressable as

$$C = F\frac{vP}{P_1} \qquad (23)$$

and equation (3) now becomes

$$V_l = \frac{F\ vP\ M\ L_2}{P_1\ gmv\ D} \qquad (24)$$

It has been found experimentally that it is better to use this last formula to check the theoretical values of (3) with a large initial volume, L_1 , and a smaller value for H, because of the greater ease of manipulation, the shorter time required for a reading, and the smaller amount of water condensed in E while the evaporator is in A. The error due to condensation can be largely eliminated by having E under evaporating conditions prior to the beginning of the experiment. Except in refined tests or at high temperatures (30 C.), the factor "t" can be neglected, and can be eliminated altogether by connecting O with a chamber of air saturated with water vapor. When the liquid

or vapors are even slightly soluble in water, the water in A should be saturated with the liquid to be tested.

The loss of liquid from E, measured by weighing, should agree within 5 per cent of that indicated by the volume increase.

Determinations based upon these formulae made possible the collection of data concerning the reactivity of the indicator mentioned above.

THE EFFECT OF CARBON DISULFIDE GAS UPON THE REGENERATION OF THE COMMON WILD MORNING GLORY ROOTS

Field tests had already shown that carbon disulfide could be used for killing the wild morning glory roots, but the causes for the variety of results had not been determined. As it seemed advisable to perform experiments which could be more carefully regulated and observed than is afforded by ordinary field conditions, combination field and greenhouse tests were used.

The field equipment for this series of experiments consisted of several Mason jars of measured volume in which the roots were treated with the carbon disulfide, a thermometer, and apparatus necessary for the application of the carbon disulfide to the fumigation chambers.

The entire set of six experiments was run during the month of November, 1927. Seven jars which could be tightly sealed were used as the carbon disulfide fumigation chambers. Each chamber contained a known quantity of soil to keep the roots moist. Freshly dug roots were cut into 10 centimeter lengths, and the ends of each root were vaselined in order that the gas would be retarded from entering the ends of the root. Six 10 centimeter lengths were placed in the bottom of each chamber. A small quantity of cotton wool was held at the top of the chamber by pinching it fast as the lid was screwed

down. The calculated volume of liquid was added to the cotton by means of a pipette, and the lids immediately adjusted. For the two lowest concentrations, the quantity of gas necessary to bring the atmosphere to the desired carbon disulfide gas concentration, was added directly to the jars by means of the apparatus shown in Figure 1.

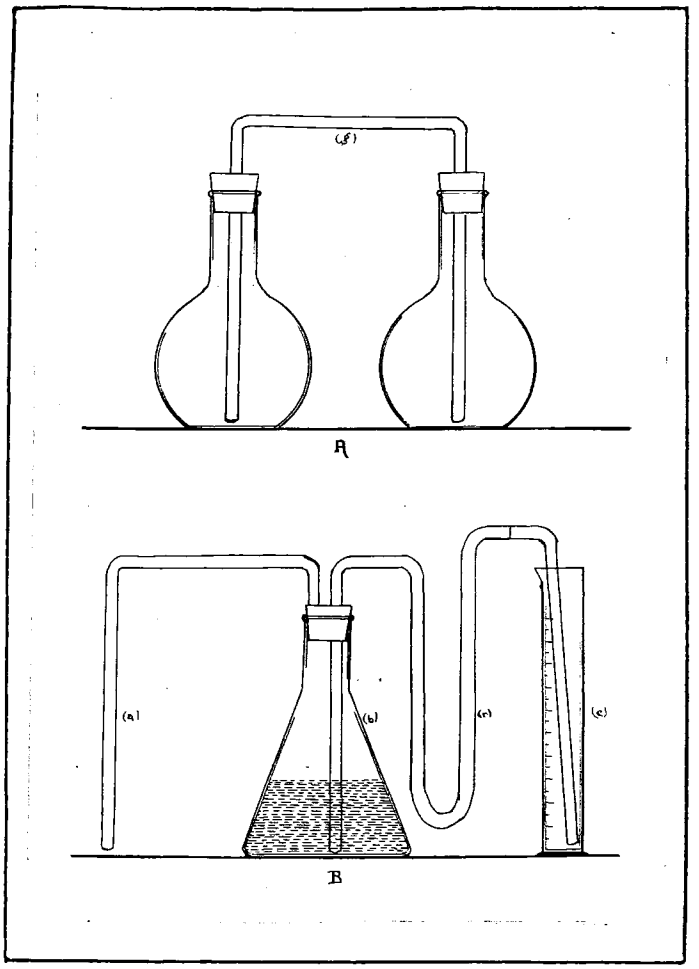

Fig. 1. Apparatus used for the injection of carbon disulfide gas into the fumigation chambers.

