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Surgical Experiments on the Differentiation of Vascular Tissue in the Shoot Apex of Carrot (*Daucus carota* L.)

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Surgical techniques were applied to the shoot apex of carrot (*Daucus carota* L.) to test the interpretation that provascular tissue is the initial stage of vascular differentiation and to localize the sources of the influences that control its differentiation. If the apex is isolated laterally by vertical incisions leaving it at the summit of a plug of pith tissue, vascular differentiation proceeds normally and an independent vascular system is formed in the pith plug. If all leaf primordia are systematically suppressed, provascular tissue continues to differentiate as an acropetal extension of the pre-existing vascular system but no further differentiation occurs. When the apex is isolated laterally and all leaf primordia are suppressed, provascular tissue continues to be formed acropetally and is extended basipetally into the pith plug by redifferentiation of pith cells, but no further differentiation occurs. This tissue reacts positively to histochemical tests for esterase indicating its vascular nature. If only one leaf primordium is allowed to develop on an isolated shoot apex, its vascular system develops normally and extends basipetally into the pith plug, but there is no extension of provascular tissue into the pith plug. These results support the interpretation that the initial stage of vascular differentiation is controlled by the apical meristem but that further maturation of vascular tissue depends upon influences from developing leaf primordia.

Key words: Provascular tissue, differentiation, carrot (*Daucus carota* L.), shoot apex, surgical techniques, leaf primordia.

INTRODUCTION

In a previous report (Xia and Steeves, 1999) it was demonstrated that, in the shoot apex of carrot (*Daucus carota*), there is a short cylinder of meristematic tissue beneath the peripheral zone of the apical meristem within which procambial strands related to developing leaf primordia subsequently differentiate. This tissue was designated provascular tissue and was interpreted as the initial stage of vascular differentiation in the shoot. A comparable tissue described in other angiosperm species has more commonly been regarded as a prolongation of undifferentiated meristem and has been referred to as residual meristem (Esau, 1965; Shininger, 1979). However, in addition to the close association of this tissue with procambium differentiation, histochemical evidence showing the presence of carboxylesterases in this tissue, as in procambium and later stages of vascular differentiation, supported the interpretation that it represents the initial differentiation of the vascular system (Xia and Steeves, 1999). This is not simply a question of terminology. Rather it has an important bearing on the understanding of the respective roles of the apical meristem and the leaf primordia that it initiates in establishing the pattern of the shoot vascular system. If the tissue in question is no more than a residuum of the apical meristem within which the leaf primordia initiate the vascular system through the differentiation of procambium, then the role of the apical meristem is at most to restrain differentiation in the region that is to become vascular. If, on the other hand, the presumptive provascular tissue represents the initial stage of vascular differentiation, the role of the apical meristem is to define the basic pattern of the system which is later acted upon by the leaf primordia. The critical issue is when, and under what influence, vascular differentiation is initiated in the shoot apex. Establishing the beginning of tissue differentiation in the shoot apex is a matter of importance in view of current molecular genetic studies in which patterns of gene expression in that region are being identified (Medford, 1992; Evans and Barton, 1997). While these patterns may simply be a reflection of the functional organization of the apex, they could also represent the first stages of the differentiation process. For example, certain of the patterns of gene expression described by Fleming *et al.* (1993) resemble the distribution of provascular tissue and the recognition of the expression of a particular gene in presumptive leaf sites has also been found (Reinhardt *et al.*, 1998).

In view of the equivocal status of the descriptive evidence relating to initial vascular differentiation, experimental analysis is required to decide among the conflicting interpretations (Xia and Steeves, 1999). There have been many experimental investigations of the shoot apex by surgical techniques but few of these have centred on the initial differentiation of vascular tissues. Studies on several ferns have resulted in a reasonably clear understanding of the respective roles of the apical meristem and leaf primordia in establishing the shoot vascular system in this group. Suppression of leaf primordia as or just before they

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emerged (Wardlaw, 1944; Soe, 1959), an operation that could be combined with the prior isolation of the meristem on a column of pith tissue by vertical incisions (Ma and Steeves, 1992), resulted in the complete differentiation of a mature vascular system but one which lacked the characteristic leaf gaps of the fern stele. These studies, coupled with histological analysis, have demonstrated a provascular stage of differentiation under the sole influence of the apical meristem which is subsequently modified by the developing leaf primordia but which can proceed to complete maturity under the control of the apical meristem alone (Ma and Steeves, 1994, 1995*a*, *b*). Furthermore, it was shown that at least part of the leaf influence can be replaced by exogenously applied auxin (Ma and Steeves, 1992).

Surgical studies conducted on a few angiosperm species have yielded a less clear picture but one that suggests significant differences from the ferns. In lupin, Ball (1952) isolated the apical meristem on a pith column by vertical incisions. Although the partially isolated meristem gave rise to a shoot with a nearly normal vascular system, in the operated shoot apex before normal leaf initiation was restored a distinct ring of apparent provascular tissue (designated procambium by the author) was observed. This was in contrast to the normal apex in which no such ring was clearly visible. A similar provascular ring was observed prior to leaf initiation in lateral regenerating shoot apices of lupin after excision of the central portion of the original apical meristem (Ball, 1950). Also in lupin, Young (1954) showed that defoliation of the shoot apex and continued suppression of leaf primordia resulted in the formation of a cylinder of meristematic tissue equivalent to provascular tissue that underwent no further differentiation. This tissue cylinder persisted in the axis to a level at which mature vascular tissue would be expected in a normal shoot. On the other hand, Wardlaw (1950), working with *Primula polyantha*, found that suppression of leaf primordia, with or without prior isolation of the apical meristem by vertical incisions, led to the differentiation of an uninterrupted ring of mature vascular tissue in the axis formed during the treatment as in the ferns. In this study, however, after a number of leaf primordia had been suppressed several were allowed to develop.

