ABSCISIC ACID AND OTHER HORMONAL EFFECTS ON GROWTH IN *SPIRODELA*

by

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ABSTRACT

The effects of abscisic acid in particular, as well well as gibberellic acid and the cytokinins, 6-benzyladenine, kinetin, and 6-dimethylallalylaminopurine, on the growth of *Spirodela oligorrhiza* were investigated.

Abscisic acid effectively arrested growth permanently at concentrations down to 10^{-1} mg/l. Normal growth tended to be resumed at concentrations of 10^{-2} and 10^{-3} mg/l between nine and twelve days after treatment. A concentration of 10^{-8} mg/l, however, resulted in a significant increase in dry weight at both eight, nine and twelve days after introduction into the culture medium. It is suggested that the resumption of growth twelve days after treatment at those concentrations which inhibit growth up to nine days, was due to a possible progressive inactivation of abscisic acid resulting in a lowering of its concentration to a level that is promotive.

It was furthermore found that the growth response of *Spirodela* in terms of dry weight production over a period

of eight days is proportional to the $\log_{10}$ concentration of abscisic acid. It is suggested that this curve can be used as a relatively reliable and easily performed bioassay to detect amounts of abscisic acid as low as 10^{-5} µg. The assay is more reliable over the range 0.01 to 10,000 µg and appears not to be affected by gibberellin, benzyladenine and kinetin.

The inhibitory effect of abscisic acid on growth in *Spirodela* was shown to be reversed by benzyladenine, kinetin and dimethylallylaminopurine, although they were not equally effective in doing so. Benzyladenine at 1.0 mg/l was the most effective in overcoming growth inhibition by abscisic acid. Gibberellic acid, however, proved ineffective in reversing the inhibitory effect of abscisic acid on *Spirodela oligorrhiza*. The apparent increases in growth obtained in some cases may have resulted more directly from gibberellic acid stimulation than from the interaction of gibberellic acid with abscisic acid.

INTRODUCTION

It is assumed that plant growth and development are regulated by a number of naturally occurring growth substances. These physiologically well-defined compounds include three major classes of plant hormones, namely, auxins, cytokinins, and gibberellins. Recently, a fourth group of substances, the abscisic acids, was added to this list (OHKUMA, SMITH, LYON and ADDICOTT, 1963 ; CORNFORTH, MILBORROW, RYBACK and WAREING, 1965). The role and action of the auxins, and especially the gibberellins and cytokinins on plant growth and development is becoming better established. As yet, relatively little is known about the mechanism(s) by which abscisic acid regulates plant growth.

Work which has been done on abscisic acid thus far, has shown that it acts as an inhibitor of growth in coleoptiles (BENNET-CLARK and KEFFORD, 1953), potato tubers (WAREING, 1968), cultures of *Lemna minor* (VAN OVERBEEK, LOEFFLER and MASON, 1967), and leaf discs and root sections (EAGLES and WAREING, 1964). It has also been established that abscisic acid is responsible for the dormancy in seeds of *Fraxinus* sp. (SONDHEIMER, TZOU and GALSON, 1968), peach seeds (LIPE and CRANE, 1966), rice seeds (DEY and SIRCAR, 1968) and apple seeds (RUDNICKI, 1969).

Isolated leaf discs of a number of species show an accelerated loss of chlorophyll after treatment with abscisic acid (EL-ANTABLY, WAREING and HILLMAN, 1967; ASPINALL, PALEG and ADDICOTT, 1967). The occurrence of senescent colouration in leaves has been found to be a common response to foliar applications of abscisic acid (SMITH, LYON, ADDICOTT and JOHNSON, 1969). When abscisic acid is applied to intact leaves, abscission is usually promoted, although the effect is variable. In *Citrus*, leaf abscission has been found to depend on the season : leaves sprayed in summer abscised but those sprayed during the winter did not (COOPER, RASMUSSEN, ROGERS, REECE and HENRY, 1968). According to ADDICOTT and LYON (1969) such variations in response are to be expected, since the levels of the hormones with which abscisic acid is presumably interacting must vary greatly with variations in environmental factors.

Contrary to these inhibitory effects, abscisic acid applied to emasculated flower buds of *Rosa sherardii* stimulated parthenocarpic development and prevented abscission of 35 per cent of the treated fruit (JACKSON and BLUNDELL, 1966). Recently CHIN, MEYER and BEEVERS (1969) clearly demonstrated abscisic acid's promotive effect on root formation in stem

cuttings of mung beans and English ivy.

Abscisic acid has been shown to counteract or inhibit gibberellin-induced responses. For example, CHRISPEELS and VARNER (1966, 1967), showed that abscisic acid inhibited gibberellin-induced synthesis of hydrolases in the aleurone layer. In *Lemna* VAN OVERBEEK *et al*, (1967), showed that gibberellic acid could not overcome the growth inhibition induced by abscisic acid.

When applied with indoleacetic acid, abscisic acid in a number of cases counteracted indoleacetate's promotion of growth. Abscisic acid also partially overcame the indoleacetic acid-induced retardation of abscission (SMITH *et al*, 1969). The abscisic acid inhibition of growth of *Lemna* (VAN OVERBEEK *et al*, 1967) could not be reversed by indoleacetic acid, but inhibition of cell division in tissue cultures by abscisic acid was partially reversed by this auxin (THOMAS, WAREING and ROBINSON, 1965).

In cultures of *Lemna*, growth could be inhibited or promoted at will, simply by interchanging culture media containing either abscisic acid or benzyladenine (VAN OVERBEEK *et al*, 1967). Whereas in barley seedlings the inhibition of coleoptile growth by abscisic acid could be overcome by kinetin and benzyl-

adenine (KHAN, 1969), the inhibition of mesocotyl growth by abscisic acid, on the other hand, could not be reversed by kinetin (MILBORROW, 1966).

It is apparent, therefore, that the interactions between abscisic acid and other plant hormones are complex and require considerable further elucidation.

A possible explanation for the action of abscisic acid on growth, is that it inhibits or accelerates some of the processes which are controlled either by the auxins, cytokinins, or gibberellins by acting as a braking mechanism, possibly by controlling protein synthesis or counteracting gibberellin-, auxin-, or cytokinin-induced responses.

In view of its apparently central role in plant growth a project was planned by which some of the effects of abscisic acid singly as well as in combination with other growth substances, could be investigated. This study was an attempt to provide a better understanding of the regulatory effects of abscisic acid on some aspects of plant growth and also to account for possible interactions between abscisic acid and gibberellin or cytokinin.

EXPERIMENTAL PROCEDURE

1.1 Plant material.

One of the problems that arose in the present investigation was the choice of suitable plant material. VAN OVERBEEK *et al*, (1967) found that bacteria, fungi and algae did not respond to treatment with abscisic acid. Only higher plants responded to treatment and, of those studied, cultures of *Lemna minor* were found to be extremely sensitive. These latter plants have an additional advantage in that they multiply vegetatively very rapidly by budding.

Lemna minor was not available and so *Spirodela oligorrhiza* (Kurz.) Hegelm., which is also a member of the Lemnaceae, was used in this investigation. *Spirodela* which has two or three roots per frond is a smaller plant than *Lemna* (Fig. 1). *Lemna* has only one root per frond.

1.2 Sterilization of *Spirodela*.

Attempts to rid the *Spirodela* plants of algae and particularly of the saprophytic fungus, *Saprolegnia*, using the technique of SMITH and CASTLE (1960) proved unsuccessful. Only after harsh treatment using a method modified from that of GORHAM (1945) were sterile fronds obtained. The plants were first submerged in 0.1 per cent $HgCl_2$ for four minutes.

