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Role of Growth Promotor and Growth Inhibitor in Foliar Senescence and Abscission of Hevea brasiliensis Muell. Arg.

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A study was made of the changes with age in the levels of endogenous growth promotor, probably indoleacetic acid (IAA), and growth inhibitor, probably abscisic acid (ABA), and of the activity of IAA-oxidase in the leaves of Hevea. *The growth promotor level decreased with ageing but the level of growth inhibitor was found to remain constant. The activity of IAA-oxidase increased with age. A hypothesis is developed to explain senescence and abscission in* Hevea *leaves based on the changes in the levels of endogenous growth promotor (IAA) and the growth inhibitor (ABA).*

It is a relatively unusual feature for trees growing in the tropical regions of the world to show anything resembling the annual cycle of defoliation and refoliation so commonly associated with the changing seasons of temperate climates. An exception is, however, presented in Malaysia by the commercial rubber tree, *Hevea brasiliensis*, in that although the leaves of juvenile trees may persist for a longer time, mature plantation trees tend to lose all their leaves at the beginning of each year (around February and March in Peninsular Malaysia). The ensuing leafless phase, commonly described as the 'wintering' period, frequently lasts for several weeks before new foliage expands. In some rubber-producing regions it is usual to discontinue 'exploitation' of the trees during this period as the yield diminishes. This fact lends added importance for studying the factors controlling leaf senescence in *Hevea brasiliensis*.

Although the underlying causes for foliar senescence and abscission are still not fully understood, they are known to be induced by such environmental factors as moisture stress, light intensity and diminishing photoperiod, by physiological process such as the formation of flowers, fruits, or younger leaves and by cytological factors. Many determinations of the changing concentrations of various leaf constituents have been published, and the disappearance of chlorophyll and other plastid pigments (WILLSTATTER AND STOLL, 1928; SCHULZE, 1956; GOODWIN, 1958, CHUA, 1970), protein (PLAISTED, 1958; CHUA, 1970), total nitrogen (COMBES AND ECHEVIN, 1927; THOMAS, 1927; CHUA, 1970) and carbohydrates (COMBES AND KOHLER, 1922; CHUA, 1970) during senescence already well established.

It is usually possible to relate premature leaf-fall in young *Hevea* trees to nutritional deficiency, moisture stress, or toxic effects; it has been supposed that the annual defoliation of older trees is in some way determined by the pattern of leaf formation and a superimposed ageing of the foliage as a whole [ageing being defined as the appearance of cytological changes as a function of time, as distinct from senescence which is a phase of progressive deterioration culminating in death of the cells (LEOPOLD, 1964)]. In

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the absence of reliable information, the immediate problem was to determine whether there was in fact a correlation between ageing and leaf-fall in *Hevea,* and the present investigation has accordingly been directed to a study of the changes which precede or accompany the onset of senescence.

MATERIALS AND METHODS

Plant Material

Leaves were obtained from rubber trees growing at the RRIM Experiment Station, Sungei Buloh, Selangor. The trees, ten from clone Tjir 1 and ten from clone GT 1, were derived from buddings made in 1951 and 1953, respectively, and under normal alternate-daily tapping on a half-spiral cut.

Sampling

Leaf samples were taken between 8 a.m. and 9 a.m. on two days each month, small branches being cut at random with the help of a pruning knife attached to a 16 ft pole; the samples accordingly came from the 16 ft level or lower. The laminae were washed with tap water, wiped dry and extracted, the extractions usually being completed within 2 h of collection.

Analyses

The procedure for the extraction of auxins is given in *Figure 1;* the final extracts were concentrated *in vacuo* at temperatures below 30°C and applied as streaks along the starting-line of chromatograms on Whatman 3 MM filter paper. Separation was achieved by ascending development in an *iso*butanol: methanol:water solvent (80:5:15, by volume). A central 2.5 cm strip was then cut from each chromatogram and divided into eleven equal sections corresponding to increasing R_f values. Auxin contents were determined by bioassay; each paper square was cut into small pieces and added to a small culture dish containing 1 ml 2% (w/v) sucrose in K_2HPO_4 – citric acid buffer (pH 5.0) and ten 2 mm *Avena* mesocotyl segments. The length of each segment was measured after incubation for 20 h at 25°C and the elongation compared with those induced by known amounts of IAA (0.1, 1.0 and 10 μg) included in each assay.

Abscisic acid was extracted from 100 g leaf extract by a modification (PIENIAZEK AND RUDNICKI, 1967) of the method used by CORNFORTH *et al.* (1965). Chromatograms were obtained by ascending development in *iso*propanol: ammonia (0.880 sp. gr.):water (10:1:1 by volume) and the oat mesocotyl assay was employed to determine the position of the inhibitor; a standard containing 10 μg synthetic abscisic acid was included on each occasion.

The extraction of IAA oxidase was carried out as described by GALSTON AND BAKER (1951), where 10 g samples of leaf tissue were each ground with sand in a chilled mortar together with 30 ml of 0.1M cold citrate-phosphate buffer, pH 6.5. The resulting homogenates were filtered through two layers of cheese cloth and centrifuged at 1000 × g to remove coarse fragments and sand. The enzyme was then precipitated by the addition of 16 ml of acetone per 40 ml of homogenate. The precipitate was centrifuged down, and then suspended overnight in half the original homogenate volume (25 ml) of cold 0.1M phosphate-citrate buffer, pH 6.6. The suspension was then recentrifuged, the clear supernatent liquid being used as the enzyme solution. The resultant enzyme preparation was stored in a refrigerator before use.