To each of the Florence flasks shown in A was added enough liquid carbon disulfide to make a saturated atmosphere. These flasks were connected by the glass tube (g) in order to allow a free passage of the gas between them, and also to retard air currents. The graduate (c) shown in B was raised until water from it completely replaced all of the air in flask (b) and the tube (a). Tube (a) was then inserted into one of the flasks A, the connection (g) being left intact. By lowering the graduate (c) the carbon disulfide vapor replaced the water in flask (b). By raising the graduate (c) above (b), it was possible to inject any desired volume of the saturated gas into the fumigation chamber.

Although various investigators were of the opinion that in field conditions the temperature was a negligable factor, efforts were made to keep the temperature as constant as possible during each experiment. To accomplish this end, horizontal tunnels were dug at the floor level of the pit from which the roots were taken (Fig. 2), and the jars containing the roots and carbon disulfide were placed within these tunnels.

Fig. 2. Photograph of pit showing constant-temperature tunnels.

The effectiveness of this method for controlling the temperature may be observed from the data given in Table I. The table shows that the difference in temperature between the time when the jars were placed in the tunnels and the time when they were removed, in only two instances exceeded 2° C. In the one case the difference was 2.5°C. and in the other the difference was 3.0°C.

The first experiment with the roots consisted of a test to determine the relation of kill to the depth from which the roots were taken. Six series of roots were used. Each series contained tests with roots taken from different depths (Fig. 3); treatments were made with carbon disulfide concentrations ranging from 100 to 0.05 per cent saturation, and each series was run for 1, 2, 4, and successive two hour intervals up to and including 16 hours. The depths from which the roots were taken for each series is shown in Table II.

TABLE I. Temperature Record (°C) During the Period of Root Fumigation.

	SERIES I		SERIES II		SERIES III		SERIES IV		SERIES V		SERIES VI	
Length of treatment in hours	Temp. when appld	Temp. when rmvd	Temp. when appld	Temp. when rmvd	Temp. when appld	Temp. when rmvd	Temp. when appld	Temp. when rmvd	Temp. when appld	Temp. when rmvd	Temp. when appld	Temp when rmvd
1	6.5	8.0	10.0	10.0	5.0	5.0	4.0	4.0	4.0	4.0	3.5	4.0
2	8.0	8.0	6.5	8.0	5.0	6.0	3.0	6.0	3.0	4.0	4.0	4.0
4	8.0	8.0	10.0	10.0	6.5	6.0	6.0	5.5	5.0	6.0	6.0	6.0
6	7.0	7.0	7.0	6.0	6.0	5.0	6.0	7.0	4.0	5.0	4.0	4.0
8	11.0	10.0	10.0	9.0	5.5	5.0	5.0	5.0	4.0	4.5	4.5	4.5
10	7.5	8.0	10.0	8.0	5.0	5.0	4.0	5.5	4.0	4.0	4.5	4.5
12	10.0	8.0	6.0	6.0	6.0	3.5	5.5	5.0	4.0	4.0	4.0	5.0
14	7.0	5.0	5.0	4.5	6.0	5.0	5.5	5.5	5.0	3.5	6.0	6.0
16	10.0	9.5	7.0	5.0	6.0	6.0	5.5	4.0	6.5	5.5	6.0	6.0

Fig. 3. Excavation showing the depths from which each series of roots was taken.

TABLE II. Relation of Series Number to Depth from Which Roots Were Taken

Series Number.	Depth from which roots were taken. (In centimeters)
I	Any Depth
II	0 - 30
III	30 - 60
IV	60 - 90
V	90 - 120
VI	120 - 150

Immediately upon the removal of the roots from the fumigation chambers, the roots were planted in the greenhouse beds at a depth of approximately 6 centimeters, (Fig.4 and 5). The criterion for regeneration was the ability of the root to send up shoots to the surface within 90 days.

The relation of the depth from which the roots were taken to the killing effect of the various concentrations of carbon disulfide, is recorded in Table III. The data are based upon greenhouse studies of root regeneration. A total of 432 roots, ten centimeters in length, were treated.

Fig. 4. Greenhouse beds showing labels and regenerating roots.

Fig. 5. Regeneration of roots indicated in more detail.

TABLE III. Relation of Depth of Root to Killing Effect of Various Atmospheric Saturations of Carbon Disulfide Gas.