These studies were extended by McArthur and Steeves (1972) using *Geum chiloense*. Here the continuous suppression of leaf primordia following lateral isolation of the apical meristem by vertical incisions resulted in the persistence of a provascular cylinder and its apparent extension into the supporting pith column by redifferentiation of parenchyma cells. Significantly, no further differentiation of this tissue occurred unless one or several leaf primordia were allowed to develop. In the latter case strands of procambium continuous with leaf traces differentiated from the provascular tissue and these ultimately matured as bundles of xylem and phloem. In the absence of leaf primordia, exogenous application of auxin to the apex, supplemented by a source of carbohydrate as a 2 % solution of sucrose, resulted in differentiation of a ring of procambium from the provascular tissue, but no further progression to mature vascular tissue occurred.

The summation of these experimental studies strongly suggests that in angiosperms, as in the ferns, the apical meristem gives rise to the initial pattern of the vascular system as provascular tissue but that, unlike the ferns, further differentiation requires the influence of developing leaf primordia. However, since these results are not entirely convincing and have not been verified by any subsequent analyses, it seemed appropriate to re-examine the question systematically. The present report describes a study in which the surgical operations of meristem isolation and leaf primordium suppression were combined in various ways to explore the respective roles of the apical meristem and its derivative leaf primordia in establishing the pattern of the vascular system.

Carrot (*Daucus carota*) was chosen for the study because histological examination revealed a distinct provascular stage in normal differentiation (Xia and Steeves, 1999) and because of the suitability of this species for experimental manipulation. The ability to carry out surgical experiments on plants with an intact root system containing a large supply of stored nutrients offers a distinct advantage. Furthermore, the very limited elongation of the subapical region in normal development reduces the complicating factor of restricted elongation when shoots with normally extended internodes are defoliated at the primordial stage. A subsequent report will explore the role of auxin in the influence exerted by the leaf primordia.

MATERIALS AND METHODS

Carrot (*Daucus carota* L. *sativa* DC. 'Little Finger') plants with roots 15–20 mm in diameter grown in an experimental garden in Saskatoon (Xia and Steeves, 1999) were used in the surgical experiments. All mature leaves were removed and the plants were sterilized for 10 min in a 1–2 % solution of Javex (5·25 % w/w solution of sodium hypochlorite, distributed by Colgate-Palmolive Canada Inc.). After washing, they were planted in sand in clean pots and placed in a growth chamber with a photoperiod of 16 h light and 8 h dark under fluorescent and incandescent light at 215–305 mol m^{-2} s^{-1}. Temperature was controlled at 25°C in the light and 22°C in the dark.

The surgical methods used in the present study followed those of McArthur and Steeves (1972). Operations and observations were carried out under a Wild M5 stereomicroscope illuminated by a cool light lamp (Fibre Optic Illuminator, Cambridge Instruments) in a Class II laminar flow cabinet to reduce contamination. Fine knives were prepared from pieces of Gillette blades cut with garden shears. Fragments having an edge 0·15–0·3 mm in width were carefully polished and thinned, held in a vice grip holder and sterilized in 70 % alcohol for 5 min before use. The shoot apex was exposed by careful removal of the overlying young leaves with a dissecting needle. After excision of the young leaves the plants with the two or three youngest primordia intact were returned to the growth chamber for 1 to 2 d to allow recovery from wounding before beginning experiments. The remaining leaf primordia were then removed.

Two surgical techniques were used individually or in combination. Isolation of the shoot apex from the original vascular system was accomplished by making four vertical incisions to a depth of about 200 μm just inside the last primordial positions (Fig. 1). This left the apex supported by a plug of differentiating and mature pith so that nutrients would still be supplied to it. The second operation consisted of puncturing successive leaf primordia as or just before they appeared on the apical dome. The position of the next primordium to emerge could be estimated by projecting the phyllotactic helix approx. 137° beyond the last formed primordium or the position of the last suppressed primordium (Xia and Steeves, 1999). After each operation the shoot apex was covered with a small cap of wet filter paper to retain moisture around the treated apex. The pot containing an experimental plant was covered with two sheets of Kleenex tissue or paper towelling and a sheet of clear plastic held in place by a rubber band. After manipulations the treated plants were returned to the growth chamber under the same conditions in which they had been grown. Following a series of preliminary trials, four experimental treatments were carried out as follows: (1) the shoot apex was isolated and newly formed leaf primordia were allowed to develop on the isolated apex; (2) all leaf primordia were removed and successive incipient primordia were punctured just before or as they emerged; (3) the shoot apex was isolated and all successive primordia on the isolated apex were punctured; and (4) the shoot apex was isolated and all successive primordia on the isolated apex were punctured except for the second (I_2) which was allowed to develop. A previous study (Xia and Steeves, 1999) had shown that in normal plants four plastochrons (12 d) are required for the first appearance of mature vascular tissue, thus the experiments were continued for a minimum of 2 weeks or approx. five plastochrons to ensure sufficient time for final maturation to occur. Twelve plants were used in each treatment and each experiment was repeated once. Because the survival rate in treatments with complete suppression of leaf primordia was low, these experiments were supplemented by additional operated plants. In total, 126 plants were treated of which 65 survived to the end of the experimental period. The major reason for the loss of experimental plants was contamination by fungi and bacteria which developed in spite of initial sterilization of the plants.

