THE EFFECT OF SOME PHYTOCIDAL SUBSTANCES ON THE METABOLISM OF LEAVES

BY

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Abstract of Thesis

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Roots were induced on isolated bean leaves by treating with 2,4-dichlorophenoxyacetic acid, which was chosen as typical of the phytocidal substances; rooting comparisons were made with leaves treated with 2.5 p.p.m. beta-indolebutyric acid and water controls. 0.5 p.p.m. 2,4-D. was found to be the most suitable concentration for use since higher concentrations caused fasciation of the roots. The number of roots induced was proportional to concentration over the range 0.1 p.p.m. to 1.5 p.p.m. 2,4-D. Dry weight per root was found to be inversely proportional to concentration. Marked decreases in root number was found when treatment was delayed 24 hours after the leaves were detached from the plant. Treatment in the dark with 2,4-D. for 24 hours before removing to the light was more effective than treatment in light for root induction.

An apparatus is described for the gasometric measurement of the assimilation by individual leaves.

2,4-D. at the concentration used for maximum rooting, did not affect the respiration of the isolated leaves. The respiration of both treated and untreated leaves showed a Q_{10} of 2.2.

Gasometric measurements of assimilation did not show significant differences between treatments although some reduction

in the assimilation was found in 2,4-D. treated leaves. Assimilation rate was found to fall with time in both treated and untreated leaves.

Net assimilation rate was also estimated by growth experiments. No significant differences were found between treatments during the first eight days; by the end of the experiment increases in increment of total dry weight per square decimetre were obtained, running parallel with the number of roots induced by the different treatments and dosages. The formation of roots tended to prevent the accumulation of assimilates in the lamina; total carbohydrate accumulation as shown by the increments in total dry weight per square decimetre tends to a maximum and the assimilation rate estimated was found to be inversely related to the initial carbohydrate content in the three experiments carried out. No significant differences were found in lamina dry weights per square decimetre between treatments. The dry weight per square decimetre of the petiole showed significant changes with time indicating that the assimilates were translocated from the lamina to the petiole.

Satisfactory agreement was obtained between the estimates of net assimilation derived from gasometric and growth experiments.

Owing to the large sampling variance it was not possible to show differences due to treatment in the relative amounts of the various carbohydrates present in the lamina. Although evidence for a shift in the starch - sugar balance was sought for, little evidence of this shift was found.

TABLE OF CONTENTS

		Page
Abstract of Thesis		i
Introduction		1
Section I.	Rooting Responses of Detached French Bean Leaves to 2,4-dichlorophenoxyacetic Acid	4
	Previous Work	4
	Methods and Materials	6
	Effect of Different Concentrations of 2,4-dichlorophenoxyacetic Acid on the Rooting of Detached French Bean Leaves	7
	Effect of Temperature on the Rooting Response	13
	Effect of Light on Rooting	17
	(a) Varying the Total Quantity of Light	18
	(b) Light - Dark Treatment Comparisons	21
	1. Effect of Delayed Treatment	27
	2. Effect of Light and Darkness	28
	3. Effect of Treatment in Light vs Treatment in Darkness	29
	4. Effect of Light given before and after Treatment	29
	Histological Studies	30
Section II.	Assimilation and Respiration Studies of Treated and Untreated Leaves by Gasometric Methods and Growth Analysis	39
	Previous Work	39
	Methods and Materials	41
	Respiration in the Chambers	42

		Page
	Apparatus for Assimilation Experiments.....	43
	Leaf Chamber.....	47
	Flow Regulation and CO_2 Absorption.....	50
	Method of Estimating the Leaf Area.....	56
	Experimental Results: Gasometric Experiments	58
	Respiration Experiments.....	59
	The Effect of Temperature on Respiration..	60
	Assimilation Experiments.....	61
	1951 Experimental Results.....	69
	1952 Experimental Results.....	72
	Estimation of Net Assimilation Rate by Growth Analysis.....	79
	1951 Experimental Results.....	83
	1952 A. Experimental Results.....	90
	1952 B. Experimental Results.....	96
	Comparison of Gasometric Results with Growth Analysis Data.....	101
Section III.	Carbohydrate Analysis on The Lamina.....	106
	Previous Work.....	106
	Methods.....	108
	Experimental Results.....	110
Summary and Conclusions.....		121
Literature Cited.....		126
Acknowledgement.....		133

INTRODUCTION

It has long been known that various plant tissues are capable of producing roots; and that rooting could be promoted by the use of many organic substances. A major part of the work with these organic substances has been concerned with the gross effects of the substance or in the more practical way, in their use with stem or leaf cuttings for propagation. In the past isolated leaves have had considerable use in the promotion of our knowledge of the physiological processes. Experiments using isolated leaves were however, limited and could not be carried on over a long period of time because of the early death or deterioration of the leaf. This disadvantage would be overcome if a root system was established on the leaf as it would enable the leaf to be kept alive over a long period of time.

Very little systematic investigation into the rooting of isolated leaves had been done until that of Gregory and Samantarai in 1950. They made a thorough investigation into the factors concerned in the rooting responses of the dwarf French bean (Phaseolus vulgaris) and ivy (Hedera helix) leaves, using beta-Indolebutyric acid as the root promoting agents. This work was extended by Murari (1950) who concentrated on the physiological functions of such leaves. These workers have demonstrated that these leaves could be kept alive over a long period of time so that studies could be extended over a considerable period. Bean leaves have been kept

alive in this laboratory for 45 days and ivy leaves for over two years without showing senescence or change.

By the use of such isolated leaves the complicating issues of translocation to other parts of the plant are largely avoided. This has many advantages since the lamina, petiole, and roots combined can be treated as a unit without the great bulk of material that arises when a whole plant is used.

Nutritional effects are confined to the lamina and any translocation that takes place is only over a very short distance after a root system has been established. It is therefore, an ideal method for studies on assimilation and carbohydrate transformations.

It was felt, therefore, that the use of rooted detached leaves would be suitable for the study of the effects of the phytocidal hormones on the metabolism of the leaf.

2,4-dichlorophenoxyacetic acid was chosen as a typical hormone of this group and was used throughout all experiments. The investigations reported here were undertaken along two lines:-

1. Rooting responses of dwarf French bean leaves to 2,4-dichlorophenoxyacetic acid as compared with those of beta-Indolebutyric acid previously studied.

2. Metabolic studies were limited to (a) growth analyses

for net assimilation; (b) carbon-dioxide assimilation as studied by direct gasometric measurement of CO_2 uptake; and (c) lamina starch-sugar analyses. Carbohydrates were estimated by chemical analysis in lamina only. Efforts were made to construct a balance sheet for carbon.

1. ROOTING RESPONSES OF DETACHED FRENCH BEAN LEAVES TO 2,4-DICHLOROPHENOXYACETIC ACID

Previous work. The reports of both, Gregory and Samantarai (1950) and Murari (1950) contain reviews of the literature dealing with the rooting responses of the leaves of various plants, and therefore, will not be dealt with here.

There is considerable literature dealing with the gross effects of 2,4-dichlorophenoxyacetic acid: Excellent reviews of the literature dealing with its action are given by Zimmerman and Hitchcock (1948); Norman, Minarik, and Weintraub (1950); and by Blackman, Templeman, and Halliday (1951). The literature concerned with the rooting responses to this substance are, however, scanty. Hitchcock and Zimmerman in 1942 tested a wide range of synthetic growth substances and reported that 2,4-dichlorophenoxyacetic acid was equal to, or more effective than beta-Indolebutyric acid in bringing about the rooting of detached Tomato and Cissus leaves. They also, noted that above the optimal strength for the plant being used (for Cissus 1.0 p.p.m.) fasciation of the root resulted. Ferri (1945) found that when Indolebutyric acid, alpha Naphthalene-acetic acid, Indoleacetic acid, and 2,4-dichlorophenoxyacetic acid was applied to the soil they were absorbed and transported upwards in sufficient amounts to induce root formation in tomato leaf cuttings taken some time afterwards. Beal (1946) reported that when 2,4-dichlorophenoxyacetic acid was applied to decapitated bean plants well formed roots developed immediately below the point of application.

Gregory and Samantarai (1950) reported that the optimal concentration for the rooting of French bean leaves with Indolebutyric acid was 2.5 p.p.m. and that best rooting was obtained when the second leaf from the apex was used. Optimal time of treatment was found to be 24 hours. Hitchcock and Zimmerman (1942) in their experiments used a similar period for the treatment of tomato leaves with 2,4-dichlorophenoxyacetic acid (2,4-D). Rice (1948) using high concentrations of 2,4-D. applied to the base of the midrib of one of the primary leaves of a kidney bean plant found that under the conditions used in his experiment, the treated primary leaves had to remain attached to the plant for 24 hours for sufficient 2,4-D. to be absorbed and translocated for maximum inhibition of plant growth.

Gregory and Samantarai found that the main point of entry for the growth substance, beta-Indolebutyric acid (I.B.A.), in isolated leaves was through the cut surface of the petiole, and that very little entered through the cuticle; i.e. that the movement was upwards through the xylem. Ferri (1945), Mitchell and Brown (1946), and Corns (1948) found that 2,4-D. could be translocated upwards through the xylem when applied to the roots. Mitchell and Brown (1946), Corns (1948) and Linder, Brown and Mitchell (1949), and Rice (1948) found that the movement of 2,4-D. was also associated with the translocation of other organic materials away from the treated area through the phloem. The movement of 2,4-D. is therefore, bipolar and will be translocated through either the xylem or the phloem in an upward or downward direction.

Methods and Materials. The dwarf French bean (Phaseolus vulgaris) was the experimental plant used throughout the work. The parent plants were grown in soil at Chelsea Physic Garden, sowings being made ten days apart to ensure that leaves of the same age should always be available throughout the summer. The leaves were harvested in the morning and brought to the laboratory with their petioles dipped in water. The method of supplying hormone to the leaves, through the petioles, was the same as used by Gregory and Samantarai and by Hitchcock and Zimmerman (1942). The leaves used in all experiments were the second leaf from the apex of nearly equal maturity.

Fresh weights of all leaves were taken as soon as possible after harvest. The petioles were then placed into 1" x 3" speciman tubes containing the appropriate solutions in which they were left for 24 hours. Following this the petioles were then rinsed and placed in tap water where they were kept until rooting was completed. The water in the tubes was changed every day. The leaves were kept in the greenhouse and on very bright days were kept in partial shade; this served to prevent wilting and damage by extreme insolation.

All experiments employing 2,4-D. were run with both water controls and a rooted comparison. This rooted comparison consisted of leaves rooted with 2.5 p.p.m. I.B.A. under the same conditions as that used for the 2,4-D.

A. Effect of different concentrations of 2,4-dichlorophenoxy-acetic acid on the rooting of detached bean leaves.

Groups of ten leaves were treated for 24 hours with one of the following range of concentrations of 2,4-dichlorophenoxyacetic acid, viz:-

2.5 p.p.m.; 1.5 p.p.m.; 1.0 p.p.m. 0.5 p.p.m.;
0.25 p.p.m.; 0.1 p.p.m.; 0.05 p.p.m.; 0.01 p.p.m.

One group treated with 2.5 p.p.m. I.B.A. and another group with tap water only, formed the controls.

Results. Table 1. summarizes the results obtained. The number of roots formed were counted after fifteen days. Roots had emerged from the petiole by the eighth day; apparently one day longer than that required for roots formed using I.B.A. A difference confirmed by later experiments.

The relation between the number of roots produced and concentrations of 2,4-D. are shown graphically in Fig. 1. It will be seen that over the range of concentrations 0.1 to 1.5 p.p.m. the $\log_e$ of the number of roots produced is directly proportionally to the $\log_e$ of concentration; the regression equation being $Y = 5.50 + (1.745)x$ with a standard error of 0.27. Above 1.5 p.p.m. the number of roots show signs of falling off but the maximum number is still very much higher than the corresponding concentrations of I.B.A. This relationship between the number of roots induced and the concentration of 2,4-D. applied to thepetiole is apparently signoidal; thus showing a similar relationship to that found by

Blackman (1952) who found that the percentage inhibition of germination of Brassica alba and Beta vulgaris bears a sigmoid relationship to the amount of toxicant in the external solution; and also that the percentage mortality of these plants showed the same relationship to the amount of toxicant in a spray applied to the plants. In this experiment water controls produced no roots.

The average dry weight of roots per leaf also reaches a maximum at 1.5 p.p.m. but the development of individual roots differs greatly in the different treatments. The weight per root plotted against concentration is shown in Fig. 2 where it is seen that the weight of individual roots produced is maximal at a concentration of 0.05 p.p.m. whereas above this concentration the individual root weight falls steadily. Their growth will, of course, be dependant upon assimilates available in the leaf and one must therefore assume that two effects are concerned; 1st. the number of roots initiated, and 2nd. the available material for their growth. In this connection Smith (1948) reported that there was a rapid mobilization of carbohydrates to the regions of meristimatic activity induced by 2,4-D; and Gregory and Samantarai (1950) found that there was a rapid mobilization of sugars and soluble nitrogen to the rooting petiole. But if these factors were the only factors concerned in rooting, one would expect that the average weight of roots per leaf should remain constant which is not the case. There must therefore, be a further effect on root growth and as the average

TABLE 1

Effect of different concentrations of 2,4-dichlorophenoxy-acetic acid on the rooting of detached bean leaves. Fresh weight and dry weight are given in Jolie Balance units, for all root weights.

Initial Treatment	Av. F.W. Leaves (gms.)	Av. No. of roots per leaf	Av. F.W. roots per leaf	Av. F.W. per root	Av. D.W. per leaf	Av. D.W. per root
2,4-D.						
2.5 p.p.m.	7.08	415	14.8	0.036	1.8	0.0043
1.5 "	6.79	471	23.9	0.051	2.4	0.0051
1.0 "	7.43	256	24.5	0.096	2.0	0.0078
0.5 "	6.95	91	23.4	0.257	2.1	0.0230
0.25 "	8.01	14.7	7.5	0.510	0.8	0.0540
0.10 "	8.08	5.1	2.2	0.430	0.2	0.0390
0.05 "	7.15	0.4	0.5	1.25	0.05	0.1250
0.01 "	8.09	1.2	0.8	0.660	0.1	0.0860
I.B.A.						
2.5 p.p.m.	5.26	232	29.7	0.128	2.7	0.0116
Water	6.87	0	0	0	0	0

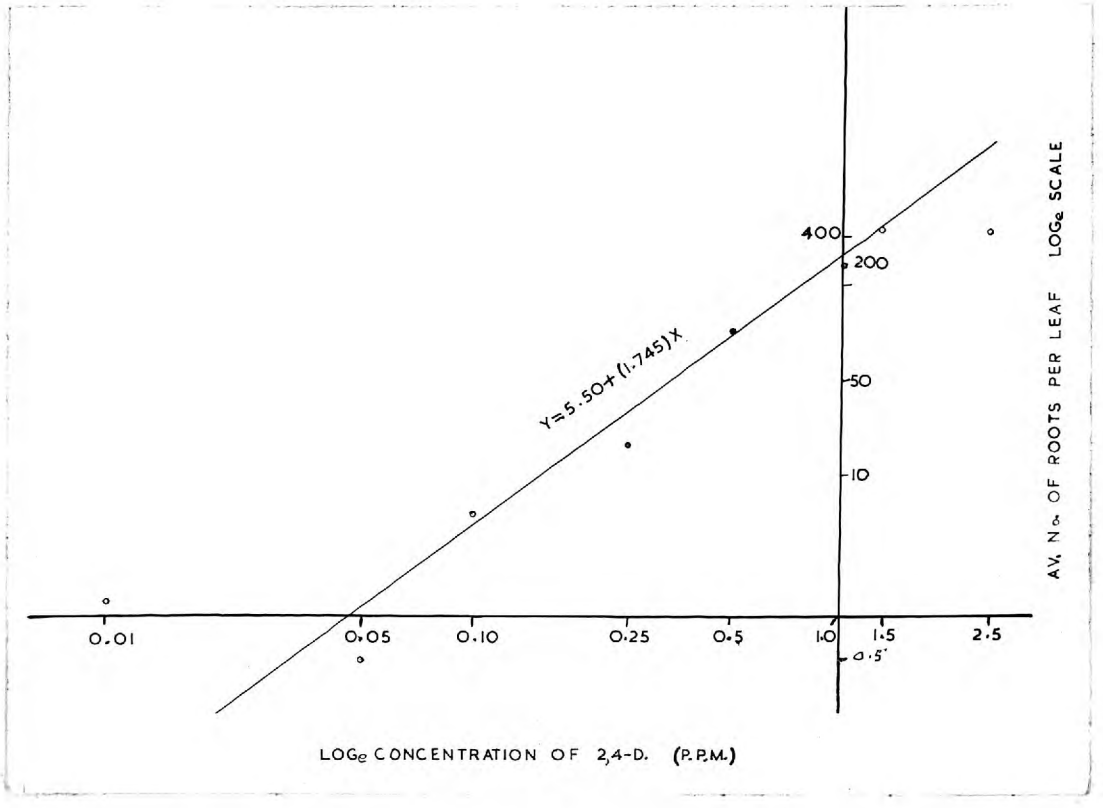


Fig. 1. Rooting response of bean leaves to various concentrations of 2,4-dichlorophenoxyacetic acid. Log_e scale.

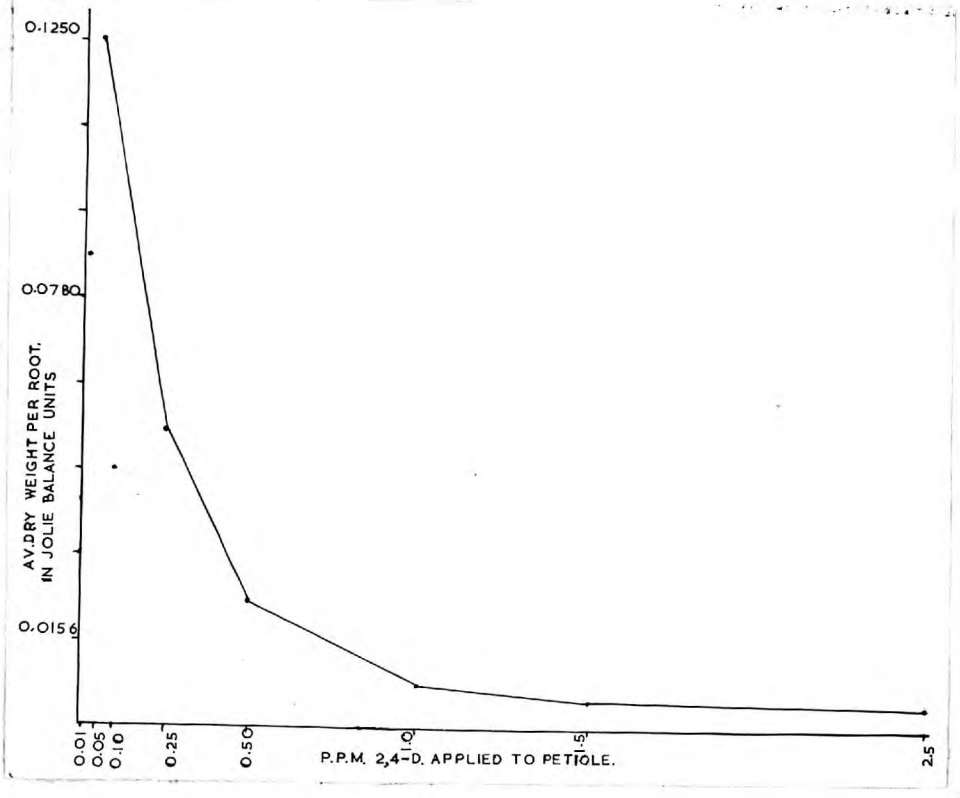


Fig. 2 Average dry weight per root induced by various concentrations of 2,4-dichlorophenoxyacetic acid.

weight per root decreases steadily with concentration, 2,4-D. must have had an inhibition effect on root growth. This fits in with the findings of Audus (1949) who, working on root growth inhibition in cress, radish, pea, and maize, found that 2,4-D. as a root inhibitor was 450 times more active than heteroauxin. Nutman et al (1945) found that when soil contained as little as one part in four million 2,4-D. root growth was inhibited in clover, sugar beet, and wheat; and that this inhibition varied with the concentration used.

The highest treatments 2.5 p.p.m.; 1.5 p.p.m.; and 1.0 p.p.m. showed abnormalities in the petiole, and 2.5 p.p.m. 2,4-D. led to considerable damage to the lamina in several cases. The abnormal petioles were swollen, twisted, and often ruptured at the junction of the lamina and petiole. This rupture often extended down the petiole for two or three centimetres. Roots were often fasciated and thickened. Only a few roots were fasciated with the 1.0 p.p.m. treatment but with the 2.5 p.p.m. 2,4-D. treatment the majority were fasciated. This fasciation often extending the whole length of the root, and as many as eight roots were united in one fasciated bundle.

0.5 p.p.m. 2,4-D. was therefore adopted as the most favourable concentration for use in further experiments. Certain precautions proved necessary at this concentration; e.g. shielding from the direct rays of the sun in the greenhouse and avoiding high temperatures during treatment, as these could result in damage to the laminas.

A difference in the rooting habit was noted between roots induced by 2.5 p.p.m. I.B.A. and those induced by 0.5 p.p.m. 2,4-D. Leaves treated with I.B.A. generally produced roots down the whole length of the petiole; many roots being produced at the top with the number gradually diminishing towards the base of the petiole. Leaves treated with 2,4-D. on the other hand, tended to induce roots that were more or less clustered at the top of petiole with only a few, if any, roots below. If there were any roots produced down the length of the petiole they were nearly always on the adaxial side of the petiole. Gregory and Samantarai found that the petiole of I.B.A. treated leaves showed six distinct rows of roots; four on the abaxial side and two on the adaxial side of the petiole. Similarly, with 2,4-D treated leaves the same number of rows of roots were produced. The majority of the roots seems to have been induced on the adaxial side. This was especially noted in those leaves producing small numbers of roots.

Water treatment in this experiment produced no roots, but a heavy callus formed at the base of the petiole. In other experiments water treated leaves did produce a few roots and in such cases they appeared from within the callus. Figure 3 and 3a illustrates the roots produced by the three treatments.

B. Effect of Temperature on the rooting response.

Marth and Davis (1945) working with high dosages of 2,4-D. found that temperatures over 65°F. were more effective for killing than

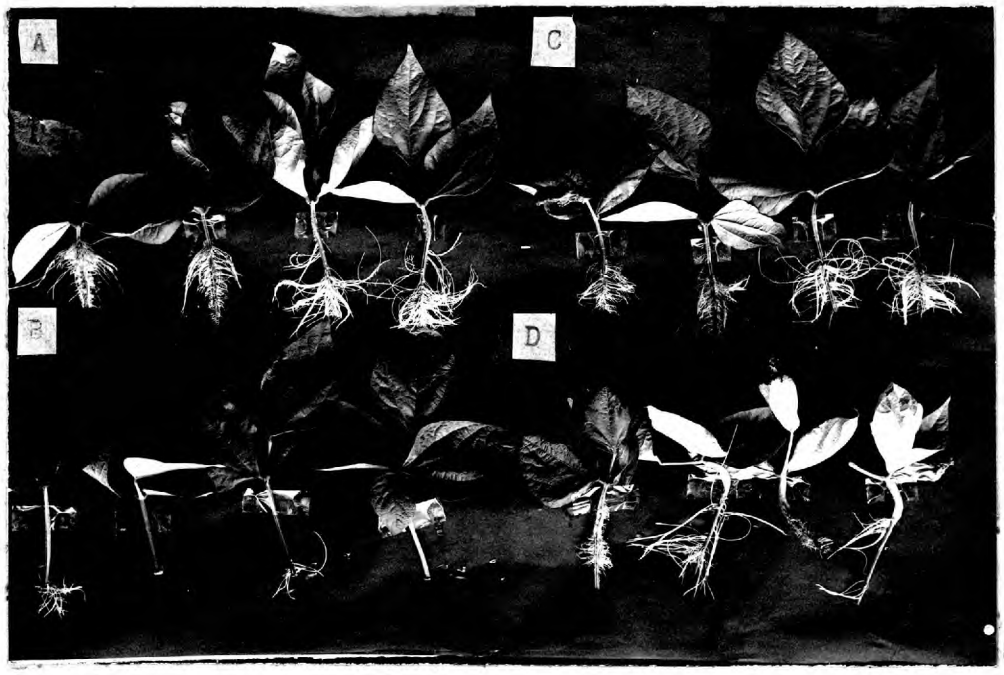


Fig. 3. Roots induced by various levels of hormone treatment.