Depth cm.	100% satur.	50 % satur.	25 % satur.	12 % satur.	6 % satur.	0.5 % satur.	0.05 % satur.	Total kill. All percentages.
Various	94.33	25.77	16.44	12.77	9.00	5.44	10.88	24.95
1-30	85.11	33.22	3.66	1.77	3.66	5.44	7.22	20.01
30- 60	96.22	38.77	7.33	10.88	12.77	10.88	9.00	26.55
60- 90	100.00	34.88	1.45	9.00	7.11	7.22	3.66	23.33
90-120	92.55	59.11	12.66	12.66	9.11	3.55	7.22	28.12
120-150	87.66	49.77	12.77	21.88	12.77	5.55	5.33	27.96
Average	92.66	40.25	9.05	11.49	9.07	6.35	7.22	25.15

In Table III, a comparison of the columns according to the percentage of saturation, or a glance at the averages as given for each percentage, will show that a saturated atmosphere of the carbon disulfide vapors is effective for killing the roots, and that a 50 per cent gives a medium kill, but that all lower concentrations are practically worthless. As will be seen from the last column the difference in the kill at each of the depths is a negligible factor. The differences that are represented by the percentages can be explained by the fact that at certain depths, the total number of roots included a large number of small-diameter roots. The size of the root evidently played some part in the effectiveness of the chemical.

This inference lead to the study of the relation of toxicity to the size of the root. Leach (8), working with carbon disulfide as a means of control for the root form of the woolly apple aphis, noted that the carbon disulfide gas killed the apple rootlets much more extensively than it did the main roots. He accounted for this by explaining that the cambium area is the area attacked by the gas, hence the less protected rootlets would be much more susceptible.

From the greenhouse records, a study was made of 2392 roots to discover the relation of the size of the root to the killing effects of the carbon disulfide gas. The

results are shown in Tables IV and V. Table IV records the results in numbers of roots planted in contrast to the number that regenerated; Table V is given in percentages figured from the actual root count.

TABLE IV. Relation of Size of Root to Killing Power of Various Saturations of the Carbon Disulfide Gas.

Root size Diameter	100 %		50 %		25 %		12 %		6 %		0.5 %		0.05 %		check	
	ptd.	reg.	ptd.	reg.	ptd.	reg.	ptd.	reg.	ptd.	reg.	ptd.	reg.	ptd.	reg.	ptd.	reg.
1 mm	115	9	153	84	171	146	167	145	182	169	193	177	194	179	35	29
1.5 mm	43	3	63	45	69	63	81	75	68	63	58	54	67	63	8	8
2 mm	24	2	45	38	47	43	42	38	35	35	40	40	31	31	2	2
2.5 mm	9	3	15	13	19	19	15	14	16	16	20	20	18	17	3	3
3 mm	6	4	4	3	10	10	12	11	7	7	2	2	7	7	2	2
3.5 mm	1	0	3	3	2	2	0	0	2	2	4	4	2	2	0	0
4 mm	0	0	2	2	2	2	1	1	1	1	1	1	0	0	0	0

TABLE V. Relation of Size of Root to Toxicity in Percentage of Kill at Various Saturations.

Root Size Diameter	%killed 100%sat.	%killed 50%sat.	%killed 25%sat.	%killed 12%sat.	%killed 6% sat.	%killed 0.5%sat.	%killed 0.05%	% died. check
1 mm	92.17	45.10	14.62	13.94	7.15	13.47	7.73	17.14
1½ mm	93.02	28.57	8.69	7.41	7.35	6.70	7.46	00.00
2 mm	91.67	15.55	8.51	9.52	0.00	0.00	0.00	00.00
2½ mm	66.67	13.33	0.00	6.67	0.00	0.00	5.55	00.00
3 mm	33.33	25.00	0.00	8.33	0.00	0.00	0.00	00.00
3½ mm	100.00	00.00	0.00	----	0.00	0.00	0.00	-----
4 mm	------	00.00	0.00	0.00	0.00	0.00	----	-----

These tables show that if roots larger than 2.5 millimeters in diameter are injured at all by the carbon disulfide vapors, the injury is only slight, and that even smaller roots are not appreciably injured when subjected to low concentrations of the gas. It will also be noted that from the 50 roots used as checks, 6 of the one millimeter in diameter roots did not regenerate. In other words, of the check roots of the one millimeter size, 17.14 per cent died. It was unfortunate that a larger number of check roots was not used, for it is probable that in such a case, one or more from the other smaller sizes would have died. It can clearly be seen that if only one of the one and one-half millimeter roots had died, the percentage of kill would have been 12.50, and if one of the other sizes had died, the percentage of kill would have been too high in proportion to the actual relationship of the unaffected roots.