Shoot apices were harvested at the end of experiments and prepared for histological examination following the methods described by Xia and Steeves (1999). Each shoot apex was excised on a tissue plug approx. 4 mm long on each side and 5 mm deep. In some cases shoot apices were fixed at intermediate stages of an experiment in order to trace the timing of developmental events. At the end of the experimental period several shoot apices from treatment 3 only were collected and frozen directly in a cryostat for histochemical identification of esterases. The procedures followed were those previously described (Xia and Steeves, 1999).

To assess the impact of surgical treatments on the shoot apex, measurements were made of the vertical growth of the shoot during the experimental period and of the size of the apical meristem at its conclusion. Vertical growth of the shoot where isolation had been carried out (treatments 1, 3 and 4) was measured in longitudinal sections as the distance from the apical summit to the lowest level of the epidermis because the isolated pith plug did not develop an epidermis. In treatment 2 where primordia had been punctured only, the distance from the apical summit to the axil of the last pre-operation primordium (P_1) was measured. To provide a basis for comparison with normal apices, the distance from the apical summit to the axil of the sixth primordium (P_6) in a random sample without regard to plastochron stage was used as an indication of vertical growth during five plastochrons. The height of the apical meristem was taken as the distance from the summit to the axil of the last formed primordium or punctured primordial position and the diameter of the meristem was measured at this level. Student's *t*-tests were used to assess the significance of differences between experimental and normal apices.

RESULTS

Isolation of the shoot apex

By 1 or 2 d after isolation the four vertical incisions surrounding the isolated shoot apex had gaped noticeably. Although this operation separated the apex from mature vascular tissue and procambium, some provascular tissue was included because of its close proximity to the terminal meristem (Xia and Steeves, 1999). In this treatment, because no leaf primordia were punctured, the development of primordia during the experimental period could be traced easily. In the 2-week experimental period, five primordia were formed. The average plastochron length of 2·8 d was thus slightly shorter than normal (3 d; Xia and Steeves, 1999). Vertical growth of the isolated shoot apex was not significantly affected by the operation (Table 1) nor were the vertical and horizontal dimensions of the apical meristem (Table 2).

The general organization of the isolated shoot apex (Fig. 2) closely resembled that found in untreated plants (Xia and Steeves, 1999). The cells of the apical dome were arranged in three or four parallel layers, and in transverse section a darker staining peripheral zone surrounded a less intensely stained central zone. The first appearance of a provascular ring resulting from the vacuolation of cortex and pith was observed at the 50–60 μm level from the apical summit (Fig. 3). This differentiation occurred as in the normal plant above the divergence of the trace of the last formed leaf primordium. The differentiation of procambial traces in relation to the emerging leaf primordia followed the normal pattern.

The most distinct reaction in the pith plug was the formation of an outer sheath by the periclinal division of cells parallel to the cut surfaces. Inside the outer sheath, pith cells redifferentiated to form a basipetal extension of the vascular system (Figs 2 and 4). Isolation appeared to accelerate vascular maturation. Mature xylem and phloem were regularly detected in the trace of the second leaf primordium in contrast to the third or fourth in normal plants. Final maturation occurred acropetally in the new

