EFFECTS OF GROWTH SUBSTANCES ON PHOTOSYNTHESIS

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(WITH ONE FIGURE)

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Introduction

The use of growth substances upon plants as herbicides and for the purpose of modifying such phenomena as dormancy, abscission, bud differentiation, and fruit set has become quite extensive. Therefore, it should be of interest to know what effect some of these compounds have upon the process of photosynthesis. Very few data have appeared in the literature in regard to this process in relation to growth substances. MITCHELL, KRAUS, and WHITEHEAD (**5**) report that the leaves of bean plants which had been depleted of sugar, starch and dextrin, by being kept in the dark, accumulated less sugar, starch and dextrin during subsequent illumination when they were treated with alpha-naphthalene-acetic acid than similar untreated control plants. The authors suggest that one possible explanation of these data may be a reduction in the rate of photosynthesis.

In the present problem in which apparent photosynthesis was measured it seemed desirable to record the rates of respiration as one basis for interpretation of the results. PRATT (**6**) found that indole-3-acetic acid at 0.1 to 100 p.p.m. increased respiration of wheat seedlings but that higher concentrations of the auxin decreased respiration. BERGER, SMITH and AVERY (**1**) concluded that indole-acetic acid at 10 mg. per liter stimulated respiration in Avena coleptiles. ZIMMERMAN and HITCHCOCK (**8**) reported that naphthalene-acetic acid caused a decrease in the rate of respiration of tomato plants during the first hour after treatment after which the rate was faster in the treated plants than in the controls. HSUEH and LOU (**4**) reported that 2,4-D at a concentration of 0.1 per cent. inhibited aerobic respiration in barley and rice. The amount of oxygen used was greatly decreased as a result of the treatment. The production of CO_2 following treatment with 2,4-D was accelerated at first and then decreased but not as much as oxygen utilization. In terms of CO_2 production BROWN (**2**) found that treatment with 2,4-D at 0.1 per cent. concentration resulted in a higher rate of respiration in bean plants. The results of TAYLOR (**7**) indicate that 2,4-D at concentrations 0.25 to 10 p.p.m. decreased respiration in wheat and mustard seedlings.

Methods

The growth substances used in this research were the acids, indole-3-acetic, gamma (indole)-butyric, beta-naphthoxyacetic, 2,4-dichlorophenoxy-

acetic, and para-chlorophenoxyacetic. Each acid was dissolved in a small amount of ethanol and then dispersed in water at a concentration of 100 p.p.m. The solutions were applied to the test plants in the form of a mist through the use of an atomizer spray.

The plants in the experiments were the common bush beans, *Phaseolus vulgaris* (var. stringless greenpod). The plants were allowed to grow in separate pots in the greenhouse until they had developed two or three compound leaves. By this time they were approximately one foot tall. The plants were selected for uniformity and arranged in pairs. One plant of each pair was sprayed with a growth substance while the others were kept for controls. Spraying was accomplished by placing the plants, one at a time, in a box, open on one side, where they were subjected to a fine mist until they were wet almost to the dripping stage. The plants were allowed to dry in the laboratory before being used. To eliminate, as far as practicable, changes in photosynthetic area during the course of the experiments all terminal and lateral buds as well as immature leaves were removed from all of the plants at the beginning of an experiment. Two matched pieces of plate glass, notched for the stem, were placed across the top of each pot and served as the bottom for a bell jar which was placed over the top of the plant. In this manner the top of each plant was enclosed in a bell jar. Photosynthesis and respiration were each determined for two-hour periods by measuring the changes in the carbon dioxide content of the air which was caused to pass through the bell jars and a series of absorption towers as previously described (**3**). Respiration was always determined immediately after photosynthesis was measured.

Data from more than 20 replicated determinations of the carbon dioxide content of air from a common source, both with and without plants, were analyzed statistically. The measurements in terms of milligrams of carbon dioxide per cubic foot of air had a standard deviation of $\pm .2$. This is equivalent to an error of from 1–2 per cent. calculated on the basis of the carbon dioxide content of the air which often varied from day to day. The apparatus was tested for accuracy regularly throughout the series of experiments. No variation in the carbon dioxide content of air around the test and control plants was considered significant unless it was more than twice the standard deviation.

Natural illumination was used in all measurements of photosynthesis. Often it was necessary to shade the bell jars with two to four layers of cheesecloth to prevent the development of excessively high temperatures around the plants. Temperature measurements with a Leeds and Northrup thermopotentiometer and light determinations with a Weston illumination meter were made several times during the course of each experiment. Light and temperature were uniform for both the control and test plants. During the measurements of respiration the bell jars were covered with several layers of black cloth.

