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ON THE ABSCISSION OF DEBLADED PETIOLES IN *COLEUS RHENALTIANUS*, ESPECIALLY IN RELATION TO THE EFFECT OF GRAVITY

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ABSTRACT

By means of experiments with *Coleus rhenaltianus* it could be demonstrated that the effect of proximally as well as of distally applied auxin (IAA) on the abscission of debladed petioles, is an indirect one. By growth processes that are induced by the application of auxin at the proximal side of the abscission zone, e.g. by the development of new adventitious roots and by the elongation of roots that are already present, a substance that retards the abscission is drained off from the petioles. This substance is produced in the petioles, and probably also in the leaf-blades, when the latter are exposed to the light, and it was found to migrate through the parenchyma. A low concentration of auxin that is distally applied, accelerates the abscission of the petioles, provided that there is growth activity in the part at the proximal side of the abscission zone.

The accelerated abscission in horizontally placed plants is due to growth processes that are induced at the lower side of the stem by the gravitational force. The difference in abscission time between the petioles of plants that are kept in the vertical position, and those that are placed horizontal, is independent of distally applied sugar and of the concentration of distally applied auxin. The abscission proves to be retarded when the petioles are debladed a week after the plants had been brought in the horizontal position or when there is no source of auxin proximal of the abscission zone (young leaves). In the horizontal position the gravitational force affects a lateral shift of auxin from the tip to the lower side of the stem, and it hampers moreover the basipetal transport of substances that are produced in the leaf blades, and by which the growth of roots, the growth at the lower side of the stem and the development of new adventitious roots are promoted.

By means of paper chromatography it was found that the substance from the petioles which retards the abscission, is most probably identical with an auxin-like substance that was extracted from full-grown leaves, and of which it could be shown that it is not an indole derivative.

In plants in the normal position the abscission is accelerated by the longitudinal component of gravity. Elimination of the longitudinal component hampers the transport of the substance which retards the abscission, a transport that is directed to centres of growth, and so it can exercise its influence on the petioles themselves, and retard their abscission.

The retardation of the abscission in plants that are rotating parallel to the horizontal clinostat axis, is influenced by the revolving speed of this axis. This retardation is greatest at a revolving speed of 1–3 min. In slowly rotating plants the retardation of the abscission is partly compensated by the effect of the transversal component of gravity which promotes the abscission. The effect of the elimination of the longitudinal component is independent of the presence of a proximal source of auxin, e.g. of the tip and of young leaves.

INTRODUCTION

It is generally known that the form in which various parts of the plant appear, may be changed under the influence of gravity. The best investigated influence is that known as geotropism. According to the so-called distribution theory of Cholodny–Went geotropic curvatures would owe their origin to an unequal distribution of auxin caused by the action of the gravitational force. For coleoptiles of *Avena* this theory found support in the experiments of a.o. Dolk (1936 1)) and Anker (1956). The latter author proved that auxin is indispensable for a geotropic response, and De Wit (1957) showed that without auxin there is even no perception of the gravitational force. The mechanism of the geotropic response in roots, however, could as yet not be explained along these lines (Audus and Brownbridge, 1957).

In other kinds of tropisms and in such processes as fertilisation, cell division, tissue differentiation, development of new adventitious roots and of flower primordia, correlative inhibition of lateral buds, the abscission of various parts, auxins appeared to be involved too, and several of these processes, moreover, are influenced in some way

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1) The original dutch text of Dolk's paper was issued as a thesis in 1930; it has posthumously been translated in english.

by gravity. For instance in horizontally placed pineapple plants the flower primordia develop earlier than in plants that are kept in the vertical position (VAN OVERBEEK and CRUZADO, 1948), and for inversely placed soybean plants the same holds true (FISHER, 1957). Since long growers of fruit-trees are applying devices, such as the bending down of young twigs, by which an earlier flowering is obtained (KNIGHT, 1822; VÖCHTING, 1884). The growth of twigs that are brought in a horizontal position is retarded as compared to that of twigs that are kept in their normal position, and, according to the observations of fruit growers, premature fruit fall too is reduced in this way.

On the other hand TERPSTRA (1956) found in debladed petioles of *Coleus* and of apple seedlings an acceleration of the abscission in horizontally placed plants. Several other investigations have shown that the abscission of petioles and of fruits is governed by an analogous mechanism. Apart from other factors that affect the abscission directly or indirectly, auxin proved to be of particular importance (ADDICOTT and LYNCH, 1955).

The results of TERPSTRA's experiments made it worthwhile to continue the study of the effect exercised by gravity on the abscission of debladed petioles. We mainly intended to obtain more information with regard to the influence exercised by gravity on the metabolism and transport of the auxin, and on the correlation existing between gravity and auxin on the one hand and between the growth phenomena and the abscission of the petioles on the other.

CHAPTER I

SURVEY OF THE LITERATURE

The earliest anatomical study of the histological changes in the abscission zone that precede the leaf abscission proper, is that of VON MOHL (1860). Experiments of MOLISCH (1886) showed that water deficiency and low light intensities accelerate the abscission of leaves, and that oxygen should be present.

KÜSTER (1916) and HEINICKE (1917) reported that abscission of petioles and pedicels can be induced experimentally by removing the leaf blade or the flower. They regard the transpiration stream as the factor by which the abscission is in the main inhibited.

HODGSON (1918) and SAMPSON (1918) made a further study of the anatomical and chemical changes which take place in the cells of the abscission zone. For details on the results of the anatomical investigations we may refer to PFEIFFER (1928), who gave a review of the whole previous literature.

The study of the effect which is exercised by hormones on the abscission process, was started by LAIBACH (1933), who found that in *Coleus* the abscission of debladed petioles is retarded by the application of auxin to their distal (leaf blade) side. This paper was followed by a number of publications on the same subject, e.g. those of La Rue (1936) and Mai (1936). The latter used pollinia of Orchids as a source of auxin. Myers (1939) believed that auxin does not primarily affect the formation of an abscission zone, but that it influences the processes leading to the hydrolysis of the primary cell wall in the latter. It appeared, moreover, that the abscission proper is not necessarily preceded by the formation of a sharply defined abscission layer (McCown, 1943; Gawadi and Avery, 1950).

From the experimental data mentioned in the preceding paragraph it was concluded that under normal circumstances abscission of leaves is prevented by auxin produced in the leaf blades. Shoji, Addicott and Swets (1951) found in leaves of Black Valentine beans that the auxin level during the vegetative phase is fairly high. Moreover, Wetmore and Jacobs (1953) found for *Coleus* that the time which elapses in leaves of different age before they are shed, is correlated with the amount of diffusible auxin produced in the leaf blade. On the other hand Myers (1940) could not demonstrate the presence of any diffusible auxin in the leaves of this plant during the winter months.

The abscission is not only influenced by indole-3-acetic acid (IAA), but also by a number of other synthetic auxins and anti-auxins. According to Gardner and Cooper (1943) the reaction on naphthalene derivatives is slower than that on indole derivatives. They believed that there is a lag in time between the application of the auxin and the moment at which the retarding action on the abscission sets in, and the difference in the retardation obtained by means of the derivatives of naphthalene and those of indole they ascribe to differences in the rate of transport of these compounds. 2,3,5-tri-iodobenzoic acid or TIBA, an anti-auxin, proved to accelerate the abscission (Whiting and Murray, 1948; Weintraub, Brown, Nickerson and Taylor, 1952; Singh, 1956). Haccius and Nies (1956) found that the effect on the abscission of debladed petioles obtained in *Ruellia* sp. by means of distally applied TIBA depends on the concentration of the latter; at low TIBA concentrations the abscission is retarded, at higher concentrations it is accelerated.

The abscission of pedicels too can be retarded by the application of auxin (Juriscec, 1939; Barlow, 1947). Under natural conditions auxin is supposed to be produced by the fruit tissue and by the developing seeds (Luckwill, 1948, 1953; Barlow, 1952). Luckwill (1953) could demonstrate chromatographically that in the endosperm of apple seeds four different compounds with auxin activity are formed. One of these compounds apparently shows a negative correlation with the fruit fall, but not with the growth of the fruit. This auxin-like compound is not confined to the endosperm, but occurs also in the pedicel. The post-blossom fall as well as the June fall appear to be negatively correlated with periods in which the auxin content of the seeds is low. In full-grown seeds the auxin content quickly decreases. The factors which prevent the abscission of fruits during the rest of the season, are still unknown (Luckwill, 1957). It is known, however, that the pre-harvest drop can be prevented successfully by spraying with auxin solutions (LEOPOLD, 1958). It is of particular interest that BATJER and THOMPSON (1948) found that in apples local application of NAA (naphthyl acetic acid) against fruit fall was most effective if the leaves too were sprayed. According to BARLOW (1950) this NAA would be absorbed by the leaves, and then transported to the fruit. From there it would migrate basipetally to the abscission zone at the base of the latter.

Several theories try to explain the effect of auxin on the abscission, but these theories leave out of account a number of other factors which may influence this process (ADDICOTT and LYNCH, 1955). Among them the theory of the hormone-ethylene balance proposed by GAWADI and AVERY (1950) and the auxin-gradient theory of ADDICOTT, LYNCH and CARNS (1955) may be mentioned. According to the first-named theory the abscission would be governed by the ratio between auxin and ethylene. GAWADI and AVERY found that the auxin content of leaves decreased with their age, but in older leaves, according to them, ethylene would be produced (just as in ripening fruits), a compound of which it is known that it strongly accelerates the abscission. This theory was endorsed by HALL (1952), who could prove an antagonism between ethylene and auxin. It appeared furthermore that after the application of defoliating agents too ethylene is set free (JACKSON, 1952).

SHOJI, ADDICOTT and SWETS (1951) concluded from their experiments that the auxin gradient across the abscission zone is of greater importance than the auxin concentration in the part that is to be shed. This finding was the starting point of the auxin-gradient theory of ADDICOTT, LYNCH and CARNS. Abscission of an organ would occur when the auxin level at the distal side becomes sufficiently low, and when that at the proximal side is high. JACOBS (1955) and TERPSTRA (1956) found support for this theory in experiments with *Coleus*. TERPSTRA, however, slightly modified it. According to her, abscission would occur when

1°. at the distal side of the abscission zone the auxin concentration is sufficiently low, and when

2°. at the proximal side either a sufficiently high amount of auxin is present, or when at that side some growth process induced by auxin, is started.

The authors mentioned above always used auxin concentrations in their experiments which from the physiological point of view must be regarded as very high. GAUR and LEOPOLD (1955) found in debladed petioles of *Coleus blumei* and of *Phaseolus vulgaris* an acceleration of the abscission when on the distal side auxin was applied in concentrations of 1–10 p.p.m. It appeared, moreover, that in explants the abscission did not depend on an auxin gradient, but on the total amount of auxin, no matter whether the latter was applied distally or proximally or at both sides. BIGGS and LEOPOLD (1958) obtained in explants of *Phaseolus* with NAA that was supplied from the proximal side, either a retardation or an acceleration of the abscission, the result depending on the concentration of the NAA. These authors believe that the effect of auxin would primarily be an effect on the abscission zone. The amount of auxin that is available in the abscission zone, would determine the result. The fact that distally applied auxin always has a greater effect than auxin that is applied proximally, would merely be due to a difference in the rate of transport.

The reaction on distally applied auxin is the same for explants and for intact plants, but so far as is known at present, it seems that this does not apply to auxin that is applied from the proximal side. A proximally applied preparation of 1 % IAA accelerates the abscission of debladed petioles in intact plants (JACOBS, 1955), but the same treatment gives a strong retardation of the abscission in explants (GAUR and LEOPOLD, 1955). It does not seem probable that this difference in behaviour might be due to mere differences in the rate at which auxin is transported in the two directions.

BEAL and WHITING (1945) could inhibit in *Mirabilis jalapa* the abscission of stem parts by means of a 2 % IAA preparation. HACCIUS (1955) found that in *Limnophila heterophylla* the abscission of internodes is induced by 2–4 D in concentrations of 20–40 p.p.m. In very high concentrations, however, this substance had no effect on the abscission.

In intact plants the abscission of debladed petioles is not only accelerated by the presence of the growing stem tip (which can be replaced by an auxin preparation of a high concentration) but also by the full-grown leaves that are found in the near vicinity (ROSETTER and JACOBS, 1953; JACOBS, 1955; TERPSTRA, 1956). ROSETTER and JACOBS first supposed that these leaves would act as a source of auxin, but as it proved to be impossible to replace them by an auxin preparation, JACOBS (1955) suggested that they supply the young leaves at the tip with substances which stimulate their growth, and indirectly their auxin production.

TERPSTRA found in decapitated plants that the abscission of debladed petioles was accelerated by the presence of leaves in their vicinity. However, she did not remove the axillary buds, which, when they begin to grow out, may replace the tip of the stem as a source of auxin. A similar effect was found by VAN STEVENINCK (1957) in *Lupinus luteus*, where the growth of the pods in the lower whorls accelerates the abscission of young ovaries in the higher ones. This is a strictly polar effect and, according to the author, it can not be ascribed to a competition for nutrients.

On the other hand, nutrients apparently may exert an influence on the abscission, so e.g. the application of sugar to debladed petioles. In explants the abscission is retarded by the application of glucose and sucrose (LIVINGSTON, 1950; BROWN and ADDICOTT, 1950; DOSTAL, 1951; BIGGS and LEOPOLD, 1957). According to WENT and CARTER (1948) in June the fall of several kinds of fruits may be prevented by spraying with sugar solutions.

The influence of gravity on abscission phenomena has been investigated by TERPSTRA (1956). She found that in *Coleus* and in apple seedlings the abscission of debladed petioles is accelerated when the plants are brought in a horizontal position. This effect can only be explained by means of the auxin-gradient theory if we assume that the auxin production is increased in the horizontal position by the action of gravity. For such increased auxin production only one indication is to be found in the literature, viz. for the articulations of grass stems. However, in Coleus too a similar accelerating effect was found when in horizontally and vertically placed decapitated plants the same concentrations of auxin were applied from the tip.

From these results and from preliminary experiments on the clinostat TERPSTRA concluded that the acceleration of the abscission in horizontally placed plants must be due to the action of the transversal component of the gravitational force. The mechanism of this action, however, remained unknown.

Although auxin would be "the principal endogenous regulator of abscission" (ADDICOTT et al., 1955), ADDICOTT and LYNCH (1955) in their review mention a large number of other agents that may produce, either directly or indirectly, an abscission. The fact that the abscission is influenced by such a variety of agents, may be regarded as an indication that this process is correlated with other physiological processes elsewhere in the plant.

The mechanism through which auxin acts upon the abscission, is still unknown. Some experiments of TERPSTRA suggest that the effect exercised on the abscission by proximally applied auxin, is an indirect one. In experiments with completely defoliated plants, for instance, no abscission of petioles was obtained when auxin was applied proximally a few days after the defoliation. This result, which could be confirmed, indicates that auxin applied from the proximal side, acts on the abscission by the intermediary of processes which have their seat in the leaves. The fact itself that in completely defoliated plants no abscission occurs, does not fit in with the auxin theory, as in this case the auxin concentration must be low at the proximal as well as at the distal side of the petioles.

From this survey of the literature it will be clear that our knowledge of the physiology of the abscission phenomena is still very incomplete. This especially holds true for the action of auxins, and the more so because conclusions often have been based on effects obtained with auxin concentrations that are much higher than those ever found in the plant. There are indications that under certain circumstances the abscission is correlated with growth processes which take place elsewhere in the plant, so e.g. the accelerating effect exerted by the stem tip and that exerted by the hypotrophy in horizontally placed plants (TERPSTRA, 1956). ADDICOTT and LYNCH (1955) and TERPSTRA (1956) leave the possibility open that growth in other parts of the plant may deprive the abscission zones of definite nutrients, and that this may lead to abscission of the parts to which they belong.

In the present paper we will assume as a working hypothesis that the abscission of debladed petioles is correlated with some meristematic activity in another part of the plant.

In the first place the effect of proximally and distally applied auxin has been investigated in more detail, especially in relation with the influence of gravity. Another item of our program was the study of the effect exercised by leaves in the vicinity of the abscission zone and of the influence of gravity on this effect. The influence of gravity has also been analysed by means of experiments on the clinostat. Finally it was tried to identify the substance which must be present in full-grown leaves, and which inhibits the abscission.

CHAPTER II

MATERIAL AND METHODS

The plant material for our experiments was obtained from cuttings from a clone of *Coleus rhenaltianus*. The plants were cultivated in the experimental part of the glass-house at a temperature of about 20° C. On bright days during the summer the temperature, however, was considerably higher. The experiments too were carried out in this glass-house, those in complete darkness excepted. The latter were made in an air-conditioned dark room at a constant temperature of 20° C and a relative air humidity of about 65 %. For the observation weak orange light was used. During the winter (November–April) in the glass-house extra light was supplied to the growing plants (TL-Philips tubes, intensity 65 W m^2 sec. at a height of 75 cm). In this way the total day length was raised to 15½ h, which prevented flower initiation in the test plants.

The plants were used for the experiments when 5 full-grown leaf-pairs had developed. The youngest full-grown leaf pairs and all the axillary buds and sprouts were removed. In all experiments, when not mentioned otherwise, the petioles of the two upper leaf pairs were used for the abscission test. The blade and the upper part of the petiole were removed, so that the part that was left, had a length of 1.5 cm.

In the experiments in which the plants were placed in a horizontal position, the pots were fixed in wooden racks. In order to prevent a bending down of the stem, they were fixed by small rings to thin sticks stuck in the earth of the pots. In this way only the tip of the stem could carry out a geotropic curvature.

The plants were taken at random from the table on which they were growing, and divided into experimental lots arranged in a latin square on the working table. The controls, i.e. the plants that were left in the vertical position, and the horizontally placed plants were put in different squares.

When not mentioned otherwise, the plants were placed horizontal one day before the petioles were debladed. Auxin (indole-3-acetic acid = IAA) was applied by means of a lanolin paste. The concentrations mentioned in the tables are those of the solution before the latter was emulgated with lanolin. To this end equal parts of solution and lanolin were mixed. For each experiment fresh pastes were prepared.

Twice a day, with an interval of 12 hours, the experimental plants were inspected. Water was spouted in a tiny jet, but under a constant pressure, against the petioles, and those that were dropped, were counted.

CLINOSTAT

For the experiments on the clinostat a modernized "DE BOUTER clinostat" was used. The experiments reported in chapter VII, i.e. those on the effect of the rotation time, required a special type of clinostat, which is described in that chapter. The De Bouter clinostat has two synchronized axes: a horizontal one and one which can rotate at any desired angle. The latter was usually used for the vertically rotating controls. The clinostat is driven by a powerful synchronous electromotor. The rotation time of both axes was 2 min. 47 sec. On each axis a round table with a diameter of 30 cm was mounted, and on each of the latter 10 pots with one plant each were fixed by means of spring clasps. The treatment of the plants was the same as that of the plants in the other experiments.

STATISTICAL TREATMENT OF THE EXPERIMENTAL RESULTS

The differences between the effect of the various treatments were checked on their significance by means of a variance analysis. In the tables and diagrams only the standard error of the difference (S.E.) and the probability that the observed difference might arise by chance (P-value) are recorded. The results plotted in the graphs have, if possible, been checked in the same way.

The significant correlation between the reaction and the physiological age of the petioles makes it indispensable to estimate the reaction for the petioles of the two different leaf pairs separately. In some experiments the effect of a difference in orientation of petioles belonging to the same leaf pair (e.g. one pointing upwards and the other downwards) has been estimated; however, it appeared that this factor has no significant effect.

Here follows an example of the way in which the mathematical analysis was applied by us.

The experiment that was chosen to this end, comprised 8 lots of 10 plants; 4 lots were left in the vertical position, 4 lots were placed horizontal, and the debladed petioles of the first leaf pair from the top were distally supplied with 1°. IAA, 2°. glucose, and 3°. sucrose. In the table x represents the abscission time of each petiole in days, and $\bar{x}$ the mean abscission time found for the 20 petioles of each lot. The sums of x and x^2 needed for the statistical analysis, and the $\bar{x}$ of each lot are given in table I. In table II the sums of x_v and of x_h are given for the calculation of the sum of the squares.

TABLE I

Treatment	Σx		Σx^2		$\bar{x}$	
	vert.	horiz.	vert.	horiz.	vert.	horiz.
control	82.0	52.0	346.0	140.0	4.10	2.60
0.1 mg IAA/1	93.0	67.0	441.0	229.0	4.65	3.35
3 % glucose	102.0	74.0	534.0	278.0	5.10	3.70
6 % sucrose	100.0	66.0	510.0	222.0	5.00	3.30

TABLE II

Totals (Σx)

Treatment / Position	control	0.1 mg IAA 1	3 % glucose	6 % sucrose	total
vert.	82.0	93.0	102.0	100.0	377.0
horiz.	52.0	67.0	74.0	66.0	259.0
total	134.0	160.0	176.0	166.0	636.0

The value of the correction factor is $(\Sigma x)/n$, being the total number of the petioles belonging to the 4 lots. The results of the analysis are resumed in Table III. For further details we must refer to manuals of statistical analysis. The symbols used in the calculations have the following meaning:

TABLE III

Analysis of variance

Source	Sum of squares	Degrees of freedom	Variance estimate	F	P
Treatments	24.10	3	8.03	20.58	< 0.001
Position	87.02	1	87.02	223.12	< 0.001
Interaction treatm./pos	0.88	3	0.29	0.74	> 0.05
Residual	60.78	155	0.39		

F = variance ratio (Table III)

Standard deviation of the total lot (δ) = $\sqrt{\text{residual variance estimate}}$ (V.E.)

or (in Table III) $\delta = \sqrt{0.39} = 0.62$

The significance of the difference between two treatments was calculated by means of STUDENT's t-test. The standard error of the difference (S.E.) was calculated according to the formula:

$$\text{S.E.} = \delta \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

where δ is the standard deviation of the total lot, and n_1 and n_2 the number of petioles, of which the mean abscission time $\bar{x}_1$ and $\bar{x}_2$ had been estimated. In the given example:

$$S.E. = 0.62\sqrt{\frac{1}{20} + \frac{1}{20}} = 0.19$$

Finally the value of t was calculated by means of the formula:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S.E.}$$

The probabilities (P) belonging to the t-values, can be found in FISCHER's t-table. The number of degrees of freedom is $n_1 + n_2 - 2 = 38$.

TABLE IV

The differences in abscission time found between the variously treated plants and the controls

Treatment	difference ($\bar{x}_1$–$\bar{x}_2$)	t	P
Vertical			
0.1 mg IAA-control	0.55	2.89	0.01–0.001
3 % glucose-control	1.00	5.26	< 0.001
6 % sucrose-control	0.90	4.73	< 0.001
Horizontal			
0.1 mg IAA-control	0.75	3.84	< 0.001
3 % glucose-control	0.90	4.73	< 0.001
6 % sucrose-control	0.70	3.68	< 0.001
Vertical-horizontal			
control	1.50	7.89	< 0.001
0.1 mg IAA/l.	1.30	6.84	< 0.001
3 % glucose	1.40	7.36	< 0.001
6 % sucrose	1.70	8.42	< 0.001

The significance of the difference that was found in this particular experiment between the various treatments and the controls and between the vertical and the horizontal plants, is given in Table IV.

CHAPTER III

THE EFFECT EXERCISED BY PROXIMALLY SUPPLIED AUXIN ON THE ABSCISSION OF DEBLADED PETIOLES IN INTACT PLANTS

In *Coleus* the abscission of debladed petioles is retarded when the stem tip and the young leaves are removed (JACOBS, 1955). When an auxin preparation of a high concentration (JACOBS, 1955: 1 %; TERPSTRA, 1956: 0.2 %) is applied to the cut surface of decapitated plants, the abscission takes place in the same way as in intact plants. The only other auxin concentration that as yet has been tried, was an

IAA solution of 2.10^{-6} g/ml, of which Terpstra reported that it had no effect. In explants the abscission is either retarded or accelerated by proximally applied auxin, the result depending upon its concentration (Gaur and Leopold, 1955); it is retarded by high and accelerated by low concentrations of IAA and NAA. The difference in the reaction on a high auxin concentration shown by intact plants and by explants, casts some doubt on the conclusion reached by Jacobs (1955) according to which in intact plants the abscission of debladed petioles would be stimulated by the application of a high auxin concentration at the proximal side. This doubt seems the more justified since the IAA concentration applied in his experiments far exceeded the normal auxin level found in the plant.

In the experiments described below we studied the effect of different auxin concentrations that were supplied from the proximal side.

1. THE EFFECT EXERCISED BY DIFFERENT IAA CONCENTRATIONS WHEN PROXIMALLY SUPPLIED

The plants were decapitated at the 5th node. The IAA concentrations of the lanolin-water preparations were resp. 100, 10, 1, 0.1 0.01 mg/l IAA. As controls we used 1°. a series of intact, not decapitated plants, and 2°. one of decapitated plants to which a plain lanolin-water emulsion had been applied. The experiment comprised 70 plants, i.e. 7 series of 10 plants each, and it was repeated three times.

The results of one of the three experiments are graphically reproduced in Fig. 1. The accelerating effect exercised on the abscission1) by the tip in combination with the young leaves is matched by that of 100 and 10 mg/l IAA. Lower concentrations do not show significant differences as compared with the controls that were supplied with plain lanolin paste.

It is noteworthy that in those cases where the abscission was found

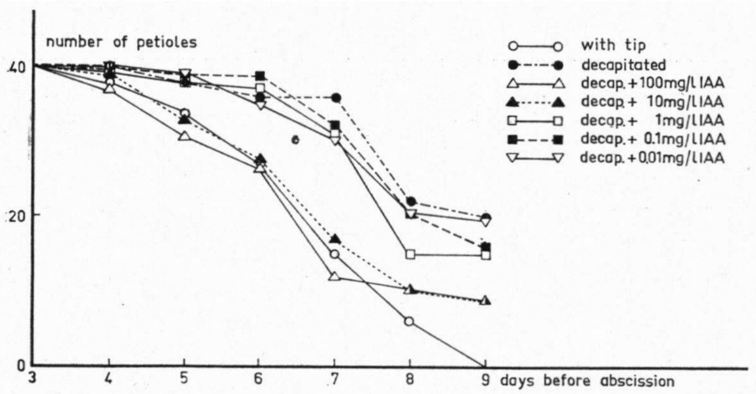

Fig. 1. Effect of proximally supplied auxin on the abscission of debladed petioles in decapitated plants.

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1) In future, unless stated otherwise, with "abscission" is meant "the abscission of debladed petioles belonging to the 1st and 2nd leaf pair".

to be accelerated, adventitious roots proved to have developed on the stem below the cut on which the IAA paste had been applied. This root formation did not occur in plants in which the auxin paste did not stimulate the abscission, i.e. at low IAA concentrations.

This observation led to a further study of the relation between the formation of adventitious roots and the abscission of the debladed petioles.

2. THE INDUCTION OF ABSCISSION AND ROOT FORMATION BY IAA IN INTACT PLANTS

When a ring of lanolin paste with a high IAA concentration is applied around the stem of intact plants, below this ring adventitious roots begin to develop. We tried to find out whether development of these adventitious roots was accompanied by an acceleration of the abscission.

The plants that were used in these experiments had three full-grown leaf pairs. The upper leaf pair was removed, and in its place a ring of 1 g/l IAA paste was applied. In the controls at the same spot a ring of plain lanolin paste was applied. The petioles of the 2nd leaf pair were debladed. The effect of the IAA on the abscission in two experiments is given in Table 1. It appears that the stimulating influence of the tip and the young leaves is increased by the development of new adventitious roots that was induced by the application of IAA.

TABLE 1

Simultaneous effect of young upper leaves and of adventitious roots whose development was induced by the action of IAA (1 g/l), applied in the form of a ring of lanolin paste on the stem between the debladed petioles and the stem tip, on the abscission time of debladed petioles.

Nr. of exp.	Number of petioles per series	Mean abscission time in days	
		Control	IAA on the stem
61	12	3.79	2.50
67	28	6.43	5.21

3. THE ABSCISSION OF PETIOLES IN DEFOLIATED AND DECAPITATED PLANTS

In completely defoliated and decapitated plants abscission of debladed petioles can be induced only when at the same time the roots are removed (TERPSTRA, 1956). This phenomenon was ascribed by TERPSTRA to an inhibiting effect exercised by the roots on the abscission. When this experiment was repeated, it was found that new roots had developed at the base of the stem. In view of the experiments described sub 1) and 2), it seems possible that here too the abscission is correlated with the development of these adventitious roots. The following experiments prove that the formation of roots is indeed always accompanied by an accelerated abscission of the debladed petioles.

a. *Abscission of petioles in non-decapitated plants of which the roots had been removed*

One lot of plants was cut off just above the roots and of another lot the soil which adhered to the roots was removed as completely as possible by washing them with tap water. The plants of both lots were put in Erlemeyer flasks filled with water, which was daily renewed. Again it appeared that (Fig. 2) the abscission was accelerated in those plants of which the roots had been removed and in which consequently new roots were developing.

