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THE PRODUCTION OF AUXIN BY TOBACCO INTERNODE TISSUES

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SUMMARY

The formation of callus at the basal end of tobacco internode tissues cultured on a basic medium has been used as an indication of the presence of auxin within the tissues. It has been shown in this way that sections of internode are capable of producing auxin. This production of auxin is related to the continued activity of the vascular cambium. If cambial activity and vascular differentiation are eliminated, auxin is no longer produced. When tissues in which cambial activity and vascular differentiation are taking place are cultured on a medium containing an inhibitor of polar auxin transport, tri-iodo benzoic acid, serried ranks of xylem tracheids are formed. It is suggested that auxin is produced as a consequence of xylem differentiation and the observations reported in this paper are interpreted in the light of this hypothesis. It is also suggested that kinins may be produced as a result of xylem and phloem differentiation, and the possibility that autolysing cells are a major source of both auxins and kinins in the plant is discussed.

INTRODUCTION

In contrast with the rapid increases in knowledge about the effects of plant hormones on growth, differentiation and morphogenesis, little is known about the cellular sites of their synthesis or the factors which control their production. One of the few specific suggestions about the site of production of auxin has been made by Torrey (1963); he reasoned from circumstantial evidence that in root tips auxin is produced by the cells of the quiescent zone, but as he pointed out the difficulties of a direct experimental test of such a hypothesis are very great. For an indirect investigation of the sites of hormone synthesis the technique of sterile tissue culture is in many ways ideal. The effects of exogenous hormones, on cultured tissues have been extensively studied and, if the culture tissue produces hormones, it should be possible to infer where they are produced from these effects on the tissue.

It has been known for some time that in the absence of exogenous growth substances tobacco internode tissues produce a callus at the morphologically basal end. This is associated with the basipetal transport of auxin; in the presence of 2-3-5 tri-iodo benzoic acid, which inhibits auxin transport, the growth of callus becomes uniformly distributed (Niedergang-Kamien and Skoog, 1956). The amount of callus formed is related to the total amount of auxin present (Niedergang-Kamien and Skoog, 1956). The callus formed at the morphologically basal end of sections of tobacco internodes can therefore be used as an indication of the presence of auxin. The results presented in this paper show that callus formation cannot be explained simply by the accumulation of auxin which was travelling through the stem at the time of explanting, but that certain tissues of the internode produce the growth factor. The cellular site of this continued production of auxin has been investigated.

MATERIALS AND METHODS

Plants and seeds of *Nicotiana tabacum* var. *java* were kindly supplied by Dr Kassanis of the Rothamstead Experimental Station. They were grown in a glasshouse and used when 2–3 months old. Lengths of stem were surface-sterilized by immersion first in 70% ethanol for 10 minutes followed by 10 minutes in dilute sodium hypochlorite (commercial solution diluted 1 in 20). They were then washed in sterile water.

Culture media

The basic medium consisted of Ca $(NO_3)_24H_2O$ (720 mg/l), KNO_3 (405 mg/l), $MgSO_47H_2O$ (245 mg/l), KH_2PO_4 (70 mg/l), NaCl (60 mg/l), $FeCl_3$ (0.7 mg/l), H_3BO_3 (0.57 mg/l), $MnCl_24H_2O$ (0.495 mg/l), $CuCl_22H_2O$ (0.268 mg/l), $Na_2MoO_42H_2O$ (0.252 mg/l), glycine (3.0 mg/l), nicotinic acid (0.5 mg/l), thiamine (0.1 mg/l), pyridoxine (0.1 mg/l), sucrose (2.5 g/l), agar (8.0 g/l). This basic medium was used for all experiments except where otherwise stated. When tri-iodo benzoic acid was used it was added to the basic medium at a concentration of 50 mg/l to give the TIBA medium. Coconut milk (100 ml/l) and α-naphthalene acetic acid (0.2 mg/l) were each added to the basic medium when exogenous growth factors were used. The media were autoclaved at 15 p.s.i. for 30 minutes before use. All operations were carried out with sterile instruments in a cabinet sterilized by ultra-violet light for 30 minutes.

Explants

Internode sections were usually placed vertically in the medium (15 ml) contained in a boiling tube, with their morphologically basal ends upwards. The terms 'apical' and 'basal' are used to refer to the morphologically apical and basal ends. In some experiments the outer tissues were stripped off down to the level of the cambium: these outer tissues are referred to as 'phloem', the inner ones as 'xylem'.

Cultures were grown in a room maintained at $25 \pm 1°$ C with dim diffuse light from a fluorescent tube for 12 hours a day.

Microscopical examination

Tissues were fixed in a chrome fixative (Johansen, 1951) or glutaraldehyde (Pickett-Heaps and Northcote, 1966), dehydrated in ethanol, cleared in tertiary butanol and embedded in paraffin wax. They were sectioned on a sliding microtome at 12 μ and stained either with safranin and picric aniline blue or Heidenhain's iron haematoxylin (Jensen, 1962). For revealing callose, dilute aniline blue and fluorescent optics were used (Jensen, 1962). Specimens were examined on a Zeiss Ultraphot. Hand-cut sections were stained with phloroglucinol (Johansen, 1940) for lignin localization.

Lignin was estimated by sodium hydroxide extraction followed by a colorimetric phenol analysis as described by Jeffs and Northcote (1966).

RESULTS

Growth of callus on internode explants on basic medium

Callus grew at the morphologically basal end of the stem sections irrespective of their orientation with respect to gravity. Most of the observations were made with explants growing with the morphologically apical end placed in the agar. However, if they were grown with the basal end placed under the surface of the agar the callus

grew at that end, showing that its submersion in the medium did not inhibit cell growth and division. Under no conditions was callus formed at the apical end on the basic medium (Plate 1, Fig. 1).

Cell divisions were apparent in the cambial region at the basal end within a few days after explanting. After 8 days (Plate 1, Fig. 2) a definite wedge-shaped area of cambial activity, the 'dynamic wedge', was apparent. Enlarged cells and both transverse and longitudinal cell divisions could be seen in the cambium, external and internal phloem and cortex. By 14 days a definite outgrowth had taken place and tracheids had differentiated in the new tissue derived mostly from the cambium and phloem. Later, in the neoformation, an arching vascular cambium developed which gave rise to lignified, pitted tracheids on the inside and phloem with sieve elements on the outside (Plate 1, Figs. 3 and 4). A cork cambium was formed in the outer tissues. Nodular zones of differentiation, usually with a cambial zone surrounding a group of tracheids, also occurred. Sometimes shoots formed in the superficial tissues (Plate 5, Fig. 24).

These observations agree with those of Sterling (1950). However, the stem sections used here were generally longer than the ones used by Sterling and were not placed horizontally on the medium, which probably explains why it was not possible to observe the extension of the 'dynamic wedge' along the whole section as he reported. If the apical end of a stem section carrying a callus was placed in 1% agar containing 1% acid fuchsin and a moving stream of air was passed over the tissue, the dye was transported into the callus and it could be seen that the tracheids formed from the arching cambium were in functional continuity with those in the 'dynamic wedge' and the xylem of the internode (Plate 3, Fig. 15).

The polar development of callus at basal cut surfaces was also shown by cutting small notches in the side of the stem explant. On the apical face, little or no cell division took place whereas on the basal face, a callus developed and often gave rise to shoots.

Growth of callus on internode explants on medium with added growth substances

On a medium containing coconut milk and α-naphthalene acetic acid, cells at the morphologically apical end were stimulated to divide and form a callus if this end was in contact with the medium (Plate 5, Fig. 23). Differentiation occurred in the new tissue formed in this way. If the stem section was placed with the apical end upwards and not in contact with the medium, no development of callus took place, presumably because growth substances were not transported acropetally.

At the basal end, callus development was strongly stimulated whether or not this end was in contact with the medium. The callus contained much less differentiated tissue than calluses produced on basic medium, no arching cambium was produced in it and the 'dynamic wedge' was much less pronounced. Nodules of tracheids appeared, and roots often arose near the vascular tissue of the explant.

Growth of callus on internode explants on water agar

If stem sections were explanted onto 0.8% agar containing nothing but distilled water, a callus was formed at the basal end. This showed that the stem section was capable of forming callus in the absence of both added growth substances and nutrients, presumably by mobilizing its stored reserves of the latter.

Formation of callus on the apical parts of bisected stem explants

Stem sections 3 cm long were bisected or trisected transversely at various times

after the initial explanting. In all cases the apical halves formed a callus at their morphologically basal end (Table 1). In other experiments it was found that the ability of the apical halves to form a callus at their basal end was retained if the original explant was bisected after 4, 6 or 9 months in culture (Plate 2, Fig. 7).