Results

It was found through preliminary experiments that even though two sets of plants were selected for uniformity of size, age and appearance they often exhibited quite different rates of photosynthesis and respiration per unit leaf area. To take care of such variability in the plants in each experiment the relative rates of photosynthesis and respiration were first determined for the plants which were to serve as controls and the test plants which were to be treated later. These results, listed in table I under the heading, "Before treatment," established the relative performance of the two sets of plants before any treatment with growth substances. Then after the test plants had been treated with a particular growth substance the measurements were repeated each day for a period of from two to four days. Any significant variations between the relative rates of photosynthesis and respiration in the test and control plants (T/C table I) before and after treatment of the test plants can be attributed to the treatment.

2,4-D treatment

With one exception the data from all experiments with 2,4-D exhibited the same trend. The results presented in table Ia and figure 1A are the means of two experiments. For photosynthesis the initial measurements established that the test plants, before treatment, had a rate of 0.9 that of the controls (T/C = 0.9). One day after treatment the ratio of the rate in the treated to the rate in the controls had decreased to 0.8. By the second day after treatment the treated plants seemed to have largely recovered, but through the third and fourth days their rate of apparent photosynthesis relative to the controls was 20 per cent. less than before the treatment (figure 1A). The data indicate that the principal effect of this concentration of 2,4-D on apparent photosynthesis is one of retardation.

With respect to respiration, 2,4-D after first causing a drop in the rate of respiration produced an acceleration of this process. The relative rate of respiration between the test and control plants was found to be 0.9 (T/C = 0.9) before treatment of the test plants. After treatment there was an initial decrease (table Ia, T/C = 0.7) in the ratio of respiration in the treated plants to that in the control plants. But through the second and third days after treatment the relative rate of respiration in the treated plants was from 10–20 per cent. (fig. 1B) more than it was before they were sprayed with 2,4-D. These data regarding the effect of 2,4-D on respiration in general confirm the conclusions of BROWN (**2**) and HSUEH and LOU (**4**).

Indole-butyric acid

The results presented in table Ib are the averages of three experiments in which gamma (indole-3)-n-butyric acid was used. The data from all experiments showed the same trend. The effects of this growth substance

upon photosynthesis and respiration in bean plants can be most easily observed by looking at figure 1A, B.

In regard to photosynthesis, the test plants, before treatment with indole-butyric acid, had a rate of apparent photosynthesis 1.1 times that of the plants which were to serve as controls. On two successive days following treatment the rate in the same test plants decreased to a value only 0.9 that of the controls. The experiments were only continued for two days but throughout this period the rate of apparent photosynthesis was depressed by the indole-butyric acid treatment. That this decrease in apparent photosynthesis was not due to a simultaneous acceleration in respiration may be observed from the graph in figure 1B. The data indicate that 24 hours after treatment with indole-butyric acid the rate of respiration remained unchanged with respect to the controls. But by the end of two days this growth substance had caused a decided drop in the rate of respiration in the bean plants.

Beta-naphthoxyacetic acid

The mean results of three experiments in which bean plants were treated with naphthoxyacetic acid are presented in table Ic. A comparison of relative rates of photosynthesis in the test and control plants before and after treatment of the test plants, figure 1A, leads to the conclusion that this growth substance first caused an acceleration in apparent photosynthesis. This increase persisted for three days, reaching a peak during the second day. By the end of four days the relative rate of photosynthesis in the treated and control plants was the same as before treatment.

From an examination of the graph, figure 1B, and the data in table Ic it is obvious that naphthoxyacetic acid also caused an increase in the rate of respiration for a period of two days following its application to the test plants. However, during the third and fourth days following treatment the relative rates of respiration in the test and control plants was the same as before treatment.

Indole-3-acetic acid

Following the treatment of the bean plants with indole-3-acetic acid there was a marked decrease in apparent photosynthesis, table Id and figure 1A, which persisted during the three-day period of the experiment. Before treatment the test plants had a rate of photosynthesis 1.5 times that of the control plants. After treatment of the test plants with this growth substance their rate of photosynthesis dropped until by the third day it was only 0.8 that in the control plants. The decrease in the rate of apparent photosynthesis in the treated plants cannot be attributed entirely to an acceleration of respiration in these plants. The data relative to respiration, table Id and figure 1B, indicate that although indole-3-acetic acid did result in a rise in respiratory rate two days after its application to the plants, by the third day it caused a drop in this process. In other words, by the end of three

days there was a decrease in both photosynthesis and respiration as a result of the treatment.