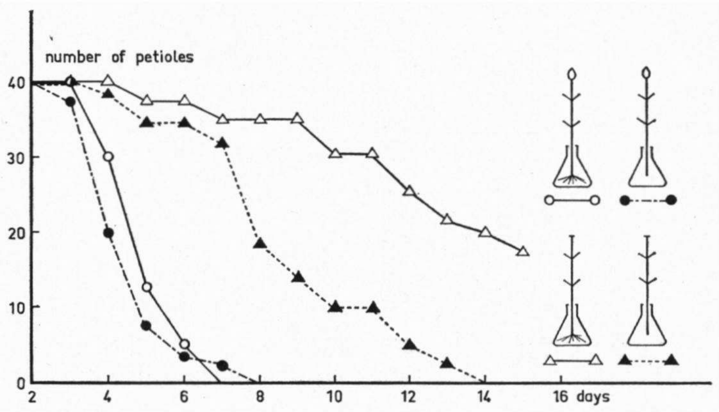

Fig. 2. The effect which removal of the olds roots and the subsequent formation of new ones at the base of the stem exercises on the abscission of debladed petioles in non-decapitated and in decapitated plants.

b. *Induction of the development of adventitious roots at the upper end and the base of decapitated plants*

The treatment of the plants was the same as sub a., but in these experiments the plants were decapitated and supplied with an auxin paste (1 g/l IAA). Here too the abscission time proved to be shorter if new roots developed either at the base or on the upper part (Fig. 3).

4. THE FORMATION OF ADVENTITIOUS ROOTS AND THE ABSCISSION OF PETIOLES IN HORIZONTALLY PLACED PLANTS

Besides a dilatation (hypotrophy) at the lower side of horizontally placed stems sometimes a development of roots is noted. This root formation can be promoted experimentally by making a transverse incision at the lower side of the stem between the first two leaf pairs and by inserting a thin blade of mica in the incision. After 5–8 days at the proximal side of the incision the development of adventitious roots can be observed with the naked eye. When a similar incision is made at the upper side of a horizontally placed stem, no roots are formed. In Fig. 4 the result is given of one of these experiments consisting of series of 10 plants each. It proves that the development of adventitious roots is accompanied by an acceleration of the abscission of the petioles.

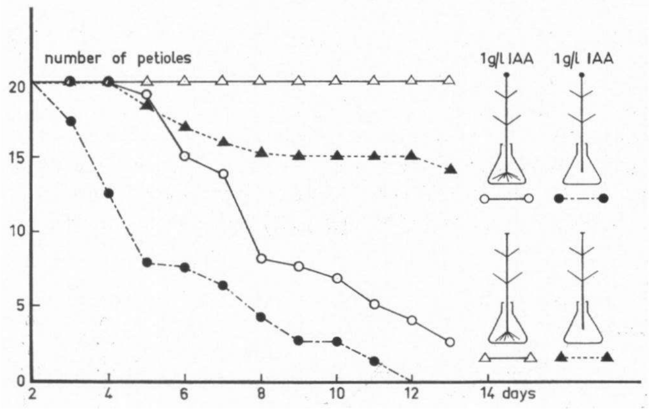

Fig. 3. The effect which a removal of the old roots and the subsequent formation of new ones at the base of the stem, eventually accompanied by a supply of auxin to the tip, exercises on the abscission of debladed petioles in decapitated plants.

As a rule the activity of meristems is increased by a local increase of the auxin concentration (SNOW, 1935; KÜNNING, 1950). The formation of adventitious roots might be caused by a lateral shift of the auxin transport under the influence of gravity, so that the accumulation of auxin at the lower side becomes high enough to induce the development of new meristems or to activate that of resting ones.

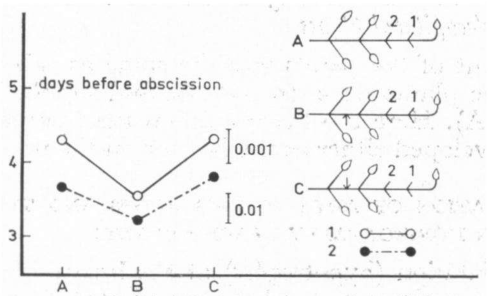

Fig. 4. Acceleration of the abscission of debladed petioles obtained by means of a transverse incision at the lower side of the stem (series B). In the plants of series C an incision was made at the upper side of the stem. Each value is the mean abscission time of 20 petioles. The statistical levels of significance (P-values) of the differences between the various lots are also shown.

5. THE INFLUENCE OF THE LEAVES ON THE DEVELOPMENT OF ROOTS AND SO, INDIRECTLY, ON THE ABSCISSION OF PETIOLES

From experiments by MOUREAU (1940) it appeared that in *Coleus* the IAA-induced formation of adventitious roots is stimulated by one or more substances that are produced in the leaves under the influence of light. If it is true that the abscission of debladed petioles is correlated with the development of new roots, it is to be expected that the abscission time too will be affected by these substances. ROSETTER and JACOBS (1953) found that the presence of young leaves at the tip has an accelerating effect on the abscission of debladed petioles when full-grown leaves are present in their vicinity. JACOBS (1955) concluded that certain substances issuing from these full-grown leaves increase the development of the young leaves, and so indirectly the auxin production in the latter. In the experiments that we will describe now, we have investigated the influence of the presence of leaves on the formation of adventitious roots that is induced by high IAA concentrations, as well as their influence on the abscission of debladed petioles, a phenomenon that is here supposed to be correlated with the formation of new roots.

a. *The influence of full-grown leaves on the abscission, when at the same time a high auxin concentration is present at the proximal side*

The plants used in these experiments had three full-grown leaf pairs. They were decapitated at the 3rd node, and supplied with auxin paste (1 g/l IAA) at the cut, i.e. proximally of the debladed petioles of the 2nd leaf pair. In half of the plants the lower leaf pair too was removed. In Fig. 5 the results of such an experiment are given.

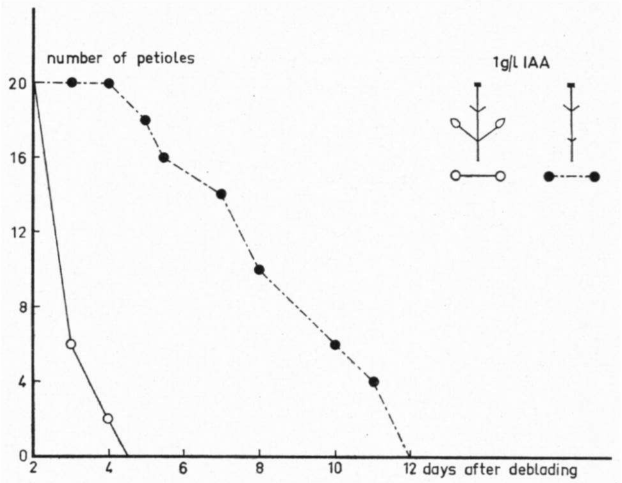

Fig. 5. Effect of the neighbouring leaves and of proximally supplied auxin on the abscission of debladed petioles. The accelerated abscission in plants in which the neighbouring leaves are left intact, is accompanied by the development of new adventitious roots at the tip.

It is evident that the abscission in plants with one full-grown leaf pair is significantly accelerated as compared to that of completely defoliated plants.

b. *The effect of proximally applied auxin in decapitated plants that are kept in the dark*

According to MOUREAU (1940) the substances which in *Coleus* promote the formation of adventitious roots are produced in the light. From the experiments sub a. it appeared that the abscission which is induced by auxin and which is accompanied by root formation, is promoted also by some influence exercised by the full-grown leaves. Since the effect of proximally applied auxin is different in entire plants and in explants, and as experiments with the latter have generally been carried out in the dark, the experiments were repeated with entire plants that were now also kept in continuous darkness. Two days before the deblading of the petioles and the decapitation of the plants, the latter were transferred to a dark room. In 10 plants auxin paste (1 g/l IAA) was applied to the cut, and in 10 plants plain lanolin paste. Fig. 6 gives the results, which prove to be the reverse of those obtained in experiments with plants that were exposed to the natural kind of illumination, for in the dark proximally supplied auxin appears to retard the abscission of the debladed petioles. At the end of the experiment in most of the plants no development of adventitious roots could be observed; in a few of them this process had just started.

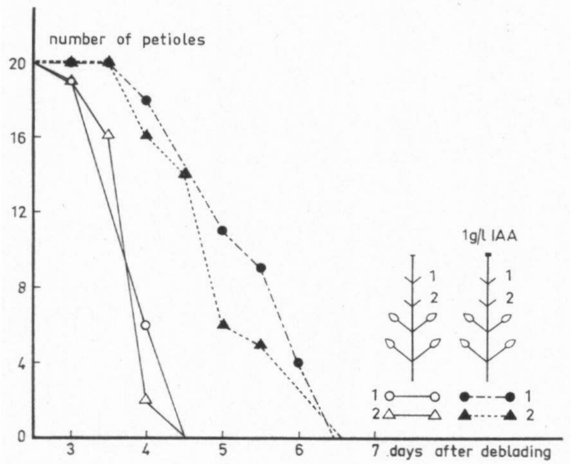

Fig. 6. Effect exercised by a high concentration of auxin that is applied proximally, on the abscission of debladed petioles in plants that are kept in the dark.

6. THE ABSCISSION IN COMPLETELY DEFOLIATED PLANTS THAT ARE KEPT IN THE DARK

The results of the experiments (5b) which show the retarding effect exercised by proximally applied auxin on the abscission of debladed petioles in plants that are kept in the dark, and the fast abscission found for the debladed petioles of the decapitated controls that received no auxin, led to a repetition of TERPSTRA's (1956) experiments with completely defoliated plants, but now the latter were kept in the dark. In these experiments too it appeared that the debladed petioles were all dropped within 4 or 5 days. This result suggests that the reason why in completely defoliated and decapitated plants no abscission occurs in the light, must be sought in the circumstance that in that case the petioles themselves produce some substance which inhibits the abscission. Apparently but a small amount of this substance is transmitted past the abscission zone, and distributed over proximally located parts of the plant.

7. THE RELATION BETWEEN THE LENGTH OF THE DEBLADED PETIOLES AND THE EFFECT WHICH LIGHT EXERCISES ON THEIR ABSCISSION

One of the phenomena which precede the abscission, is a loss of colour in the petioles caused by a decomposition of the chlorophyll. This is observed in intact plants as well as in explants. In the stem no loss of colour is visible.

The decomposition of the chlorophyll apparently is retarded by a substance that is produced in the petioles as a result of the illumination. It is not impossible that this substance is transported from the petioles in the direction of the stem tip towards sites that are involved in some meristematic activity. Quantitatively the production of this substance must depend on the volume of the petioles and, as the diameter of the latter may be regarded as constant, on their length. It is therefore to be expected that in completely defoliated and decapitated plants abscission of debladed petioles will occur in the light too if the deblading is carried out in such a way that but a small part of the petiole is left.

This was tested in the next experiments, which consisted of two series of 12 plants each; the latter were all completely defoliated and decapitated. The length of the petioles was 2 cm in the plants which served as controls, and 0.3 cm in the other series. Fig. 7 shows the result of one of these experiments. It appeared that in the plants with very short petiole stumps the illumination was unable to prevent the abscission. From this it may be concluded that the retardation of the rate of abscission mainly depends on the amount of a substance which is produced under the influence of the illumination at the distal side of the abscission zone, i.e. in the petiole and in intact leaves presumably also in the leaf blade. The rate of abscission is probably also influenced by the rate at which this substance is utilized by meristematic activity in the parts that are situated proximal of the abscission zone.

8. DISCUSSION

The results of the experiments thusfar described, do not support the auxin-balance theory of JACOBS (1955), according to which the abscission of debladed petioles would be directly promoted by a high auxin level at the proximal side. This auxin, which under natural circumstances is produced by the young leaves and by the tip, would be replaceable by a strongly concentrated, artificially supplied auxin. According to Jacobs (1958) the abscission of complete leaves would also be promoted by auxin produced by axillary shoots. He tried to prove this by applying an 1 % IAA-lanolin paste on decapitated lateral shoots; 82 days later the dropped leaves were counted. It was found that the percentage of dropped leaves was somewhat higher for the plants that had been treated with auxin, and then only for the 2nd and 3rd leaf pair (counted from the base.) The long interval between the treatment and the inspection, and also the very high concentration of the IAA paste used by him, make this experiment inconclusive, and it is doubtful whether the difference that was found by him, may be ascribed to a direct effect of the auxin.

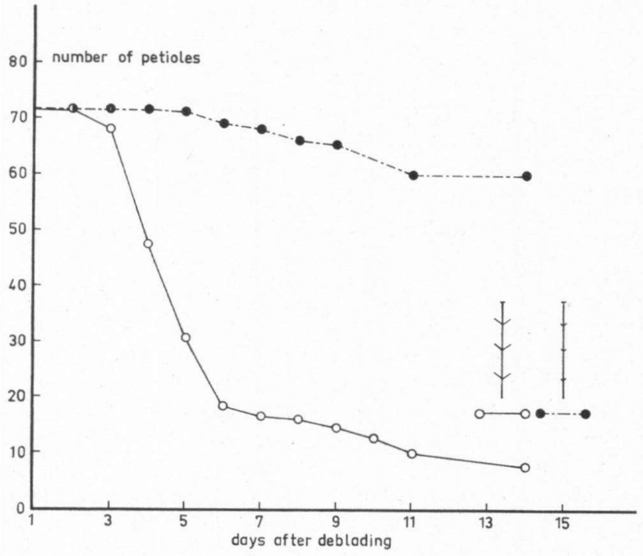

Fig. 7. Abscission of debladed petioles in decapitated, completely debladed plants exposed to natural conditions of light. The length of the petioles was 2 and 0.3 cm.

Therefore, in a few experiments auxin (0.1 % IAA-paste) was supplied to decapitated axillary shoots in order to test their influence on the abscission of debladed petioles. The result was that in all the plants that had been treated with auxin, the abscission proved to be retarded as compared to the controls, no matter whether the latter were provided with lateral shoots or not.

The experiments described above seem to justify the conclusion that the effect exercised on the abscission of debladed petioles by auxin that is proximally applied in high concentrations, is an indirect one, i.e. that auxin only acts via a meristematic activity of some kind such as the induction of the development of new adventitious roots.

Especially from the experiments on the influence which the leaves exercise on the abscission, we may conclude that the abscission and the development of new adventitious roots are strongly correlated. If the auxin concentrations at the proximal side are the same, these processes are markedly influenced by the presence of leaves. Under natural light conditions a high auxin concentration can induce the formation of adventitious roots also in entirely defoliated and decapitated plants where no abscission ever occurs. In this case, however, this happens at a much slower rate, and the number of roots remains much smaller. These observations are not based on exact estimations of the number of roots or root primordia, as the latter are not well possible since the root formation often is attended and obscured by a strong development of callus.

Under natural auxin conditions the development of new roots was also observed in horizontally placed plants, in which the transport in the lower part of the stem had been blocked. According to the CHOLODNY–WENT theory the meristematic activity at the lower side of the stem should be attributed to an increased auxin concentration at this side, resulting from a lateral auxin transport under the influence of gravity.

MOUREAU (1940) already showed that in *Coleus* the development of adventitious roots induced by auxin also depends on "leaf factors". The latter are produced in the leaves under the influence of light. Sucrose as well as amino acids could exert a promoting effect on the root formation, but they could not entirely replace the influence exercised by the leaves (see chapter VI).

In the dark the effect of a high auxin concentration proximally of the abscission zone is in entire plants the same as that found in explants by GAUR and LEOPOLD (1955). Therefore, the effect which the auxin supplied from without exercises on the abscission, must depend on the induction of a meristematic activity elsewhere in the plant. For the latter leaf factors [1]) produced by the leaves in the light, apparently are indispensable.

FISCHNICH (1935) could show that the basipetal transport of auxin is blocked at those sites where root meristems develop. This would mean that proximally applied auxin can not reach the abscission zones as soon as roots start to develop in the stretch between the place where the auxin is supplied, and the petioles. If this is right, it would also account for the difference in the effect exercised by proximally supplied auxin on the abscission in plants that are kept in the light and in other ones that are kept in the dark, for in the latter no root meristems develop, or if they do they do so much later and in a smaller number.

It may be concluded that in entire plants the effect of high concentrations of auxin which are present proximally of the abscission zone, is an indirect one. In the light it acts through some meristematic

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1) In accordance with MOUREAU (1940) the term "leaf factors" is adopted in this paper to indicate the substance or substances that are produced in the leaves in the light, and that have a promoting effect on the development of roots.

activity which is induced by auxin itself. We must assume that some essential substance is withdrawn from the petioles by this meristematic activity. Consequently in the abscission zone processes are initiated that lead towards a hydrolysis of the primary cell walls in that region. Probably the young leaves and the formation of adventitious roots witdraw the same essential substance from the petioles. This essential substance was indicated as "A.R.S." (Abscission Retarding Substance) [1]). It was shown that in the debladed petioles too this A.R.S. is formed in the light. This would account for the difference in behaviour which completely defoliated and decapitated plants provided with roots show in the light and in the dark. From the observation that in such plants in the light after a removal of the roots abscission did occur, TERPSTRA (1956) concluded that the roots exert an inhibiting influence on the abscission. It has been argued that this inhibiting influence must have been due to the fact that at the base of her plants no new roots were formed.

In the absence of meristematic activity at the proximal side, in the light a sufficient amount of A.R.S. is produced in the debladed petioles to make them self-supporting, i.e. to assure that no abscission takes place. As soon, however, as a meristematic activity starts at the proximal side, this A.R.S. is withdrawn from the petioles, and the hydrolytic processes become prevalent. In the long run the stem, and presumably the roots too, drain this A.R.S. off from the petioles; this follows from experiments with decapitated plants and with explants (in long-lasting experiments with explants of *Coleus* often root formation can be observed, especially after the application of auxin). An indication that the hydrolytic processes which precede the abscission, are not confined to the primary cell walls of the abscission zone, is the decomposition of chlorophyll in the petioles, which, however, remains confined to the latter, the stem remaining green. In *Tropaeolum majus* MEYER (1918) found that the hydrolysis of starch and of chloroplast proteins was promoted by the presence of the growing tip; after decapitation the loss of colour of the leaves proved to be retarded. The formation of roots induced by auxin is also accompanied by a transport of carbohydrates and nitrogen compounds which must owe their origin to such a hydrolysis, towards the centres of meristematic activity (STUART, 1938). GREGORY and SAMANTARAI (1950) found in isolated leaves of dwarf French beans and of ivy that the development of roots which could be induced by auxin, could be reduced by the use of lower light intensities as well as by a removal of the leaf blade; by the supply of sucrose and asparagin this reduction could be eliminated.

Although there is strong evidence that the rate of abscission depends on the rate at which the A.R.S. is withdrawn from the petioles by meristematic activity at the proximal side, there is no indication

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[1]) In this paper, the abbreviation "A.R.S." will be used to indicate the substance from the petioles that has a retarding influence on the abscission. This A.R.S. is produced in the petioles and most probably also in the leaf blades, in the light. The term "leaf factors" therefore includes also the A.R.S.

whatsoever as to the chemical nature of this substance. It is even unknown whether it is only a question of some nutrient or whether a substance of a hormone-like character is involved.

A retarded abscission caused by proximally applied auxin has also been reported, e.g. by PORTHEIM (1941) in debladed petioles of bean leaves and by BARLOW (1950) when 2–4 D and NAA was applied from the proximal side in pedicels of apples. This last result has also been obtained by EDGERTON and HOFFMANN (1948) for the abscission not only in the case of pedicels but also in that of petioles of the apple. With these plants the effect of auxin might be explained by assuming that high auxin concentrations do not induce meristematic activities, so that in their case the auxin transport towards the abscission zones was not interfered with. In that case its action could be compared with that of distally applied auxin (see next chapter).

SUMMARY

1. Under normal light conditions the abscission time depends on
   a. the length of the debladed petioles, and
   b. the meristematic activity at the proximal side of the abscission zone.
2. Under natural light conditions no abscission occurs
   a. when the debladed petioles have a sufficient length, and
   b. when the growth activity at the proximal side of the abscission zone is strongly reduced.
3. When plants are kept in the dark, the abscission proceeds readily under all conditions.
4. The promoting effect of high auxin concentrations at the proximal side is an indirect one; it acts only through the intermediary of some meristematic activity induced by the auxin, e.g. the development of new adventitious roots.
5. Evidence is given of the production in the light of a substance (A.R.S.) in the petioles (and leaves), which inhibits the abscission. Only when this A.R.S. is deviated to sites with meristematic activity at the proximal side of the petioles, the abscission can proceed.

CHAPTER IV

THE EFFECT OF DISTALLY SUPPLIED AUXIN AND SUGARS ON THE ABSCISSION IN PLANTS KEPT IN THE VERTICAL AND IN A HORIZONTAL POSITION

According to the most generally accepted view the abscission of leaves and fruits, is governed by the auxin that is produced by these parts themselves. During the period immediately preceding the abscission no diffusible auxin could be found either in leaves (GAWADI and AVERY, 1950) or in the endosperm of the seeds, which is to be regarded as the main centre of auxin production in fruits (LUCKWILL, 1948,1957). Moreover, abscission of petioles and pedicels can be obtained by removing the part that is borne by them, i.e. the source of auxin. Another argument in favour of this view is that by application of auxin from the distal side the abscission is retarded. However, there are indications that in the absence of an auxin producing part (e.g. leaf blades) abscission of the debladed petioles need not to occur, namely, as shown in chapter III, under the following conditions, 1°. that there is no meristematic activity at the proximal side of the latter, 2°. that they are kept under natural light conditions, and 3°. that they are not too short.

The abscission time further appears to depend strongly on meristematic activity at the proximal side of the abscision zone. The accelerated abscission in horizontally placed plants was ascribed by TERPSTRA (1956) to growth induced by the action of gravity at the proximal side, i.e. to hypotrophy, a thickening at the lower side of the stem. To test the validity of this explanation it seems desirable to study the effect of distally supplied auxin in horizontally placed plants.

1. THE EFFECT EXERCISED BY DISTALLY SUPPLIED AUXIN ON THE ABSCISSION IN VERTICALLY AND IN HORIZONTALLY PLACED PLANTS

Each experiment comprised 100 plants, 50 of which were placed horizontal one day before the test petioles were debladed. Both groups of plants were divided in 5 lots of 10 plants each. In 4 lots auxin was supplied from the distal side in concentrations of 1., 0.1, 0.01 and 0.001 mg/l IAA. The 5th lot served as a control, and here the tips of the petiole stumps were provided with plain lanolin paste.

The result of 3 of the experiments are represented in Fig. 8. The P-values were calculated from the variance analysis of these 3 experiments. Each value is the mean abscission time of 60 petioles.

The abscission in the vertically as well as in the horizontally placed plants is distinctly retarded by 1 mg/l IAA, but significantly accelerated by 0.01 mg/l IAA as compared to the controls. This matches the results obtained by GAUR and LEOPOLD (1955). Further it is evident

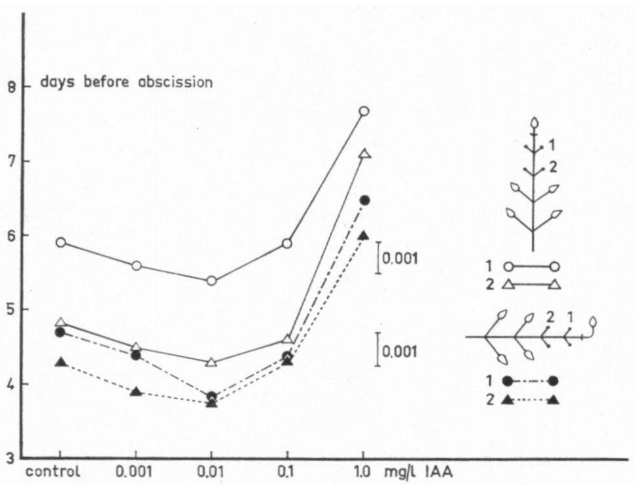

Fig. 8. Effect exercised by distally supplied auxin on the abscission of debladed petioles in plants kept respectively in the vertical and in a horizontal position. Each point represents the mean abscission time of 60 petioles. The statistical level of significance (P-value) of the differences between the various series is shown also.

that the difference in abscission time found between the plants in the vertical and those in a horizontal position, more precisely the acceleration of the abscission in the horizontal position, is independent of the auxin supply. This indicates that the abscission is not governed exclusively by the auxin produced by the part that is to be shed, but that it depends also on other factors. It is known from the literature that the abscission is retarded when sugar is supplied, and since with the leaf blade the main centre of sugar pruduction is removed, the effect of sugar supplied from outside had to be investigated too.

2. THE EFFECT EXERCISED BY DISTALLY SUPPLIED SUGAR ON THE ABSCISSION IN VERTICALLY AND IN HORIZONTALLY PLACED PLANTS

From preliminary experiments in which solutions of 3 % glucose and 6 % sucrose were emulgated with lanolin and distally applied on the petioles, it appeared that at least in intact plants the abscission is delayed.

In the next experiments the effect exercised on the abscission by 0.1 mg/l IAA, 3 % glucose and 6 % sucrose was tested in vertically and in horizontally placed plants. For each treatment 10 plants were used, and each experiment was repeated three times. From Fig. 9, in which the results are given, it is clear that the abscission is retarded by sugar as well as by 0.1 mg/l IAA supplied from the distal side. The acceleration of the abscission in the horizontal position, however, is not influenced by the supply of sugar or of IAA.

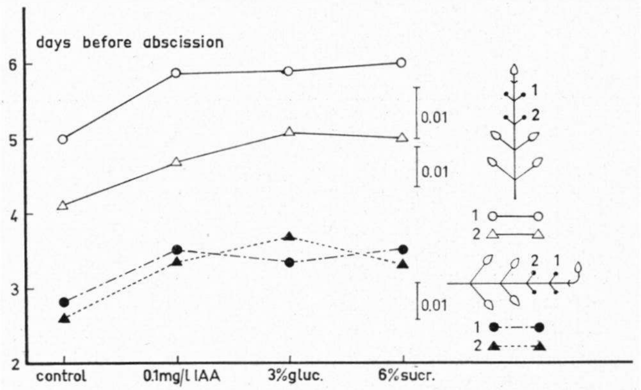

Fig. 9. Effect exercised by distally supplied auxin and by glucose and sucrose on the abscission of debladed petioles in plants kept respectively in the vertical and in a horizontal position. Each point represents the mean abscission time of 20 petioles. The statistical level of significance (P-value) of the difference between the various series is shown also.

3. THE EFFECT EXERCISED ON THE ABSCISSION IN PLANTS THAT ARE KEPT IN THE VERTICAL AND IN A HORIZONTAL POSITION, BY SIMULTANEOUSLY SUPPLIED AUXIN AND SUGAR

Each experiment comprised 6 series of 10 plants standing vertical and 6 series of 10 plants that were kept in a horizontal position. The lanolin paste that was applied distally on the petioles, was emulgated with

series 1: water (control)
,, 2: 1 mg/l IAA
,, 3: 3 % glucose solution
,, 4: 6 % sucrose solution
,, 5: 1 mg/l IAA + 3 % glucose
,, 6: 1 mg/l IAA + 6 % sucrose

The experiment was repeated three times and similar experiments were carried out with auxin concentrations of 10 and 100 mg/l IAA. In Fig. 10 the results are given of an experiment in which 1 mg/l IAA had been used. By applying sugar and IAA simultaneously, the retarding effect of IAA is increased. This result was, however, not obtained in all experiments; so, for instance, it was not found when during the winter months solutions of 10 mg/l IAA, and during the summer solutions of 100 mg/l IAA were used. In these instances the very strong retarding effect of auxin was not increased by a simultaneous dose of sugar. Fig. 11 gives the result of one of these experiments; the latter was carried out with plants in the vertical position.

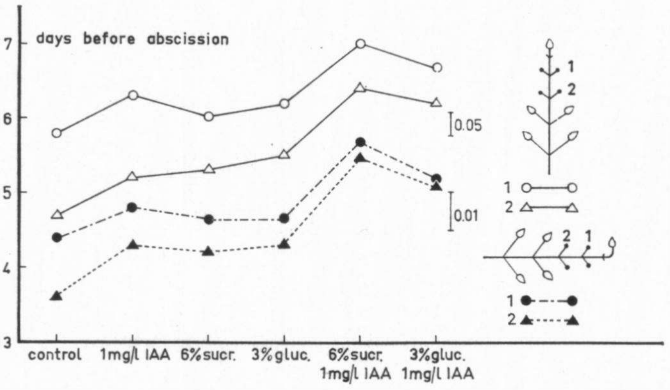

Fig. 10. Effect exercised by a simultaneous supply of auxin and sugar to the distal side, on the abscission of debladed petioles in plants that were kept respectively in the vertical and in a horizontal position. Each point represents the mean abscission time of 20 petioles (see also fig. 9).

It appears once more that the difference in the abscission time found between plants in the vertical and in a horizontal position, is independent of a simultaneous supply of sugar and auxin.

It must be stressed that in none of the experiments thusfar dealt with, a significant difference in the abscission time was found between the petioles at the lower and those at the upper side of the plants that were placed in the horizontal position.

4. THE EFFECT EXERCISED ON THE ABSCISSION IN DECAPITATED PLANTS BY DISTALLY SUPPLIED AUXIN

TERPSTRA (1956) did show already that the difference in the abscission time found between plants in the vertical and in a horizontal position, is independent of the auxin level at the proximal side. It now was found to be independent of the auxin concentration at the distal side too.

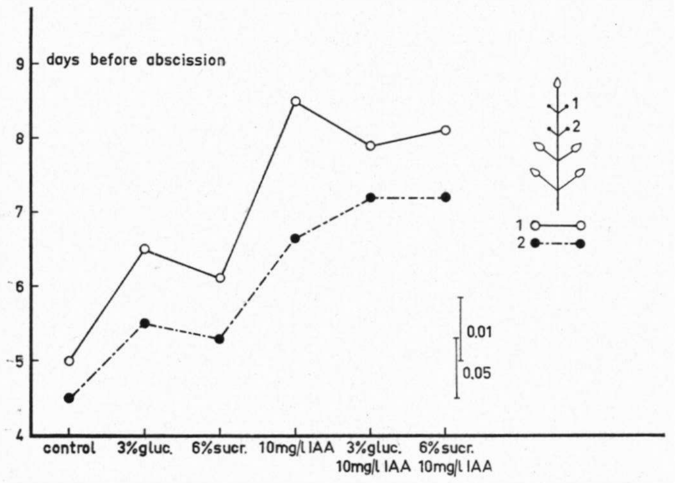

Fig. 11. Effect exercised by a simultaneous supply of auxin in a high concentration and of sugar on the abscission of debladed petioles. Each point represents the mean abscission time of 20 petioles (see also fig. 9).