Incubation mixtures containing 20 ml enzyme solution (in 0.1M phosphate-citrate buffer, pH 6.6) and 10 ml of 50 μg/ml IAA were illuminated (500 ft candles) at 25°C ± 1°C. Samples (2.0 ml) were taken at 20 min intervals and residual IAA content deter-

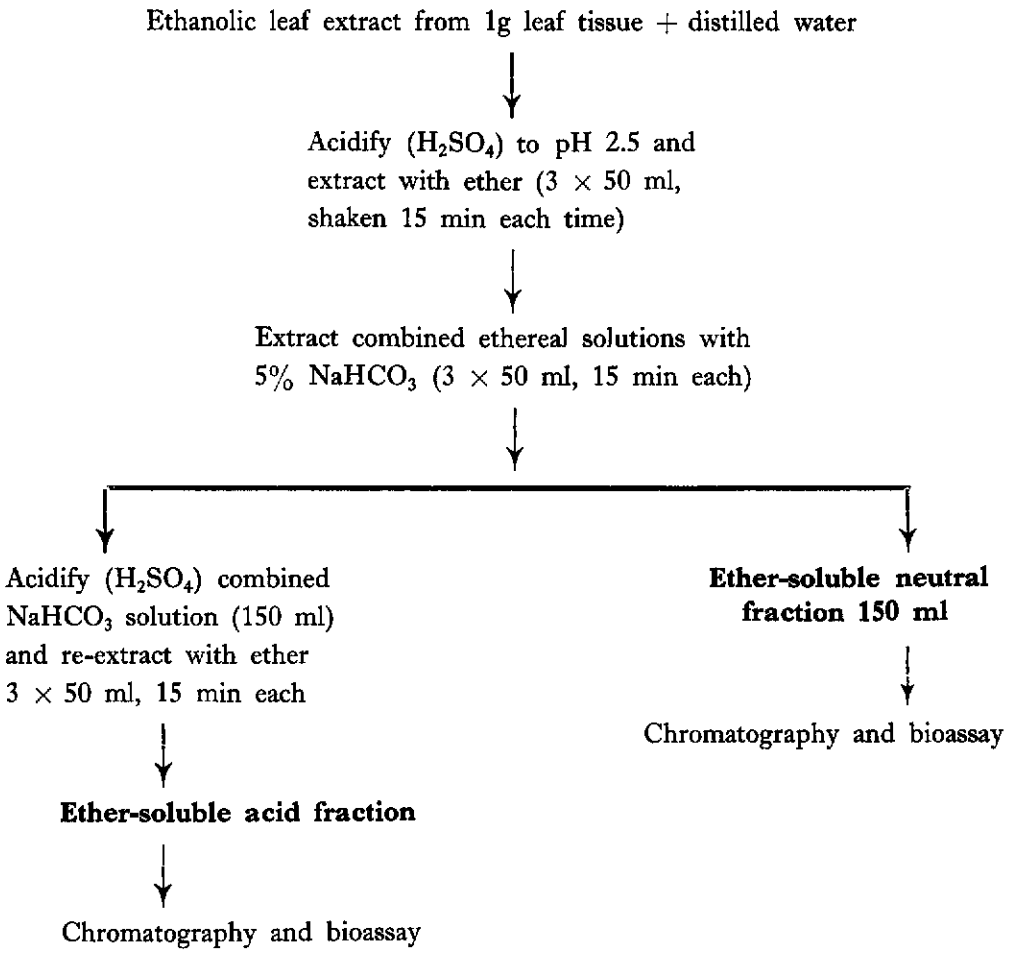

Figure 1. Procedure for isolation of auxin from Hevea *leaf extracts.*

mined colorimetrically by the method of GORDON AND WEBER (1951): each sample was mixed thoroughly with 4.0 ml reagent (50 ml 35% $HC10_4$ + 1 ml 0.5M $FeCl_3$) and optical absorption at 530 mμ was measured after 25 min by which time the readings were maximal.

Since colour development is not proportional to concentration at high IAA levels, calculations were made by reference to a standard curve for the range 0.2 – 50 μg/ml IAA.

An estimate of the amount of protein 'bound' auxin in young and old *Hevea* leaves was obtained with the help of peptidase. One-gram samples of leaf tissue were ground in a mortar with 10 ml of 0.1M phosphate-citrate buffer, pH 6.6, and incubated at room temperature overnight with 1 mg peptidase (obtained from Nutritional Biochemicals, U.S.A.). At the end of the reaction, the auxin was extracted and bioassayed as described above using *Avena* mesocotyl segments.

Changes in Growth Promotor and Growth Inhibitor

The growth promotor contents of the *Hevea* leaves were determined in Apiil when they were young, in October when they were well matured and in March when senescence had set in. Chromatography of auxin extracts confirmed the finding of BOLLE-JONES (1954) that the IAA is probably the acidic growth hormone of *Hevea* leaves *(Figures 2* and *3)*. As the leaves aged the concentration of the acidic hormone decreased, whereas the neutral component increased. The concentration of extractable hormone was determined each month for both clones *(Figure 4a)*; the concentration of presumed IAA was lowest during the period October – December, after which it tended to increase upto the time of abscission. This late increase was, however, slight when compared to the increase during the period of leaf expansion in April.

