CAMBIAL REGENERATION IN APPROACH GRAFTS BETWEEN PETIOLES AND STEMS

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Summary

This study of the positions in which cambia regenerate in grafts between petioles and stems aims at discovering whether principles of cambial regeneration proposed for organs with radial symmetry are applicable also to dorsiventral petioles.

It is shown for three species that the cambia regenerating in approach grafts tend to unite the cut stem and petiolar cambia into a single cambial ring passing through both organs, though this ring remains interrupted in certain grafts because cambia regenerate only in callus and not in differentiated ground tissue of the petiole.

From the results of various types of approach graft it is argued that: (i) the behaviour of these petioles in grafting is equivalent to the behaviour of half-stems; (ii) the capacity of differentiated tissues to control the position of cambia regenerating in adjacent callus depends on the presence of living vascular strands in the differentiated tissues. These points are discussed in terms of the previously proposed "gradient induction" hypothesis.

I. Introduction

An approach graft between two stems is made by bringing them together side by side, cutting away their opposed faces over a distance of a few centimetres, and tying them together. Normally, the stems are cut away to about one-half of their thickness, so that each cambial cylinder is interrupted and appears in transverse section as a half-ring (Fig. 1b); in such a graft, cambia regenerating in the callus formed between the cut stems join the two half-rings into a single ring (Fig. 1d). If, on the other hand, one of the two stems is cut only superficially ("scraped") leaving the cambial cylinder intact as in Figure 1c, the regenerating cambium arises in a different position, joining together the cut ends of the cambium in the deeply cut stem (Fig. 1e).

A feature common to both these types of graft is that cambia regenerate in such a position that the interruptions of cambial continuity caused at grafting are removed: regeneration results in the formation of continuous cambial rings, as seen in transverse section. This tendency for cambial regeneration to close interruptions in cambial rings is found in stems, hypocotyls, and roots that have been grafted, split, or wounded in a variety of ways; it is explicitly stated in the laws of cambial regeneration proposed by Janse (1921) ("Le cambium, qui tend à constituer un anneau fermé, . . . "), and it is implicit in the various theories of regeneration set out by other authors (see Warren Wilson and Warren Wilson 1961).

Stems, hypocotyls, and roots have radial symmetry, and cambial regeneration following grafting or wounding tends to restore the normal condition: that of a

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closed ring. Petioles of many species differ fundamentally from these axial organs in having dorsiventral symmetry: the vascular tissue and cambium, as seen in transverse section, are in the form not of a closed ring but of an arc that is open on the adaxial side. Hitherto, no attempt seems to have been made to find out whether the principles of cambial regeneration that apply to organs with radial symmetry are true also for dorsiventral petioles. Accordingly we have examined cambial regeneration in approach grafts between stems and petioles, and in this paper we present the results and discuss them in relation to principles of cambial regeneration.
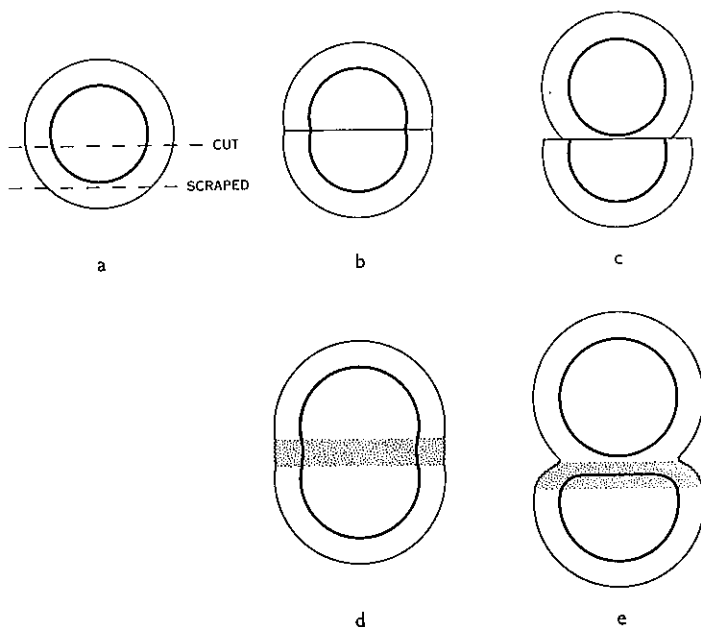

Fig. 1.—Diagrammatic transverse sections of: (a) stem before operation, with broken lines to indicate amounts cut away for cut stem and scraped stem grafts; (b) cut stem approach graft immediately after the operation; (c) scraped stem approach graft immediately after the operation; (d) cut stem approach graft after regeneration has occurred; (e) scraped stem approach graft after regeneration. In this and subsequent figures the cambium is indicated by a heavy line, and regenerated tissues by stippling.

