A Technique for the Anatomical Study of Root Parasitism

BY

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With Plate V

STEPHENS (1912) was the first to study the structure and development of the haustoria of *Striga lutea* Lour. parasitic on maize roots. Later, Saunders (1933) carried out more extensive studies on the parasitism of this plant and found certain differences between resistant and susceptible strains of *Sorghum vulgare* in regard to the rapidity and extent of penetration of the parasite.

In our investigations on the attack of Striga, a root parasite on *Sorghum vulgare* and on several other hosts (Kumar and Solomon, 1941), we have examined three species of the parasite, viz. *Striga lutea*, *S. densiflora*, and *S. euphrasioides*. Any attempt to study by the usual methods of section cutting the differences in the type of attack by different species of Striga on different varieties of Sorghum possessing varying degrees of resistance to parasitism would be most laborious and time-consuming. It was therefore found necessary to devise a technique by which the whole material is made nearly transparent and then any desired tissue, in the present case lignified tissues, stained with an almost transparent stain. The method had to be rapid and easy of manipulation in order to deal with the large amount of material under study.

Various techniques and their modifications were tried but the results were not satisfactory. The method suggested by Debenham (1939) for the microscopic examination of the xylem of whole plant organs, besides taking too long, did not give very transparent preparations. After several trials it was found that staining by the Feulgen technique after properly clearing the material gave very satisfactory results. Subsequently, to test the possibility of wider application of this method, the technique was tried on a variety of other materials including fern fronds, Selaginella stem, roots of seedlings, flower buds, leaves, &c. The results showed that if the material is not very thick and can be well cleared, the lignified elements take on a very characteristic, bright, reddish-violet colour.

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Material

Striga seeds were sown in a Petri dish with moist blotting-paper at the bottom and one or two grains of Sorghum were placed in the centre and surrounded by a wad of cotton to keep them moist. The Petri dish is covered with a piece of cardboard, slit on one side to allow the Sorghum leaves to come up, and the dish is kept in an incubator at 30° C. in the dark. Within two to three days the Sorghum grains germinate and the main root grows horizontally on the blotting-paper, which is kept moist by the addition of a little water every day. The main root gives rise to many lateral roots. The Striga seeds which lie near the host roots germinate and establish contact with them. The dishes are examined every day and material collected for fixation. It was found possible to procure enough material in the different stages in about two weeks.

Fixation

As materials are to be mounted whole, they should be of convenient size and not larger than is necessary for the particular study. Small lengths, about 0·5 to 1 cm., of the host root with the parasite attached are cut with a pair of sharp scissors and immediately transferred to the fixative. Hillary (1939) has shown that for successful application of the Feulgen technique a fixative containing chromic acid should be used. We have used, with uniformly good results, Nawaschin's fluid both for cytological and morphological studies, and as materials may be left in this fixative for a week or two without any damage, it was used throughout the investigation.

Washing and Clearing

After keeping the material in the fixative for at least 24 hours it is washed in running water for one hour and then passed to 70 per cent. alcohol after keeping in 30 per cent. and 50 per cent. alcohol for one hour each. The material is left in 70 per cent. alcohol at least overnight; this hardens the material and prevents maceration during subsequent treatment. Material which cannot be handled immediately may be stored in 70 per cent. alcohol.

The fixed material is brought down to water after passing through 50 per cent. and 30 per cent. alcohol and given a few changes of water. The method then used is that devised by Schultz (1897) for demonstration of bone in mammalian embryo in which the soft tissues are cleared with potassium hydroxide. The material is left in 1 per cent. potassium hydroxide solution for one to two days. A higher concentration of hydroxide tends to macerate soft tissues. When the material is properly cleared the vascular strands should be easily visible through the outer tissues, which should be almost transparent. The cleared material is then washed in running water for about 30 minutes and is ready for staining.

Staining

This is done by a modification of the Feulgen reaction now widely used for chromosome studies (de Tomasi, 1936). The Feulgen reaction as applied to chromatin involves a chemical reaction in which as a result of a mild hydrolysis of thymonucleic acid contained in the chromosomes an aldehyde is liberated. This aldehyde when brought into contact with decolorized fuchsin sulphurous acid restores the natural colour of fuchsin and thus stains the chromosomes containing the liberated aldehydes. Margolena (1932) has stated that lignin, suberin, and cutin also give a persistent positive reaction with the Feulgen test. Relying on the observations of Mehta (1925), she infers that this positive reaction is due to the presence of aldehydes in the molecules of these three substances. In the present technique this positive reaction of lignin to the Feulgen test is applied in staining the lignified elements of both host and parasite. The most important points on which a successful reaction depends are (1) use of an absolutely colourless 'leuco basic fuchsin', and (2) correct hydrolysis.