Reference to Tables IV and V will show that a saturated atmosphere of the carbon disulfide gas was deleterious for all sizes of roots subjected to the gas. The 50 per cent atmosphere seemed also to have toxic effects. Below 50 per cent, however, no significant kill was obtained even for the smallest roots. This last statement is justified by the fact that in no case did the percentage of kill for the one millimeter roots equal or surpass the percentage of death of the check roots. It is obvious that any kill

under 50 per cent is insignificant.

A study was also made of the 2392 roots to determine the relation of kill to the range of concentrations of the carbon disulfide gas and to the time of exposure of the roots. Table VI records the results of these observations. The figures in the table are given in the percentage of kill of the roots for the different concentrations of carbon disulfide. Although there is a fluctuation in the maximum kill for the different concentrations at the successive periods of exposure, these differences lose their abruptness when based upon the total number of roots. The last column in Table VI reveals that the variation or fluctuation of killing for the successive hours, becomes neglegable when the total number of roots is considered in the calculations.

A comparison of the percentage kill for any time interval, with the corresponding average kill for the concentration of carbon disulfide used, will show that the deviation exceeds 15 per cent in only a few cases. Thus in the first column that records the kill, even where there was one hundred per cent kill, the difference from the average is only 7.17, and the lowest kill is just 9.5 from the average.

TABLE VI. Relation of Kill to the Range of Concentrations of the Carbon Disulfide Gas and to the Number of Hours Exposed.

Hours Exposed	100 % atmos.	50 % atmos.	25 % atmos.	12 % atmos.	6 % atmos.	0.5% atmos.	0.05% atmos.	Total kill-All concentrations
1	83.33	22.00	16.50	10.83	24.83	13.66	16.33	26.78
2	97.16	11.00	10.83	10.83	13.50	2.66	8.16	22.02
4	100.00	38.66	11.00	22.00	13.50	8.00	10.83	29.14
6	86.00	52.50	5.33	27.66	2.66	5.50	2.66	26.04
8	100.00	58.33	5.33	2.66	2.66	10.83	5.33	26.45
10	94.33	49.83	10.83	2.66	8.16	0.00	5.33	24.45
12	94.33	35.83	11.00	10.83	0.00	0.00	11.00	23.28
14	91.50	19.33	27.50	8.00	8.00	16.50	2.66	24.78
16	88.83	74.83	2.66	8.00	8.33	0.00	2.66	26.40
Average	92.83	40.26	11.22	11.50	9.07	6.35	7.22	25.48

From the foregoing regeneration studies, centain conclusions may be drawn. Although the studies did not include tests made at relatively high temperatures, the low temperature treatments gave results comparable to those of other experimenters, namely - that temperature is not a limiting factor in relation to the killing effect of carbon disulfide.

Further results show that roots from any depth and of similar size are equally susceptible to the carbon disulfide gas. The effectiveness of the gas, however, is modified by the size of the root. Roots over 2.5 mm. in diameter were seldom killed, even in a saturated atmosphere of the carbon disulfide gas.

In order to be effective for destroying the common wild morning glory roots, high concentrations of the carbon disulfide gas must reach the roots. A few hours of exposure is as effective as longer periods of time.

FIELD AND LABORATORY TESTS WITH CARBON DISULFIDE

Pertinent studies have already been made by several investigators concerning the underground use of carbon disulfide. Mayhew (2) recommended that the liquid carbon disulfide "be applied by making holes three feet apart each way over the entire surface of the morning glory covered patch". The holes were to be made 18 inches deep by means of a bar. Four ounces of carbon disulfide per hole was the dose suggested, and the holes were then to be filled with soil. Gas penetration would be best when the soil was "quite dry".

It is interesting to note the direct contract of recommendations suggested by Ayres et al. (1) for Idaho conditions. These recommendations included the application of 2 ounces of the chemical to the hole; the holes were to be 18 inches deep and two feet apart each way. The carbon disulfide should be applied at a time when the soil is "quite moist". The statement is made that negative results almost always followed the use of the material in dry soils.

Although Leach (8) was interested in the use of carbon disulfide as a control for the woolly apple aphis, his studies of the behavior of the chemical when injected into the soil, show some interesting results concerning temperature, moisture, soil types, and methods of application.