Table 1. *Growth of callus on inverted tobacco stem section bi- and tri-sected at various times after explanting (observations made 43 days after the beginning of the experiment)*

Days before bi- or tri-secting	Position of section	Growth of callus at basal end	Growth of shoots at basal end
1	Upper	+ +	–
	Lower	+ +	–
1	Upper	+ + +	–
	Lower	+ +	–
2	Upper	+ +	–
	Lower	+ + +	Shoots
2	Upper	+ + +	–
	Lower	+ + +	–
3	Upper	+ +	–
	Lower	+ + + +	Shoots
3	Upper	+ + +	–
	Lower	+ + +	–
5	Upper	+ + +	Shoots
	Lower	+ +	Shoots
7	Upper	+ +	–
	Lower	+ +	Shoots
7	Upper	+ + +	–
	Lower	+ + +	–
14	Upper	+ + +	–
	Lower	+	Shoots
14	Upper	+ + +	–
	Middle	+ +	Shoots
	Lower	+ +	–
23	Upper	+ + + +	–
	Lower	+	–
23	Upper	+ + +	–
	Middle	+	–
	Lower	+	Shoots

+ + + +, very prolific growth of callus;
+, small growth of callus.

Stem sections 2 cm and 4 cm long were explanted, the basal 1 cm was removed from both after 2 days and the two parts were cultured separately. The two halves of 2 cm explants formed approximately equal amounts of callus and the 3 cm apical parts of the 4 cm explants produced more callus than the 1 cm basal parts (Plate 2, Figs. 5 and 6).

Continued activity of the vascular cambium in internode explants

As the stem grows as a result of secondary thickening, the number of rows of xylem cells increases. This number was found to increase in successively old internodes and also to increase from the apical to basal part of a given internode.

Stem sections from the top and bottom of an internode (but taken at least 1 cm from the nodes) were fixed for microscopical examination at the time the middle 4 cm section was explanted and left to form a callus. After 29 days the upper and lower parts of this middle stem section were fixed and the centre portion (1 cm) was re-explanted. This centre portion in turn produced a callus and was fixed after a further 45 days. The number of

rows of xylem vessels was counted in transverse sections of all these samples. The representative results given in Fig. 11 show that the number of xylem rows had increased, and indicated continued vascular cambial activity in the explant.

If the outer tissues of the internode were stripped off down to the level of the cambium the remaining cylinders, containing xylem, internal phloem and pith, formed a callus at the basal end. This represents a similar system to that used by Niedergang-Kamien and Skoog (1956) and Skoog and Miller (1957). At the cambial surface along these explants only a few cell divisions took place producing wound cells and no differentiation occurred (Plate 3, Fig. 13). The vascular cambium had therefore been eliminated.

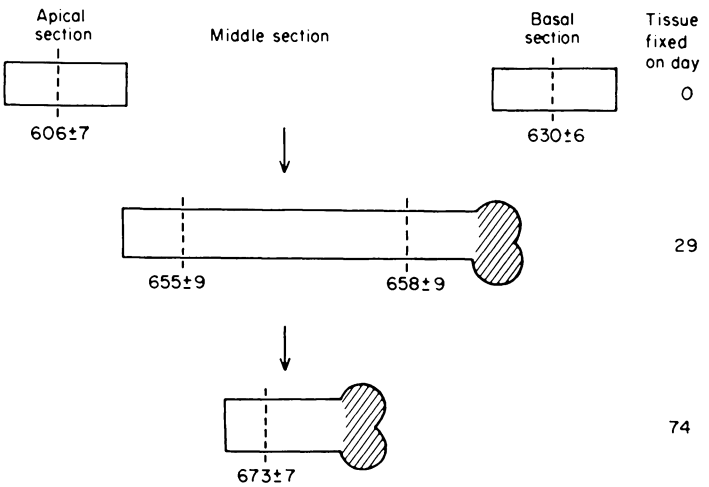

Fig. 11. Continued activity of the vascular cambium in cultured internode sections. The dotted lines indicate the position at which transverse sections were cut in order to count the number of xylem cell rows. The figures each represent the mean of eight counts of the number of xylem rows, with the standard deviation.

Three originally adjacent internode sections were explanted and left for 1 day to allow free-moving auxin to accumulate at the basal end and the basal 1 cm was then removed. One of these three stem sections was then stripped of its outer tissues down to the cambial region, another was similarly stripped for only half its length and the third was explanted intact. The resulting growth of callus (Plate 2, Fig. 8) was least on the stripped section, greater on the half stripped section and greatest on the intact one.

Separately cultured 'xylem' tissues

Immediately after stripping off the outer tissues as described above, the cells at the newly exposed surface of the inner tissue were mostly cells already differentiating into xylem or ray parenchyma (Plate 3, Fig. 12). A few cell divisions occurred at this surface producing wound cells (Plate 3, Fig. 13). No further changes were observed. At the basal end a callus developed which contained an arching zone of tracheids surrounded by a cambial zone; outside this zone phloem was formed (Plate 3, Fig. 14).

When these 'xylem' explants were bisected after different lengths of time in culture and the two halves explanted separately, a new callus was formed at the basal end of the apical half if the original sections were bisected within about 7 days. However, on the

apical parts obtained by bisection of explants after about 7 days, no new callus appeared (Plate 2, Fig. 9).

Separately cultured 'phloem' tissues

Immediately after the outer tissues were stripped from the internode section, the newly exposed surface of the outer tissues consisted of thin walled cambial cells (Plate 4, Fig. 16). No xylem cells were present.

After 3 days in culture some cell division had taken place all along the exposed surface of the explant (Plate 4, Fig. 18). A callus then developed at the basal end and a cambial zone appeared along the whole length of the explant cutting off new phloem elements and xylem tracheids. This cambium was much more active at the basal end than at the apical end. The new cambial zone was in continuity with an arch of cambium which formed in the callus (Plate 4, Fig. 19). Ordered rows of differentiated phloem and xylem cells were formed (Plate 4, Fig. 17). Shoots were sometimes formed on the callus.

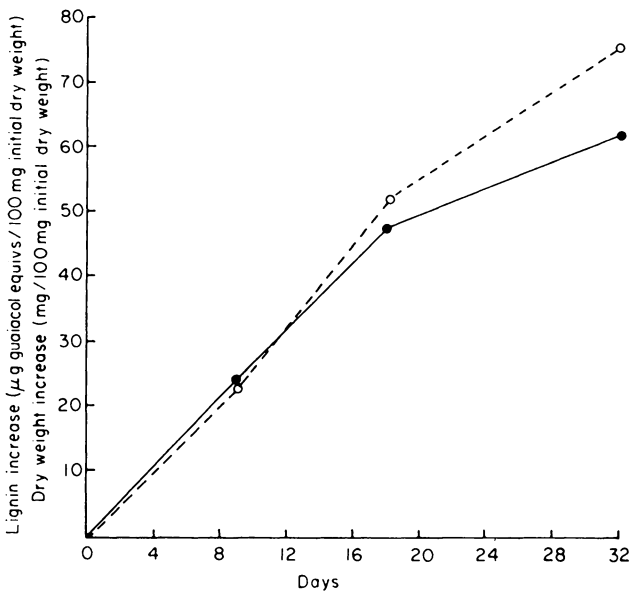

Fig. 20. Relationship between xylem differentiation (expressed as increase in total lignin) and growth of 'phloem' explants. ○, Lignin; ●, dry weight increase.

If these 'phloem' explants were bisected at various times after explanting, a new callus was formed at the basal end of the apical halves (Plate 2, Fig. 10). The ability to form a new callus on the apical halves persisted in explants kept for at least 4 months.

No new phloem fibres appeared so that the increase in lignin content of the tissue could be used as an indication of the amount of xylem formed. The relationship between xylem differentiation and total growth could therefore be studied by comparing the amount of lignin with the dry weight of the specimen. The results (Fig. 20) show that differentiation and total growth are closely related.

The first appearance of differentiating xylem tracheids was studied by examining microscopically whole phloem explants stained with phloroglucin/HCl. During the first 2 days no differentiation could be observed, but by the third day lignified, reticulately thickened cells appeared at the basal end (Plate 5, Fig. 21). From these cells

new differentiation occurred acropetally and usually by the fourth day several files of lignified cells were found to stretch right to the apical end of a 2 cm long 'phloem' explant. New areas of differentiation appeared at the basal end and from these new acropetal files of differentiating cells arose (Plate 5, Fig. 22). Cells also differentiated in lateral continuity with these so that bands of lignified cells were found along the explant.

The above results were obtained when the 'phloem' explants were placed with their basal ends uppermost in the medium but the results were similar when they were placed horizontally, cambial side down or with their basal ends in the medium. The differentiation, however, took longer to appear and was less extensive.