According to TERPSTRA the acceleration of the abscission in horizontally placed plants did not occur when the plants were decapitated and the petioles debladed two days after the plants had been brought in the horizontal position. This suggests that the acceleration of the abscission by the action of gravity depends on the presence of the tip and the young leaves. In horizontally placed plants an increased growth is noted at the lower side, and this increased growth is obviously induced by auxin produced by the tip and the young leaves. In vertical plants too the abscission is, as we have seen, accelerated by meristematic activity (development of roots) at the proximal side of the debladed petioles. On the other hand the abscission proved to be accelerated also after the application of auxin in a low concentration (0.01 mg/l IAA) from the distal side. The question arises whether the latter effect also occurs in the absence of meristematic activity at the proximal side, i.e. in decapitated plants. Therefore, in a number of experiments the effect of distally supplied auxin in concentrations of 0.1, 0.01 and 0.001 mg/l IAA was tested in decapitated plants. In none of these experiments a consistent difference in abscission time could be found between the petioles that had been treated with auxin, and the controls.

5. DISCUSSION

Since LAIBACH (1933) discovered that in *Coleus* the abscission of debladed petioles could be retarded by the application of auxin, it is generally believed that intact leaves (WETMORE and JACOBS, 1953), and young fruits too (LUCKWILL, 1948, 1953), are protected against abscission by the auxin that is continuously produced by these parts themselves. This view found support in the discovery that the auxin production of the latter is strongly reduced in the period just prior to the abscission. The experiments described above indicate that besides IAA (and analogous auxins) there must be other substances which exercise an essential influence. In chapter III it has already been reported that under natural light conditions no abscission occurs when there is no meristematic activity at the proximal side, notwithstanding the fact that no auxin is provided at the distal side.

From the experiments whose results were reported in this chapter, it appeared that the difference in abscission time between vertically and horizontally placed plants is not influenced by the various concentrations of auxin and by the sugars that were supplied from the distal side.

In analogy to the acceleration of the abscission caused in vertical plants by meristematic activity at the proximal side (e.g. in the tip of the stem or at the sites where new adventitious roots are developing), the abscission in horizontally placed plants seems to be accelerated by growth induced by the action of gravity. This growth consists in a thickening at the lower side and sometimes also in the formation of adventitious roots at that side.

The acceleration of the abscission in horizontally placed plants occurs only in the presence of the tip and the young leaves or, in decapitated plants, when auxin is supplied from the proximal side (TERPSTRA, 1956). In both cases the auxin acts on the abscission through the development of new adventitious roots, but in the horizontal position a lateral shift of the auxin to the lower side might cause a further increase of the auxin concentration at that side, and so enhance the meristematic activity.

There are three arguments for our assumption that in the horizontal position too the acceleration of the abscission is only indirectly affected by the auxin. In the first place, the abscission time of petioles inserted at the lower side of the stem, where the auxin concentration at the proximal side presumably is higher, is the same as that of the petioles inserted at the opposite side. Secondly, the difference in the abscission time between vertically and horizontally placed plants was found to be independent of distally applied auxin at all tested concentrations. Thirdly, the acceleration of the abscission by the application of a low auxin concentration (0.01 mg/l IAA) at the distal side was only found in intact, non-decapitated plants.

The abscission in the vertical as well as in a horizontal position is governed primarily by meristematic activity at the proximal side. By this activity the petioles are deprived of the A.R.S. which inhibits the metabolic changes leading to abscission. It was shown in chapter III that this A.R.S. is produced in the petioles in the light. It is not identical with IAA or with a sugar, though these substances too may delay the abscission. Already in 1916 KÜSTER has found that the leaf contains a substance that inhibits the abscission, and that this substance remains active even when the vascular system of the petiole has been clogged. This indicates that this substance can migrate through the parenchyma towards the abscission zone. In the next chapter this question will be treated in more detail.

It is still obscure in which way auxin acts on the abscission. It does not seem unlikely that IAA and analogous auxins affect the migration of the A.R.S. that is present in the petioles. Auxin might cause changes in the physico-chemical system of the protoplasm, e.g. in the latter's permeability for water.

The permeability of the protoplasm for water shown by the epidermal cells of *Rhoeo discolor* is increased by low concentrations of auxin (10^{-5} — 10^{-7} g/cc IAA) and strongly decreased by high ones (10^{-4} g/cc IAA) (VON GUTTENBERG and BEYTHIEN, 1951). Ethylene, a substance that, although no auxin, causes a strong acceleration of the abscission (HALL, 1952), enhances the permeability for water at any concentration. SACHER (1957) came to the conclusion that auxin (IAA and NAA) retards the abscission in explants of *Phaseolus* because it exercises an effect on the permeability of the protoplasmic membranes. The intercellular spaces of explants that were treated with auxin, were after 2½–3 days filled with air, whereas those of the controls had become infiltrated with fluid. He was of opinion that auxin maintains the selective permeability of the membranes, and so prevents exosmosis of cellular solutes to the intercellular spaces.

ANKER (1953) pointed out that auxin, once it has entered into the protoplasm, might act also upon lipophilic phases inside the latter (e.g. on the membranes of mitochondria and of microsomes), and might change in this way the enzymatic activity and consequently the metabolism.

The way in which carbohydrates exert an influence on the abscission, is also completely unknown. ADDICOTT and LYNCH (1955) mention the possibility that a sufficient supply with carbohydrates might reinforce the cell walls of the tissue, thus making abscission less easy. This does not seem likely, since the difference in the abscission time found between plants in the vertical and in a horizontal position, proved to be independent of distally supplied sugars.

In explants there is no abscission when there is a deficiency of carbohydrates. BIGGS and LEOPOLD (1957) found that abscission took place when sucrose was supplied in low concentration at the proximal side; high concentrations of sucrose, however, had a retarding effect. Several authors believe that carbohydrates would yield the energy required for the abscission process (SAMPSON, 1918; CARNS *et al.*, 1951; ADDICOTT *et al.*, 1955). This, however, can not account for the difference in effect between low and high sucrose concentrations (BIGGS and LEOPOLD, 1957). The supply of carbohydrates to tissues which are deficient in this material, might activate some growth activity at the proximal side of the petioles, and deprive the latter in this way of the A.R.S.

Often in the abscission zone more starch is present than in the tissue in the neighbourhood (LIVINGSTON, 1950; GRIESEL, 1954). In some plants the starch disappears during the abscission, but in other plants on the contrary starch is actually deposited during the abscission. Nothing is known of the way in which the transport of carbohydrates is regulated, nor of the way in which starch is utilized in the abscission zone, but the irregularity found in the behaviour of this substance in various plants suggests that these processes are of secondary importance only.

Prior to the abscission the chlorophyll in the petioles is decomposed (GAWADI and AVERY, 1950; BROWN and ADDICOTT, 1950). According to OLMSTED (1951) the decomposition of chlorophyll itself does not initiate the abscission. Ethylene, which strongly accelerates the abscission, also accelerates the decomposition of chlorophyll (HALL, 1952). The products of the decomposition of chlorophyll probably do not influence the abscission itself, since the shorter the stumps of the petioles and the smaller, therefore, the amount of these products, the more rapidly the abscission proceeds.

In leaves the breakdown of chlorophyll is closely related to that of the proteins. The decomposition of chlorophyll in the leaves of *Tropaeolum* can temporarily be retarded by the supply of glucose (MICHAEL, 1935/1936). Possibly an excess of (soluble) carbohydrates could temporarily inhibit the progress of hydrolytic processes, and by doing so also retard the abscission.

Simultaneous supply of IAA and sugar causes a stronger retardation of the abscission than a supply of IAA or of sugar alone does. Perhaps the transport of auxin is accelerated by the presence of a sufficient amount of carbohydrates (WEINTRAUB and BROWN, 1950). The retarding effect of sugar on the abscission, however, depends on the IAA concentration. If this concentration is very high, the effect of auxin is not increased by sugar.

MEINL and VON GUTTENBERG (1952) found that the protoplasm in the epidermal cells of *Rhoeo discolor* became practically impermeable for glucose when these cells were supplied with a high concentration of auxin (10^{-4} g/cc IAA). This is the same concentration at which the simultaneous supply of sugar caused no longer an additional inhibiting effect on the abscission. The uptake of sucrose from a nutrient solution by isolated pea roots of which the growth was inhibited by a 10^{-8} Mol IAA solution, was only 27 % of that of the controls which received no auxin (BANDI, 1957); this indicates a decrease of the permeability for sucrose too.

SUMMARY

1. The abscission is retarded when the distal side is supplied with auxin at high concentrations (1 mg/l IAA or more), eventually in combination with sugar.

2. By supplying the distal side with auxin in low concentrations (0.01 mg/l IAA) the abscission is accelerated, but only when there is at the proximal side a sufficient meristematic activity.

3. The accelerated abscission noted in plants that are brought in a horizontal position, must be due to growth activity induced at the lower side of the stem by the action of gravity.

4. The difference in abscission time between plants in the vertical and in a horizontal position is independent of the auxin concentration at the distal side and also of the effect of distally supplied sugar.

5. New indications were obtained for the presence of a substance (A.R.S.) (see item 5 of the summary of chapter III, p. 23) which is produced in the light in the petioles (and leaf blades). The abscission zone may be deprived of this A.R.S. by meristematic activity at the proximal side.

In the next chapter further investigations with regard to the A.R.S. are reported.

CHAPTER V

EXTRACTION FROM FULL-GROWN LEAVES OF A SUBSTANCE WHICH RETARDS THE ABSCISSION

The results of some of the experiments discussed in the preceding chapters have given evidence of the presence in the petioles of a substance (A.R.S.) that retards the abscission, and that is synthesized in the light. This A.R.S. may presumably be withdrawn from the petioles by meristematic activity at the proximal side and is not identical with IAA.

Küster (1916) already showed that a substance which retards the abscission, may be transported from the leaf blade to the abscission zone independently of the vascular strands. In our own experiments it was found that the same holds true for the A.R.S. that is formed in the petioles.

In these experiments the petioles of the 2nd and 3rd leaf pair were debladed; the length of these two pairs of petioles was 0.5 and 3 cm respectively. At the lower side of the petioles of 3 cm and at a distance of a 0.5 cm from the abscission zone a ± 2 mm wide incision was made; this meant that only a bridge of parenchyma and of epidermis was left. To prevent a too strong loss of water the plants were placed under a glass-bell.

The mean abscission time of the short petioles proved to be 3 days, whereas of the long petioles with interrupted vascular bundles after 3 weeks 80 % were still attached to the stem. Part of the A.R.S. is doubtless produced in the upper part of the petioles, and the experiment proves that this part must be able to reach the abscission zone via the parenchyma, otherwise there could have been no retardation of the abscission in the longer petioles.

By means of paper chromatography Luckwill (1956, 1957) could separate from the leaves and seeds of the apple four substances with auxin activity, none of which was identical with IAA. Only one of these substances had a retarding effect on the abscission. This so-called „auxin-2" also gave positive reactions in the coleoptile-cylinder test and in the bean-rooting test.

During the growth period the fruit fall in *Ribus nigrum* proved to be negatively correlated with the concentration of one of these auxin-like substances produced in the fruits (Wright, 1956). This auxin showed a deferred Salkowski reaction, and probably is an indole derivative.

The concentration in which this substance occurred in the fruits, was not correlated with meristematic activity.

The indications thusfar obtained by indirect means for the assumption that the leaves and petioles of *Coleus* too would contain a substance not identical with IAA but exercising a retarding influence on the abscission, were checked in the next series of experiments, in which an attempt was made to extract this substance and to isolate it by means of paper chromatography.

1. METHOD

The method of LUCKWILL (1957) was applied, though with a few alterations. The leaves were not dried at 70°, but directly sliced and immersed in peroxide-free ether.

In each experiment circa 200 g leaves (fresh weight) were extracted during 18 hrs at 4° C. After filtering, the ether was evaporated, and the residue extracted 8 times with 25 ml aq. dest. at ± 40 ° C. The water extracts were collected in the same vessel, and the entire amount was shaken 8 times with ether. Each of the ether fractions then was shaken 5 times with 10 ml of a 8 % $NaHCO_3$ solution. The remaining fraction contains the neutral auxins, as the acid auxins have passed into the alkaline solvent. The combined bicarbonate fractions were acidified with 1 N HCl to a pH = 4. LUCKWILL used to this purpose tartaric acid, and at first this was also used in our own experiments. However, in this case in the chromatograms typical "tails" occurred. From the startline up to a Rf = 0.32 a substance which retards the abscission, was present, though the maximum activity was found between Rf = 0.28 and 0.32. However, when the acidifying was carried out with HCl, there were no "tails" whatsoever.

The acidified solution was shaken 4 times with ether. This ether extract was dried over $MgSO_4$ anhydride and then evaporated again. This residue was dissolved in 0.1 ml ethanol and brought on the start line of the paper (Whatman No. 1). The paper strip was 9 cm wide. Parallel to the chromatogram strip proper we brought on the paper, as a marker, ca 2 μg IAA dissolved in an aliquot of the same extract. When after a few hours equilibrium had been reached, the chromatogram was developed by means of the ascending method with a butanol-water-ammonia mixture as a solvent. After 18 hours, the front then having reached a distance of about 25 cm from the start line, the chromatogram was dried, and the control strip removed. By means of cinnamic aldehyde and HCl vapour (WIELAND and BAUER, 1951) the IAA was spotted, and its Rf determined.

According to the prescription of LUCKWILL (1951), the chromatogram proper was tested by means of the *Coleus* abscission-test on its faculty to retard the abscission. To this end the chromatogram was divided parallel to the start line in strips with a width of 1 cm. Out of these strips paper discs of 0.5 cm diameter were punched. As a control we used paper discs of a blank chromatogram, in which only the solvent had run. After the discs had been wetted with aq. dest., as a rule 2 of them were placed on the top of a petiole of an explant. The latter were all of the same physiological age. The explants were split longitudinally, and one of the halves was used as control (blank paper discs). By these precautions the variability of the test material was strongly reduced. By this method 6 explants could be treated with discs from a single 1 cm strip (i.e. 2 × 6 petioles, viz. 6 with discs of the blank and 6 with discs of the real chromatogram). The explants were placed in petri dishes on wet filter paper, and the dishes stood in an air-conditioned dark room at 22° C.

The neutral ether fraction was treated in a similar way. However, this fraction can be left out of consideration, since it did not contain any substance that retarded the abscission. The reported results therefore refer only to the acid fraction of the ether extract.

2. THE ACTIVITY OF THE ACID FRACTION

Fig. 12 shows what influence the different zones of the chromatogram exercised on the abscission of petioles in vitro. This result was obtained in three successive experiments. The maximum retardation of the abscission is found just in front of the IAA spot. In fig. 13 the result is given of an experiment in which the strip between Rf = 0.20 to 0.36 was washed out with ether, after which the eluate was chromatographed a second time. By this treatment the Rf of the unknown substance had not changed. It was not changed either when the eluate was chromatographed by means of another solvent (isopropanol-water). In this case too the maximum activity was found just in front of the IAA spot. The acceleration of the abscission indicated in Fig. 12 for the strips with the Rf values 0.40–0.50 will probably have been caused by some toxic pigments that were present in this part of the chromatogram.

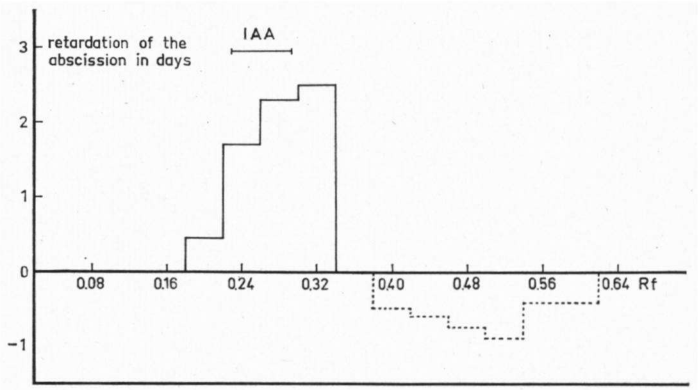

Fig. 12. Activity of the successive zones of a paper chromatogram obtained from the acid fraction of an ether extract from full-grown leaves of *Coleus*, as found in the *Coleus* abscission-test. The zero line represents the abscission time of the petioles found in the explants which served as controls. The Rf of IAA was determined in the same chromatogram by means of a colour reaction.

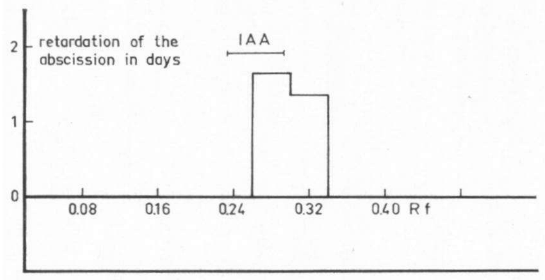

Fig. 13. Activity of the successive zones of a paper chromatogram obtained from the eluate that was made from a part of a chromatogram (Rf 0.24–0.36) obtained from the acid fraction of an ether extract from full-grown leaves, as found in the *Coleus* abscission-test (see Fig. 12).

3. NEGATIVE RESULT OF OUR ATTEMPT TO IDENTIFY THE SUBSTANCE THAT MUST BE PRESENT IN THE ACID FRACTION OF THE ETHER EXTRACT AS AN INDOLE DERIVATIVE.

In the next experiment the physiological activity of the acid fraction of an ether extract of 200 g leaves (fresh weight) was estimated in combination with chemical reactions on indole derivatives. The zone between Rf = 0.20 and Rf = 0.40 of the first chromatogram was washed out with ether. The paper for a second chromatogram was divided in 4 longitudinal strips with a width of 2.5 cm. On the start line were brought respectively 5 μg IAA, half of the eluate, 5 μg IAA and the other half of the eluate. After developing the chromatogram, the four strips were separated. On one strip with IAA and on one with eluate the reaction with cinnamic aldehyde/HCl vapour was carried out. Only the strip with IAA gave a positive reaction. The two other strips were tested in the *Coleus* test in the way described above. The results are given in Fig. 14. Again the Rf of the unknown substance is found to be slightly higher than the Rf for IAA. Its activity in retarding the abscission is at least equal to, if not higher than that of 5 μg IAA. From the fact that the unknown substance has at least the same activity as 5 μg IAA, but that it does not give the reaction with cinnamic aldehyde /HCl vapour, it follows that it

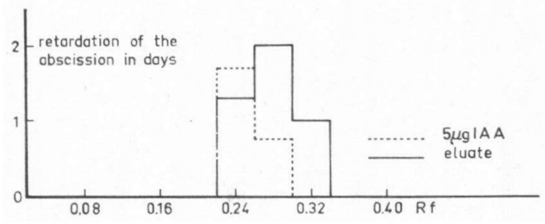

Fig. 14. Activity of the successive zones of a paper chromatogram obtained from the eluate that was made from a part of a chromatogram (Rf 0.24–0.36) obtained from the acid fraction of an ether extract from full-grown leaves, and of 5 μg IAA, as found in the Coleus abscission-test (See Fig. 12).

cannot be identical with IAA, and that it is most probably not an indole derivative either.

In order to find further evidence in favour of the latter conclusion a number of reactions on indole derivatives were applied. In successive tests the residue of the acid fraction of an ether extract of 250 g leaves was dissolved in ethanol, and brought as a spot on the paper for chromatography. After the chromatogram had been developed and dried, the following reactions were tried; they all yielded a negative result:

1°. the SALKOWSKY-test according to GORDON and WEBER (1951) with $FeCl_3$ and $HClO_4$.
2°. EHRLICH's test on indole-scatole compounds (LINSER and KIERMAYER, 1957) with p-dimethylamino-benzaldehyde +HCl.
3°. the nitrose reaction according to VON DENFFER *et al.* (1952) with a 1 % ethanol solution of $NaNO_2$ / HCl vapour. By means of this test an amount of 0.1 μg IAA is still identifiable.

The conclusion, therefore, seems justified that the substance which retards the abscission, can not be identified with an indole derivative. Since the Rf value of this substance is almost the same as that of IAA, it is not excluded that small quantities of IAA are present at the same spot.

4. THE EFFECT EXERCISED BY A SIMULTANEOUS SUPPLY OF IAA AND OF THE SUBSTANCE CONTAINED IN THE LEAF EXTRACT ON THE ABSCISSION IN EXPLANTS

In order to find out whether the action of the substance which retards the abscission, is obscured or not by the presence of IAA, the zone which in the chromatogram obtained from the acid fraction of the ether extract is found between Rf = 0.28 and Rf = 0.30, was washed out with ether. After evaporation the residue was dissolved in 0.1 ml ethanol. By means of a micropipet (of 5 μl) paper discs were soaked with:

a. 5 μg of the extract
b. 0.05–0.1 μg IAA (ethanol solution)
c. 5 μg of the extract + 0.05 — 0.1 μg IAA (*a* + *b*)
d. 5 μg of blank ethanol.

After these discs had been dried and subsequently wetted with aq. dest., they were placed in the way described above on the top of petioles of longitudinally split explants. The results of these experiments are given in Table 2. The extracts were always prepared from 200–250 g leaves (fresh weight).

It appears that the effect on the abscission exercised by different concentrations of IAA is not enhanced by the addition of the extract to such a degree as might have been expected if the substance with the retarding effect in the latter was identical with IAA. Only in the experiments 4 and 5, in which the retarding effect of the extract alone was stronger than in the other experiments, the effect of 0.1 and 0.2 μg IAA was slightly enhanced. This extra retardation might have been

TABLE 2

The influence exercised on the abscission of *Coleus*-petioles in vitro by a simultaneous supply of IAA and of an extract from full-grown leaves of *Coleus* (eluate from a chromatogram of the acid fraction)

	Mean abscission time in days (of 6 petioles)																	
Exp.	5 mm³ eluate	control	0.05 μg IAA	control	0.1 μg IAA	control	0.2 μg IAA	control	0.5 μg IAA	control	0.05 μg IAA	0.05 μg IAA + 5 mm³ eluate	0.1 μg IAA	0.1 μg IAA + 5 mm³ eluate	0.2 μg IAA	0.2 μg IAA + 5 mm³ eluate	0.5 μg IAA	0.5 μg IAA + 5 mm³ eluate
1	4.3	3.3							8.3	3.3							8.5	8.3
2	2.5	1.3			3.3	1.5			8.8	1.5			3.8	4.1			10.5	9.5
3	3.2	2.0	3.2	2.8	4.3	3.2					3.0	3.2	3.2	3.7				
4	3.3	1.0			3.0	1.0							3.0	4.0				
5	3.3	1.3			3.6	2.3	5.6	1.3					5.6	6.3	4.6	6.0		

The values in each pair of columns refer to petioles of the same explants.

caused by small quantities of IAA that were present in the part of the chromatogram of which the effect was tested.

5. THE ACTIVITY OF THE ABSCISSION-RETARDING SUBSTANCE FROM THE LEAF EXTRACT IN THE STANDARD AVENA-TEST

From the mere fact that a substance retards the abscission, can not be concluded that this substance is an auxin. Therefore, the zone of the chromatogram corresponding with the Rf of IAA and the zone just in front of this Rf were washed out and tested on auxin activity in the standard *Avena* curvature-test; the latter was carried out according to the method used by TERPSTRA (1953).

For each experiment the acid fraction of an ether extract obtained from 200 g leaves (fresh weight) was chromatographed in butanol-water-ammonia. Of both eluates a series of dilutions was made, since it was possible that the auxin concentration of the eluate would be supra-optimal.

In both zones of the chromatogram a strong auxin activity was demonstrated. The highest activity was found once more in the zone just in front of the IAA spot. This appeared from the fact that for the eluate obtained from the IAA spot the largest curvature was obtained with a dilution of 1:10, and for the eluate obtained from the zone in front of the latter with a dilution of 1:100.

6. ULTRA-VIOLET ABSORPTION BY THE ABSCISSION-RETARDING SUBSTANCE FROM THE LEAF EXTRACT

The acid fraction of the ether extract obtained from 200 g leaves (fresh weight) was chromatographed in the way described above (in butanol-water-ammonia). The zone corresponding with the spot of IAA and that just in front of the latter (1.5 cm wide), were washed out separately with ether. From a second chromatogram, in which 5 μg IAA had run, the zone with the Rf of IAA and a 1.5 cm blank zone in front of the latter were washed out in the same way. After evaporation of the ether each residue was dissolved in 2.5 ml ethanol. The solutions obtained in this way are perfectly clear. The coloured compounds that are present in the chromatogram, were apparently strongly adsorbed to the paper, and could not be washed out with ether.

The U.V. absorption was determined by means of a ZEISS spectrophotometer. The solution of the blank eluate was used as zero. The absorption spectra are reproduced in Fig. 15.

The maximum absorption of the washed out IAA was found at $\lambda = 2700$ Å, that is at a shorter wave length than that at which it was found by NITSCH (1956) and VAN DE VEERDONK (1956), who found the peak at $\lambda = 2800$ Å.

The absorption spectra of the parts of the acid fraction of the ether extract that were washed out from the zone of the IAA spot and from the zone just in front of the latter (1.5 cm wide), are virtually identical. The quantitative differences indicate that the concentration of the absorbing compound is higher in the zone in front of that of the IAA-spot than at the IAA-spot itself. This matches the results of the abscission test and of the standard *Avena*-test, for in the latter too the highest activity was found in this zone of the chromatogram.

The absorption spectrum of this substance shows several maxima; there are peaks at $\lambda = 2400$ Å, $\lambda = 2800$ Å and $\lambda = 3150$ Å. It can not be decided whether this is the absorption spectrum of a single compound or of a mixture of substances. However, it is obvious that the spectra of both Rf zones are identical, which means that in both zones the same compound or compounds are present. The peak at $\lambda = 2800$ Å might indicate the presence of IAA, although the maximum of pure IAA, chromatographed and washed out in the same way as the extract, was found at $\lambda = 2700$ Å. The higher Rf

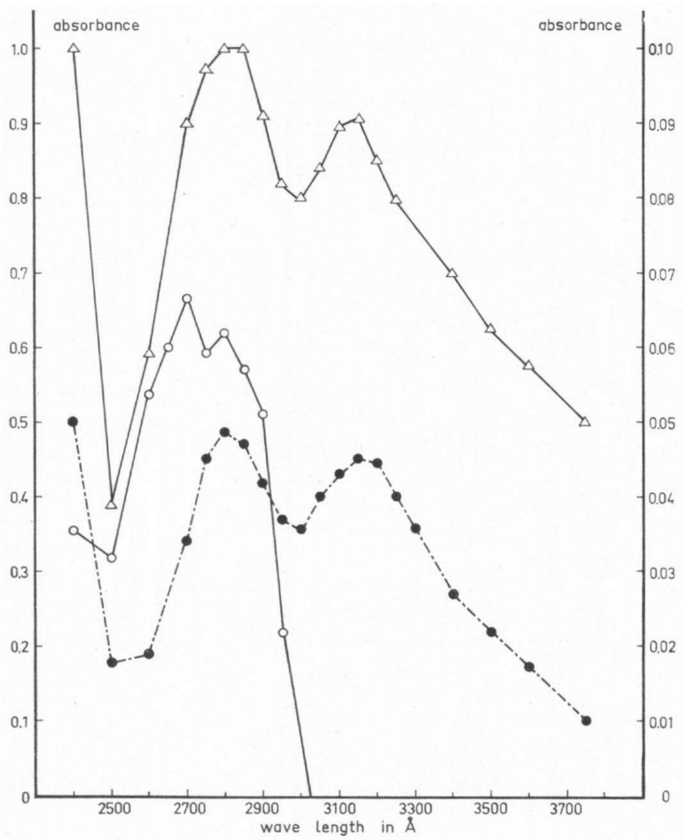

Fig. 15. Ultra-violet absorption spectra of the eluates obtained from parts of a chromatogram; ○——○: eluate of 5 μg chromatographed IAA (right scale); ●- - - -●: eluate from a part of the chromatogram obtained from the acid fraction of an ether extract from full-grown leaves of *Coleus*, viz. from the part corresponding with the spot of IAA (left scale); △——△: eluate from the part of the chromatogram just in front ($1^1/_2$ cm) of the spot of IAA (left scale).

value and the negative results of all reactions on indole compounds, however, are not in favour of the identity of the preparation obtained from the leaves with IAA.

7. Discussion

It was shown by means of paper chromatography that in ether extracts of *Coleus* leaves an auxin-like substance is present that is not identical with IAA, and most probably not with any other indole derivative. The Rf value, however, differs only slightly from the Rf of IAA. Since all reactions on the indole nucleus were negative, it had to be concluded that this substance is not an indole compound. The negative reactions can not be caused by a too low concentration of the substance in the extract. A known quantity (5 μg) of IAA gave positive reactions. The unknown substance caused a retardation of the abscission which is at least as strong as that of this quantity of IAA (Fig. 14), but it failed to give any indole reaction. Elution did not change the Rf of this substance; in a new chromatogram it was found at the same place.