In the light of the experimental work already done, it seemed that methods should be evolved that would aid in the determination of the proper soil conditions for maximum results when the carbon disulfide was injected into the soil. The experiments by Leach (8) and O'Kane (9) showed similar results concerning the relation of temperature to carbon disulfide diffusion, and produced data to uphold the belief that the rate of diffusion varied according to the soil types. The work of these two men, and that of Fleming (5), noted that the moisture content of the soil was extremely important and also a factor which limited the diffusion of the gas.

Two preliminary field tests in Colorado may be mentioned here, for they are suggestive of the need of a careful study of the diffusion problem. The one experiment was performed May 13, 1927. The soil was a heavy upland clay. Holes were made 18 inches deep and every 2 feet apart, and they were staggered in the alternate rows. Two ounces of the liquid carbon disulfide were applied to each hole, and the openings were immediately closed. The following observations are recorded:

May 13 - - Carbon disulfide applied.

May 13 - - Vines started to wilt.

May 16 - - Tops turning yellow and dying.

June 5 - - Tops dead and brown.

July 7 - - Tops dead. No regeneration.

Aug. 1 - - No regeneration.

Oct.28 - - No regeneration.

Conclusions - - Perfect kill.

Four ounces applied in holes 3 by 3 feet apart, under the same conditions as the above experiment, gave an imperfect kill.

The second example is a direct contrast to the one just quoted. The experiment was conducted in the same locality. The carbon disulfide was applied on May 16, to open, dry, sandy, bottom soil. Two-ounce doses in holes 2 feet apart and 4-ounce doses in holes three feet apart, both applied ten inches deep, failed to kill.

As experimenters have always obtained variable results, intensive plans were made to run a number of field tests. Screen containers as shown in Figure 6, were equipped with strips of indicator paper previously described, and the containers were placed in holes as shown in Figure 7. The holes were so arranged as to give five indicator tests at each radial distance from the dosage hole. The radial distances were 4, 8, 12, and 16 inches. Experiments were set up at various times and in different types of soil, but the soil moisture was too high to permit diffusion of the gas.

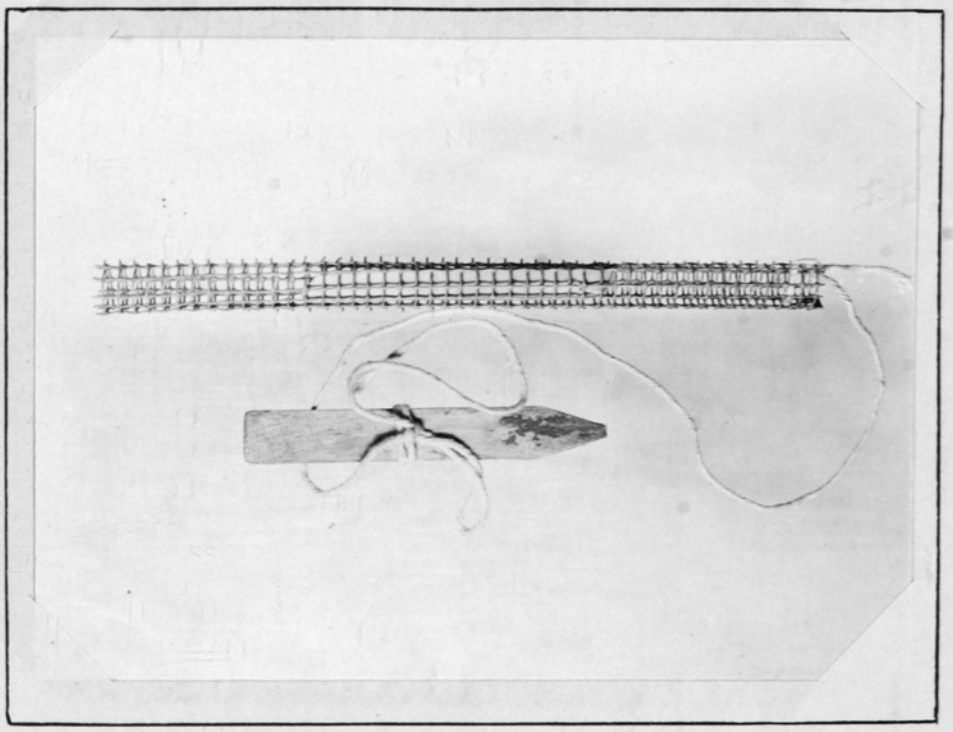

Fig. 6. Screen indicator containers used in field tests for carbon disulfide diffusion.