'Phloem' explants were placed horizontally, cambium face down, on medium containing TIBA. Under these conditions a few cell divisions took place along the cambial face and the cells in contact with the medium enlarged but no differentiation occurred and no callus was formed. The explants turned yellow and died within a week or two. If 'phloem' explants were placed vertically in medium containing TIBA with the basal end upwards, differentiation was again observed at the basal end within 3 days. If, on the other hand, they were explanted apical end upwards, differentiation occurred not at the basal end but in the middle of the explant at the surface of the medium.

In a further series of experiments, 'phloem' sections were explanted basal end upwards in normal medium and subsequently bisected, and the two halves were placed horizontally, cambial face down, on medium containing TIBA. If bisected two days after explanting, the basal half subsequently developed a considerable growth of callus containing a cambial zone and numerous differentiated tracheids, while the apical half underwent no further development, turned yellow and died. If bisected after 3 days or more, both halves remained green and healthy and formed a callus containing differentiated cells, though the amount of callus formed on the basal half was always considerably greater than that formed on the apical half.

The effects of TIBA on explants with continued cambial activity

Niedergang-Kamien and Skoog (1956) showed that TIBA caused a uniform distribution of callus on the 'xylem' sections with which they worked. In our experiments it also abolished the polarity of development of callus on 'phloem' and intact stem sections, in which a vascular cambium was active.

Stem sections were placed horizontally on medium containing TIBA after they had been cultured for several days on normal medium and the basal end had been removed. Specimens were examined microscopically at various times afterwards and these showed that cambial activity took place all along the explant and that numerous new xylem cells were formed. So remarkable was this effect that in some cases the width of the new xylem formed exceeded by several times the width of the original xylem (Plate 6, Figs. 25 and 26). Some of these lignified cells still contained cytoplasm and nuclei but in the majority the cell contents had disappeared (Plate 6, Fig. 27). 'Phloem' sections were explanted horizontally onto medium containing TIBA after a week or so in normal medium when a growth of callus had already developed. These specimens differed from control explants which were placed horizontally on normal medium in that they showed a more pronounced cambial activity, with more xylem differentiation, along the explant. The callus at the basal end (in which there was presumably little polar transport of auxin for TIBA to inhibit) developed similarly in explants on medium containing TIBA and in controls. The effects of TIBA were more spectacular on 'phloem' explants placed on it before a marked basal callus had developed, but after xylem differentiation

had occurred in them. The development of callus on the basal part of a phloem explant placed on TIBA medium 3 days after it was explanted onto normal medium is shown in Plate 6, Fig. 28. An outgrowth occurred along the whole length of the specimen and this consisted largely of lignified xylem with an active cambial zone external to it.

DISCUSSION

Niedergang-Kamien and Skoog (1956) showed that the majority of free auxin in sections of internode at the time of excision accumulated at the basal end of the section within 3 hours. Therefore, in internode sections bisected a day or more after explanting, any auxin accumulating at the basal end of the apical halves and giving rise to a new callus is likely to have been produced in the tissues. The apical halves of internode sections gave rise to a callus at their basal end if the original sections were bisected at any time from one day to several months after explanting. This indicated that the auxin present at the time of explanting accumulated at the basal end of the explant and also that the internodes continued to produce auxin. The relative importance of free auxin which was in the stem at the time of explanting and auxin which was subsequently produced is shown in the experiment in which sections 2 cm and 4 cm long had their basal 1 cm removed after 48 hours. The results indicated that the initial free auxin which accumulated at the basal end was relatively unimportant; the amount of callus formed was approximately equal on the two 1 cm sections derived from the 2 cm explant and much greater on the 3 cm apical part than the 1 cm basal part of the 4 cm explant. That the amount of callus formed was related to the length of the apical part of the explant indicated that the auxin was produced all along it and not merely at the cut surfaces. These observations recall those of Zimmermann (1936) who reported that the yield of diffusible auxin from *Fraxinus* and *Acer* internodes was proportional to the length of the internode, and the experiments of Gunckel and Thimann (1949) which showed that auxin was formed in the older internodes of the long shoots of *Ginkgo*.

This continued production of auxin by internode sections was associated with continued cambial activity as shown by the increase of xylem cell rows. If the cambium was eliminated by stripping off the outer tissues, the production of callus was diminished.

The 'xylem' explants did not retain the ability to regenerate a callus on their apical halves if they were bisected more than a week or so after explanting. This indicated that they did not form auxin; thus the mature xylem tissues, pith and mature internal phloem are unlikely to be the sites of auxin production in the whole internode sections. The 'phloem' explants on the other hand did retain this ability to regenerate callus on their apical halves if they were bisected at periods up to several months after explanting. They also formed a vascular cambium which continued to give rise to new xylem and phloem derivatives. It therefore seems likely that the production of auxin was a consequence of vascular cambial activity. This means that auxin must be produced by any one or all of the tissues present; that is the dividing cambial cells, the differentiating xylem or the differentiating phloem.

The formation of differentiating xylem cells at the basal end of the 'phloem' explants within 3 days of explanting was probably due to the accumulation of free auxin at that end. This was shown by the experiments in which 'phloem' tissue was placed basal or apical end up in medium containing TIBA which acted as an inhibitor of auxin transport. Basipetal transport of auxin could still occur in the part above the level of the medium, and if the basal end was uppermost, lignified differentiated cells first formed

at the basal end, as in the controls. If the basal end was in the medium with TIBA, lignified cells appeared not at the basal end but in the middle of the explant at the surface of the medium, presumably due to auxin accumulation there. When the 'phloem' tissue immediately after stripping was placed horizontally on a medium containing TIBA, the accumulation of auxin at the basal end was prevented although the wound response still took place. Under these conditions there was no xylem differentiation, no callus was produced and the explants turned yellow and died within a week or two. If this explanting onto TIBA medium occurred after auxin had been allowed to accumulate and vascular differentiation had been initiated, callus was produced; this indicated that the differentiation of vascular tissue was associated with auxin production.

Dividing cells are unlikely to produce growth factors as a consequence of their division since many callus cultures cease to grow in the absence of exogenous growth factors and the wound response is self-limiting. The cell division which occurred on the stripped surface of the 'xylem' explants and on the 'phloem' explants laid horizontally on medium containing TIBA ceased within a few days. If the dividing cells of the cambium produce auxin, as was suggested by Söding (1937), they do so not because it is a general property of dividing cells to produce auxin, but because they are in close proximity to differentiating vascular tissue. Our observations would best be explained by suggesting that differentiating xylem and/or phloem cells produce auxin as they differentiate, or produce precursors of auxin such as tryptophan which are converted into auxin by adjacent parenchyma or cambial cells. The observations on internode sections and 'phloem' tissues explanted after several days on to medium containing TIBA indicate that the differentiating xylem rather than the phloem is the source of auxin or auxin precursors. Auxin is known to stimulate cambial activity (Wareing, Hanney and Digby, 1964) and xylem differentiation (Wetmore and Rier, 1963; Jeffs and Northcote, 1966) and if it is also produced as a consequence of xylem differentiation then in the absence of the polar transport mechanism for removing auxin, a positive feedback effect would be expected. The production of serried ranks of xylem cells under these conditions represents just such a phenomenon. When polar transport is not inhibited, some of the auxin produced might well lead to a certain amount of cambial activity and vascular differentiation while the remainder would be transported to the basal end of the explant stimulating callus formation. The hypothesis that auxin is produced as a consequence of xylem differentiation would thus explain all the observations reported in this paper. The possibility that phloem differentiation might also lead to the production of auxin seems less likely, although it cannot be excluded.

It is known that tryptophan can be converted to the auxin indolylacetic acid by a variety of tissues in plants (Kulescha, 1952; Gordon, 1961) and also in bacteria (Stowe, 1955) and animals (Weissbach *et al.*, 1959). As cells autolyse, the breakdown of cell structure might be expected to release free tryptophan from cell compartments such as vacuoles and also lead to the production of free tryptophan from the enzymatic hydrolysis of proteins. This tryptophan could then be converted to indolylacetic acid. It has been shown that autolysing tissues do in fact produce auxin (Sheldrake and Northcote, 1968*a*). In detached senescing leaves in which proteins break down (Chibnall, 1939) auxin is also produced (Sheldrake and Northcote, 1968*b*). It is well known that when xylem cells differentiate, their cell contents break down and disappear, presumably as a result of an autolytic process. Some of the products of this breakdown and the enzymes responsible for it might well be carried away in the xylem sap passing through the newly differentiated xylem cells, and an analysis of bleeding sap and guttation fluid

has in fact shown the presence of proteases, nucleases and phosphatases, as well as sugars and a large range of amino acids, and in the case of *Avena* guttation fluid, small amounts of auxin (Sheldrake and Northcote, 1968*c*).