A. 2.5 p.p.m. I.B.A. B. 0.5 p.p.m. 2,4-D.

C. Water D. 0.25 p.p.m. 2,4-D.

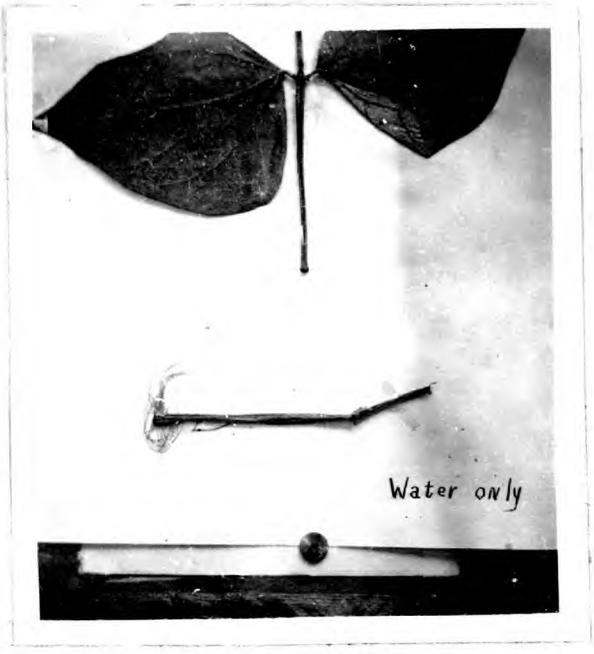
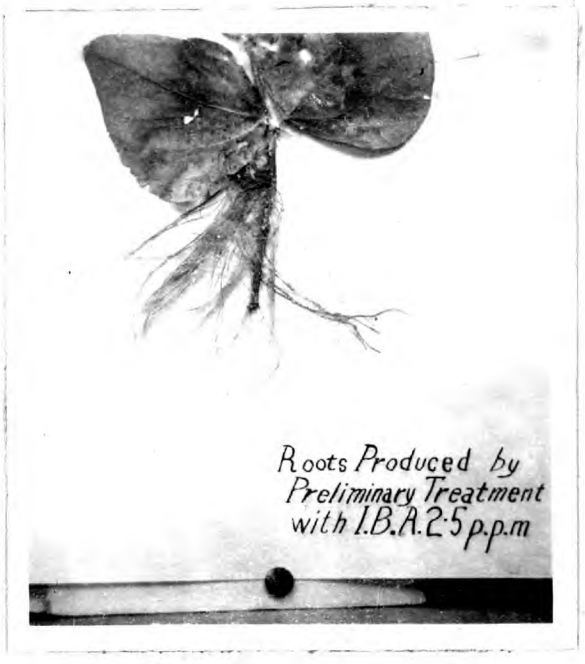
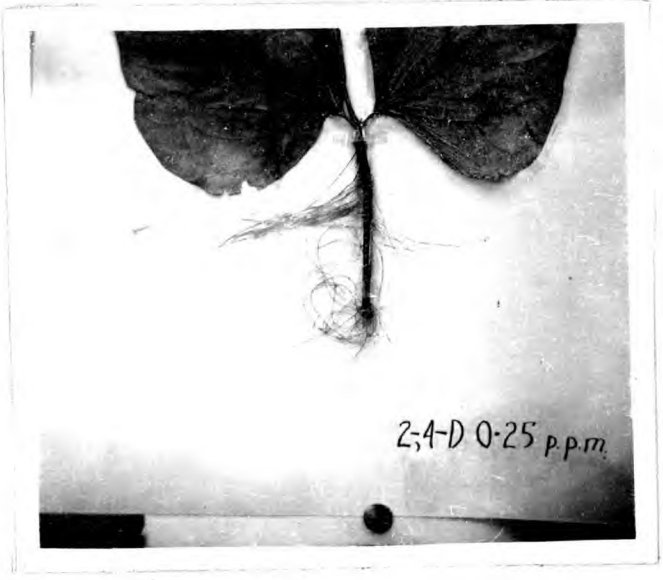
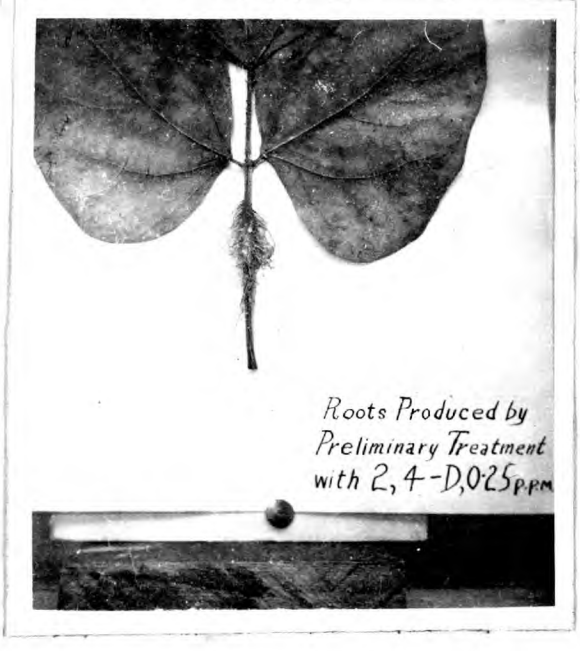


Fig. 3a
Typical water induced roots



Typical I.B.A. induced roots





Typical 2,4-D. induced roots

lower temperatures. Rice (1948) using 46° - 58°F. as his low range of temperatures found no stem curvature, while in his higher ranges every treated bean plant showed curvature. He did not, however, report on temperatures between 58° and 79°F. Kelly (1949) found that when physiological processes are slowed down as by low temperature, the plants are less susceptible to 2,4-D. Weintraub and Brown (1950) obtained a constant repression of the trifoliate leaf expansion due to a particular dose over the range 22° - 34°C. when 2,4-D. is applied directly to the bud of bean seedlings; when however, the 2,4-D. is applied to the primary leaves the dose response curve is composed of two distinct segments; the first is where the response is a function of dosage and is connected by a plateau in which the response is independent of dose, and is temperature sensitive. Gregory and Samantarai (1950) while doing no systematic studies on the effect of temperature, noted that there was a very large temperature effect.

No systematic examination of the temperature effects were undertaken in this work, but it was thought worthwhile to report some of the observations made from experiments set up for other purposes.

A duplicate of the preceding experiment was set up in July 1950 in an endeavour to repeat the results obtained. During the treatment period of this experiment the weather was cloudy and cool. These temperature conditions remained for all the early part of the experiment, rarely rising above 65°F. No roots were produced on any of the petioles at any of the levels of treatment used. This effect was

noted in other experiments where light conditions were poor and the temperature low, although conditions had been favourable during the preliminary period of treatment.

A seasonal effect, should also, be noted here. It was found very difficult to get the petioles to root after the end of August, when the day length and temperatures were falling. This severely restricted the period in which experiments could be carried out successfully.

C. Effect of light on rooting.

Mitchell and Brown (1946) found that the 2,4-D. stimulus is not readily translocated from bean leaves in darkness. Linder, Brown and Mitchell (1949) found that treated plants did not show curvature when kept in the dark, but that this curvature developed when the plants were removed to the light. Penfound and Minyard (1948) found that bean plants and water hyacinth were killed more readily when treated in darkness or diffuse light than when treated in direct sunlight. Felber (1948) found that 2,4-D. treated bean plants developed "protuberances" on the lamina, but that the development of these intumescences were independent of light and temperature. Thimann (1947) reports that a slightly greater kill of woody plants by 2,4-D. was obtained in the light than in the shade; but Zimmerman and Hitchcock (1948) found that this was not always the case as they obtained a better kill of Japanese honeysuckle in the shade than in the light. Elger (1950) reports that he obtained a better kill of

broad-leaved herbs in the shade than in the light. Rice (1948) found that while no stem curvature developed in plants treated in the dark, significantly greater amounts of the ammonium salt of 2,4-D. was absorbed by bean plants treated in the dark, than in those treated in the light. Gregory and Samantarai (1950) found that very few roots were induced on isolated bean leaves kept in the dark and treated with I.B.A., and that the number of roots induced on leaves kept in the light was almost proportional to the total light received.

The light effect was investigated by two methods. The first was an examination of the amount of light required for rooting using a similar method to that used by Gregory and Samantarai; and the second was to test the effect of light and darkness during the period of feeding with 2,4-D.

(a) Varying the total quantity of light.

To vary the light condition leaves were rooted in 18 cm. cardboard cubes with varying sized apertures, thus controlling the total amount of light received by each leaf. The size of aperture cut into the top of each cube was as follows: 15 x 15 cm.; 10 x 10 cm.; 5 x 5 cm.; 4 x 2.5 cm.; 1 x 1 cm. and 0. Samples of ten leaves were given each light treatment. A further set was kept alongside, fully exposed to the light. This experiment was carried out in the greenhouse and the leaves were given the standard treatment of 0.5 p.p.m. 2,4-D. under the stated conditions. A further set of leaves was placed in the dark for 24 hours while being treated and later removed

to the greenhouse until rooted. And yet another set was placed in a controlled temperature darkroom and there allowed to remain for the same period of time. Roots were counted at the end of 15 days.

The results obtained in this experiment are summarized in Table II. Leaves receiving less light than that allowed in by the 5 x 5 cm. apertures were all dead at the end of the experiment. As found by Gregory and Samantarai, there was an increasing number of roots induced with increasing amounts of light received by the laminas which was roughly proportional to the total amount of light available. In this experiment those leaves under the 15 x 15 cm. apertures received only part of the light available, as there was a certain amount of shading from the sides of the cardboard box and from the lip of the cover. This would, in effect, limit the amount of direct light received. This partial shading seemed to act beneficially, but reducing the light on one-fifth of full aperture (5 x 5 cm.) acted adversely and still smaller amounts of light led to death, without rooting. Those leaves that received full daylight had fewer average roots than those that received their light from the 10 x 10 cm. apertures. The leaves that were kept in the dark in a controlled temperature darkroom although dead at the end of the experiment, had produced a great many minute roots; in this way differing from the leaves kept in the greenhouse under cardboard shades. The reason for this is unknown and the matter was not pursued further. The set treated with hormone in the dark for 24 hours, produced more than the controls treated in full light; and therefore it appeared possible that treatment in the dark

TABLE II

Number of roots induced under varying light conditions.

Light Treatment Size of Aperture in cms.	Av. No. of roots per leaf	Av. F.W. roots Jolie Balance Units per leaf	Av. D.W. roots Jolie Balance Units per leaf
15 x 15	173	16.7	1.5
10 x 10	48	6.4	0.6
5 x 5	8	1.0	0.1
4 x 2.5	0	All dead at end of experiment.	
1 x 1	0	" " " " "	"
0 (Greenhouse)	0	" " " " "	"
Full Light (Greenhouse)	22	9.2	0.7
24 Hours Dark	58	9.8	0.8
Continuous Dark (Constant Temp. Room 26°C.)	These leaves were all dead at the end of the experiment but all petioles were bearing many small roots.		

was more favourable. This was tested in further experiments.

(b) Light - Dark treatment comparisons

In the first experiment ten leaves were treated under one of the following conditions: -

A. All leaves were treated immediately after harvest.

i. Treated in the light for 24 hours and then kept in the dark for 24 hours, and then placed in the light until rooted.

ii. Treated in the light for 24 hours, and then kept in the light until rooted.

iii. Treated in the dark for 24 hours, and then kept in the light until rooted.

iv. Treated in the dark for 24 hours and kept in the dark a further 24 hours before removal to the light.

B. All leaves kept off the plant 24 hours before treatment.

v. In dark 24 hours before treatment. Treated in the dark 24 hours and then removed to light.

vi. In dark 24 hours before treatment then treated for 24 hours in the light. Left in the light after treatment until rooted.

vii. In light for 24 hours and then treated in the dark for 24 hours, then removed to light until rooted.

In this experiment the term light refers to the light received under natural daylight conditions in the greenhouse, as all light treatments were given there. Dark treatments are those

received in a dark room. Water controls received no treatment other than the varying light-dark regimes.

The results obtained are given in Table III. Leaves from group iii, i.e. those leaves treated in the dark for the first 24 hours, produced a greater number of roots than any other light treatment given. The next highest group were those that were given the treatment in the dark during the second 24 hours. This confirms the previous observation in Table II that treatment in the dark was more favourable for root induction by 2,4-D. Water treated controls produced slightly more roots when given a preliminary dark treatment.

As this experiment was not completely balanced for all factors and that some of this rooting may have been due to temperature factors, this experiment was repeated with a view to removing the temperature factor during the initial treatment phase and to provide a better balance to the light phases. All leaves were kept in a controlled temperature room for the first 48 hours. Light was provided by two 1000 watt lamps above a water screen, to remove as much of the radiant heat as was possible. This gave a light intensity of 250 f.c. at the surface of the leaves. The leaves receiving dark treatments were kept under dark covers in the same room, in close proximity to those receiving the light treatment. The temperature under the covers was only one degree cooler than under the lights. The temperature in proximity to the leaves kept in the light was maintained at 26°C.

TABLE LII

Preliminary Light-Dark Experiment

All light treatments refer to normal light in the greenhouse.

	Treatment First 48 Hours	Av. No. of roots per leaf	Av. D. W. Roots per leaf (J.B. units)
0.5 p.p.m. 2,4-D. Treated Leaves			
A. All leaves were treated immediately after harvest.			
i.	Treated in the light 24 hours and then placed in the dark for 24 hours.	100	1.3
ii.	Treated in light 24 hours and kept in light.	72	1.1
iii.	Treated in dark 24 hours and then removed to light.	126	2.0
iv.	Treated in dark 24 hours, kept in dark a further 24 hours and then removed to light.	99	1.5
B. All leaves kept off the plant 24 hours before treatment.			
v.	In dark for 24 hours and then treated in dark a further 24 hours.	82	1.6
vi.	In dark 24 hours and then treated in light for 24 hours.	65	1.2
vii.	In light 24 hours and then treated in dark for 24 hours.	108	1.1
	Water treated controls		
	in light for 24 hours and then dark for 24 hours.	2.4	0.2
	In light continuously (natural day).	2.2	0.1
	In dark 24 hours and then removed to light.	4.8	0.3
	In dark 48 hours and then removed to light.	5.2	0.3

The leaves were divided into three sets; the first was treated with 0.5 p.p.m. 2,4-D.; the second receiving 2.5 p.p.m. I.B.A.; and the third set was the water controls. Each set was divided into groups of five leaves; each group receiving one of the light treatments shown in the following factorial diagram of the experiment.

Factorial design for light-dark experiment

Preliminary treatment before removal to the greenhouse.

	A. Immediate treatment after removal from the plant.		B. All leaves kept off the plant 24 hours before treatment.	
	Treated in Light	Treated in Dark	Treated in Light	Treated in Dark
Kept in Light	i. Treated in light 24 hrs. and then kept in light a further 24 hrs.	iii. Treated in dark 24 hrs. and then kept in light 24 hours.	v. Kept in light 24 hrs. then treated in light 24 hrs.	vii. Kept in light 24 hrs. then treated in the dark 24 hours.
Kept in Dark	ii. Treated in light 24 hrs. and then kept in dark 24 hrs.	iv. Treated in dark 24 hrs. and then kept in dark a further 24 hrs.	vi. Kept in dark 24 hrs. then treated in light 24 hrs.	viii. Kept in dark 24 hrs. then treated in dark 24 hrs.

TABLE IV

Light - Dark Experiment. Average number of roots per leaf.

	A. Immediate Treatment		B. Treatment Delayed 24 Hrs.	
	Light	Dark	Light	Dark
Water treated leaves				
Light	i 0.3	iii 0	v 1.0	vii 2.5
Dark	ii 3.0	iv 0	vi 0	viii 3.0
Mean	1.6	0	0.5	2.8
2.5 p.p.m. I.B.A. treated leaves				
Light	i. 320	iii 238	v 103	vii 49
Dark	ii 246	iv 184	vi 137	viii 8
Mean	283	211	120	28.5
0.5 p.p.m. 2,4-D. treated leaves				
Light	i 69	iii 164	v 5	vii 1
Dark	ii 8	iv 33	vi 0	viii 7
Mean	38.5	98.5	2.5	4.0

TABLE IV-a

Light - Dark Experiment. Average D.W. per leaf and per root in mgs.

	A. Immediate Treatment		B. Treatment Delayed 24 Hrs.	
	Light	Dark	Light	Dark
Water treated leaves				
Light	i	iii	v	vii
Root D.W. per leaf	2.0	0	16.0	5.0
per root	6.6	0	16.0	2.0
Dark	ii	iv	vi	viii
Root D.W. per leaf	12.0	0	0	11.0
per root	4.0	0	0	3.6
2.5 p.p.m. I.B.A. treated leaves				
Light	i	iii	v	vii
Root D.W. per leaf	179	128	76	40
per root	0.56	0.54	0.74	0.82
Dark	ii	iv	vi	viii
Root D.W. per leaf	155	137	150	42
per root	0.73	0.85	1.09	4.75
0.5 p.p.m. 2,4-D. treated leaves				
Light	i	iii	v	vii
Root D.W. per leaf	61.8	86.0	16.8	1.0
per root	0.90	0.52	3.4	1.0
Dark	ii	iv.	vi	viii
Root D.W. per leaf	12.5	54.0	0	9.6
per root	1.5	1.7	0	1.4

From the results of the experiment presented in Table IV, the effect of various factors on rooting can be estimated.

1. The effect of delayed treatment. The overall effect of delaying treatment can be estimated from the totals i to iv vs v to viii.

	Immediate treatment	Delayed treatment	
Mean I.B.A.	247	74	Root No. per leaf.
Mean 2,4-D.	69	3.2	
Mean I.B.A.	149	79	Mean D.W. of roots
Mean 2,4-D.	53	6.9	per leaf in mg.
Mean I.B.A.	0.67	1.85	Mean D.W. per root
Mean 2,4-D.	1.16	1.45	in mg.

The effect of delay of treatment is very great on the number of roots per leaf and on the mean weight of roots induced. This effect is much greater on leaves treated with 2,4-D. than on leaves treated with I.B.A. On the mean weight per root the effect is reversed, both with regard to delayed treatment and substance used, but this is due to the fact that mean weight of roots produced is inversely related to the number of roots produced. Evidently, there is a marked competition between the growing roots and the rate of supply of some nutrient, probably carbohydrates, limiting the growth of individual roots. That some such effect on individual roots is occurring is shown by the fact that, the mean weight per root is much higher in the water controls, as seen in Table IV-a,

than in any of the treated leaves.

2. Effect of Light and Darkness. The treatments i and v received a total of 48 hours of light during the first two days of the experiment; treatments ii and iii, and vi and vii received 24 hours of light during the same period, while iv and viii were in darkness throughout this period. The results are shown below.

	48 Hours Light	24 Hours Light	0 Hours Light	
Mean I.B.A.	212	168	96	Root No. per leaf.
Mean 2,4-D.	37	43	20	
Mean I.B.A.	128	116	89	Mean D.W. of roots
Mean 2,4-D.	40	25	32	per leaf in mg.
Mean I.B.A.	0.65	1.59	2.80	Mean D.W. per root
Mean 2,4-D.	2.15	0.75	1.55	in mg.

As far as I.B.A. is concerned the mean number of roots is almost linearly related to the number of hours light given in the 48 hours. The mean weight of roots per leaf also show an increase, but which is not proportional to the light given. The mean weight per root is related in the reverse sense as shown before. The effect of light after treatment with 2,4-D. is much less pronounced. The very high value for the number of roots produced when 24 hours of light are given is largely due to the high value in treatment vi.

3. Effect of Treatment in Light vs Treatment in Darkness. The mean effects of treatment in light and darkness are shown below.

	Treatment In Light	Treatment In Dark	
Mean I.B.A.	202	120	Root No. per leaf
Mean 2,4-D.	21	56	
Mean I.B.A.	143	87	Mean D.W. of roots per leaf in mg.
Mean 2,4-D.	23	38	

In I.B.A. treated leaves, treatment is more effective in light than in dark, while for 2,4-D. the reverse is true.

4. Effect of Light given before and after Treatment. Leaves treated with I.B.A. immediately after isolation show no difference between treatments ii and iii, which would indicate that with such leaves it is immaterial whether the dark period is given before or after treatment. In the delayed treatment however, treatment vi is much more effective than treatment vii, i.e. a preliminary period in the dark followed by treatment in the light leads to a greater response than a preliminary period in the light followed by treatment in the dark. The reason for this is not known. In the case of 2,4-D. treatment iii, i.e. treatment in the dark followed by a light period is much more effective than the reverse order of treatments. In the delayed treatments, vi and vii, no rooting occurs, and therefore, the effect of darkness enhancing the rooting as seen in the previous table is almost entirely due to treatment iii.

However, these results are in agreement with previous work; Rice (1948) found that the uptake of 2,4-D. was greater in the dark than in the light; and Penfound and Minyard (1948) found that bean plants were more readily affected by 2,4-D. when given in the dark than in direct sunlight. Gregory and Samantarai (1950) have shown with I.B.A. preliminary starvation progressively reduced the rooting response; and that rooting is greater when treatment with I.B.A. is given in the light than in darkness.

The water controls in this experiment produced a few roots, these were more or less at random within the treatments. A dark period, as in the previous experiment seemed to be more favourable, but this may have been due only to chance. The water induced roots by the end of the rooting period, were very long in comparison to the roots induced by either 2,4-D. or I.B.A.

These experiments have shown that treatment in the dark with 2,4-D. gives optimal rooting, and therefore this procedure was followed in all the remaining experiments.

Histological Studies

Gregory and Samantarai (1950) made a careful examination of the development of root tissues in the isolated bean leaf; and found that within three days after treatment the parenchymous cells, just outside the phloem and medullary rays, enlarge and show periclinal division forming a ring of meristimatic tissue inner to cortex; by the fourth day the phloem has proliferated and smaller

bundles were formed in the medullary rays, and that the formation of roots primordia by groups of derivatives takes place very rapidly; by the fifth day roots were seen through the disorganized cortical tissue, vascular strands have developed and established connection with the xylem and phloem of the adjacent primary bundles of the petiole; by the sixth day root formation is complete.

Beale (1946) treating bean plants with 2,4-D. found that cell proliferation occurred and that this response was chiefly in the endoderm, cambium, phloem and ray parenchyma; and that well formed roots were formed internal to the cambium. Smith (1948) found extensive cell divisions in the cambium, phloem and medullary rays by the third day; and a band of active tissue extending from the cambium to the endodermis was formed by the fifth day. Numerous root primordia were found from this active tissue by the ninth day and the cortical cells were crushed. Murray and Whiting (1949) applying 2,4-D. to young maize plants, found that the meristimatic zone at the periphery of the stelar area was stimulated to excessive proliferation which resulted in the production of numerous small individual roots or in fasciation of roots. Eames (1950) treated bean plants with 125 p.p.m. 2,4-D. found that all tissues between the cortex and primary xylem proliferated; the first divisions being in the endodermis, quickly followed by divisions in the inner pericycle, primary phloem and all immature cambium derivatives of the hypocotyle. In all tissues, the first divisions are periclinal soon followed by anticlinal and transverse divisions

until a continuous sheath of proliferating tissue is formed. He also found that in the primary xylem the parenchyma proliferated freely.