Terpstra (1953) found that the Rf value of IAA was affected by one or more components of the extract from *Avena* coleoptiles. The activity found by her at a somewhat higher Rf, always was lower than that of the spot corresponding with the Rf of IAA. However, in her experiments the IAA used as marker was not chromatographed together with the extract. In the present experiments the IAA was always chromatographed mixed with the same amount of extract as ran through the chromatogram proper. An effect exercised on the Rf of the IAA by other substances that may have been present in the extract, therefore can not account for the difference in Rf between the IAA used as marker and the abscission-retarding substance from the leaves, the auxin character of which was shown in the *Avena* curvature-test.

It seems that the abscission is affected by this substance from the leaves in a way that is different from that in which it is affected by IAA. Luckwill (1956) found by means of the same method as was used in our own experiments, a linear relation between the applied quantities of IAA and NAA and the retardation of the abscission. For IAA this rectilinear relation was found for quantities of 1 to 10 μg IAA per petiole. Between 0.01 and 0.1 μg IAA per petiole the test failed. The material used in our own experiments apparently was more sensitive to IAA, since a consistent retardation of the abscission was found with 0.1 μg IAA. The addition of a certain amount of the abscission-retarding substance from the leaves did not or did only slightly increase the effect of IAA. If IAA and the substance from the leaves were identical or even if they were only synergistic, a simultaneous application of IAA and of the abscission-retarding substance from the leaves would have caused a much more pronounced retardation of the abscission than IAA alone did.

From experiments with intact plants it was concluded in chapter IV that IAA applied at the distal side affects the abscission only indirectly, viz. via an influence on the transport of the A.R.S. It is possible that after simultaneous application of IAA and of the abscission-retarding substance from the leaves, the IAA hampers the migration of the latter. For the effect of a simultaneous supply of sugars and of a high concentration of IAA a similar explication was proposed.

The fact that the abscission-retarding substance from the leaves gives a positive reaction in the standard *Avena*-test, increases the probability of our hypothesis according to which the A.R.S. would be involved in other processes, viz. in meristematic activity elsewhere. The extracted abscission-retarding substance most probably is identical with the A.R.S., although there is no definite evidence that this is so. In the next chapter it will be shown that the A.R.S. mainly acts upon the development of root primordia and probably also on the growth of roots.

Just like "auxin-2" from the leaves and seeds of the apple (LUCKWILL, 1957) and "auxin-2" from the fruits of *Ribes nigrum* (WRIGHT, 1956), the production of the A.R.S. does not seem to be associated with meristematic activity. According to several authors (VAN OVERBEEK, 1933; NAVEZ, 1933; THIMANN and SKOOG, 1934) auxins acting upon cell elongation are synthesized only in the light. WIEDOW and VON GUTTENBERG (1953), however, believed that the synthesis of IAA does not require light, whereas another acid-proof auxin would be produced only in the light, although independently of the photosynthesis.

In young explants of *Phaseolus vulgaris* the abscission of the petioles is retarded under the influence of light (BIGGS and LEOPOLD, 1957). This retardation of the abscission, however, was also obtained by a supply of sucrose at a high concentration. For this reason the effect of light was regarded by these authors as indirect; light would act by the intermediary of the products of photosynthesis. In explants of medium-aged plants the abscission is retarded only by a supply of sucrose, but not by light. As pointed out by the authors, light might not only act by the intermediary of photosynthesis but also in another way. In the petioles of young *Phaseolus* plants some auxin might be synthesized in the light, and the effect of sugars in normal explants might have a secondary meaning. In old explants the synthesis of auxin would no longer be possible; in the latter a supply of sugars too is ineffective. The secondary meaning of photosynthesis also follows from the results of other experiments carried out by BIGGS and LEOPOLD. By the application of 10^{-5}–10^{-4} Mol NAA the abscission was accelerated only in the light. Light alone, however, had a retarding influence. At higher concentrations, however, NAA retards the abscission to the same extent in the light as in the dark, independently, therefore, of photosynthesis.

As the results of our own experiments with IAA prove, at low concentrations NAA may accelerate the abscission by affecting the A.R.S. in the petioles. Since in intact plants this accelerating effect on the abscission by low concentrations of IAA applied from the distal side, appeared to depend on meristematic activity at the proximal side of the petioles, it was concluded that low concentrations of IAA and NAA enhance the migration of the A.R.S. from the petioles.

In an investigation aimed at the solution of a quite different problem, BOTTELIER (1954) found in the petioles an interaction between a substance produced in the light and the IAA supplied from outside. When petioles of *Ageratum houstonianum* were submersed in a solution of IAA, an epinastic curvature was produced, but in young petioles this happened only in the light. However, in old petioles this curvature was produced in the light as well as in the dark. If the leaf blade was not removed, the epinastic curvature of the young petioles was produced also in the dark (BOTTELIER, 1956). It seems probable that this substance, which is produced in the light and which is present in the leaf blades, is identical with the A.R.S.

It was not chromatographically tested whether in the apical meristem and in the young leaves still other auxin-like substances occur. There are indirect indications that this actually is so. The development of adventitious roots under the influence of an auxin from the young leaves as well as under that of IAA supplied from outside, for instance, proved to be correlated with an accelerated abscission. Although IAA is present in excess, the A.R.S. is still withdrawn from the petioles.

It was not possible to identify the abscission-retarding substance from the leaves. The shift of this substance from an acidified aqueous solution to ether is an indication of its acid character. Its stability against acid and alkali was not tested. In this regard there is no uniformity of opinion. According to the early work of KÖGL, HAAGEN SMIT and ERXLEBEN (1934), IAA is not acid-proof, but it is stable in an alkaline medium. TERPSTRA (1953), on the other hand, concluded from results obtained by the aid of paper chromatography that in extracts made from the tips of *Avena* coleoptiles IAA was the only auxin whose presence could be demonstrated. In this case the extracts had been heated for 3–6 hours in 1.66 N HCl, but the IAA still showed a considerable auxin activity. Synthetic IAA, on the other hand, was completely inactivated after the same treatment. If IAA really is the only auxin which occurs in extracts obtained from *Avena* coleoptiles, no much value can be attributed to the criterion of stability in acid or alkaline media.

The so-called "red-light factor" (RLF) discovered by BLAAUW-JANSEN (1959) in tips of *Avena* coleoptiles that had been irradiated with red light ($\lambda = 6600$ Å), was also stable in acid. This RLF has the same Rf value as the substance extracted from the leaves of *Coleus*, but in the *Avena* curvature-test it is inactive.

Taking all these data in consideration one may conclude that in the leaves of *Coleus* a substance is produced that gives a positive reaction in the *Avena* curvature-test and that retards the abscission in Coleus; its Rf value is slightly higher than that of IAA, and it is not an indole derivative. The way in which the abscission is affected by this substance must be different from that in which it is affected by IAA. This substance is most probably identical with the A.R.S. from the petioles.

CHAPTER VI

THE INFLUENCE EXERCISED ON THE ABSCISSION BY THE NEIGHBOURING LEAVES

Jacobs (1955) explained the acceleration exercised on the abscission by the neighbouring leaves by assuming that these leaves would produce a substance or substances which favour the growth of the young leaves at the tip. According to Jacobs and Bullwinkel (1953) the auxin production of young leaves would increase with an increase of the surface of their leaf blade. By means of the auxin-balance theory the influence exercised on the abscission by the neighbouring leaves could be explained.

It was shown in chapter iii that the development of adventitious roots as well as the abscission of the petioles are accelerated by leaf factors produced by leaves in the vicinity, and that the effect of IAA is only an indirect one.

In order to obtain information 1°. with regard to a possible promotion of the growth, e.g. of the normal growth of the roots, by these leaf factors, and 2°. with regard to a correlation between that growth and the abscission of the petioles, experiments were carried out with decapitated plants. Here the young leaves are missing, and it seemed worth while to know whether in this case too the abscission is accelerated by leaf factors produced by leaves in the vicinity of the debladed petioles.

Further the effect exercised on the growth of leaves by the neighbouring ones was investigated in horizontally placed plants. In such plants, as a rule, the growth of the stem is reduced, but nothing is known with regard to the growth of the leaves. If we assume that the increase in surface of the young leaves is accompanied by an increase in the production of auxin, estimations of the growth of the leaves would give us indirectly some information with regard to the production of auxin. Finally, the effect of gravity on the transport, and that of light on the production of the leaf factors were studied.

1. The effect of gravity on the growth of young leaves

Jacobs and Bullwinkel (1953) found that in *Coleus* the longitudinal growth of the leaf blade is correlated with the increase in surface. By the use of their method of estimation the presence of this correlation could be confirmed for the plant material used in our own experiments. It therefore is sufficient to measure the longitudinal growth of the leaves in order to obtain an approximative idea of their increase in size.

In a number of experiments the longitudinal growth of the blades of a young leaf pair was estimated in plants in the vertical and in a horizontal position for a period of 7–10 days. In 3 experiments this was done in plants of which the axillary buds and sprouts had been removed. In the other experiments two leaf pairs were debladed in the usual way, and here the growth of the next higher leaf pair was measured. In Table 3 the results are given.

TABLE 3

Growth of young leaf blades in plants that are kept respectively in the vertical and in a horizontal position.

Nr. of exp.	Number of leaves per series	Mean increase in length (mm) during 7 consecutive days			
		Vertical		Horizontal	
		with the neighbouring leaves intact	with the neighbouring leaves removed	with the neighbouring leaves intact	with the neighbouring leaves removed
209	30	3.1	4.6	2.1	3.1
228	30	9.0	10.4	6.5	7.7
182	40	8.8		5.5	
184	40	9.0		4.5	
188	40	7.0		3.6	

Removal of leaves does not reduce the growth rate of young leaves in their vicinity, but often causes a slight increase, at least so long as the plants are kept in the normal position. In horizontally placed plants, however, the growth rate of the young leaves is strongly reduced by this measure. This reduction was found also in plants that were rotating parallel to the horizontal axis of the clinostat (Table 4).

TABLE 4

The growth of young leaf blades in plants rotating vertically and horizontally round the clinostat axis (1 rotation in 2 min. 47 sec.)

Nr. of exp.	Number of leaves per series	Mean increase in length (mm) for 7 days			
		Vertical		Horizontal	
		with the neighbouring leaves intact	with the neighbouring leaves removed	with the neighbouring leaves intact	with the neighbouring leaves removed
185	10	9.2	9.2	3.8	4.1
186	10	6.0	4.3	3.1	2.1

The acceleration of the abscission in horizontally placed plants therefore is not positively correlated with the growth rate of the young leaves at the tip. In horizontally rotating plants the abscission as well as the growth of the young leaves are strongly retarded.

2. THE INFLUENCE OF NEIGHBOURING LEAVES ON THE ABSCISSION IN PLANTS IN THE VERTICAL AND IN A HORIZONTAL POSITION

In these experiments the effect of gravity on the accelerating influence which neighbouring leaves exercise on the abscission, was studied. The petioles were debladed immediately after the plants had been placed horizontal. The diagram which accompanies Fig. 16 illustrates the mode of treatment. The graph represents the results of one of the four experiments.

In plants in the normal position as well as in horizontally placed plants the abscission is accelerated by the presence of leaves in the vicinity. In plants in the normal position the effect, however, is stronger, especially on the petioles of the upper leaf pair.

In another experiment the effect of the lowest leaf pair was investigated. Fig. 17 shows that these leaves accelerate the abscission only in plants in the normal position. The same experiment was repeated

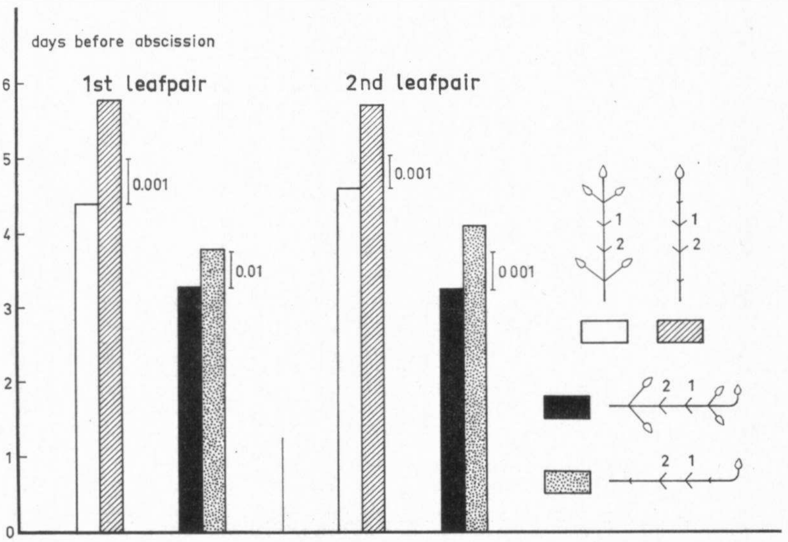

Fig. 16. The effect exercised by the presence of leaves in the vicinity on the abscission of debladed petioles in vertically and horizontally placed plants. The petioles were debladed immediately after the plants had been brought in the horizontal position. Each rectangle represents the mean abscission time of 30 petioles. The P-values of the differences are shown too.

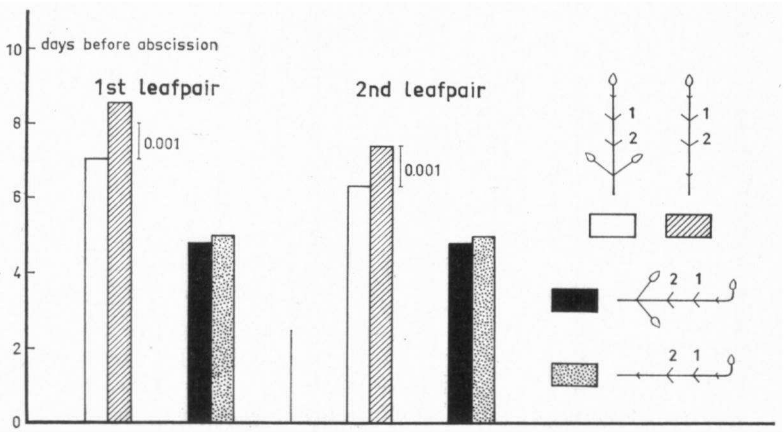

Fig. 17. The effect exercised on the abscission of debladed petioles by leaves, inserted below the latter in vertically and in horizontally placed plants. Each rectangle represents the mean abscission time of 20 petioles (see also Fig. 16).

with decapitated plants (Fig. 18). It appears that in the vertical position the abscission is also accelerated when the tip and the young leaves are missing.

From these experiments it appears that in the horizontal position the effect which is exercised on the abscission by the presence of leaves in the vicinity, is notably reduced. In these experiments the petioles were debladed immediately after the plants have been brought in a horizontal position. In connection with this result it seemed desirable to investigate whether the time which elapses between the moment at which the plants are placed horizontal and that at which the petioles are debladed, affects the length of the abscission time.

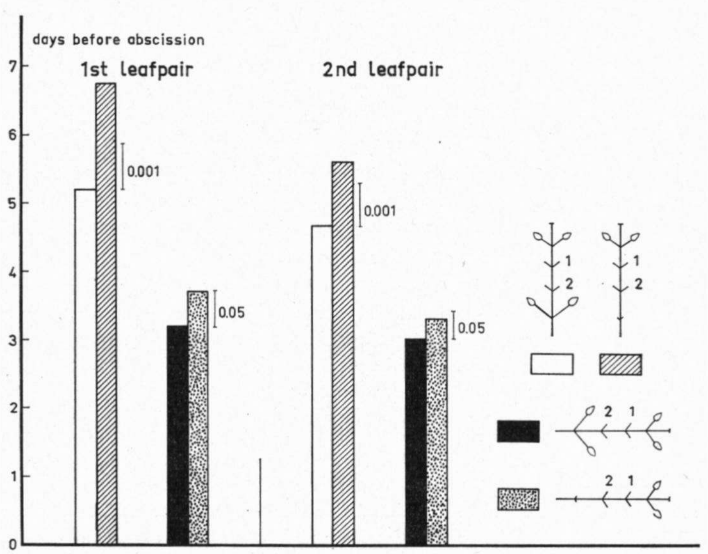

Fig. 18. The effect exercised by the presence of leaves in the vicinity on the abscission of debladed petioles in vertically and horizontally decapitated plants. Each rectangle represents the mean abscission time of 30 petioles (see also Fig. 16).

3. EXPERIMENTS ON THE EFFECT EXERCISED ON THE ABSCISSION BY THE LENGTH OF TIME WHICH ELAPSES BETWEEN THE MOMENT AT WHICH THE PLANTS ARE BROUGHT IN A HORIZONTAL POSITION AND THE MOMENT AT WHICH THE PETIOLES ARE DEBLADED

In horizontally placed plants adventitious roots often develop at the lower side of the stem in consequence of an increased growth activity at that side. The growth of these roots mostly stops after a short time. If the accelerated abscission in horizontally placed plants really is correlated with the activation of the growth at the lower side of the stem, it is to be expected that the length of time which elapses between the moment at which the plants are brought in the horizontal position and the moment at which the petioles are debladed, can not be without importance. In plants that had been kept already for some time in the horizontal position before the petioles were debladed, the abscission time might have been influenced by substances produced by these blades; in plants that are kept in the normal position these substances would have been drained off from the petioles, but in the plants in the horizontal position the substances supplied by these leaf blades would be available to the petioles.

Each experiment comprised 5 series, each of 10 plants:
a) plants placed horizontal 4 days before the deblading
b) ,, ,, ,, 3 ,, ,, ,, ,,
c) ,, ,, ,, 2 ,, ,, ,, ,,
d) ,, ,, ,, 0 ,, ,, ,, ,,
e) ,, kept vertical (control)

On the day "zero" in all plants the petioles that were to be used in the experiment, were debladed. After deblading the plants of series a) – d) remained in the horizontal position. The experiment was repeated 3 times.

The result of one of the experiments is recorded in Table 5. The abscission time indeed proved to depend upon the time elapsing between the moment that the plants were brought in a horizontal position and that at which the petioles were debladed. When the plants were placed horizontal 4 days prior to the moment at which the petioles were debladed, the abscission times in horizontal and vertical plants proved to be equal, and by this delay the accelerating effect of the horizontal position therefore had completely disappeared.

TABLE 5

The effect exercised by different intervals elapsing between the moment the plants were brought in the horizontal position and the moment at which the petioles were debladed, on the abscission time of the petioles. Each value is the mean abscission time of 20 petioles.

Series	Interval in days between the moment the plants were placed horizontal and that at which the petioles were debladed	Mean abscission time in days		S.E.
		leaf pair 1	leaf pair 2	
a	4	4.12	3.85	
b	3	3.75	3.30	
c	2	3.77	3.17	
d	0	3.07	2.97	
e	vertical	4.17	3.85	0.17

In a number of other experiments, which have been summarized in Table 6, the interval between the moment at which the plants were brought in the horizontal position and that at which the petioles were debladed, was 8 days. It appears that in these cases too the abscission time of the horizontal plants is equal to that of the vertical controls; it even may be longer.

The question arises whether this result is due to a temporarily increased growth activity at the lower side or perhaps to the influence of the leaves in the vicinity of the petioles, as these leaves are different in plants in a horizontal and in the vertical position. The retarded

TABLE 6

The abscission time of debladed petioles in plants in the vertical and in a horizontal position. The plants were placed horizontally 8 days before the petioles were debladed.

Nr. of exp.	Number of plants per series	Leaf pair	Mean abscission time in days		Difference	S.E.	P
			vertical	horizontal			
160	20	1	4.03	4.45	0.42	0.12	< 0.001
		2	3.76	4.11	0.35		0.01–0.001
167	10	1	3.75	4.17	0.42	0.18	0.02
		2	3.85	4.00	0.15		> 0.05
182	10	1	4.80	4.95	0.15	0.24	> 0.05
		2	4.15	4.40	0.25	0.11	0.05–0.02
192	10	1	4.92	5.27	0.35	0.23	> 0.05
		2	4.35	5.05	0.70	0.21	< 0.001

abscission in plants which had already been kept for a long time in the horizontal position before the deblading took place, and the results of the experiments reported sub 2) suggest that in the horizontal position the effect exercised by the neighbouring leaves either on the abscission itself or on growth processes correlated with the latter, is affected by the action of gravity.

4. THE EFFECT OF NEIGHBOURING LEAVES ON THE ABSCISSION WHEN THERE ELAPSES AN INTERVAL OF 4 DAYS BETWEEN THE MOMENT AT WHICH THE PLANTS ARE BROUGHT IN A HORIZONTAL POSITION AND THAT AT WHICH THE PETIOLES ARE DEBLADED

From the experiments reported sub 3) it appeared that the accelerating effect which a sojourn in the horizontal position exercises on the abscission, disappears when there is an interval of 4 days between the moment at which the plants are brought in the horizontal position, and that at which the petioles are debladed. Since in these plants, in which always leaves were present in the vicinity of the debladed petioles, the abscission time in the horizontal and in the vertical position is the same, they offer a possibility to analyse the effect exercised by neighbouring leaves on the abscission in relation to the influence of gravity.

In the next experiments the petioles were debladed 4 days after the plants had been brought in the horizontal position. The plants were decapitated and defoliated at the same moment, as indicated in the diagram which accompanies Fig. 19. The graphs which represent the results of one of these experiments, show that the acceleration of the abscission by the presence of leaves in the vicinity is found only in the vertical position. On the other hand, the abscission in horizontally placed plants in which the neighbouring leaves are removed, proves to be accelerated in comparison with that in similarly treated vertical controls. From the fact that the abscission times in horizontally placed plants are not influenced by the presence or absence of neighbouring leaves, it must be concluded that the mechanism through the inter-

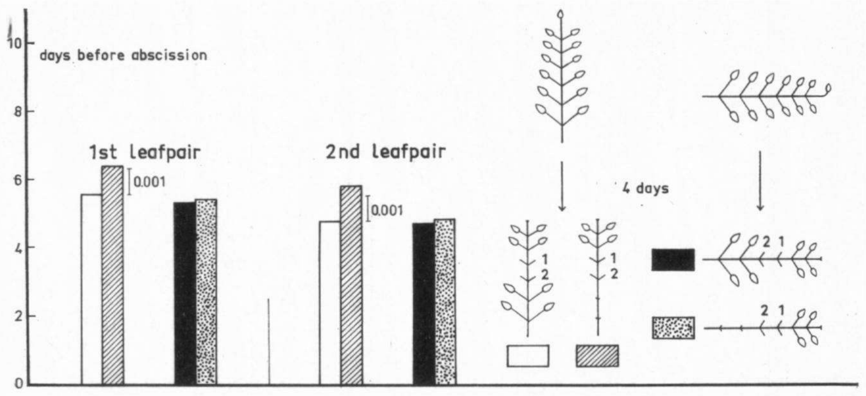

Fig. 19. The effect exercised by the presence of leaves in the vicinity on the abscission of petioles that were debladed 4 days after the plants had been brought in the horizontal position. Each rectangle represents the mean abscission time of 30 petioles. The P-values of the differences are shown too.

mediary of which the leaf factors produced in these leaves accelerate the abscission, acts in the normal, i.e. the vertical position only.

This result can be explained by assuming that in the vertical plants the leaf factors promote a process A. In horizontally placed plants this process A is not affected. In the latter, on the other hand, these leaf factors would activate a process B (growth activity at the lower side of the stem). By both processes, by A as well as by B, the A.R.S. is drained off from the petioles.

If the amount of the A.R.S., drained off by the processes A and B is about the same, the abscission time in plants in which the leaves which produce the leaf factors, are left intact, must be the same in a horizontal and in the vertical position.

The process B in horizontally placed plants takes place during the 4 days preceding the deblading of the petioles; thereafter the leaf factors exert no longer any influence on it. This follows from the fact that in the horizontal position the abscission time in plants with and without neighbouring leaves is the same.

In vertical plants, no matter whether they are intact or decapitated, the growth of young leaves is not promoted by the presence of other ones. However, it might be that the leaf factors produced by the latter act upon the growth of the roots (this might be the process A). In that case the growth of the roots would depend on the leaf factors, and this growth, therefore, would increase with the number of leaves.

The result of the next experiment is in good agreement with this hypothesis. It consisted of 4 series of 10 plants each:

series A: plants decapitated above the 2nd internode from the tip and supplied on the cut with a concentrated auxin paste (1 g/l IAA).

series B: plants decapitated as in A, but treated with plain lanolin paste.

These plants (A and B) had one pair of intact leaves besides the 2 pairs of debladed petioles.

series C: plants decapitated above the first internode from the tip, and supplied with auxin as in A.

series D: plants decapitated as in C, but supplied with plain lanolin paste.

These plants (C and D) had two leaf pairs besides the 2 pairs of debladed petioles.

Fig. 20 gives the result of this experiment. In the series B and D abscission at a determinable rate was only found in the petioles of the lower leaf pair, i.e. the leaf pair directly above the intact leaves.

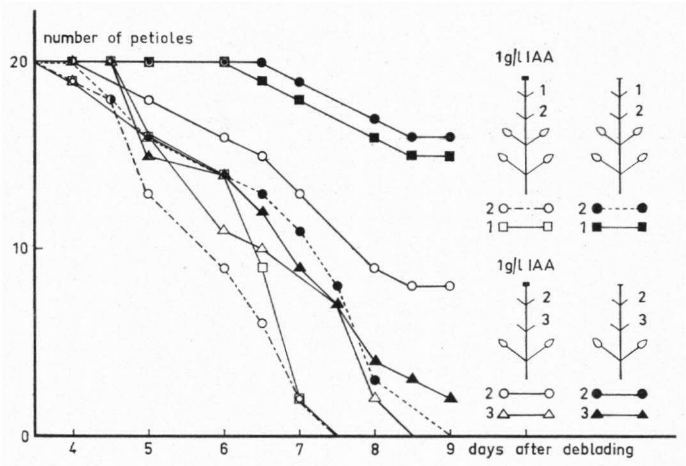

Fig. 20. The effect exercised by the number of leaves in the vicinity of the debladed petioles on the abscission of the latter in plants that were decapitated at different distances from the tip, and supplied either with a blank lanolin or with an IAA-preparation on the cut.

Although the petioles of this leaf pair 2 have the same physiological age, their abscission is different in these two series, and this difference depends upon the number of leaves that are left intact. According to our working hypothesis this result ought to be due to a stronger growth of the roots in series D.

In the series A and C below the place where the auxin is applied, adventitious roots are formed. In series C the petioles of the leaf pair 2 are dropped earlier than they are in series A. Apparently there is some competition for the leaf factors between the new roots near the tip and the old ones at the base of the stem.

In horizontally placed plants either the longitudinal transport of the leaf factors is inhibited, or their production is impeded in some way. If the growth process induced at the lower side of horizontally placed plants is also influenced by the leaf factors, and if the longitudinal transport of these leaf factors is inhibited, this inhibition will be less pronounced if the plants are debladed spirally, as in that case an intact leaf is left just opposite each debladed petiole.

In order to check this, the plants were placed horizontal 4 days prior to the moment at which the petioles were debladed. During this interval the growth activity at the lower side of the stem has started. If the further course of this growth activity would depend partly on the leaf factors produced by the intact leaves, it is to be expected that it will be promoted in the immediate neighbourhood of the nodes.

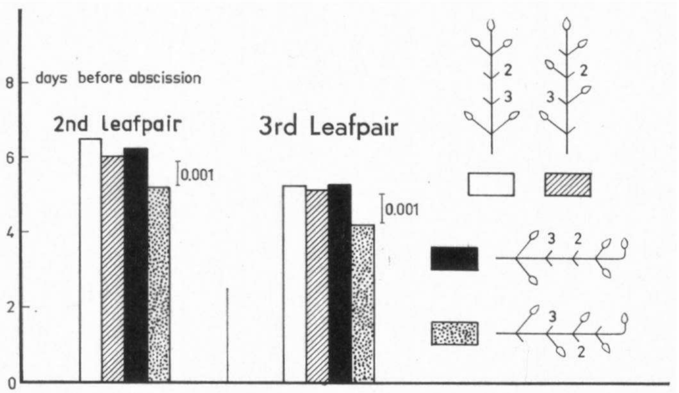

Fig. 21. The effect exercised by leaves in the vicinity of debladed petioles on the abscission of the latter in plants in the vertical and in the horizontal position. The leaves were either opposite or arranged in a spiral. The petioles were debladed 4 days after the plants were brought in the horizontal position. Each rectangle represents the mean abscission time of 15 petioles. The P-values of the differences are shown too.

The way in which the deblading was carried out, is shown in the diagram appended to Fig. 21; the graphs represent the results of one of these experiments. Only if there is an intact leaf opposite the debladed petiole, the abscission may prove to be accelerated in the horizontal position. When this leaf occupies a position at the upper side of the stem (leaf pair 1 in the diagrams), this result is always inconsistent.

A study of the vascular supply of the leaves reveals that there are no vascular connections between the various bundles of a leaf pair. The course of the vascular bundles has been studied by the aid of the method of Jacobs (1952), i.e. consecutive sections through the stem were made transparent with NaOH and chloral hydrate, and then stained with alkaline fuchsin in ammonia.

The course of the vascular system proved to be the same as that of other *Coleus* varieties (Fischnich, 1935); in the node however, no transverse connections could be detected. Fischnich mentions in his paper that he actually did find them. However, even under the microscope I have not been able to detect such connections.

If the leaf factors would migrate only through the vascular system, the reported results would be easily explainable. In order to study this transport a number of experiments was carried out.

45 plants were placed horizontal in such a way that the petioles

of the 2nd and 4th leaf pair were in the vertical plane. After 4 days the leaves of the 1st and 3rd leaf pair were entirely removed, and the petioles of the other leaf pairs debladed, as indicated in the diagram added to Fig. 22. The graphs show that the intact leaves accelerate the abscission in a higher degree when they are inserted at the lower side of the stem. In the absence of leaf blades there is no difference in the abscission time between petioles at the upper and at the lower side.

