

Further Observations on the Wound Reactions of the Petioles of *Pteris aquilina*.

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With four Figures in the Text.

IN 1912 I recorded in this journal¹ some observations on the wound responses of filicinean petioles. Whilst in Cumberland during the summer of 1914 the opportunity was taken of collecting material of *Pteris aquilina*, in which specimens showing wound-scars of greater or less extent are not uncommon. This material was supplemented by a further supply collected at Oxtun (Notts.). Specimens such as these suffer, of course, from the disadvantage that one cannot determine either the cause of the injury or the age of the wound, but, on the other hand, they afford some evidence as to how far the wound response exhibited under natural conditions agrees with the results obtained experimentally. The injured petioles may be grouped, for purposes of description, under two heads as follows:

(i) Those in which the wound did not penetrate below the sub-epidermal sclerenchyma. These were very common.

(ii) Those in which the sclerenchyma had been penetrated. The wounds of this second type naturally varied in severity, and in some cases were fairly deep seated.

Whilst differing in points of detail, the whole of the specimens examined showed a certain number of well-marked features in common, namely:

(i) The occurrence of a bright yellow substance in the walls of the zone of cells abutting on the wound. This discoloration, which was due to the deposition of a tannin-like substance,² became more pronounced and darker in tint in the most superficial layers.

¹ Holden, H. S. : Some Wound Reactions in Filicinean Petioles. *Ann. Bot.*, 1912, p. 777.

² It gives a greenish-black coloration with neutral ferric chloride, and a red coloration with an aqueous solution of iodine in KI mixed with a little 10 % ammonia. (Cf. Haas and Hill, *Chemistry of Plant Products*, pp. 190-7.)

(ii) The partial or complete degeneration, in the wound area, of the lignified elements constituting the sub-epidermal armour.

(iii) A more or less pronounced thickening of the cell-walls, sometimes of a purely cellulose nature, but often accompanied by partial or complete lignification.

Taking now the first of the two types of wound referred to above, it is found that the nature of the response is somewhat variable. Where the wound is extremely slight—that is to say, when the wound surface does not penetrate below the fourth or fifth layer of cells—there is very little obvious effect, except for the yellow discoloration alluded to. Microchemical tests demonstrate, however, that in the majority of cases the most superficial cells of the sclerenchyma have become delignified, and give the cellulose reaction with chlor-zinc-iodine.¹ If, on the other hand, the sclerised armour is almost penetrated, there is very generally a compensatory thickening of the cortical elements immediately below the wound surface, such thickening being normally of a cellulose or ligno-cellulose character. In about 20 per cent. of the cases examined, however, lignification appeared to be complete. These differences may of course be due to differences in the age of the wounds, but, judging from appearances, this is unlikely. The walls of the affected cells are abundantly pitted and do not appear in any way degenerate, except possibly for the yellow colour (cf. Fig. 1, v). The effect on the sclerised elements themselves, apart from delignification, is also of some interest, as in many cases a considerable increase in the thickness of the wall accompanies this process. This feature is well illustrated in Fig. 1, iii and iv, in which the walls of the sclerenchyma on the flanks of the wound show a marked increment which is entirely of a cellulose nature and is often obviously stratified. With regard to wounds of the second type, namely, those which penetrate the sub-epidermal sclerenchyma, the reaction is found to be extremely variable. In a large number of cases the plant exhibits merely a somewhat extensive local thickening of the cells in the affected area (Figs. 1, i, ii; 2, i, &c.). The modified cells always extend more deeply into the tissues of the petiole in the neighbourhood of the vascular strands (Figs. 1, ii; 2, ii), and in many cases the latter are wholly or partly flanked by patches of thickened tissue which are quite disconnected from the main mass (Figs. 2, iv; 4, i). As in the less severe types of wound described above, the amount of lignification varies considerably, but the superficial uninjured cells practically always give a cellulose reaction, whilst those below stain pink or red with phloroglucin after acidification with HCl. The cause of the cellulose reaction of these more superficial cortical elements is something of a puzzle, but it may be that their nearness to the wound surface acts deleteriously upon them, and thus prevents their reacting as fully as those which are

¹ See Appendix.

deeper seated. In addition to the reaction to phloroglucin given by the obviously thickened elements, a red coloration frequently occurs in the adjoining cortical tissue in which there appears to be no thickening, so that it is evident that lignification is produced to some extent in these tissues also.