Murray and Whiting (1947) made a complete study of the histological changes in decapitated bean plants to which 50 p.p.m. 2,4-D. had been applied to the cut surfaces. They found that proliferation had started by the end of the first 24 hours in the endoderm and in the pericycle within 2 or 3 days; and by the third day the phloem had formed a wide band of proliferated tissue. Parenchyma outside the interfascicular cambium had responded similarly to the phloem parenchyma. In what they called the root zone they found that the organization of a primordia generally occurred within each medullary ray ; and by the fifth day root histogens were distinguishable, but developed in a fasciated manner with no separation into individual roots. In no instance did they get roots to emerge through the outer surface as the root tips collapsed before they were able to do so.

In this work no systematic histological studies were made on the development of the root system. Sections were made, however, from petioles in which roots had been induced by 0.5 p.p.m. 2,4-D. and 2.5 p.p.m. 2,4-D. The petioles were fixed in Billing's modified Navashin fluid and after usual procedure were embedded in paraffin. Sections were cut in both longitudinal and transverse section, and stained with safranin and Ehrlich's haematoxylin.

A transverse section of a petiole showing two roots induced

by 0.5 p.p.m. 2,4-D. is given in Fig. 4; with the longitudinal section in Fig. 5. The root vascular tissue is seen to arise adjacent to the vascular bundle of the petiole, and connected up with the phloem and xylem. Tissues inner to the cortex had become meristimatic and the cortex had been disrupted. These roots had therefore developed in a similar manner to the roots induced by I.B.A. as found by Gregory and Samantarai. A slight fasciation is shown in the transverse section at the base of the two roots.

Figs. 6 and 7 are a transverse section and a longitudinal section, respectively, of petioles in which roots had been induced by 2.5 p.p.m.. Here extensive proliferation external to the cambium and inner to the cortex had taken place. Many small meristimatic cells of the parenchyma are in evidence. The transverse section (Fig. 6) shows three root bundles fasciated into one root with the parenchyma cells forming a bond between the three vascular systems; these vascular systems arise adjacent to the vascular bundles of the petiole as found in the roots induced by 0.5 p.p.m. 2,4-D. Both xylem and phloem tissues are in evidence in all three root bundles. In the longitudinal section (Fig. 7) extensive proliferation of the phloem parenchyma is shown; there is also evidence that proliferation of the xylem has occurred to a lesser extent. A thick band of small-celled meristimatic cells lies external to the cambium with some disruption of the cortical layer occurring.

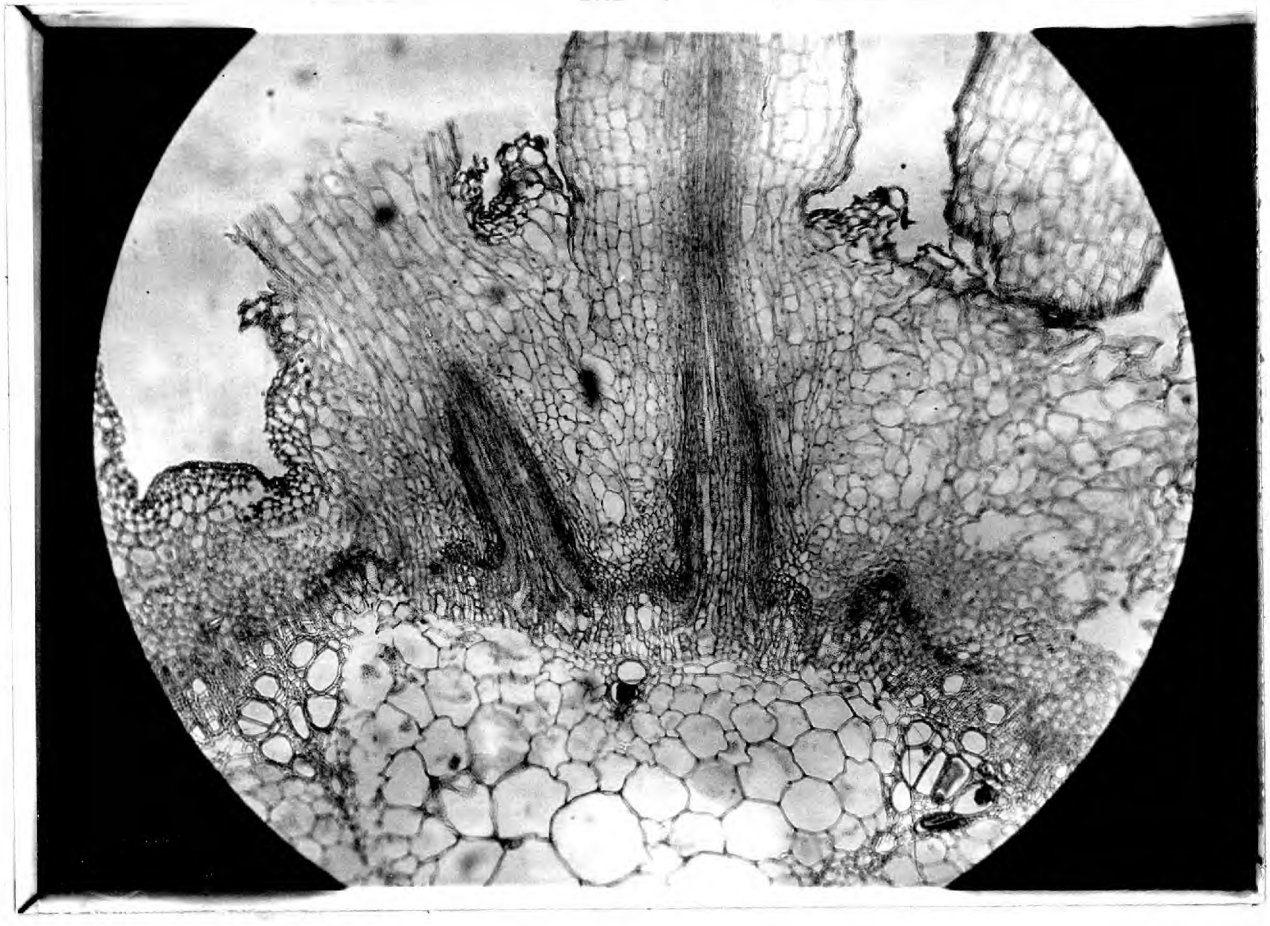


Fig. 4. Transverse section of bean petiole treated with 0.5 p.p.m. 2,4-D., showing two fully emerged roots joined up to the vascular bundle.

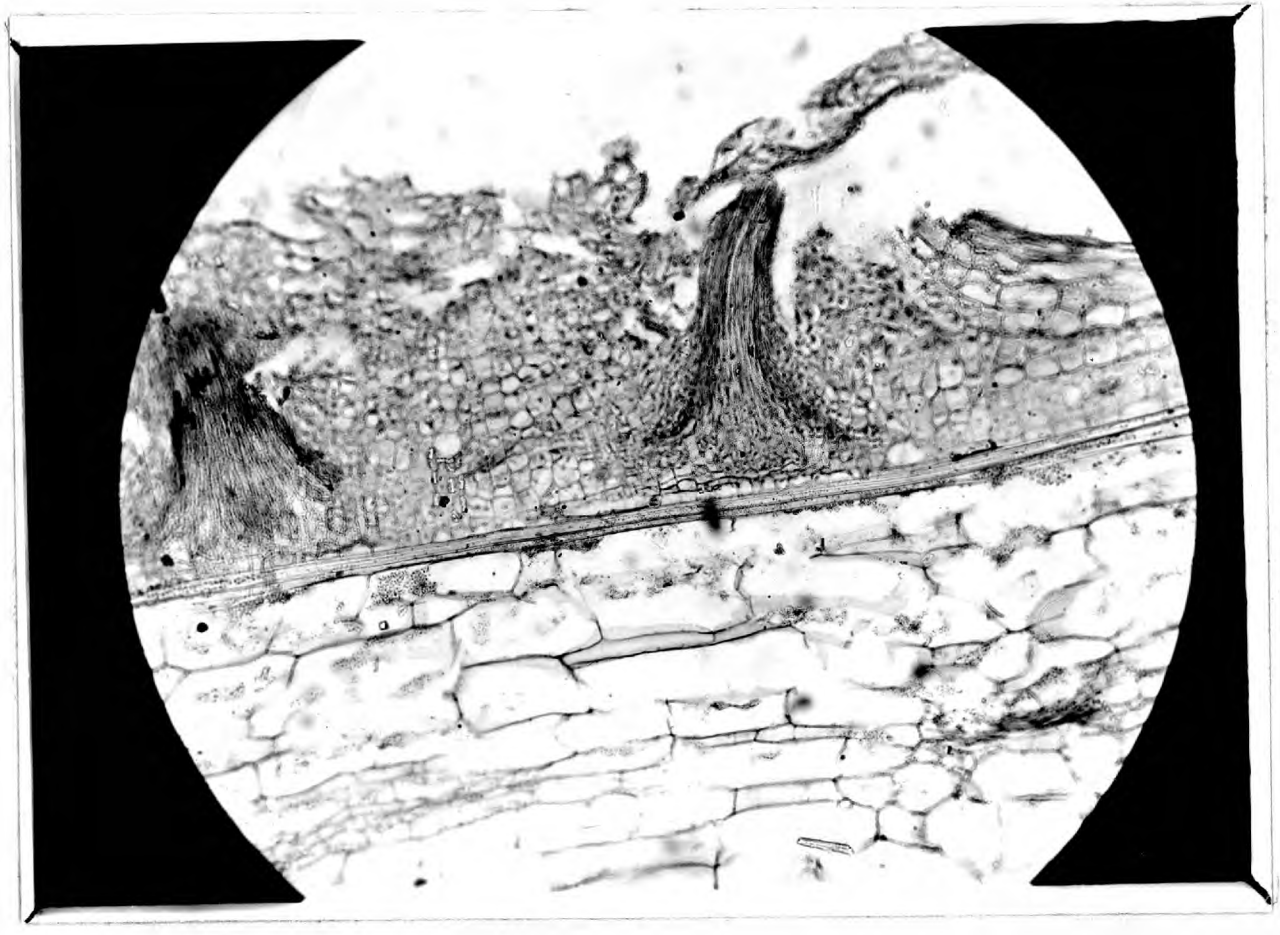


Fig. 5. Longitudinal section of bean petiole treated with 0.5 p.p.m. 2,4-D. showing fully emerged adventitious root.

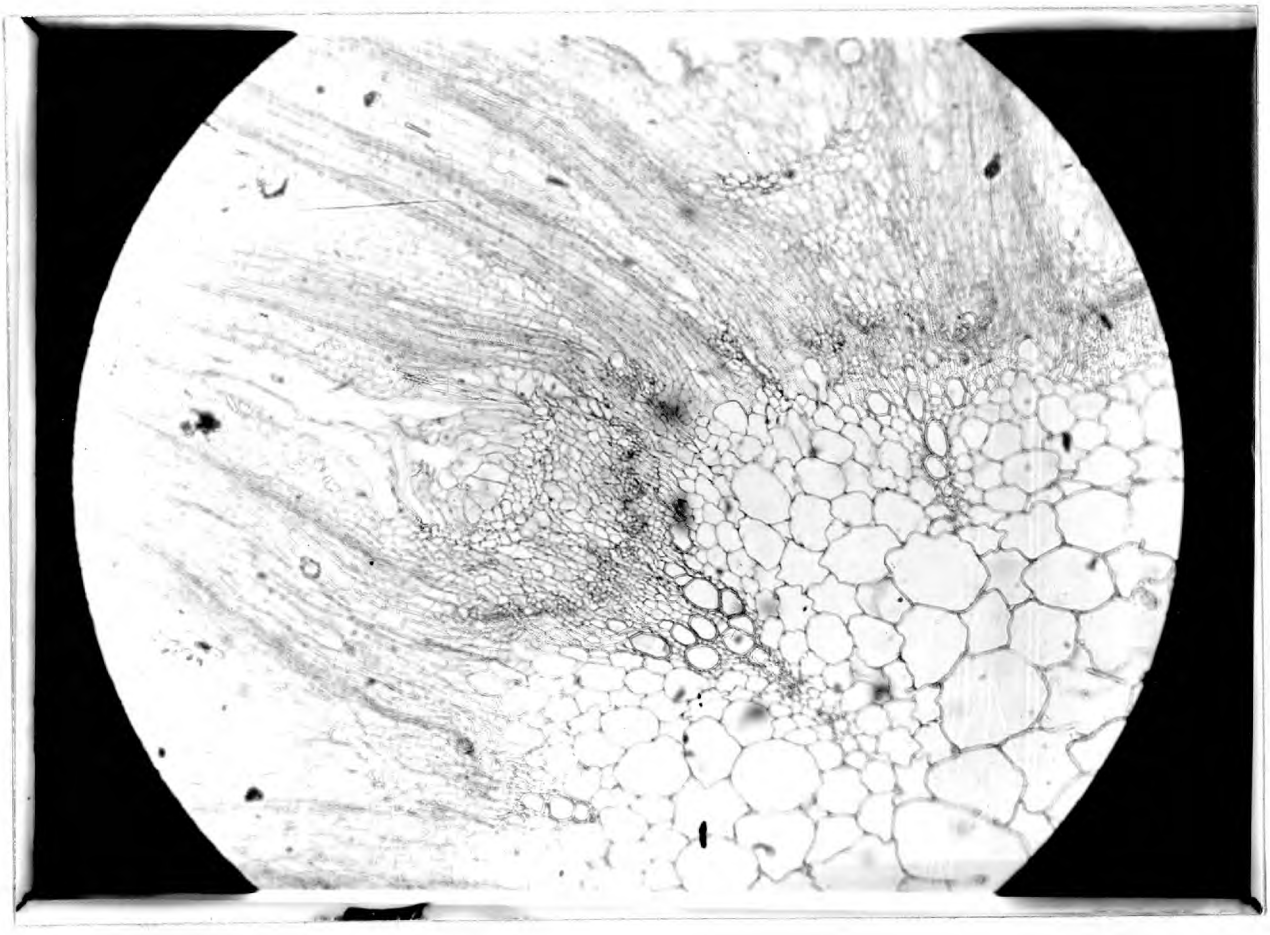


Fig. 6. Transverse section of bean petiole treated with 2.5 p.p.m. 2,4-D showing fasciation of three root bundles into one fasciated root.

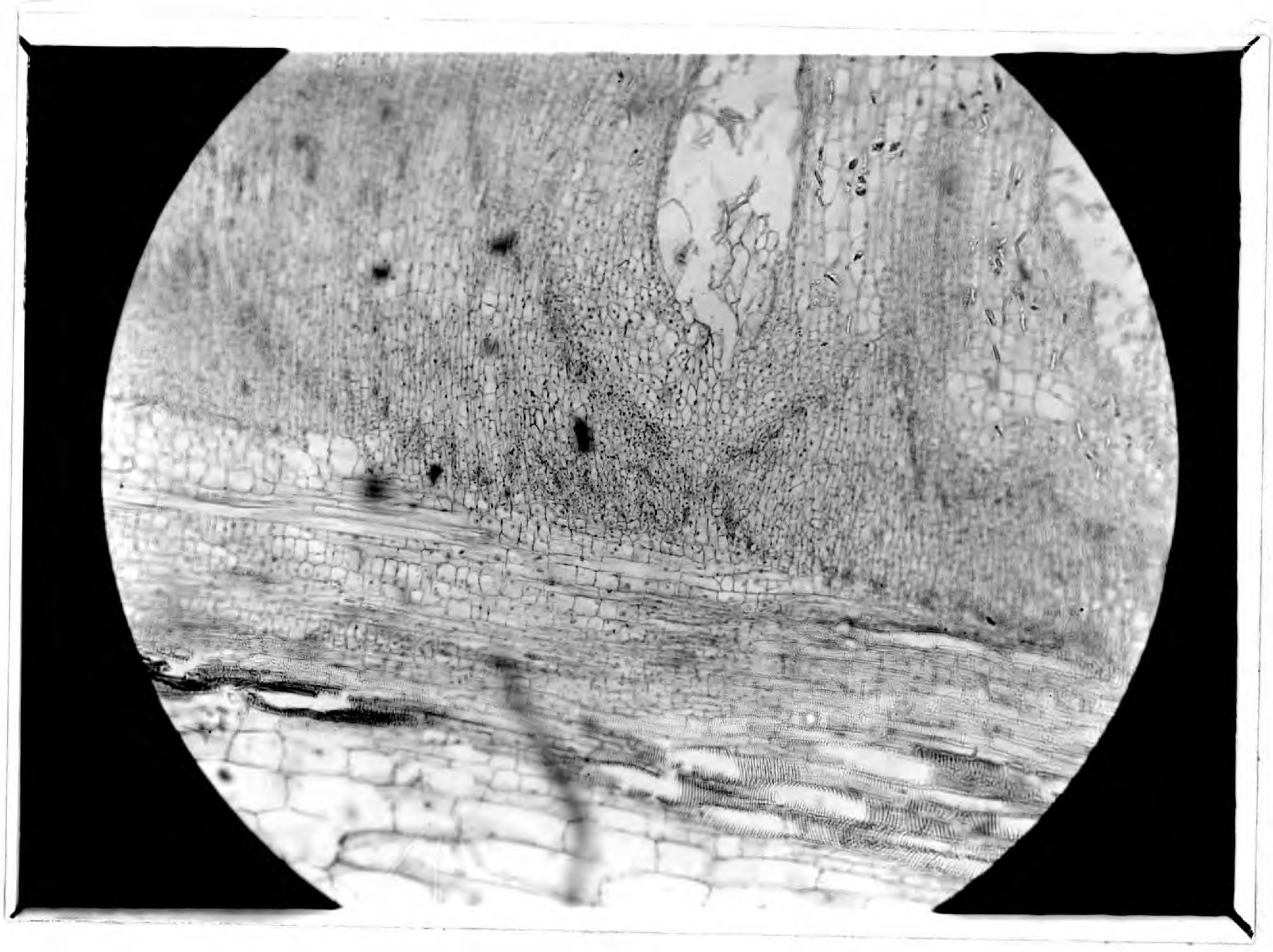


Fig. 7. Longitudinal section of a bean petiole treated with 2.5 p.p.m. 2,4-D showing extensive proliferation of primary tissues and parenchyma.

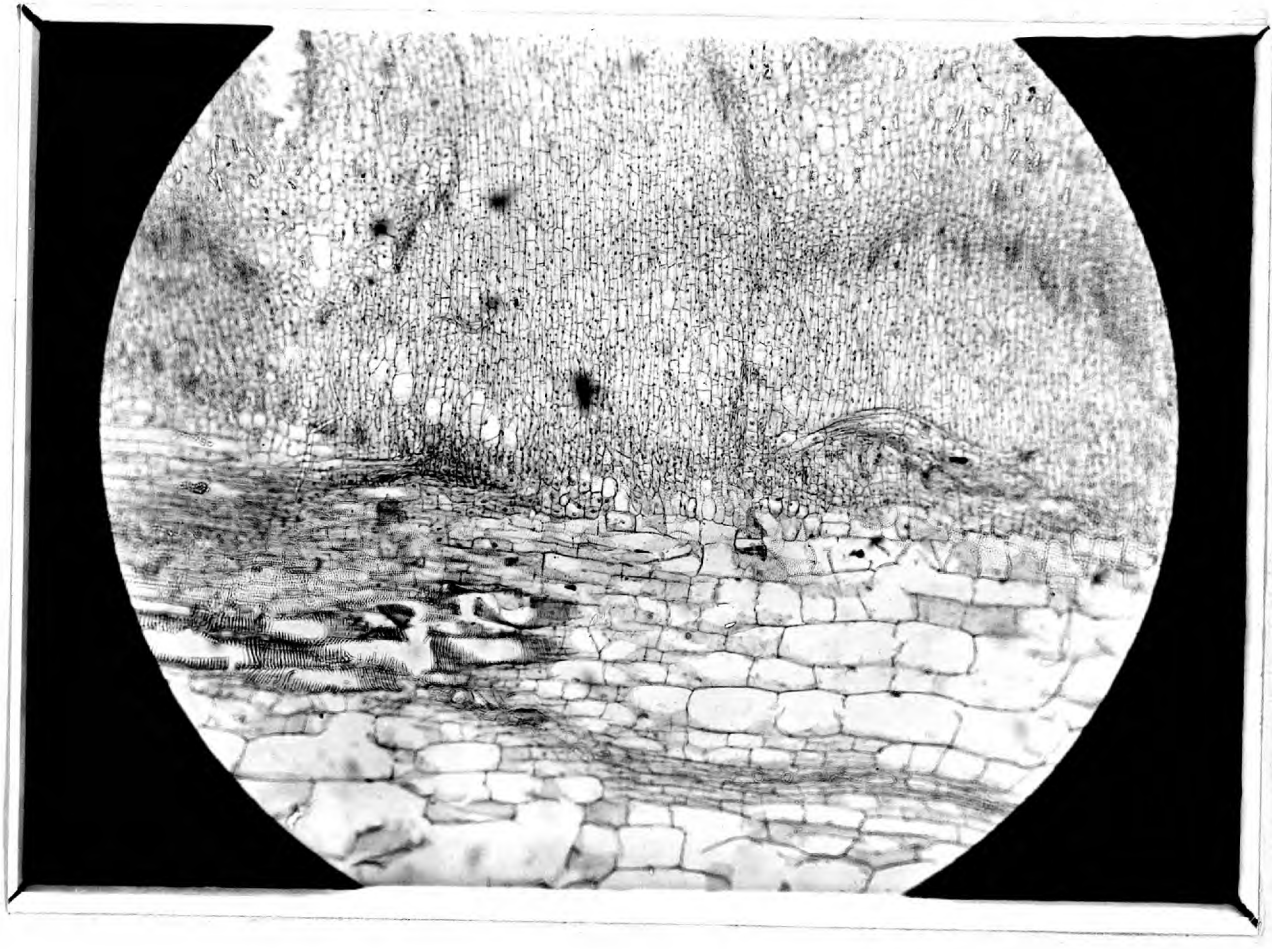


Fig. 7a. Longitudinal section of a bean petiole treated with 2.5 p.p.m. 2,4-D. showing extensive proliferation of primary tissues and parenchyma.

Section II

Assimilation and Respiration Studies of Treated and Untreated Leaves by Gasometric Methods and Growth Analyses.

Murari (1950) measured the assimilation rates of leaves rooted with I.B.A. over a period of thirteen days and came to the following conclusions; (1) assimilation rate is unaffected by the accumulation of carbohydrates in the leaf; and (2) treatment with I.B.A. has no effect on CO_2 assimilation. Untreated leaves showed no decline in assimilation rate until the leaves started to show signs of senescence. He concluded that the assimilation rate is independent of treatment.