The presence of the growth inhibitor, probably abscisic acid (abscission II), was isolated and bioassayed by measuring the inhibitory activity of the compound on oat mesocotyl segments grown in the dark. The extraction was carried out on both young and senescent leaves obtained from juvenile and mature *Hevea* trees. *Table 1* shows that there were no marked differences in amount of this compound between young and old leaves from juvenile and mature

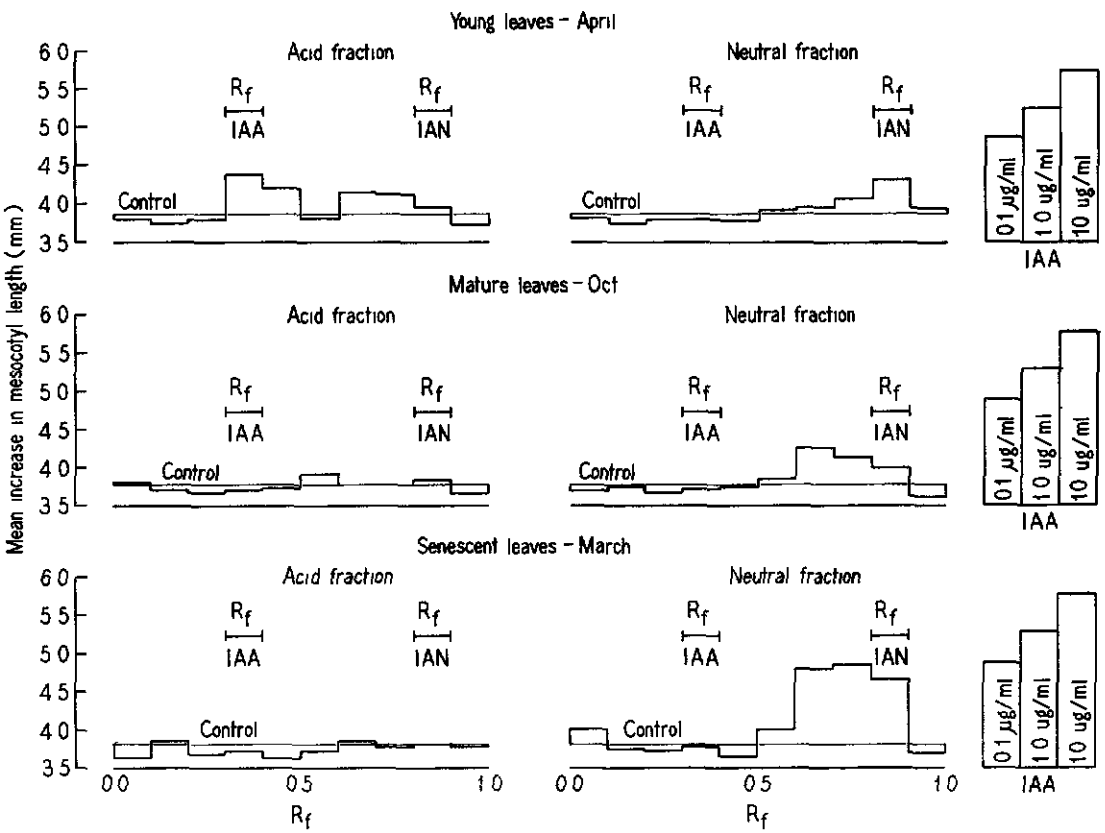

Figure 2. Chromatographic separation of the growth substances of Hevea *leaf extracts of clone Tjir 1. Activities assayed by oat mesocotyl test.*