Section III of the paper describes and discusses the course of cambial regeneration in normal petiole : stem grafts; Section IV considers regeneration in some special types of petiole : stem graft in relation to the control of the positions in which cambia regenerate.

II. METHODS

Three species, with differing vascular distribution in their petioles, have been used. In *Atropa belladonna* most of the vascular tissue occurs in a continuous, shallow

arc, though there are also a few small traces towards the wings of the petiole. *Datura stramonium* is rather similar, except that: (i) the arc is more extensive, forming roughly a semicircle; and (ii) the arc is not continuous, but is broken at several points by ground tissue (rays), which remains parenchymatous throughout the normal life of the petiole. In *Helianthus annuus* the vascular tissue lies along a shallow arc, but consists of three large and several smaller bundles that are discrete and remain separated by wide parenchymatous rays.
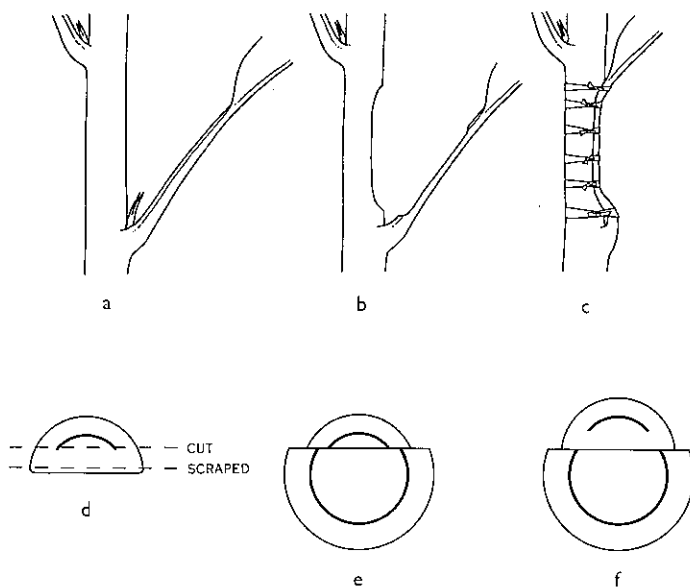

Fig. 2.—(*a, b, c*) Diagrams showing method of approach grafting petiole and stem. Below, diagrammatic transverse sections of: (*d*) petiole before operation, with broken lines to indicate amounts cut away for cut petiole and scraped petiole approach grafts; (*e*) cut petiole : stem approach graft immediately after the operation; (*f*) scraped petiole : stem approach graft immediately after the operation.

The plants were grown on John Innes compost in pots in a greenhouse, and the grafts were made between young, rapidly growing stems and petioles during May, June, and early July. In grafting, the stem and petiole were cut away on their opposed faces and tied together firmly with wet raffia (Fig. 2, *a–c*). The ties were renewed as the grafted tissues expanded in growth, and were removed after 3 or 4 weeks when a firm union had formed.

Two types of approach graft were made, differing in an important detail: in one, the adaxial face of the petiole was cut away deeply so as to expose the cambium of the vascular arc ("*cut*" petiole, Fig. 2e); in the other, the petiole was cut away superficially or merely scraped and stripped of epidermis, its vascular tissue being left intact ("*scraped*" petiole, Fig. 2f). In both cases the stem was cut quite deeply, so as to remove about one-third of its thickness and interrupt its cambial ring.

Graft unions were sampled after an interval sufficient for the complete regeneration of new cambium and for the formation by it of phloem and xylem. The unions were preserved in 70% alcohol and later sectioned by microtome, without embedding, at about 70 μ. Sections were stained in safranin and light green.