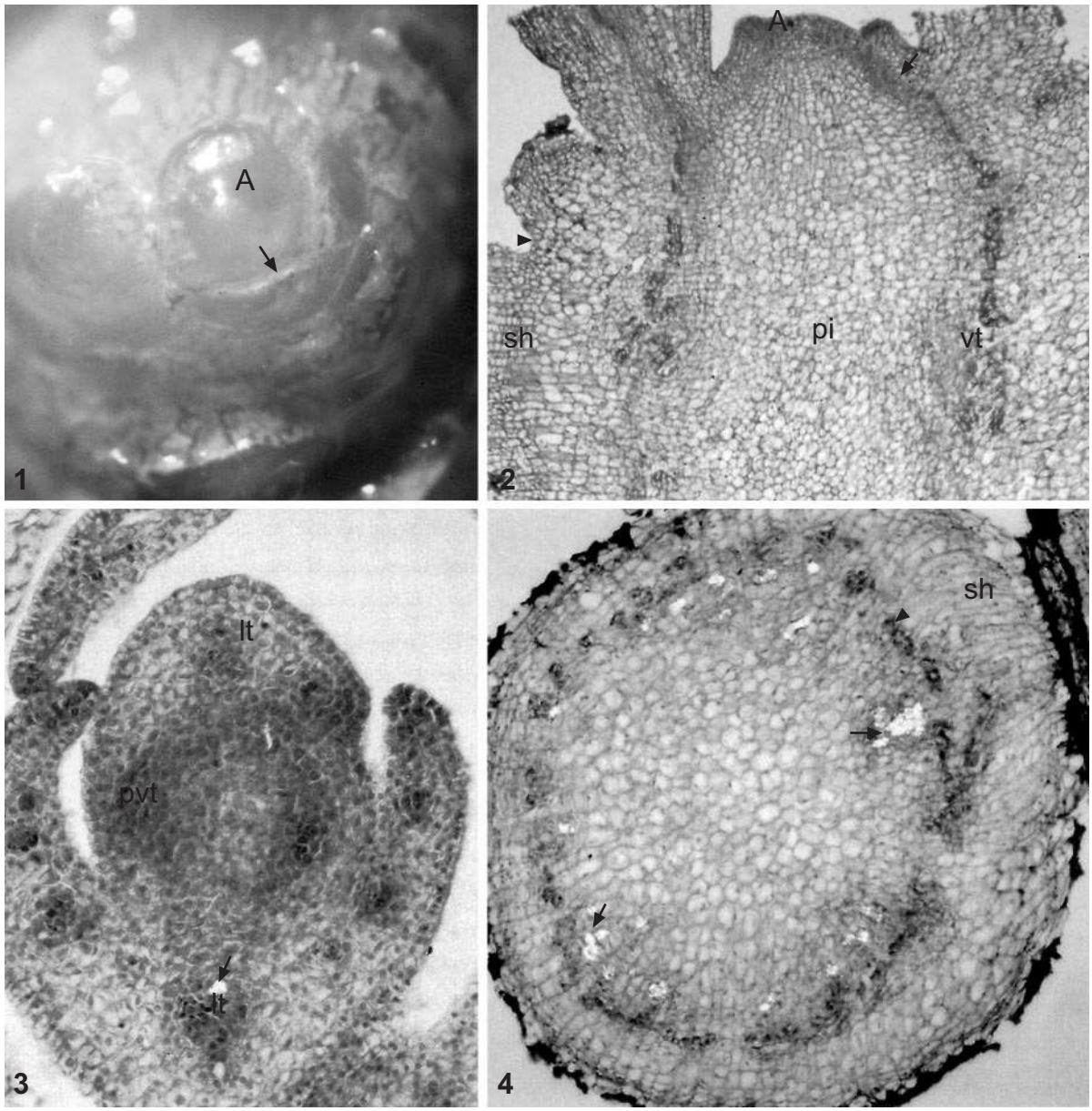


FIG. 1. Top view of an exposed shoot apex illustrating the isolation of the apical meristem by vertical incisions. A, Apical dome; arrow, incision. ×76.

FIG. 2. Longitudinal section of an isolated shoot apex 2 weeks after the operation, showing a newly formed vascular system extended in the pith plug. A, Apical meristem; arrow, provascular tissue; vt, vascular tissue; pi, pith; sh, outer sheath; arrowhead, base of new growth. ×106.

FIG. 3. Transverse section 50 μm from the summit of an isolated shoot apex 2 weeks after the operation, photographed in partially polarized light, showing a distinct provascular ring. pvt, Provascular tissue; lt, leaf trace; arrow, mature xylem. ×270.

FIG. 4. Transverse section of the pith plug 390 μm below the summit of an isolated shoot apex 2 weeks after the operation, showing a differentiating vascular system. Photographed in partially polarized light. Arrows, Mature xylem; arrowheads, phloem; sh, outer sheath. ×120.

growth formed by the isolated apex and extended basipetally into the pith plug. Near the base of the pith plug there was xylem only and the shape and size of these tracheary elements indicated that they had been converted directly from parenchyma cells rather than differentiating from procambium. Although the vascular system in the pith plug was essentially independent of the original vascular system, the converted xylem elements at the base in some cases established a connection between the two systems.

Suppression of leaf primordia

Because incipient leaf primordia were suppressed at or prior to emergence, the plastochron length could only be

TABLE 1. *Vertical growth of shoot apices in different treatments and controls*

Treatment	Vertical growth (μm)
1. Isolation only	506 ± 96 (5)
2. Complete puncturing	195* ± 29 (6)
3. Isolation followed by complete puncturing	177* ± 14 (9)
4. Isolation with only I_2 allowed to develop	193* ± 12 (5)
5. Controls	426 ± 67 (13)

All values are means ± s.d.; sample sizes used in the experiments are shown in parentheses.
* Significantly different from control value at $P \leqslant 0{\cdot}05$

TABLE 2. *Dimensions of the apical meristem of shoot apices from surgical treatments and controls*

	Apical meristem	
Treatment	Height (μm)	Diameter (μm)
1. Isolation only	37 ± 5 (5)	133 ± 22 (5)
2. Complete puncturing	33 ± 6 (6)	133 ± 49 (6)
3. Isolation followed by complete puncturing	38 ± 10 (9)	145 ± 42 (9)
4. Isolation with only I_2 allowed to develop	36 ± 3 (5)	117 ± 22 (5)
5. Controls	35 ± 5 (13)	150 ± 50 (13)

All values are means ± s.d.; sample sizes used in the experiments are shown in parentheses. All treatment means are not significantly different from control value ($P \geqslant 0{\cdot}05$)

estimated. As in the case of the isolation experiments, it appeared to be slightly shorter than in normal plants. This was verified by those instances in which a primordium had just begun to emerge at the time of puncturing. Following puncturing of a primordial position there was usually a swelling of the remaining tissue indicating where the primordium would have formed. Initially the formation of leaf primordia was not interrupted by the operations but only rarely were more than four initiated during the experimental period. In contrast to the isolation treatment, vertical growth of the operated apices was substantially reduced in comparison to normal plants (Table 1). The organization of the apical meristem appeared relatively normal (Fig. 5) in spite of the extensive wounding caused by the operations, and its dimensions were only slightly reduced (Table 2).