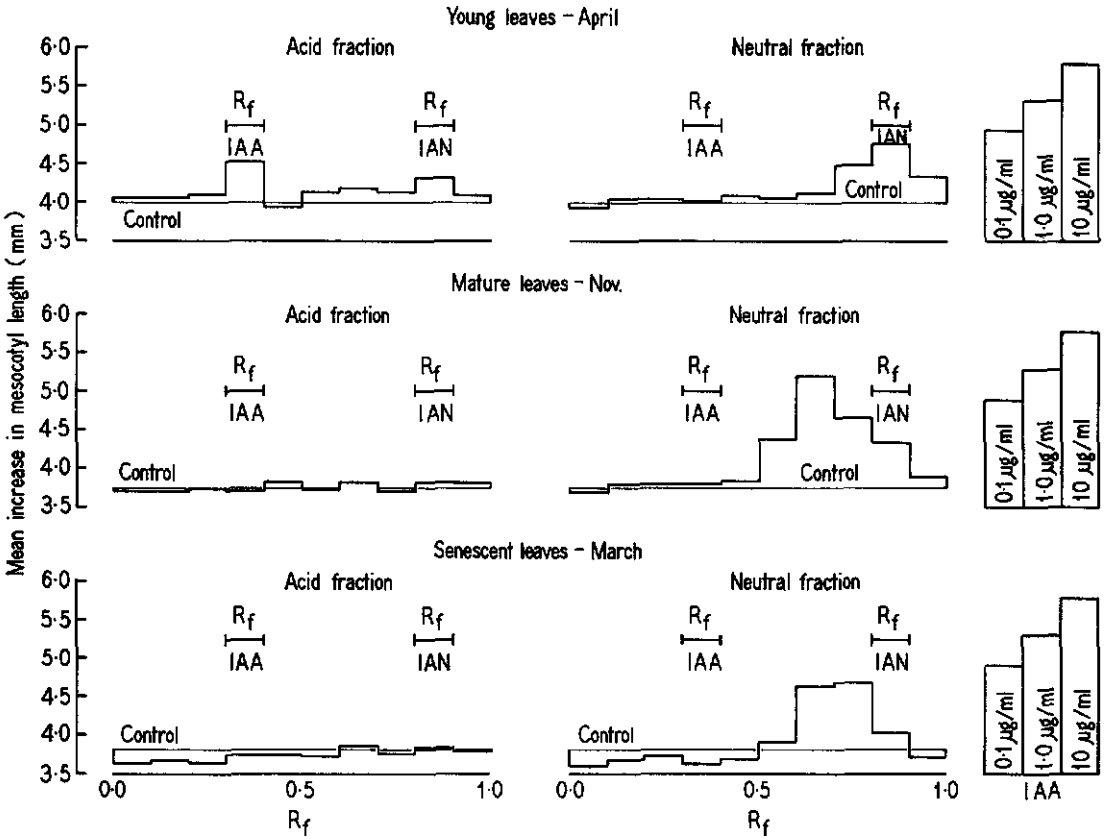

Figure 3. Chromatographic separation of the growth substances of Hevea *leaf extracts of clone GT 1. Activities assayed by oat mesocotyl test.*

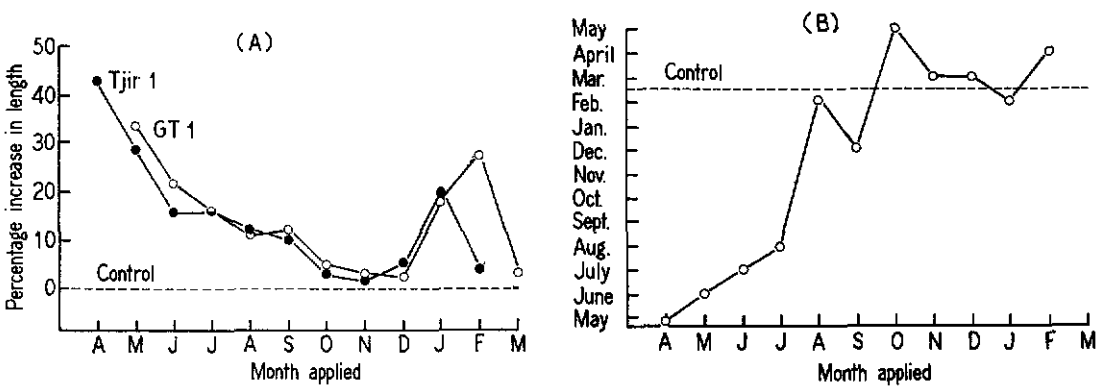

Figure 4. Variation in the content of free auxin (acid fraction, probably IAA) of leaves (A), and effects of IAA (100 p.p.m.) on delaying senescence of Hevea *leaves of clone Tjir 1 from time of leaf expansion until leaf-fall (B).*

TABLE 1. LEVEL OF GROWTH INHIBITOR IN LEAVES OF DIFFERENT AGES FROM JUVENILE AND MATURE *HEVEA* TREES

Age of leaves	Percent inhibition of oat mesocotyl sections by growth inhibitor (abscisic acid) from leaves (R_f 0.7 – 0.8)
Young leaves (2 months) from juvenile trees	82
Old leaves (10 months) from *juvenile trees*	84
Young leaves (2 months) from mature trees	82
Old leaves (10 months) from mature trees	83

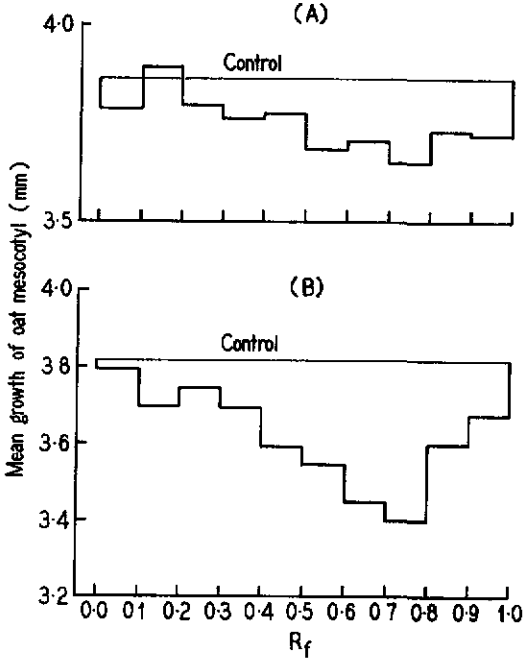

Figure 5. Bioassay of (A) abscisic acid (abscission II) standard (10 μg/ml) and (B) growth inhibitor (probably abscisic acid) from Hevea *leaf extracts.*

trees. From *Figure 5b,* it is seen that the peak of the inhibition was at R_f 0.7 – 0.8 coinciding with that of the R_f of the synthetic abscisic acid when the activity of the eluates was bioassayed with oat mesocotyl segments grown in the dark for 20 hours.