In all the drawings below (made by camera lucida) xylem is hatched with radial lines, and vascular cambium (whether original or regenerated) is shown
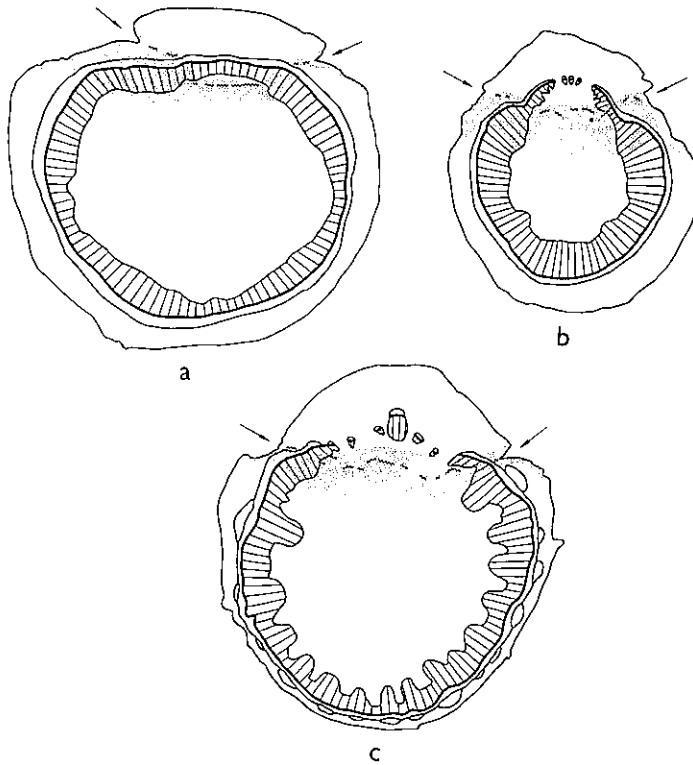

Fig. 3.—Transverse sections of cut petiole: stem approach grafts in
(a) *Atropa*, (b) *Datura*, (c) *Helianthus*.

by a heavy line. Stippling is used to indicate regenerated tissues, namely, callus, wound parenchyma, the regenerated cambium and tissues derived from it, and periderm. The internal phloem of *Atropa* and *Datura* has been omitted for clarity. "Scar line" is shown by small zigzag lines; it consists of the remains of cells damaged at grafting, and helps to indicate the interface between regenerated tissues derived from stem and petiole respectively. The position of this interface at the periphery of the unions is marked by arrows in the drawings.

III. Normal Petiole : Stem Grafts

(a) "Cut" Petiole : Stem Grafts

Two grafts of this type were made with *Atropa*, three with *Datura*, and two with *Helianthus*; they were sampled 3 to 8 weeks after grafting. In every case it

was found that, throughout the length of the union where the petiolar cambium had been exposed at grafting, regenerating cambia had joined together the cut ends of the petiolar and stem cambia on each side (Fig. 3, *a–c*). Thus the petiole had participated in regeneration in the same way as a half-stem; regeneration had tended to unite the cut petiolar and stem cambia into a single ring.

In *Atropa*, this ring was perfectly continuous. In *Datura* and *Helianthus*, on the other hand, a complete ring was not formed; although interfascicular cambia occasionally arose near the ends of the vascular arc, most or all of the rays remained parenchymatous. Consequently, the cambial ring was interrupted at these points even when regeneration was complete. This failure to regenerate an uninterrupted ring across the petiolar rays can hardly have been due to lack of time, for it has been observed in a cut petiole : stem graft of *Lycopersicon esculentum* which was not sampled until $19\frac{1}{2}$ weeks after grafting.

In this especially old union the amount of secondary xylem produced by the petiolar cambium where it joined the regenerated cambium was many times greater than the normal xylem production of the same petiole just below the union; this effect was found generally in cut petiole : stem grafts, but was less marked when the unions were examined at a younger stage.

(b) "Scraped" Petiole : Stem Grafts

Four grafts of this type were made with *Atropa*, two with *Datura*, and four with *Helianthus*; they were sampled 3 to 11 weeks after grafting (mean period 6 weeks). In these grafts the petiolar cambium normally showed no unusual activity, and never became joined into a ring with the stem cambium (Fig. 4). Nor, however, did the stem cambium regenerate independently an uninterrupted ring, as it would have done in the absence of the petiole (Fig. 9a); it remained open beneath the petiole. Limited lengths of regenerating cambium extended from the cut ends of the stem cambium as if to form a ring with the cambial arc of the petiole; but they were confined to the callus. In *Atropa* and *Datura* they ended in the petiolar callus close to the petiolar ground tissue; in *Helianthus* the same structure tended to arise, but massive proliferation of the callus resulted in distortion, so that it was often impossible to trace the course of the cambium clearly through the callus. Even in the oldest unions examined the regenerating cambium did not extend into the petiole; the cambial ring remained interrupted here in grafts of all three species.