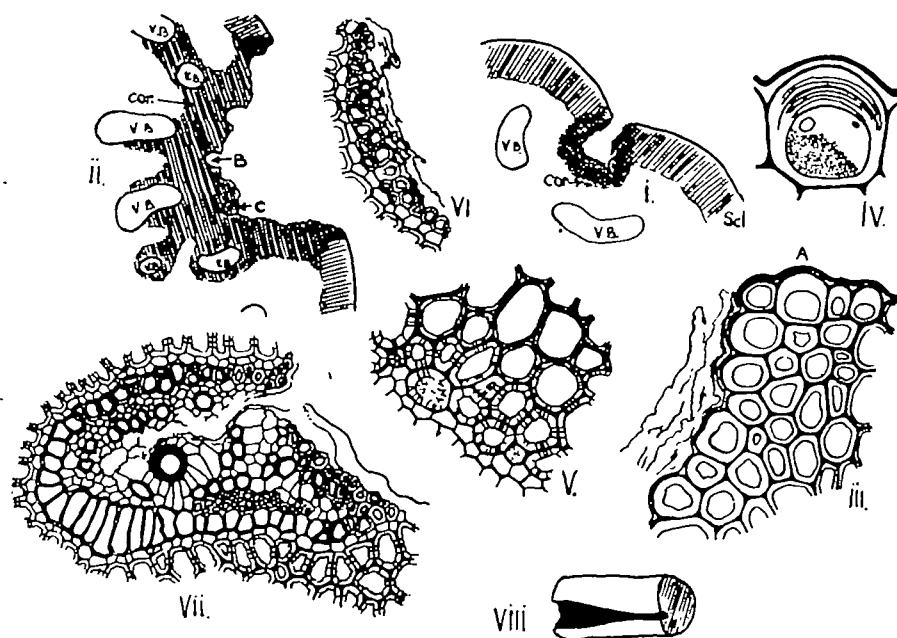


FIG. 1. i. Diagrammatic transverse section of wound area showing compensatory thickening of cortex. ii. Diagrammatic transverse section near middle of wound area shown in i. Note the spread of the thickened tissues in the neighbourhood of the vascular strands. iii. Small portion of i, showing the delignified sclerenchyma on the flank of the wound and its increased thickness. iv. A single cell (A) from iii, showing the stratification of the wall. v. A small portion of the thickened cortex in the vicinity of a bundle. vi. Remains of bundle ii, B, showing the thickened cells of the starch sheath. vii. Remains of bundle ii, C, showing thickening and in some cases elongation of cells of the starch sheath. viii. Portion of wounded petiole from which i and ii were cut, i being from the narrow end, ii from the broad end. i and ii $\times 12$, iii $\times 350$, iv $\times 650$, v $\times 250$, vi and vii $\times 500$, viii $\times 1$. scl. sub-epidermal sclerenchyma; cor. thickened cortical cells.

A second and more pronounced type of reaction which occurs fairly frequently is that of the radial elongation of the cortical cells in the wound area (Fig. 2, iv). This phenomenon is usually accompanied by an increase in the thickness of the cell-walls, as in the cases previously described. Between the two types of reaction, namely, thickening with elongation, and thickening alone, no sharp line of division can be drawn, since in many the more vigorous type of response occurs in the middle of the wound area whilst absent from the two ends. Moreover, there are instances in which a local patch of cortical parenchyma has elongated and become thickened, whilst throughout the rest of the wound no such elongation is manifest (Figs. 3, i; 2, ii, c). Local growth of this character seems in no sense

dependent on the proximity of the vascular strands, and there appears to be no satisfactory explanation of its occurrence.

In some few cases the stimulus of wounding seems to have produced very far-reaching results, and to have caused elongation through quite a large part of the cortex. Such a case is illustrated by Fig. 3, i, iii, iv.