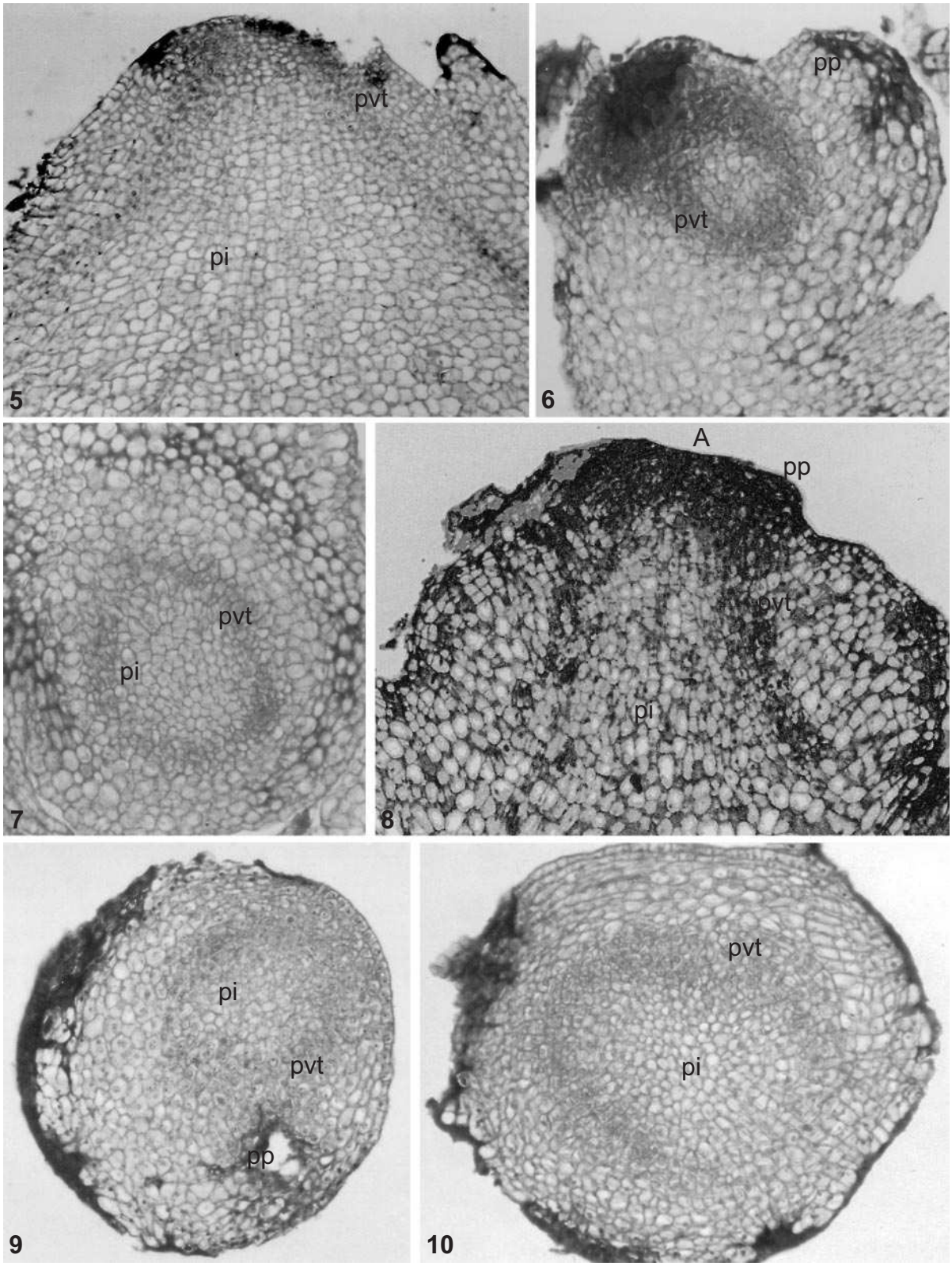
Removal of leaf primordia had little effect on the formation of provascular tissue other than to enhance its distinctiveness. Provascular tissue first appeared as a ring at the 50 μm level from the summit of the apex and was clearly evident at the 70 to 80 μm level (Fig. 6). In the absence of leaf primordia, no axial procambial bundles interrupted the ring which could be traced down from the apical summit to a level at which mature phloem and xylem elements would be expected in a normal plant (Fig. 7). In the few instances in which a primordium had been incompletely suppressed, procambium and even mature vascular tissue was sometimes observed in the swollen leaf base. Otherwise no procambium or mature vascular tissue was found in the new growth above the last pre-existing primordium. Because the shoot apices were not isolated by vertical incisions provascular tissue was continuous with the original vascular system below.

Isolation of the shoot apex and suppression of all leaf primordia

As in the treatment in which leaf primordia were suppressed without isolation of the apex, there was no initial impact on the formation of primordia. Only rarely, however, was there evidence for the initiation of more than four primordia during the 2-week experimental period. This limitation is similar to that observed in the suppression only treatment. In comparison with normal shoot apices or those isolated without leaf suppression, vertical growth was distinctly reduced (Table 1). The reduction in vertical growth agrees with the result of leaf suppression only and suggests that it is a response to the absence of leaf primordia rather a consequence of isolation. The organization of the apical meristem was essentially normal (Fig. 8) and its dimensions were not adversely affected by the combined operations (Table 2).

In the new growth a ring of provascular tissue appeared at the level of 50 to 70 μm from the apical summit. In the absence of leaf primordia the provascular tissue underwent no further differentiation but persisted throughout the newly formed segment (Fig. 9). As in the isolation only treatment, the pith plug developed an outer sheath of periclinally arranged cells. Within this sheath provascular tissue was formed by the redifferentiation of pith tissue so that the provascular cylinder was extended basipetally from the new growth into the plug (Figs 8 and 10). This extension did not establish a connection with the pre-existing vascular system and remained suspended in the pith plug. Nowhere in the provascular cylinder was there any evidence of further differentiation to the procambial or mature vascular tissue stages.

The identification of provascular tissue in the new growth formed by the isolated apex in the absence of leaf primordia and extending through redifferentiation into the pith plug was based on the size, shape and cytological characteristics of the cells. In order to provide substantiating evidence for this interpretation, a histochemical test for the presence of esterases was carried out on fresh frozen longitudinal sections of several treated plants at the end of the experimental period. In contrast to the weak general colouration of other tissues, an intense positive reaction was observed in the putative provascular tissue extending from near the peripheral zone of the meristem to the base of the suspended cylinder (Fig. 14). Although a lack of material prevented extensive inhibitor tests, comparison with results obtained with normal plants (Xia and Steeves, 1999) supports the conclusion that this reaction is due to carboxylesterases, a recognized indicator of vascular differentiation (Gahan, 1981).