(c) Other Petiole : Stem Grafts

In one *Datura* and two *Helianthus* grafts, the cut in the petiole at grafting was made deliberately on a slant, deeper at one end than the other. As a result, the petiolar cambium was exposed over part of the union (cut petiole) but not in the rest (scraped petiole). Serial sections showed that in all three unions the transition from scraped petiole to cut petiole types of regeneration—described above—coincided with the level at which the cut at grafting had first exposed the petiolar cambium. A similar relationship has been observed near the upper and lower ends of several cut petiole grafts, where the grafting knife has entered and left the petiole. Some other petioles have happened to be cut on one side of the vascular arc and scraped

on the other over a short distance; in such cases regenerating cambia joined the stem and petiolar cambia on the cut petiole side, but ended in the petiolar callus on the scraped petiole side, so that the cambial ring remained interrupted here. This correspondence within single unions between type of petiolar operation and course of regeneration makes it especially clear that the factor deciding which of these two types of regeneration shall occur is the depth of the cut at grafting: if ground tissue remains beyond the ends of the vascular arc in the petiole it prevents regenerating cambium from joining the petiolar and stem cambia.
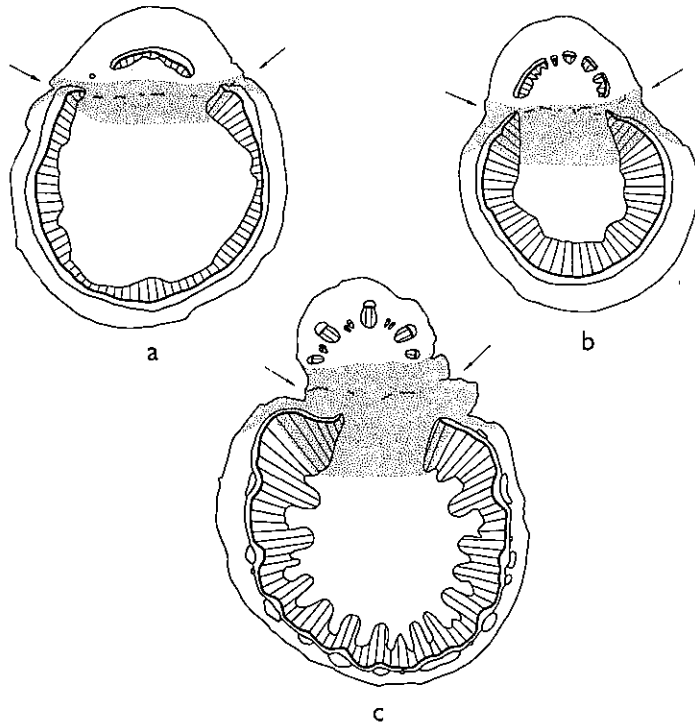

Fig. 4.—Transverse sections of scraped petiole:stem approach grafts in (a) *Atropa*, (b) *Datura*, (c) *Helianthus*.

(d) Discussion

The positions of regenerated cambia in petiole : stem grafts of the two main types described above are summarized diagrammatically in Figure 5. These diagrams refer to petioles having arcs that are continuous; for species with discontinuous arcs the diagrams would be similar except that the discontinuities present in the original petiolar arcs would remain after regeneration in each type of graft union.

These results seem to be most simply interpreted by the following two generalizations:

(i) Cambial regeneration tends to occur along a path that unites the original cambia of both stem and petiole into a single ring passing through both organs.

      (ii) Cambia regenerate in the callus formed at the cut surfaces of stem and petiole; they do not regenerate in the differentiated ground tissue of the petiole.

A general hypothesis to explain the positions in which cambia regenerate in wounds and grafts has been proposed previously (Warren Wilson and Warren Wilson 1961). We now discuss the application of this "gradient induction" hypothesis to the results described above. The hypothesis states that:

      (1) In undifferentiated tissue a gradient in some factor as yet unknown tends to arise perpendicular to the exposed surface.

      (2) A vascular cambium can form at only one position on this gradient, where the factor is at an appropriate level.