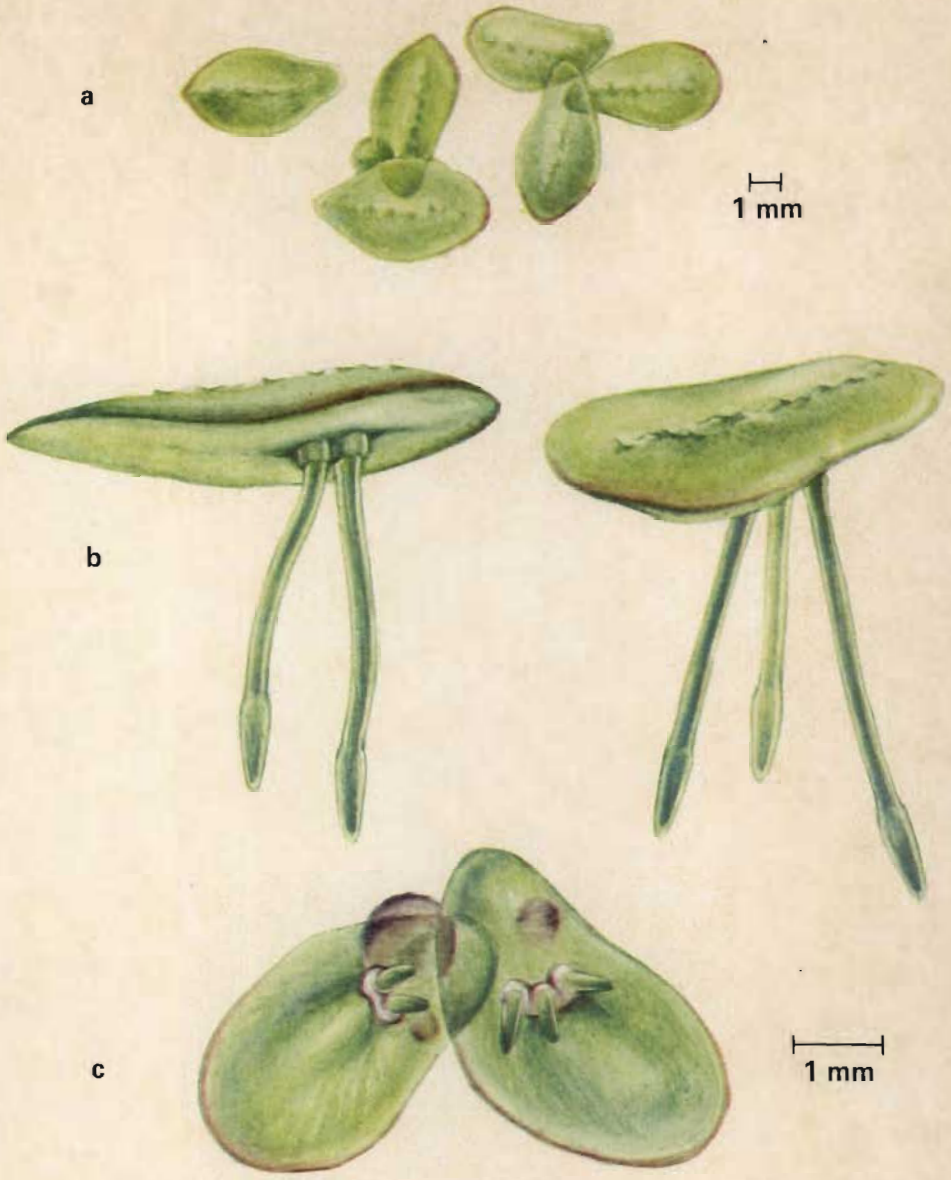


FIG. 1. *Spirodela oligorrhiza* (Kurz.) Hegelm.

a. floating thalli (fronds)

b. thalli in profile showing roots

c. under-surface showing young roots and young thalli

This was followed by a brief rinsing in sterile water and submergence of the plants in 50 per cent ethanol for 30 seconds. The plants were then rinsed twice in sterile nutrient solution before being transferred to a 500 ml erlenmeyer flask containing sterile nutrient medium. Only 25 per cent of the plants survived these treatments and these plants were eventually sub-cultured and used in the different experiments.

1.3 *In vitro* culture of *Spirodela*.

Plants of *Spirodela* were grown in 50 ml erlenmeyer flasks, each containing 30 ml full strength nutrient solution. Except for replacing $FeSO_4$ with NaFe-EDTA the nutrient solution was the same as that used by **HOAGLAND** and **ARNON (1950)**. The composition of the macro- and micro-element fractions of the nutrient solution are given in Tables 1 and 2.

Table 1. Composition of nutrient solution for the *in vitro* culture of *Spirodela*

Stock solution molar	Amount stock solution in nutrient solution ml/l
KNO_3	5
$Ca(NO_3)_2$	5
$MgSO_4$	2
KH_2PO_4	1
NaFe-EDTA (0.4%)	4
Micro-element solution	2

Table 2. Composition of micro-element solution

Salt	Element supplied	Concentration of salt g/l
H_3BO_3	B	2.86
$MnSO_4$	Mn	1.54
$CuSO_4.5H_2O$	Cu	0.08
$ZnSO_4.7H_2O$	Zn	0.22
$H_2MoO_4.H_2O$	Mo	0.02

All the stock solutions, except NaFe-EDTA were prepared by dissolving the salts in the required volume of deionised water. The NaFe-EDTA solution was prepared as outlined by **VAN AS (1959)**. Before the addition of the appropriate growth substance(s) the pH of the nutrient solution was adjusted to 4.6. The different growth substances used in these investigations were made up with the nutrient solution.

Each flask contained either three or six uniform fronds depending on the nature of the experiment conducted, and was aerated twice daily for 45 minutes. The air was sterilized by passing it through a Swinnex filter-unit fitted with a GS 0.22 µ filter disc (Fig. 2). The air flow was regulated as far as was possible by first passing the air through a container with side-arms, which served as a

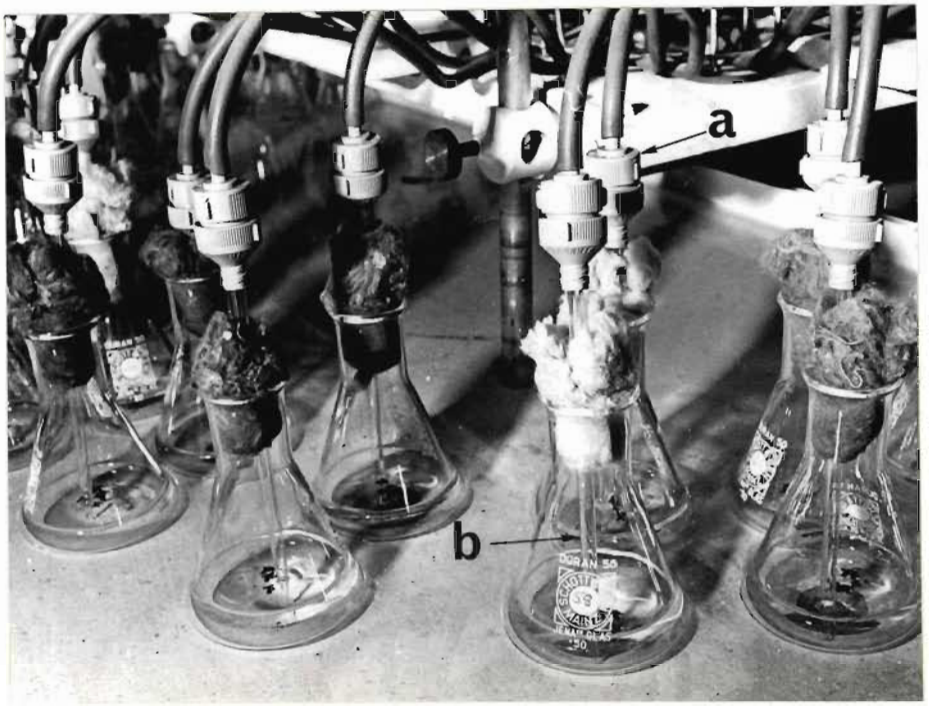


FIG. 2. Apparatus used for culturing *Spirodela*. a, Swinnex filter-unit fitted with GS 0.22μ filter disc; b, capillary tube.

pressure equaliser (Fig. 3) and then by passing it through a glass tube drawn to a capillary.

In order to obtain the appropriate concentrations of growth substances, these were dissolved directly in the nutrient solution. Subsequent dilutions of the stock solution were also carried out with nutrient solution.

Gibberellic acid (GA) solutions were prepared by dissolving its potassium salt in a few drops of ethanol before making up to volume with nutrient solution. Solutions of the cytokinins, namely, kinetin (6-furfurylaminopurine), benzyladenine (BA) (6-benzylaminopurine), and 6-dimethylallalylaminopurine (DMAAP), were prepared by adding the respective substances to ca. 200 ml nutrient solution and autoclaving at 1.057 kg/cm^2 for 15 minutes. After cooling, the cytokinin solutions were made up to volume with nutrient solution. (RS)-Abscisic acid (ABA) was dissolved in a few drops of chloroform followed by a few drops of ethyl acetate and sufficient boiling nutrient solution to bring it almost up to volume. After cooling the solution was made up to volume with nutrient solution.

The culture media (nutrient solution plus the appropriate dilution of growth substances) were sterilized by autoclaving the flasks together with the Swinnex filter-units at 1.057 kg/cm^2 pressure for 15 minutes.

FIG. 3. Half of apparatus used for culturing *Spirodela*. a, air inlet; b, pressure equaliser; c, side-arms.

Plants were transferred from their sterile cultures to the experimental flasks in a sterile transfer chamber. The transfer chamber was sterilized before each batch of transfers by wiping the working surface and spraying the atmosphere with 1 per cent thymol in 95 per cent ethanol and then radiating the chamber with a UV germicidal lamp for 30 minutes.

Cultures were maintained at a temperature of $22 \pm 4^{\circ}C$ under a 16-hour photoperiod of 3.0×10^{3} lm/m^{2}. The light was supplied by "cool white" 40W fluorescent tubes. Each experiment was repeated at least twice with six replicates in each treatment. Growth was measured as the increase in dry weight over a period of time which varied in the different experiments from eight to nine to twelve days. The dry weight was determined by oven drying the material at $105^{\circ}C$ and then weighing it on a semimicro balance to five decimal places.