Freeland (1949) measuring the carbon dioxide content of the air under a bell jar covering a 2,4-D. treated bean plant, reports that he found an immediate decrease in photosynthesis with complete recovery after 48 hours. In 1950, using O_2 evolution as the criterion he found that there was partial to complete recovery after treatment depending upon the concentration used. Pickett el al (1951) found that apple leaves sprayed with 10 p.p.m. 2,4-D. showed no significant difference in the amount of photosynthate manufactured, from the control leaves. Other evidence in the literature dealing with these processes in relation to 2,4-D. was limited to the work done on respiration. Taylor (1947) found that 2,4-D. at concentrations from 0.25 to 10 p.p.m. decreased respiration in wheat and mustard seedlings, and stated

that the differential effect does not seem to be primarily concerned with a distinct difference in response of the energy metabolism. Brown (1946) found that in terms of CO_2 production, bean seedlings treated with 0.1% (100 p.p.m.) 2,4-D. resulted in a higher rate of respiration; which was 19 to 80% greater than that of the controls. Hseuh and Lou (1947) reported that production of CO_2 was at first accelerated and then decreased; and that 0.1% (100 p.p.m.) inhibited respiration of barley and rice seedlings. Kelly and Avery (1949; 1949A; and 1951) found that 2,4-D. had both a stimulatory and inhibitory effect upon the respiration of pea and oat tissue depending upon the concentration used; that physiologically old tissue was relatively inert to 2,4-D.; and that starved tissue showed a greater effect on the respiration rate than was shown by fresh tissue. Corns (1950) reports a similar stimulation or inhibition of the respiration rate of Bean leaves depending upon soil moisture content; concentration of 2,4-D. used; and time after treatment. Nickell (1949; 1950) also reports a similar stimulation and inhibitory effect upon the respiration by virus tissue of Rumex acetosa. West and Henderson (1950) found that as little as 0.01M 2,4-D. inhibited the respiration of Lupinus roots and that 0.001M. 2,4-D. showed no inhibition. Weintraub et al (1950) using 2,4-D. labelled with C^{14} found that detached bean plants respired less than did attached plants; Carboxyl labelled CO_2 was respired off during the first day or two coming off in greater amounts than the methoxy labelled CO_2 which followed the carboxy group. Labelled CO_2 was not respired from the ring position.

In view of these conflicting results it appeared important to study systematically the effect of 2,4-D. on the assimilation and respiration rates of bean leaves supplied with an optimal concentration of the substance for rooting. It appeared unwise to carry the concentration to high levels which are known to lead to injury of the plant. It was thought that the optimal concentration for rooting would represent the most interesting concentration for study.

Methods and Materials. Detached bean leaves were treated with 0.5 p.p.m. for 24 hours and then at various periods during the rooting process the CO_2 uptake was measured by normal gasometric methods. The experiments were made in two sections; the first, to measure the CO_2 uptake during the day, at various intervals up to fifteen days after treatment; and secondly, to measure the CO_2 respired at night by the same leaves.

During the first season (1951) the leaves were treated, in situ, while in the leaf chambers used for the assimilation experiments. In certain of the experiments measurements were made during the period that the leaves were being treated. In 1952 the leaves were treated in the dark since it had been found in previous experiments more suitable for inducing roots. The apparatus used in 1951 was that previously used by Murari. This was the modified version of that described by Porter et al (1949). It will not be described here as it is essentially the same in principle to the apparatus set up for use in 1952 which will be described later.

Respiration in the Chambers; Respiration experiments are much simpler to carry out than assimilation experiments, since the rate of flow of the air stream need only to be quite slow; and the CO_2 respired is independent of the rate of flow. The difficulties mentioned later in connection with assimilation experiments did not arise, and a reliable estimation of CO_2 could be made.

A stream of CO_2 - free air was passed through the chambers at a uniform rate; and absorbed in spiral bubblers containing N/2 NaOH. These spiral bubblers were first weighed dry; 50 cc of NaOH was then run in from an automatic pipette; the whole again weighed and placed on the apparatus. A weight correction at the end of the experiment, was made for changes in water content of the soda through absorption of water vapour by again weighing the spiral bubblers. The spiral bubblers were then allowed to drain into 250 cc. volumetric flasks. No effort was made to wash out all the soda from the bubblers. After draining the spiral bubblers were again weighed. In this way errors due to incomplete washing were avoided, and the fraction of the total soda in the bubbler actually used for titration was known and corrections applied. 10 cc. of 10% $BaCl_2$ was added to the flask to precipitate the carbonate, and the flasks were made up to the mark with distilled water, well shaken to thoroughly mix the soda and the water. The precipitate was then allowed to settle out. 50 cc. aliquots were removed from the supernatant liquid and titrated in the usual may with N/10 HCl.

Apparatus for Assimilation Experiments. Modification of the apparatus used in 1951, was found necessary for use in 1952. The leaf chamber used by Murari was found to have several disadvantages. It was found that it was practically impossible to place the two trifoliate bean leaves used in the test into the leaf chamber without overlapping to a greater or lesser extent, and thus shading portions of the lamina. The amount of overlapping could not be accurately estimated. It was also found that while the chambers appeared to be too deep to allow all the air in the chamber to come into contact with the laminas, they were too shallow to place the leaflets in the chamber without sometimes damaging the petiole; or the lamina being held in close contact with the lid and preventing the air circulating over the upper surface of the lamina. There was however, good turbulence in the air within the chamber and most of the air must have come in contact with the lower surface of the lamina at least, since there was a fairly rapid depletion of CO_2. The water treated leaves used up approximately 63% of the available CO_2 during these experiments. This brought the residual in the air down to 0.011% which is very close to the maximum that can be used by the leaf. This will be discussed later when the data from these experiments is presented; and due to this depletion it would be impossible to make a correction on the assimilation for the average CO_2 available in the chamber.

The apparatus designed in 1952 (See. Figs. 8 to 11) was the same in principle to that described by Chenoy (1938); Porter et al

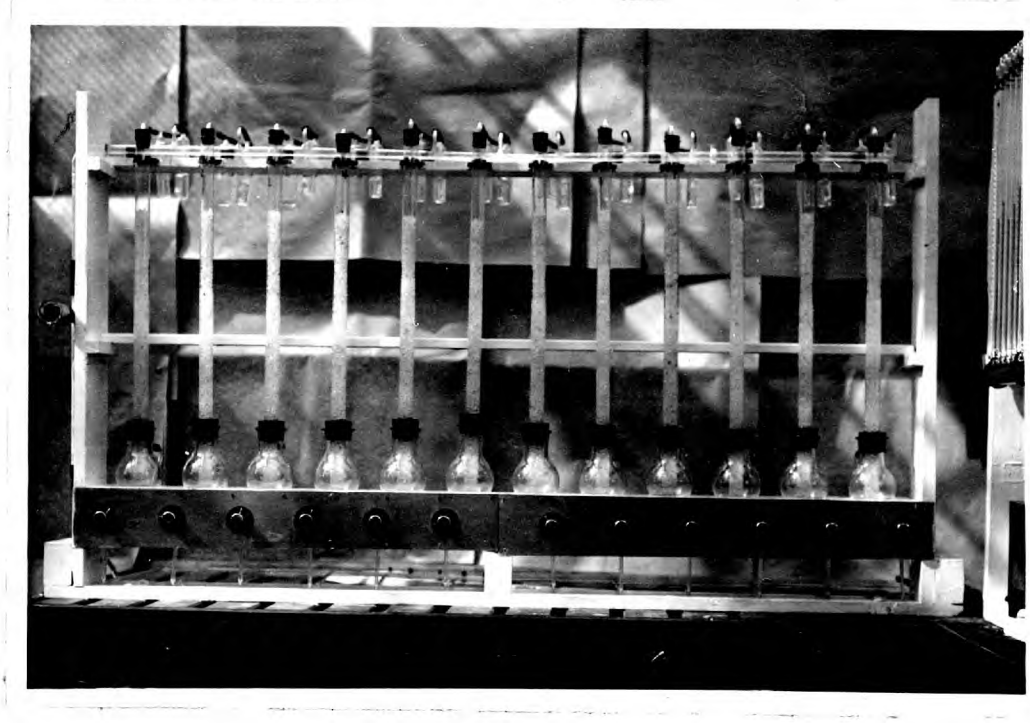


Fig. 8. Photograph of the absorption section of the assimilation apparatus, with towers in position.

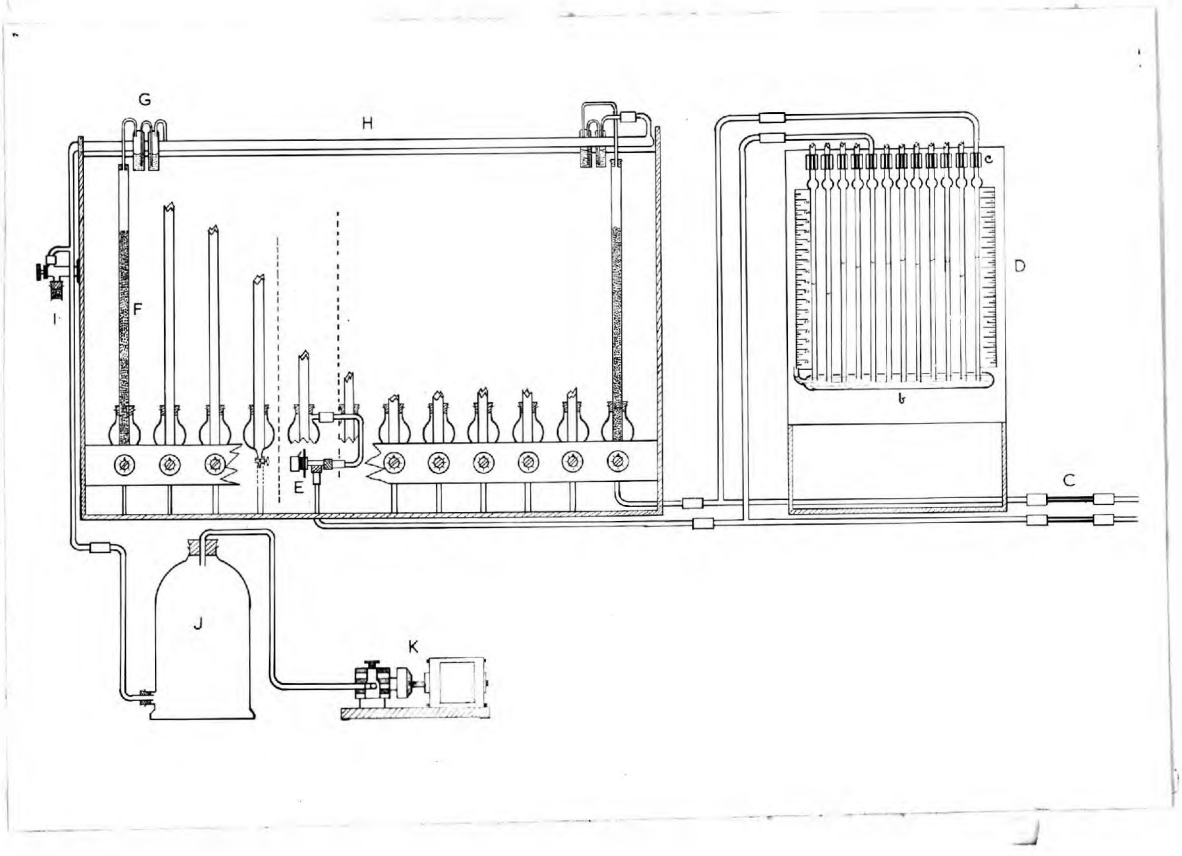


Fig. 9. Diagramatic representation of the absorption section of the assimilation apparatus showing the arrangement of the towers, manometers, and flow resistances.

C. Capillary resistances
D. Multiple manometers
   b. reservoirs
   c. dampers
E. Variable needle valves
F. Absorption towers
G. Baryta traps
H. Common lead to electric suction pump
I. Leak valve
J. Surge tank
K. Electrical suction pump

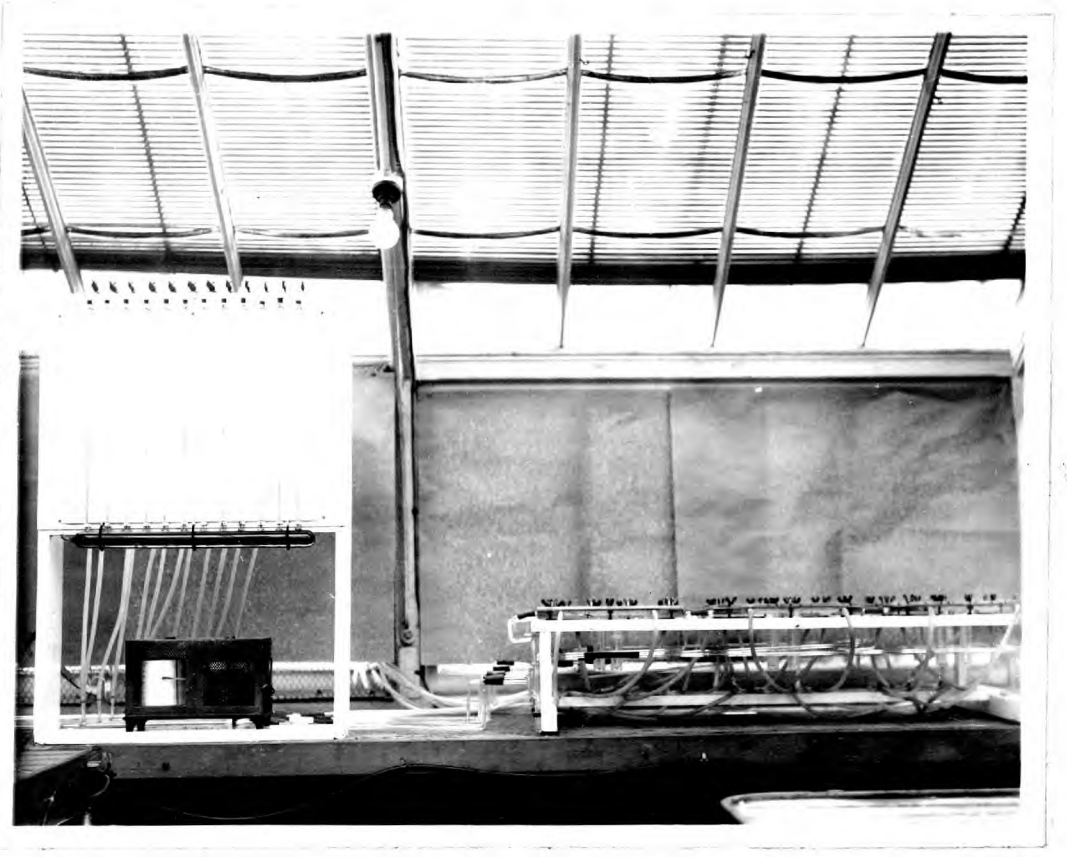


Fig. 10. Photograph of the leaf chambers and manometers of the assimilation apparatus. Capillary resistances are located just behind thermograph.

(1949); and Murari (1950). The major change was that twelve leaf chambers each holding one leaf were now used in place of six chambers each holding two leaves; requiring therefore, twelve absorption towers (F), and twelve manometers (D); the whole apparatus was therefore, redesigned to accommodate these new requirements.

Leaf Chamber (A). The original leaf chamber, as used by Murari was made of perspex with external dimensions: 12" x 12" x 9/10". It was made in two pieces; the lower section formed the chamber, and consisted of a perspex base plate with laminations of perspex around the rim which gave internal measurements to the chamber of $10\frac{1}{2}$" x $10\frac{1}{2}$" x 3/16"; the upper section consisted of a flat piece of perspex which formed the lid to the chamber. Two 3/4" circular holes in the base plate and in close proximity to opposite walls, were fitted with rubber stoppers to receive the petioles of the leaves; these rubber stoppers also served to hold tubes on the underside of the chamber, into which the petioles dipped.

These chambers were modified by dividing them into two with a 3/4" perspex insert across the diagonal and sealed to the base plate; thus making two triangular leaf chambers 10" x 10" x 3/16". To raise the lid and to eliminate the use of grease in sealing the lid to the base, cork gaskets were cut to fit around the rim and along the diagonal; this raised the lid $\frac{1}{4}$", making the depth of the chamber 5/16". The original 3/4" circular holes were sealed and appropriate new $\frac{1}{4}$" holes were drilled in the centre of the chamber, one on each

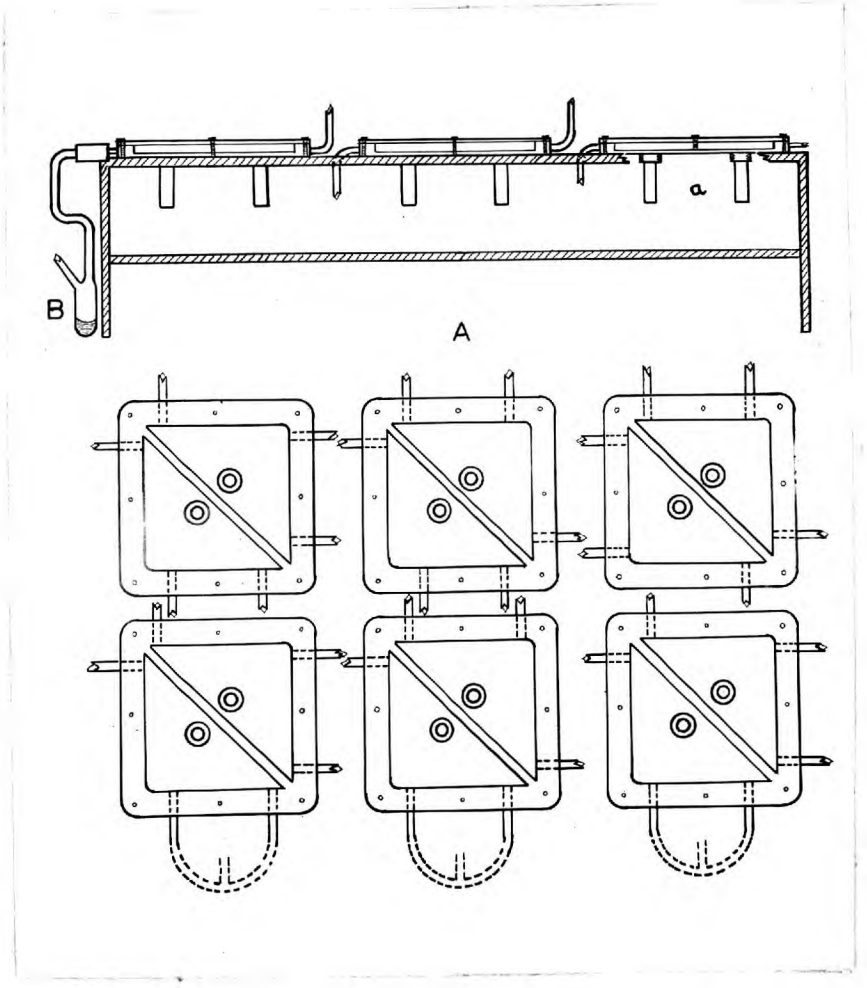


Fig. 11. Diagramatic representation of the leaf chamber arrangement of the assimilation apparatus.

A. Leaf chamber
   a. perspex tubes, into which the petioles dipped.

B. Moisture traps.

side of the partition. The petioles just rested through the holes which were sufficiently large to allow for any swelling that may occur at the neck of the petiole without becoming restricted as sometimes happened in the older chambers. Two threaded perspex rings were cemented to the underside of the chamber, in such a position that the centre of the ring was in line with the centre of the petiole hole. A perspex tube (a) 4" x 1" with internal diameter of 3/4" and threaded at the top, was fitted into the circular ring. This tube was threaded to facilitate the changing of the water daily, and to ensure ease in making an air tight joint. Eight brass screws were threaded through the rim of the chamber and two through the partition. Wells were cut into the rim around the screws to a depth of $\frac{1}{4}$". The upper perspex plate which formed the lid had holes drilled to correspond to the location of the brass screws. On the underside of the lid, small circular rings were cemented around the holes. These rings fitted through the cork gasket and down into the wells in the rim of the chamber, serving to hold the gasket in place and as a support to the lids when they were screwed into place, thus helping to prevent fracturing of the lid.

Through the rim three air tubes were fitted in each chamber; two serving as inlets for the air stream and were placed on one side of the chamber; the third tube served as the outlet and was located on the opposite side of the triangle to the other two. The tubes were arranged in this manner to ensure tubulence in the air, and to decrease the possibility of any blind spaces, thus ensuring a supply of air to all

parts of the lamina. These new chambers had one disadvantage, being slightly small in size they were unable to hold the average trifoliate leaf used without some crowding. The extent of this crowding of the leaflets was small and was not thought great enough to influence the final results.

Each chamber had its own air supply. The two inlet tubes of each chamber were connected by plastic tubing to a Y-piece and from the Y-piece to an inlet placed outside the greenhouse. The leaf chambers were supported in parallel on a wooden frame; i.e. three double chambers down each side. The outlet tubes were connected by glass tubing to moisture traps (B) located at one end of the wooden frame. From the moisture traps glass tubes lead to capillary resistances.

Flow Regulation and CO_2 Absorption. Assimilation rate, being estimated by the difference in CO_2 content between incoming and outgoing air, equal flow to each leaf chamber was essential. This was assured in the following manner; 0.5 mm capillary tubes (C), approximately 9 cm. long, and of as equal and uniform resistance as possible, were inserted in the circuit after the moisture traps. The pressure drop between the atmosphere and the inner end of these tubes was recorded on a multiple manometer (D). This manometer was placed between the resistances and the needle valves and in measuring this pressure drop, gave the rate of air flow through each circuit. A large tube 2" in diameter and sealed at each end formed the reservoir (b).

Twelve holes were made in a line along the length of the tube and were of a slightly larger size than required to take the manometer tubing. Manometer tubes were suspended with their bases inserted through these holes. These tubes were each 50 cm. long with a bulb blown near the top which acted as a trap to prevent any fluid rising too high in the tubes while the flow rate was being adjusted. On the upper end of each tube was placed a short length of pressure tubing into which had been inserted a piece of thermometer tubing (c); this served to damp out excessive oscillations in the manometer. This multiple manometer had the advantage that the pressure in each manometer was more or less independent of the others; and in consequence the cutting out of one of the manometers had no appreciable effect on the others. The fluid used in the manometers was liquid paraffin dyed red with Sudan III. The whole was suspended on a frame, the tubes being held in place by spring clips, and the reservoir being held up in place by a coiled spring. The tubes were backed by squared millimetre paper marked with the correct height necessary to ensure the correct flow of air through each circuit after previous calibration.

From the resistances the air passed by glass tubing to the absorption towers (F) through variable needle valves (E). These needle valves were used to make minor adjustments in the flow of air through each circuit with corresponding changes in the height of the column of liquid in the manometers. These had to be inspected at regular intervals, as fluctuations in the rate of flow occurred from time to time due to various reasons, such as voltage drops in the electricity

supply to the pump drawing the air into the apparatus.

The carbon dioxide in the air was removed in the absorption towers. These towers consisted of a tube 75 cm. long and 2.5 cm. in diameter, which was fitted by means of a rubber stopper in a glass bulb (200 cc. capacity). This glass bulb was fitted with a side arm through which the air entered, and a fine tubulure below which was closed with a glass tap. The tube rested on the bottom of the bulb and was filled to a height of 60 cm. with glass beads. A known weight of N/1 NaOH was introduced into the bulb, and when air was passed through it the liquid was raised to within 3 or 4 cm. of the top layer of beads, and the gas stream was broken up into many small bubbles. The top of the tube was closed with a rubber stopper which was kept in place until the tower was fitted to the apparatus. It was then removed and an L-shaped tube fitted with a rubber stopper was inserted in its place. This L-shaped tube connected the top of the tower to Baryta traps (G) which were incorporated to serve as a check on the completeness of the absorption of CO_2 by the towers. The Baryta traps were in turn connected to a common lead (H) which lead to an electric suction pump (K). Between the last tower and the pump, a large valve (I) was located which provided an air leak as only a part of the suction developed by the pump was needed to maintain the appropriate flow in the towers. It also provided a gross control of the rate of flow, the final adjustments being made on the needle valves. A surge tank (J) was provided to iron out minor fluctuations in the flow caused by the pump.

Each circuit was calibrated for flow resistance by the method outlined by Barr (1934) in which the movement of a soap film over a standard distance in a glass tube was measured. A regression line was obtained from a series of readings for each manometer, made at different rates of flow, and from this the correct manometer setting for the desired flow rate was found. A blank run was made at 20 litres per hour. The results show that the amount of air that passed through each tower was in very close agreement. The average was found to be 13.37 mg. per hour (theoretical for 20 litres being 13.2 mg. CO_2). The greatest deviation from this value was only 1.87% and the standard deviation was calculated to be 0.22 mg. This can be considered as adequate for this type of experiment.