      (3) Cambial orientation, as regards phloem and xylem formation, is determined by the direction of the gradient.

      (4) When cambium, phloem, or xylem have differentiated, the level of the unknown factor becomes fixed around them.

      (5) The gradient arising in undifferentiated tissue that immediately adjoins tissue in which a gradient is fixed tends to conform to this established gradient.

(Undifferentiated tissue may be placed so that the gradient in it is determined jointly by both surface effect (1) and by adjacent established gradient (5).)
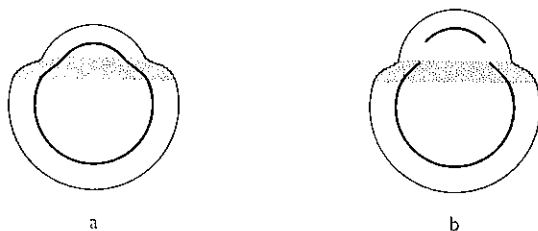

Fig. 5.—Diagrammatic transverse sections of: (a) cut petiole:stem approach graft after regeneration has occurred, (b) scraped petiole:stem approach graft after regeneration.

      In discussing the application of this hypothesis it is convenient to indicate the trend of the gradients by using arbitrarily the symbols — to represent the level near the exposed surface, and + for the level away from it; cambium may then form in the plane between — and + signs, producing phloem on the — side and xylem on the + side.

      It is helpful first to consider approach grafts between stems (Fig. 1). In a normal stem we suppose the gradient to be established beneath the surface, with the cambium occurring between the outer (—) and the inner (+) levels, as in Figure 6a. When two cut stems are approach grafted (Fig. 1b) the gradient arising in the callus formed between them is determined in part by the exposed surfaces, but (since the exposed surfaces are small) to a larger extent by the established gradients in the adjacent, differentiated tissue of the original stems. These influences

in combination produce the gradients shown in Figure 6*b*, and the regenerating cambia, lying between − and + signs, join the cut ends of the original cambia. In the cut stem : scraped stem graft the gradients in the callus are again determined mainly by those in the original stems on each side. Since one of these is + and the other − over the central region, a gradient from + to − arises across the callus as shown in Figure 6*c*, where a regenerated cambium is indicated between + and − symbols.
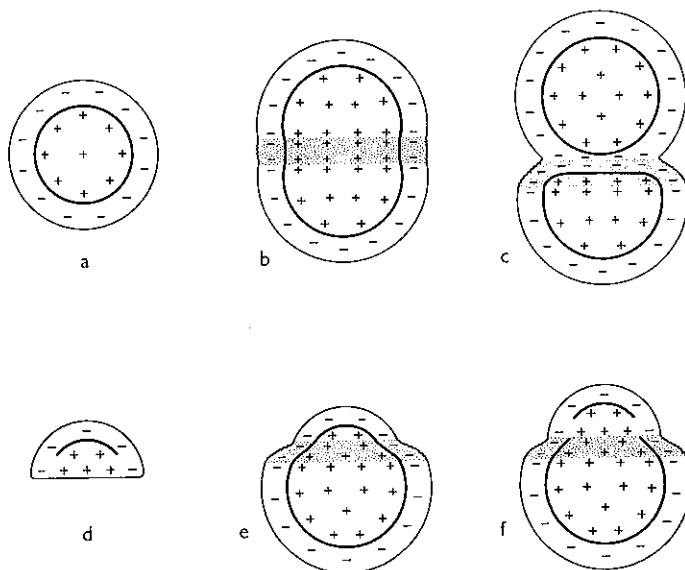

Fig. 6.—Diagrams showing pattern of hypothetical gradients in: (*a*) stem before operation; (*b*) cut stem approach graft after regeneration; (*c*) scraped stem approach graft after regeneration; (*d*) petiole before operation; (*e*) cut petiole : stem approach graft after regeneration; (*f*) scraped petiole : stem approach graft after regeneration.