The production of auxin as a consequence of the autolysis of differentiating xylem cells could therefore explain the production of auxin by internodes in which the vascular cambium is active. A similar explanation would account for the well-known sites of auxin production in root and shoot tips and in expanding leaves, in all of which vascular tissue is differentiating. Indeed in the case of expanding leaves Jacobs and Morrow (1957) have shown a close correlation between auxin production and xylem differentiation. The production of auxin by autolysing tissues in general could also account for its formation in developing embryos (Nitsch, 1950) and seeds (Went and Thimann, 1937) in both of which nutritive tissues such as the endosperm break down. The presence of auxin in pollen and orchid pollinia (Went and Thimann, 1937) could also be explained on this basis since in pollen development there is also the breakdown of a nutritive tissue, the tapetum (Esau, 1953). Thus the majority of the auxin in the plant may be produced as a consequence of cell breakdown, although it may also be synthesized by cells such as those in *Avena* coleoptile tips (van Overbeek, 1941; Wildman and Bonner, 1948) and in the quiescent zone of the root tips as suggested by Torrey (1963).

It seems possible that kinins may be produced in a similar manner. Kinetin was first isolated from aged DNA preparations and from fresh autoclaved DNA (Miller *et al.*, 1956). In an autolysing cell the breakdown of nucleic acids would be expected to release purine bases. Adenine itself has kinin-like effects (Skoog and Miller, 1957) and some of the adenine might be transformed spontaneously or enzymatically into more active kinins. Furthermore, recent reports that *t*-RNA contains purine bases with strong kinin activity (Skoog *et al.*, 1966; Hall *et al.*, 1967) make it seem likely that kinins would be released directly from such RNA on autolysis. Thus kinins may be formed in the plant by the breakdown of nutritive tissues, by differentiating xylem cells and also differentiating phloem elements, in which the nucleus breaks down. This would explain the presence of kinins in bleeding sap (Kende, 1964) and the presence of kinins in vascular tissue which stimulated division of sterile tobacco pith in the experiments of Jablonski and Skoog (1954).

Turing's diffusion–reaction theory of morphogenesis, discussed by Wardlaw (1965) postulates that patterns of differentiation can arise and maintain themselves by stable patterns of distribution of metabolites which can be thought of by analogy with a stationary wave. The vascular cambium and its differentiating derivatives represents such a pattern of differentiation. The release of substances by differentiating xylem and phloem cells and the presence of sucrose and other metabolites which are conducted along mature phloem elements will provide different environments for the cambium itself, and for its derivatives on either side. Cambial derivatives adjacent to the xylem may be stimulated to differentiate into xylem by auxin produced by already differentiating cells. The cambial cells may be stimulated to divide by auxin from the differentiating xylem and by kinins which may be produced by both differentiating xylem and phloem; both kinins and auxin have been shown to be necessary for the division of certain types of cell in sterile culture (Skoog and Miller, 1957; Miller, 1963). Derivatives on the phloem side may differentiate into phloem under the influence of high sucrose:auxin ratios (Wetmore and Rier, 1963). Such a system would be self-catalysing and self-perpetuating. This hypothesis would not only account for the pattern observed in transverse sections of stems and roots, but also help to explain how xylem and phloem

cells differentiate in longitudinal continuity with each other in the intact plant and also in regenerating xylem strands such as those observed in *Coleus* (Sinnott and Bloch, 1945) and in the continuous files of xylem cells which develop in the tobacco phloem explants described above.

What seems beyond dispute, however, is that much more attention should be paid to the substances released by differentiating xylem and phloem cells. We have shown that auxin is probably produced as a consequence of xylem differentiation, and postulate that kinins may also be produced during xylem and phloem formation. We suggest that these substances are produced as a result of autolysis of the cell contents during differentiation and that these and other substances released affect the cells in their immediate environment and are probably of the greatest importance in the initiation and maintenance of patterns of growth and differentiation.

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REFERENCES

CHIBNALL, A. C. (1939). *Protein Metabolism in the Plant*. New Haven.
ESAU, K. (1953). *Plant Anatomy*. New York.
GORDON, S. A. (1961). The biogenesis of auxin. *Handb. PflPhysiol.*, **14**, 620.
GUNCKEL, J. E. & THIMANN, K. V. (1949). Studies of development in long shoots and short shoots of *Ginkgo biloba*. *Am. J. Bot.*, **36**, 145.
HALL, R. M., CSONKA, L., DAVID, H. & MCLENNAN, B. (1967). Cytokinins in the soluble RNA of plant tissues. *Science, N.Y.*, **156**, 69.
JABLONSKI, J. R. & SKOOG, F. (1954). Cell enlargement and cell division in excised tobacco pith tissue. *Physiologia Pl.*, **7**, 16.
JACOBS, W. P. & MORROW, I. B. (1957). A quantitative study of xylem development in the vegetative shoot apex of *Coleus*. *Am. J. Bot.*, **44**, 823.
JEFFS, R. A. & NORTHCOTE, D. H. (1966). Experimental induction of vascular tissue in an undifferentiated plant callus. *Biochem. J.*, **101**, 146.
JENSEN, W. A. (1962). *Botanical Histochemistry*. San Francisco.
JOHANSEN, D. A. (1940). *Plant Microtechnique*. New York.
JOHANSEN, D. A. (1951). *Manual of Phycology* (Ed. by G. M. Smith), Appendix B. Chronica Botanica, Waltham, Mass.
KENDE, M. (1964). Preservation of chlorophyll in leaf sections by substances obtained from root exudate. *Science, N.Y.*, **145**, 1066.
KULESCHA, Z. (1952). Récherches sur l'élaboration de substances de croissance par les tissues végétaux. *Revue gén. Bot.*, **59**, 19.
MILLER, C. O. (1963). Kinetin and kinetin-like compounds. *Mod. meth. Pl. analysis*, **6**, 194.
MILLER, C. O., SKOOG, F., OKUMURA, F. S., VON SALTZA, M. H. & STRONG, F. M. (1956). Isolation, structure and synthesis of kinetin, a substance promoting cell division. *J. Am. chem. Soc.*, **78**, 1375.
NIEDERGANG-KAMIEN, E. & SKOOG, F. (1956). Studies on polarity and auxin transport in plants. *Physiologia Pl.*, **9**, 60.
NITSCH, J. P. (1950). Growth and morphogenesis of the strawberry as related to auxin. *Am. J. Bot.*, **37**, 211.
OVERBEEK, J. VAN (1941). A quantitative study of auxin and its precursors in coleoptiles. *Am. J. Bot.*, **28**, 1.
PICKETT-HEAPS, J. D. & NORTHCOTE, D. H. (1966). Relationship of cellular organelles to the form and development of the plant cell wall. *J. exp. Bot.*, **17**, 20.
SHELDRAKE, A. R. & NORTHCOTE, D. H. (1968*a*). (In preparation).
SHELDRAKE, A. R. & NORTHCOTE, D. H. (1968*b*). (In preparation).
SHELDRAKE, A. R. & NORTHCOTE, D. H. (1968*c*). (In preparation).
SINNOTT, E. W. & BLOCH, R. (1945). The cytoplasmic basis of intercellular patterns in vascular differentiation. *Am. J. Bot.*, **32**, 151.
SKOOG, F., ARMSTRONG, D. J., CHERAYIL, J. D., HAMPEL, A. R. & BOCK, R. M. (1966). Cytokinin activity: Localization in transfer RNA preparations. *Science, N.Y.*, **154**, 1354.
SKOOG, F. & MILLER, C. O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. *Symp. Soc. exp. Biol.*, **11**, 118.
SÖDING, H. (1937). Wuchsstoffe und Kambiumtätigkeit der Bäume. *Jb. wiss. Bot.*, **84**, 639.
STERLING, C. (1950). Histogenesis in tobacco stem segments cultured *in vitro*. *Am. J. Bot.*, **37**, 464.

STOWE, B. B. (1955). The production of indoleacetic acid by bacteria. *Biochem. J.*, **61**, ix.
TORREY, J. G. (1963). Cellular patterns in developing roots. *Symp. Soc. exp. Biol.*, **17**, 285.
WARDLAW, C. W. (1965). *Organisation and Evolution in Plants*. London.
WAREING, P. F., HANNEY, C. E. A. & DIGBY, J. (1964). The role of endogenous hormones in cambial activity and xylem differentiation. *The Formation of Wood in Forest Trees* (Ed. by M. H. Zimmermann), p. 323. New York.
WEISSBACH, H., KING, W., SJAERDSMA, A. & UDENFRIEND, S. (1959). Formation of indole-3-acetic acid and tryptamine in animals. *J. biol. Chem.*, **234**, 81.
WENT, F. W. & THIMANN, K. V. (1937). *Phytohormones*. New York.
WETMORE, R. H. & RIER, J. P. (1963). Experimental induction of vascular tissue in callus of angiosperms. *Am. J. Bot.*, **50**, 418.
WILDMAN, S. G. & BONNER, J. (1948). Observations on the chemical nature and formation of auxin in the Avena coleoptile. *Am. J. Bot.*, **35**, 40.
ZIMMERMANN, W. A. (1936). Untersuchungen über die räumliche und zeitliche Verteilung der Wuchsstoffes bei Bäumen. *Z. Bot.*, **30**, 209.