Activity of IAA-oxidase

From *Figure 6* a difference in activity of IAA-oxidase in young and senescent *Hevea* leaves is apparent. The breakdown of IAA by IAA-oxidase was slightly increased by light but this effect was mainly associated with senescent leaves.

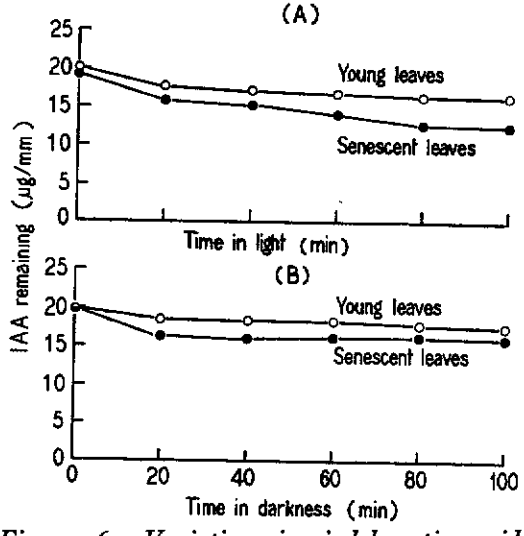

Figure 6. Variation in indoleacetic acid oxidase (IAA oxidase) activity in Hevea *leaf tissues (A) in light and (B) in darkness of clone Tjir 1.*

Bound 'Auxin' of Hevea *Leaves*

It is generally accepted that 'free' auxin is that which is available by diffusion or rapid extraction and 'bound' auxin as that obtained only after enzymatic activity, gentle hydrolysis or prolonged extraction. The occurrence of a protein 'bound' auxin in *Hevea* leaves of different ages has been determined. The first batch of leaves was extracted for the protein 'bound' auxin and assayed using the oat mesocotyl straight test at the end of April when the leaves were very young (about one month old), the

second batch in August when the leaves were mature and the last batch in January when they were senescent. From *Table 2* it is seen that young leaves contained very little 'bound' auxin. As the leaves aged, the amount increased very markedly particularly during the period of the onset of senescence.

TABLE 2. LEVEL OF 'BOUND' AUXIN IN *HEVEA* LEAVES OF DIFFERENT AGES EXTRACTED BY PEPTIDASE ENZYMES

Age of leaves	Percent growth of *Avena* mesocotyl segments induced by 'bound' auxin extracted from *Hevea* leaves[a]
Young (April)	3.5
Mature (August)	8.5
Senescent (January)	32.5

[a]Percent growth above untreated leaves

Effect of Exogenous Application of Growth Promotor and Growth Inhibitor

A batch of leaves (approximately 500 each month) was chosen at random from the lower branches of Tjir 1 trees and treated with IAA from the period two weeks after refoliation in April to the time when the trees were beginning to shed their leaves in February. Indoleacetic acid (IAA) was chosen for use because it was found to be one of the most likely major endogenous growth promotors found in *Hevea* leaves as indicated in *Figures 2* and *3*. The auxin, IAA, was mixed with lanolin in a water-bath (temperature 50°C). Three concentrations of IAA were prepared and they were 10 p.p.m., 100 p.p.m. and 1000 p.p.m. The warm IAA-lanolin paste was cooled to room temperature, taken out to the field and applied to the *Hevea* leaves by smearing the upper leaf surface of each batch with concentrations of 10 p.p.m., 100 p.p.m., 1000 p.p.m. IAA-lanolin paste respectively. Each leaflet received in the case of 10 p.p.m. an approximate amount of 0.04 mg, for 100 p.p.m. an amount of 0.4 mg and for 1000 p.p.m. an amount of 4 mg of IAA. The object of the experiment was to determine the effect of IAA on the life span of the *Hevea* leaves.

Figure 4a shows that the concentration of endogenous growth promotor (IAA) was high when the leaves were young in April and gradually declined to a very low level during October to December and began to rise slightly again in January before leaf-fall in February and March. When the application of 100 p.p.m. exogenous IAA was done between April and September, the life span of *Hevea* leaves was shortened and senescence and abscission were accelerated *(Figure 4b)*. This may be because of the toxic effect caused by an overdose of the auxin. On the other hand when 100 p.p.m. IAA was applied during the month of October when the endogenous auxin content of the leaves was just depleting *(Figure 4a)*, the life span of the leaves was lengthened and senescence and abscission were delayed upto the following May as seen in *Figure 4b*. The 10 p.p.m. of IAA had no effect on the life span of the leaves when applied monthly from the time of refoliation to that of defoliation, whereas the 1000 p.p.m. of IAA accelerated the senescence and abscission of the leaves within two weeks after application each month. These results indicated that 10 p.p.m. of IAA was ineffective because of its low dosage and in the case with the 1000 p.p.m. of IAA, the accelerated onset of senescence and abscission might be due to an overdose of the hormone.