Cambial regeneration in the cut petiole : stem graft (Fig. 5*a*) is essentially similar to that in the cut stem : stem graft (Fig. 1*d*), and can be explained in terms of the "gradient induction" hypothesis along similar lines. On the other hand, regeneration in the scraped petiole : stem graft (Fig. 5*b*) is fundamentally different from that in the scraped stem : stem graft (Fig. 1*e*). We suggest that this difference arises because gradients in the entire petioles are arranged as in Figure 6*d*, reflecting their dorsiventral nature and contrasting with the radially symmetrical arrangement of gradients in the entire stem (Fig. 6*a*). Thus the entire petiole is effectively like a half-stem; and when it is merely scraped before grafting to a stem, it is to be expected that regenerating cambia will lie along the same path as in an approach graft between two cut stems, i.e. uniting the original cambia of the approach-grafted parts into a single ring. In fact a continuous ring is not formed, since cambia fail to regenerate where this path passes through the petiolar ground tissue, the cells of this tissue being for unknown reasons not normally "competent" to form cambial initials.

IV. EFFECTS OF ELIMINATING THE ABAXIAL PART OF THE PETIOLE FROM PETIOLE : STEM GRAFTS

(a) Death of the Petiole

In a cut petiole : stem graft of *Lycopersicon* which was not sampled until 19½ weeks after grafting, the lamina had died of age long before sampling. However, the whole of that length of the petiole where the cambium had become joined by regeneration into a single ring with the remaining stem cambium was still alive and active at the time of sampling, several weeks after the lamina and the rest of the petiole had shrivelled and died. Thus the death of the lamina had no apparent effect on the structure of the union.
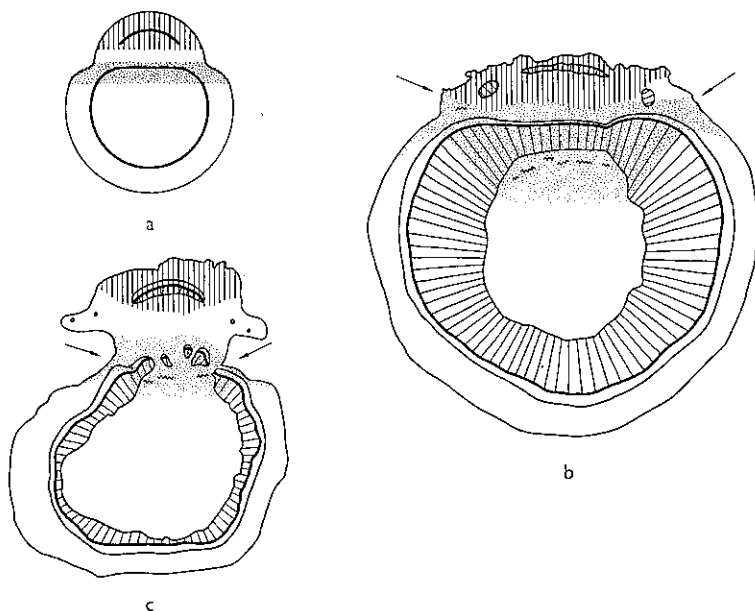

Fig. 7.—(a) Diagrammatic transverse section of scraped petiole : stem approach graft after death of the abaxial part of the petiole. Transverse sections of scraped petiole : stem approach grafts after death of the abaxial part of the petiole in (b) *Datura*, (c) *Atropa*.

In scraped petiole : stem grafts, on the other hand, death of the leaf resulted in regeneration of additional cambium. Four examples of this situation were examined; in three of them (one *Atropa*, one *Datura*, and one *Helianthus* graft) the death of the leaf was due to normal aging, the grafts having been made late in the season and sampled after rather long intervals, and in the fourth (*Atropa*) the lamina died because growth of the union caused the raffia ties to cut into the petiole above and below the union so that its vascular supply was restricted. The processes of death and cambial regeneration had proceeded to different stages at different levels in each graft, and serial sections of the four unions allow the following general account to be given.