2. RESULTS AND DISCUSSION

2.1 The influence of abscisic acid on growth in *Spirodela*.

Recent investigations on the growth substance abscisic acid point clearly to its role as a major inhibitor of plant growth. VAN OVERBEEK *et al*, (1967) reported that *Lemna minor* is extremely sensitive to ABA. An ABA concentration of 1.0 mg/l was sufficient to keep it indefinitely in a state of dormancy, and could inhibit 95 per cent of the growth in this species. Apparently this reduction in growth is a result of the inhibiting effect of ABA on nucleic acid metabolism.

It was thought that it would be highly instructive to determine (1) whether *Spirodela oligorrhiza* is equally sensitive to ABA, (2) over what concentration range ABA effects the growth of this species - especially whether lower concentrations than those used by VAN OVERBEEK *et al*, (1967) had any effect on growth, and (3) to observe the effect of different culturing periods on the growth of this plant.

In order to determine the effect of ABA on *Spirodela*, six uniform, sterile plants were grown as described in the experimental procedure. The concentrations of ABA investigated

were 10.0, 1.0, and 10^{-1} through 10^{-9} mg/l, respectively.

Figure 4 shows that growth in *Spirodela* measured over nine- and twelve-day periods was significantly inhibited by the application of 10.0, 1.0, and 10^{-1} mg/l ABA. The control treatment gave an approximately five-fold increase in dry weight during the same period. These results are very similar to those of **VAN OVERBEEK** *et al*, (1967) using *Lemna*, and shows that *Spirodela* is equally as sensitive to treatment with ABA. However, after twelve days the cultures treated with 10^{-2} and 10^{-3} mg/l ABA resumed normal growth. **VAN OVERBEEK** *et al*, (1967) reported a resumption of normal growth after nine days of culturing at concentrations of 10^{-1} mg/l or lower of ABA. Figure 4 furthermore shows a significant stimulation of growth in *Spirodela* cultured in the presence of 10^{-8} mg/l ABA after both nine and twelve days of growth.

These results seem to suggest that the function of abscisic acid in plant growth should not be categorically regarded as solely that of inhibition, although its major action probably is analogous to that of a growth retardant. Contrary to its usual inhibiting effects, ABA does appear to have promotive effects, such as in the acceleration of abscision **(BORNMAN, SPURR and ADDICOTT, 1967)** where the synthesis of certain degradative enzymes of the pectic constituents

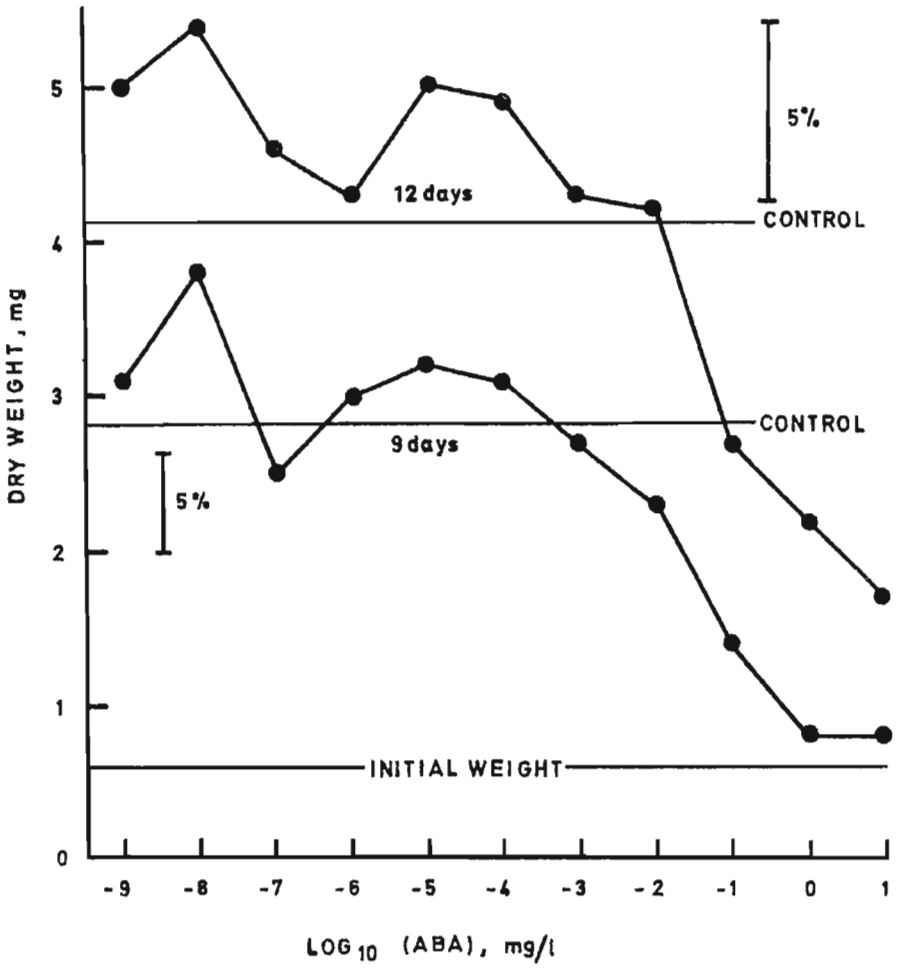


FIG. 4. Effect of abscisic acid (ABA) on growth of Spirodela after 9 and 12 days of culturing.

of the cell wall is probably stimulated by ABA. Flowering in certain short-day species, e.g. *Ribes nigrum* is also promoted by ABA (WAREING, 1968).

It is suggested that the resumption of apparently normal growth twelve days after treatment with concentrations of the order of 10^{-2} and 10^{-3} mg/l could be due to the gradual inactivation of ABA down to those concentrations low enough to allow growth to take place. The results of the work of RUDNICKI (1969) on apple seeds would support this supposition since it was found that during stratification of the seeds the concentration of ABA gradually decreases with a consequent improvement in germination.

2.2. A possible bioassay for abscisic acid.

In order to study effectively the role of ABA on the regulation of plant growth it is essential that reliable and easily performable bioassays are available for the determination of amounts of this growth substance in plant material. At the commencement of this research a number of methods were available for determining the amounts of ABA in plant tissue. OHKUMA *et al*, (1963) reported on the use of the standard cotton explant bioassay to test the phenomenal abscission-accelerating properties of ABA. However, this bioassay, which employs the

cotyledonary petioles of 14-day old cotton seedlings, also responds to ethylene and gibberellic acid. MILBORROW (1967) described a bioassay in which the effect of ABA on coleoptile length in dissected wheat embryos was measured. He also developed a sophisticated racemate dilution method which, unfortunately, requires rather specialized equipment.

While the present investigation was underway, DAVIS, HEINZ and ADDICOTT (1968) described a chemical assay using a gas-liquid chromatographic method to measure the trimethylsilyl derivatives of ABA.

From the results such as those presented in Figure 4 it was clear that over a certain concentration range (10.0 - 10^{-5} mg/l ABA) there was a linear relationship between the $\log_{10}$ concentration of ABA and the dry weight of *Spirodela*. It was then argued that if the culturing technique could be improved upon and if the effects of other hormones such as the gibberellins and cytokinins could be eliminated, the possibility did exist that *Spirodela* might be effectively used in a bioassay for ABA.

In the following experiments an attempt was made to demonstrate more clearly the relationship between the concentration of ABA and the dry weight of *Spirodela*. This was done first by decreasing the length of the culturing period

as preliminary experiments had shown clearly that longer periods of time (e.g. up to 21 days) gave rise to excessive variability. Secondly, the number of plants per flask was reduced from six to three. This reduction in plant number was regarded as essential in order to decrease the time taken to transfer the plants into the flasks. Even with the use of fewer plants, separation and transfer took between eight and ten hours. The time factor is of importance as observations showed that new bud formation took approximately 24 hours. RIMON and GALUN (1968) also showed that at the time of protrusion of a new bud from the reproductive pocket of its mother frond, this bud is already a carrier of at least two later generations.