FIGS 5–10. For legends see facing page.

Isolation of the shoot apex with one leaf primordium

This treatment was similar to that just described except that the second primordium to be initiated after the operation (I_2) was allowed to develop normally. As in the complete suppression experiments, only rarely were more than four primordia initiated. Vertical growth during the experimental period was reduced in comparison with normal plants or those in which the apex was simply isolated, and was equivalent to that achieved when all primordia were suppressed (Table 1). The organization of the apical meristem at the end of the experiment was essentially normal (Fig. 11) but its diameter was somewhat reduced possibly because of encroachment by the single vigorously developing leaf primordium (Table 2).

The provascular ring first appeared at the 50 μm level, and at the 90 μm level could be observed adjacent to the traces of the unpunctured leaf primordium (Fig. 12). It became less distinct at lower levels and was not prolonged basipetally into the pith plug as was the case when all primordia were suppressed. In contrast, procambium of the median and two lateral traces of the developing leaf primordium differentiated normally and extended basipetally into the pith plug inside the outer sheath of periclinally arranged cells (Fig. 13). Mature xylem and phloem differentiated in these traces and extended basipetally through the pith plug. At the base of the pith plug only xylem was present, apparently redifferentiated from parenchyma cells, and these elements in some cases established a connection with the pre-existing vascular system.

DISCUSSION

In the present study surgical operations on the shoot apex of carrot have been used to extend histological observations on the initial stage of vascular differentiation and to localize the source(s) of the influences that control its differentiation. Two basic operations have been applied separately and in combination. Isolation of the apical meristem by vertical incisions on a plug of maturing pith tissue is expected to eliminate the influence of the subjacent vascular system while allowing a sufficient supply of nutrients and water to reach the meristem (Wardlaw, 1950; Ball, 1952; McArthur and Steeves, 1972). The suppression of leaf primordia, preferably at the incipient stage, on the otherwise intact apex should remove all, or at least most, of the leaf influence on the differentiation process (Wardlaw, 1950; McArthur and Steeves, 1972). When the two operations are combined, the apical meristem, supplied only with basic essentials, functions with a high degree of independence from both the subjacent vascular system and developing leaf primordia. Finally, the development of a single leaf primordium on an apex subjected to the combined operations facilitates a more precise analysis of leaf influence on the differentiation process.

In any study in which drastic experimental techniques are employed to target a particular process it is important to exercise caution in the interpretation of results. There is always the possibility that indirect consequences of the procedure may obscure its direct effects upon the targeted process. In this surgical investigation the impact of wounding coupled with the overall effect of an altered supply of nutrients and water must be considered when evaluating the results of isolation and organ removal. Since the survival rate of experimental plants for the duration of the experimental period was only approx. 50 %, it is imperative to examine the status of those that did persist and upon which the interpretations are based.

Histological examination at the termination of the experiments indicated that in all treatments the basic construction of the apical meristem had been retained in spite of some distortion resulting from wounding and the development of scar tissue. Likewise the dimensions of the meristem, although highly variable, remained within the range of variation of the normal apex (Xia and Steeves, 1999). When the shoot apex only was isolated, the vertical extent of new growth was essentially equivalent to that in a normal plant indicating that the meristem was adequately supplied. However, in all treatments in which leaf primordia were suppressed there was a substantial reduction in vertical growth. Moreover, it appeared that in the absence of leaf primordia the capacity of the meristem for continued growth was restricted and only rarely were more than four new primordia initiated. A similar loss of vigour was noted by Wardlaw (1950) and McArthur and Steeves (1972). This adverse effect of leaf suppression could be the result of the repeated infliction of damage on the apical meristem. On the other hand, in studies of excised shoot apices of several angiosperms in sterile culture, it has been found that successful development depends upon the inclusion of several leaf primordia

FIG. 5. Longitudinal section of a shoot apex on which all leaf primordia were suppressed for 2 weeks showing the continued development of provascular tissue. pvt, Provascular tissue; pi, pith. ×190.

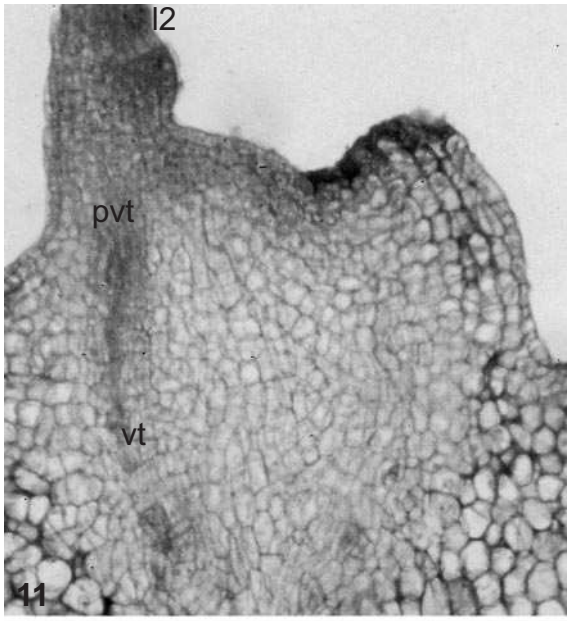
FIG. 6. Transverse section 70 μm from the summit of a shoot apex on which all leaf primordia were suppressed for 2 weeks, showing a distinct provascular ring. pvt, Provascular tissue; pp, punctured leaf primordium. ×255.