In that length of the petiole which had been grafted to the stem, death of its tissues as shown by microscopic examination occurred first on the abaxial side and spread towards the stem through the abaxial ground tissue, then the vascular arc, and finally the adaxial ground tissue. Meanwhile, however, additional cambial regeneration occurred, generally starting at the ends of the cambia originally regenerated when the leaf was living, and extending along a path tending to join them together and so form a complete cambial ring (Fig. 7, *a*, *b*). The result of this was generally to form a complete cambial ring; but in one case (*Atropa*, Fig. 7*c*) the regeneration was only spasmodic, though along the normal path. In *Helianthus* this path lay through callus derived from the stem, but in the other grafts it lay in petiolar callus, since fragments of scar line were present on the inner side of the xylem formed by the newly regenerated cambium.
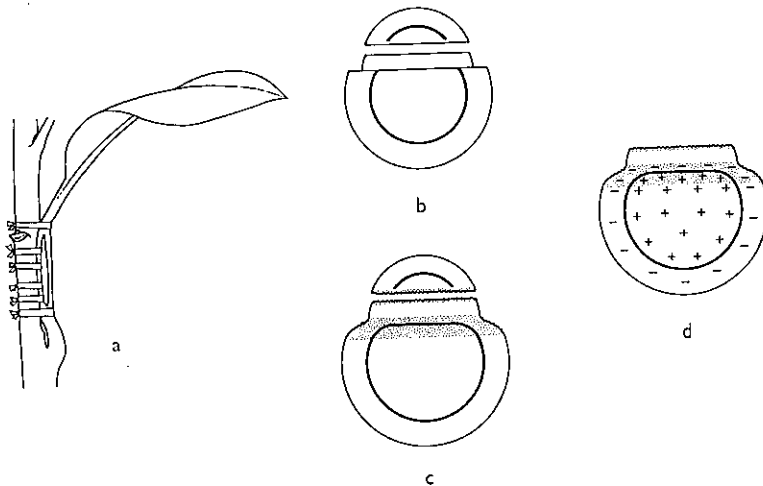

Fig. 8.—(*a*) Diagram showing method of making split scraped petiole : stem approach graft. Right, diagrammatic transverse sections of this graft : (*b*) immediately after the operation, (*c*) after regeneration, (*d*) showing pattern of gradients.

(b) Split "Scraped" Petiole : Stem Grafts

A situation rather similar to that resulting from death of the leaf can be obtained by means of an operation. A stem and petiole are prepared for a scraped petiole : stem graft, and the petiole is then split by a tangential, longitudinal incision just adaxial to the vascular arc. This split isolates the adaxial ground tissue of the petiole which is now tied to the stem (Fig. 8, *a*, *b*), and if the graft is successful this petiolar tissue remains alive and becomes united to the stem. The other part of the petiole, containing the vascular arc, maintains a vascular supply to the lamina, so that the latter remains alive. Certain petioles of *Atropa* have an especially large mass of adaxial ground tissue, and five successful split scraped petiole : stem grafts have been made with this species. They were sampled 5 to 7 weeks after grafting.
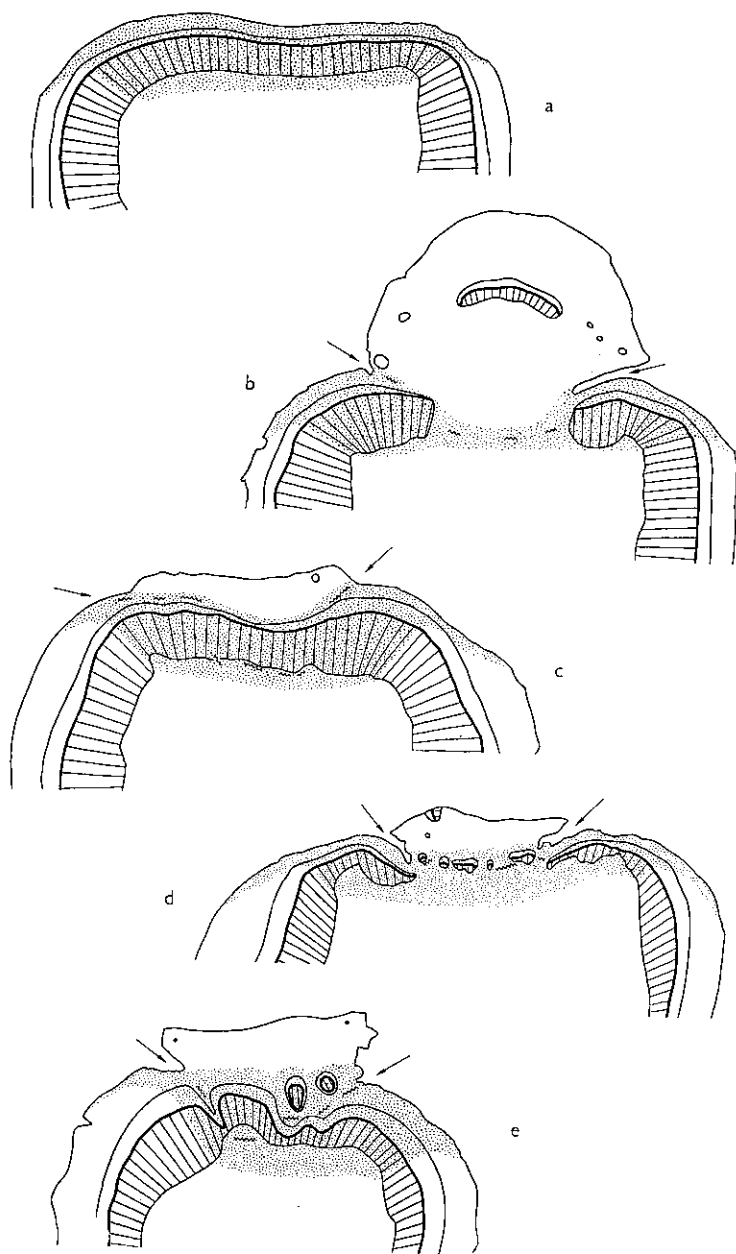
Fig. 9.—(*a–e*) Transverse sections of split scraped petiole : stem approach grafts in *Atropa*; explanation in text (half-stem away from grafted side not shown).