Figure 5 shows that the growth response obtained in the case of three plants per flask was essentially the same when compared with six plants per flask. This appeared to be the case irrespective of the culturing period (Fig. 6). Figure 6 also shows that the growth response of *Spirodela* in terms of dry weight production is proportional to the $\log_{10}$ concentration of ABA. A linear regression of data from an eight-day growth period gave a better fit over the range of concentrations of ABA from 10^{-5} to 10.0 mg/l than over the whole range

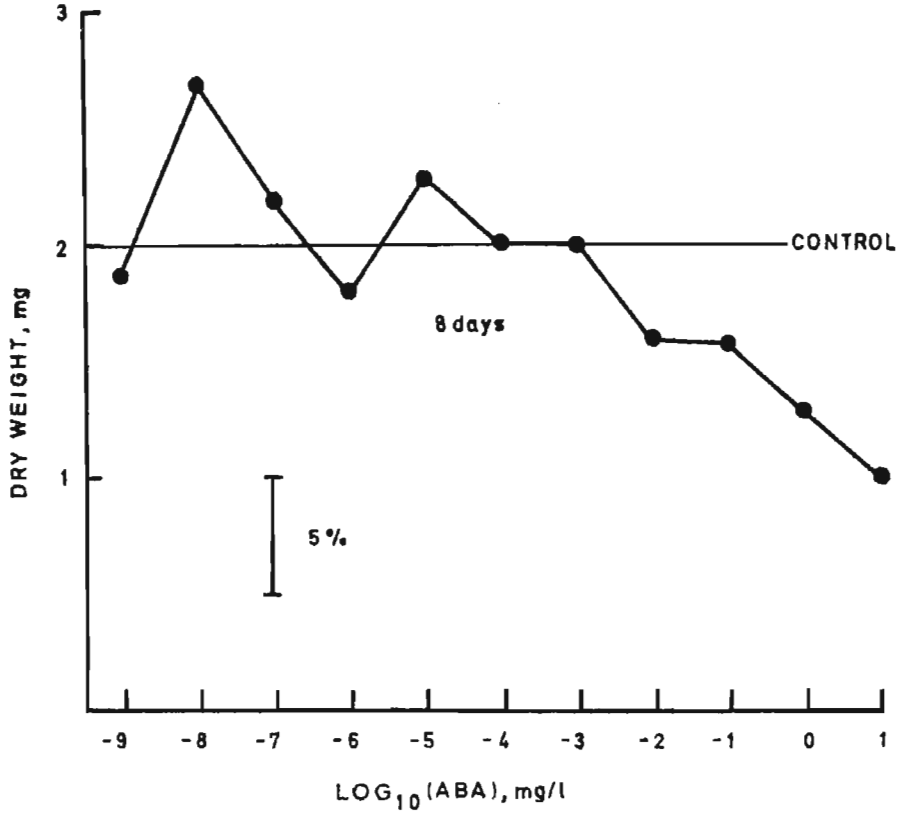


FIG. 5. Effect of abscisic acid (ABA) on growth of Spirodela after 8 days of culturing.

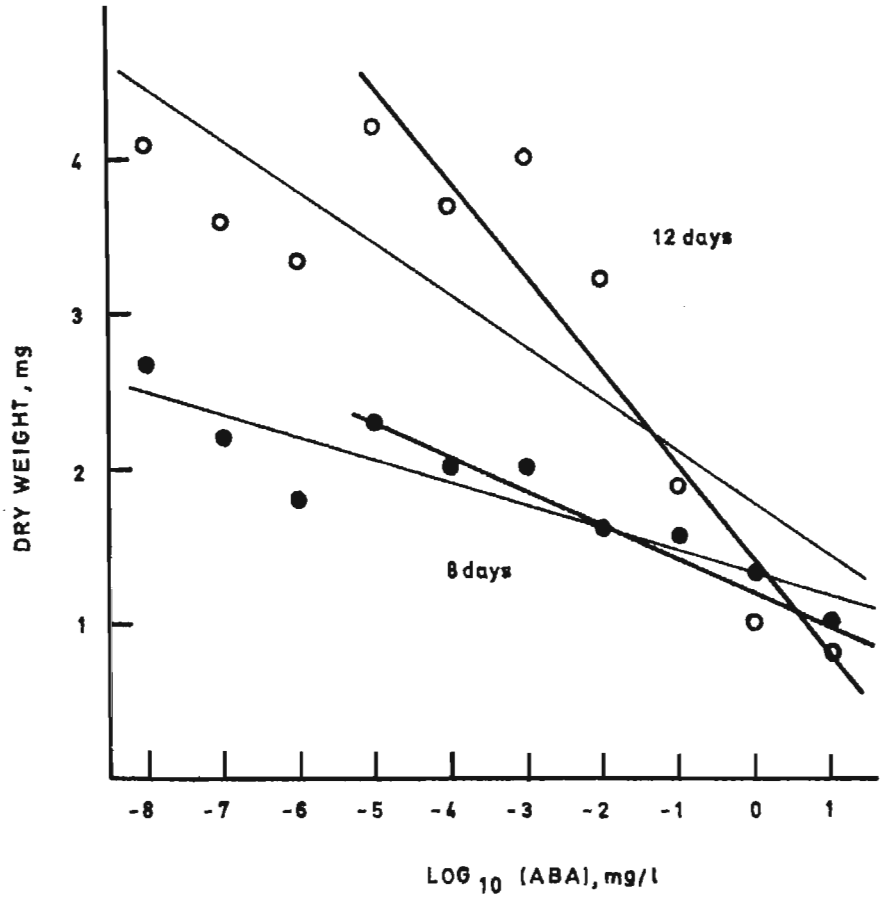


FIG. 6. Regression lines showing the relationship between the dry weight of Spirodela and the concentration of abscisic acid (ABA).

under consideration, namely, 10^{-8} to 10.0 mg/l. The same applied to the results obtained for a twelve-day growth period. It should be noted that the apparent break between 10^{-6} and 10^{-5} mg/l of ABA was obtained repeatedly. (Fig. 4). The usable range of this assay is therefore from 0.01 to 10,000 µg, which is reasonable if it is taken into account that in the test of **DAVIS *et al*, (1968)** the lowest concentration of ABA detectable was 0.025 µg.

The results of GA, BA and kinetin over the same range of concentrations are rather variable (Fig. 7). It cannot be categorically stated at this stage that their combined presence in an extract would not seriously affect the results of a bioassay on ABA. For the present bioassay it would be necessary to use extracts which have been purified as much as possible, for example by employing **RUDNICKI'S (1969)** extraction techniques. **DAVIS *et al*, (1968)**, however, were able to use hormones extracted with a minimum of preliminary purification. It must be pointed out that the present bioassay cannot be used for identifying ABA. Its identity has to be established by other methods, such as those of **MILBORROW (1967) and RUDNICKI (1969).**

The slope of the regression line for the relationship between the concentration of ABA (10^{-5} - 10.0 mg/l) and

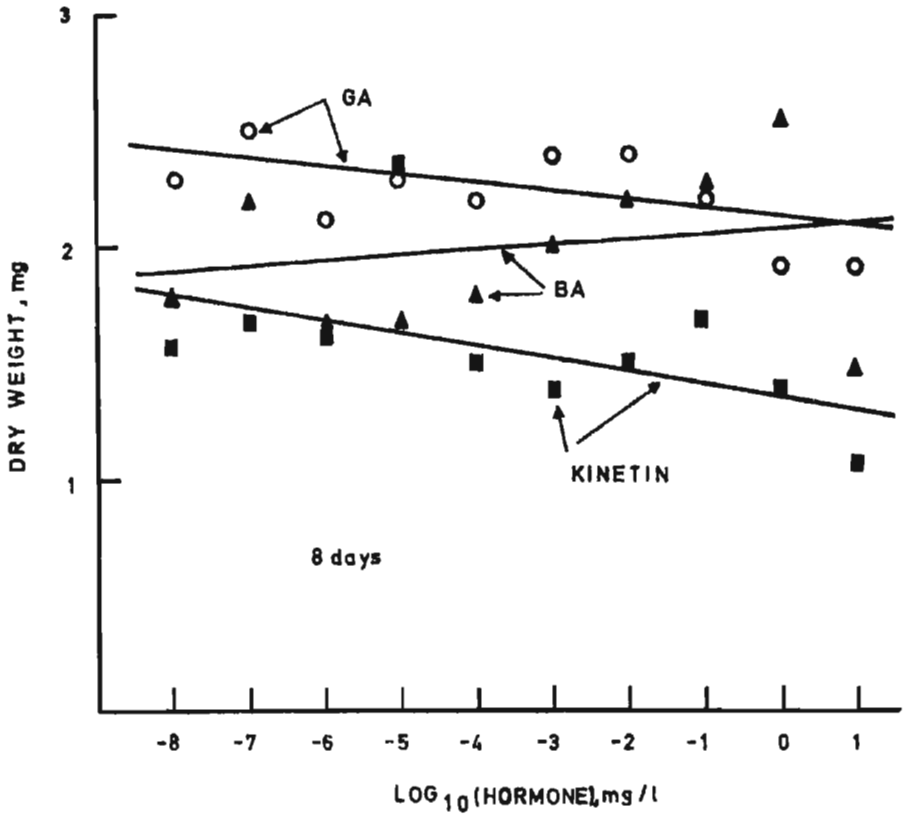


FIG. 7. Regression lines showing the relationship between the dry weight of Spirodela and the concentration of kinetin, benzyladenine (BA) and gibberellic acid (GA).

the dry weight of *Spirodela* over a twelve-day growth period, is greater than that for the eight-day growth period. This indicates a potentially more sensitive detection of ABA in a bioassay taken over a twelve-day period than over an eight-day period. The goodness of fit of the eight-day results, however, was better than those for the twelve-day, indicating that an eight-day period gives more reliable results.