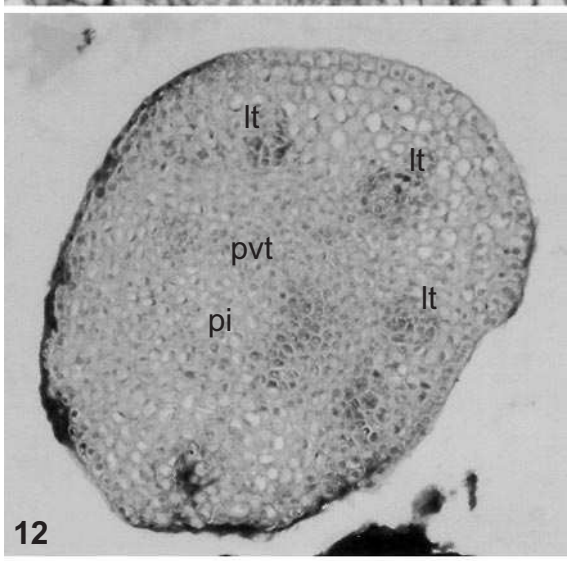
FIG. 7. Transverse section 140 μm from the summit of a shoot apex on which all leaf primordia were suppressed for 2 weeks, showing a continuous provascular ring. pvt, Provascular tissue; pi, pith. ×224.

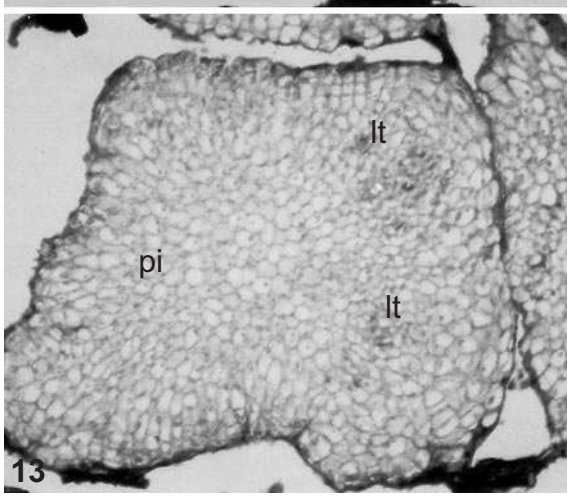
FIG. 8. Longitudinal section of an isolated shoot apex on which all leaf primordia were suppressed for 2 weeks showing the continued development of provascular tissue. A, Apical meristem; pp, punctured leaf primordium; pvt, provascular tissue; pi, pith. ×164.

FIG. 9. Transverse section 120 μm from the summit of an isolated shoot apex on which all leaf primordia were suppressed for 2 weeks showing a distinct provascular ring. pp, Punctured leaf primordium; pvt, provascular tissue; pi, pith. ×200.

FIG. 10. Transverse section 200 μm from the summit of an isolated shoot apex on which all leaf primordia were suppressed for 2 weeks showing a ring of provascular tissue in the pith plug, pvt, Provascular tissue; pi, pith. ×208.







in the explant unless hormonal supplements are included in the medium (Ball, 1960; Smith and Murashige, 1970). This suggests a significant interaction of the apical meristem with its most recent derivative organs (Shabde and Murashige, 1977). Thus, although the development of the experimental plants was certainly not normal, it does not appear that there was a drastic disruption of development at least during the experimental period. Even the possible impact of reduced vertical growth on the differentiation process was minimized by the fact that in normal development this growth is very limited.

The observations contained in this report lend strong support to the recognition of an initial stage of vascular differentiation preceding procambium and formed under the exclusive control of the apical meristem. This initial or provascular stage was identified both histologically and histochemically in the normal shoot apex of carrot (Xia and Steeves, 1999) but the early differentiation of procambium in relation to developing leaf primordia made it difficult to establish with certainty that this tissue was not merely a residuum of the apical meristem, a residual meristem, within which the procambium represented the first stage of vascular differentiation (Esau, 1965). The removal of leaf influence by the systematic suppression of primordia in the present study demonstrated that the apical meristem continued to produce provascular tissue as an uninterrupted cylinder which extended basipetally to a level at which, in a normal shoot apex, not only procambium but also the first appearance of mature vascular tissue would be expected. Thus the production of a provascular cylinder that effectively blocks out the future vascular system is shown to be a function of the apical meristem independent of leaf influence. This result confirms earlier observations on *Primula* (Wardlaw, 1950), *Lupinus* (Young, 1954) and *Geum* (McArthur and Steeves, 1972).

Of even greater significance with regard to the question of the role of the apical meristem in vascular differentiation was the result of combining leaf primordium suppression with the isolation of the apical meristem. In this case the apical meristem, separated both from the influence of developing leaf primordia and from that of the subjacent vascular system, gave rise to a distinctive cylinder of provascular tissue. Moreover, provascular tissue differentiation was extended basipetally into the supporting plug of pith tissue through the redifferentiation of tissues formed prior to the operation, confirming the earlier

FIG. 11. Longitudinal section of an isolated shoot apex on which all leaf primordia except one (I_2) were suppressed for 2 weeks showing provascular tissue and the developing vascular system of I_2. I_2, Leaf primordium; pvt, provascular tissue; vt, vascular traces of leaf primordium. ×264.

FIG. 12. Transverse section 90 μm from the summit of an isolated shoot apex on which all leaf primordia except one (I_2) were suppressed for 2 weeks, showing a provascular ring and leaf traces of I_2. pvt, Provascular tissue; lt, leaf traces; pi, pith. ×200.

FIG. 13. Transverse section in the pith plug 200 μm from the summit of an isolated section apex on which all leaf primordia except one (I_2) were suppressed for 2 weeks, showing the traces of I_2 and the absence of provascular tissue. pi, Pith; lt, leaf trace. ×177.