Fig. 7. Holes used for testing carbon disulfide diffusion.

Having met with these adverse field conditions, experiments were run in the laboratory using soil tubes and air-dried sand of different sized particles. Thymol blue indicator paper was placed at 4-inch intervals along the inner surface of the tubes. Cotton wool which contained enough liquid carbon disulfide to maintain a high atmospheric saturation of the gas, was inserted in one end of the tubes, and cork stoppers were then placed behind the cotton. The first experiment was run at 12 degrees centigrade, but diffusion had not taken place in the check tube within the first twelve hours, and so subsequent experiments were conducted at higher temperatures. Table VII records the data obtained when tests were made in the tubes at a temperature of 23°C.

It is obvious that the conditions under which the soil-tube tests were made, were not comparable to field conditions, but they do give results which suggest the following conclusions:

1 - Although it was previously noted that temperature is a negligible factor in regard to the usefulness of the carbon disulfide gas as a killing agent for the common wild morning glory roots, it can be seen clearly that the temperature will affect the rate of diffusion of the gas through the soil.

TABLE VII. Distance of Carbon Disulfide Gas Diffusion in 90 Minutes, as Shown by Thymol Blue Indicator.

Air-dry sand. Size mesh	Distance of carbon disulfide diffusion (In inches)
1 - 28	36
28 - 48	36
48 - 100	36
100 - 200	28
Less than 200	20
Check	36

2 - The rate of diffusion is proportional to the size of the particles, the reason being that soils of coarser texture have larger air spaces around the particles.

3 - The rate of diffusion is affected by the presence of moisture in these air spaces. The greater the water content of the soil, the less air space available for the diffusion of carbon disulfide vapors.

MICROSCOPIC OBSERVATIONS OF THE WILD MORNING GLORY ROOTS

Observations had often been made by experimentors relative to the effect upon plants of carbon disulfide when injected into the soil as a control measure against root aphids. Leach (8) reports that his studies of 200 apple trees showed that the carbon disulfide vapor killed the cambium. He also found that rootlets were killed under circumstances that seemed to have little or no effect upon the main roots.

At the time of the regeneration studies upon the morning glory roots, microscopic observations were conducted, the attempt being made to locate the portion of the root attacked by the vapors. The results checked accurately with the data recorded in Tables IV and V. For roots less than 2.5 millimeters in diameter, the cambium region showed a distinct coloration, whereas the larger roots did not show this change. Comparison of the cortex of the various sized roots seemed to bring out the chief reason for the difference in effect of the carbon disulfide upon the roots of unequal diameters. Figure 8 is a photograph of a cameralucida drawing of a normal root of the 1.5 - millimeter size, and Figure 9 is a photograph of a free hand section.

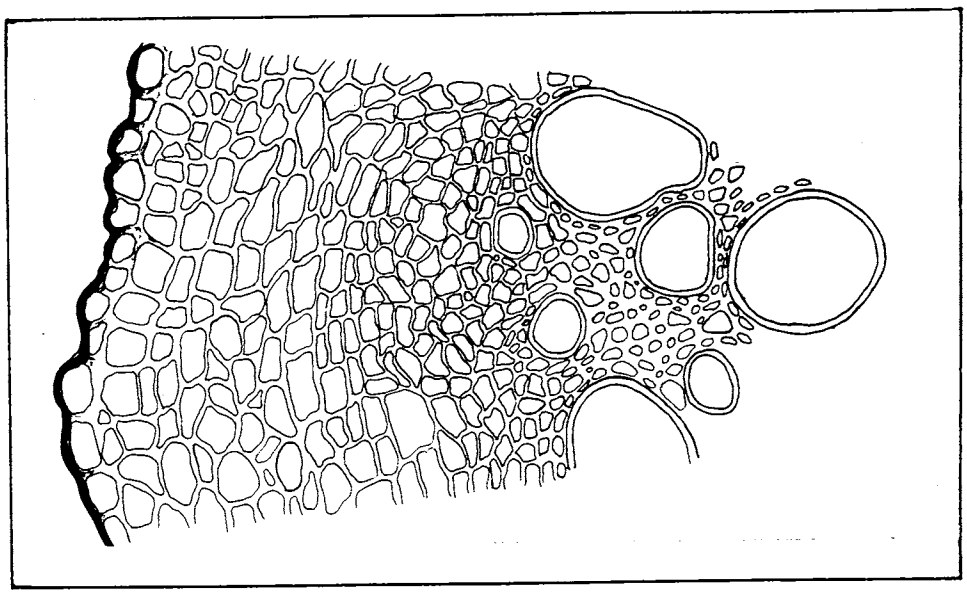

Fig. 8. From a cameralucida drawing of a portion of a normal root of the 1.5-millimeter size.