EXPLANATION OF PLATES 1–6

PLATE 1

Longitudinal sections of tobacco internode sections cultured on basic medium.
Fig. 1. Apical end 53 days after explanting. No callus has been formed. × 34.
Fig. 2. Basal end 8 days after explanting. A wedge-shaped area of division has appeared in the cambial region and divisions can also be seen in the internal and external phloem and cortex. × 30.
Fig. 3. Callus formed at the basal end 50 days after explanting. An arching zone of tracheids can be seen, outside which is a cambium. In the superficial part of the callus a cork cambium has been formed. × 30.
Fig. 4. Part of an arching cambial zone in a callus similar to that shown in Fig. 3. Stained with aniline blue and photographed with ultra-violet light. The cambium lies between the tracheids and the phloem in which the callose of the sieve plates (S) fluoresces. × 150.

PLATE 2

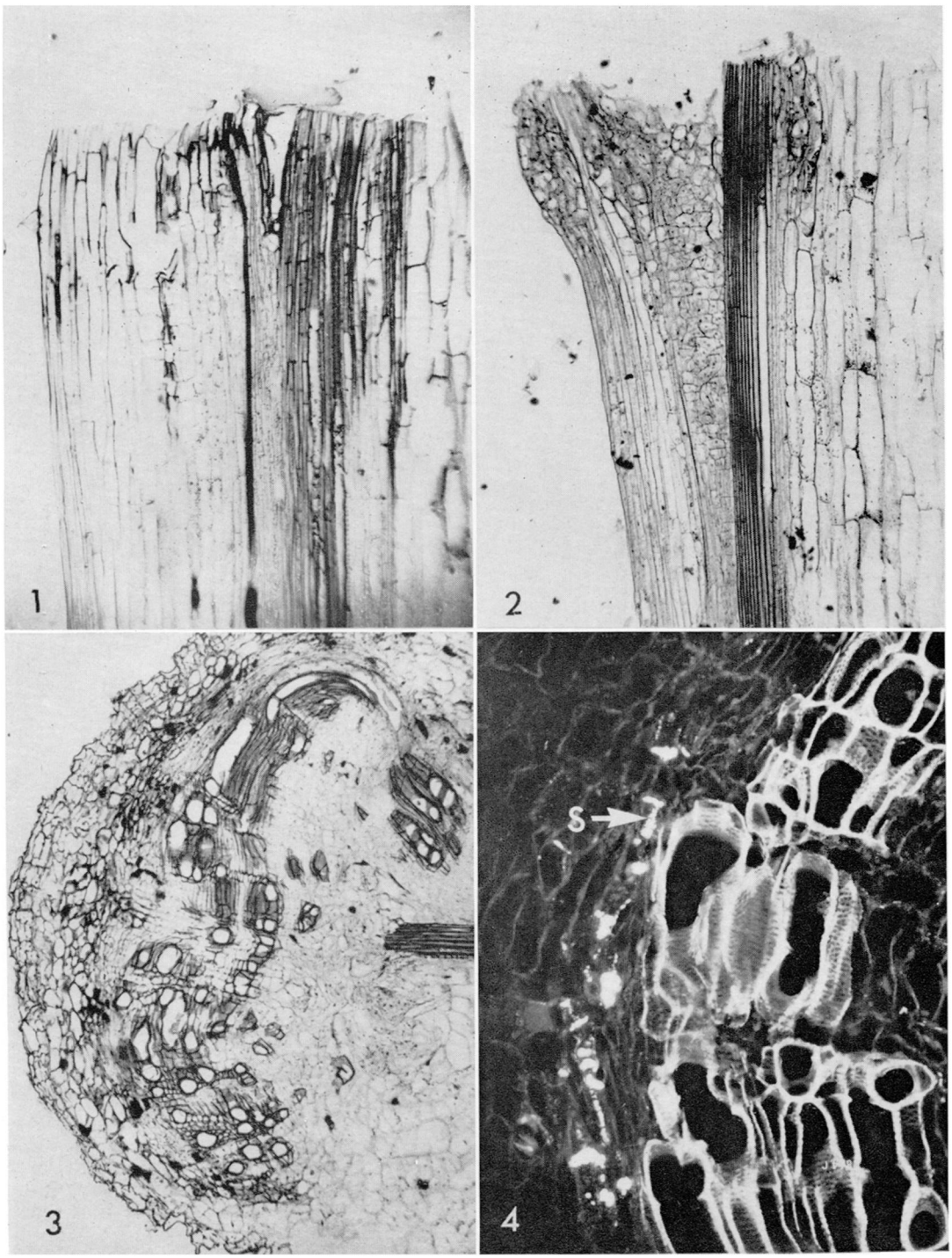
Figs. 5 and 6. Callus growing on two pieces of the same explant which was divided after 2 days in culture and further cultured for 50 days. a, Apical portion of the original explant; b, basal 1 cm portion.
Fig. 5. The original explant was 4 cm long. × 1.35.
Fig. 6. The original explant was 2 cm long. × 1.35.
Fig. 7. Callus growing on the two halves of an internode section which had been bisected after 6 months in culture. Each half was cultured for a further 40 days. a, Apical half; b, basal half. × 1.35.
Fig. 8. Three adjacent internode sections explanted for 1 day and basal 1 cm removed, each was re-explanted: 1, intact; 2, stripped of its outer tissues along half its length; 3, stripped of its outer tissues along its whole length. Cultured for 45 days. × 1.35.
Fig. 9. 'Xylem' explant bisected after 11 days in culture and each portion further cultured for 34 days. No callus growth has developed on the apical part a. × 1.8.
Fig. 10. Callus growing on the basal ends of the apical halves of 'phloem' explants which had been cultured for 3, 5, 9, 13 and 19 days before bisection. Photographed together 35 days after the beginning of the experiment. × 0.9.

PLATE 3

Figs. 12–14. Inner 'xylem' tissues of the stem.
Fig. 12. Transverse section immediately after stripping off the outer tissues. × 482.
Fig. 13. Transverse section after 20 days in culture. A few large wound cells have been produced at the exposed cambial surface. × 150.
Fig. 14. Longitudinal section of the basal end of a xylem explant cultured for 25 days. In the callus which has formed, an arching zone of tracheids can be seen outside which is a cambium. × 30.
Fig. 15. Longitudinal section of the basal end of a 40-day-old internode explant whose apical end had been placed in acid fuchsin for several hours. The dye can be seen in the xylem of the explant, the dynamic wedge and callus. × 30.