At the start of an experiment the absorption tower was weighed dry with the rubber stopper in the top of the tube, and the side arm closed with a glass rod. 50 cc. of N/1 NaOH was then run in from an automatic pipette, and the whole weighed again. This served to determine the amount of NaOH delivered by the pipette. At the end of the experiment the absorption tower was removed from the apparatus, the side arm was again closed and the tube stoppered. It was then weighed to determine changes in weight due to water loss or gain. The NaOH was allowed to drain from the tower into the bulb, and then through the lower tap on the bulb into a 250 cc. volumetric flask containing 10 cc. of 10% $BaCl_2$ and 100 cc. of distilled water. After the tower was drained it was reweighed to give the residual amount of

NaOH left in the tower. After weighing, the tower and beads were washed and dried, ready for the next experiment. This weighing technique eliminated the necessity for quantitative removal of the NaOH from the tower since the results can be adjusted to the original quantity of NaOH.

The $BaCl_2$ and water precipitates the carbonate and rapidly dilutes the excess NaOH. The volumetrics were then diluted to the 250 cc. mark; shaken well to ensure thorough mixing of the NaOH and water, and then allowed to stand overnight to allow the precipitate to settle out. After settling, 50 ml. aliquots of the supernatant liquid was pipetted into a conical flask; 50 ml. of N/10 HCl was run into the conical flask from an automatic pipette, the remaining NaOH being titrated with a further quantity of N/10 HCl and the amount of carbonate absorbed found by substracting the total titration from the appropriate blank.

A specimen calculation from an actual experiment is given below: -

	Weight of absorption unit in gm.	Weight of NaOH in gm.
1. Weight of empty tower	656.15	
2. + 50 ml. N/1 NaOH before experiment	708.30	52.15
3. + 50 ml. N/1 NaOH after experiment	705.75	49.60
4. After running out the NaOH	662.15	43.60

43.60 gm. NaOH made up to 250 ml. and 50 ml. aliquots titrated with N/10 HCl. (actual strength HCl used in this experiment was 0.114N.)

The results therefore, were adjusted to 0.1N).

ml. N/10 HCl required for 50 ml. NaOH = 50 / 29.22 = 79.22 ml.

ml. N/10 HCl required for excess NaOH in 52.15 gm. taken =

$$79.22 \times 5 \times \frac{49.60}{43.60} \times 1.14 = 450.60 \text{ ml.}$$

ml. required for blank = 484.00

Hence ml. N/10 HCl equivalent to CO_2 absorbed = 33.40 ml.

Duration of experiments 10 hours.

Therefore mg. of CO_2 absorbed in 10 hours = 33.40 x 2.2 = 83.77 mg.
or 8.38 mg. per hour.

CO_2 in the air for this experiment was measured as 13.22 mg. per hr.

Hence CO_2 assimilated by leaf = 13.22 - 8.38 = 4.84 mg. per hour.

Leaf area in this chamber was 1.541 dm^2.

CO_2 assimilated per dm^2 = 4.84/1.541 = 3.14 mg. /dm^2/hour.

Leaf Areas

Murari (1950) found that dry weight was an inadequate criterion for the estimation of assimilation. He also found that very little change in leaf area occurs in isolated bean leaves; from 103 to 105 cm^2 in the first 7 days as compared to an increase from 103 to 202 cm^2 on the attached leaf. He pointed out that the estimation of the assimilation rate would be greatly affected by the thickness of the leaf, which would be reflected in the dry weight. This can best be illustrated by the following examples taken from one of the assimilation experiments of 1952.

TABLE V

	Initial leaf F.W.gm.	Leaf Area dm^2	Lamina D.W. $gm./dm^2$
1.	4.73	1.786	0.393
2.	4.78	1.587	0.472
3.	4.81	1.782	0.388
4.	4.74	1.425	0.428

These leaves were harvested at the same time, from neighbouring plants of the same seeding date. Comparisons show these leaves to have initial fresh weights that were nearly equal, but the leaf areas varied considerably, and this was reflected in the lamina dryweight per square decimetre. Differences in thickness of the lamina would therefore account for the variations in the leaf area per leaf and this in turn would be reflected in the assimilation rate. Leaf 1. and 2. in the above table had identical pre-treatment as did leaves 3. and 4.

Since all the CO_2 entering the leaf passes through the surface, it would appear on general grounds that the estimation of assimilation on an area basis would be more satisfactory, and this method has therefore been adopted.

Method of Estimating Leaf Area: Various methods were considered for estimating the leaf area, such as, tracing the outline of the leaf and weighing the leaf tracing, the use of the planimeter, and etc; but all were rejected because of the length of time needed for

the measurements to be made. It was felt desirable that these measurements should be made as rapidly as possible so that the leaves could be placed in the ovens for drying, for dry weight estimations before any serious loss in weight could occur; and also to be able to deal with large samples of leaves. The method as outlined below was found to be very satisfactory and fairly rapid.

The method settled upon and used throughout all experiments was to obtain the leaf areas by shadowgraphs made on blueprint paper. A large sheet of glass was covered with blueprint paper and fastened at the corners by Selotape. A standard area was marked on the paper; the method used was to lay a piece of cardboard 12 x 20 cm. at one corner of the sheet. The leaves were rapidly weighed to obtain fresh weights, and the laminas then detached and placed on the paper. The separate leaflets were marked in such a way that they could afterwards be identified. The leaves were kept in contact with the blueprint paper by covering with a sheet of glass. The length of time the paper was exposed to the diffuse light in the laboratory while this was being done did not materially affect it. The whole was then taken into the bright sunlight and exposed. A very short period was all that was necessary to expose the paper, about five minutes. It was then brought back into the laboratory where the leaves were removed and the paper developed. After the paper had dried the leaf outlines were cut out together with their test area. These were then weighed and the leaf area determined by proportion. An example of the method used in calculating the leaf area is given below:

Area of test strip		240 cm^2 (2.40 dm^2)
Weight of test strip		2.184 gm.
1 gm. of paper	=	1.099 dm^2
Weight of leaf area		1.1706 gm.
Therefore leaf area	=	1.1706 x 1.099 = 1.286 dm^2.

Test areas were taken with each sheet so that an average for the day's weighings could be made. This is necessary as small errors may be made in cutting the test strips, and also to allow for variations in the weight of the paper from roll to roll, and within the roll. Variations in the weight of the test strip were relatively small in any one day's weighings; maximum difference found in any one day was 2% of the area (0.02 dm^2); the majority of the test strips had less than 0.5% difference from the mean.

Experimental Results. Two types of assimilation measurements were made; 1. by direct gasometric methods; and 2. from changes in the dry weight per unit area of leaves before and after a given interval of time.

During 1951 both assimilation and respiration experiments were repeated with an average of four estimations made on single samples of two leaves during the course of the experiment; a total of twenty-one runs were made.

Certain difficulties were encountered in these experiments which affected the life of the leaves in the chambers.

1. It was found that if the surfaces of the chambers were not kept flooded with water on bright days, radiant heat would cause the internal temperature of the chambers to rise as high as ten degrees above that of the external air of the greenhouse, resulting in injury to the leaves. If in the course of the experiment such injury occurred the experiment was stopped, or fresh samples of comparable leaves were used to replace those injured. Flooding the surface of the chambers reduced this risk and became standard practice for all later experiments.

2. To seal the chambers, Resin Cerate was used. Certain precautions were necessary to prevent any excess from coming into contact with the leaves within the chambers as it was liable to damage the laminas.

3. Season of the Year. It was found practically impossible to make successful runs during the later part of the year, as the leaflets were liable to dehisce. This occurred with the controls as well as with the treated leaves.

Respiration Experiments. The summary of the respiration results is given in Table VI, in which mean respiration rates in milligrams per hour, per square decimetre are entered. Each figure entered is the mean value for four replicated leaves on four occasions; that is the mean of sixteen leaves. As the leaves were in pairs in the chambers, figures represent the mean of eight estimations. The standard deviations in the Table as will be seen, are large. The variability is however, due to the difference between different

occasions, and is largely accounted for by the prevailing differences in temperature. The high correlation between the standard deviation of the two sets (water treated and 2,4-D. treated leaves) is to be expected. The analysis of variance appended to the table show that the treatment effect is quite insignificant, whereas the variance due to occasions is above the 5% point and therefore significant. There is therefore, no evidence that the treatment with 2,4-D. has in any way affected the respiration rate. The significant occasion variance is due to very high respiration rates in both series, associated with the dark period immediately following treatment. In this experiment the leaves were treated, in situ, in the chambers.

The effect of Temperature on Respiration. In Fig. 12 the respiration rates are plotted against temperatures prevailing during the night. The regression line calculated from all individual data is entered in the figure with fiducial limits. It will be noted that there are four aberrant points; the two high values refer to experiments carried out during the first night of treatment; the low values are for two nights at the end of an experiment. The figures for these nights are given in Table VII and are marked with an asterisk. The correlation co-efficient including these aberrant points has been found to be 0.45 with a t. value of 1.95 (P. for 0.10 = 1.75). Since the aberration of these points are evidently not random, a second correlation co-efficient was worked out for the data omitting these

values. The correlation co-efficient is now found to be 0.61 which is significant with a t. value of 2.55 (P. for 0.05 = 2.20). From the data it is clear that the temperature co-efficient Q_{10} for respiration in these experiments, between temperatures of 10° and 20°C. is of the order $Q_{10} = 2.2$.

The results presented do not confirm the findings of Taylor (1947), Brown (1946), and Hseuh and Lou (1947) that the respiration rate is effected by treatment with 2,4-D. at the optimal concentration for rooting.

Assimilation Experiments. Since these experiments were done during two different years, and on two different sets of apparatus, the results for the two years will be discussed separately.

Carbon dioxide assimilation was measured as the difference between the amount of CO_2 in the air drawn through blank chambers; i.e. representing the CO_2 supplied to the leaves; and residual CO_2 in the air emerging from chambers after having passed over leaves. In this way the apparent assimilation was estimated. The calculations used to give assimilation rate was as follows:

CO_2 in air	Residual CO_2	Actual Assimilation	Assimilation mg./hr./dm^2
C_1	C_2	$C_1 - C_2$	$\frac{C_1 - C_2}{\text{leaf area}}$

This last figure gives the assimilation rate of the leaves in the chamber for the CO_2 concentration available to the leaf. A figure obtained in this way represents the actual carbon dioxide uptake

TABLE VI

Summary of Respiration Results for Water controls and 2,4-D. treated leaves. All results are in mg./hr./dm^2 and are the mean of four experiments. Each experiment having two leaves per chamber and two chambers per treatment.

Treatment	Water-controls CO_2 respired	Standard Deviation	2,4-D. Treated Leaves CO_2 respired	Standard Deviation
Night of treatment	1.22	0.27	1.11	0.33
Night after treatment	0.87	0.16	0.85	0.15
2 nights after "	0.61	0.28	0.75	0.23
3 nights after "	0.60	0.15	0.60	0.15
5 nights after "	0.60	0.23	0.65	0.22
8 nights after "	0.47	0.14	0.50	0.13
12 nights after "	0.56		0.54	
Weighted mean of treatments	0.70		0.73	
Standard Error of Mean	0.09		0.08	

Analysis of Variance

	D.F.	S.S.	Variance	F.	5% point
Treatment	1	0.0004	0.0004	0.02	5.99
Occasions	6	0.5420	0.0903	5.34	4.28
Error	6	0.1075	0.0169		
Total	13	0.6499			

Significant difference between occasions 0.32 mg./hr./dm^2.

TABLE VII

Average respiration rate and temperature for each individual experimental run made. Respiration rate given is the mean of the rates found for the water controls and 2,4-D. treated leaves combined. Mean temperatures were obtained from thermograph recordings made on the night of the experiment.

Date	Mean Temperature °C.	Mean respiration mg./hr./dm^2.
12/9/50	14.7*	1.11*
13/9/50	13.4	0.71
15/9/50	14.0	0.43
17/9/50	11.2	0.37
5 /7/51	18.5	0.81
6 /7/51	18.6	0.86
8 /7/51	18.4	0.78
10/7/51	18.3	0.80
17/7/51	22.1*	1.48*
18/7/51	20.0	0.94
24/7/51	16.3	0.90
25/7/51	18.9	0.98
27/7/51	18.8	0.78
1 /8/51	19.0*	0.45*
2 /8/51	19.3*	0.55*
10/8/51	16.1	0.45
15/8/51	16.5	0.49

Correlation Co-efficient (1) $r_{xy} = 0.45$ $t = 1.95$ ($P_{0.10}=1.75$)

Regression Co-efficient $b_{yx} = 0.049$ Standard Error 0.17

Equation for regression line $Y = -0.10 + 0.049x$

* These four aberrant points are eliminated from the second correlation co-efficient.

Correlation Co-efficient (2) $r_{xy} = 0.61$ $t = 2.55$ ($P_{0.05} = 2.20$)

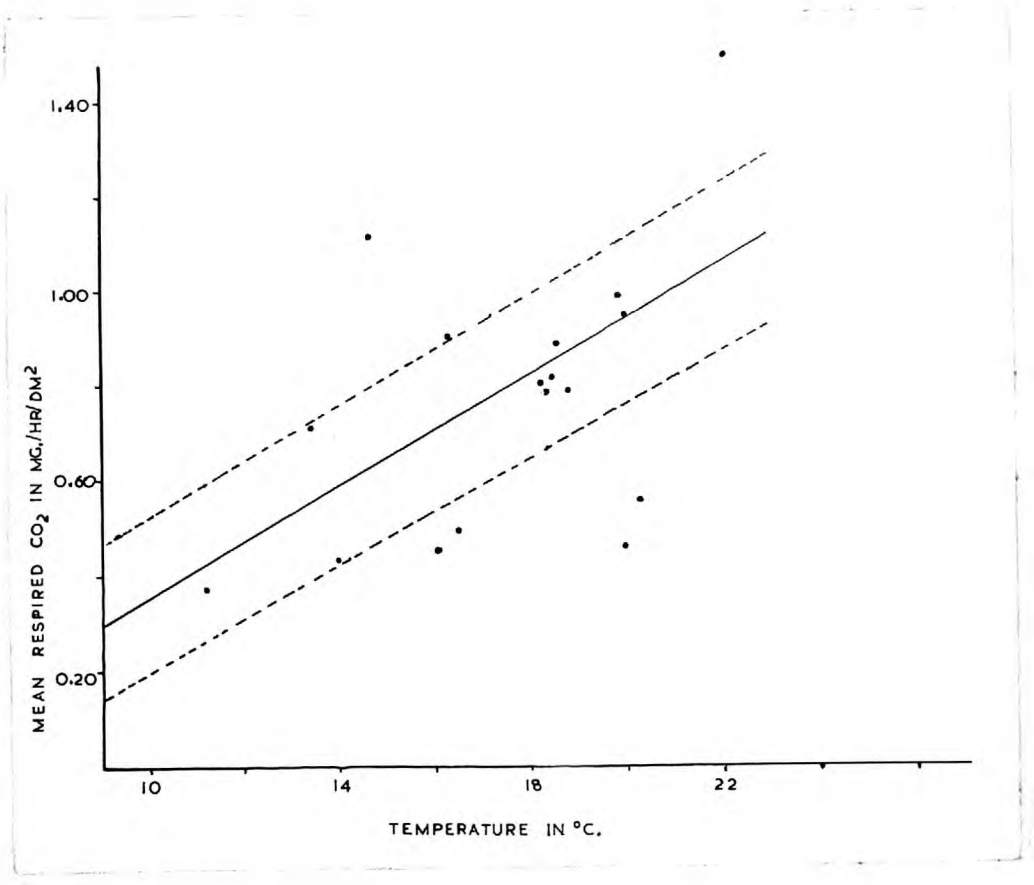


Fig. 12. The effect of temperature on respiration. The solid line is the regression line found from the equation:

$$Y = -0.10 + 0.049x$$

The dotted lines show the fiducial limits.

per unit area of the leaves; but takes no account of the prevalent concentration of carbon dioxide in the chamber and therefore, assimilation rates cannot be adjusted to CO_2 supplied. The distribution in the chamber must, under the conditions of these experiments, have been very variable. Before any direct comparison can be made between different experiments some estimate of the carbon dioxide content of the air in the chambers must be made; and further, the assumption made that the assimilation rate was limited by the carbon dioxide supply, and therefore proportional to the carbon dioxide content of the air. In order to minimize the magnitude of this correction it is, of course, desirable that depletion as the air passes through the chamber should be as small as possible; i.e. a high flow rate must be maintained. The actual rate in 1951 was 20 litres per hour, and this was increased to 25 litres per hour in 1952. To estimate the average concentration of the carbon dioxide in the chamber the following relation was assumed; that the average assimilation is equal to that derived by the following equations.

Mean CO_2	Corrected actual assimilation to 0.03%	Corrected assimilation rate
$\frac{C_1 - C_2}{\text{Log}_e C_1 - \text{Log}_e C_2}$	$\frac{\text{Observed A.A.} \times 13.2}{\text{Mean } CO_2}$	$\frac{\frac{\text{Observed A.A.} \times 13.2}{\text{Mean } CO_2}}{\text{Leaf Area}}$

The corrected assimilation rate obtained by the use of this formula cannot however, be accepted without reservation, as can be shown by consideration of the following experimental data.

Date	CO_2 in Air	Residual CO_2	Observed A.A.	Leaf Area dm^2	Assim. hr/dm^2	Mean CO_2	Corrected Assim.	Assim.dm^2
18/7/51	12.42	4.10	8.32	4.179	2.09	7.53	14.67	3.68

The air entering the leaf chamber contained 12.42 mg. CO_2 in 20 litres, while the air emerging from the leaf chamber contained 4.10 mg., the concentration of carbon dioxide had thus been reduced from approximately 0.03% to 0.01%. As the work of Gabrielson (1948) and of Heath and Milthorpe (1950) has shown, it appears probable that the carbon dioxide content of the air cannot be reduced to less than 0.01% by assimilation which would indicate in this experiment that all carbon dioxide available for assimilation had been used. If these low concentrations in the air stream were reached at some time before the air emerged it is clear that the estimated average carbon dioxide content available for assimilation was underestimated, and therefore the corrected values for assimilation rate would be too high. From the data given it is seen that the corrected assimilation rate, at this rate of flow, was greater than the total amount of carbon dioxide passed into the chamber, and therefore an over-correction had been made. It was a realization of this possibility which led to the increased rate of flow in 1952. The summary of assimilation results are entered in Table VIII and VIIIa. It will be seen that in general, the residual carbon dioxide emerging from the chamber is greater than 1/3 of the carbon dioxide entering, indicating that the concentration of carbon dioxide in the chamber was not reduced below the minimal

TABLE VIII

Summary of Assimilation results for Season 1951 in mgs. per hour.

Date of Exp't	CO_2 in Air	Residual CO_2	Observed assimilated CO_2 per chamber	Observed assimilated CO_2 per dm^2	Mean CO_2	Corrected Assimilation per chamber	Corrected Assimilation per dm^2
A. Water treated leaves preliminary experiment 1950.							
6/9	12.02	6.31	5.71	1.48	9.59	7.91	2.10
7/9	14.09	7.04	7.05	1.86	10.16	9.16	2.43
13/9	11.74	6.24	5.50	1.98	8.75	8.29	2.94
14/9	11.76	6.16	5.60	2.02	8.61	8.62	3.04
16/9	13.37	8.40	4.97	1.76	10.66	6.20	2.18
18/9	11.46	8.30	3.16	1.12	9.70	4.30	1.52
20/9	12.11	7.48	4.63	1.64	9.62	6.36	2.25
1951 experiments							
6/7	11.61	8.06	3.55	2.04	9.77	4.81	2.79
7/7	11.16	6.60	4.56	2.95	8.62	6.98	4.11
9/7	12.69	10.09	2.60	1.51	11.45	3.00	1.74
11/7	9.99	6.67	3.32	1.94	8.23	5.34	3.13
18/7	12.42	4.10	8.32	2.09	7.53	14.67	3.68
19/7	11.68	3.00	8.68	2.15	6.32	18.22	4.56
25/7	12.46	5.84	6.62	2.00	9.27	10.02	3.02
26/7	12.44	5.95	6.49	1.96	8.80	8.77	2.94
28/7	9.77	5.15	4.62	1.38	5.44	8.72	2.61
2/8	11.14	6.66	4.48	1.33	8.75	6.78	2.03
4/8	13.82	7.54	6.28	1.88	10.36	8.01	2.39
10/8	10.46	3.92	6.54	1.30	6.66	12.95	2.59
15/8	9.62	5.48	4.14	0.80	7.37	7.41	1.46
Means	11.79		5.36			8.39	2.68

Observed assimilated CO_2 is equal to 46.4% of measured CO_2.

Calculated assimilation rate is equal to 63.6% of theoretical CO_2 content in 20 litres of air.

TABLE VIII a

Summary of Assimilation results for Season 1951 cont'd in mgs. per hour.

Date of Exp't	CO_2 in Air	Residual CO_2	Observed assimilated CO_2 per chamber	Observed assimilated CO_2 per dm^2	Mean CO_2	Corrected Assimilation per chamber	Corrected Assimilation per dm^2
B. 2,4-D. treated leaves Preliminary 1950							
6/9	12.02	6.78	5.24	1.27	9.18	7.54	1.83
7/9	14.09	8.57	5.52	1.34	11.08	6.57	1.59
13/9	11.74	5.77	5.97	2.10	8.56	9.22	3.23
14/9	11.76	5.99	5.77	2.02	8.50	8.96	3.14
16/9	13.37	6.27	7.10	2.46	9.30	10.34	3.57
18/9	11.46	7.70	3.76	1.32	9.37	5.32	1.89
20/9	12.11	6.90	5.21	1.82	9.27	7.45	2.60
1951							
6/7	11.61	7.20	4.41	2.78	9.23	6.38	4.00
7/7	11.16	7.02	4.14	2.59	8.93	6.05	3.84
9/7	12.69	10.80	1.89	1.20	11.85	2.12	1.34
11/7	9.99	7.18	2.81	1.80	8.54	4.34	2.78
18/7	12.42	5.08	7.34	2.06	8.21	12.15	3.39
19/7	11.68	7.04	4.64	1.30	9.13	6.72	1.88
25/7	12.46	8.16	4.30	1.20	10.11	5.74	1.68
26/7	12.44	7.06	5.38	1.63	9.50	7.50	2.24
28/7	9.77	7.53	2.24	0.70	8.53	3.45	1.06
2/8	11.14	7.66	3.48	1.07	9.34	4.91	1.51
4/8	13.82	7.80	6.02	1.84	10.53	7.57	2.28
10/8	10.46	7.22	3.24	0.87	8.75	4.89	1.32
15/8	9.62	7.07	2.55	0.72	8.31	4.05	1.16
Mean	11.79		4.55			6.56	2.32

Observed assimilated CO_2 is equal to 38.6% of measured available CO_2.

Calculated assimilated CO_2 is equal to 49.7% of theoretical available CO_2.

(0.01%) and therefore corrected values for assimilation rates are not vitiated by this fact. There are however, occasions such as the one already cited of the 18.7.51 where the lower limit had been reached, and the experiment of the 19.7.51, in which the carbon dioxide fell below this limit. Therefore the calculated assimilation rate in these experiments is likely to be overestimated.