Abscisic acid has been reported to accelerate senescence and abscission of leaves. Experiments were set up to study the effect of exogenous application of abscisic acid on *Hevea* leaves. Three batches of two-month-old leaves (approximately 500 per batch) were chosen at random from the lower branches of Tjir 1 trees and treated with 10 p.p.m., 100 p.p.m. and 1000 p.p.m. of abscisic acid. Four months later, another three batches of six-month-old leaves

(approximately 500 per batch) were again treated with 10 p.p.m., 100 p.p.m. and 1000 p.p.m. abscisic acid. It was observed that in all cases the treated leaves senesced and fell within two weeks. This result is not surprising because abscisic acid is known to cause senescence and abscission of leaves. From *Table 1* it is seen that there is no difference in the level of growth inhibitor (abscisic acid) in young and old leaves, but on the other hand, from *Figure 4a*, the endogenous auxin is seen to decline as the *Hevea* leaves age. Since the level of abscisic acid remained relatively constant and the level of auxin declined with age, it is very probable that an imbalance in concentration of growth inhibitor (abscisic acid) and growth promotor (IAA) may have been brought about during the ageing process of *Hevea* leaves. This imbalance in favour of growth inhibitor may therefore be responsible for foliar senescence in *Hevea*. Thus, in order to prove this hypothesis, three experiments were set up. In the first experiment, batches of leaves of two months and six months of age were treated with 10 p.p.m. abscisic acid in lanolin alone and 10 p.p.m. abscisic acid plus equal amount of IAA in lanolin. The other two experiments were also conducted using the same technique as above but the concentrations of abscisic acid and IAA (growth promotor) used were increased to 100 p.p.m. and 1000 p.p.m. respectively. The results of the experiments are shown in *Table 3*.

The results *(Table 3)* show that when the balance was upset by artificially raising the level of abscisic acid, the senescence and abscission processes in the two-month and six-month-old leaves were initiated and the leaves fell within fourteen days. This effect of abscisic acid was found to be true for concentrations from 10 p.p.m. to 1000 p.p.m. The results also demonstrate that at each of these concentrations, abscisic acid can be rendered inactive in accelerating senescence by application of equal amounts of IAA. The balance between growth promotor and growth inhibitor in leaves has therefore a major role in leaf senescence and abscission in *Hevea* leaves.

DISCUSSION

Endogenous Growth Regulators and Hevea *Leaf Senescence*

Auxin formation in *Hevea* leaves appears to be associated with growth; the amount of detectable growth promotor, most likely IAA, was high when the leaves were young and began to decrease as the leaves aged *(Figure 4)*. The pattern resembles that found in bean (SHOJI *et al.*, 1951), *Coleus*, Aster and *Solidago* leaves; an exception is provided by *Ginko* (GUNCKEL AND THIMANN, 1949), although it has yet to be established that this is not a result of the simultaneous effect of growth inhibitors.

'Free auxin' moves freely in the polar transport system and appears to be imme-

TABLE 3. INTERACTION OF ABSCISIC ACID (ABA) AND INDOLEACETIC ACID (IAA)

Age of leaves (month)	Average days to abscission						
	Control	ABA (10 p.p.m.) in lanolin	ABA (10 p.p.m.) + IAA (10 p.p.m.) in lanolin	ABA (100 p.p.m.) in lanolin	ABA (100 p.p.m.) + IAA (100 p.p.m.) in lanolin	ABA (1000 p.p.m.) in lanolin	ABA (1000 p.p.m.) + IAA (1000 p.p.m.) in lanolin
2	300	14	300	14	300	14	300
6	180	14	180	14	180	14	180

diately effective as a growth hormone. CHUA (1970) has shown that 'free auxin' does move freely in the *Hevea* plant and this movement of 'free auxin' may be responsible for the active growth of the young plant. When the auxin supplies become inadequate the whole level of metabolism slows down as the plant ages. This may also be the reason why MCINDOE (1958) and WYCHERLEY (1961) found that the rooting capacity of *Hevea* cuttings declined with age. Since in young *Hevea* plants new shoots and new leaves appear at a faster rate than mature *Hevea* plants, the auxin from the leaves and shoots will maintain the plant in a juvenile stage and thus no complete senescence and total defoliation occur in young juvenile *Hevea* plants, as in the case of mature *Hevea* plants which exhibit the 'wintering' phenomenon. GUNCKEL *et al.* (1949) reported that the capacity of *Ginko* trees to produce long shoots, which depends on auxin supply, declined with age. This suggests decreased auxin supply in the older trees.

'Bound' auxin, on the other hand, sometimes possesses hormonal activity and sometimes not. LARSEN (1951) recognises four different types of 'bound' auxin: auxin-protein complex, neutral precursors of auxin, precursor complexes and auxin associated with structural protein. The 'bound' auxin fraction of *Hevea* leaves falls in the last category since it is not released by boiling or under strongly acid or alkaline conditions, but is liberated by proteases *(Table 2)*.

From *Figure 4a* it is seen that there is a late appearance of extra growth promotor (IAA) immediately before leaf-fall. This increase is not as high as the auxin content of young leaves in April and May. This small increase in growth promotor IAA before leaf-fall may merely result from the release of conjugated IAA as a consequence of protease activity in the senescent *Hevea* leaves (CHUA, 1970). In detached senescing leaves protein breakdown takes place (CHIBNALL, 1939) with the subsequent production of auxin (SHELDRAKE AND NORTHCOTE, 1967). It is known that tryptophan can be converted to auxin, IAA, by a variety of plant tissues (KULESCHA, 1952; GORDON AND WEBER, 1951). As cells autolyse, the breakdown of cell structure in the old leaves might be expected to release 'free' auxin from cell compartments, such as vacuoles, and also lead to the production of free tryptophan from enzymatic hydrolysis of proteins. This tryptophan could then be converted to IAA. The small increase in growth promotor (IAA) *(Figure 4a)*, as a result of the release of 'bound' auxin by protease action or as a result of IAA formed from tryptophan produced by enzymatic hydrolysis of proteins, could induce the formation of abscission layer in the *Hevea* leaves.