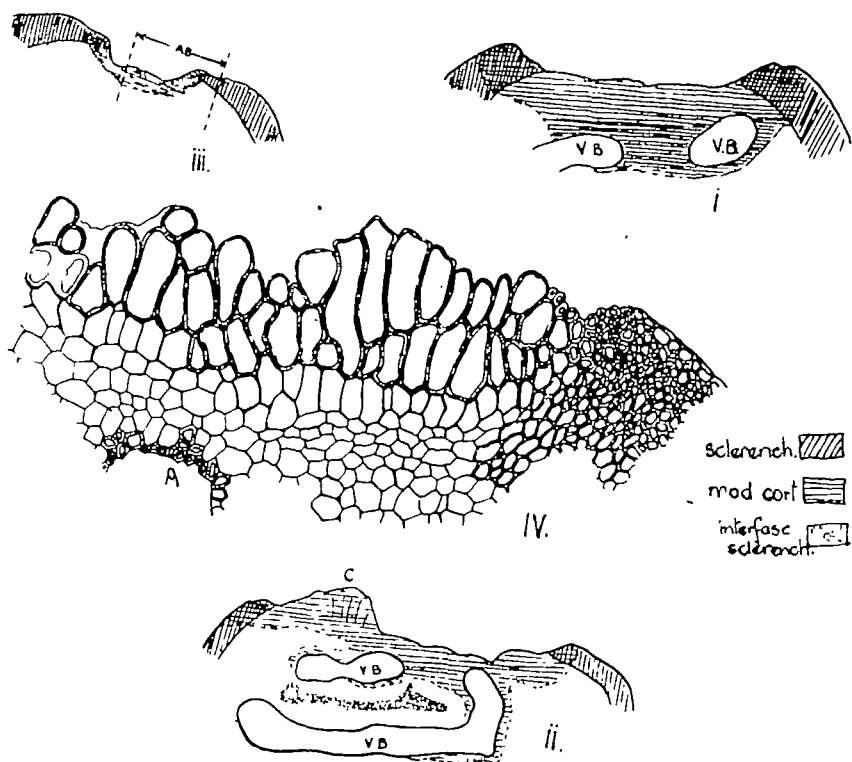


FIG. 2. i. Diagrammatic transverse section of wound area, showing a specimen in which thickening of the cortical cells has taken place to a considerable depth. ii. Diagrammatic transverse section, showing extension of thickened cells in the region of the bundles, and at c, local elongation of the cells. iii. Diagrammatic transverse section in which a relatively shallow cortical zone has been affected. iv. The portion AB of iii, showing elongation of the cells with thickening. Note at A the local thickening in the bundle region. i, ii, and iii $\times 12$; iv $\times 300$.

Here it will be seen that fully half the cortical parenchyma is affected, and that the wholesale elongation shows some very interesting features. The radial extension of the cells seems to have developed what may almost be termed 'lines of flow', some spreading fanwise from the vascular strands, others flowing round these obstructions (Fig. 3, ii). As is to be expected, these lines of flow come into contact in various parts of the petiole, this resulting in their mutual arrestment. Such contact regions are indicated by a separation line of gummy deposit of a yellow or brown colour (Fig. 3, iii and iv). Occasionally contact occurs between several lines of

flow, as in Fig. 3, iv, in which case the gummy deposit appears branched in a triradiate or quadrate manner, as seen in transverse section.

The discussion of the effect of wounding on the vascular bundles has been deferred till last, because it seems to have little or no connexion with