1951. Tables VIII and VIIIa separately present the detailed data obtained in 1951 for the water controls and the 2,4-D. treated leaves. In the last column of the tables the corrected values for the assimilation rate in milligrams per hour, per square decimetre are shown. The results given are the mean assimilation rates for two replicated pairs of leaves. In Table IX the means are entered for the various days after treatment, for both water controls and 2,4-D. treated leaves. The mean values for the assimilation rate as given in this table show that on the average the water controls have the higher assimilation rate. It is also apparent that there is a drift downwards in both series from the beginning to the end of the experiments. It appears from the data that the fluctuating values for the assimilation rate in the two series, are correlated. To investigate this correlation further the individual values for the assimilation rate (20 values) were plotted against each other. The results are shown in Fig. 13. The correlation co-efficient between the values of the two series were calculated and gave an

$$r_{xy} \text{ of } \neq 0.45 \text{ with a t. of } 2.13 \ (P_{0.05} = 2.10)$$

The correlation therefore is highly significant indicating that the

TABLE IX

Summary of Assimilation Results by Days from Treatment in mg./hr./dm^2.

Days from Treatment	Treatment	
	Water Control	2,4-D. treated
Day of Treatment	3.21	2.77
Day after treatment	3.00	2.40
2nd day after treatment	3.26	2.76
3rd day after treatment	2.18	1.99
5th day after treatment	2.06	1.94
8th day after treatment	2.14	2.04
12th day after treatment	2.39	2.28
Weighted Mean	2.68	2.32

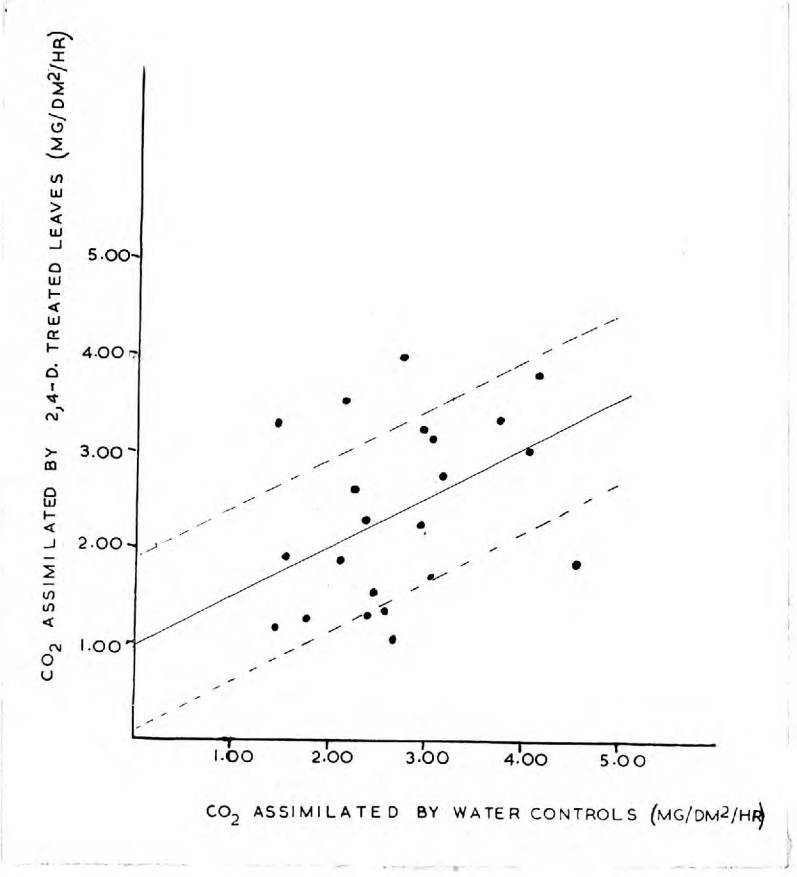


Fig. 13. Correlation diagrams for assimilation experiments 1951 Assimilation rate for water treated leaves plotted against the assimilation rate for 2,4-D. treated leaves.

$r_{xy} = \pm 0.45$ regression equation $Y = 0.91 \pm 0.525x$

Dotted lines represent the fiducial limits.

fluctuating rates obtained in these experiments may be attributed to variations in the level of external factors, light and temperature, obtaining on the different days. The regression of the assimilation rate of 2,4-D. treated leaves on the assimilation rate for the water controls was calculated, giving the following equation: $Y = 0.91 + 0.525x$; y is the assimilation rate of the treated leaves and x is the controlled values. If the assimilation rates were uneffected by treatment one would expect the regression co-efficient to be 1.0; the value obtained (0.525) confirm the fact already indicated by the mean values in Table IX, that 2,4-D. treatment has consistently reduced the assimilation rate of the leaves.

1952. Similar data for the experiments in 1952 are in Tables X and XI. So far as summary Table XI is concerned, full data were only available for the first three occasions. The means of these three represent the results of four experiments each, employing four replicate experiments. The last experiment for 15 days after treatment was not complete in the sense that only one experiment with four replicates was available. The weighted mean for the assimilation rates in the control and treated leaves are entered, and show a small difference in favour of the 2,4-D. treated leaves. Analysis of variance using the first three occasions for which complete data are available are shown in Table XIa. The treatment effect is quite insignificant whereas the variance between occasions is very highly significant, as is also the variance between experiments. The first order interactions are all significant to the 5% point.

TABLE X

Summary of Assimilation Results 1952, in mgs. per hour.

Date of Exp't	CO_2 in Air	Residual CO_2	Observed Assimilated CO_2 per chamber	Observed Assimilated CO_2 per dm^2	Mean CO_2	Calculated Assimilation per chamber	Calculated Assimilation per dm^2
A. Water treated leaves							
28/6	12.94	8.95	3.99	2.67	10.91	4.91	3.29
30/6	13.22	8.84	4.38	2.86	10.92	5.32	3.48
5/7	13.35	11.22	2.13	1.35	11.44	2.50	1.57
12/7	12.34	12.09	0.25	0.15	11.30	0.28	0.17
8/8	14.63	9.55	5.08	4.06	11.90	7.18	5.70
10/8	14.39	12.24	2.15	1.82	13.32	2.69	2.29
14/8	15.00	12.86	2.14	1.70	13.97	2.64	2.09
20/8	15.15	9.91	5.24	3.66	11.86	7.36	5.13
22/8	14.59	12.61	1.98	1.30	13.84	2.42	1.50
25/8	18.35	15.14	3.21	2.19	16.52	3.22	2.19
28/8	14.91	9.31	4.60	4.10	11.52	8.06	5.89
30/8	16.40	12.95	3.45	2.27	14.57	3.92	2.57
3/9	16.23	14.38	1.85	1.09	15.43	1.98	1.29
B. 2,4-D. treated leaves							
28/6	12.94	9.65	3.29	2.50	11.43	3.86	2.88
30/6	13.22	9.54	3.68	2.69	11.32	4.26	3.10
5/7	13.35	11.48	1.86	1.43	11.74	2.10	1.60
12/7	12.34	11.20	1.14	0.72	11.97	1.30	1.11
8/8	14.63	11.29	3.34	2.88	12.98	4.39	3.72
10/8	14.39	12.66	1.73	1.53	13.54	2.12	1.87
14/8	15.00	13.12	1.88	1.47	13.42	2.60	2.02
20/8	15.15	11.60	3.55	2.75	12.78	4.72	3.68
22/8	14.59	12.12	2.47	1.75	13.38	3.12	2.21
25/8	18.35	14.16	4.19	2.99	15.92	4.35	3.10
28/8	14.91	8.00	6.91	4.71	11.10	10.38	7.26
30/8	16.40	12.62	3.78	2.29	14.32	4.54	2.73
3/9	16.23	10.65	5.58	3.53	13.23	7.25	4.09
Means							
Water Treated Leaves			3.11	2.25		4.04	2.88
2,4-D. Treated Leaves			3.34	2.40		3.63	3.03

TABLE XI

Summary of Assimilation Results by Days from Treatment. 1952. Means of four experiments, each with four leaves per treatment, except for 15 days after treatment in which only one experiment was carried out. The results are given in milligrams per hour per square decimetre.

Days from Treatment	Water controls	Leaves treated with 0.5 p.p.m. 2,4-D.
Day after treatment	5.00	4.38
3 days after treatment	2.46	2.48
5 days after treatment	1.79	2.70
15 days after treatment	0.17	1.11
Weighted mean	2.86	3.03

TABLE XI a

Analysis of Variance on Assimilation results, 1952. The results for the fifteenth day after treatment are not included in the analysis.

	D.F.	S.S.	Variance	F.	5% Point
Treatment	1	0.2450	0.2450	0.18	3.96
Occasions	2	122.2779	61.1389	48.50	3.11
Experiments	3	22.2515	7.4172	5.39	2.72
T x O	2	10.9768	5.4884	3.98	3.11
T x E	3	17.2450	5.7483	4.18	2.72
O x E	6	40.2949	6.7158	4.88	2.21
Error	78	108.1679	1.3738		
T x O x E	6	9.5551	1.5925	1.16	2.32
Error	72	98.6128	1.3696		
Total	95	321.4590			

Occasions represent a series of increasing ages of the leaf, and the total effect has already been shown to be very highly significant. The significant interaction with treatment (T x O) indicates that the change in the assimilation rate with age varies with treatment. The treatment - experiment interaction (T x E), which is highly significant, reflects the effect of the time of the year at which the plants were grown. The even more highly significant occasion - experiment interaction (O x E) is a consequence of the varying levels of external factors on the different occasions on which the experiment was carried out. The second order interaction (T x O x E) is not significant when compared with the error variance and therefore, to estimate the error in the main effects and first order interactions, has been included in the error variance.

The treatment occasion interaction has been analysed one step further and the relative analysis of variance is entered in Table XII. The data in Table XI based on mean rates, shows that on the fifth day of treatment there is a large difference between treatments, and the analysis for that day confirms that this difference is significant above the 5% point.

TABLE XII

Analysis of Variance

Fifth day after treatment 1952.

	D.F.	S.S.	Variance	F.	5% point
Treatment	1	7.2200	7.2200	5.42	4.41
Experiment	3	6.2578	2.0859	1.54	3.55
T x E	3	11.4424	3.8141	2.82	3.55
Error	24	32.4054	1.3052		
Total	31	57.3346			

The mean value for the assimilation rate includes the very high values for the treated leaves on the 28/8 and 3/9; and these two values result in the interaction between experiments and treatment already mentioned. The values cannot therefore be discarded; but since they contribute largely to the value of the mean, the latter cannot be regarded as indicating any general relation between the assimilation rates of the control and the treated leaves.

In order to determine the general relation between the assimilation rates the individual values have been plotted against each other in Fig. 14. A correlation co-efficient has been determined and the value for r_{xy} of $\neq 0.74$ with a t. value of 3.65 and $P_{0.01}$ of 3.11; and is thus significant at the 1% point. Again therefore the fluctuation in assimilation rate indicated by the scatter of points, is not fortuitous, and is largely due to variation in other factors.

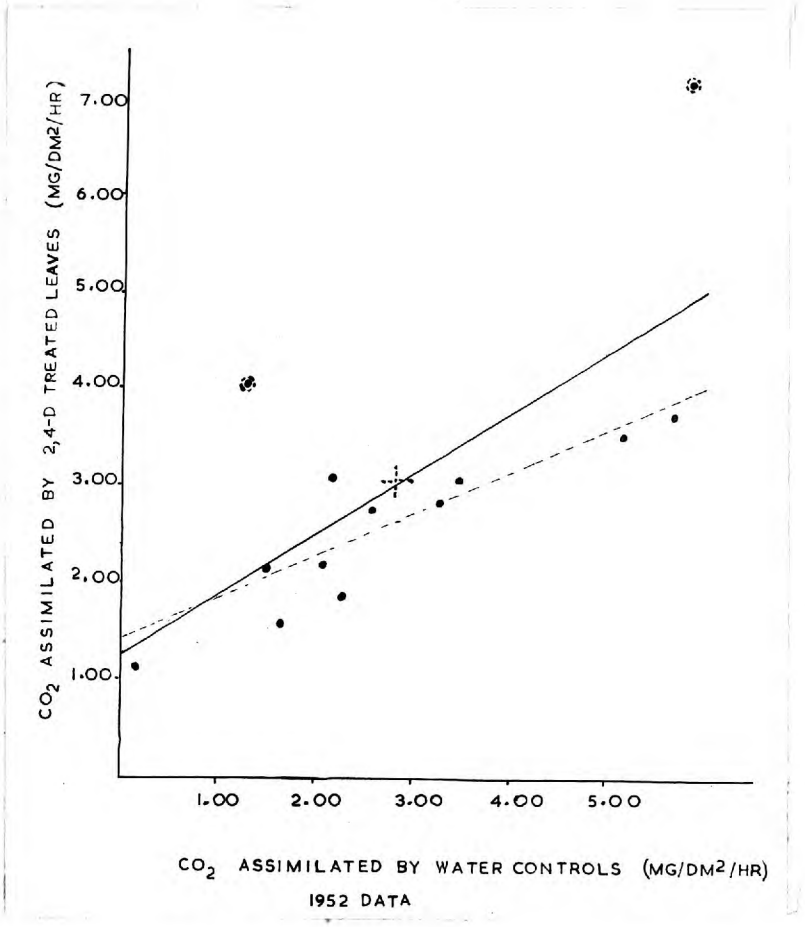


Fig. 14. Correlation Diagram for assimilation experiments 1952.

Assimilation rate for water treated leaves plotted against the assimilation rate for 2,4-D. treated leaves.

Solid line is the regression line including all data.

$r_{xy} = \pm 0.74$ Regression equation $Y = 1.17 \pm 0.625x$

Dotted line represents the regression line for all data except the two high aberrant values.

$r_{xy} = \pm 0.83$ $Y = 1.46 \pm 0.438x$

The regression line including all values is given by the equation, $Y = 1.17 + 0.625x$ where y is the assimilation rate for 2,4-D. treated leaves and x for the control values.

This regression co-efficient of the 1952 experiments thus agrees with the values obtained in the 1951 experiments shown in Fig. 13 and again indicates that the assimilation rate after 2,4-D. treatment has been reduced in spite of the two very high values already mentioned. Omitting these values the correlation co-efficient now rises to = 0.83 and the regression equation now becomes $Y = 1.46 + 0.438x$. The dotted line in Fig. 14 represents this equation.

Finally, in Fig. 15 all data for 1951-1952 are entered, together with the regression line (full line) which can be represented by the equation $Y = 0.93 + 0.631x$. The correlation co-efficient was found to be 0.64. The dotted line represents the regression line omitting the aberrant values obtained in 1952, and which were obtained in the last experiment. The regression equation for this line is $Y = 1.07 + 0.507x$.

It can therefore be concluded that the 2,4-D treatment has reduced the assimilation rate of the leaves. The fact that the regression line does not pass through zero may indicate that the regression is actually curvilinear but it was not considered worthwhile establishing this point.

It should be pointed out that the average assimilation in

1952 was higher than in 1951 and may have well been due to the fact that the rate of flow had been increased from 20 litres to 25 litres per hour; and each chamber contained only one leaf compared with two in the previous year, and thus avoided mutual shading. This improvement in technique was reflected by the fact that the average CO_2 in the air emerging from the chamber was increased from 37% in 1951 to 73% in 1952 of the original CO_2 content of the air entering the chamber.

From the data in Table XI it is evident that the assimilation rate tends to fall with time. This decline in the rate of assimilation closely approaches a hyperbola, as can be seen from Fig. 16 in which the $\log_e$ assimilation rate has been plotted against time and treatment, for both control and treated leaves. It will be noted that the experimental values closely approximate to a straight line, indicating that the assimilation rate is steadily falling throughout the experiment. From the data in Fig. 16 one may conclude that there is no differential effect between treatment with water or 2,4-D on the assimilation rate in this respect.

B. Estimation of Net Assimilation Rate by Growth Analysis. In addition to the gasometric methods for the measurement of the assimilation rate already described a simpler and more direct method for the estimation of the net assimilation rate was used, based upon the increments of dry weight in rooted leaves. Three such experiments were carried out, and will be referred to as 1951; 1952A; and 1952B.

These experiments were done at the same time as the gasometric experiments; the leaves were not enclosed in a chamber as in the gasometric experiments; but were exposed to the open air of the greenhouse in a situation alongside, so that the external conditions were similar.

The method employed was as follows: - A suitable number of dwarf French bean leaves, grown out of doors, were collected and brought to the laboratory with their petioles in water. Fresh weights were immediately determined, and each individual leaf was then placed in a numbered specimen tube containing the appropriate solution, viz:- tap water, 2,4-D; or I.B.A. A sample of the leaves was taken at this time to form the initial sample; shadowgraphs of these leaves were made to obtain the leaf area of the lamina, and then they were dried down to constant weight in an oven, controlled at 80 degrees centigrade. The period of time used to dry the leaves down was overnight. The remainder of the leaves were, in the meanwhile, placed in the dark for 24 hours with their petioles dipped in the appropriate solution. After this period the hormone solution was discarded and replaced by tap water, and the leaves removed to the greenhouse; the water in the tubes was changed daily. The treated leaves and the water controls were divided into two groups which constituted two series harvested at two periods of time. At the time of harvest, fresh weights were again taken; leaf areas determined by making shadowgraphs; and fresh weights of the separate parts of the leaf obtained.

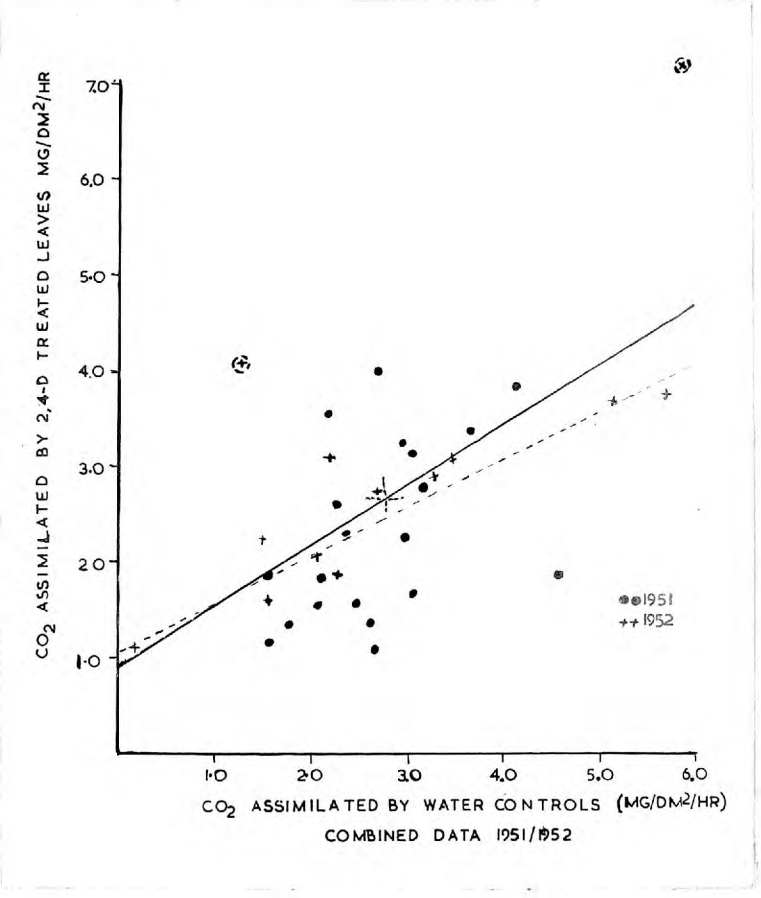


Fig. 15. Correlation diagram for all assimilation data (1951 and 1952 combined).

Full line represents the regression line for all data.

$r_{xy} = 0.64$ $\qquad Y = 0.93 \pm 0.613x$

Dotted line represents the regression line omitting the 1952 aberrant values.

$Y = 1.07 \pm 0.507x$

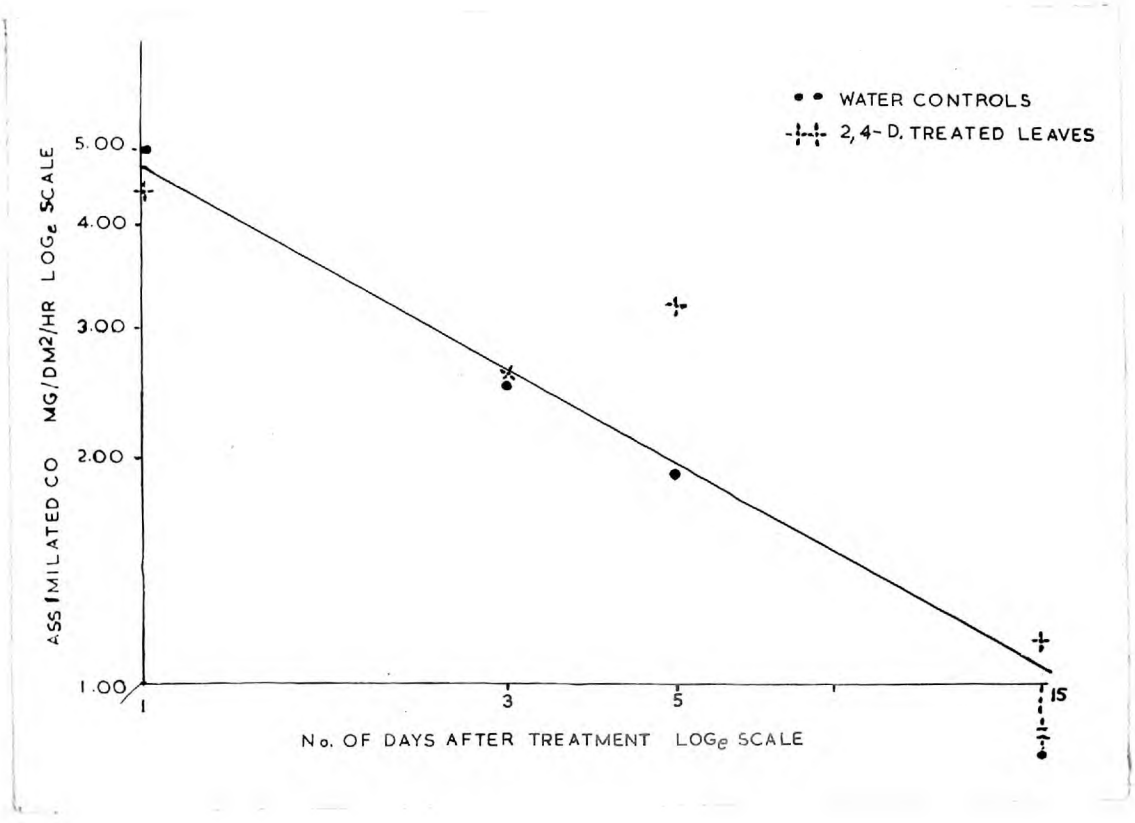


Fig. 16. Regression line showing the fall in assimilation rate with time.

The equation of the regression line is

$$Y = 1.572 + (-0.567)x$$

Data from Table XI.

Making the shadowgraphs necessitated the removal of the lamina from the petiole. Petiole and root fresh weight were measured and the weight of the lamina obtained by difference from the total. The various parts of the leaves were then dried down at 80°C. and the dry weights determined. Varying numbers of leaves were used in the different experiments as shown by the following table:-

TABLE XIII

Experiment designation	Date of starting the experiment	No. of Leaf replicates per sample	No. of days after treatment before harvesting	
			1st. series	2nd series
1951	31 July '51	10	11	21
1952 A	18 June '52	15	8	16
1952 B	9 July '52	40	8	15

In the following Tables XIV to XVI the primary data are entered. All the data have been reduced to comparable basis by stating all values in terms of changes per square decimetre of leaf area.

Results - 1951. As was found in previous work with the isolated French bean leaves, Murari (1950), very little growth in the leaves occurred after isolation. This is seen from the very small increases in fresh weight in all series. Water content of the leaves tends to fall during the course of the experiment; this effect being much less marked in the leaves treated with 2.5 p.p.m. I.B.A. than in the other two treatments.

TABLE XIV

Summary of information obtained 1951

All datum are the average of ten measurements. All weights are in grams.