The typical result of this operation, found at some levels of four out of the five grafts, was the regeneration of a cambium joining together the cut ends of the stem cambium, and re-forming a continuous cambial ring (Fig. 8c). The position of the scar line indicated that this new cambium in every case passed through callus derived from the petiole. The three sections shown in Figure 9a, b, and c, taken from different levels of a single union, are particularly instructive. In all three the stem has been treated similarly, about one-third of its thickness being cut away. Figure 9a lies above the graft; the cut stem has regenerated in the normal manner, re-forming a complete cambial ring. Figure 9b lies at the upper end of the graft, above the level where the petiole was split; here there is effectively a scraped petiole graft, and cambial regeneration has not closed the interruption in the stem cambium. Figure 9c lies in the region where the petiole was split; and here cambial regeneration has closed the cambial ring by regeneration through the petiolar callus.

In one of the split petiole grafts which showed this pattern of regeneration the regenerated cambium was not continuous, but was present only spasmodically along the usual path (Fig. 9d, cf. Fig. 7c). It is possible that rounding off of such fragments of cambium was responsible for the development of small rings of cambium, formed in the petiolar callus at certain levels in three of the five split petiole grafts (e.g. Fig. 9e); wherever these rings were formed, a regenerated cambium occurred in the stem callus beneath them, joined to the cut end of the original stem cambium.

(c) Discussion

The typical course of regeneration is essentially similar in the two special cases of scraped petiole grafts discussed above: the dying petiole and the split petiole. In each case a regenerating cambium closes the interruption in the stem cambium which would have persisted with an entire and living scraped petiole. This suggests that the influence of the petiole on regeneration depends on the abaxial part of the petiole (which dies first, or is separated off by splitting); and it seems likely that this is because it is the vascular tissue which is responsible.

However, the regeneration occurring when this abaxial part of the petiole is eliminated is not exactly the same as in the complete absence of the petiole; for instead of passing wholly through callus derived from the stem, the regenerating cambium runs on the outer side of the scar line, that is, through callus formed from the petiole. Thus, the presence of the remaining, adaxial part of the petiole causes an outward displacement of the position of the cambium.

We suggest, as an interpretation of these results in terms of the gradient induction hypothesis, that the maintenance of a gradient by differentiated tissue depends on the presence in it of living vascular tissue. Thus when the petiolar vascular tissue dies or is separated off from the adaxial ground tissue, the original gradient in the latter is no longer maintained. Effectively there results therefore a new exposed surface—on the outer side of the adaxial ground tissue—and a gradient is induced in the mingled petiolar and stem calluses lying a short distance beneath this, with − tissue towards the petiolar surface and + tissue on the other side, adjacent to the + tissue of the cut stem (Fig. 8d). A cambium then regenerates between − and + regions.

To sum up, we suggest that:

(i) Gradients in dorsiventral petioles are arranged approximately as are those in a half-stem.

(ii) No cambium is formed where the path along which cambia tend to regenerate crosses petiolar ground tissue; this tissue is not "competent" to form cambium.

(iii) The capacity of differentiated tissues to induce gradients in adjacent callus depends on the presence in them of living vascular strands.

V. ACKNOWLEDGMENTS

VI. REFERENCES

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