Para-chlorophenoxyacetic acid

Four experiments were performed with chlorophenoxyacetic acid. In general all of the results showed the same trend. The data presented in table Ie for this growth substance are the averages from two experiments

TABLE I

PHOTOSYNTHESIS AND RESPIRATION IN BEAN PLANTS BEFORE AND AFTER TREATMENT WITH GROWTH SUBSTANCES. C-PLANTS = CONTROL PLANTS WHICH WERE NOT TREATED. T-PLANTS = TEST PLANTS WHICH WERE TREATED AFTER A PRELIMINARY DETERMINATION OF THE RATES OF PHOTOSYNTHESIS AND RESPIRATION

		CO_2 EXCHANGE IN MG./DM.^{2} OF LEAF/HR.				
	TIME IN DAYS	Before treatment	After treatment			
			1	2	3	4
a. 2,4-D						
Photosynthesis:	C-plants	2.2	6.3	2.7	4.1	4.4
	T-plants	1.9	4.9	2.5	2.8	3.0
	Ratio T/C	0.9	0.8	0.9	0.7	0.7
Respiration:	C-plants	1.8	1.8	1.5	1.0	
	T-plants	1.7	1.3	1.6	1.0	
	Ratio T/C	0.9	0.7	1.1	1.0	
b. Indole-butyric acid.						
Photosynthesis:	C-plants	2.7	6.1	5.4		
	T-plants	2.9	5.4	4.8		
	Ratio T/C	1.1	0.9	0.9		
Respiration:	C-plants	1.2	1.0	1.7		
	T-plants	1.2	1.0	1.0		
	Ratio T/C	1.0	1.0	1.6		
c. Naphthoxyacetic acid.						
Photosynthesis:	C-plants	3.9	5.7	6.1	4.5	5.0
	T-plants	3.1	5.1	6.2	3.9	4.2
	Ratio T/C	0.8	0.9	1.0	0.9	0.8
Respiration:	C-plants	2.0	1.3	2.2	1.9	2.1
	T-plants	1.0	0.8	1.4	0.9	1.1
	Ratio T/C	0.5	0.6	0.6	0.5	0.5
d. Indole-acetic acid.						
Photosynthesis:	C-plants	1.6	4.4	4.0	10.0	
	T-plants	2.4	5.0	4.7	8.0	
	Ratio T/C	1.5	1.1	1.2	0.8	
Respiration:	C-plants	3.3	2.0	0.8	1.4	
	T-plants	2.6	1.5	0.8	0.8	
	Ratio T/C	0.8	0.8	1.0	0.6	
e. Chlorophenoxyacetic acid.						
Photosynthesis:	C-plants	3.5	5.4	3.4	5.8	
	T-plants	4.7	4.7	3.3	2.2	
	Ratio T/C	1.3	0.9	1.1	0.4	
Respiration:	C-plants	1.2	1.7	1.0	1.6	
	T-plants	1.3	2.5	1.4	1.5	
	Ratio T/C	1.1	1.5	1.4	0.9	

which were continued for the longest time. The relative effects may be readily observed from figure 1A, B. At the concentration used, chloro-

phenoxyacetic acid resulted in a pronounced decrease in apparent photosynthesis in the bean plants. This effect became more pronounced with time. It seems probable that part of the initial drop in apparent photosynthesis in the treated plants may be due to a simultaneous increase in respiration. For two days after treatment the rate of respiration was accelerated in the treated plants. By the third day this effect had disap-