Abscission accelerators are known to play a part in leaf senescence in a variety of genera, and a growth inhibitor (abscisic acid) has been detected in *Hevea* leaves *(Figure 5b)*. The growth inhibitor (abscisic acid) level remained generally constant *(Table 1)*. From *Table 3* it is seen that as soon as the level of abscisic acid was artificially raised, the *Hevea* leaves turned yellow and fell within two weeks.

OSBORNE (1967) has shown that abscisic acid not only induces yellowing of *Xanthuim* leaves but also inhibits protein synthesis, as measured by the incorporation of 14-leucine in the protein fraction. Similarly, MADISON AND RAPPAPORT (1968) attributed the ability of abscisic acid to prevent sprouting of potato buds to an inhibition of protein and nucleic acid synthesis, and depression of nucleic acid synthesis has been demonstrated by CHRISPEELS AND VARNER (1967) and VAN OVERBEEK AND LOEFFLER (1967).

Growth Regulator Control of Senescence and Abscission

The changing composition of senescent *Hevea* leaves can to a large extent be attributed to a diminishing RNA availability

and less efficient protein synthesis. There are already abundant indications (SILBERGER AND SKOOG, 1953; FLETCHER AND OSBORNE, 1965) that both aspects are subject to auxin control, and it is accordingly significant that senescence is marked by a parallel decrease in the growth promotor (IAA) concentration in the *Hevea* leaves *(Figure 4a)*. The correlation is emphasised by the way in which auxin application delays leaf abscission for periods of upto three months; the timing and concentration of the application were obviously critical, late treatment having little effect and excessive dose simply being toxic, accelerating senescence and defoliation as seen in *Figure 4b*. In order to retard the process of senescence and abscission, it seems that growth regulator concentration has to be maintained within narrow limits. Various concentrations of IAA ranging from 10 p.p.m. to 1000 p.p.m. were tried and the effective concentration was found to be 100 p.p.m. When the application of auxin was done in April to September, the life span of the *Hevea* leaves was shortened and senescence and abscission were accelerated as seen in *Figure 4b*. This may be due to an overdose of the auxin because during this period the endogenous growth promotor was high *(Figure 4a)*. On the other hand, when the 100 p.p.m. of IAA was applied during the month of October, when the endogenous growth promotor content of the leaves was just depleting *(Figure 4a)*, the life span of the leaves was lengthened and senescence and abscission were delayed upto the following May as seen in *Figure 4b*. Application of the 100 p.p.m. IAA during the months of November, December and January, after the endogenous auxin has been at a minimal level for some time, resulted in a slight retardation of abscission.

According to ADDICOTT AND LYNCH (1955), abscission is not controlled by the amount of auxin in the abscission area but rather by the auxin gradient across the abscission zone. Abscission can be stimulated by applying synthetic auxin to the petiole between the abscission zone and the stem, and it can be inhibited by applying auxin distal to the abscission zone. On the other hand, GAUR AND LEOPOLD (1955) stated that high concentrations of auxin inhibit abscission while low concentrations promote abscission. They concluded that abscission is controlled primarily by the quantity of auxin rather than the auxin gradient. The results obtained in the present investigation *(Figures 4a* and *b)*, without taking growth inhibitor (abscisic acid) into consideration, are clearly in accordance with the latter idea.

In view of the isolation of abscisic acid as a leaf abscission factor (ADDICOTT *et al.*, 1955) and the implication of a related substance (dormin) in the cessation of growth of *Acer* leaves (CORNFORTH *et al.*, 1965; WAREING, 1965), it is noteworthy that there was no increase in the presumed abscisic acid contents of ageing *Hevea* leaves *(Table 1)*. Since the level of growth inhibitor (abscisic acid) remained relatively constant and the level of growth promotor declined with age *(Figure 4a)*, it is very probable that an imbalance in concentration of growth inhibitor (abscisic acid) and growth promotor (IAA) may be brought about during the ageing process of *Hevea* leaves. Experiments were conducted to test this hypothesis. The results in *Table 3* show that the balance was upset by artificially raising the level of abscisic acid. This imbalance initiated the senescence and abscission processes in the two- and six-month-old leaves, and they fell within fourteen days. This effect of abscisic acid was found to be true for concentrations from 10 p.p.m. to 1000 p.p.m. The results also demonstrate that at each of these concentrations, abscisic acid can be rendered inactive in accelerating senescence by application of equal amounts of IAA. The fact

that yellowing can be prevented by the simultaneous application of appropriate amounts of auxin even when there is a hundred-fold increase in abscisic acid concentration is suggestive of a neutralisation effect. It also introduces the probability that the dominating factor in *Hevea* leaf senescence is not so much the absolute growth promotor (IAA) content but rather the balance between the growth promotor and the remarkably constant growth inhibitor (abscisic acid) level. Essentially, similar results have been obtained by VAN OVERBEEK (1968) with respect to *Lemna,* which is exceedingly sensitive to abscisic acid. Concentrations as low as one part in 10^9 reduce the rate of growth and 1 p.p.m. induces permanent dormancy, but the inhibition was entirely abolished by subsequent cytokinin conditions. In this particular instance, auxin and gibberellin were not effective.