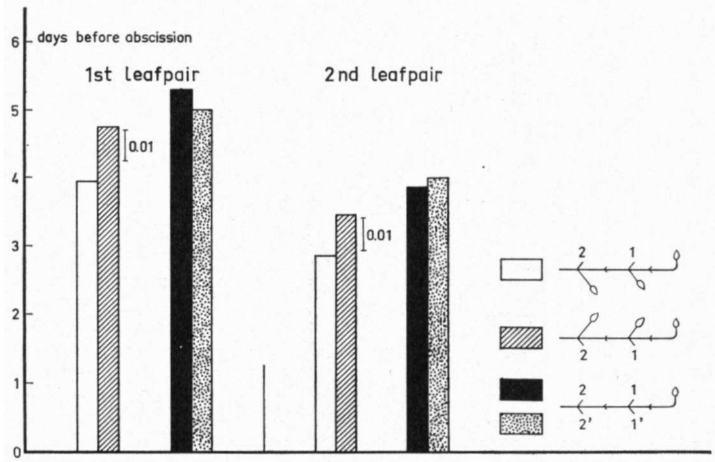

Fig. 22. The effect exercised by intact leaves situated resp. at the lower side and at the upper side of the stem in horizontally placed plants, on the abscission of debladed petioles. The plants were placed in the horizontal position 4 days before the petioles were debladed. Each rectangle represents the mean abscission time of 15 petioles. The P-values of the differences are shown too.

It is therefore the transport, not the production of the leaf factors, which is influenced in horizontally placed plants.

The results thusfar obtained can be summarized as follows:

1) From the moment at which the plants are brought in a horizontal position, at the lower side of the stem a growth process is induced by a substance produced at the tip and in the young leaves; this substance can be replaced by IAA.
2) This growth process is promoted by one or more leaf factors produced in the leaves, of which the transport takes place through the vascular system only.
3) By this growth process the A.R.S. (see chapter v) is drained off from the petioles.

From the results of the experiments reported in this chapter it can also be concluded that the leaf factors which are involved in this growth process, are not identical with the A.R.S., since the latter can also migrate through the parenchyma. This conclusion is confirmed by an experiment, the results of which are given in Fig. 23. In a series of plants with intact leaves at the lower side of the stem (B), a horizontal incision was made through the node, and a thin blade of mica was inserted; in this way the transverse transport of the A.R.S. was blocked.

By this blocking the abscission of the petioles at the upper side proved to be retarded. In other experiments the same incision was made, but then the two halves of the node were pressed together by binding raffia round the stem. In this case the abscission proceeds at the same rate as in the controls without incision. The wound itself therefore does not cause the retardation of the abscission which occurs when the transverse transport of the A.R.S. is actually blocked.

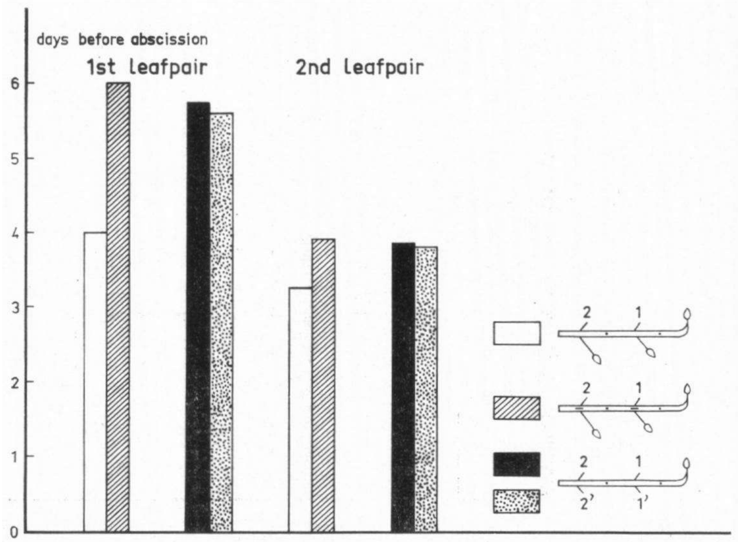

Fig. 23. The effect exercised by a blocking of the transversal track of transport used by the A.R.S. from the petioles on the abscission of the latter. Each rectangle represents the mean abscission time of 10 petioles.

The leaf factors can not be replaced by sugars. This was proved in experiments in which the petioles at the lower side of the stem were debladed too (length of the petioles about 4 cm); the latter were placed in small tubes which contained a sugar solution, about 5 mm of the petiole stump being submerged. The sugar solutions were renewed every day, and each time a fresh cut was made by removing a small part of the petioles. The concentrations of the sugar solutions were 1, 2, 3, and 5 % glucose or sucrose. In none of the experiments the abscission of the petioles at the opposite side was accelerated. This was not so either when a mixture of amino acids (composition according to Rijven (1952), 1 g/l in phosphate buffer) had been added to the sugar solution.

5. THE EFFECT OF LIGHT ON THE PRODUCTION OF THE LEAF FACTORS

In the next experiments it was tested whether the leaf factors which indirectly accelerate the abscission, are produced in the light only. If these leaf factors would be identical with those that promote the development of adventitious roots, and probably that of the normal roots too, the presence of the leaves that produce these factors, would not be able to accelerate the abscission in the dark.

a. *The effect of the leaf factors in the dark*

The experiments were carried out in the dark room. In half the plants the 1st and the 4th leaf pair were removed, and in all plants the petioles of the 2nd and 3rd leaf pair were debladed in the usual way. This deblading took place either immediately after the plants had been transferred to the dark room or resp. 2 and 3 days later. The results are summarized in Table 7. In the dark the abscission is accelerated by the leaf factors only when the petioles are debladed immediately after the transfer of the plants to the dark.

TABLE 7

The effect of neighbouring leaves on the abscission of debladed petioles in the dark. Before deblading the petioles, the plants were placed in the darkroom for a different number of days.

Nr of exp.	Number of plants per series		Number of days in the dark before the petioles were debladed	Mean abscission time in days		difference	S.E.	P
		leaf pair		with neighbouring leaves	without neighbouring leaves			
196	10	1	0	2.10	2.83	0.73	0.13	< 0.001
		2		1.77	2.20	0.43		< 0.001
200	10	1	0	3.57	4.00	0.43	0.06	< 0.001
		2		3.27	3.90	0.63		< 0.001
198	15	1	2	4.55	4.25	—0.30		
		2		3.92	3.85	—0.07		
200	10	1	3	3.42	3.52	0.10		
		2		3.22	3.40	0.18		
206	15	1	4	3.76	3.66	—0.10		
		2		3.41	3.65	0.24		

b. *Experiments under natural light conditions*

For these experiments vertical and horizontally placed plants were used. The petioles were debladed in a spiral way. Each experiment comprised 60 plants, half of which were placed horizontal. In each position the leaves of 10 plants were wrapped in black paper, and so shut off from the light; of 10 other plants the leaves were removed. The results are given in Table 8, and they prove that when the leaf blades are darkened, no longer an acceleration of the abscission by leaf factors produced by neighbouring leaves occurs.

6. THE EFFECT EXERCISED BY NEIGHBOURING LEAVES ON THE ABSCISSION IN PLANTS THAT ARE ROTATING ON THE CLINOSTAT

It appeared from previous experiments that the longitudinal transport of the leaf factors is distinctly hampered in plants that have

TABLE 8

The effect of neighbouring leaves with darkened leaf blades on the abscission of debladed petioles in plants in a vertical and in a horizontal position.

Nr. of exp.	Number of plants per series	leaf pair	Mean abscission time in days					
			vertical			horizontal		
			with neighbouring leaves		without neighbouring leaves	with neighbouring leaves		without neighbouring leaves
			control	leaf blades darkened		control	leaf blades darkened	
212	10	1	6.60	7.20	7.50	5.00	6.00	5.90
		2	5.80	6.70	7.20	4.70	6.00	5.50
		3	5.30	5.80	6.00	4.10	4.80	4.80
		4	4.30	4.80	4.90	4.00	4.20	4.30
213	10	1	7.25	7.70	7.85	5.70	6.40	6.40
		2	6.50	7.25	7.10	5.15	6.15	6.15
		3	5.40	6.05	6.15	4.65	5.35	5.35
		4	4.80	5.60	5.35	4.50	4.90	4.90

been brought in a horizontal position. If this inhibition would be due to a unilateral effect of gravity, it should not occur in plants that are rotating parallel to the horizontal axis of a clinostat.

a. *Experiments on the clinostat under natural light conditions*

On both axes of the clinostat, on the vertical as well as on the horizontal axis, 5 plants with and 5 plants without intact leaves in the vicinity of the debladed petioles were fixed. Moreover, in one series the plants were decapitated just above the youngest, not yet full-grown leaf pair. The results are given in Table 9. They show that

TABLE 9

The effect of neighbouring leaves on the abscission of debladed petioles in plants rotating either vertically or horizontally on the clinostat axis (natural light conditions). Rotation time: 2 min. 47 sec.

Nr. of exp.	Number of plants per series	Leaf pair	Mean abscission time in days			
			with neighbouring leaves		without neighbouring leaves	
			vertical	horizontal	vertical	horizontal
183	5	1	4.60	5.45	6.95	7.85
(decap.)		2	4.25	4.45	5.45	5.65
185	5	1	4.85	5.20	6.60	7.30
		2	4.05	4.65	4.95	6.85
186	5	1	4.65	5.40	5.90	6.10
		2	4.25	4.90	5.05	5.20
194	5	1	3.90	4.45	4.75	6.45
		2	3.35	4.55	4.40	6.00

Mean acceleration of abscission in plants with neighbouring leaves:
Rotating vertically: 1.27 days
Rotating horizontally: 1.55 days

the acceleration of the abscission caused by leaf factors produced in the neighbouring leaves is found to be the same in vertically (control) and in horizontally rotating plants. However, the abscission itself proceeds slower in the plants that are rotating horizontally, than in those that are rotating vertically. This point will be considered more extensively in chapter VII.

b. *Experiments on the clinostat in the dark*

In order to eliminate an occasional difference in illumination between plants rotating vertically and those rotating horizontally, the same experiments were repeated in the dark. In this case both axes of the clinostat rotated in the horizontal plane, and the controls stood vertical. In this way in one experiment 10 plants with and 10 plants without intact leaves in the vicinity of the debladed petioles could rotate horizontally. The petioles were debladed immediately after the plants had been transferred to the dark room. Table 10 shows that in this case too in horizontally rotating plants the abscission is accelerated by leaf factors.

TABLE 10

The effect of neighbouring leaves on the abscission of debladed petioles in plants rotating horizontally on the clinostat axis in the dark. Rotation time: 2 min 47 sec.

Nr. of exp.	Number of plants per series	Leaf pair	Mean abscission time in days		Difference	S.E.	P
			with neighbouring leaves	without neighbouring leaves			
218	10	1	5.02	5.55	0.53	0.23	0.01–0.02
		2	4.40	5.07	0.67		0.01–0.001
219	10	1	4.80	5.22	0.42	0.15	0.01–0.001
		2	4.30	4.85	0.55		< 0.001

7. DISCUSSION

In their review ADDICOTT and LYNCH (1955) mention the possibility, that auxins would affect the abscission indirectly, viz. by deviating nutrients to the sites where the auxins are active. Auxins present at the proximal side of the abscission zone then would withdraw nutrients from the petioles in case the auxin level in the latter would be low. The abscission thus would be accelerated by a deficiency of nutrients. A similar possibility was also mentioned by TERPSTRA (1956), who suggests that all organs producing auxin, would attract nutrients, and consequently would cause a deficiency of nutrients in organs with a low auxin content. JACOBS (1955) came to the conclusion that the growth of the young leaves at the tip is promoted by leaf factors produced by the other leaves. In another paper JACOBS and BULLWINKEL (1953) reported for the same variety of *Coleus* that after removal either of the young leaves at the tip or of the axillary shoots the remaining leaves show compensatory growth.

Nutrients certainly play an important role in all kinds of growth processes, and so indirectly also in the abscission. However, it was found (chapter v) that the abscission does not only depend on growth processes taking place at the proximal side of the petiole, but also on the A.R.S., which is produced in the light in the petiole itself, and which may be drained off from the petiole by growth processes elsewhere.

As reported in chapter III the acceleration of the abscission is correlated with the development of adventitious roots that is induced by a high auxin concentration. Since the abscission appears to be accelerated also by leaf factors, it was supposed that these leaf factors would promote the formation of new roots and the growth of those that are already present. In this respect there is some resemblance with the theory of JACOBS, according to which substances produced in the older leaves would promote the growth of the young ones; in our opinion they act in this way on the growth of roots.

The fact that the abscission is accelerated by the presence of young leaves as well as by the development of new roots and by the growth of roots that are already present, does not implicate that the effect exercised by young leaves should be identical with that of the growth and development of roots. It is to be expected that in the young leaves in the light the same A.R.S. will be produced as in the petioles (and in full-grown leaf blades), but it is not to be expected that this A.R.S. is produced in the roots too. The A.R.S., therefore, will not be withdrawn from the petioles by the young leaves, as the latter produce this A.R.S. themselves.

Some more light was shed on the difference in the way in which young leaves and roots exercise their influence, by the experiments reported in this chapter; the latter showed that:

1. the growth of young leaves is independent of leaf factors produced in the other leaves,
2. the abscission is accelerated by the older leaves also when the plants are decapitated, i.e., when the young leaves are removed.

From these data it was concluded that the mechanism through which the abscission of the petioles is accelerated by the presence of young leaves and by that of the full-grown leaves in their immediate vicinity is a similar one; in both cases it depends on the production of substances of which it is known that they regulate the growth of non-green parts (mainly of the roots).

LAUDI and GEROLA (1956) found that in *Coleus* the abscission of debladed petioles is accelerated by the presence of leaves in their vicinity only if the latter are inserted at their proximal side. According to JACOBS (1955) the abscission would also be accelerated by the presence of leaves that are inserted at the distal side. This was also found in our own experiments.

The suggestion that the leaf factors especially promote the growth of non-green parts and tissues, is corroborated by the results obtained with plants that has been placed in a horizontal position. By an auxin from the tip and the young leaves the abscission is accelerated, and a growth activity is induced at the lower side of the stem which may be accompanied by the formation of adventitious roots. These processes are promoted by leaf factors produced in the full grown leaves. The effect of the latter, however, depends on their position in relation to the petioles and to the tissue whose growth is to be stimulated by these leaf factors. Only when the intact leaf is situated opposite the debladed petiole, and when it has in addition a direct vascular connection with the lower half of the stem, the abscission is accelerated. Therefore, if the leaf is pointing upward, the leaf factors that are produced by it, have no effect.

From this it was concluded that the unilateral effect of gravity rests on a blocking of the longitudinal transport of the leaf factors. In plants that are rotating parallel to the horizontal axis of the clinostat, the abscission is accelerated by these leaf factors to the same extent as in plants in the normal position. Since no growth activity is induced in the stems of horizontally rotating plants, the leaf factors are most probably used for the same growth processes for which they are used in plants in the normal position. It is supposed that these growth processes lead to the development of new roots and to the elongation of the existing ones.

In horizontally rotating plants the abscission is retarded as compared to that found in plants in the normal position. This phenomenon, however, is independent of the leaf factors produced in the neighbouring leaves.

Several authors (VAN DER LEK, 1925; BOUILLENNE and WENT, 1933; BOUILLENNE, 1935, 1938; COOPER, 1936; DELARGE, 1941; GAUTHERET, 1942, 1944; BOTTELIER, 1956) have reported that certain substances produced in buds and leaves promote the growth of the roots as well as the formation of new ones. These substances are synthesized in the light, and transported in the basipetal direction only. WENT (1938) assumed that there was but one substance, for which he proposed the name rhizocaline. As it appeared from numerous later investigations that root formation may also be induced by auxins, and especially by IAA, the term rhizocaline was less and less used, since this substance was generally believed to be identical with auxin.

BOUILLENNE and BOUILLENNE-WALRAND (1955), however, showed that the question of root formation should not be simplified in such a way. It appeared that auxin could not induce root formation when a definite intracellular factor was deficient. According to these authors rhizocaline comprises three different constituents, viz.

1. a specific substance with ortho-di-phenol nuclei which is produced in the leaves,
2. a non-specific substance, viz. an auxin that is transported at low concentrations,
3. an enzyme system localized in particular cells or in tissues such as the pericycle, the phloem and the cambium.

Only in the cells mentioned in (3) the rhizocaline would be synthesized in a reaction between the ortho-di-phenol compound and the auxin which is catalised by the enzyme system.

In the experiments dealt with in this chapter the development of adventitious roots proved to depend on leaf factors, which can not be replaced by nutrients. The transport of these leaf factors, which takes place through the vascular system only, is moreover influenced by gravity. If the stem is kept in a horizontal position, the longitudinal transport is blocked or at least strongly retarded.

Since the transport of the leaf factors according to the authors mentioned above, would be a polar one, this retardation in the horizontal position might mean that the polarity is changed under the influence of gravity. Several authors (TITTMANN, 1895; SIMON, 1908; LOEB, 1926) have assumed the existence of such a relation between gravity and the changes in the polarity that were observed by them. This idea usually arose from experiments with isolated parts of plants. By GAUTHERET (1944) differences in air humidity between the upper and the lower side were mainly held responsible for the changes in the polarity.

The blocked or retarded transport of the leaf factors that was observed in horizontally placed plants, appeared to be caused by the unilateral reaction to the gravitational force. No blocking or retardation was found in plants that were rotating parallel to the horizontal axis of the clinostat.

The development of adventitious roots near the tip of decapitated plants which takes place after the latter has been supplied with a high concentration of IAA, does not favour the idea of a polar i.e. a basipetal transport of the leaf factors. However, according to GAUTHERET (1944) the polarity would be annulled by high concentrations of IAA, since at these concentrations IAA would have a toxic effect.

As has been shown, the abscission is accelerated by leaf factors produced in young leaves (the auxin produced in these leaves included) as well as in full-grown leaves in the vicinity of the debladed petioles, because these leaf factors promote the development of roots. By this growth process the A.R.S. is drained off from the petioles.

However, since the abscission proved to be correlated with several other phenomena, one should be cautious when interpreting the results of experiments in which e.g. the leaves in the vicinity of the debladed petioles have been replaced by some auxin preparation.

LAUDI and LAMBRI (1956) carried out experiments with branched plants of *Coleus bicolor*. All petioles were debladed and, with the exception of those of one lateral branch, provided with an IAA preparation. By this treatment the abscission of the petioles that received no IAA, appeared to be accelerated. According to the theory developed in this paper, this should be explained as follows. Leaf factors produced by the young leaves at the tip promote the growth of the roots; by this growth the A.R.S. is drained off from the petioles; in the petioles treated with IAA the migration of the A.R.S. is hampered, and the withdrawal of the A.R.S. from the petioles that received no IAA, is consequently increased.

In a similar way the results of the experiments of Jacobs (1958) can be explained.

The retardation of the abscission found in plants that had been kept for a long time (one week) in the horizontal position, must be due to:

1. the transient character of the growth process induced at the lower side of the stem; this process reaches its peak during the first days that are passed in the horizontal position, and then gradually decreases;
2. the blocking or hampering of the basipetal transport of the leaf factors by the unilateral reaction to the gravitational force.

By this the growth of the plant as a whole is indirectly retarded.

The retarded growth that has often been observed in stems in a horizontal position, can be explained by a decrease in the development of the roots, a decrease that is caused by the fact that the transport of the leaf factors which are essential for this development, is hampered. Recently, Wareing and Nasr (1958) found such a reduced growth in length of the stem in their experiments with horizontally placed plants of apple, prune, cherry and black currant.

The theory of Went (1938) according to which the roots would provide the stem with a specific hormone called caulocaline, which would be essential for its development, has been criticized by several authors (e.g. by Gautheret, 1944). In this regard the capacity of the root system to supply the stem with nutrients would be at least of equal importance.

It is more difficult to account for the retarded growth (retarded increase of the blade surface) of the leaves that is observed in horizontally placed plants as well as in plants that are rotating round a horizontal axis. There may be a correlation between the retarded abscission that is found in such plants, and the reduced growth rate of the leaves. It has been shown that the abscission is retarded in plants that are kept in a horizontal position for a long time (e.g. a week), as well as in horizontally rotating plants (see also next chapter) by a delayed migration of the A.R.S. from the petioles. If this retarded migration would result in an accumulation of this A.R.S., the concentration of the latter might perhaps attain a level that would prove to exercise a retarding influence on the further growth of the leaf. This possibility might be tested by quantitative estimations of this substance.

SUMMARY

The results obtained in this chapter can be summarized as follows:

1. The abscission of debladed petioles is indirectly accelerated by leaf factors produced by leaves in their vicinity. The direct action of these leaf factors is a promotion of growth processes, most probably in the roots, by which the A.R.S. (see chapter v) is drained off from the petioles.
2. The leaf factors are produced in the light. They can not be replaced by nutrients.
3. For their transport they are dependent on the vascular system.
4. The acceleration of the abscission in horizontally placed plants is correlated with growth activity at the lower side of the stem. This growth process is promoted

also by the leaf factors; and it was shown that the latter indirectly accelerate the abscission of debladed petioles.

5. In the horizontal position the basipetal transport of these leaf factors is blocked or strongly hampered by the unilateral effect of the gravitational force.
6. In plants rotating parallel to the horizontal clinostat axis there is no decrease in the transport of the leaf factors.
7. If the petioles are debladed in plants that had been kept in the horizontal position for a week, the abscission in the horizontal position is retarded to the same extent as in plants that are rotating parallel to the horizontal clinostat axis.
8. In plants placed in a horizontal position as well as in plants that are rotating parallel to the horizontal clinostat axis, the growth rate of the leaves is reduced.

CHAPTER VII

THE EFFECT OF THE LONGITUDINAL AND THAT OF THE TRANSVERSAL COMPONENT OF GRAVITY ON THE ABSCISSION

The accelerated abscission of debladed petioles in plants that are kept in a horizontal position, was ascribed by TERPSTRA (1956) to the unilateral effect of the transversal component of gravity. This conclusion was based on preliminary experiments on the clinostat, by which it was found that the abscission time was the same for plants rotating parallel to the horizontal axis of the clinostat and for the controls, i.e. for plants in the normal position. The revolution time of the clinostat axis was 4 hours.

From some of our own experiments described in chapter VI, it appeared that at a much shorter revolution time (2 min. 47 sec.) the abscission was retarded. This aberrant result suggested that the revolution time of the clinostat axis is a factor that should not be neglected. It seemed probable that the revolution time is related in some way to the time during which gravity can exert an unilateral effect. Furthermore, it was possible that the longitudinal component of gravity too affects the abscission. In the next experiments the effect of the rotation speed of the clinostat axis as well as the effect of the longitudinal component of gravity have been studied.

1. THE ABSCISSION IN PLANTS THAT ARE ROTATING VERTICALLY AND HORIZONTALLY ON THE CLINOSTAT

a. *Experiments under natural light conditions*

In each experiment 10 plants were fixed to the horizontal and 10 plants to the vertical axis of the „DE BOUTER" clinostat (rev. time 167 sec.). After 24 hours rotation the petioles of 2 leaf pairs were debladed. In a few experiments the plants were debladed prior to their being mounted on the clinostat. The results are given in Table 11.

b. *Experiments in the dark*

In order to eliminate an effect exercised by occasional differences in illumination on the plants that were mounted on the two axes of the clinostat, some experiments were carried out in a dark room.

TABLE 11

Abscission of petioles in plants rotating either horizontally or vertically on the clinostat axis (1 rotation in 2 min. 47 sec. and natural light conditions). Each value is the mean abscission time of 20 petioles.

Nr. exp.	Leaf pair	Mean abscission time in days		Differ-ence	S.E.	P
		vertic. rot.	horiz. rot.			
140*a*	1	4.20	4.92	0.72	0.21	< 0.001
	2	3.65	4.32	0.67		< 0.001
144*a*	1	5.02	5.45	0.43	0.18	0.02
	2	4.60	5.02	0.42		0.02
149*b*	1	4.12	4.95	0.83	0.14	< 0.001
	2	4.02	4.47	0.45		0.01–0.001
155*b*	1	4.75	5.97	1.22	0.19	< 0.001
	2	4.50	5.67	1.17		< 0.001

a) petioles debladed before the plants were placed on the clinostat
b) petioles debladed after one day rotation.

After a rotation during 24 hours in the dark the petioles were debladed. The results of two experiments are given in Table 12.

It appears that in the plants that are rotating round the horizontal axis, the abscission, in the light as well as in the dark, is retarded as compared to that in the controls that are rotating round the vertical axis.

TABLE 12

Abscission of petioles in plants rotating either horizontally or vertically on the clinostat axis in the dark (one rotation in 2 min. 47 sec.). Each value is the mean abscission time of 20 petioles.

Nr. of exp.	Leaf pair	Mean abscission time in days		Differ-ence	S.E.	P
		vertic. rot.	horiz. rot.			
195	1	2.70	3.35	0.65	0.10	< 0.001
	2	2.60	2.97	0.37		< 0.001
197	1	3.57	4.02	0.45	0.13	< 0.001
	2	3.30	3.82	0.52		< 0.001

2. EXPERIMENTS WITH ROTATION DURING A SHORT TIME

Now the question arose how long the rotation round the horizontal axis should last in order to produce this retarding effect on the abscission that was described above. In order to find an answer to this question plants that were treated in a similar way, were rotated for 1 or 2 days only. After the rotation both series of plants were placed either in the normal or in a horizontal position.

The results of some of these experiments are given in Table 13. It appears that the abscission is already consistently retarded after rotation of one day round the horizontal axis, and that this retardation is independent of the position in which the plants are placed after

TABLE 13

Abscission of petioles in plants rotating during 1 or 2 days either vertically or horizontally. After the rotation all plants were placed either vertical or horizontal. Each value is the mean abscission time of 20 petioles.

Nr. of exp.	Vertic. and horiz. rotation during	Position after rotation	Leaf pair	Mean abscission time in days after having rotated		Difference	S.E.	P
				vertic.	horiz.			
118	1 day	horiz.	1	4.47	5.02	0.55	0.25	0.05–0.02
			2	4.02	4.75	0.72		0.01–0.001
92	2 days	horiz.	1	3.67	4.37	0.70	0.18	< 0.001
			2	4.17	5.00	0.83		<0.001
141	1 day	vertic.	1	4.80	5.80	1.00	0.21	< 0.001
			2	4.32	4.97	0.65		0.01–0.001
122	2 days	vertic.	1	4.87	5.67	0.80	0.22	< 0.001
			2	4.60	5.25	0.65		0.01–0.001
125	2 days	vertic.	1	4.37	5.12	0.75	0.20	< 0.001
			2	3.87	4.45	0.58		0.01–0.001

the rotation. It follows from these data that the changes which take place in the petioles during the first days after the deblading, are decisive for the length of the abscission time.

The retardation of the abscission in plants that are rotating round the horizontal axis can not be due to the centrifugal force, since at this rotation speed the latter is negligeable as compared to the gravitational force. On the other hand, it is not excluded that the abscission may be affected by the longitudinal component of gravity. This component is eliminated in plants rotating horizontally, whereas the transversal component acts successively on each longitudinal sector. The difference between the results of TERPSTRA's experiments and of those reported here, must probably be due to the fact that the transversal component is "perceived" only by slowly rotating plants, or, in other words, that it has to act for a certain time on each longitudinal sector of the stem to exert its influence. In that case the abscission would be accelerated in the same way as in horizontally placed plants. In the mean time the elimination of the longitudinal component has a retarding influence on the abscission, as appeared from the following experiments.

After some of the petioles had been debladed the plants were treated as follows:

1. placed in the inverse position (upside down),
2. rotated under various angles to the vertical,
3. fixed at angles of 45° and 135° to the vertical.

3. ABSCISSION IN THE PLANTS THAT WERE PLACED INVERSELY

The first preliminary experiments were carried out in the experimental glass-house. It appeared that a plant in the upside-down position is in a very labile condition, and tends to curve up geotropically. This could be prevented by decapitating the plants. As shown in Fig. 24 the abscission in such plants is retarded as compared to that in the controls which were kept in the normal position. Since it might be objected that the illumination of the inversely placed plants was not the same as that of the controls, the experiments were repeated in the dark room. However, in the dark too the abscission in inversely placed plants proved to be retarded. This retarding effect can be due either to an elimination of the action of the longitudinal component or to its action in the inverse direction.

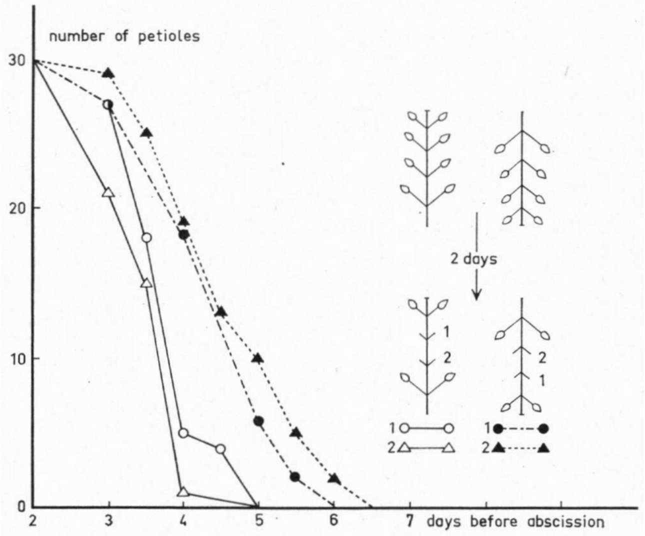

Fig. 24. The abscission of debladed petioles in plants in the normal and in the inverse position.