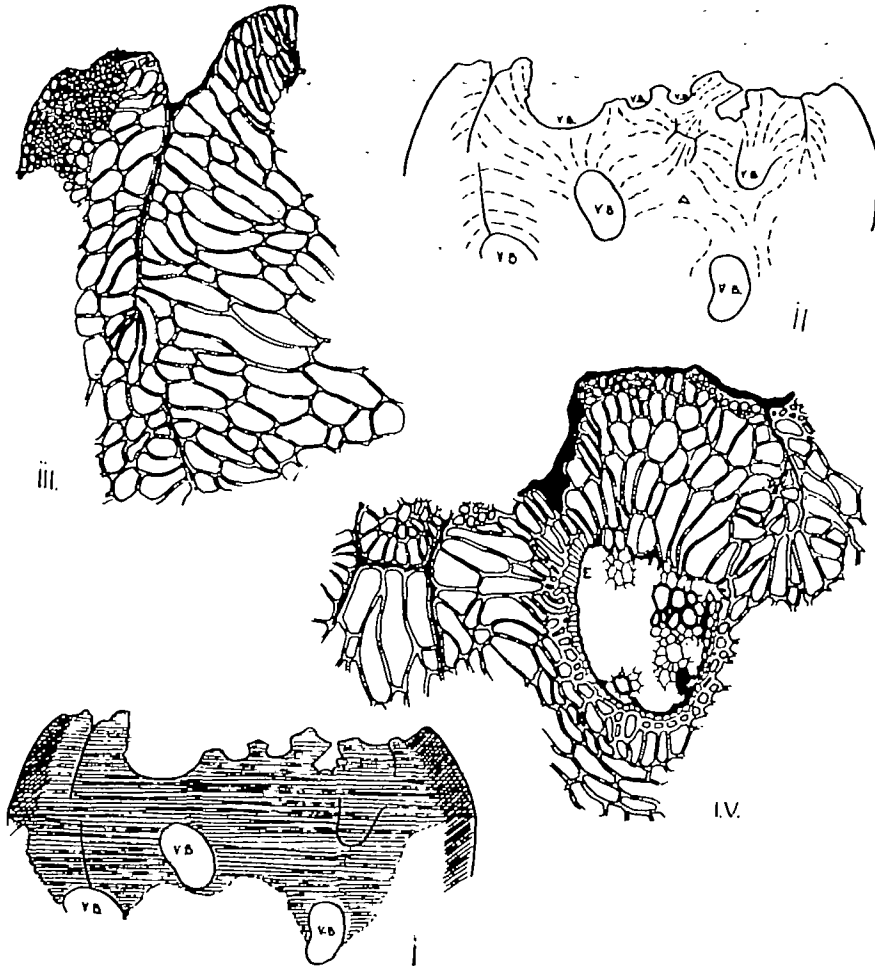


FIG. 3. i. Diagrammatic transverse section of wound area, showing the cortical cells affected to an unusually large extent. ii. Tracing from i, indicating the 'lines of flow' exhibited by the elongated cortical cells and also the lines of contact. iii. Portion of ii, more highly magnified to show detail. This is from the left of ii. iv. Portion from the right of ii. Note the elongation of the endodermal cells at E. i and ii $\times 12$, iii and iv $\times 300$.

the nature of the traumatic response in the general ground-tissue. The tissues¹ comprised in the vascular bundles show no reaction when the wound is purely superficial and does not penetrate the sclerenchyma. Apart from the occasional discoloration of the xylem and phloem elements,

¹ The endodermis is included here.

the deeper wounds also appear normally to have no effect, although, as in the case of the petiole illustrated in Fig. 3, iv, disintegration of the bundle may occur, but this is comparatively rare. In the few instances in which there is an obvious reaction, the vascular bundles affected either abut on or are adjacent to the wound surface. The traumatic response is always confined to the non-specialized constituents of the bundle, such as the starch sheath and the conjunctive parenchyma, the only exception noted during

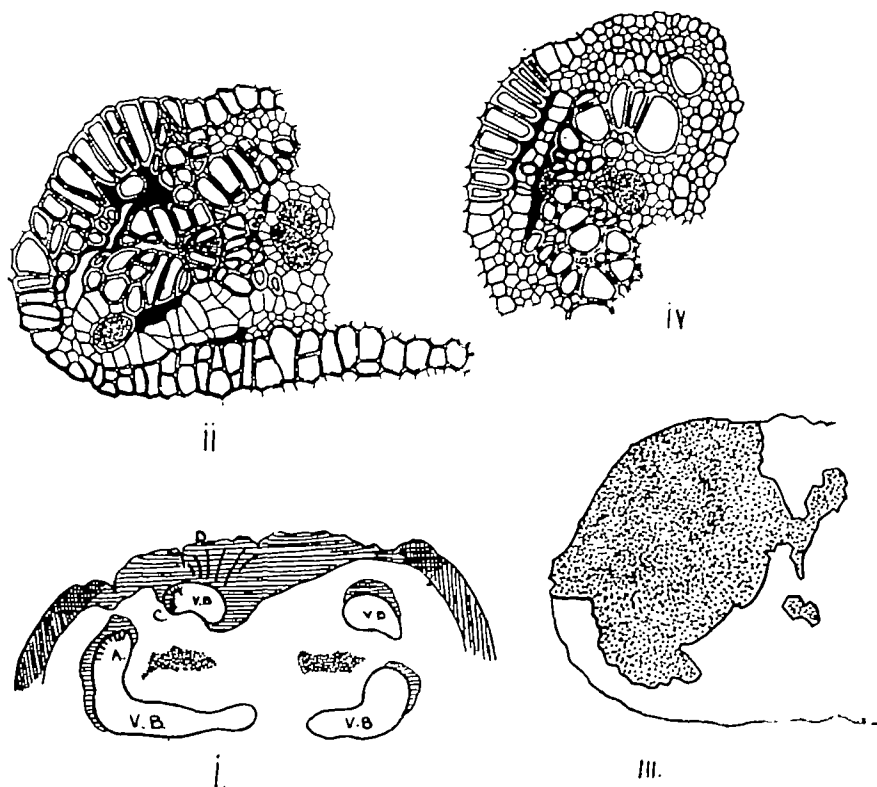


FIG. 4. i. Diagrammatic transverse section of wound area, showing pad of thickened cortical cells at wound surface and also isolated masses of thickened tissue on the flanks of the bundles. Local elongation of the cortical elements occurred at D, and the tissues of the vascular strands were affected at A and C. ii. Affected portion of C, showing effect of wound on starch sheath and conjunctive parenchyma. iii. Tracing from ii. The dotted area represents the cells which were discoloured by tannin. iv. Affected portion of C. i $\times 12$; ii, iii, and iv $\times 500$.