It is concluded that the growth response of *Spirodela oligorrhiza* in terms of dry weight production over a period of eight days is proportional to the $\log_{10}$ concentration of ABA and can be used as a sensitive, reliable and easily performed bioassay to detect amounts as low as 10^{-5} µg. The assay is particularly reliable over the range 0.01 to 10,000 µg.

2.3 Cytokinin and gibberellin effects on abscisic acid induced inhibition of growth in *Spirodela*.

A number of investigations have shown that the inhibitory effects of abscisic acid on protein synthesis, germination and growth can be overcome by the cytokinins (VAN OVERBEEK *et al*, 1967; KHAN, 1968; KHAN and DOWNING, 1968; SANKHLA and SANKHLA, 1968; KHAN, 1969). When applied together with abscisic acid, benzyladenine and other purine derivatives have been found to reverse to a large extent the

inhibitory effect of abscisin on the growth of *Lemna minor* (VAN OVERBEEK *et al*, 1967; VAN OVERBEEK, 1968). However, this antagonism was not shown by gibberellic acid which appeared to have no promotive effect on the growth of *Lemna* in either the presence or absence of abscisic acid. KHAN (1968) showed that the gibberellin-induced germination of Grand Rapids lettuce seed in the dark was inhibited by abscisic acid. This inhibition was reversed by kinetin and benzyladenine, but not by an excess of gibberellin. Likewise, gibberellin failed to counteract the inhibition by abscisin of α-amylase synthesis and coleoptile growth in intact barley seeds, although in combination with kinetin it caused a nearly complete reversal of α-amylase synthesis. This combination of kinetin and gibberellin, however, was unable to bring about the complete reversal of the abscisic acid-inhibited coleoptile growth. Kinetin possibly acts by removing the abscisic acid inhibition of enzyme specific sites thereby allowing gibberellic acid to function to produce α-amylase (KHAN and DOWNING, 1968).

CHRISPEELS and VARNER (1967), on the other hand, have reported that gibberellin-induced α-amylase synthesis in excised barley aleurone is inhibited by abscisic acid but

partially reversed by excess gibberellin. VILLIERS' (1968) microautoradiographic study of the effects of abscisic acid and gibberellin on dormancy in *Fraxinus* lends support to the suggestion by THOMAS *et al*, (1965) and ASPINALL *et al*, (1968) that abscisic acid acts antagonistically towards gibberellin.

In view of the antagonisms which have been established and the apparent anomalies in connection with the actions and interactions of abscisic acid, gibberellin and the cytokinins, it was decided to examine some of the effects of these hormones, applied alone and in combination with one another, on the growth of *Spirodela*.

Six sterile two-frond plants of *Spirodela* were grown as described earlier. Each treatment consisted of six replications. Growth was measured as the increase in dry weight 12 days following the introduction of the different hormones into the culture medium.

The effects on the growth of *Spirodela* of ABA, GA, BA, kinetin and indoleacetic acid (IAA) applied singly at different concentrations are shown in Figure 8. Abscisic acid strongly inhibited growth at concentrations of 0.1 to 10.0 mg/l and as previously reported it accelerated growth at a concentration of 10^{-8} mg/l (VAN STADEN and BORNMAN, 1969).

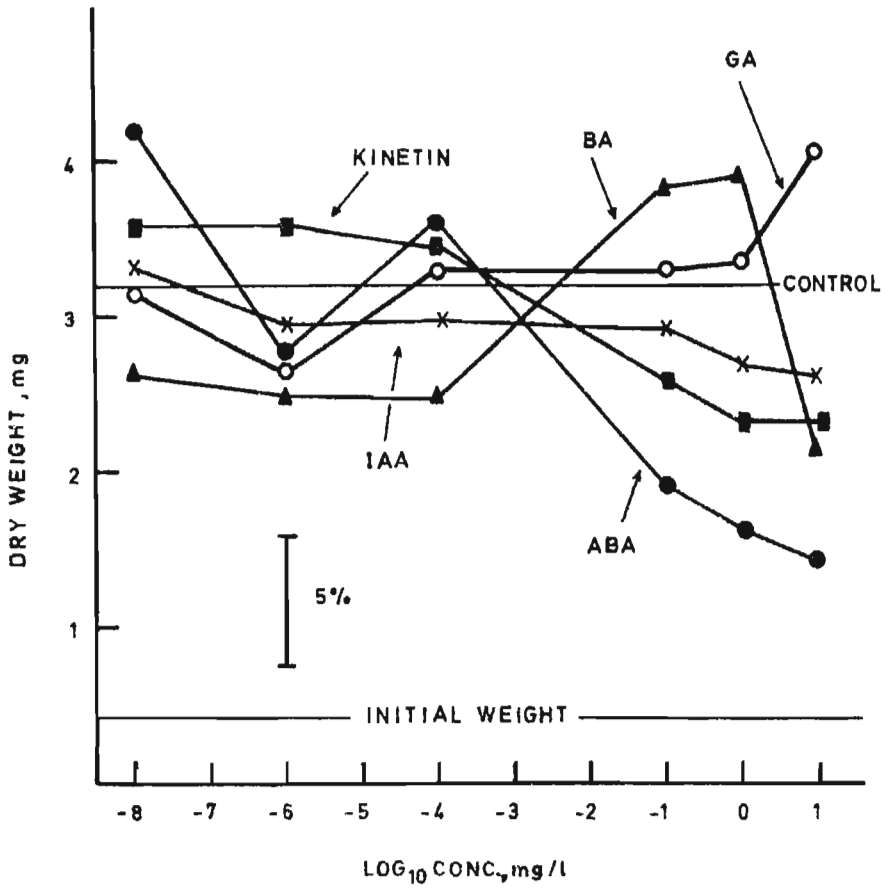


FIG. 8. Effect of increasing concentrations of abscisic acid(ABA), gibberellic acid (GA), kinetin, indoleacetic acid(IAA), and benzyladenine(BA) on the growth of Spirodela. Each point on the curves represents the mean of six replications.

At a level of 10.0 mg/l, kinetin, BA and IAA reduced growth while GA resulted in an increase in dry weight. VAN OVERBEEK *et al*, (1967), however, reported that GA had no such effect on the growth of *Lemna minor*. Concentrations of 0.1 mg/l and 1.0 mg/l of kinetin also resulted in decreases in the dry weight of *Spirodela* whereas at the same levels BA accelerated growth by about 22 per cent over that of the control. These results obtained for IAA and BA are in agreement with those reported by VAN OVERBEEK *et al*, (1967) for *Lemna*. It must, however, be pointed out that in those cases where relatively high concentrations (1.0 and 10.0 mg/l) of IAA were applied the auxin was apparently photo-inactivated.. This severely affected the growth of the plants. It was therefore decided not to use IAA in further experiments. Concentrations of the hormones below 0.1 mg/l generally appeared to have very little effect on growth.

As shown in Figure 8 abscisic acid greatly inhibited growth at levels of 10.0, 1.0 and 0.1 mg/l. The comparative effects of some cytokinins and GA, on ABA-inhibition of growth at different levels were investigated and the results are shown in Figure 9.

Figure 9A shows that the inhibitory effect of 0.1 mg/l ABA was significantly reversed by 1.0 mg/l kinetin but not by