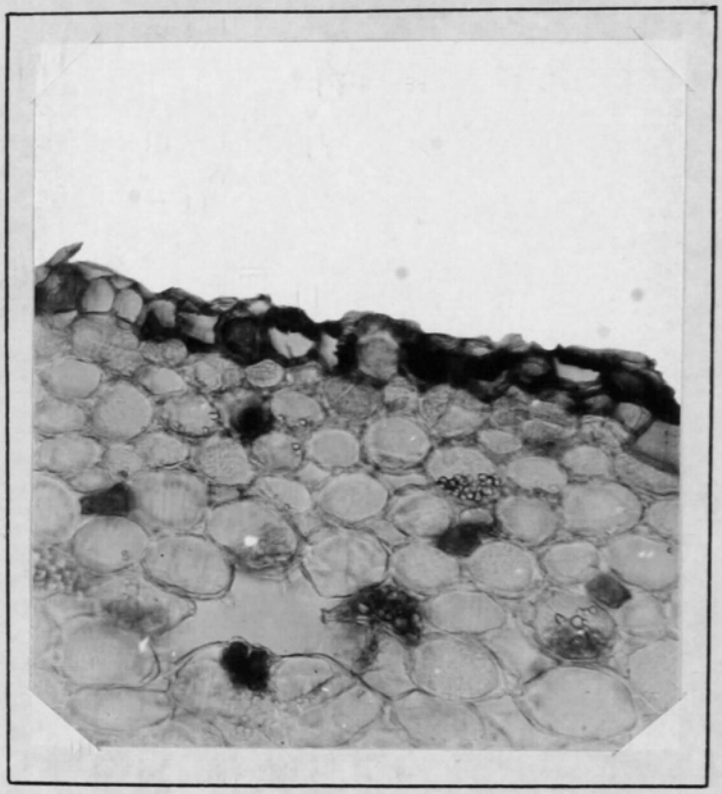

Fig. 9. Photograph of root section showing epidermis and cortex.

The drawings in Figure 10 are from sections of the epidermis of roots of 1, 2.5, and 4 millimeters diameter. The shaded areas show the difference in thickness of the epidermis of these roots.

The relation of the epidermis and cortex to the stele is made apparent in Figure 11. Microscopic observations revealed that in the smaller roots the diameter of the central, conducting area is approximately a third of the diameter of the whole root, whereas in the larger roots the proportion of conducting area to the storage region is much smaller. The cortical region consists of large cells used for storage. The relative thickness of the storage region is a limiting factor in the penetration of the carbon disulfide gas into the root.

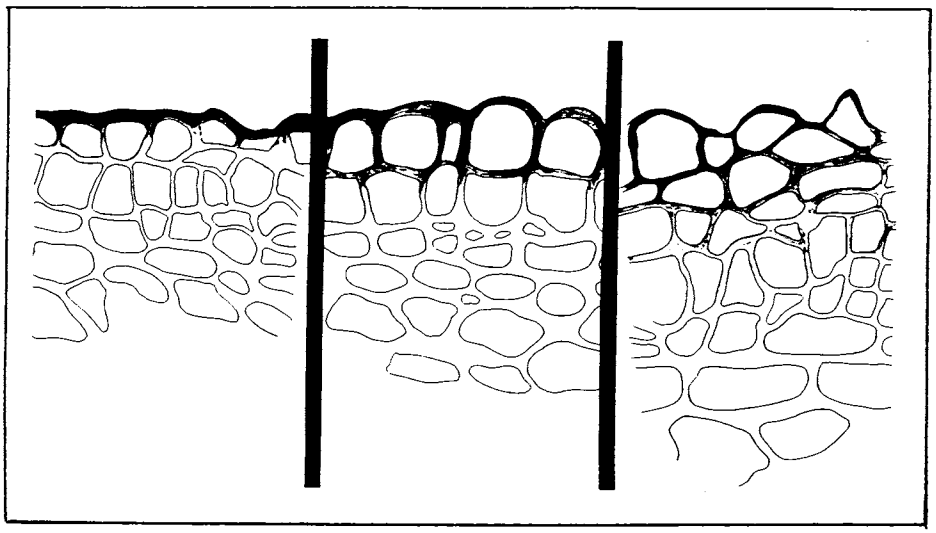

Fig. 10. Sections from different sized roots showing variation in thickness of epidermis.