4. THE ABSCISSION IN PLANTS ROTATING ROUND AXES INCLUDING VARIOUS ANGLES WITH THE VERTICAL

If we assume that at the revolution time that was used by us, the transversal component of gravity exercises no influence on the abscission time, changes in the latter that are found in plants that are rotated round axes including various angles with the vertical, will have to be ascribed to differences in the magnitude of the longitudinal component. The different angles could be obtained by varying the position of one of the two axes of the clinostat. One of the control series rotated horizontally, and the other stood in the normal position. The effect of the longitudinal component must decrease proportional to cos α, when α is the angle of deviation from the vertical. At $\alpha = 90°$ (rotation parallel to the horizontal axis) the longitudinal component is eliminated (cos $\alpha = 0$).

The experiments in which the plants were rotated at angles of 30° and 45°, consisted of 3 series of 10 plants each:

series a: axis of the clinostat horizontal,
series b: axis of the clinostat at an angle of 30° or of 45°,
series c: vertical.

One day before the petioles of which the abscission was to be studied, were debladed, the plants were transferred into the dark room and mounted on the rotating clinostat axes, resp. kept in the vertical position.

TABLE 14

Abscission of petioles in plants rotating either horizontally or at an angle of 30° and in vertical plants. Each value is the mean abscission time of 20 petioles.

Nr. of exp.	Leaf pair	Mean abscission time in days					S.E.
		vertic.	horiz. rot.	difference horiz. rot.-vertic.	rotation at an angle of 30°	calculated for rotat. at an angle of 30°	
272	1	4.42	4.90	0.48	4.55	4.66	0.11
	2	3.95	4.52	0.57	4.15	4.23	
275	1	3.30	3.80	0.50	3.52	3.55	0.07
	2	3.05	3.67	0.62	3.25	3.36	
277	1	3.12	3.70	0.58	3.30	3.41	0.10
	2	2.70	3.30	0.60	3.00	3.00	

TABLE 15

Abscission of petioles in plants rotating either horizontally or at an angle of 45° and in vertical plants. Each value is the mean abscission time of 20 petioles.

Nr. of exp.	Leaf pair	Mean abscission time in days					S.E.
		vertic.	horiz. rotat.	difference horiz. rot.-vertic.	rotation at an angle of 45°	calculated for rotat. at an angle of 45°	
259	1	2.65	3.50	0.85	3.40	3.24	0.15
	2	2.45	2.97	0.52	2.72	2.81	
261	1	3.05	3.32	0.27	3.42	3.24	0.11
	2	2.70	3.10	0.40	3.20	2.98	
262	1	3.02	3.92	0.90	3.60	3.65	0.10
	2	2.70	3.55	0.85	3.27	3.29	
264	1	4.15	4.52	0.37	4.57	4.41	0.12
	2	3.72	4.20	0.48	4.10	4.05	
266	1	3.57	4.47	0.90	4.10	4.20	0.18
	2	3.32	4.25	0.93	3.95	3.97	

The results of the experiments are summarized in Tables 14 and 15. The retardation of the abscission caused by a partial elimination of the effect of the longitudinal component of gravity must be related in some way to the difference in abscission time found between horizontally rotating ($t_{hor.}$) and vertical ($t_{vert.}$) plants. Theoretically this retardation caused by a rotation round an axis including an angle α with the vertical, should be:

$\sin \alpha \times$ retardation of horizontally rotating plants.

The abscission time (t_a) of plants rotating at $\alpha = 30°$ and $\alpha = 45°$ then can be calculated by means of the equation:

$$t_\alpha = t_{vert.} + \sin\alpha\,(t_{hor.} - t_{vert.})$$

The results of these calculations are given in column 7 of Tables 14 and 15. There is evidently a close agreement between the data that were found experimentally and those that were calculated theoretically for $\alpha = 30°$ and $\alpha = 45°$.

In the previous experiments it was assumed that the retardation of the abscission in horizontally rotating plants must be due to the elimination of the longitudinal component of gravity. In inversely placed plants too the abscission is retarded (Fig. 24). If elimination of the longitudinal component has the same effect as its action in the inverse direction, the abscission times of petioles in plants rotating round axes including angles with the vertical between $\alpha = 90°$ and $\alpha = 180°$ should remain the same, also of course those of horizontally rotating and inversely placed plants.

This was checked in the next experiments, in which 4 series of 10 plants each were used:

series a: plants vertical,
series b: plants rotating horizontally,
series c: plants rotating at angles $\alpha = 120°$, $\alpha = 135°$ or $\alpha = 150°$,
series d: plants placed upside down.

From Tables 16, 17 and 18, which give the results of these experiments, it appears that no distinct difference was found between the abscission time of horizontally rotating and inversely placed plants. In the majority of the experiments the difference found between the abscission time of petioles in plants rotating at angles of $\alpha = 120°$, $\alpha = 135°$ and $\alpha = 150°$ and that of horizontally rotating and inversely placed plants was not significant either.

Consequently the retardation of the abscission in plants rotating at angles between 120° and 150° can not be expressed by one of the two following formulas:

1. cos α × inversely acting longitudinal component
2. sin α × effect of eliminated longitudinal component.

This, however, would not be necessary, if the retardation reached its maximum value in the neighbourhood of $\alpha = 90°$.

TABLE 16

Abscission of petioles in plants rotating either horizontally or at an angle of 120° and in plants in the normal and in the inverse position.
Each value is the mean abscission time of 20 petioles.

Nr. of exp.	Leaf pair	Mean abscission time in days							S.E.
		norm.	horiz. rotat.	difference hor. rot. -norm.	rotat. at an angle of 120°	difference rot. at an angle of 120° -hor. rot.	inverse	difference inverse-hor. rot.	
281	1	3.55	3.90	0.35	4.30	0.40	4.15	0.25	0.12
	2	3.32	3.72	0.40	3.92	0.20	3.92	0.20	
282	1	3.85	3.95	0.10	4.27	0.32	4.07	0.12	0.10
	2	3.47	3.77	0.30	4.00	0.23	3.90	0.13	

TABLE 17

Abscission of petioles in plants rotating either horizontally or at an angle of 135° and in plants in the normal and in the inverse position.
Each value is the mean abscission time of 20 petioles.

Nr. of exp.	Leafpair	Mean abscission time in days							S.E.
		norm.	horiz. rotat.	difference hor. rot. -norm.	rotat. at an angle of 135°	difference rot. at an angle of 135° -hor. rot.	inverse	difference inverse- hor. rot.	
255	1	3.65	3.97	0.32	3.97	0.00	3.82	—0.15	0.13
	2	3.27	3.67	0.40	4.17	0.50	3.92	0.25	
256	1	4.30	4.22	—	4.45	0.23	4.47	0.25	0.14
	2	3.75	3.95	0.20	4.17	0.22	4.17	0.22	
258	1	3.17	3.75	0.58	3.60	—0.15	3.25	—0.50	0.12
	2	2.90	3.25	0.35	3.45	0.20	3.25	0.00	
268	1	3.70	4.15	0.45	4.22	0.07	4.15	0.00	0.13
	2	3.30	3.72	0.40	3.85	0.13	3.97	0.25	
269	1	3.70	4.27	0.57	4.50	0.23	4.12	—0.15	0.18
	2	3.55	3.85	0.30	4.17	0.32	3.77	—0.08	

TABLE 18

Abscission of petioles in plants rotating either horizontally or at an angle of 150° and in plants in the normal and in the inverse position.
Each value is the mean abscission time of 20 petioles.

Nr. of exp.	Leaf pair	Mean abscission time in days							S.E.
		norm.	horiz. rotat.	difference hor. rot. norm.	rotat. at an angle of 150°	difference rot. at an angle of 150° -hor. rot.	inverse	difference inverse- hor. rot.	
278	1	3.67	3.80	0.13	3.95	0.15	3.92	0.12	0.11
	2	3.17	3.55	0.38	3.75	0.20	3.55	0.00	
279	1	3.10	3.72	0.62	4.05	0.33	3.67	—0.05	0.13
	2	2.62	3.40	0.78	3.82	0.42	3.27	—0.13	

The conclusion is that the effect of elimination of the longitudinal component and that of its action in the inverse direction is approximately the same, and further that the abscission is accelerated most, and that the abscission time therefore is shortest, in plants in the normal position.

The geotropical reaction of the tip of inversely placed plants has apparently no effect on the abscission time. If it really had such an effect, then a difference should have been found between the abscission time of inversely placed plants and that of plants rotating e.g. at $\alpha = 135^\circ$, which do not curve geotropically. From the data of Table 17 it is clear that the differences are inconsistent.

5. THE ABSCISSION IN PLANTS FIXED AT ANGLES OF 45° AND 135°

In the experiments described in section 4, in which the plants rotated round axes including various angles with the vertical, the transversal component of gravity presumably had no effect at all. However, in plants fixed at an angle to the vertical the abscission must be affected by this component, which is proportional to sin α.

It was shown that the accelerating effect of gravity on the abscission is strongest when the petioles are debladed immediately after the plants have been brought in the horizontal position. Since the longitudinal component is eliminated at angles between 90° and 180°, it might be expected that the effect which is exercised on the abscission by this elimination, will be more pronounced in plants fixed at 135° (in general at $\alpha > 90°$) than in those fixed at 45° ($\alpha < 90°$).

In the next experiments a set of plants was fixed at an angle of 45°. These experiments too were carried out in the dark. Two days prior to the start of the experiments the plants were transferred to the dark room. After the petioles of which the abscission was to be studied, were debladed, the plants, 10 in each series, were kept or placed in the following positions:

series a: vertical,
series b: horizontal,
series c: fixed at an angle of 45°.

Series b and c were mounted on the axes of the resting clinostat. The results of some of the experiments are given in Table 19. In plants in the horizontal position the abscission was either accelerated or retarded as compared to that in plants in the normal position, but the differences were generally small. In all experiments in plants fixed at $\alpha = 45°$ the abscission proved to be accelerated as compared to that in the vertical plants. This accelerating effect was most pronounced in those cases were it was also noticeable in the horizontal controls.

TABLE 19

Abscission of petioles in plants in vertical (normal) and horizontal position and in plants at an angle of 45°.
Each value is the mean abscission time of 20 petioles.

Nr. of	Leaf pair	Mean abscission time in days					S.E.
		vertic.	horiz.	difference horiz.-vert.	at an angle of 45°	difference at an angle of 45°-vert.	
241	1	5.02	4.70	— 0.32	4.42	— 0.60	0.18
	2	4.80	4.92	+ 0.12	4.67	— 0.13	
243	1	4.10	4.47	+ 0.37	3.77	— 0.33	0.12
	2	3.87	3.90	+ 0.17	3.52	— 0.35	
247	1	4.50	4.15	— 0.35	3.87	— 0.63	0.12
	2	4.05	4.00	— 0.05	3.67	— 0.38	

From these results it follows that the abscission time is influenced

a. in horizontally placed plants:
   1. by a retarding effect of the elimination of the longitudinal component,
   2. by an accelerating effect of the unilateral action of the

transversal component of gravity. This effect depends on a supply of auxin from the tip and the young leaves, the concentration of which probably undergoes a steady decrease in the dark (OORTWIJN BOTJES, 1938).

These opposite effects seem to compensate each other to a larger or less extent. Consequently the abscission time is only slightly different from that of the vertical controls.

b. in plants fixed at an angle of $\alpha = 45^\circ$:

1. by a retarding effect of the partly eliminated longitudinal component, the retarding effect being (:) $\cos \alpha$.
2. by a promoting effect of the transversal component, being (:) $\sin \alpha$.

The resulting effect, a promotion of the abscission, is stronger than the promotion by the longitudinal component in the vertical controls alone.

Similar experiments were made with plants fixed at an angle of $\alpha = 135^\circ$. One experiment consisted of 3 or 4 series of 10 plants each in the following positions:

series a: vertical,
series b: horizontal,
series c: fixed at an angle of $\alpha = 135^\circ$,
series d: upside down.

TABLE 20

Abscission of petioles in plants in normal, horizontal and inverse position and in plants at an angle of 135°.
Each value is the mean abscission time of 20 petioles.

Nr. of exp.	Leaf pair	Mean abscission time in days							S.E.
		norm.	horiz.	difference hor.-norm.	at an angle of 135°	difference at an angle of 135°-norm.	inverse	difference inverse-norm.	
227	1	6.02	5.60	—0.42	6.40	+0.38			0.20
	2	5.60	5.07	—0.53	6.07	+0.47			
230	1	4.50	4.70	+0.20	5.07	+0.57			0.22
	2	4.15	4.32	+0.17	5.05	+0.90			
233	1	4.90	5.22	+0.32	5.67	+0.77			0.17
	2	4.57	4.97	+0.40	5.45	+0.88			
249	1	3.97	3.65	—0.32	4.05	+0.09	4.30	+0.33	0.11
	2	3.37	3.42	+0.05	3.82	+0.45	4.27	+0.90	
250	1	3.70	3.40	—0.30	3.75	+0.05	3.87	+0.17	0.23
	2	3.02	3.20	+0.18	3.47	+0.42	4.20	+1.18	

As it appears from Table 20 in all experiments the abscission in plants fixed at an angle of $\alpha = 135^\circ$ proved to be retarded as compared to the vertical controls. The retardation however, was less than that in inversely placed plants. Also at $\alpha = 135^\circ$ the accelerating effect of the transversal component being (:) $\sin 135^\circ$ is manifest. The final effect is once more the difference between the retarding effect of the inversely acting (= elimination of the) longitudinal component and the accelerating effect of the unilaterally acting transversal component.

6. THE EFFECT EXERCISED BY THE LONGITUDINAL COMPONENT IS INDEPENDENT OF THE AUXIN LEVEL AT THE PROXIMAL SIDE OF THE PETIOLES

The acceleration of the abscission in horizontally placed plants was found to depend on the auxin level at the proximal side, no matter whether the auxin was produced by the tip and the young leaves, or artificially supplied. It now was investigated whether this holds true also for the retarding effect caused by the elimination of the longitudinal component of gravity.

For these experiments the plants were decapitated, and the petioles of 3 leaf pairs were debladed in the usual way. The other leaves were removed. Since in such plants under natural light conditions no abscission occurs, the experiments were carried out in the dark room. In each experiment one series of plants was left in the vertical position, whereas the other one was placed horizontally.

TABLE 21

Abscission of petioles in decapitated, wholly debladed plants in vertical (normal) and horizontal position (in the dark).

Nr. of exp.	Leaf pair	Number of petioles	Mean abscission time in days		Difference	S.E.	P
			vertic.	horiz.			
210	1	20	4.68	5.22	0.54	0.10	< 0.001
	2	20	4.25	4.75	0.50		< 0.001
	3	20	4.12	4.57	0.45		< 0.001
214	1	28	4.53	4.83	0.30	0.12	0.02–0.01
	2	28	4.08	4.59	0.51		< 0.001
	3	28	3.27	3.98	0.71		< 0.001

In Table 21 the results of two experiments are given; they show that under these conditions the abscission is retarded in the horizontal position. If this retardation would be caused by the elimination of the longitudinal component, it should also be found in plants rotating horizontally on the clinostat. This was tested in experiments in which the plants rotated round the horizontal axis of the clinostat instead of being kept in a horizontal position. Table 22, in which the results of a number of these experiments are given, shows that in horizontally rotating plants too the abscission is retarded.

In the absence of an auxin source at the proximal side of the petioles the retarding effect obtained in horizontally placed and horizontally rotating plants by the elimination of the longitudinal component is the same.

The effect of the longitudinal component therefore is independent of the auxin level at the proximal side. It seems probably that this

TABLE 22

Abscission of petioles in decapitated, completely debladed plants rotating either vertically or horizontally on the clinostat axis (one rotation in 2 min. 47 sec.) in the dark.

Nr. of exp.	Leaf pair	Number of petioles/ series	Mean abscission time in days		Difference	S.E.	P
			vert. rot.	hor. rot.			
199	1	20	4.00	4.52	0.52	0.12	< 0.001
	2	20	3.87	4.22	0.35		0.01–0.001
	3	20	3.52	3.82	0.30		0.02–0.01
207	1	20	5.12	5.72	0.60	0.14	< 0.001
	2	20	4.75	5.37	0.62		< 0.001
	3	20	4.45	4.50	—		—
208	1	20	5.77	6.35	0.58	0.20	0.01–0.001
	2	20	5.60	6.02	0.42		0.05–0.02
	3	20	4.82	5.10	0.28		> 0.05
210	1	40	4.68	4.96	0.28	0.10	0.01–0.001
	2	40	4.25	4.77	0.57		< 0.001
	3	40	4.12	4.31	0.19		0.05

effect is related to the A.R.S. and especially to the transport of this substance. It was shown that abscission is possible only when the A.R.S. disappears from the abscission zone, i.e. when it is withdrawn from the latter to other parts of the plant where it is used in some growth process. Therefore, it seems that the transport of this A.R.S. is governed by the longitudinal component, and that the elimination of the latter hampers it.

In the dark there is no synthesis of the A.R.S., at least under all conditions tested by us, and here, therefore, no abscission should have occurred unless the A.R.S. had been withdrawn from the petioles, or unless the substance had been consumed by the petioles themselves. In the latter case no difference in abscission time should be found between the petioles of plants in different positions.

One might argue that the abscission process itself might be influenced by gravity. In order to check this possibility, an experiment was carried out in which 20 plants were left vertical, and 20 other ones were rotated horizontally on the clinostat. The length of the

TABLE 23

Abscission of petioles in vertical plants and in plants rotating horizontally on the clinostat axis (one rotation in 2 min. 47 sec.) in the dark. Length of the petioles: 2 mm. Each value is the mean abscission time of 40 petioles.

Nr. of exp.	Leaf pair	Mean abscission time in hours	
		vertical	horiz. rotat.
215	1	48.4	50.8
	2	45.4	48.5
	3	37.0	39.4

petiole stumps was only 2 mm. Every 6 hours the abscission was controlled. Table 23, which gives the result of this experiment, shows that the greatest retardation of the abscission found in the rotating plants was 3 hours, a value of no statistic significance. The longitudinal component therefore does not affect the abscission process itself.

7. THE EFFECT OF GRAVITY ON THE ABSCISSION IN PLANTS THAT WERE KEPT IN THE HORIZONTAL POSITION DURING A LIMITED TIME

It seemed worth while to know how long plants should be exposed to the unilateral action of the transversal component of gravity, in order to obtain an accelerated abscission. It has been reported at an earlier occasion (Vendrig, 1958) that a sojourn of 12 hours in the horizontal position suffices to produce an acceleration of the abscission, but that this acceleration is obtained only when the sojourn in the horizontal position is preceded by a dark period (night). In these experiments the plants that after a dark or light period were kept for 12 hours in the horizontal position, did not belong to the same culture. When the experiments were repeated with plants belonging to one and the same lot, the earlier obtained results could not be reproduced. Table 24 shows that a sojourn of 12 hours in the horizontal position always causes an acceleration of the abscission, no matter whether the plants in the preceding period were exposed to the light or kept in the dark. The absence of this effect after the plants had been exposed for 12 hours to the transversal component of gravity, will probably have been caused by the particular condition of part of the plants (auxin level) and to seasonal variability of the latter.

Table 24

The influence of gravity on the abscission of petioles in plants placed in a horizontal position for 12 hours after a dark (night)- or light (day) period.
Each value is the mean abscission time of 40 petioles.

Leaf pair	Mean abscission time in days				Difference	
	After a light period		After a dark period		vertical (control) – 12 hours horizontal	
	vertical control	12 hours horiz.	vertical control	12 hours horiz.	after a light period	after a dark period
1	7.81	7.22	8.75	8.45	0.59	0.30
2	6.87	6.35	7.63	7.03	0.52	0.60
1	7.80	6.65	8.34	7.73	1.15	0.61
2	5.94	5.32	7.53	6.17	0.62	1.36

8. THE EFFECT OF THE REVOLVING SPEED OF THE CLINOSTAT AXIS ON THE ABSCISSION IN HORIZONTALLY ROTATING PLANTS

The revolving speed of the "De Bouter" clinostat, that was used in the experiments on which we have thusfar reported, is 167 seconds, and can not be altered. In order to study the effect of the revolving speed on the abscission, a special clinostat was required. This instrument was ably designed by Mr P. A. De Bouter, who, in cooperation with Messrs A. van Garderen and C. Montijn, also built it in the laboratory workshop (Fig. 25 and 26). A three-phase synchronous motor of $\frac{1}{2}$ HP at the base of the instrument drives, by the intermediary of a transmission mechanism submerged in an oil bath, a vertical axis which carries at the top a horizontal table of 60 cm diam. By a system of pinions and (partly conical) cog wheels 6 horizontal axes mounted under the horizontal table, are driven, each at a different revolving speed; these speeds were 55 sec., 110 sec., $9^1/_3$ min., 28 min., 55 min. and 110 min. On each axis a table was mounted of 30 cm diam., on each of which 10 small pots with one plant each could be fixed by means of spring strings and hooks.

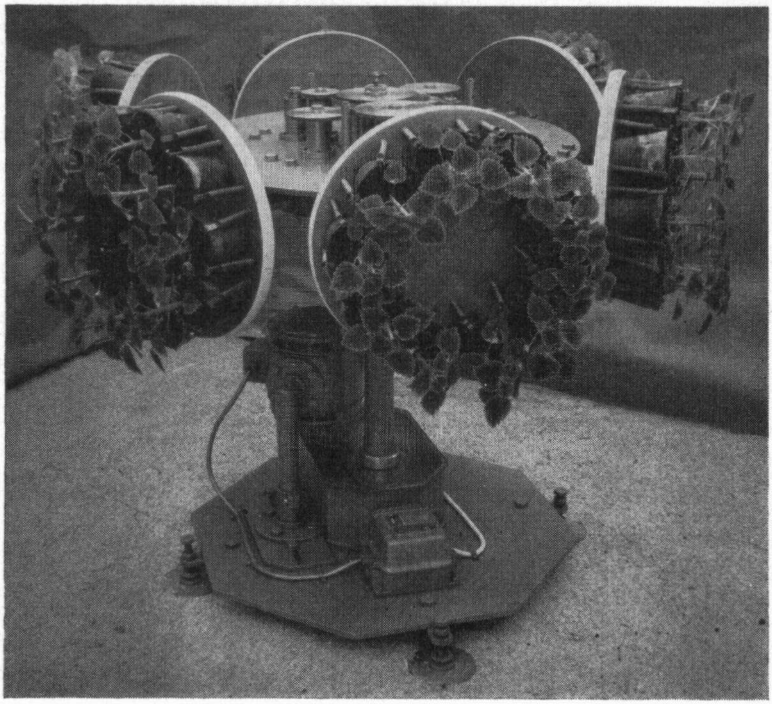

Fig. 25. Photograph of the new clinostat provided with 6 horizontal axes at different revolving speeds.

It should be mentioned that the horizontal table at the top too rotates (one revolution in 10 min.). This is essential, since in this way all possible differences in the external conditions, especially in the illumination to which the plants may become exposed, are ruled out.

By means of this new clinostat the following experiments were carried out. Each experiment comprised 70 plants, 10 of which were kept in the normal position and served as controls. On each of the horizontal axes another group of 10 plants was mounted. After a sojourn of one day on the clinostat, in all plants the petioles of 2 leaf pairs were debladed. The experiments were carried out in the experimental glass-house under natural light conditions during the months January to March 1959. The results of 5 similar sets of experiments are shown in Fig. 27, in which the mean values that were found for the retardation of the abscission on the six different horizontal axes, are plotted together with the mean abscission time found in the vertical controls. It appears that the retardation of the abscission is the smaller, the slower the speed of rotation, one revolution time, viz. that of 55 min., excepted, but at all the revolving speeds that were tested, the abscission proved to be retarded. At a still slower revolving speed, viz. one of 4 hours, Terpstra (1956) did not find any difference at all in the abscission time between the test plants and the vertical controls.

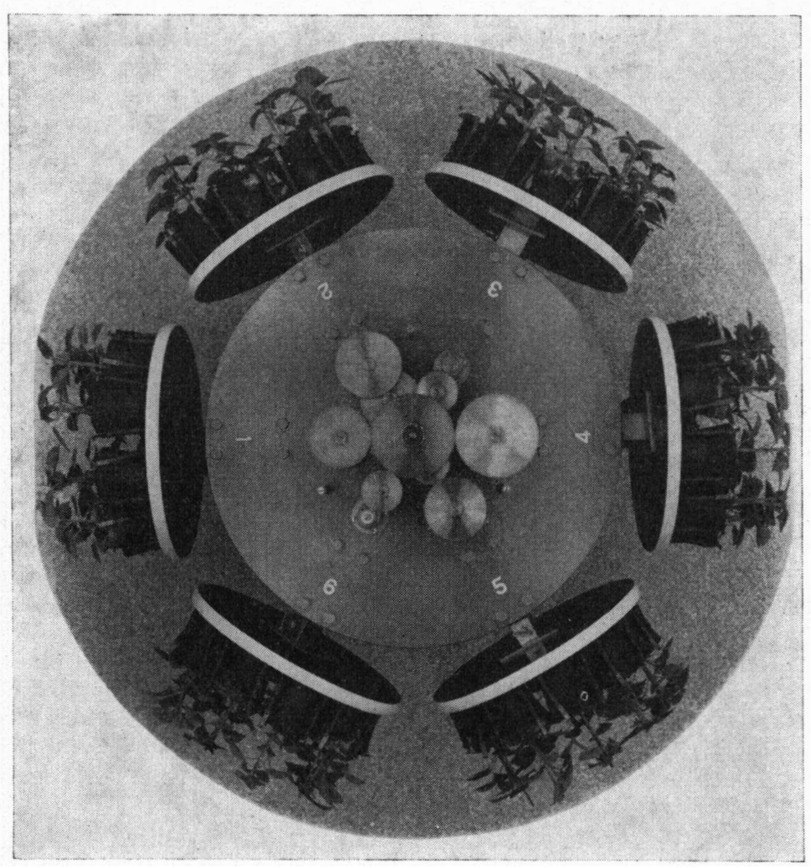

Fig. 26. The clinostat of Fig. 25 seen from above.

It may therefore be concluded that in horizontally rotating plants the abscission is, on the one hand, retarded by the elimination of the longitudinal component of gravity, but that it may, on the other hand, be accelerated by the all-sidedly acting transversal component. The latter effect, however, manifests itself only at a low revolving speed, and is absent when the revolving time is shorter than about 3 minutes.

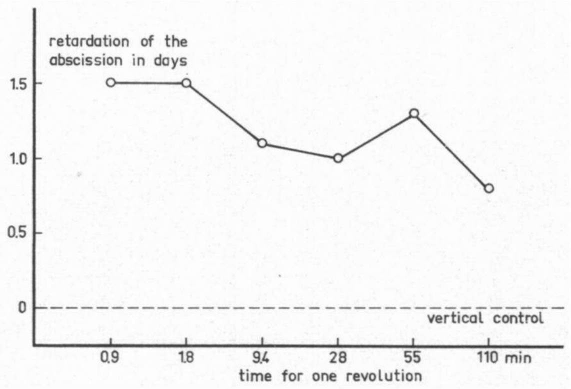

Fig. 27. The effect of the revolving speed of the horizontal axes of the clinostat on the retardation of the abscission of petioles in horizontally rotating plants. Each point represents the mean abscission delay of 200 petioles; for comparison the abscission time of the vertical controls is shown too.

It has been shown that the effect of the transversal component largely depends on the auxin level at the proximal side of the debladed petioles. Theoretically it is therefore to be expected that the speed of rotation will have no influence on the abscission in decapitated plants, because in such plants there is no auxin supply from the proximal side so that the transversal component can not act. To check the correctness of this conclusion some experiments were carried out on the new clinostat.

The plants were decapitated above the upper full-grown leaf pair.

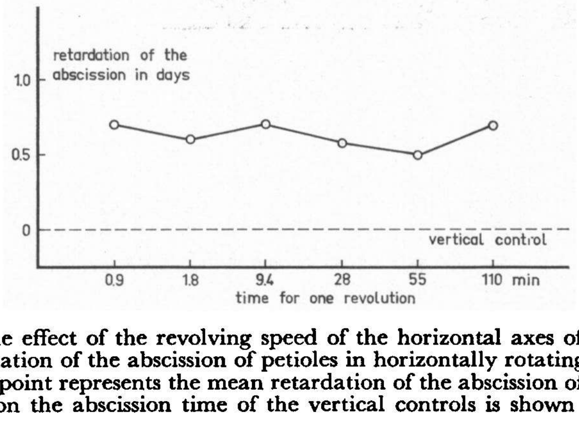

Fig. 28. The effect of the revolving speed of the horizontal axes of the clinostat on the retardation of the abscission of petioles in horizontally rotating, decapitated plants. Each point represents the mean retardation of the abscission of 120 petioles; for comparison the abscission time of the vertical controls is shown too.

The petioles of the next two leaf pairs were debladed. The plants then had one intact leaf pair left. After 2 days the petioles were shortened to a length of 4 mm, and in this state the plants were mounted on the clinostat, on each axis 10 plants, 10 plants remaining in the normal position to serve as controls. The results (the mean values of 3 experiments) are shown in Fig. 28.

As we had expected, the retardation of the abscission in the horizontally rotating plants now appeared to be independent of the speed of rotation.