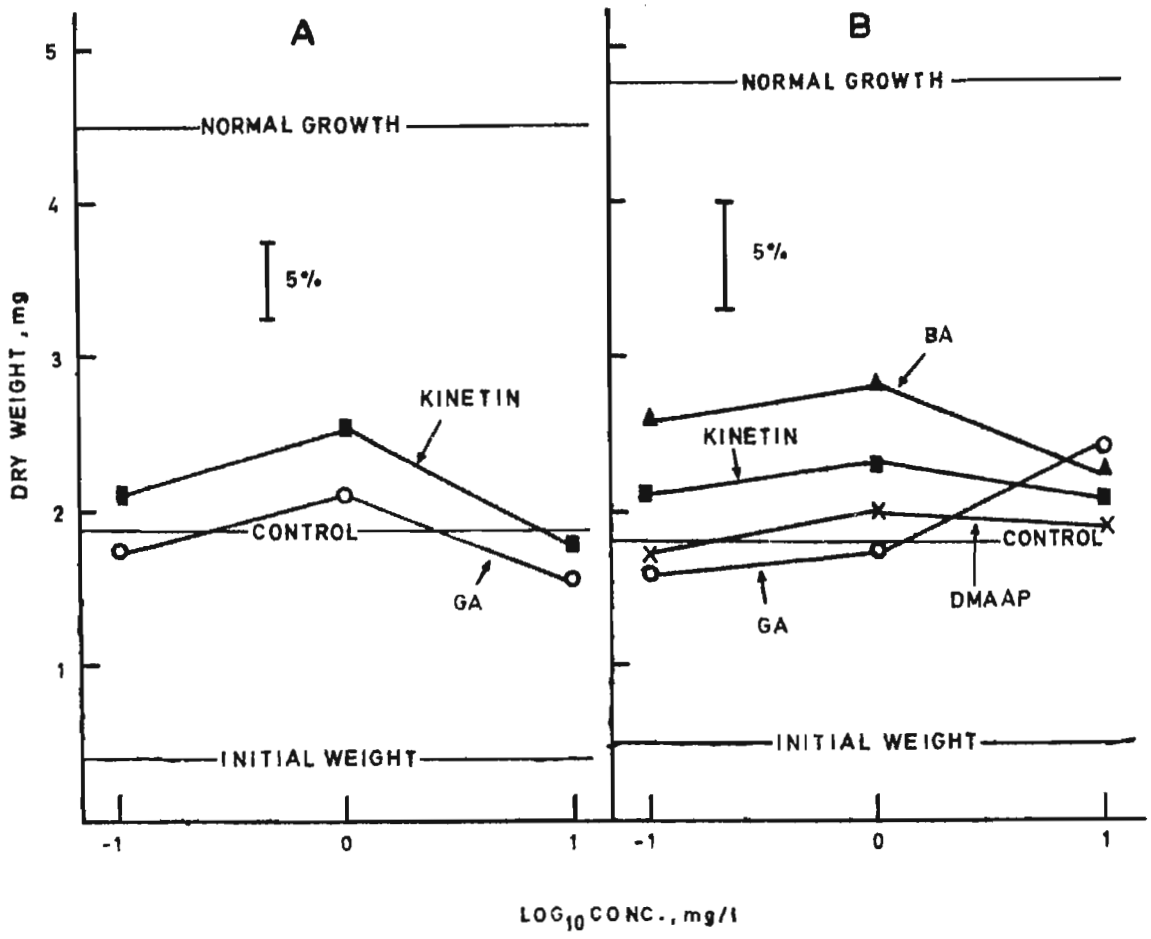


FIG. 9. A. Effects of three concentrations of gibberellic acid (GA) and kinetin on the dry weight of Spirodela in the presence of 0·1 mg/l abscisic acid (ABA).

B. Effects of three concentrations of gibberellic acid (GA) and cytokinins (benzyladenine, kinetin, dimethylallalylamino purine) on the growth of Spirodela in the presence of 1 mg/l abscisic acid (ABA).

10.0 mg/l. This decrease in the response to kinetin can probably be attributed to the fact that kinetin at 10.0 mg/l inhibits the growth of *Spirodela* (Fig. 8). Where ABA was supplied at the rate of 1.0 mg/l its inhibitory effect on growth was reversed only by BA at 0.1 and 1.0 mg/l (Fig. 9B).

Otherwise, the compounds tested had no significant effect on the growth inhibition by ABA. Another interesting observation, also reported by **KHAN and DOWNING (1968)**, is that with an increase in ABA concentrations kinetin becomes less effective in reversing the growth inhibitory properties of this growth regulator.

Of the three cytokinins investigated, BA was the most effective while DMAAP was the least effective. A very interesting observation is the striking similarity shown by the curves for kinetin, DMAAP and BA (Fig. 9B). These curves agree closely with those published by **KHAN (1969)** for the reversal of ABA-inhibited growth by kinetin and BA in barley seed. **KHAN (1969)** postulated that the slight increase in coleoptile growth of barley by GA plus ABA over ABA alone may reflect an overall stimulation of growth by GA and is probably not a true reversal of ABA inhibition by GA. The present results may perhaps be interpreted similarly (compare Figures 8 and 9B).

Because no decisive result was obtained as to whether GA could overcome the inhibitory effect on growth by ABA, it was decided to investigate the combined effects of ABA, BA, and GA on the growth of *Spirodela*. This was done in order to determine whether the inhibitory effect of ABA could be overcome by GA if applied in combination with the cytokinins.

If *Spirodela* was grown in the presence of 1.0 mg/l each of ABA and BA an increase in the concentration of GA to 1.0 mg/l, significantly increased the dry weight production of the plants over that of the control (Fig. 10A). KHAN and DOWNING (1968) reported that a combination of GA and kinetin did not bring about the reversal of ABA inhibition of coleoptile growth to any greater extent than that achieved by the two hormones separately. The present findings seem to indicate that although GA is unable to overcome the inhibitory effect of ABA on its own it may do so in combination with the cytokinins. It is, however, also possible that this increase in dry weight was the result of increased growth and is not a reflection of an interaction between ABA and GA. SANKHLA and SANKHLA (1968) reported that although GA was completely ineffective in reversing the inhibition of germination of lettuce seeds caused by ABA it exerted a stimulating effect

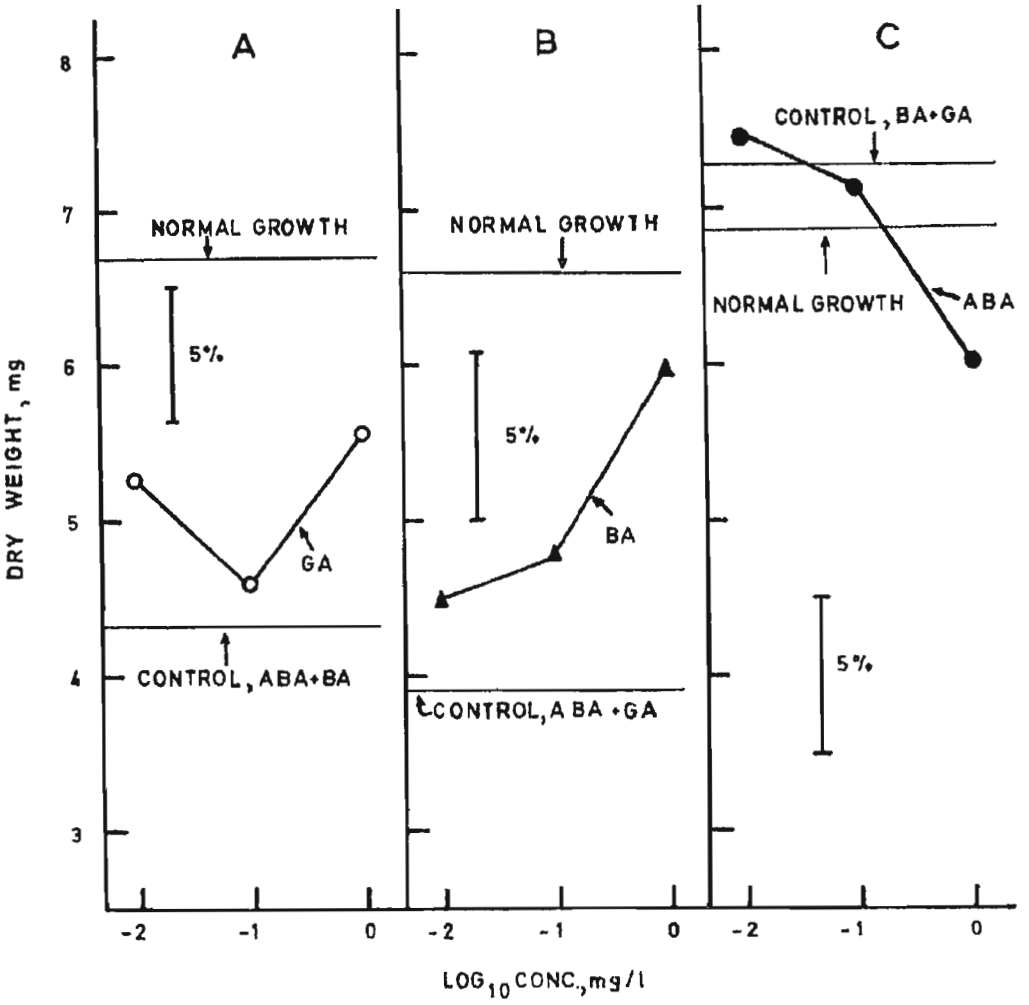


FIG. 10. The effect of increasing concentrations of: A, gibberellic acid (GA) in the presence of 1 mg/l each of abscisic acid (ABA) and benzyladenine (BA); B, benzyladenine in the presence of 1 mg/l each of abscisic acid and gibberellic acid; and C, abscisic acid in the presence of 1 mg/l each of benzyladenine and gibberellic acid; on the dry weight of Spirodela after 12 days, growth. Each point on the curves represents the mean of six replications.

on seedling growth as a result of which the seedlings exhibited an increased hypocotyl elongation.

Where *Spirodela* was grown in the presence of 1.0 mg/l each of ABA and GA an increase in the concentration of BA progressively increased growth (Fig. 10B). Benzyladenine and GA (1.0 mg/l of each) stimulated growth to above normal levels. Abscisic acid at concentrations of 10^{-2} and 10^{-1} mg/l did not counteract the combined effects of BA and GA. However, it was effective at 1.0 mg/l (Fig. 10C).