the present observations being that shown in Fig. 3, iv, in which the endodermis had elongated at one point. With regard to the starch sheath, this may simply develop thicker walls, or may become both elongated and thickened. These stages are well illustrated in Fig. 1, vi and vii. The wound in this particular case had caused the total destruction of some of the vascular strands and the partial destruction of two others (Fig. 1, ii, B and C respectively). Of the strand B only a portion of the starch sheath

was left, and this had thickened considerably (Fig. 1, vi), whilst of the strand C about half remained. In this the starch sheath had also thickened, and in addition elongation of the cells had occurred on the side remote from the wound surface (Fig. 1, vii). The remaining xylem and sieve-tubes showed a bright yellow coloration, and there were some indications of elongation in the case of the conjunctive parenchyma. A further illustration is afforded by the vascular bundles shown in Fig. 4, i, ii, and iv. The reaction only affected one end in each of the two bundles and, as in the previous instance, the elements composing the starch sheath had elongated and thickened, but in addition many of the cells had divided by a transverse wall. The conjunctive parenchyma was much more obviously active in the smaller and more superficial strand than in any other instance noted. Many of the cells were thickened and displaced, and in one small portion, in which the cells had remained thin-walled, a small cambiform patch was developed. All the thickened cells were bright yellow with tannin, and gave a cellulose reaction.

One other point remains to be mentioned, namely, the absence of the gum deposit which was so constant a result of the traumatic stimulus in artificially wounded forms.¹ In the present series, gum deposits only occurred in the cavity parenchyma of affected bundles and in occasional tracheides. Apart from this one feature, the examination of the petioles confirms the results arrived at experimentally in the earlier paper with regard to ferns of the type of *Pteris aquilina*.

SUMMARY.

1. Petioles of wild *Pteris aquilina* often show wound-scars. The majority of the wounds are very superficial, not penetrating the sub-epidermal sclerenchyma; others are deeper seated.

2. The wound reactions are somewhat variable, but are characterized by (i) a compensatory local thickening, and partial or complete lignification of the cortical parenchyma, which may or may not be accompanied by elongation, (ii) the local delignification of the sub-epidermal sclerenchyma, (iii) a deposit of tannin in the cell-walls in the affected area.

3. Wound reactions in the tissues composing the vascular strands are rare, and where they do occur are confined to the starch sheath and conjunctive parenchyma, which thicken and may elongate and divide.

4. The results obtained are confirmatory of those produced experimentally.

¹ Holden, loc. cit.

APPENDIX.

The phenomenon of local delignification in the sub-epidermal sclerenchyma is so characteristic and so unexpected a feature of wounds of all types, that a few remarks on the methods employed are perhaps advisable. It was found that the discoloration of the cells interfered with the micro-chemical study of the tissues, and it was therefore removed by treatment with eau de javelle. For this purpose the section to be studied was mounted in a drop of that liquid, and heated on the slide over a Bunsen flame. As a rule, from five to ten seconds of this treatment were sufficient to remove all colour. A little fresh eau de javelle was then added to dissolve the crystals formed by the partial evaporation of the first supply, and the whole was then absorbed with filter-paper. The section was next treated with a series of drops of spirit until all traces of the eau de javelle were removed. (About twenty drops successively applied and absorbed were found to work well in practice.) It was then treated with either chlor-zinc-iodine or with phloroglucin followed by either HCl or H₂SO₄. The walls referred to as delignified invariably gave a positive cellulose reaction with chlor-zinc-iodine, and a negative lignin one with phloroglucin and acid. It appears to be a general impression¹ that treatment with eau de javelle has a delignifying effect on plant tissues, and this is probably the case after more prolonged treatment, but the establishing of efficient controls demonstrated that no error had arisen from this source. In the first place, the whole of the sub-epidermal sclerenchyma remote from the wound and the xylem elements in the stele gave with phloroglucin a positive reaction after treatment, indistinguishable from that given by untreated sections, whilst sections of uninjured *Pteris* petioles, after identical treatment for the short time necessary in these experiments, showed no difference in their reactions. Other control experiments were made with the young stem of *Lycopodium alpinum*, in the thickened cortex of which the lignification is incomplete. In such specimens, treatment with phloroglucin and an appropriate acid produces a pink (ligno-cellulose) rather than a red coloration, and it was found here also that the brief period of treatment with eau de javelle had no delignifying effects. It will thus be seen that the inference that delignification is a traumatic response is amply justified.

¹ Cf. Zimmerman, Botanical Microtechnique: Cavers, Pract. Botany, &c.