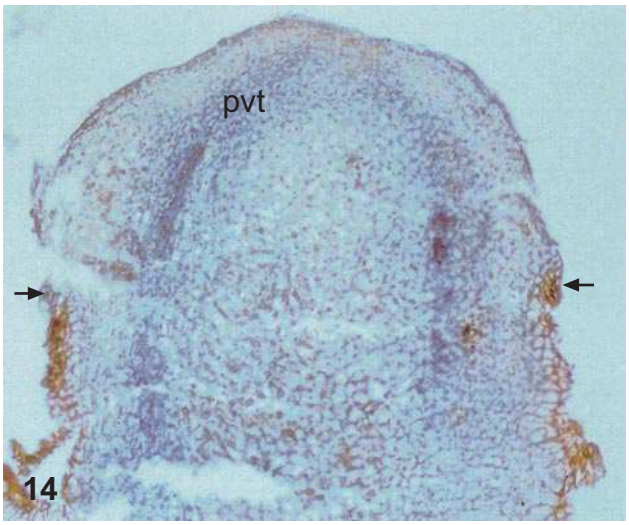


FIG. 14. Longitudinal section of an isolated shoot apex on which all leaf primordia were suppressed for 2 weeks showing esterase activity in the provascular tissue in the new growth and its extension in the pith plug. pvt, Provascular tissue. Arrows indicate boundary between new growth and pith plug. ×200.

observations of McArthur and Steeves (1972). This extended provascular cylinder was clearly the result of a positive control exerted by the apical meristem and not a residuum of undifferentiated meristematic tissue, a residual meristem. It is, therefore, significant that histochemical tests showed a strong esterase reaction in this tissue as in the provascular tissue in the newly formed region, indicating that differentiation had occurred.

These experiments have delineated the important role of the apical meristem in blocking out the pattern of the vascular system. They have also demonstrated that, in the absence of leaf primordia, the differentiation of that system cannot proceed beyond the initial or provascular stage. Whereas an isolated and defoliated apical meristem initiated only a provascular cylinder, if leaf primordia were allowed to develop after the isolation an independent and essentially normal mature vascular system resulted, as previous workers have shown (Wardlaw, 1950; Ball, 1952; McArthur and Steeves, 1972). The essential role of leaf primordia in shaping the ultimate form of the vascular system was demonstrated with particular clarity in those instances in which a single primordium was allowed to develop during the course of the experiment. In the portion of the provascular cylinder confronting the primordium, a typical leaf vascular supply was differentiated and extended into the subjacent pith plug. It is of particular interest to note that as the leaf vascular supply differentiated the remainder of the provascular cylinder below the level of leaf trace departure became indistinct and was not extended into the pith plug. This suggests that the differentiation of the procambial strands of the leaf system promotes the parenchymatization of the remaining provascular tissue. Alternatively, the developing leaf vascular system may act as a sink drawing away some substance(s) essential for the maintenance of provascular tissue. This observation sheds further light on the report by Young (1954) that in lupin the suppression of all leaf primordia resulted in the retention of a cylinder of meristematic tissue, but that if only one primordium was eliminated it was confronted by a parenchymatous gap in the vascular system.

The present investigation provides a systematic confirmation of earlier studies on dicotyledons which concluded that the apical meristem plays a significant role in vascular differentiation by blocking out the system in the initial or provascular stage. However, development proceeds no further without the contribution of the most recently formed leaf primordia whose influence causes procambial strands and ultimately mature xylem and phloem to differentiate from the provascular tissue to form the characteristic eustelic mature vascular system. An exception to the second part of this conclusion has been Wardlaw's (1950) report that an isolated and defoliated apical meristem of *Primula* could produce a fully mature vascular system. In this experiment, however, after a period of defoliation several leaf primordia were allowed to develop possibly because, as in carrot, the continuous suppression of leaf primordia reduced the vigour of growth. The further differentiation of vascular tissue beyond the provascular stage may thus be interpreted as the result of this leaf influence as in the present study when one primordium was allowed to develop, and thus does not contradict the general conclusion.

The confirmation of this conclusion emphasizes the contrast with the results of comparable studies on ferns. Several surgical investigations on ferns have shown that an isolated and defoliated apical meristem not only initiates a provascular stage of differentiation but also promotes its further development into a fully mature vascular system. The contribution of leaf primordia, while not essential for complete differentiation, nonetheless significantly modifies the form of the vascular system through an expansion of parenchymatous tissues including the formation of characteristic leaf gaps (Ma and Steeves, 1992, 1995*b*). The direct impact on actual vascular tissue differentiation was found to be minimal. Thus the role of the apical meristem in dicotyledons in initiating the vascular system is similar to that in ferns, but does not extend beyond that initial step, and the contribution of developing leaf primordia is substantially greater.

There is a large body of evidence showing that auxin plays a major role in the differentiation of vascular tissue in seed plants (Aloni, 1987; Steeves and Sussex, 1989). Sachs (1981) has interpreted the organized vascular system as the result of the pattern of auxin flow through the plant, but there have been few attempts to test this hypothesis in the shoot apex where the pattern is actually established. In their study of *Geum*, McArthur and Steeves (1972) applied exogenous auxin to the isolated and defoliated shoot apex in the expectation that maturation beyond the provascular stage might thus be promoted. The results were suggestive but not conclusive. Auxin alone appeared to enhance the cytological distinctiveness of provascular tissue and, when combined with a supplemental supply of sugar, promoted its differentiation to a recognizable procambial stage. Maturation, however, did not proceed beyond this point. The incentive to pursue this experimental approach in the

carrot system is compelling and the results of such a study will be reported in a subsequent communication.

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