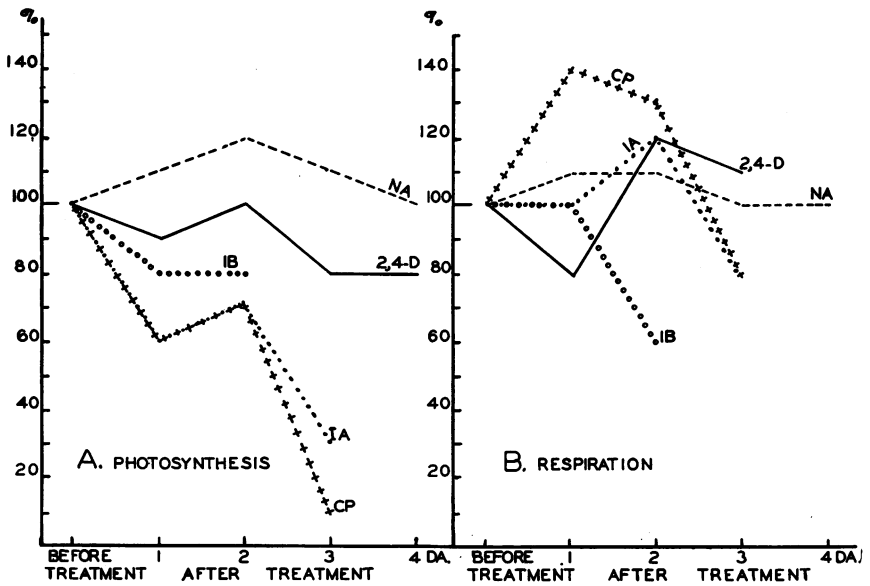

FIG. 1. Relative rates of photosynthesis and respiration in bean plants before and after treatment with growth substances when the rates before treatment are arbitrarily set at 100 per cent. of the rate in the control plants. NH, naphthoxyacetic acid; IB, indole-butyric acid; IA, indole-3-acetic acid; CP, chlorophenoxyacetic acid.

peared and there was even a 20 per cent. decrease in the respiratory rate of the treated plants relative to the controls on the basis of their comparative rates before treatment.

Discussion

In testing the effects of growth-regulating substances on the process of photosynthesis it was impossible because of time and facilities to try a large number of such compounds or many concentrations. At the start of the problem several compounds and concentrations were examined before the ones used in this paper were selected. Largely from the practical point of view it was decided to use a few of the more common growth regulators and to test their effects at a concentration of 100 p.p.m. upon carbon dioxide exchange in plants. This concentration seemed to be reasonably close to what other investigators had often found to be desirable or beneficial with respect to such phenomena as fruit set and rooting.

Of the five growth substances tested only naphthoxyacetic acid produced

an acceleration in the rate of apparent photosynthesis in beans. This beneficial effect, although quite marked for three days, did not persist and was not in evidence on the fourth day following treatment.

Apparent photosynthesis was decreased in the plants when they were sprayed with either 2,4-dichlorophenoxyacetic acid, indole-butyric acid, indole-acetic acid, or chlorophenoxyacetic acid. This effect lasted throughout the course of the experiments which ran from two to four days. The results with indole-acetic and chlorophenoxyacetic acid were much more pronounced than those from either 2,4-D or indole-butyric acid treatment. With respect to 2,4-D this seems strange in view of its well known drastic effects upon certain growth phenomena. A study of the curves in figure 1A, B discloses that the effects produced by these growth substances on apparent photosynthesis cannot be accounted for entirely on the basis of their effects upon respiration. With the exception of indole-butyric acid all of the growth substances sooner or later caused an increase in the rate of respiration. Naphthoxyacetic acid resulted in an increase in both apparent photosynthesis and respiration for two days following its application. The low rate of apparent photosynthesis for the first two days following treatment with chlorophenoxyacetic acid may be due to a corresponding acceleration of respiration but on the third day both processes were depressed. Indole-acetic acid resulted in lowered rate of apparent photosynthesis throughout the experiment but only on the second day could an accelerated rate of respiration have been partly responsible. Indole-butyric acid caused a sharp drop in respiration on the second day following its use while apparent photosynthesis was depressed from the start. On the first day after treating the plants with 2,4-D both respiration and photosynthesis were depressed. Then the rate of respiration was accelerated which may account in part for the low rate of photosynthesis after the second day.

That the growth substances used do effect the process of photosynthesis in bean plants, mostly adversely, there is no doubt. Whether the effects are direct or indirect is an unsolved problem. Preliminary investigation indicates that the effects are not explainable on the basis of the effects of the growth substances upon the opening and closing of stomates.

Summary

At a concentration of 100 p.p.m. the effects of the acids of 2,4-dichlorophenoxyacetic, indole-3-acetic, gamma (indole-3)-n-butyric, beta-naphthoxyacetic and para-chlorophenoxyacetic were determined upon apparent photosynthesis and respiration in bean plants. Each growth substance was applied as a mist spray.

With the exception of naphthoxyacetic acid all of these growth substances caused a decrease in the rate of apparent photosynthesis in beans which continued throughout the duration of the experiments which lasted from two to four days. Naphthoxyacetic acid resulted in an acceleration

of photosynthesis over a period of three days after which the rate relative to that of the controls became the same as before treatment.

In regard to respiration all of the growth substances, except indole-butyric acid, caused a temporary increase in the rate of respiration. With indole-acetic and chlorophenoxyacetic acids the initial accelerated rate of respiration was followed by a drop in the rate. The first effect of 2,4-D was a drop in respiration. Naphthoxyacetic acid caused a slight increase in carbon dioxide production for two days after which there was no acceleration. Following the treatment with indole-butyric acid the rate of respiration decreased after the first day.

The effects of the growth substances used upon the rate of apparent photosynthesis in beans cannot be accounted for entirely upon the basis of their effects upon respiration.

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