9. DISCUSSION

A. *The influence of the longitudinal component of gravity*

In the literature that deals with this subject, there is a good deal of controversy and confusion. Most of the investigations were confined to the influence exercised by the longitudinal component on the size of the geotropic curvature (see RAWITSCHER, 1932). Those authors who advocate an effect of the longitudinal component, often speak of a "tonic" effect of some kind, since this component can not have a tropistic effect. The term "tonus" had been borrowed by PFEFFER (1893) from human and comparative physiology, but he did not precisely define its meaning. Generally speaking "tonus" is a somewhat vague indication of a particular state of the protoplasm. In order to avoid this vagueness, PFAELTZER (1934) replaced the term "tonus" by "the reaction capacity of the plant"; in his paper the new term was used especially for the capacity of plants to react upon a geotropic stimulus. Since the effect of a "stimulus" can be known only by its reaction, this concept did not enlighten us with regard to the nature of the action exercised by the longitudinal component.

Positive indications of such an action were found in a retarded growth shown by inversely placed plant parts, e.g. in the branches of the weeping willow (VÖCHTING, 1884), in the coleoptiles of *Avena* (CZAPEK, 1898), in seedlings of different other plant species (HERING, 1904) and in shoots of *Pharbitis hispida* (BREMEKAMP, 1912).

On the other hand HERING (1904), ZOLLIKOFER (1921) and ZIMMERMANN (1924) found an increased rate of growth in inversely placed plants, resp. in the sporangiophores of *Phycomyces nitens*, in coleoptiles of *Avena* and in roots of *Lepidium*.

In horizontally placed plants a decreased growth rate was found by SACHS (1887) in roots of *Vicia faba* and of *Aesculus hippocastanum*, by SCHTSCHERBAK (1910) in hypocotyls of *Lupinus albus*, and by WAREING and NASR (1958) in seedlings of different fruit trees and shrubs. On the other hand an increased growth has also been reported in such plants, e.g. by NOLL (1888) in stems of *Hippuris vulgaris*, by COPELAND (1900) in epi- and hypocotyls of *Helianthus*, *Lupinus*, *Cucurbita*, *Pisum* and *Phaseolus*, and by ZOLLIKOFER (1921) in the coleoptile of *Avena*.

NEMEČEK (1922) found the strongest decrease of the growth rate in stems of *Phaseolus* and *Helianthus* when the plants were forced to grow at an angle of 135° from the vertical.

A retarded growth in plants rotating horizontally on the clinostat was found by BREMEKAMP (1912) in *Pharbitis hispida*, and this was confirmed by RAWITSCHER (1924). Most authors, however, could not observe an influence of a horizontal rotation on the rate of growth, e.g. ELFVING (1880) in roots of *Lupinus* and *Pisum*, HERING (1904) in seedlings of different cereals, DOLK (1936) in coleoptiles of *Avena*. KONINGSBERGER (1922) who was the first to register the growth on the clinostat, reported that during a long lasting rotation the growth rate of *Avena* coleoptiles gradually decreased. After replacing the plants in the vertical position an acceleration of the growth was reported, preceded by a short-lasting retardation. From this it was concluded that the longitudinal component promotes the rate of growth. After a short-lasting elimination of this component obtained by rotation round the horizontal axis of the clinostat, a growth reaction was recorded. Using the same auxanometer DOLK, however, could not reproduce these results, which he ascribed to slight autotropic curvatures in the plane of the shorter diameter of the coleoptile. Finally, BRAIN (1935) studied the growth rate of a number of plants species which rotated during long times on the clinostat (one rotation) hour). In some of his objects a retardation, in other ones an increase of the rate of growth was found.

This brief survey, in which no mention is made of experiments with centrifugal forces acting perpendicularly to the direction of gravity, clearly shows how uncertain the effect of the longitudinal component often is. Many authors believe that this component has no physiological effect or meaning, or, more plainly expressed, that it does not exist.

By the reported experiments it could be ascertained that the longitudinal component at any rate does accelerate the abscission. However, its effect on the abscission process, i.e. on the hydrolysis of the primary cell walls in the abscission zone, is not a direct one. The substance (A.R.S.) that retards this process is, as had been shown (chapter V), an auxin-like substance produced in the green parts of the petioles (and leaf blades) when these parts are exposed to the light. This A.R.S. may be drained off from the petioles by growth processes which take place in other parts. The effect of the longitudinal component probably consists of an influence exercised on the migration of this A.R.S.

In chapter VI we already concluded that the A.R.S. is probably transported in a basipetal direction, and that this transport is blocked or seriously impeded in horizontally placed plants and in plants rotating round a horizontal axis. The most plausible explanation seems to be that the longitudinal component promotes the transport of the A.R.S. Consequently the abscission is retarded by the elimination of the longitudinal component, and by a partial or total inversion of the direction of its action.

In horizontally placed plants the longitudinal component is eliminated. In this case, however, the abscission is only retarded when the source of the auxin supply at the proximal side of the debladed petioles is taken away by decapitation and by the removal of all young leaves. These leaves produce a substance replaceable by IAA, which induces growth activity at the lower side of the stem. So long as this growth process lasts, the elimination of the longitudinal component seems to exercise no influence; in this case the A.R.S. has to travel only in a transversal and practically not in the longitudinal direction in order to reach the sites where the growth activity is found.

The accelerating effect exercised by the longitudinal component on the abscission was also evident in plants that were rotating at various angles to the vertical, but only when the deviation from the latter was less than 90°. From the fact that rotation at angles larger than 90° gave the same retardation of the abscission as was found in horizontally rotating and in inversely placed plants, we had to conclude that the inversion of the longitudinal component has the same effect as its elimination.

A significant retardation of the abscission was already found when the plants had rotated round the horizontal axis for 1 or 2 days only. This indicates that the conditions which are present during the first days after the deblading of the petioles, are decisive for the length of the abscission time. The same holds true for the supply of auxin from the distal side, which in order to be effective on the abscission should take place within 2–3 days after the deblading (Barlow, 1950, confirmed by our own experiments).

In the abscission time two phases can be distinguished, viz.

1. a phase during which the A.R.S. prevents the abscission proper (2). This factor may be drained off from the abscission zone by growth processes going on or started elsewhere in the plant. This withdrawal can be promoted or retarded by an application of auxin from the distal side, the effect of the latter depending on its concentration. In the stem the rate of longitudinal transport of the A.R.S. is promoted by the longitudinal component of gravity, which means that it is most effective in the normal position;
2. the abscission process proper, consisting of a hydrolysis of the primary cell walls in the abscission zone.
   This process starts only when the concentration of the A.R.S. has sunk to a rather low level. This phase of the process is neither affected by auxin nor by gravity.

The transport of the A.R.S. is not confined to the vascular system, but takes place also in the parenchyma.

In this connection the physico-chemical state of the protoplasm and of its boundery membranes must be of importance. In the older literature numerous changes in the physico-chemical properties of the protoplasm have been ascribed to the transversal action of gravity, such as changes of the osmotic value, of the viscosity, of the sugar content, of the pH, of the enzyme activity (catalase) and of the permeability (see e.g. Metzner, 1937). For future research in this field it seems advisable to extend the study to plants rotating parallel to the horizontal axis of the clinostat, at a not too slow rate as in such plants the complications caused by the unilateral action of the transversal component are eliminated.

B. *The influence of the transversal component of gravity*

The conclusion of TERPSTRA (1956) that the acceleration of the abscission in horizontally placed plants is caused by the unilateral action of the transversal component of gravity, was confirmed in our own experiments. The process, however, proved to be a rather complicated one. The acceleration of the abscission is obtained only if the petioles are debladed within a few days after the plants have been brought in the horizontal position. TERPSTRA had found already that in order to obtain the acceleration of the abscission either the presence of a substance produced in the tip of the stem or else that of artificially supplied auxin, are indispensable. Most probably the young leaves are the source of the substance that is produced in the tip and which is replaceable by auxin. According to the CHOLODNY–WENT theory this auxin-like substance would be transported laterally, and so accumulate at the lower side of the stem, where it causes a stronger growth (hypotrophy).

Hypotrophy as well as epitrophy are well-known phenomena in horizontally placed plants. Hypotrophy is most common in conifers, epitrophy in broad-leaved trees. In perennials both epi- and hypotrophy occur; *Corchorus* and *Cannabis*, for instance, show epitrophy in the horizontal position (SATO, 1956). According to MOLOTKOVSKY *et al.* (1955), hypotrophy is found in *Asclepias* and epitrophy in the chestnut, and the hypotrophy of *Asclepias* is accompanied by an increase in the amount of ascorbic acid in the lower side, whereas the epitrophy of the chestnut is accompanied by an increase in the amount of the same substance in the upper side.

In the literature many data are to be found on the induction of meristematic activity by a local accumulation of auxin (a.o. FISCHNICH, 1935; MOUREAU, 1939), an activity which results in the development of adventitious roots. This development of new roots is also often met with at the lower side of horizontally placed plants.

In horizontally placed *Corchorus* plants (SATO, 1956), the epitrophy could be prevented by supplying the upper side of the stem with auxin (NAA). This can be explained by assuming that at the upper side the auxin level is lowered because this substance is deviated to the lower side, the lowering of the auxin level being responsible for the epitrophic growth.

Like the development of adventitious roots, the activation of the growth at the lower side of the stem too is in horizontally placed stems promoted by leaf factors. In horizontally placed plants the longitudinal transport of these leaf factors is hampered (chapter VI). By the induction of local growth at the lower side of horizontally placed plants the A.R.S. is drained off from the petioles. The longitudinal transport of the A.R.S. too is hampered in the horizontal position. The A.R.S. is not identical with the substance produced in the young leaves, as appeared from the fact that the abscission time of the petioles at the lower side of the stem is the same as that of the petioles at the upper side; since the auxin-like substance from the young leaves is supposed to accumulate at the lower side, it would, if it were identical with the A.R.S., cause a retarded abscission of the petioles at the lower side.

The experiments on the clinostat prove that the lateral transport of the auxin-like substance produced in the tip and in the young leaves (and which is replaceable by auxin) as well as the retardation of the longitudinal transport of the leaf factors, are caused by the unilateral action of the transversal component of gravity. However, if the rotation speed of the clinostat axis is sufficiently high ($<$ 3 min.), neither an activation of growth nor a retardation of the transport of the leaf factors are met with.

The effect exercised by the unilateral action of the transversal component was also recognizable in experiments in which the plants were fixed in a position which deviated from the normal one. The abscission time then proved to depend on the resultant of the simultaneous effects of the longitudinal and of the transversal components. This meant that at an angle of 45° the abscission was still accelerated, whereas it was retarded at an angle of 135° (Tables 19 and 20). As the accelerating effect of the transversal component at 45° and at 135° is the same, this can be explained by the fact that the accelerating effect exercised by the longitudinal component in the normal position, is reduced to zero when the deviation from the normal position reaches a value of 90°.

The transient character of the growth activity at the lower side of horizontally placed plants follows from the fact that an acceleration of the abscission is obtained only if the petioles are debladed within a few days after the plants have been brought in the horizontal position. When the plants were kept for a longer time in the horizontal position, a retardation of the abscission was often observed (chapter VI). On the other hand, a sojourn in the horizontal position of 12 hours already sufficed to cause an acceleration of the abscission (Table 24).

C. *On the physiological effect of the rotation on the clinostat*

According to SACHS (1873), the successive reactions on the unilateral geotropic stimuli would compensate each other. In his opinion, therefore, there would be a perception of the gravitational force, but there would be no reaction, since the successive effects would neutralize each other.

CZAPEK (1898), on the other hand, was of opinion that there is no perception of the gravitational force by horizontally rotating plants, at least if the rate of rotation is not too low. At very low speeds the successive stimuli would be perceived, and would cause some kind of reaction.

In later years but little attention has been paid to a possible influence of the speed at which the horizontal clinostat axis rotates. TALTS (1932) and REISS (1934) found that in roots the previously induced geotropic curvature was the larger, the higher the revolving speed of the clinostat axis (one revolution in ½–53 min.). The way in which shoots reacted on the revolving speed of the clinostat axis, was just the reverse. The authors ascribed this result to some specific "geotonic" effect of the rotation. Larsen (1953) too found in roots of *Artemisia* an effect of the revolving speed on the rate of growth and on the size of a previously induced geotropic curvature; at all speeds the rate of the growth was retarded (one revolution in $^1/_4$–128 min.), but the retardation was smallest at revolving speeds between $^1/_2$–2 minutes and of 64 min.; in the inverse position too the growth was retarded.

In our experiments with a new clinostat the revolving speed proved to affect the abscission time in horizontally rotating plants. As compared to the abscission time in vertical controls the greatest retardation was found at speeds of 55 and 110 seconds. It was smaller at speeds of 9 and 28 min. and smallest at a speed of 110 min. At a speed of 55 min. a second peak of the retardation was found (Fig. 27), which can not be accounted for. With a speed of one revolution in 4 hours Terpstra (1956) did not find any difference at all between the abscission time of the plants that were rotating horizontally and that of the vertical controls.

When we compare the results of these experiments with those of Larsen (1953), it appears that the speed of rotation effects the abscission in a way that is the exact opposite from that in which it influences the growth of the *Artemisia* roots. More or less similar speeds of rotation, viz. in Larsen's experiments ½ or 2 min. and 64 min., in our own experiments 55 or 110 sec. and 55 min., cause a *minimum* decrease of the growth rate in the *Artemisia* roots and a *maximum* decrease of the acceleration of the abscission in the *Coleus* petioles. This may perhaps be regarded as a difference that in some way is related to the different ways in which stems and roots react on the gravitational force.

The effect of the speed of rotation on the abscission proved to depend, just like the acceleration of the abscission in horizontally placed plants, on the presence of the tip and the young leaves. This probably means that in slowly rotating plants the auxin-like substance produced in the young leaves induces the same growth processes, though now successively at all sides, as it does at the lower side of horizontally placed plants. For this induction it is apparently necessary that each side is exposed to the gravitational force during a period of sufficient length. It can not be decided whether the stimuli themselves are combined into a single one or whether each stimulus itself causes a reaction. The peak in the decrease of the acceleration of the abscission found at a revolving speed of 55 min. gives an indication of the complexity of the physiological effects of the rotation round the horizontal axis of the clinostat. On the other hand the reported results are strongly in favour of Czapek's theory on the clinostat.

De Wit (1957) has shown that in the *Avena* coleoptile the gravitational force which ultimately finds its expression in a geotropic curvature is perceived only when free auxin is available. Brauner and Hager (1958), on the other hand, believe that in seedlings of

Helianthus annuus the gravitational force is perceived also in the absence of auxin. They noted that three days after decapitation the plants had lost the capacity to react geotropically. If they had passed in this condition 14 hours in the horizontal position, and were brought back in the vertical position and provided with IAA, a strong curvature was produced whose direction corresponded with that of an ordinary geotropic one. They concluded that the transversal polarisation induced by gravity does not cause a lateral migration of auxin, but of another substance, and that the latter causes a long-lasting physiological asymmetry.

The question whether the induction of a transversal polarity causes the lateral transport of auxin, or whether the lateral migration of auxin itself is a direct effect of the action of gravity, is as yet undecided. The reaction on the transversal action of gravity that is actually observed, postulates the availability of auxin. This holds true for geotropism as well as for the activation of growth at the lower side of the stem.

In plants provided with distinct nodes (grasses) the production of auxin is resumed in the nodes of full-grown stem parts when the plants are placed horizontal or when they are rotating horizontally on the clinostat (SCHMITZ, 1933; VAN OVERBEEK *et al.*, 1945). BRAIN (1942) found an increase of diffusible auxin in plants of *Lupinus albus* that had rotated on the clinostat (1 revolution/hour). Here the growth rate too increased on the clinostat (BRAIN, 1935).

In *Coleus* no increase of the auxin production was found in horizontally placed plants. An unilateral effect of gravity was recognizable only when the auxin-like substance produced in the young leaves or when artificially supplied auxin was available.

The transport of the auxin-like substance produced in the tip and in the young leaves and that of externally supplied IAA shows a fundamental difference with that of the A.R.S. (produced in the petioles and leaf blades). The transport of the first substances is affected by gravity only in horizontally placed plants, in which the unilateral action of gravity is accompanied by a lateral migration of these substances. The longitudinal transport of the A.R.S., on the other hand, is governed by the longitudinal component of gravity.

SUMMARY

1. The abscission is accelerated (in plants in the normal position) by the longitudinal component of gravity.

2. Elimination of the longitudinal component impedes the basipetal transport of the A.R.S. and this, therefore, retards the abscission in comparison with that found in the plants in the normal position.

3. The longitudinal component is eliminated in horizontally placed or horizontally rotating plants, and the same effect is found in plants fixed at or rotating under angles of more than 90° to the vertical and in plants that are placed upside down.

4. In horizontally placed plants the retardation of the abscission (2) is neutralized by the acceleration caused by the induction of growth processes at the lower side of the stem.

5. When the plants had been kept in the horizontal position for a week before

the petioles were debladed, this growth process had been completed, and the abscission, therefore was, (2) retarded.

6. This effect was also obtained in the horizontally placed plants when there was no source of auxin at the proximal side of the debladed petioles.
7. In horizontally rotating plants the abscission time depends on the revolving speed of the clinostat axis. The retardation of the abscission by the elimination of the longitudinal component reaches its maximum value at a revolving speed of 1–3 min.
8. At a low revolving speed the transversal component causes an acceleration of the abscission (4), which partly compensates the retardation caused by (2).
9. The effect of the revolving speed of the clinostat axis on the abscission is independent of the presence of a source of auxin proximal of the debladed petioles.

CHAPTER VIII

GENERAL DISCUSSION

1. THE RELATION BETWEEN ABSCISSION AND AUXIN

In their review of the literature dealing with the physiology of abscission ADDICOTT and LYNCH (1955) came to the conclusion that the abscission is governed by the auxin gradient found along the axis of the abscission zone. The results of experiments carried out by other authors (JACOBS, 1955, 1958; LAUDI and LAMBRI, 1956; TERPSTRA, 1956), who replaced either the tip (i.e. the proximal auxin source) or the leaf blade (i.e. the distal auxin source) by concentrated preparations of auxin (IAA), fitted fairly well in the frame of this theory. The results of some of TERPSTRA's experiments, however, did not agree with the theory, and the finding of HALL and LIVERMAN (1956) according to which in *Phaseolus* and cotton seedlings removal of the tip had no effect on the abscission, did not do so either. It appeared, moreover, that in explants the abscission is not governed by the auxin gradient, but by the height of the auxin concentration in the abscission zone, and that it does not matter whether the auxin is obtained from the proximal or from the distal side (GAUR and LEOPOLD, 1955; BIGGS and LEOPOLD, 1958).

In the present paper it was shown that in *Coleus* the acceleration of the abscission found after relatively high concentrations of IAA had been supplied from the proximal side, is to be regarded as an indirect effect of this supply. If leaf factors were not lacking or deficient, the locally high concentration of IAA induced the development of adventitious roots, and the abscission appears to be correlated with the development of these new roots. If for some reason no new roots were developed, the abscission was found to be retarded. The same result was obtained with decapitated plants that were supplied with a relatively high auxin concentration from the proximal side.

This proves that the theory of JACOBS (1955), according to which in intact plants of *Coleus* the acceleration of the abscission is a direct effect of the auxin produced in the young leaves at the tip, is untenable.

Several other facts too seem to indicate that the acceleration of the abscission obtained by leaf factors that are produced by the young leaves as well as by full-grown leaves in the vicinity of the debladed petioles, is an indirect one, and that this acceleration is in reality obtained by the intermediary of their effect on the growth of roots or of a part of the stem.

ADDICOTT and LYNCH (1955) and TERPSTRA (1956) mention the possibility that a growth which is induced somewhere in the plant by auxin, may drain off nutrients from other parts, also, therefore, from the petioles. The acceleration of the abscission then would be due to a shortage of nutrients. It is generally accepted that nutrients are preferably directed towards sites where the auxin concentration is relatively high (a.o. WENT, 1938; EMSWELLER and STUART, 1948).

In *Coleus* the abscission proved to depend not only on growth processes going on or initiated at the proximal side of the debladed petioles but also on the length of the latter. It could be shown that in debladed petioles which are exposed to the light, a substance (A.R.S.) is formed which retards the abscission. This A.R.S. can migrate towards the abscission zone through the parenchyma, i.e. independently of the vascular system (chapter v).

GARDNER and COOPER (1943) reported that the effect of distally supplied IAA and NAA was greater when the petioles had a length of 2 cm than when they were only 0.5 cm long. However, the abscission time of the controls that had not been supplied with auxin, was the same. This aberrant result probably is to be ascribed to the relatively high temperature (27° C) at which the experiments were carried out. At this temperature the abscission proceeds very quickly, and since the authors controlled the petioles only once in 24 hours small difference in the abscission time may have escaped their attention. The retarding effect exercised on the abscission by distally supplied auxin might, according to these authors, depend on or be governed by a second substance or set of substances that are already present in the petioles. This might be the substance (A.R.S.) mentioned above.

Although IAA when distally applied to the petioles, may retard the abscission at higher concentrations and accelerate it at low ones, this effect too is in *Coleus* an indirect one. This had to be concluded from the fact that the difference in the abscission time found between plants in the normal position and plants that had been brought in the horizontal one, has a constant value, and is independent of the concentration of the IAA that is supplied from the distal side. Moreover, the abscission is only accelerated by the distal application of low concentrations of IAA when the plants are intact, and not when they have been decapitated. This means that this acceleration occurs only when there is a sufficiently active growth at the proximal side of the debladed petioles, a growth that is made possibly by auxin and other leaf factors produced in the young leaves. In decapitated plants the A.R.S. is apparently drained off but slowly from the abscission zone, and the IAA apparently can not lower the concentration of the A.R.S. to such an extent that abscission can take place. Under these circumstances the balance between the production of the A.R.S. in the petioles and its withdrawal in a proximal direction is maintained on a level that is not so low that the abscission can proceed.

In the dark there is in the petioles of explants no production of the A.R.S. Therefore, in such explants the abscission can be accelerated by low concentrations of IAA (Biggs and Leopold, 1958).

The retarding effect on the abscission obtained by the elimination of the longitudinal component of gravity, is of fundamental importance. This effect proved to be independent of auxin and of growth activity induced by auxin at the proximal side of the debladed petioles; it is connected with the A.R.S. produced in the petioles (and leaf blades). Since most of these experiments were carried out in the dark, no new supply of the A.R.S. could be produced, and therefore only the residual amount can have played a part. As the abscission time primarily depends on the rate at which the A.R.S. is withdrawn from the petioles, we will have to conclude that the elimination of the longitudinal component must hamper the transport of the A.R.S., and that in plants in the normal position this transport is facilitated by the longitudinal component.

There is no evidence that IAA exercises an effect on the synthesis of the A.R.S. in the petioles, nor is there any evidence that the activity of the A.R.S. depends on the presence of IAA. If there were some antagonistic or synergistic interaction between IAA and the A.R.S., the latter would be independent of the presence of growth processes at the proximal side of the debladed petioles. This, however, is not so. IAA and other substances acting in boundery interphases, might cause physico-chemical changes in the protoplasm, and via such a mechanism they might perhaps affect the migration of the A.R.S. from the abscission zone.

The transportability of the A.R.S. through the parenchyma and the effect exercised by the longitudinal component of gravity on that transport, suggest an auxin-like character of the A.R.S.

From extracts obtained from full-grown leaves an auxin could be isolated of which it was shown that it could not be an indole derivative. This auxin proved to retard the abscission, and gave a positive reaction in the standard *Avena* curvature-test. This is no proof that the isolated auxin actually is identical with the A.R.S. produced in the petioles. However, since this factor is drained off from the petioles and from the abscission zone by growth activity at their proximal side, it could well be an auxin. If that were so, there would be no reason to doubt the identity of the auxin from the full-grown leaves with this A.R.S. produced in the petioles, and then the latter too might promote the growth processes induced at their proximal side.

It must be concluded from the experiments of which the results are reported here, that neither IAA nor other auxins exercise a direct effect on the hydrolysis of the primary cell walls in the abscission zone. Barlow (1950) found for pedicels that soon after the removal of the part that is borne by them, a critical phase sets in during which the abscission is induced. When NAA was applied two days after the removal of that part, a retardation of the abscission was no longer noticeable. Moreover, under other circumstances the effect of NAA appeared to be the greater, the shorter the stumps of the pedicels were. In *Coleus*, on the other hand, the retarding effect of NAA and IAA that is applied from the distal side, is the greater, the longer the petiole stumps are (GARDNER and COOPER, 1943). From our own (not published) experiments it appears that application of IAA to the petioles 1–2 days before their abscission had no further effect. Auxin apparently does not affect the later phase of the abscission process.

Our conclusion that the effect of IAA on the abscission is an indirect one, is not fully compatible with the view of those authors who wish to connect the part played by the auxin in the abscission process with some enzyme activity. According to FACEY (1950) IAA would act upon the enzyme system which is involved in the system of esterifications and de-esterifications. At high concentrations IAA would favour the formation of the insoluble pectic acid and of its Ca-salt.

OSBORNE (1958*a*) studied in explants of *Phaseolus vulgaris* the localisation of the pectin-methylesterase (P.M.E.) and the changes which its activity undergoes in the abscission zone. The P.M.E.-activity appeared to decrease rapidly during the period preceding the abscission, no matter whether the latter took place in the normal course of events or whether it was accelerated by the administration of ethylene or of endothal. The application of 2–4 D and of 2.4.5.T retarded the abscission, and maintained or enhanced the P.M.E.-activity.

In *Nicotiana tabacum* the abscission of non-fertilized pistils is accelerated by a supply of methionine and retarded by a supply of IAA (YAGER and MUIR, 1958*b*). Methionine would act as a methyl-donor, and thus favour the esterification of pectic and pectinic acids leading to the formation of water-soluble pectin. The explanation of the effect of methionine as a methyl-donor, however, can not be valid for the accelerating effect it exercises on the abscission, an effect that was found by YAGER and MUIR (1958*a*) in other amino-acids too.

By a number of other substances the abscission can be either accelerated or retarded, e.g. by TIBA (WEINTRAUB *et al.*, 1952; HACCIUS and NIES, 1956), by substances blocking sulphydryl groups (VAN OVERBEEK *et al.*, 1955; HOTTA and OTA, 1956), and by some substances that are present in leaves that have been shed (OSBORNE, 1955, 1958). It does not seem likely that such substances would have a specific effect on the abscission. For the maintenance of a part, and for changes of its condition too, it is indispensable that the enzyme systems involved in its metabolism function normally. The abscission generally is retarded by inhibitors of the respiration (ADDICOTT and LYNCH, 1955), whereas in explants it is accelerated by an increased oxygen tension (CARNS *et al.* 1951). The metabolism of the involved part therefore is doubtless essential for the abscission process. LEINWEBER and HALL (1959) found for cotton that the respiration of the leaf blade decreased with an increase of age, whereas in the abscission zone the respiration increased up to the moment at which the abscission took place.

The problem whether the retardation caused by auxin in the hydrolysis of the primary cell walls in the abscission zone is due to a direct or to an indirect action of the auxin, therefore, is apparently as yet unsolvable.

As the processes leading to the abscission of a pedicel are apparently started as soon as the part borne by the pedicel has been removed, we may conclude that the abscission of pedicels, like that of the petioles, is governed by auxin that is produced in the part that is borne by them.

In black currants (WRIGHT, 1956) and in apples (LUCKWILL, 1957) this auxin would be produced in the seeds. According the WRIGHT, the synthesis of auxin is bound to that of proteins, and depends therefore on a supply of nutrients. Periods of fruit fall proved to be correlated with a low auxin content of the endosperm. However, especially for the apple this correlation appeared to be present only during a definite period in the development of the fruit. During the first two weeks after the fall of the petals no auxin was found in the seeds, and after the June fall the seeds could be removed from the fruits without causing abscission (LUCKWILL, 1957*b*). The auxin by which the abscission is governed, is therefore most probably not only produced in the endosperm of the seeds, but also in the pericarp. However, although in the pericarp the presence of auxin could be demonstrated, there appeared to be no relation between the auxin content of the latter and the abscission. When the auxins were isolated by means of paper chromatography, it appeared that those which were found in the full-grown leaves of the apple, are the same as those that were found in the seeds (LUCKWILL, 1957). The production of auxins in the leaves seems to depend, as a rule, on light (SÖDING, 1952). In the seeds, on the other hand, the synthesis of auxin seems to proceed independently of light, but it should be realized that in this case the precursors are produced in the green parts of the plant.

As mentioned by WRIGHT, the production of auxin in the endosperm depends on a sufficient supply with nutrients. The number of fruits and seeds and the activity of the vegetative growth determine the quantity of nutrients that is needed. In the apple the vegetative growth decreases, as a rule, strongly after the June fall. Just as in the debladed petioles of *Coleus*, the substance which retards the abscission is apparently produced in a sufficient amount to prevent abscission in the period in which the vegetative growth decreases or stops.

VAN STEVENINCK (1958) found for *Lupinus luteus* that the number of flowers which are lost by abscission, is strongly reduced when the main stem and/or the lateral branches are partly defoliated. This reduction, however, was even stronger when the terminal buds had been removed. This phenomenon matches that in *Coleus*, where the abscission of the petioles is strongly retarded by the same manipulations i.e. by defoliation and decapitation. In *Lupinus* in a later stage the abscission of young pods proved to depend on the number that on account of the preceding abscission of the flowers were left, and further on the photosynthetic capacity of the plant. Moreover, the growth of the pods in the lower whorls appeared to lead to the abscission of young pods in the upper whorls (Van Steveninck, 1957). According to the same author (1959), the abscission of these very young pods would be caused by some anti-auxin produced in the older ones. This was concluded from a paper-chromatographic analysis carried out with an ether extract from young pods (prepared 7 days after the fall of the petals); in this extract a substance which retards the abscission as well as one which accelerates the latter, were detected. Both substances would be transported from the young pods in the acropetal direction. However, such a conclusion would only be justified when it had been proved that these substances actually do migrate acropetally, and that they do not affect the abscission of the pods in which they are produced.