From the above data, the suggested interaction between growth promotor and growth inhibitor in determining leaf senescence and abscission in *Hevea* leaf is given in *Figure 7.* As may be seen, abscission does not occur when both the endogenous growth promotor and growth inhibitor are in equilibrium. As the *Hevea* leaves progress

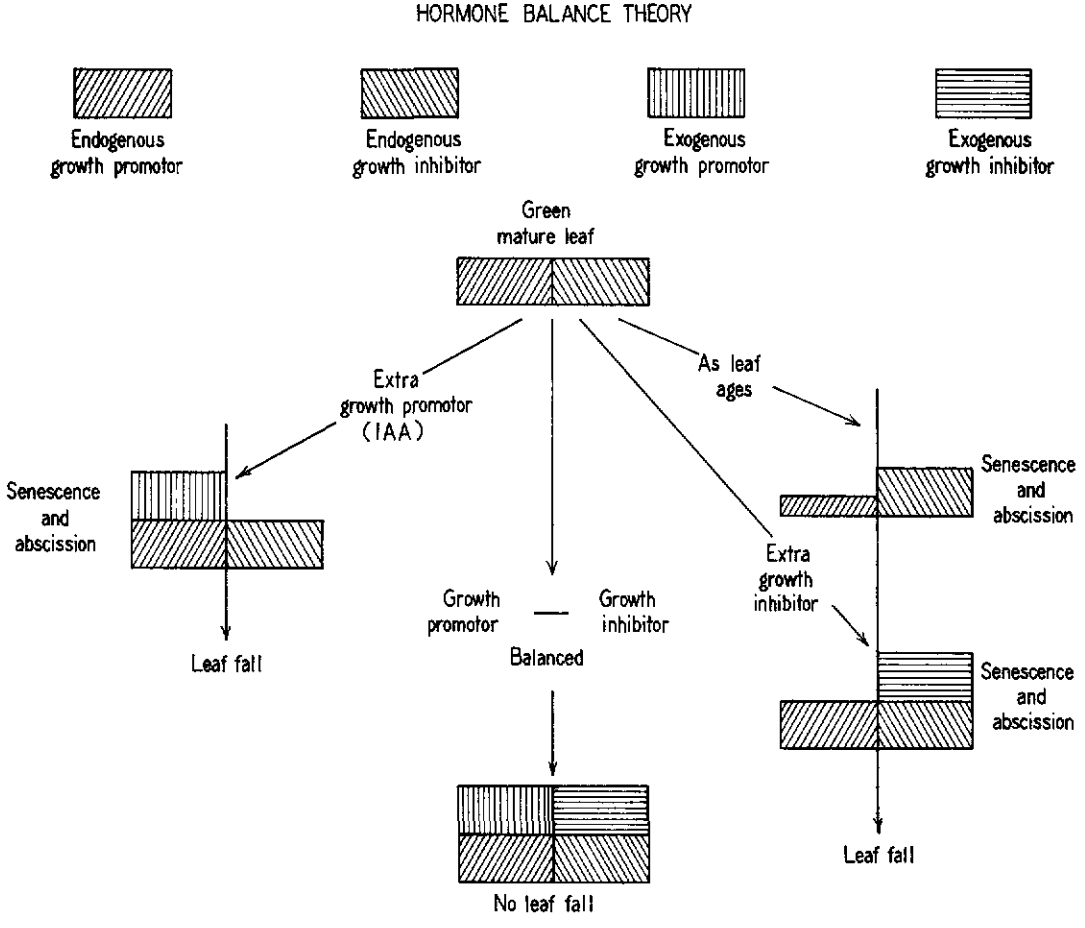

Figure 7. Suggested interaction of growth promotor (IAA) and growth inhibitor (ABA) determining leaf senescence and abscission.

in age, the endogenous growth promotor content declines, but not the endogenous growth inhibitor. When this imbalance situation in favour of growth inhibitor (abscisic acid) is created, the leaves senesce and fall. This imbalance can also be created by raising the level of exogenous growth promotor or growth inhibitor. By so doing, senescence and abscission processes are initiated and the leaves will turn yellow and fall. Application of equal amounts of growth promotor and growth inhibitor to *Hevea* leaves will prevent the onset of senescence and abscission.

Fate of IAA in Hevea *Leaves*

From *Figures 6a* and *b*, it is seen that the IAA-oxidase activity increases with age of the *Hevea* leaves. According to HARE (1964), it appears that the enzymatic destruction of IAA is important in regulating the amount of growth substance in the plant. Thus, as the cells of *Hevea* leaves age and lose their ability to grow and produce auxin, the increase in IAA-oxidase activity may be partly responsible for the destruction and decrease in IAA content of *Hevea* leaves *(Figure 4a)*. However, this is only a suggestion because the role of IAA-oxidase in the physiology of the plant still remains obscure.

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