2.4 Interaction between abscisic acid, gibberellic acid and benzyladenine on growth in *Spirodela*.

As a consequence of the results of the preceeding experiments it was felt that it would be of value to investigate the possible interactions between ABA, GA, and BA on the growth of *Spirodela*. This was done firstly because a much wider range of hormone concentrations could be investigated and secondly because the experiments could be designed so as to indicate at which concentrations the interactions (if any) between these take place.

For the purpose of these experiments six sterile two-frond plants were grown for a period of 12 days.

To test the effects in combination of ABA and benzyl-

adenine on the growth of *Spirodela*, a 3 x 6 factorial experiment with three replications was designed. Table 3 shows that the inhibitory effect of ABA is most pronounced at a concentration of 1.0 mg/l, although there is a certain amount of inhibition at lower concentrations (Fig. 11B). It had previously been reported by VAN OVERBEEK *et al*, (1967) and KURAISHI, KASAMO, TEZUKA, USHIJIMA and TAZAKI, (1968) that benzyladenine

TABLE 3. The effect of various concentrations of abscisic acid, and benzyladenine on the dry weight (mg) of *Spirodela*.

Benzyladenine concentration (mg/l)	Abscisic acid concentration (mg/l)					
	0	10^{-8}	10^{-6}	10^{-4}	10^{-2}	1
1.0	5.9	5.3	6.4	5.3	5.0	1.3
10^{-2}	6.3	5.6	4.4	5.0	4.6	1.0
10^{-4}	4.5	4.9	5.0	4.8	3.9	1.2

L.S.D. (P = 0.05) = 0.89 L.S.D. (P = 0.01) = 1.19

accelerated growth of *Lemna* and *Raphanus sativus* respectively. The present data indicates that it is most effective at 1.0 mg/l and least effective at a concentration of 10^{-4} mg/l (Fig. 11A and 13A). These results seem to be in agreement with those obtained earlier (Figures 8 and 10B), indicating that the inhibiting effect of ABA on growth is most effectively

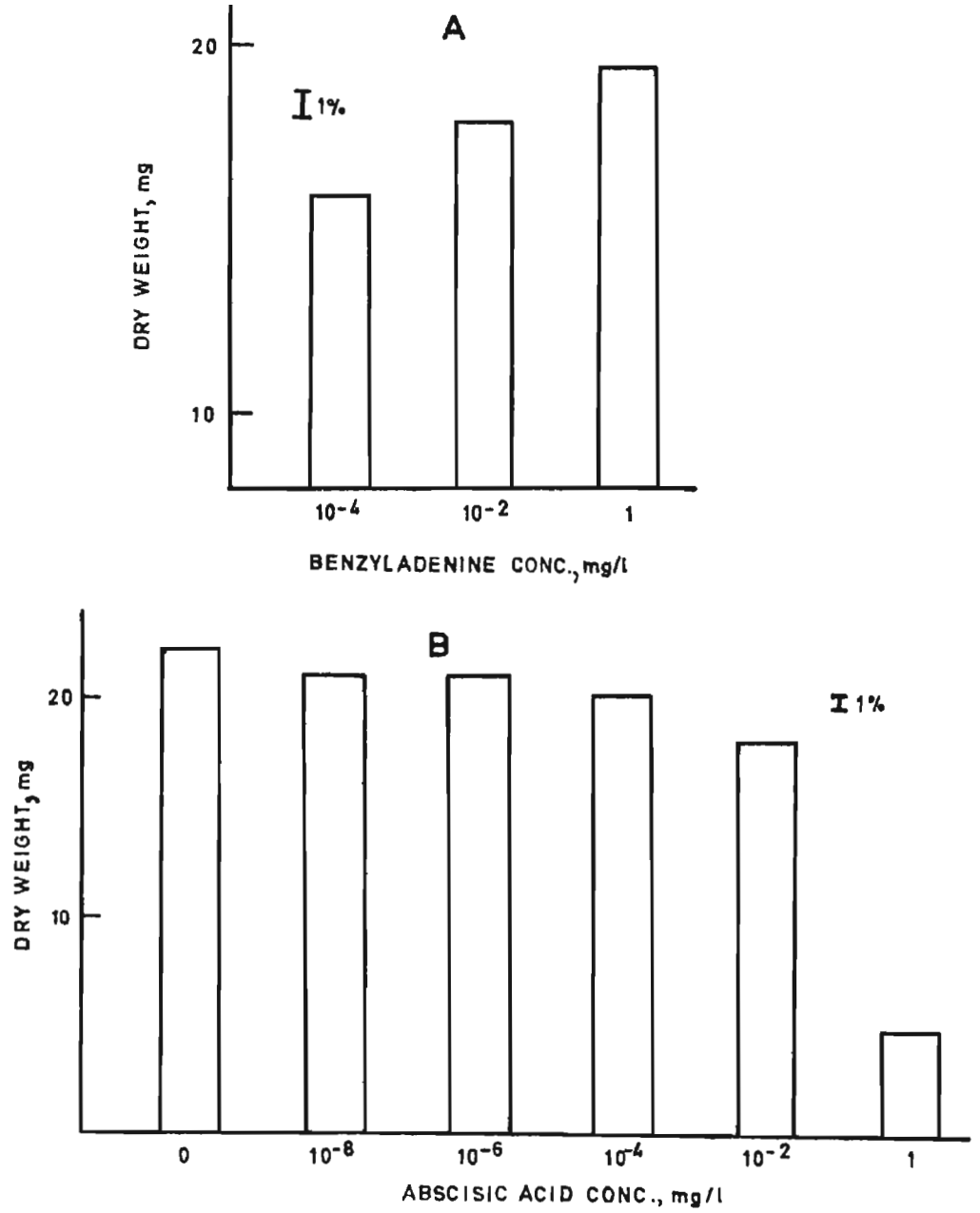


FIG. II. A. The effect of benzyladenine (BA) on the dry weight of Spirodela (12 days).

B. The effect of abscisic acid(ABA) on the dry weight of Spirodela (12 days).

overcome by 1.0 mg/l of BA.

In a second 3 x 6 factorial experiment with three replications the influence of ABA and gibberellic acid on the growth of *Spirodela* was investigated (Table 4). With an

TABLE 4. The effect of various concentrations of abscisic acid and gibberellic acid on the dry weight (mg) of *Spirodela*.

Gibberellic acid concentration (mg/l)	Abscisic acid concentration (mg/l)					
	0	10^{-8}	10^{-6}	10^{-4}	10^{-2}	1
1.0	1.6	2.1	2.1	2.3	1.4	0.9
10^{-2}	2.3	2.7	2.0	2.0	1.9	0.8
10^{-4}	2.4	2.3	2.0	2.3	2.5	0.8
L.S.D. (P = 0.05) = 0.75 L.S.D. (P = 0.01) = 0.99						

increase in the concentration of GA above 10^{-4} mg/l there is a decrease in the dry weight of *Spirodela*, showing that these higher concentrations of GA retard the growth of *Spirodela* (Fig. 12A). The optimum GA concentration for stimulation of growth appears to be in the vicinity of 10^{-4} mg/l, because lower concentrations of GA had no effect on the growth of this species (Fig. 13B). From Table 4 it would appear as if a certain amount of interaction takes place between ABA and