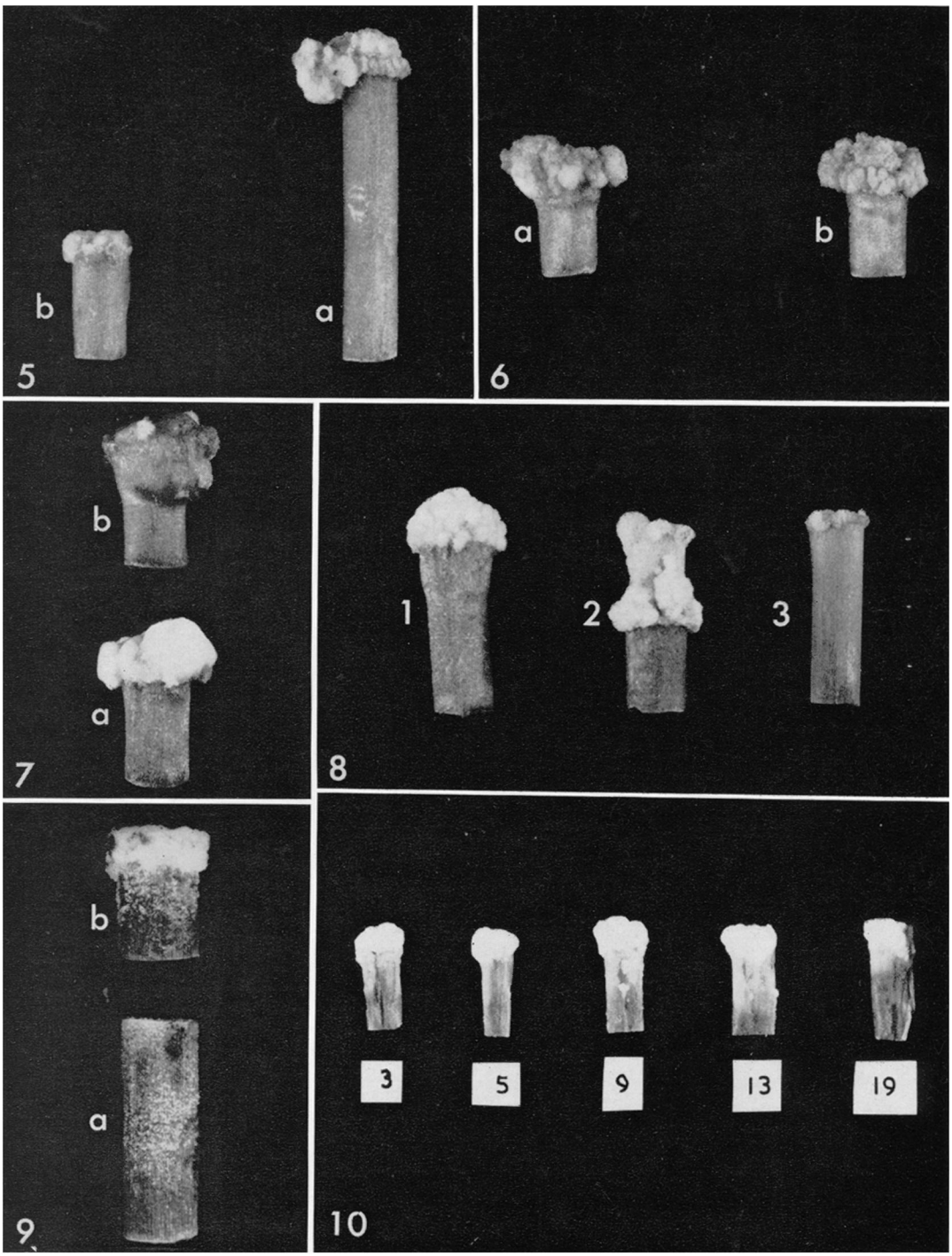
PLATE 4

Figs. 16–17. Tranverse sections of outer 'phloem' tissues of the stem stained with aniline blue and photographed with ultra-voilet light.
Fig. 16. Immediately after stripping. The phloem fibres and callose in the sieve plates of the phloem fluoresce. At the newly exposed cambial surface there are thin walled cambial cells: no xylem cells are present. × 120.
Fig. 17. After 30 days in culture. At the bottom of the picture are the original phloem fibres, above them the original and newly formed phloem, the cambial zone and differentiated xylem cells. × 120.



A. R. SHELDRAKE AND D. H. NORTHCOTE—*AUXIN PRODUCTION IN INTERNODES*

(facing page 12)



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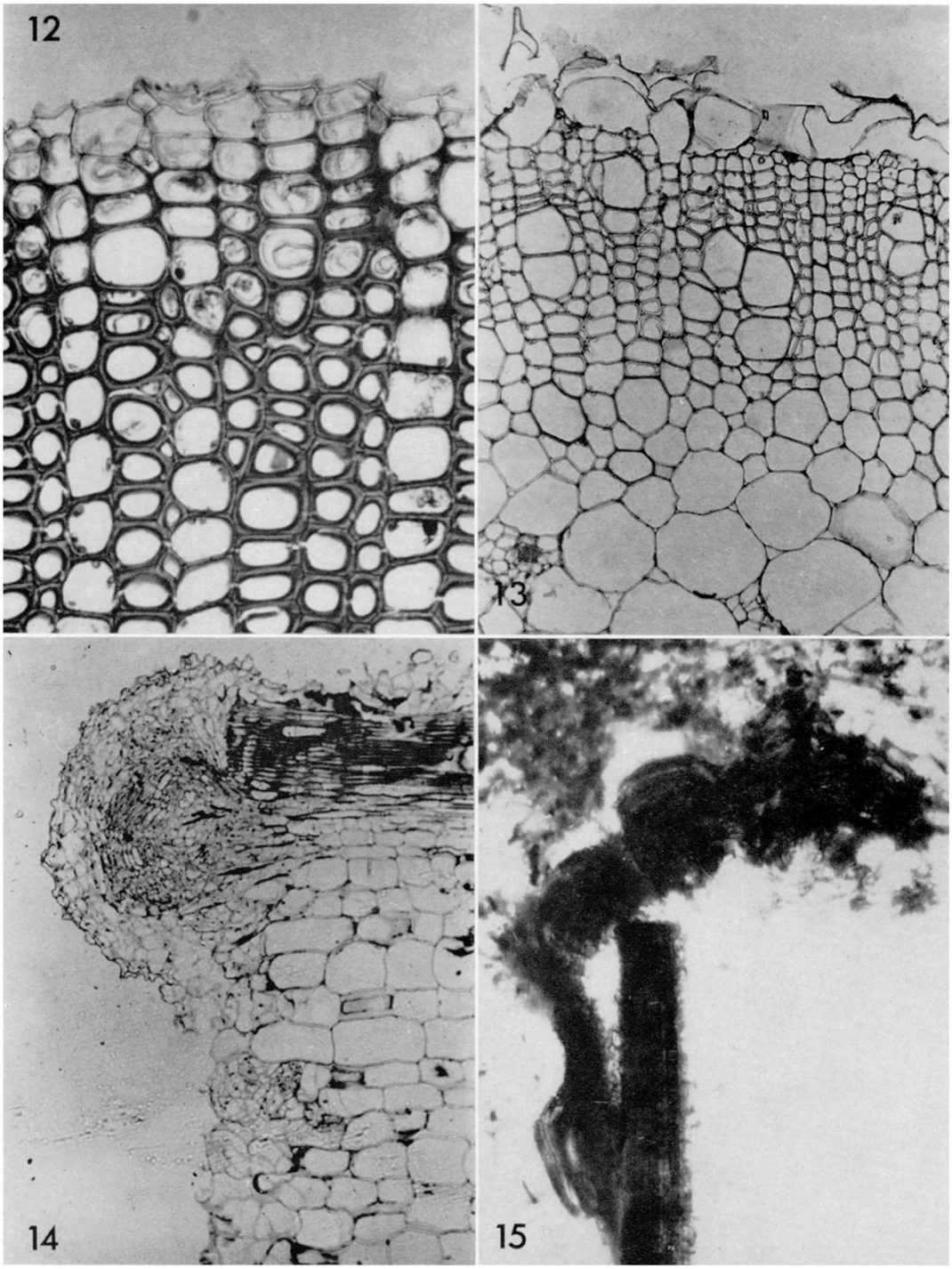

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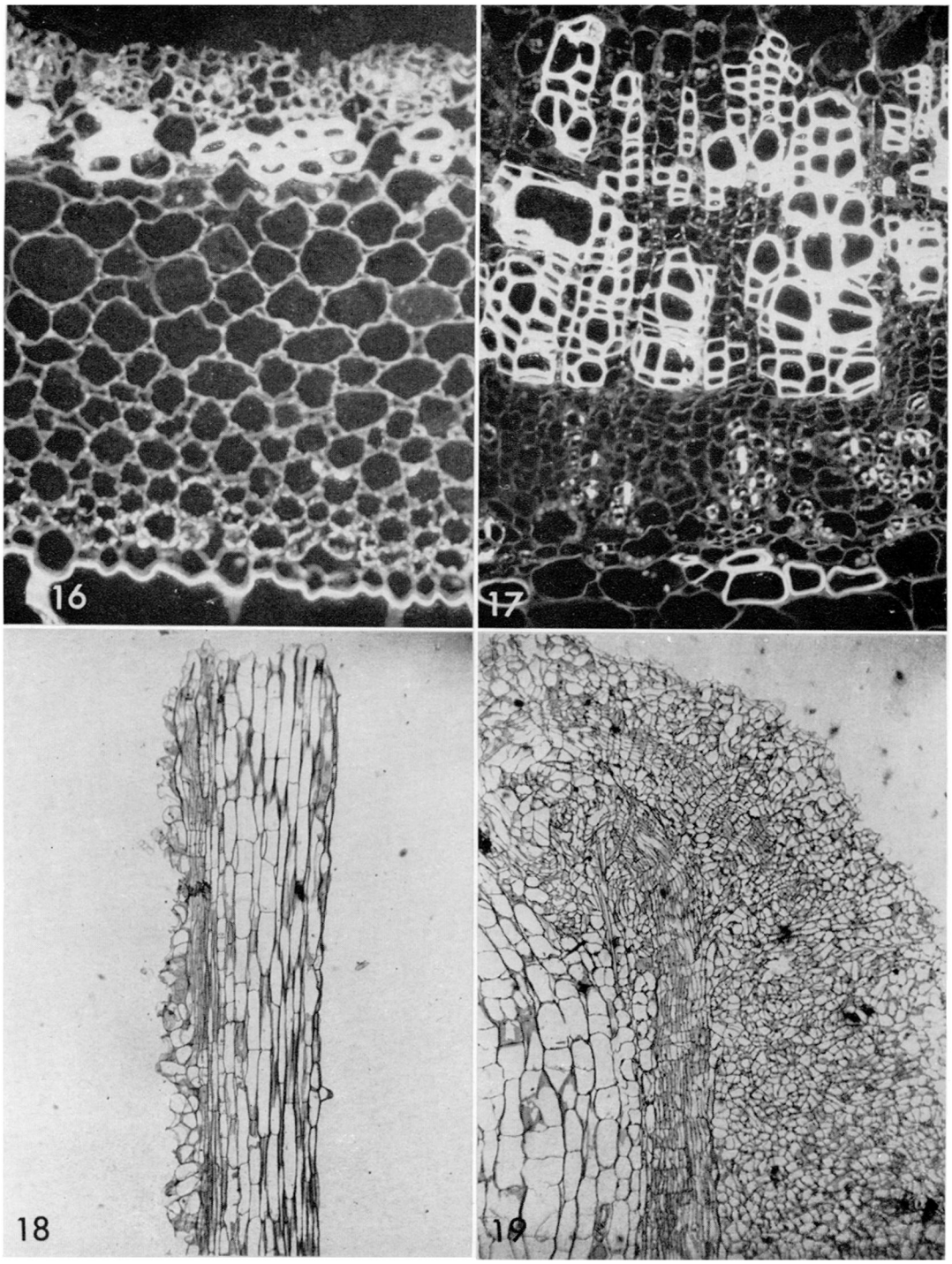