A. Fresh Weight.

Treatment	Initial F.W./dm^2	Final F.W./dm^2	Total D.W./dm^2	Water Content H_2O/D.W. as %	Leaf area dm^2
Initial leaves	3.03	-	0.443	584	1.913
Water controls					
11 days	3.14	3.18	0.479	564	2.078
21 days	3.11	3.33	0.550	505	2.211
0.5 p.p.m. 2,4-D. treated leaves					
11 days	3.00	3.13	0.474	561	1.700
21 days	3.02	3.70	0.613	504	1.826
2.5 p.p.m. I.B.A. treated leaves					
11 days	3.16	3.34	0.500	568	1.798
21 days	3.07	3.90	0.586	565	1.959

B. Dry Weights

Treatment	Total D.W./dm^2	Lamina D.W./dm^2	Petiole D.W./dm^2	Root D.W./dm^2
Initial leaves	0.443	0.356	0.087	-
Water controls				
11 days	0.479	0.357	0.130	-
21 days	0.550	0.390	0.158	0.002
0.5 p.p.m. 2,4-D treated leaves				
11 days	0.474	0.336	0.133	-
21 days	0.613	0.388	0.190	0.033
2.5 p.p.m. I.B.A. treated leaves				
11 days	0.500	0.367	0.137	-
21 days	0.586	0.378	0.167	0.039

Necessary difference for significance Total D.W./dm^2 0.060 gms.

Lamina D.W./dm^2 0.056 gm. Petiole D.W./dm^2 0.019 gms.

TABLE XV

Summary of information obtained 1952 A.

Each datum is the average of 15 measurements. All weights given are in grams.

A. Fresh Weight

Treatment	Initial F.W./dm^2	Final F.W./dm^2	Total D.W./dm^2	Water content H_2O/D,W, as %	Leaf Area dm^2
Initial leaves	3.20	-	0.387	727	1.429
Water controls					
8 days	3.15	3.79	0.482	687	1.617
16 days	3.16	3.96	0.579	585	1.543
0.25 p.p.m. 2,4-D. treated leaves					
8 days	2.89	3.65	0.481	660	1.521
16 days	3.03	4.00	0.604	573	1.442
0.5 p.p.m. 2,4-D. treated leaves					
8 days	3.29	3.78	0.513	639	1.516
16 days	3.29	3.99	0.611	554	1.314
2.5 p.p.m. 2,4-D. treated leaves					
8 days	3.55	4.41	0.533	728	1.346
16 days	3.98	5.15	0.685	654	1.217
2.5 p.p.m. I.B.A. treated leaves					
8 days	3.29	4.34	0.522	732	1.484
16 days	3.35	4.60	0.621	640	1.383

TABLE XV a

Summary of information obtained (in gms.)

Experiment 1952 A. cont'd.

B. Dry Weights.

Treatment	Total D.W./dm^2	Lamina D.W./dm^2	Petiole D.W./dm^2	Root D.W./dm^2
Initial Leaves	0.400	0.326	0.075	-
Water treated leaves.				
8 days	0.482	0.352	0.129	
16 days	0.579	0.382	0.190	
0.25 p.p.m. 2,4-D. treated leaves.				
8 days	0.481	0.334	0.141	
16 days	0.604	0.375	0.210	0.020
0.5 p.p.m. 2,4-D. treated leaves.				
8 days	0.513	0.361	0.149	
16 days	0.611	0.384	0.204	0.020
2.5 p.p.m. 2,4-D. treated leaves.				
8 days	0.533	0.374	0.163	
16 days	0.685	0.380	0.228	0.074
2.5 p.p.m. I.B.A. treated leaves.				
8 days	0.522	0.365	0.148	
16 days	0.621	0.383	0.195	0.039

Necessary difference for significance Total D.W./dm^2 = 0.069 gms.

TABLE XVI

Summary of information obtained. 1952 B. Each datum is the average of 40 measurements. All weights are given in grams.

A. Fresh Weight

Treatment	Initial F.W./dm^2	Final F.W./dm^2	Total D.W./dm^2	Water Content H_2O./D.W. as %	Leaf Area dm^2
Initial	3.10		0.466	565	1.349
Water controls					
8 days	2.94	3.36	0.545	518	1.445
15 days	2.95	3.50	0.580	505	1.397
0.5 p.p.m. 2,4-D. treated leaves					
8 days	2.98	3.43	0.545	532	1.447
15 days	2.95	3.86	0.592	552	1.511
2.5 p.p.m. I.B.A. treated leaves					
8 days	3.03	3.52	0.530	564	1.325
15 days	3.00	4.25	0.613	593	1.339

B. Dry Weights

Treatment	Total D.W./dm^2	Lamina D.W./dm^2	Petiole D.W./dm^2	Root D.W./dm^2
Initial leaves	0.466	0.372	0.090	-
Water treated leaves				
8 days	0.545	0.417	0.123	-
15 days	0.580	0.412	0.163	0.002
0.5 p.p.m. 2,4-D. treated leaves				
8 days	0.545	0.408	0.136	-
15 days	0.592	0.382	0.199	0.028
2.5 p.p.m. I.B.A. treated leaves				
8 days	0.530	0.397	0.133	-
15 days	0.613	0.398	0.174	0.033

Necessary difference for significance

Total dry weight	0.029 gm.
Petiole D.W./dm^2	0.017
Lamina D.W. /dm^2	0.028

The changes in dry weight for the various parts of the leaf and total changes in dry weight per square decimetre are entered in the lower part of the table. Analysis of variance shows that the increase in total D.W./dm^2 over the initial D.W./dm^2 was highly significant, with an F. value of 6.25 with a 5% point of 3.13; the necessary difference for significance being 60 milligrams. The gain in total dry weight per square decimetre for the different periods and treatments are entered in the following table.

TABLE XVII

Gain in total dry weight per square decimetre. Experiment 1951. All figures are in milligrams.

Treatment	Gain in weight per period and per day.					
	1st 11 days		2nd 10 days		Total 21 days	
	per period	per day	per period	per day	per period	per day
Water treated leaves	36	3.3	71	7.1	107	5.1
0.5 p.p.m. 2,4-D. treated leaves	31	2.8	139	13.9	170	8.1
2.5 p.p.m. I.B.A. treated leaves	57	5.2	86	8.6	143	6.8

It will be seen from this table that none of the increments over the first eleven days are significant. This is probably due to adverse weather conditions during this part of the experiment; the days being cloudy and cool with a maximum temperature of 21°C. during the day and with a minimum night temperatures as low as 10°C. During the second period of the experiment, significant increases in dry weight are found for all treatments; and the difference between

the water controls and the 0.5 p.p.m. 2,4-D. treated leaves are significant over the whole period of the experiment. The value for the 2.5 p.p.m. I.B.A. treated leaves does not differ significantly from the water controls. The results of this experiment tend to confirm the findings from the gasometric experiments.

The rate of increase per square decimetre, per day, as shown in the table and averaged over the whole period are very low; and are far below the rates reported by Heath and Gregory (1938) in which the dry weight increases per square decimetre for various species averages 79 mg./dm^2/day; and Goodhall (1945) found for Tomato a net assimilation rate of 69 mg./dm^2/day in the summer, and 17 mg./dm^2/day in the winter. None of the assimilation rates reached even the winter rates found by Goodhall for Tomato. The whole scale of the experiment was too limited and not much weight can be attached to these results.

As far as the changes in weight in the different parts of the leaf are concerned it will be noted that the changes in dry weight per square decimetre of the lamina are very small, and quite insignificant. Analysis of variance given an F. value of 0.76 with the required difference for significance being 55.6 mgs. This agrees with the findings of Pickett et al who found that there was no significant difference in the amount of photosynthates accumulated by apple leaves sprayed with 10 p.p.m. 2,4-D. and the control leaves.

In contrast with the Lamina, the petiole dry weights have

increased significantly with time over all treatments. Analysis of variance gives an F. value between treatments of 9.21 with the 5% point equal to 2.74; the necessary difference for significance being 18.8 mgs. No significant difference between treatments was found on the 11th day after treatment, but at the 21 day level the 0.5 p.p.m. 2,4-D. treatment showed a significantly greater difference from the water controls. I.B.A. treated petioles were not significantly different from the water controls. In this they parallel the results found from the total dry weights. The increases in weight with time are significant at both levels for all treatments. This effect was due to the accumulation of assimilates in the petiole as found by Gregory and Samantarai.

The number of roots produced were in accordance with earlier experiments. 0.5 p.p.m. 2,4-D. treated leaves had about half the number of roots shown by the I.B.A. treated leaves. (64 as compared with 160). Water treated leaves produced an average of 11 roots per leaf; this result being entirely due to two of the ten leaves with large numbers of roots; one of these two leaves had 78 roots and the other had 26; the remainder of the leaves had not more than 3 very short roots.

1952 A. In view of the small assimilation rate obtained in the previous experiment of 1951, in which the second leaf from the apex was used throughout, it was considered possible that this leaf was not the most actively assimilating. In the present experiment therefore, three series of leaves were used, namely, 1. the uppermost ex-

panded leaf on the main axis; 2. the uppermost leaf on the first axillary branch; and 3. the next leaf below the uppermost leaf on the main axis. Samples of five leaves of each kind were used at each level of the experiment. In Table XV mean values are entered for fifteen observations and include all three series. The differences between the values obtained for the sets of leaves used are in the main not significant, as shown by the data set out in the table below.

TABLE XVIII

Mean increments of weight per leaf series in gms./dm^2
All treatments combined.

Leaf Series	Initial D.W./dm^2	D.W./dm^2 at the end of 8 days	D.W./dm^2 at the end of 16 days	Gain in weight from the Initial leaves 8 days	16 days
1.	0.323	0.474	0.597	0.151	0.274
2.	0.405	0.500	0.660	0.095	0.255
3.	0.434	0.545	0.604	0.111	0.170
Mean	0.387	0.506	0.621	0.119	0.234

Necessary difference for significance 0.069 gms. The only significant difference being in the increment in dry weight between leaf No. 3 which was the lower leaf on the main axis, and leaf No. 1 at the end of the 16th day.

As was shown in the 1951 experiment the water content of the leaves fell throughout the course of the experiment, at all treatment levels, With 2.5 p.p.m. I.B.A. and 2,4-D. the fall in

water content did not begin until after the first eight days. At the end of 16 days these two treatments still had the highest water content of the different treatments and 0.5 p.p.m. 2,4-D. with the lowest.

In the 2.5 p.p.m. levels of both hormones the increment in growth as indicated by increments in final fresh weight, was greater than in all other series; but the high water content at the end of the eight days was not due to lack of assimilates as the data for total dry weight per decimetre squared show, which in these two series are the highest values. In the 2.5 p.p.m. 2,4-D. series the leaf area progressively falls and this reflects the marginal damage caused by the high hormone treatment; the damage did not, however, affect the assimilation rate. It should be stated that the dead portions of the lamina were removed before the final dry weight was taken, otherwise, by including the dead material and calculating the assimilation on living portions a spurious increase in the assimilation rate would be recorded.

Analysis of variance for the total dry weight per decimetre squared gives an F. value of 6.21 with a 1% point of 3.41 for treatment effects. Necessary difference for significance being 0.069 grams. Leaves in all treatments gained significantly in weight per decimetre squared from that of the initial leaves by the end of the eighth day and a further significant gain in weight was made by the end of the sixteenth day. Increases in weight per

square decimetre are shown in Table XIX.

At the end of the eighth day there was no significant difference between treatments in total dry weight per decimetre squared; all series of treatments showed significant increases in weight from the initial dry weight per square decimetre.

TABLE XIX

Increases in Total D.W./dm^2 Experiment 1952 A in grams.

Treatment	First 8 Days	Second 8 Days	Total gain in weight 16 days
Water controls	0.095	0.097	0.192
0.25 p.p.m. 2,4-D.	0.094	0.122	0.217
0.5 p.p.m. 2,4-D.	0.126	0.098	0.224
2.5 p.p.m. 2,4-D.	0.146	0.152	0.298
2.5 p.p.m. I.B.A.	0.135	0.099	0.234

Necessary increase for significance 0.069 gms.

By the end of the sixteenth day the treatment difference had increased with the 2.5 p.p.m. 2,4-D. reaching a level significantly higher than the water controls. From this experiment therefore, there is no evidence of a decrease in assimilation rate due to hormone treatment; on the contrary, there appears to be an increase in the assimilation rate due to treatment with 2,4-D., the effect becoming greater the higher the concentration. The effect of the I.B.A. in spite of it's greater rooting response, is about equal to that of 0.5 p.p.m. 2,4-D; and is less than that of the corresponding strength of 2,4-D. in both rooting response and increase in weight.

The increase in dry weight per decimetre squared following hormone treatment is not seen when the lamina alone is considered, in no instance did the increment changes from that of the initial increment reach a significant level.

Petiole dry weights per square decimetre, on the other hand, all show significant increases, both at the end of the 8 day and 16 day periods, thus indicating that the major part assimilated by the lamina is translocated to the petiole. So far as treatment effects are concerned there was no significant differences between treatments in petiole dry weight per square decimetre, but when root weight was included the 2.5 p.p.m. 2,4-D. treatment becomes significantly higher than the water controls.

The increase in assimilation rate, with dosage, of 2,4-D. treated leaves noted in the previous Table runs parallel with the amount of root produced in these treatments, as is shown in Table XX.

TABLE XX

Number of roots produced by the various treatments in Experiment 1952 A. and their Dry Weight in milligrams.

Treatment	Number of roots induced Average	D.W. of roots induced (mgs.) per leaf
Water controls	2	0.4
0.25 p.p.m. 2,4-D.	21	17.0
0.5 p.p.m. 2,4-D.	52	27.0
2.5 p.p.m. 2,4-D.	131	57.0
2.5 p.p.m. I.B.A.	85	44.0

This might be attributed to the fact that translocation and utilization of the assimilated material in root formation tends to prevent the accumulation of assimilates in the lamina and thus reduce the net accumulation rate. Data to be presented later, on total carbohydrate content in the lamina (Table XXVIII) lends support to this view; the total carbohydrates in terms of milligrams per gram fresh weight, for the three levels of 2,4-D., at the end of 16 days, being 36.3; 31.2 and 29.8 mgs. respectively.

Net Assimilation Rate. Since the area of the leaves change very little during the course of the experiment, and indeed, in some cases owing to sampling errors the leaf area was less than the original, the net assimilation rate could not be calculated from the standard formula employed in growth analysis:

$$\frac{(W_2 - W_1)(\log_e L_2 - \log_e L_1)}{L_2 - L_1}$$

instead the net assimilation rate could be calculated directly by increments in dry weight per decimetre squared in which such changes in area as occur have been eliminated. Net assimilation figures are entered in Table XXI. In column 2 the assimilation rates as mg./dm^2/period are entered; in column 3 the assimilation is entered as mg./dm^2/day; and in column 4 the assimilation in terms of CO_2 taken up are calculated on the assumption that all increases in weight are due to carbohydrates. In this experiment the assimilation rate in the two periods showed no consistent differences, unlike the experiment in 1951. The general level of this experiment was much higher than in the earlier experiment as the enviromental conditions were more favourable. In this respect growth experiments resembled the gasometric determinations already presented.

Blackman (1952) treating young Helianthus annuus plants with various concentrations of Na-2,4-D., applied either as a spray or by individual drops, found that with increasing dosages different effects on the relative growth rate were produced in different parts of the plant. The data presented in Table XVIII, in which the increments in dry weight are given for three different sets of leaves, tends to bear this out. This difference in effect will be reflected in the results presented in Table XV, each datum represents a mean found from the data for the three sets combined. This will in part account for some of the differences noted in the Net Assimilation rates given in Table XXI between the different dosages at both time levels.

1952 B. The experiance gained in the two previous experi-

ments showed that to obtain definitive results it would be necessary to increase the number of replicated leaves to the maximum possible extent. In this experiment, the treatments were reduced to three namely; water controls; 0.5 p.p.m. 2,4-D. and 2.5 p.p.m. I.B.A.; the latter two treatments being used to obtain maximum rooting effects. The leaves were sampled after 8 and 16 days; 40 replicated leaves were used for each sample. The improvement in technique is shown by the standard errors of the sample, for in this experiment the difference in total dry weight per square decimetre necessary for significance was 29 mgs., as compared to 69 mgs. in the experiment 1952 A and 60 mgs. in experiment 1951; and the standard error of the initial fresh weight did not exceed 5% of the mean.

In this experiment the water controls showed a progressive decrease in water content; the 0.5 p.p.m. 2,4-D. showed first a fall in water content followed by a recovery, while the leaves treated with I.B.A. gained in water content during the second period. The hormone treated leaves again showed more growth than the water controls as shown by the increases in fresh weight per square decimetre.

There is again no significant differences between treatments in the dry weights per square decimetre of the lamina. F. value is 1.56 with a 5% point of 1.98. All treatments showed a significant increase in lamina dry weights over the initial value; this value being the most marked in the water controls.

Petiole weight per square decimetre showed a very significant increase in weight in all treatments over the initial weights; and significant treatment effects now appear between treatments with the highest value occurring in the 0.5 p.p.m. 2,4-D. treated leaves which differs significantly from both the water controls and the I.B.A. treated leaves.

In total dry weight per square decimetre the increment over the initial value is highly significant for all treatments and on all occassions, but the differences between treatments is not significant except for I.B.A. at 15 day level which shows significant increase over the water controls. Increases in total dry weight per square decimetre are shown in Table XXII. It will be seen that the uniform high rate of the 1952A experiment is associated with low initial dry weight per square decimetre. The low values obtained in the 1952B experiment was not due to unfavourable weather conditions as the conditions during this experiment were good throughout, but on the other hand, the initial dry weight per square decimetre was considerably higher than in experiment 1952A. In the 1951 experiment where the initial weight was also high, together with unfavourable weather conditions led to a very low assimilation rate. Taking the data as a whole therefore, it would appear that the total dry weight per square decimetre tends to a maximum and therefore the higher the initial value the less the increment obtained. As already has been pointed out the total carbohydrate in the lamina falls with the increasing amount of root produced, but the translocation to the root system

TABLE XXI

Mgs. of assimilated CO_2 equivalent to increases in increments of total dry weight. Experiment 1952 A. 1 Mg. carbohydrate is equivalent to $\frac{264}{180}$ or 1.47 mg. assimilated CO_2.

Treatment	Increase in dry weight per period mg./dm^2	per day	mg./dm^2/day of equivalent CO_2 assimilated
Water controls			
1st 8 days	95	11.9	17.5
2nd 8 days	97	12.1	17.8
Total 16 days	192	12.0	17.6
0.25 p.p.m. 2,4-D. treated leaves			
1st 8 days	94	11.8	17.4
2nd 8 days	122	15.2	22.3
Total 16 days	217	13.6	20.0
0.5 p.p.m. 2,4-D. treated leaves			
1st 8 days	126	15.8	23.2
2nd 8 days	98	12.2	17.9
Total 16 days	224	14.0	20.6
2.5 p.p.m. 2,4-D. treated leaves			
1st 8 days	146	18.2	26.8
2nd 8 days	152	19.0	28.0
Total 16 days	298	18.6	27.4
2.5 p.p.m. I.B.A. treated leaves			
1st 8 days	135	16.9	24.9
2nd 8 days	99	12.4	18.2
Total 16 days	234	14.6	21.5

TABLE XXII

Increases in Total Dry Weight per Square Decimetre Experiment 1952 B.

Treatment	Increases in Total D.W./dm^2 in mgs. per period	per day
Water controls		
1st 8 days	79	9.9
2nd 7 days	35	5.0
Total 15 days	114	7.6
0.5 p.p.m. 2,4-D. treated leaves.		
1st 8 days	79	9.9
2nd 7 days	47	6.7
Total 15 days	126	8.4
2.5 p.p.m. I.B.A. treated leaves		
1st 8 days	64	8.0
2nd 7 days	83	11.9
Total 15 days	147	9.8

It will be noted that the assimilation rate varied considerably in the different experiments. The average values for assimilation in treatments common to all three experiments together with their initial dry weight per square decimetre are entered in the table below.

TABLE XXIII

Experiment	Initial D.W./dm^2 in mg.	Net Assimilation in mg./dm^2/day Water Controls	0.5 p.p.m. 2,4-D.	2.5 p.p.m. I.B.A.
1951	443	5.1	8.1	6.8
1952 A	387	12.0	14.0	14.6
1952 B	466	7.6	8.4	9.8

over the short period as used in these experiments is not sufficient to counteract the effect of accumulated assimilates in the lamina which tends to limit further assimilation.

This tendency for the assimilation rate to decrease as the experiment proceeds is shown clearly in experiment 1952 B, and this decline is least where the root system has developed to the greatest extent.

Comparison of gasometric results with growth analysis data.

An effort has been made to compare the values for the 1952 B experiment obtained by growth analysis and the gasometric determinations. The relevant data are entered in Table XXIV. From the meteorological data obtained from the Meteorological Office South Kensington, the average number of hours sunshine over the period of the experiment was found to be 7.0 hours per day. The average assimilation rate obtained gasometrically for water controls and 0.5 p.p.m. 2,4-D. treated leaves are entered as obtained for Table XI, for the first 8 days and the total period; assuming that the gasometric figures are representative of the rates under the average conditions of sunshine prevailing during the experiments. The assimilation of CO_2 per day has been calculated using the average value for seven hours sunshine, and these are entered in the table.

To compare with these figures, the amount of CO_2 required to be assimilated to account for the observed gain in dry weight

TABLE XXIV

Comparison of gasometric results with growth analysis Experiment 1952 B.

Total number of hours sunshine during the experiment. 111.4

Average number of hours sunshine per day. 7.0

	Leaf pre-treatment	
	Water	0.5 p.p.m. 2,4-D. in mg./dm^2
Average CO_2 assimilated per hour as recorded by gasometer from Table XI		
1st. 8 days	3.08	3.19
Total 15 days	2.86	3.03
,; assimilation during the hours of sunshine will equal		
1st. 8 days	21.56	22.33
Total 15 days	20.02	21.21
Av. number of hours dark 7' 50"		
Av. number of hours daylight 16' 10"		
Respiration during the dark per hour from Table VI	0.70	0.73
,; total respiration during hours of darkness will equal	5.48	5.92
Mg. CO_2 equivalent to gain in weight per day from Table XXV		
1st 8 days	14.55	14.55
Total 15 days	11.17	12.35
Mg. CO_2 required for gain in weight plus respiratory losses		
1st 8 days	20.0	20.5
Total 15 days	16.7	18.3
Difference between gasometric estimations and net assimilation estimations		
1st 8 days	1.6	1.8
Total 15 days	3.3	2.9 mg/day/dm^2

TABLE XXV

Mg. CO_2 equivalent to net assimilation as found from the total D.W./dm^2 increases in weight. 1 mg. Carbohydrate is equivalent to 1.47 mgs. CO_2.

Treatment	Increases in dry weight in mg./day/dm^2	mg./dm^2/day of equivalent CO_2 assimilated
Water treated leaves		
1st 8 days	9.9	14.55
2nd 7 days	5.0	7.35
Total 15 days	7.6	11.17
2.5 p.p.m. I.B.A. treated leaves		
1st 8 days	8.0	11.76
2nd 7 days	11.9	17.49
Total 15 days	9.8	14.40
0.5 p.p.m. 2,4-D. treated leaves		
1st 8 days	9.9	14.55
2nd 7 days	6.7	9.85
Total 15 days	8.4	12.35

per day have been used (Table XXV) and are entered in the table. A correction needs to be made for the respiration losses during the hours of darkness, and this correction has been derived from the actual respiration rates obtained from gasometric experiments (Table VI). Adding the mg. CO_2 lost by respiration to the CO_2 assimilated in growth experiments, the estimated gross assimilation per day has been calculated for the water controls and the 2,4-D. treated leaves for which the values obtained are 20.0 mg. and 20.5 mg. CO_2 respectively for the first eight days; and 16.7 mg. and 18.3 mg. over the whole period.