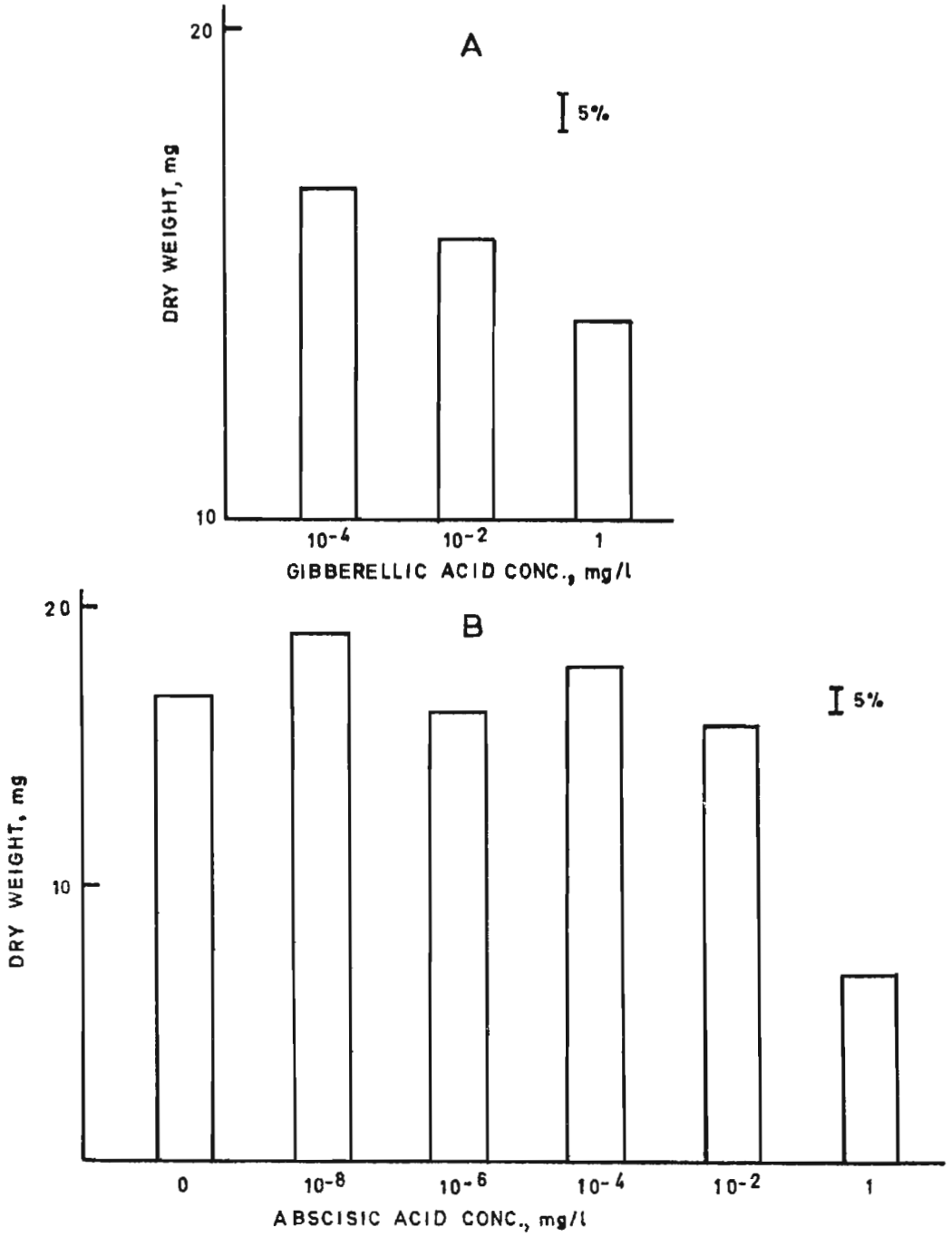
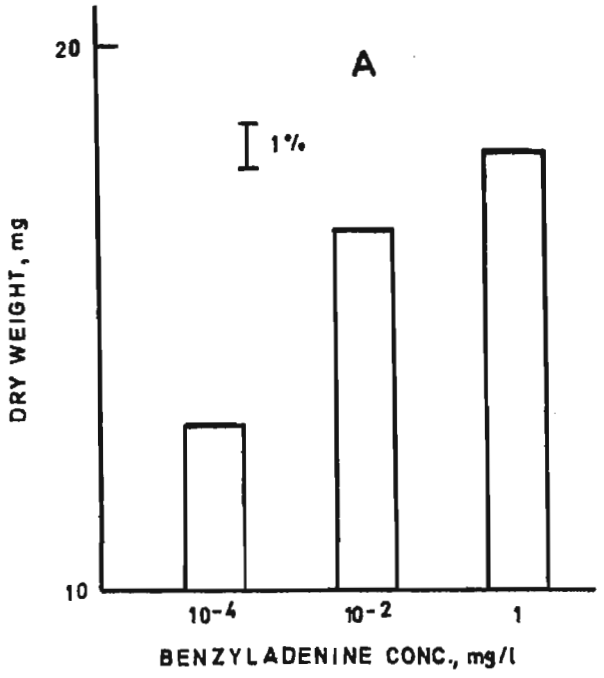


FIG.12.A. The effect of gibberellic acid (GA) on the dry weight of Spirodela (12 days).

B. The effect of abscisic acid (ABA) on the dry weight of Spirodela (12 days).



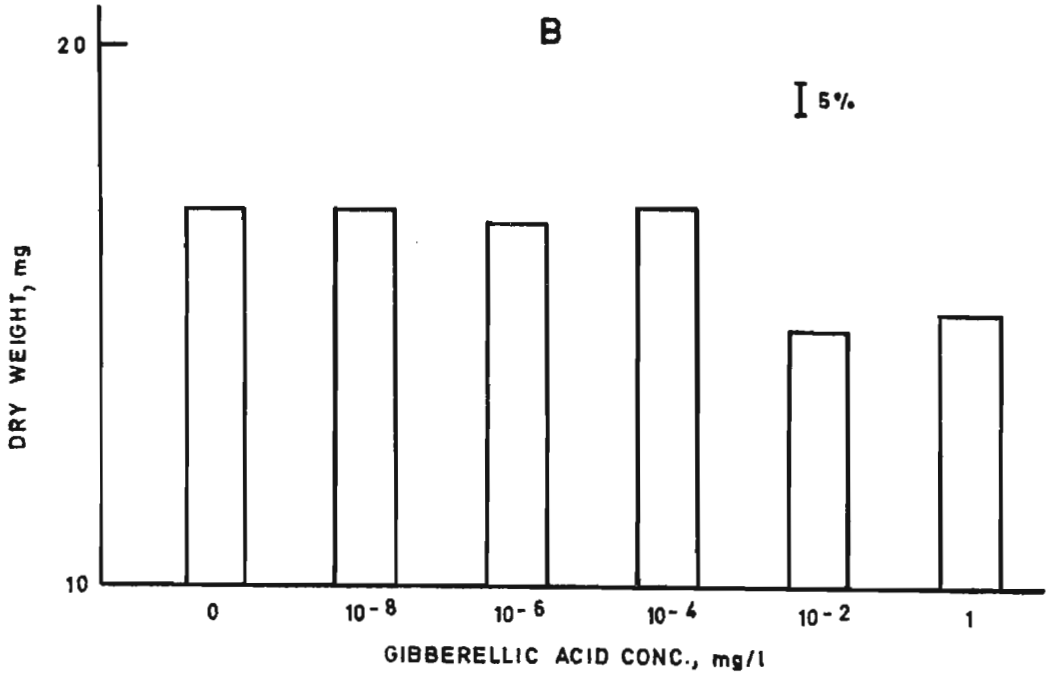


FIG .13.A. The effect of benzyladenine(BA) on the dry weight of Spirodela (12 days).
B. The effect of gibberellic acid (GA) on the dry weight of Spirodela (12 days).

GA. The results are, however, not conclusive. It would seem that the effect of GA on ABA-inhibited growth in *Spirodela* cannot be attributed to the reversal of the effect of ABA but is due to an acceleration of growth with increased GA concentrations.

CONCLUSIONS

The fact that different concentrations of abscisic acid affect the growth of *Spirodela* in different ways is an interesting one, and seems to lend support to VAN OVERBEEK'S (1968) and VILLIERS', (1968) use of the term hormone in reference to abscisic acid.

Although abscisic acid has been found to accelerate some growth processes, for example flowering in a number of short-day plants (WAREING, 1968) and root formation in mung bean and English ivy (CHIN *et al*, 1969) the present findings are of particular interest because it appears to be one of the first indications that low concentrations of this hormone (10^{-8} mg/l) may promote plant growth. The findings of RUDNICKI (1969) and SONDHEIMER *et al*, (1968) with apple and *Fraxinus* seeds respectively also point to this fact, since during stratification of the seeds there is a gradual decrease in their abscisic acid content while at the same time the germinative percentage increases. One speculates as to whether this increase in germination is not brought about by a concentration of abscisic acid too low to be determined by the bioassays presently at our disposal. It would seem that abscisic acid must not be regarded exclusively as an in-

hibitor of plant growth although at present there are very few indications that it promotes plant growth.

The use of *Spirodela* in a bioassay for abscisic acid holds promise, but further experiments need to be carried out so as to investigate the combined effects of gibberellin, cytokinins, and auxins on the growth of this plant. It is, however, possible to extract and purify abscisic acid from plant material before commencing with a bioassay (RUDNICKI, 1969).

The inhibitory effect of abscisic acid on the growth of *Spirodela* can be overcome by the cytokinins, benzyladenine being the most effective of the three cytokinins investigated. The manner in which this reversal is brought about is not known.

From the present investigation it is concluded that gibberellic acid cannot counteract the inhibitory effect of abscisic acid on *Spirodela*. Increases in growth obtained in a number of cases may be due to gibberellin-stimualted growth rather than an interaction with abscisic acid. A number of investigators (VILLIERS, 1968 and THOMAS *et al*, 1965) have found that gibberellic acid acted antagonistically to abscisic acid. This could possibly be explained in that treatment with gibberellic acid and abscisic acid induces different responses

in different plant material. It may thus prove worthwhile to investigate the relationship between gibberellic acid and abscisic acid more fully in different plant material. Tissue culture experiments may prove particularly useful in this regard because the overall effect on growth and differentiation of a wide range of combinations of hormone treatments can be studied.

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