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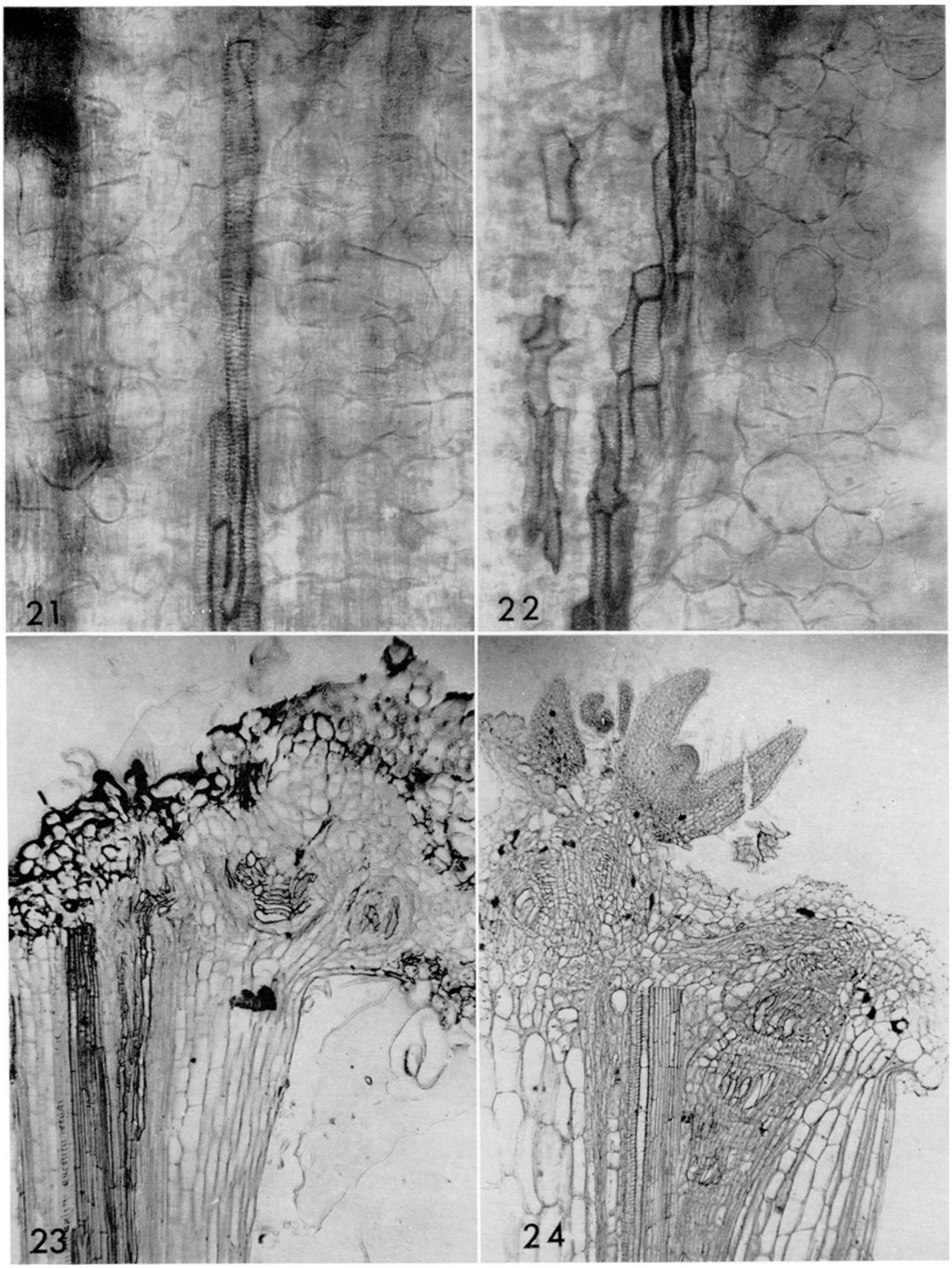

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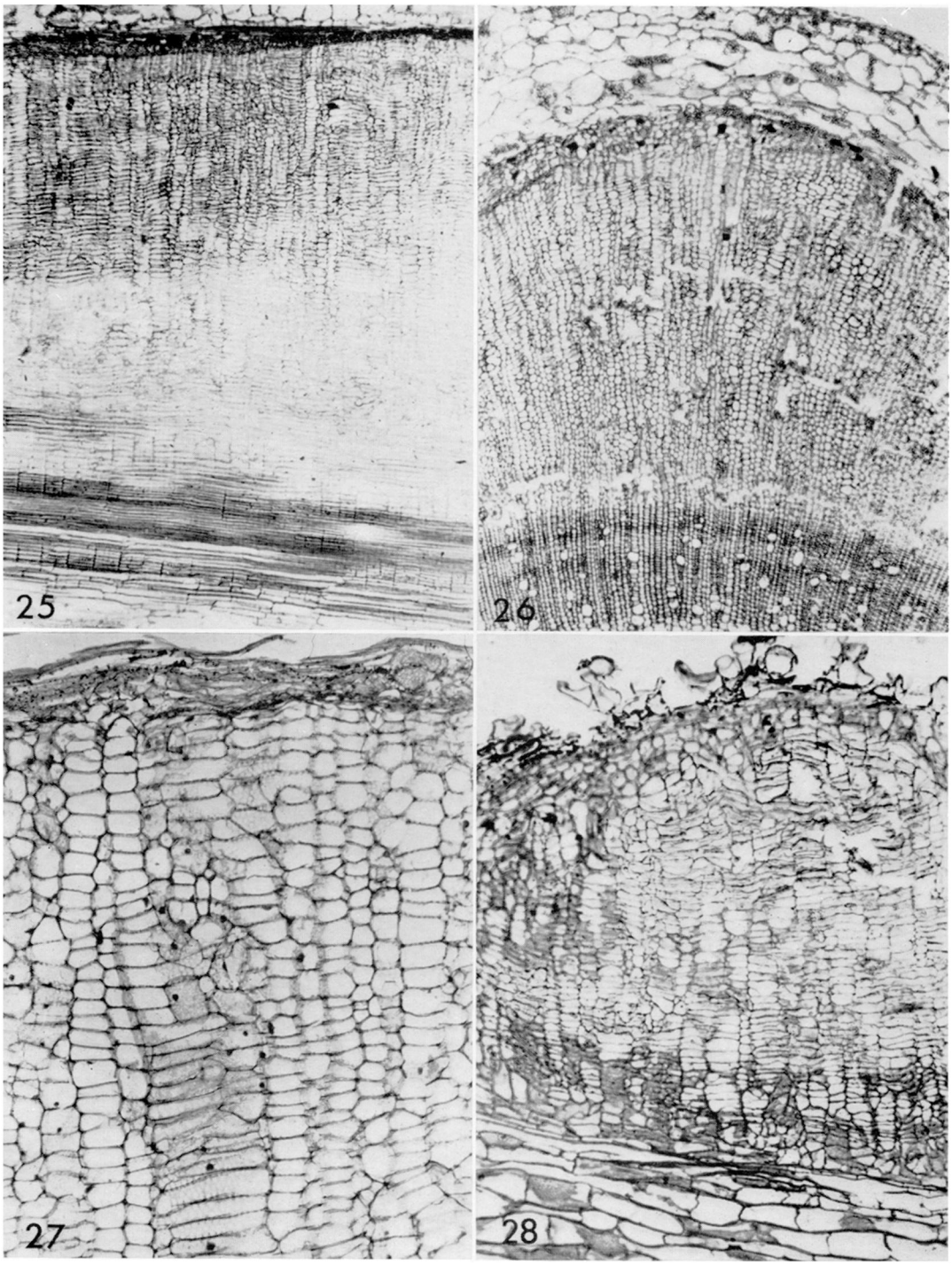

A. R. SHELDRAKE AND D. H. NORTHCOTE—*AUXIN PRODUCTION IN INTERNODES*

Figs. 18–19. Longitudinal sections of the basal ends of cultured phloem explants.
Fig. 18. After 3 days. Enlarged and dividing cells are visible all along the recently exposed cambial surface, on the left. × 30.
Fig. 19. After 30 days. The active cambial zone is in continuity with an arching cambial zone which has formed in the callus. × 30.