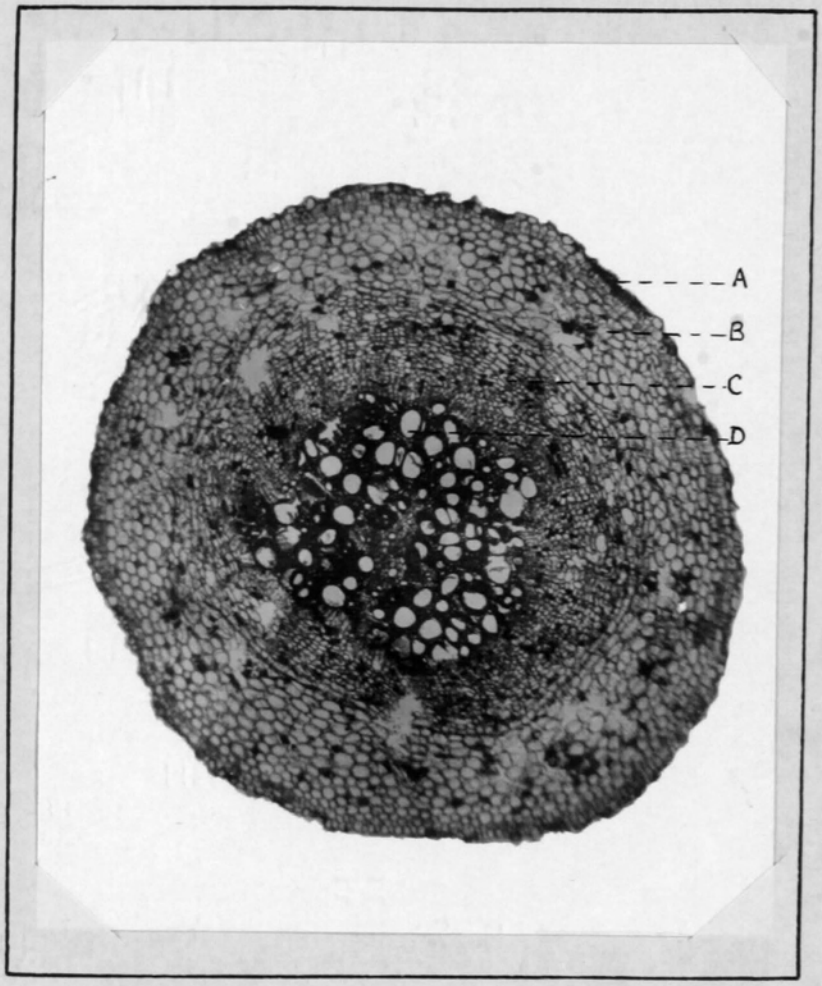

Fig. 11. Cross section of root showing:

A - Epidermis.
B - Cortex.
C - Cambium region.
D - Conducting area.

SUMMARY

One of the first considerations in the study of the effect of carbon disulfide upon the roots of the common wild morning glory, was the development of methods for ascertaining the behavior of the chemical. This demanded a formula which would express the liquid-to-gas volume change, and showed the need of a chemical indicator which would record the presence of the gaseous substance. A formula was evolved and experimentally checked. Thymol blue proved to be a useful indicator for detecting the presence of the carbon disulfide gas in concentrations greater than 10 per cent.

Root regeneration tests showed that roots from any depth were equally susceptible to the effects of the carbon disulfide, but the kill was directly proportional to the size of the root. There was very little difference in the percentage of kill for either long or short periods of exposure of the roots to the carbon disulfide gas, but there was a marked effect according to the concentrations of gas used. A saturated atmosphere gave a good kill, and a 50 per cent saturated atmosphere gave a medium kill, but concentrations lower than 50 per cent were practically valueless.

The rate of diffusion of the gas through the soil is dependent upon the soil temperature and the texture of the soil. Coarser soils permit more rapid diffusion due to the larger air spaces surrounding the soil particles. Soil moisture is a direct factor in diffusion, for its presence in the soil either partially reduces the air spaces, or completely fills them, thus retarding or stopping the diffusion of the gas.

Microscopic observations showed that the carbon disulfide attacked the cortex and cambium regions of the root. This attack was directly influenced, however, by the size of the root and the thickness of the epidermis. Roots less than 2.5 millimeters in diameter were visibly affected by the chemical, whereas roots larger than 2.5 millimeters seemed to be unaffected.

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