The agreement between the estimated assimilation rate derived from the growth data and the gasometric data respectively agree reasonably well for the first eight days of the experiment, but over the whole period the assimilation rate from the gasometric figures are somewhat high. Giving due regard to paucity of the gasometric data over the whole period it may be claimed that the agreement between the two methods of estimating the assimilation rate is satisfactory.

With regard to the effect of treatment with 2,4-D. on the assimilation rate, both methods show that there is no decrease in assimilation due to hormone treatment, but on the contrary, there is some indication that the assimilation rate is actually increased by hormone treatment. In view of the analysis of the gasometric data previously given which indicate that 2,4-D. treatment reduced

the assimilation rate, neither of these conclusions can be regarded as definitive, but obviously more weight should be attached to direct observations in Table XXIV; the evidence points to the conclusion that 2,4-D. has no direct effect on the assimilation rate. 2,4-D. treatment has already been shown to have no effect on the respiration rate either.

Sec. III

Carbohydrate analysis on the lamina

The position with regard to the effects of growth hormones on carbohydrate metabolism is somewhat conflicting. The following data has been found in the literature.

Using 2,4-D. as a spray, Tukey et al (1945) found a complete disappearance of starch granules from Bindweed and Sowthistle rhizomes. Mitchell and Marth (1944) observed that starch hydrolysis was more rapid during the ripening of bananas when they were treated with 2,4-D. than in the controls. Smith et al (1947) found that in Bindweed treated with 2,4-D. there was a rapid increase in total sugars at first and then a drop to the same level as the controls; and that Starch-dextrins decreased to 1/3 of the controls by the tenth day. D. E. Wolfe et al (1950) treated soybeans with 2,4-D. and found that total sugars was higher, reducing sugar was higher and that starch content was lower. Sell et al (1949) obtained a depletion of the reducing and non-reducing sugars; a decrease in acid hydrolysible polysaccharides and a reduction in carbohydrate reserves in bean plants treated with 2,4-D. Beauchamp (1950) obtained an increase in the sugar content of the sugar cane equal to 1% of the cane weight by spraying with 2,4-D. Weller et al (1950) found a depletion in the 2,4-D. treated bean plant of non-reducing sugars but no significant change in the reducing sugars; the percentage starch was practically the same in the leaves and roots of treated and untreated plants. Wort (1950) treating buck-

wheat with 2,4-D. found a decline in both total sugars and starch dextrins of Buckwheat, and that this effect increased with dosage. Smith (1948) using bean tissue cultures noted a reduction in both starches and sugars when they were treated with 2,4-D. and Gall (1948(obtained a marked degredation of starch in 2,4-D. treated bean tissue cultures. The general consensus of opinion being that there was a general reduction in starches and sugars although the results noted for sugar content varied under the different conditions used by the various workers. It must also, be noted that all the above mentioned workers used much stronger dosages than those used in these experiments.

Previous work carried out on isolated and rooted French bean leaves by Gregory and Samantarai and by Murari are obviously more comparable with the present work; first, as the same leaf material was used, and second that the dosage of hormone employed was such as to give maximum rooting response. In these experiments I.B.A. was used throughout at a dosage of 2.5 p.p.m.

Gregory and Samantarai, working with the bean leaf, estimated the soluble carbohydrates in the lamina, and all conclusions as to starch metabolism were drawn from iodine stained preparations. They found a marked fall in starch content immediately after treatment until the third day after which, the starch content rose together with the soluble sugars, but that the starch content in the treated leaves never reached the same value as in the untreated controls.

This work was carried further by Murari who analysed the whole leaf using the lamina and petiole combined; and found that the total carbohydrate increased throughout the whole experiment, at first rapidly and then at a progressively lower rate until the end of the experiment; this was true of both treated and control leaves. In the untreated leaves the starch content rose until the sixteenth day while in the leaves treated with I.B.A. the starch content rose at a slower rate until the eighth day and then declined. The conclusion drawn from this work was that the balance of soluble carbohydrates and starch was shifted by I.B.A. treatment in the direction of decreasing starch and increasing sucrose, the level of reducing sugars being much less affected.

It appeared important that the starch-sugar balance in isolated rooting leaves should be investigated further. In the present work therefore, certain experiments were undertaken to make further study into this relationship, using I.B.A. at the same level as used by previous workers (2.5 p.p.m.), and 2,4-D. at a level giving optimal rooting (0.5 p.p.m.).

Methods. Four disks one centimetre in diameter were removed from each leaflet at random, but taken so that the main veins were not included. These disks were removed immediately after the fresh weights of the leaves were obtained from each set of leaves used in the growth experiments; i.e. from the initial leaves and at the eighth and fifteenth day weighings. They were then weighed immediately and extracted in 95% alcohol for five minutes. The disks and alcohol were then placed in bottles which were sealed with paraffin

wax, and in this way stored until the analysis were made. Before analyses were made the disks were drained and re-extracted; the two lots of alcohol combined and concentrated under reduced pressure. The residue after reduction was taken up in water at the rate of 25 or 50 cc. distilled water for each gram of initial fresh weight in the disks; depending upon the amount of material used and the amount of sugar present in the sample. The amount of reducing sugar in the sample was estimated by the iodometric technique described by Somogyi (1945) using his phosphate buffered copper tartrate reagent; 5 cc. aliquots being used for each determination. Total sugars were estimated by the same technique after inverting the non-reducing sugars with invertase. Non-fermentable reducing substances were found by digesting an aliquot with bakers yeast and estimated by the above method. The amount of non-fermentable reducing substance obtained was deducted from the total sugars and reducing sugars obtained in this way to give the amount of sugars actually present.

Starches were estimated by the method described by Hanes (1936). After draining and washing of the disks with alcohol the disks were allowed to air-dry; and then ground in a mortar to which a few grains of cleaned quartz sand had been added. The starch was then solubulized with hot alcoholic HCl under a reflux condensor for 15 minutes. The starch was then dissolved out with boiling water and the water made up to a standard quantity after extraction. Two aliquots were taken for the estimation of starch; to one of the

aliquots one cc. of 1% beta amylase solution was added along with two cc. of pH4.6 acetate buffer; to the other, one cc. of distilled water and two cc. of the buffer was added. They were then digested overnight at 20°C. along with two standard starch solutions to give the hydrolysis limit of the amylase. Starch was estimated as maltose, with the titration value found as the difference between the digested and undigested aliquots. Somogyi's solution was again used.

All values found were adjusted to the original fresh weight as milligrams per gram fresh weight.

These analyses were made for all levels of the three growth experiments described in the preceding pages.

Results. The primary data for carbohydrate analyses for the three experiments, are entered in Tables XXVI to XXIX. All values given are in milligrams per gram fresh weight. To compare the values of the different experiments it has been necessary to calculate the data on a fresh weight basis. This was due to the fact that the analyses were carried out on sampling disks cut out of the leaf; and of these only the fresh weights were known.

It will be noted that the experiment 1952 B (Table XXIX) differs markedly from the other two experiments by the very high total carbohydrates present initially in the leaves.

Comparisons between the three experiments in so far as the changes in the carbohydrate fractions are concerned, for the

three comparable levels of treatment, are shown in Table XXX and XXX a. The following ratios are entered; total sugars over starch; invert sugar over starch; and reducing sugar over starch. The fraction, invert sugar, measures the sucrose content of the leaf. The relative amount of sucrose and reducing sugar can therefore be derived directly from the data in the table as both the terms are stated in terms of the starch present.

The ratio data for invert sugar over starch and total sugar over starch are shown in the table below.

TABLE XXXI

Invert Sugar over Starch Summary data from Experiments 1951 and 1952 A.

		Water	0.5 p.p.m. 2,4-D.	2.5 p.p.m. I.B.A.
1951	8 days	1.15	1.83	1.58
"	16 "	0.76	4.23	0.89
1952 A	8 "	1.58	2.04	1.41
"	16 "	1.80	2.04	0.84
TOTAL -		5.29	10.14	4.72

TABLE XXVI

Lamina Carbohydrate Analyses. Experiment 1951 Summary.

Treatment	Total Carbo-hydrates	Starch	Total Sugars	Invert Sugars	Reducing Sugars
	in milligrams per gram Fresh Weight				
Initial	4.59	2.26	2.33	1.16	1.17
Water treated leaves.					
Immediately after treatment.	3.91	1.83	2.08	1.09	0.99
24 Hours after	9.43	1.74	7.69	5.29	2.40
11 days after	19.19	5.71	13.48	6.60	6.88
21 days after	22.79	5.10	17.69	3.80	13.89
0.5 p.p.m. 2,4-D. treated leaves.					
Immediately after treatment.	4.05	1.09	2.96	1.89	1.07
24 Hours after	7.76	1.28	6.48	3.29	3.19
11 days after	19.51	3.57	15.94	6.55	9.39
21 days after	26.75	3.33	23.42	13.89	9.58
2.5 p.p.m. I.B.A. treated leaves.					
Immediately after treatment	4.23	1.33	2.90	1.30	1.60
24 Hours after	7.73	1.09	6.64	4.22	2.42
11 days after	17.74	4.26	13.47	6.71	6.76
21 days after	20.86	5.47	15.38	4.85	10.53

TABLE XXVII

Lamina Carbohydrate Analyses. Experiment 1952 A.

All data in mgs. per gram fresh weight.

Leaf Group	Total Carbo-hydrates	Starch	Total Sugars	Invert Sugars	Reducing Sugars
Initial.					
1.	6.24	1.06	5.18	2.30	2.88
2.	13.28	6.21	7.07	2.70	4.37
3.	3.79	0.60	3.19	1.86	1.33
Av.	7.77	2.62	5.15	2.29	2.86
Water - 8 days.					
1.	29.09	5.49	23.60	7.86	15.74
2.	28.70	8.43	20.27	13.04	7.23
3.	26.65	8.06	18.59	13.53	5.06
Av.	28.15	7.33	20.82	11.47	9.34
Water - 16 days.					
1.	32.17	7.44	24.73	16.10	8.63
2.	25.95	7.83	18.12	2.80	15.32
3.	22.16	3.00	19.16	13.69	5.49
Av.	26.76	6.09	20.67	10.86	9.81
0.25 p.p.m. 2,4-D. - 8 days.					
1.	52.96	17.63	35.33	18.25	17.08
2.	40.07	11.49	28.58	18.20	10.38
3.	20.12	4.38	15.74	11.22	4.52
Av.	37.72	11.17	26.55	15.89	10.66
0.25 p.p.m. 2,4-D. - 16 days.					
1.	62.72	12.04	50.68	27.30	23.38
2.	25.41	13.98	11.43	4.69	6.74
3.	20.69	2.12	18.57	12.53	6.04
Av.	36.27	9.38	26.89	14.84	12.05
0.5 p.p.m. 2,4-D. - 8 days.					
1.	30.29	11.86	18.43	7.02	11.41
2.	16.49	6.95	9.54	2.85	6.69
3.	17.92	4.01	13.91	10.26	3.65
Av.	21.57	7.61	13.96	6.71	7.25

TABLE XXVII

Lamina Carbohydrate Analyses continued. Experiment 1952 A.

Leaf Group	Total Carbo-hydrates	Starch	Total sugars	Invert sugars	Reducing sugars
	in mgs. per gm. fresh weight				
0.5 p.p.m. 2,4-D. - 16 days					
1.	43.99	8.87	35.12	23.46	11.66
2.	25.28	10.59	14.69	8.20	6.49
3.	24.20	3.17	21.03	14.40	6.63
Av.	31.16	7.54	23.61	15.35	8.26
2.5 p.p.m. 2,4-D. - 8 days					
1.	39.68	4.36	35.32	8.00	27.32
2.	15.13	6.33	8.80	3.00	5.80
3.	16.56	2.61	13.95	9.86	4.09
Av.	23.79	4.43	19.36	6.95	12.40
2.5 p.p.m. 2,4-D. - 16 days					
1.	43.82	2.38	41.44	23.62	17.82
2.	25.33	12.49	12.84	8.98	3.86
3.	20.15	9.06	11.09	4.83	6.26
Av.	29.77	7.98	21.79	12.48	9.31
2.5 p.p.m. I.B.A. - 8 days					
1.	44.76	10.10	34.66	21.85	12.81
2.	25.25	10.57	14.68	3.91	10.77
3.	14.05	3.25	10.80	8.05	2.75
Av.	28.02	7.97	20.05	11.27	8.78
2.5 p.p.m. I.B.A. - 16 days					
1.	28.74	13.40	15.34	9.91	5.43
2.	25.37	8.31	17.06	8.90	8.16
3.	14.59	5.36	9.23	3.80	5.43
Av.	22.90	9.02	13.88	7.54	6.34

TABLE XXVIII

Lamina Carbohydrate Analyses.

Experiment 1952 A Summary in milligrams per gram fresh weight.

Treatment	Total Carbo-hydrates	Starch	Total Sugars	Invert Sugars	Reducing Sugars
Initial	7.77	2.62	5.15	2.29	2.86
8 days after treatment					
Water	28.15	7.33	20.82	11.47	9.34
0.25 p.p.m. 2,4-D.	37.72	11.17	26.55	15.89	10.66
0.5 p.p.m. 2,4-D.	21.57	7.61	13.96	6.71	7.25
2.5 p.p.m. 2,4-D.	23.79	4.43	19.36	6.95	12.40
2.5 p.p.m. I.B.A.	28.02	7.97	20.05	11.27	8.78
16 days after treatment					
Water	26.76	6.09	20.67	10.86	9.81
0.25 p.p.m. 2,4-D.	36.27	9.38	26.89	14.82	12.05
0.5 p.p.m. 2,4-D.	31.16	7.54	23.61	15.35	8.26
2.5 p.p.m. 2,4-D.	29.77	7.98	21.79	12.48	9.31
2.5 p.p.m. I.B.A.	22.90	9.02	13.88	7.54	6.34

TABLE XXIX

Lamina Carbohydrate Analyses.

Experiment 1952 B. in milligrams per gram fresh weight.

Treatment	Total Carbo-hydrates	Starch	Total sugars	Invert sugars	Reducing sugars
Initial	24.12	9.66	14.46	7.16	7.30
8 days after treatment					
Water	29.41	18.33	11.08	3.17	7.91
2.5 p.p.m. I.B.A.	29.05	16.42	12.63	5.19	7.44
0.5 p.p.m. 2,4-D.	30.76	17.55	13.21	4.74	8.47
15 days after treatment					
Water	29.46	10.42	19.04	9.53	9.51
2.5 p.p.m. I.B.A.	23.11	9.28	13.83	6.96	6.87
0.5 p.p.m. 2,4-D.	27.34	13.24	14.10	6.02	8.08

TABLE XXX

Lamina Starch - sugar ratios for all three experiments.

Only comparable treatments entered.

Treatment and Time		Total sugars / Starch	Invert sugars / Starch	Reducing sugar / Starch
Initial leaves				
1951		1.04	0.52	0.52
1952 A		1.97	0.88	1.09
1952 B		1.50	0.74	0.76
Water treated leaves				
1951	11 days	2.36	1.15	1.21
1952 A	8 days	2.85	1.58	1.27
1952 B	8 days	0.61	0.17	0.44
0.5 p.p.m. 2,4-D. treated leaves				
1951	11 days	4.45	1.83	2.62
1952 A	8 days	3.14	2.04	1.10
1952 B	8 days	0.75	0.27	0.48
2.5 p.p.m. I.B.A. treated leaves				
1951	11 days	3.16	1.58	1.58
1952 A	8 days	2.52	1.41	1.11
1952 B	8 days	0.77	0.32	0.45

TABLE XXX a

Lamina starch - sugar ratios continued.

Treatment and Time		Total sugars / Starch	Invert sugars / Starch	Reducing sugar / Starch
Water treated leaves				
1951	21 days	3.48	0.76	2.72
1952 A	16 days	3.41	1.80	1.61
1952 B	15 days	1.83	0.92	0.91
0.5 p.p.m. 2,4-D. treated leaves				
1951	21 days	7.15	4.23	2.92
1952 A	16 days	3.14	2.04	1.10
1952 B	15 days	1.07	0.46	0.61
2.5 p.p.m. I.B.A. treated leaves				
1951	21 days	2.82	0.89	1.93
1952 A	16 days	1.54	0.84	0.70
1952 B	15 days	1.49	0.75	0.74

TABLE XXXI Cont'd.

Summary. Total sugar over starch ratios.

Data from Experiment 1951 and 1952 A.

	Water	0.5 p.p.m. 2,4-D.	2.5 p.p.m. I.B.A.
1951 8 days	2.36	4.45	3.16
1951 16 days	3.48	7.15	2.82
1952 A 8 days	2.85	3.14	2.52
1952 16 days	3.41	3.14	1.54
Total --	12.10	17.88	10.04

The variability in the data is so great that mean values have little weight. It appears however, that so far as 0.5 p.p.m. 2,4-D. treatment is concerned there is evidence for a change in the ratio of soluble sugars to starch, and in this respect these results agree with the previous results found by Murari for I.B.A. treated leaves. The I.B.A. treated leaves in this experiment present no evidence for a change in the ratios of sugars to starch.

The 1952 B Experiment shows very low ratios of sugar to starch. These are summarized in Table XXXII.

TABLE XXXII

Invert sugar over starch summary . Experiment 1952 B.

	Water	0.5 p.p.m. 2,4-D.	2.5 p.p.m. I.B.A.
8 days	0.17	0.27	0.32
16 days	0.92	0.46	0.75
Total -	1.09	0.73	1.07

Total sugar over starch

	Water	0.5 p.p.m. 2,4-D.	2.5 p.p.m. I.B.A.
8 days	0.61	0.75	0.77
16 days	1.83	1.07	1.49
Total -	2.44	1.82	2.21

There is no evidence of change of ratios in this experiment and here, the 2,4-D. shows the lowest ratio. It must therefore, be concluded that the findings of Murari have not been on the whole confirmed.

SUMMARY AND CONCLUSIONS

In this work, experiments were carried out to investigate (1) the rooting response of isolated French bean leaves to phytocidal substances applied to the petiole in non-lethal concentrations; and (2) the effects of these substances on the assimilation by the lamina, using both gasometric and growth experiments. 2,4-dichlorophenoxyacetic acid (2,4-D.) was chosen as being typical of these substances. 2.5 p.p.m. beta-Indolebutyric acid (I.B.A.) was used as a control giving similar rooting response but without toxic effects; water controls were used in addition.

(1) The following features of the rooting response were investigated to determine the treatment conditions for maximal rooting with 2,4-D.;

A. optimal concentrations of the growth substance for rooting; and B, the light effect on rooting.

(2) Over the range of concentrations 0.1 p.p.m. to 1.5 p.p.m. 2,4-D. the number of roots induced was directly proportional to the $\log_e$ of concentration. A fall in the number of roots induced by a higher concentration (2.5 p.p.m.) was noted; the number of roots induced by the two higher concentrations exceeded that induced by 2.5 p.p.m. I.B.A.. Mean dry weight of roots per leaf attained a maximum at 1.5 p.p.m. 2,4-D. and decreased above and below this concentration. Since the assimilation rate is not much affected by treatment, and if carbohydrate supply were the only factor concerned in determining root growth and root dry weight, it would mean that

the dry weight per leaf should remain constant and the product of root number and weight per root should also be constant. At high concentration the product of root number and weight falls below that of 1.5 p.p.m., but this is not compensated by increases in weight per root indicating an inhibition of root growth by higher concentrations. At concentrations below the optimum total root weight also falls. The total weight of a root system will depend upon the growth of individual roots, and presumably there was not sufficient time during the experiment to enable sufficient branching to take place, so that, the root weight per leaf was limited by the growth rate of the roots.

Abnormalities occurred in the roots induced by the concentrations 1.0 to 2.5 p.p.m.; the number of these abnormal roots increased with concentration. 0.5 p.p.m. was therefore, chosen as the most suitable concentration to use in further experiments.

(3) No rooting response was observed when leaves were treated under adverse weather conditions such as low temperature.

(4) An increasing number of roots was obtained with increasing amounts of light, during the rooting period after treatment. Leaves receiving light below a minimum or kept in darkness died before the end of the experiment.

(5) A marked decrease was found in the number of roots induced when treatment with either 2.5 p.p.m. I.B.A. or 0.5 p.p.m. 2,4-D. was delayed 24 hours. The root length was again inversely related to the number of roots produced, suggesting that there is a strong

competition between growing roots and the supply of some nutrient, probably carbohydrates, which tends to limit the growth of individual roots.

(6) The mean number of roots induced by 2.5 p.p.m. I.B.A. was inversely related to the number of hours light received during the first 48 hours. Root induction was best when treatment with I.B.A. was given in the light. The effect of light on root induction by 0.5 p.p.m. 2,4-D. was less pronounced; the greater number of roots were produced where only a 24 hour light period was given during the first 48 hours. 2,4-D. treatment in the dark during the first 24 hours was more effective than treatment in the light for root induction.

(7) An apparatus is described for the gasometric measurement of the assimilation by individual rooting leaves.

(8) No evidence was found that 2,4-D. at the concentration used for maximal rooting (0.5 p.p.m.) affected the respiration of isolated leaves. Respiration of both water controls and the leaves treated with 0.5 p.p.m. 2,4-D. was found to be correlated with temperature with a Q_{10} between 10°C. and 20°C. of 2.2 .

(9) During the 1951 assimilation experiments, measured gasometrically, the assimilation rate of leaves treated with 0.5 p.p.m. 2,4-D. was consistently reduced. There was some evidence to show that the CO_2 content of the air leaving the root chambers could not be reduced below the minimum of 0.01% by the assimilating leaves.

No significant difference was found from the 1952 assimi-

lation experiments, between the assimilation rates for the water controls and the 2,4-D. treated leaves. A correlation diagram was made from the two sets of data shows that some reduction in the assimilation rate had probably occurred. This may in part, be due to the paucity of data for the latter part of the experimental period. The assimilation rate for both the water controls and the 2,4-D. treated leaves fell with time; little difference being found between the two treatments in this respect.

(10) Three experiments were carried out to measure the net assimilation rate by growth experiments where changes in increment of total dry weight per square decimetre were used as a measure of the assimilation rate. No significant difference was found between treatments at the end of the first period (8 or 11 days) in any of the experiments. At the end of the experiment all treated leaves had higher assimilation rates than the water controls. This increase in the assimilation rate paralleled the increase in the number of roots induced by the different treatment and dosages. This suggests that the translocation and utilization of assimilated materials in root formation tends to prevent the accumulation of assimilates in the lamina and thus allowing the assimilation rate to be maintained. Total carbohydrate in the isolated leaves tended towards a maximum; leaves with a high initial total dry weight per square decimetre had low assimilation rates, with the reverse being true where the leaves had a low initial dry weight per square decimetre.

(11) No significant differences were found between treatments in the increments of lamina dry weight per square decimetre. In the 1952 experiments the increments in the dry weight of the lamina were significant, the water treated leaves showing the largest increments in weight. Owing to adverse weather conditions during the first period of the 1951 experiments no significant changes in the lamina dry weights per square decimetre occurred.

(12) Petiole dry weights per square decimetre all increased significantly over the initial dry weights, the differences between successive periods of the experiment reached significance. Significant changes also occurred between treatments, 2,4-D. having the greatest change in petiole weights per square decimetre.

(13) Water content fell with all treatments except in the last experiment where treated leaves showed an increase in the water content.

(14) Satisfactory agreement was found between gasometric measurements and the net assimilation measurements for the first eight days. Gasometric measurements were higher for the whole sixteen days, than the net assimilation measurements. This was probably due to the paucity of gasometric measurements made during this period.

(15) No definite conclusion as to the relative amounts of the various carbohydrates could be drawn from the carbohydrate analyses, owing to the great variability between experiments. In the lamina there was no evidence of a shift in the starch/sugar balance due to hormone treatment.

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