PLATE 5

Figs. 21–22. The cambial face of 'phloem' explants stained with phloroglucin HCl.
Fig. 21. After 3 days. The basal end of the explant is just off the top of the picture. Differentiating tracheids have appeared and files of tracheids in longitudinal and lateral continuity are differentiating acropetally. × 120.
Fig. 22. The apical part of a 7-day-old explant. Note the longitudinal files of lignified tracheids. × 120.
Fig. 23. Longitudinal section of the apical end of an internode explant cultured in a medium containing growth substances for 53 days. Note the outgrowth of callus. × 30.
Fig. 24. Longitudinal section of the basal end of an internode explant cultured for 39 days. Shoots have arisen in the superficial part of the callus. × 30.

PLATE 6

Figs. 25–27. Stem explants cultured on medium containing TIBA after they had been cultured on normal medium for several days and the basal end had been removed.
Fig. 25. Longitudinal section of an internode explant cultured for 35 days. The original xylem can be seen at the bottom of the picture, the cortex at the top. The intervening tissue consists of new cambial derivatives many of which are lignified, pitted xylem tracheids. × 30.
Fig. 26. Transverse section of similar tissue to that shown in Fig. 25. × 38.
Fig. 27. Longitudinal section. New cambial derivatives in a tissue similar to that shown in Fig. 25. Most of the cells are lignified pitted tracheids which have lost their cell contents although in a few cytoplasm and nuclei are still visible. × 150.
Fig. 28. Longitudinal section of the basal part of a 'phloem' explant transferred after 3 days in culture to TIBA medium and grown for 35 days. Rows of new cells have appeared, many of which are lignified tracheids. × 49.

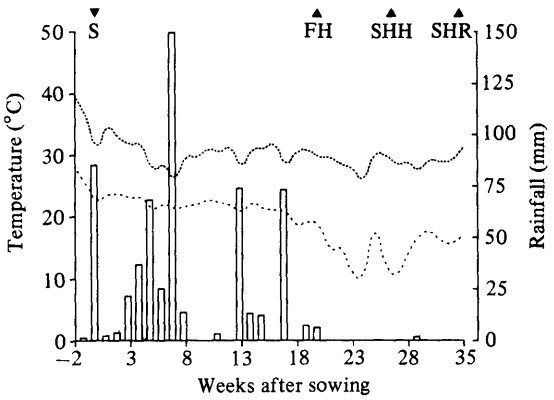

Fig. 1. Meteorological data for the growing season of short-duration pigeonpea genotypes at ICRISAT Center, 1984–5. □, Rainfall (mm); ------, maximum temperature (°C); ···, minimum temperature (°C). Time of sowing (S), first-flush harvest (FH), second-flush harvest in hand picking (SHH), and ratooning (SHR) treatments are also shown.

31 WAS in both genotypes. This delay can be attributed to the fact that the flowers in the ratooning treatments developed on new shoots, whereas on intact plants, flowering began on existing shoots soon after the maturity of the first flush.

The error variances for effect of harvest method on yield of first and second flush, total yield, total dry matter at the second flush maturity, and the yield loss due to pod dropping on both the alfisol and the vertisol were homogeneous, so data for both soils were analysed together. The interaction between soil type and harvest method was not significant for these variables. The mean values for the two soils are therefore presented in Table 1. The first-harvest yield of ICPL 81 was significantly lower than that for ICPL 87. The poor yield of ICPL 81 may be due to its poor emergence, which was 57% on the vertisol and 32% on the alfisol, compared with 80% of ICPL 87 on both soils. Nevertheless, the first-harvest yield of ICPL 81 did not differ significantly between the two soils; this may be due to its plasticity. In an experiment using different plant population densities, seed yield increase of only 5% was observed in ICPL 81 when its density was increased from 16 to 42 plants/m^2 (Chauhan *et al.* 1984). In both genotypes, the first-harvest yield was similar for both ratooning and hand picking. For the second-harvest yield, the interaction between the harvest method and genotype was highly significant. The second-harvest yield of ICPL 87 was significantly lower when harvested by ratooning than by hand picking, whereas for ICPL 81 there was no significant difference between first-flush harvest methods (Table 1). ICPL 87 has a greater leaf area than ICPL 81 at maturity of the first flush (unpublished results) and may have consequently suffered more from the ratooning.

Tayo (1985) reported the opposite results in a study of the effects of ratooning and hand picking on the second-harvest yield of a dwarf pigeonpea variety in the lowland tropical environment of Ibadan, Nigeria. Here, under non-limiting moisture conditions, ratooned plants grew better and yielded more than intact plants from which pods were hand picked. The higher yield of the ratooned plants was attributed to the greater physiological efficiency of leaves on the new shoots as compared with older leaves on intact plants. The fact that in the lowland tropical environment a longer period of regrowth, about 23 weeks, was available for the realization of this vigour compared with 13 weeks at ICRISAT Center, may partly explain the different responses to ratooning in the two environments. The regrowth period at ICRISAT Center, which is in an essentially semi-arid tropical environment, was perhaps insufficient for the compensatory regrowth of the ratooned plants. This was reflected in the lower dry matter of ratooned plants at the second-flush maturity than plants in the hand-picking treatment (Table 1). Whether a longer regrowth period would enable higher yields in ratooned plants than in hand-picked plants in a semi-arid tropical environment is not known. However, it seems important to examine this, particularly since ratooning was much less labour intensive than hand picking. In the present study, the labour requirement (number of man

Table 1. *Mean seed yields in ratooning (R), hand picking (H), and single-harvest (S) treatments of two short-duration pigeonpea genotypes*

	ICPL 81			ICPL 87			
	R	H	S	R	H	S	S.E.
First-flush yield (t/ha)	1·33	1·35	NH	2·19	2·03	NH	0·052
Second-flush yield (t/ha)	0·55	0·67	NH	0·78	1·47	NH	0·050
Total yield (t/ha)	1·87	2·00	2·06	2·97	3·50	3·52	0·078
Total dry matter (t/ha)	2·67	3·25	5·65	3·89	5·28	7·66	0·162
Yield loss (%)	0·40	1·30	4·70	0·90	2·30	7·30	0·430

NH, not harvested separately.

days/ha) for harvesting the first flush by ratooning was 31 for ICPL 81 and 56 for ICPL 87, as compared with a hand picking requirement of 243 for ICPL 81 and 211 for ICPL 87.

In the treatment where harvesting of the first flush of pods was delayed until the second flush of pods had matured, in both genotypes the total yield obtained in the single harvest was similar to the yield of two separate harvests in the hand picking treatment (Table 1). In ICPL 87 it was significantly more than the total yield of the ratooning treatment. This suggests that presence of mature first-flush pods does not affect the formation of pods in the second flush. This harvest method, therefore, had an advantage over hand picking and ratooning, as the yield was not lowered, while there was no labour requirement for a first-flush harvest. Thus, unless one wants to harvest the crop earlier, both flushes may be harvested together. However, in the single-harvest treatment there was a slightly greater yield loss in the form of increased dropping of pods (Table 1). There is also a possibility of rain, diseases, and insects damaging the crop when mature pods are left on the plants.

REFERENCES

CHAUHAN, Y. S., VENKATARATNAM, N. & SHELDRAKE, A. R. (1984). The high yield potential of extra-early pigeonpeas in peninsular India. *International Pigeonpea Newsletter* **3**, 28–31.

SHARMA, D., SAXENA, K. B. & GREEN, J. M. (1978). Potential for ratooning in pigeonpea. *Field Crops Research* **1**, 165–172.

SUAREZ, J. J. & HERREARA, D. (1971). Response of pigeonpea (*Cajanus cajan* Millsp.) at different populations submitted to different heights of cutting. *Revista Cubana de Ciencia Agricola* **5**, 71–75.

TAYO, T. O. (1985). Assessment of the effect of ratooning pigeonpea (*Cajanus cajan* (L.) Millsp.) in the lowland tropics. *Journal of Agricultural Science, Cambridge* **104**, 589–593.

VENKATARATNAM, N. & SHELDRAKE, A. R. (1985). Second harvest yields of medium duration pigeonpea (*Cajanus cajan*) in peninsular India. *Field Crops Research* **10**, 323–332.