Wright (1956) and Luckwill (1957) too found growth inhibitors in seeds, viz. in those of black currant and of apple. However, no relation between these substances and the abscission could be found.

It is not known whether the abscission of young pistils, whether fertilized or not fertilized, is governed by auxin. The abscission of young apples and pears can temporarily be prevented by means of spraying with NAA. In a later stage the fruit fall proved to be increased by this treatment. In the orchards this practice sometimes is applied to reduce the number of fruits. In *Nicotiana tabacum* too a spraying with auxin could temporarily prevent the abscission of non-fertilized pistels (Garden and Marth, 1937). However, as soon as a second period of growth had started, al non-fertilized pistels were shed. During the period in which the fruits develop, the supply with nutrients is apparently more apt to play the part of a limiting factor than the auxins are apt to do.

2. Gravity and correlative phenomena

It appeared that in *Coleus* the effect which is exercised on the abscission by gravity, is indirect only. The abscission is correlated with growth processes at the proximal side of the petiole. This made it possible to study the effect which gravity exercises on the growth, without the need of difficult and tedious direct measurements, simply by using the abscission as an indicator: the shorter the abscission time, the stronger the growth activity.

The unilateral action of the transversal component of gravity induces in *Coleus* at the lower side of the stem a swelling and occasionally also root formation. This can be explained by the aid of the Cholodny-Went theory, according to which auxin would migrate laterally and accumulate at the lower side. The auxin itself is mainly produced in the tip and in the young leaves; in decapitated plants it can be replaced by IAA.

The development of adventitious roots, and probably also the normal growth of the roots, are promoted by leaf factors produced in the full-grown leaves. The transport of these leaf factors takes place only by means of the vascular system. In the longitudinal direction this transport can be blocked or at least hampered by the unilateral action of the transversal component of gravity.

In the literature the promotion of the development of new roots and of the growth of those that are already present, is often ascribed to "rhizocaline". Libbert (1956/1957) concluded from the literature that rhizocaline comprises several distinct substances, among which auxin would be the only one that is polarly transported; the other substances would be nucleic acid compounds, a factor "X" found in peas, and vitamins. On the other hand, Bouillenne and Bouilenne-Walrand (1955) claim that rhizocaline is a compound of auxin and a specific substance produced in the leaves by the activity of an enzyme; this enzyme would occur only in special tissues (cambium, pericycle and phloem).

Gravity must change the distribution of at least one of the leaf factors, but although it was as yet impossible to decide of what substance or substances the distribution is changed, some evidence was obtained which indicates that not only the transport of auxin (IAA), but also that of some other substances are governed by this force.

The abscission is accelerated by growth processes which take place at the proximal side of the petioles and which are induced by auxin produced in the tip and in the young leaves and promoted by leaf factors produced in the full-grown leaves. This acceleration can be explained by assuming that by these growth processes the A.R.S. is drained off from the abscission zone. This substance itself is also an auxin, and is produced in the light in the petioles and probably also in the leaf blades.

The lateral transport of the auxin produced in the tip and in the young leaves, and the longitudinal transport of the leaf factors are respectively caused and hampered by the unilateral action of the transversal component, but the transport of the A.R.S. depends on the longitudinal component of gravity. This follows from the retardation of the abscission in horizontally rotating plants: elimination of the longitudinal component hampers the migration of this factor from the abscission zone. The physiological action of the longitudinal component is completely eliminated not only in horizontally placed or horizontally rotating plants but also in plants fixed at or rotating at angles of more than 90° to the vertical, and in plants placed upside down. This effect is independent of auxin, and of the growth processes induced by the latter at the proximal side of the petioles (chapter vii).

The present study has revealed that gravity exercises an effect on the transport of several substances, and so its action must be quite complicated. However, quite a number of phenomena that have been observed in the field of plant physiology, and in which gravity is known to play a part, can already be interpreted as correlative effects.

The effect of gravity on the elongation of plant parts mainly becomes apparent in relatively long-lasting experiments. Contradictory statements which are not rarely found in the literature, may be ascribed partly to the time factor and partly to the choice of the object. Most of the studies on the effect exercised by gravity on the elongation, were made with coleoptiles, hypo- and epicotyls or with the roots of seedlings. Such organs probably will be less dependent on correlative factors than plants in a latter stage of development, in which e.g. the growth of the roots depends on substances produced in the leaves.

Recently in branches of the apple, cherry, prune and black currant that were kept for a long time in a horizontal position, the elongation appeared to be strongly reduced (Wareing and Nasr, 1958). This statement agrees with practical experience.

The growth of the stem presumably depends on substances produced in the leaves and in the roots. It was shown for *Coleus* that in plants which are kept in a horizontal position, the basipetal migration of leaf factors, which are supposed to promote the growth of normal roots, is seriously hampered. In this way not only the growth of the roots, but also their capacity to absorb mineral nutrients may be decreased, and the last-named effect would in its turn cause a reduction of the growth of the aerial parts.

In the praxis of fruit growing vegetative growth is reduced artificially by grafting on poorly growing stocks, by removing part of the roots or of a ring of the phloem, and by inversing parts of the phloem (Dickson and Samuels, 1956; Sax, 1957). The last-named manipulation, viz. the inversion of the phloem, is an interesting one, since this presumably will hamper the polar transport of particular substances.

However, the reduction of the vegetative growth is not the only effect that is obtained by means of such manipulations, the development of the flower primordia too is affected and takes place at a much earlier stage. This phenomenon has already been mentioned by Knight as early as 1803. According to this author, in twigs that were bent in a horizontal position, the basipetal transport of assimilation products would presumably be hampered so strongly that a larger amount of them would be available for the development of flowers. Wareing and Nasr (1958) experimentally demonstrated this early flowering in young apple trees that were brought in a horizontal position. In the second year only a few flowers developed in the plants that grew in the normal position. In the horizontally placed plants, however, numerous flowers were developed on the spurs of 1 and 2 years old twigs. Longman and Wareing (1958) found a similar promoting effect on the development of flowers in horizontally and inversely fixed twigs of *Larix leptolepis*. In the inversely fixed twigs the flowers by far outnumbered those in the horizontally fixed ones.

In inversely placed soybean plants not only an early flowering occurred, but flowers developed also at older internodes (Fisher, 1957). According to this author the development of the flowers was delayed by a relatively high auxin level. In inversely placed plants a hampered transport of auxin would favour the development of flowers at the older internodes too.

According to De Zeeuw (1954) in short-day plants a relatively high auxin level would promote vegetative growth, a low auxin level generative activity. Removal of the young leaves (Fisher, 1955; Fisher and Loomis, 1954) promotes here the development of flowers.

In plants that are not short-day plants, the available products of photosynthesis would be limiting to flowering. The natural auxin level would be so high that it would be impossible to control it. Flowering could only be promoted by increasing the concentration of the products of photosynthesis, and this was obtained by removing part of the young leaves (De Zeeuw, 1954). It is, however, liable to doubt whether the available quantity of photosynthates actually can be raised by the removal of young leaves. If the growth of young leaves depended directly on the products of photosynthesis obtained from the full-grown leaves, removal of the full-grown leaves would decrease the growth rate of the young leaves, which it apparently does not do. At least it did not do this in *Coleus*. On the other hand, the removal of the young leaves was found to cause a compensatory growth of the older ones (Jacobs and Bullwinkel, 1953). According to these authors, this might indicate the presence of a competition between young and old leaves for some deficient substance.

From this brief survey it appears that different authors ascribe the promotion of flowering to a number of substances and other factors. According to Söding (1952), the formation of roots, the activity of the cambium, the development of flowers, etc. would all depend on a certain balance between the amounts in which a number of hormones are available. The hormones would have no specific activity, neither individually nor in combination. For each specific action the specific disposition and potency of the tissue that is involved, would be determining. The physiological regulation of the specific action would depend on the factor that in this particular case is the limiting one, especially on the factor that becomes available at the latest moment or that disappears first.

Although these considerations do not actually contribute to a solution of the problem, they may stimulate some further speculations. We may suppose e.g. that the growth of the roots and the development of the flowers are regulated by the same factors, though in a different proportion. In that case it would be possible to promote the one process by inhibiting the other. The transport of certain substances that govern growth, can be hampered by a ring-wound or by placing the plants horizontal or inverse. By doing so these substances would become available for the development of flower primordia. The removal of young leaves probably decreases the growth rate of the roots, which consequently need a smaller amount of the leaf factors, and in that case these substances too would become available for the development of flower primordia.

From an observation made by Resende (1948) it appears that the formation of roots may retard flowering. In horizontally placed plants of *Bryophyllum* the formation of adventitious roots was accompanied by a retarded flowering. Skoog and Miller (1957) too found by studies in vitro that all types of growth, from cell elongation to the differentiation of organs, are governed by some universal mechanism, a quantitative interaction between growth substances, especially between auxins and kinetins, and between these and other substances. Eventually kinetins could functionally replace the substances which according to other authors (Gautheret, 1944; Bouillenne, 1950) are responsible for the development of special parts.

The retardation of the development of flower primordia (Fisher, 1957; De Zeeuw, 1954) as well as the acceleration of the abscission of flowers (Van Steveninck, 1957) and that of the abscission of debladed petioles by substances produced in the young leaves, are probably all correlated with the same growth processes; these growth processes would take place elsewhere in the plant, and by them these substances would be needed.

Finally, it should be mentioned that Wareing and Nasr (1958) introduced the term "gravimorphism" for the effect of gravity on correlative processes leading to certain morphogenetic phenomena.

SUMMARY

1. The experiments that are described in this paper, were performed with plants belonging to a clone of *Coleus rhenaltianus*. The effect exercised on the abscission of debladed petioles by auxin (IAA) that was applied either at the distal or at the proximal side of the abscission zone, was studied under various circumstances. The main object of this study was to find out the effect that is exercised on the abscission by gravity.

2. The abscission time proved to be dependent on the presence of growth activity at the proximal side of the abscission zone and on the length of the petioles. (chapter III).

3. Under natural light conditions the abscission proved to be strongly retarded or even indefinitely postponed when the petioles exceeded a certain length, or when the growth activity at their proximal side was strongly reduced. When such plants are kept in the dark the abscission takes place in the normal way. (chapter III).

4. The abscission is indirectly accelerated when auxin is applied in a high concentration at the proximal side of the abscission zone. This auxin acts through the induction of a meristematic activity (e.g. the development of new adventitious roots). This growth, and indirectly also the abscission time of the petioles, is dependent on some substances produced by the leaves in their vicinity. In plants that are kept in the dark, the abscission of the petioles is retarded by proximally applied auxin (high concentration). (chapter III).

5. Distally applied auxin in a concentration of 1 mg l IAA or more, as well as distally applied sugar have a retarding effect on the abscission. (chapter IV).

6. The abscission is accelerated by a low concentration (0.01 mg l IAA) of auxin that is applied distally, provided that there is a sufficient growth activity proximal of the abscission zone. (chapter IV).

7. The accelerated abscission in plants that are placed in a horizontal position, is correlated with growth processes at the lower side of the stem. These growth processes (hypotrophy, development of new adventitious roots) are induced by the transversal component of gravity, but only in the presence of a substance that is produced in the tip and in the young leaves. This substance is replaceable by auxin (IAA). (chapter IV).

8. The difference in abscission time between petioles of plants in the normal and in the horizontal position is not influenced by different concentrations of auxin nor by sugar if the latter are applied distally of the abscission zone. (chapter IV).

9. It is concluded that the petioles contain a substance by which the abscission is retarded and that this substance may be drained off from them by growth processes

which are going on proximal of the abscission zone. This substance is produced in the petioles, and probably also in the leaf blades, when these parts are exposed to the light. The migration of this substance is probably impeded by high concentrations of auxin (IAA) that are distally applied, and promoted by low auxin concentrations that are applied in the same way. (chapter IV).

10. The substance from the petioles which retards the abscission, is able to migrate through the parenchyma. (chapter V).

11. By means of paper chromatography an auxin-like substance was isolated from the acid fraction of an ether extract that had been prepared from full-grown leaves. This substance shows auxin-activity in the *Avena* curvature-test and retards the abscission in *Coleus*. The maximum activity in the chromatogram is found just in front of the place that is occupied in the same chromatogram by IAA. All colour reactions on indole derivatives that were carried out on this substance were negative. In explants this substance does not enhance the retardation of the abscission that is caused by IAA.

From these facts and from the ultra-violet absorption spectrum it is concluded that the substance can not be an indole derivative. Most probably it is identical with the substance that is present in the petioles and of which it was shown also that it retards the abscission. (chapter V).

12. The abscission of the debladed petioles is indirectly promoted by substances produced by leaves in their vicinity. These substances are produced in the light in the leaf blades and in their action on the abscission, they can not be replaced by nutrients. The way in which they promote the growth of chlorophyll-free parts (roots) and tissues (e.g. the growth at the lower side of the stem in horizontally placed plants), is a direct one. By these growth processes the auxin-like substance which retards the abscission is drained off from the petioles. (chapter VI).

13. The transport of the substances meant in 12 takes place only through the vascular system. The basipetal transport is strongly hampered by the transversal component of gravity. (chapter VI).

14. In horizontally placed plants as well as in horizontally rotating ones, the growth rate of young leaves proves to be reduced. (chapter VI).

15. In horizontally placed plants the abscission is only accelerated when the petioles are debladed within a few days after the plants have been brought in the horizontal position. The abscission was retarded in plants that had been kept in the horizontal position already for a week before the petioles were debladed; this retardation of the abscission is caused by the decrease of the growth activity proximal of the abscission zone; the growth processes at the lower side of the stem have stopped and the growth of the roots is probably reduced because the transport of the substances meant in 12, is hampered by the action of gravity. (chapter VI).

16. In plants that are kept in the normal position, the longitudinal component of gravity has a promoting effect on the abscission. Elimination of the longitudinal component hampers the basipetal transport of the auxin-like substance from the petioles by which the abscission is retarded, and under these circumstances this substance therefore can exercise its retarding influence on the petioles. (chapter VII).

17. The longitudinal component of gravity is eliminated in horizontally placed and in horizontally rotating plants. The effect of the longitudinal component is also eliminated in plants fixed or rotating at angles of more than 90° to the vertical, and in inversely placed plants. (chapter VII).

18. When the petioles are debladed within a few days from the moment at which the plants were brought in the horizontal position (induction of growth processes at the lower side of the stem), the retardation of the abscission caused by the elimination of the longitudinal component is compensated by an acceleration of the abscission by the transversal compenent of gravity.

In horizontally placed plants the abscission is retarded when there is no source of auxin (e.g. young leaves) proximal of the abscission zone or when the petioles are debladed a week after the plants have been brought in the horizontal position. (chapter VII).

19. In plants rotating horizontally on the clinostat the abscission time of the debladed petioles is dependent on the revolving speed of the clinostat axis. The retardation of the abscission by the elimination of the longitudinal component is partly compensated by an acceleration of the abscission caused by the transversal

component. This effect of the transversal component of gravity is dependent on the presence of a source of auxin proximal of the abscission zone. (chapter VII).

20. The effect which is exercised on the abscission of debladed petioles by the elimination of the longitudinal component of gravity is independent of the presence of a source of auxin proximal of the abscission zone. (chapter VII).

21. The results are discussed in connection with our knowledge of the influence which gravity exercises on the growth in length and on the formation of flowers.

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REFERENCES

Addicott, F. T. and R. S. Lynch. 1955. Ann. Rev. Plant Physiol. **6**: 211.
————, ———— and H. R. Carns. 1955. Science **121**: 644.
Anker, L. 1953. Acta Bot. Neerl. **2**: 22.
————. 1956. Acta Bot. Neerl. **6**: 1.
Audus, L. J. and M. E. Brownbridge. 1957. J. Exp. Bot. **8**: 105.
Bandi, P. 1957. Mitt. d. Naturf. Ges. Bern N.F. **15**: 93.
Barlow, H. W. B. 1947. Ann. Rep. East Malling Res. St. p. 121.
————. 1950. J. Exp. Bot. **1**: 264.
————. 1952. Rep. 13th Int. Hortic. Congr. p. 145.
Batjer, L. P. and A. H. Thompson. 1948. Proc. Am. Soc. Hort. Sci. **51**: 77.
Beal, J. M. and A. G. Whiting. 1945. Bot. Gaz. **106**: 420.
Biggs, R. H. and A. C. Leopold. 1957. Plant Phys. **32**: 626.
———— and ————. 1958. Am. Journ. Bot. **45**: 547.
Blaauw-Jansen, G. 1959. Acta Bot. Neerl. **8**: 1.
Bottelier, H. P. 1954. Ann. Bogor. **1**: 185.
————. 1956. Ann. Bogor. **2**: 175.
————. 1956. Ann. Bogor. **2**: 183.
Bouillenne, R. 1935. Congr. Intern. Bot. Amsterdam.
————. 1950. Année biol. **54**: 597.
———— and M. Bouillenne. 1938. Bull. Soc. Roy. Bot. Belg. **29**: 43.
———— and M. Bouillenne-Walrand. 1955. Rep. 14th Int. Hort. Congr. Netherl. p. 231.
———— and F. W. Went. 1933. Ann. Jard. Bot. Buitenzorg **43**: 25.
Brain, E. D. 1935. New Phyt. **34**: 97.
————. 1942. New Phyt. **41**: 81.
Brauner, L. and A. Hager. 1958. Planta **51**: 115.
Bremekamp, C. E. B. 1912. Rec. des Trav. bot. neerl. **9**: 281.
Brown, H. S. and F. T. Addicott. 1950. Am. Journ. Bot. **37**: 650.
Carns, H. R., F. T. Addicott and R. S. Lynch. 1951. Plant Phys. **26**: 629.
Cooper, W. C. 1936. Plant Phys. **10**: 789.
Copeland, E. B. 1900. Bot. Gaz. **29**: 185.
Czapek, F. 1898. Jahrb. f. wiss. Bot. **32**: 145.
Delarge, L. 1941. Mem. Soc. Roy. Sc. Liège **5**: 1.
Denffer, D. von, M. Behrens and A. Fischer. 1952. Naturwiss. **39**: 258.
Dickson, A. G. and E. W. Samuels. 1956. J. Arn. Arbor. **37**: 307.
Dolk, H. E. 1936. Rec. des Trav. bot. neerl. **33**: 509.
Dostal, R. 1951. Acta Ac. Sc. Nat. Mor. Sil. **23**: 67.
Edgerton, L. J. and M. B. Hoffmann. 1948. Proc. Am. Soc. Hort. Sc. **62**: 159.
Elfving, F. 1880. Acta Soc. Sc. Fennica **12**: 23.
Emsweller, S. L. and N. L. Stuart. 1948. Proc. Am. Soc. Hort. Sc. **51**: 581.

Facey, V. 1950. New. Phyt. **49**: 103.
Fischnich, O. 1935. Planta **24**: 552.
Fisher, J. E. 1955. Bot. Gaz. **117**: 156.
———. 1957. Science **125**: 396.
——— and W. E. Loomis. 1954. Science **119**: 71.
Garden, F. E. and P. C. Marth. 1937. Bot. Gaz. **99**: 184.
Gardner, F. E. and W. C. Cooper. 1943. Bot. Gaz. **105**: 80.
Gaur, B. K. and A. C. Leopold. 1955. Plant Phys. **30**: 487.
Gautheret, R. J. 1942. Bull. Soc. Chim. biol. **24**: 13.
———. 1944. Rev. Cytol. Cytophysiol. Veget. **7**: 45.
Gawadi, A. G. and G. S. Avery Jr. 1950. Am. Journ. Bot. **37**: 172.
Gordon, S. A. and R. P. Weber. 1951. Plant Phys. **26**: 192.
Gregory, F. G. and B. Samantarai. 1950. J. Exp. Bot. **1**: 159.
Griesel, W. C. 1954. Phytomorpholog. **4**: 123.
Guttenberg, H. von and A. Beythien. 1951. Planta **40**: 36.
Haccius, B. 1955. Beitr. Biol. d. Pfl. **31**: 207.
——— and H. Nies. 1956. Planta **47**: 613.
Hall, W. C. 1952. Bot. Gaz. **113**: 310.
——— and J. L. Liverman. 1956. Plant Phys. **31**: 471.
Heinicke, A. J. 1917. Cornell Univ. Agr. Exp. St. Bull. **393**: 45.
Hering, G. 1904. Jahrb. f. wiss. Bot. **40**: 499.
Hodgson, R. W. 1918. Univ. Calif. Publ. Bot. **6**: 417.
Hotta, Y. and T. Ota. 1956. Bot. Mag. Tokyo **69**: 126.
Jackson, J. M. 1952. Arkansas Ac. Sc. **5**: 73.
Jacobs, Wm. P. 1952. Am. Journ. Bot. **39**: 301.
———. 1955. Am. Journ. Bot. **42**: 594.
———. 1958. Am. Journ. Bot. **45**: 673.
——— and B. Bullwinkel. 1953. Am. Journ. Bot. **40**: 385.
Jurisic, J. 1939. Anz. Akad. Wiss. Wien, Sitz. Math. Naturwiss. kl. 14.
Knight, T. A. 1803. Roy. Soc. London Phil. Trans. p. 277.
———. 1822. Trans. Hort. Soc. London **4**: 159.
Kögl, F., H. Erxleben and H. J. Haagen Smit. 1934. Hoppe Seyl. Z. physiol. Chem. **228**: 90.
Koningsberger, V. J. 1922. Rec. des Trav. bot. neerl. **19**: 1.
Künning, H. 1950. Planta **38**: 36.
Küster, E. 1916. Ber. dtch. bot. Ges. **34**: 184.
Laibach, F. 1933. Ber. dtch. bot. Ges. **51**: 336.
———. 1933. Ber. dtch. bot. Ges. **51**: 386.
Larsen, P. 1953. Physiol. Plant. **6**: 735.
La Rue, C. D. 1936. Proc. Nat. Ac. Sc. **22**: 254.
Laudi, G. and F. M. Gerola. 1956. Nuovo Giorn. Bot. It. **63**: 336.
——— and L. Lambri. 1956. Nuovo Giorn. Bot. It. **63**: 324.
Leinweber, C. L. and W. C. Hall. 1959. Bot. Gaz. **120**: 144.
Lek, H. A. A. van der. 1925. Thesis Utrecht.
Leopold, A. C. 1958. Ann. Rev. Plant Physiol. **9**: 281.
Libbert, E. 1956/57. Wiss. Zeitschr. der Humboldt Univ. Berlin **6**: 315.
Linser, H. and O. Kiermeyer. 1957. Methoden zur Bestimmung pflanzlicher Wuchsstoffe. Wien.
Livingston, G. A. 1950. Plant Phys. **25**: 711.
Loeb, J. 1926. Les bases physico-chimiques de la régéneration. Paris.
Longman, K. A. and P. F. Wareing. 1958. Nature **182**: 380.
Luckwill, L. C. 1948. J. Hort. Sc. **24**: 32.
———. 1953. J. Hort. Sc. **28**: 14.
———. 1956. J. Hort. Sc. **31**: 89.
———. 1957*a*. J. Hort. Sc. **32**: 18.
———. 1957*b*. Symp. Soc. Exp. Bot. **9**: 63.
Mai, G. 1936. Jahrb. f. wiss. Bot. **79**: 681.
McCown, M. 1943. Bot. Gaz. **105**: 212.
Meinl, G. and H. von Guttenberg. 1952. Planta **41**: 167.
Metzner, P. 1937. Ber. d. dtch. bot. Ges. **55**: 1.
Meyer, A. 1918. Flora. **111**: 85.

Michael, G. 1935/36. Z. f. Bot. **29**: 385.
Mohl, H. von. 1860. Bot. Zeit. **18**: 1, 9, 273.
Molisch, H. 1886. Bot. Centralbl. **25**: 393.
Molotkovsky, G. Ch. and J. G. Molotkovski. 1956. Ber. Wiss. Biol. **102**: 181.
Moureau, J. 1939. Bull. Soc. roy. Sc. Liège **8**: 561.
————. 1940. Bull. Soc. roy. Sc. Liège **9**: 41.
Myers, R. M. 1939. Illinois State Ac. Sc. **32**: 80.
————. 1940. Bot. Gaz. **102**: 323.
Navez, A. E. 1933. Proc. Nat. Acad. Sc. U.S.A. **19**: 636.
Nemeček, R. 1922. Österr. bot. Zeitschr. **71**: 255.
Nitsch, J. P. 1956. In R. L. Wain and F. Wightman, The chemistry and mode of action of plant growth substances. London.
Noll, F. 1888. Arb. der bot. Inst. Wurzburg. III: 496.
Olmsted, Ch. E. 1950/51. Bot. Gaz. **112**: 365.
Oortwijn Botjes, J. 1938. Proc. Kon. Ned. Akad. Wet. Amsterdam **C 41**: 161.
Osborne, D. J. 1955. Nature **176**: 1161.
————. 1958*a*. Trop. Agr. **35**: 145.
————. 1958*b*. J. Exp. Bot. **9**: 446.
Overbeek, J. van. 1933. Rec. des Trav. bot. neerl. **30**: 538.
————, R. Blondeau and V. Horne. 1955. Am. Journ. Bot. **42**: 205.
———— and H. J. Cruzado. 1948. Am. Journ. Bot. **35**: 410.
————, G. Davila Olivo and E. M. Santiago de Vazquez. 1945. Bot. Gaz. **106**: 440.
Pfaeltzer, J. W. 1934. Thesis Utrecht.
Pfeffer, W. 1893. Verh. d. Ges. Naturf. und Aerzte **65** I: 68.
Pfeiffer, H. 1928. In K. Linsbauer, Handbuch der Pflanzenanatomie V. Berlin.
Portheim, L. 1941. Ann. Bot. N.S. **5**: 35.
Rawitscher, F. 1924. Zeitschr. f. Bot. **16**: 1.
————. 1932. Der Geotropismus der Pflanzen. Jena.
Reiss, E. 1934. Planta **22**: 543.
Resende, F. 1948. Port. Acta Biol. **2**: 251.
Rosetter, F. N. and Wm. P. Jacobs. 1953. Am. Journ. Bot. **40**: 276.
Rijven, A. H. G. C. 1952. Acta Bot. Neerl. **1**: 157.
Sacher, J. A. 1957. Science **125**: 1199.
Sachs, J. 1873. Arb. des Bot. Inst. Wurzburg **I**: 385.
————. 1887. Vorlesungen über Pflanzenphysiologie. Leipzig.
Sampson, H. C. 1918. Bot. Gaz. **66**: 32.
Sato, I. 1956. Jap. J. Bot. **15**: 249.
Sax, K. 1957. Proc. Am. Soc. Hort. Sc. **69**: 68.
Schmitz, H. 1933. Planta **19**: 614.
Schtscherbak, J. 1910. Beih. z. bot. Centr.Blatt. **25** I: 358.
Shoji, K., F. T. Addicott and W. A. Swets. 1951. Plant Phys. **26**: 189.
Simon, S. 1908. Jahrb. f. wiss. Bot. **45**: 351.
Singh, J. P. 1956. Indian J. Hort. **13**: 165.
Skoog, F. and C. O. Miller. 1957. Symp. Soc. Exp. Bot. **9**: 118.
Snow, R. 1935. New Phyt. **34**: 347.
Söding, H. 1952. Die Wuchsstofflehre. Stuttgart.
Steveninck, R. F. M. van. 1957. J. Exp. Bot. **8**: 373.
————. 1958. J. Exp. Bot. **9**: 372.
————. 1959. Nature **183**: 1246.
Stuart, N. W. 1938. Bot. Gaz. **100**: 298.
Talts, J. 1932. Planta **16**: 178.
Terpstra, W. 1953. Thesis Utrecht.
————. 1956. Acta Bot. Neerl. **5**: 157.
Thimann, K. V. and F. Skoog. 1934. Proc. Roy. Soc. London **114**: 317.
Tittmann, H. 1895. Jahrb. f. wiss. Bot. **27**: 164.
Veerdonk, F. C. G. van de. 1956. Thesis Utrecht.
Vendrig, J. C. 1958. Proc. Kon. Ned. Akad. Wetensch. Amsterdam **C**, **61**: 288.
Vöchting, H. 1884. Über Organbildung im Pflanzenreich II: 40. Bonn.
Wareing, P. F. and T. Nasr. 1958. Nature. **182**: 379.

WEINTRAUB, R. L. and J. W. BROWN. 1950. Plant Phys. **25**: 140.
———, J. W. BROWN, J. C. NICKERSON and K. N. TAYLER. 1952. Bot. Gaz. **113**: 348.
WENT, F. W. 1938. Plant Phys. **13**: 55.
——— and M. CARTER. 1948. Am. Journ. Bot. **35**: 95.
WETMORE, R. H. and WM. P. JACOBS. 1953. Am. Journ. Bot. **40**: 272.
WHITING, A. G. and M. A. MURRAY. 1948. Bot. Gaz. **109**: 447.
WIEDOW, H. L. and H. VON GUTTENBERG. 1953. Planta **41**: 589.
WIELAND, TH. and L. BAUER. 1951. Angew. Chem. **63**: 511.
WIT, J. L. DE. 1957. Acta Bot. Neerl. **6**: 1.
WRIGHT, S. T. C. 1956. J. Hort. Sc. **31**: 196.
YAGER, R. E. and R. M. MUIR. 1958*a*. Science **127**: 82.
——— and ———. 1958*b*. Proc. Soc. Exp. Biol. and Med. **99**: 321.
ZEEUW, D. DE. 1954. Med. Landb. Hogeschool Wageningen **54**: 1.
ZIMMERMANN, W. 1924. Ber. d. dtch. bot. Ges. **42**: 39.
ZOLLIKOFER, CL. 1921. Rec. des Trav. bot. neerl. **18**: 237.