Basic fuchsin suitable for this purpose is difficult to obtain. We tried four samples of basic fuchsin, and of these Grubler's special basic fuchsin gave the best result, being superior to the certified stain supplied by Coleman & Bells (certificate No. CF. 17). The stain is prepared in the following way: pour 100 c.c. of boiling distilled water over 0·5 gm. of powdered basic fuchsin in a flask; shake vigorously and allow to cool to 50° C.; filter into a bottle with a ground glass stopper and add 10 c.c. of N/HCl; cool to 25° C. and add 0·5 gm. of potassium metabisulphite. Leave the bottle always well stoppered and in a dark place. The liquid becomes colourless within 24 hours and can be used till it begins to assume a reddish tint due to oxidation.

As shown by Hillary (1939), hydrolysis is a critical part of the procedure and the optimum period of hydrolysis varies with the fixative used and sometimes also with the material. With Nawaschin's fixative, 15 to 20 minutes hydrolysis in dilute HCl (8 c.c. of concentrated HCl of s.g. 1·19 to 100 c.c. of distilled water) at a constant temperature of 60° C. in an electric incubator was found the best for the materials used in the present study. Material which is ready for staining is transferred from water to cold HCl of the same strength as used for hydrolysis. After 1 or 2 minutes it is transferred to dilute HCl at 60° C. and hydrolysed for 15 mins. Then the acid is poured off and some decolorized fuchsin solution, prepared as detailed above, is added. The material is left in this solution from 6 hours to overnight. Then it is washed in running tap-water for about 10 minutes and then given two or three changes of water made slightly alkaline by the addition of a few drops of 1 per cent. KOH solution; this treatment intensifies the stain. It is found advantageous to omit the usual washing in SO_2 water, as the retention of a very light stain in the cellulose walls is found desirable. The material is then rinsed in distilled water and passed up rapidly through alcohol grades of 30, 50, 70, and 95 per

cent. strength, allowing about 3 minutes in each. Then it is changed to absolute alcohol, then to xylol-alcohol, keeping in each for about 5 minutes and finally taken to pure xylol. After leaving in xylol for 1 hour the material is mounted in thin canada balsam. The slide is placed horizontally on a hot plate at about 40° C. for a few hours with a 5 gm. weight on the coverslip. This helps to flatten slightly the material and is useful for certain types of observations. More balsam is added at the edge of the coverslip if necessary.

Comments

Previous studies on the structure and development of haustoria of *Striga* give only details of certain stages, as they have been mainly based on serial sections (cf. illustrations of Stephens (1912) and Saunders (1933)). The advantages of the present technique are: (1) it is possible to follow all the stages from germination of the *Striga* seed to the development of the adult parasite; (2) a complete and clear idea of the type and degree of haustorial penetration at any stage of development of the parasite on the host root can be obtained; and (3) it is easy to make comparative studies under controlled conditions. However, it is not to be expected that this technique, though facilitating anatomical examination of unsectioned material, will completely eliminate the need for section cutting; it reduces considerably, however, the latter type of work and is distinctly superior to it for the type of observations mentioned above. Another advantage is that material which has been stained and brought to the xylol stage can be examined under the microscope, rough diagrams made of the haustoria, and then, if desired, can be taken to paraffin for embedding in the usual manner and serial sections made which can be counterstained with light green or any other suitable stain and examined. These observations together with those made prior to embedding the material give a much clearer idea of the anatomy than can be obtained from serial sections alone.

It is hoped that this technique, with modifications where necessary, will prove to be of wider application. Plate V shows photomicrographs of preparations made by this method. Brief notes on the anatomical features are given in the explanation to the plate. A detailed anatomical account will be published when the studies now in progress are completed.

Summary

A new technique for the anatomical study of root parasitism is described. It involves fixation of the material in Nawaschin's fluid, clearing in 1 per cent. potassium hydroxide solution and then staining by a modification of the Feulgen technique.

The advantages of this technique over the usual method of sectioning are indicated.

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EXPLANATION OF PLATE V

Illustrating the article by L. S. S. Kumar, A. Abraham, and S. Solomon, on 'A Technique for the Anatomical Study of Root Parasitism'.

The photomicrographs are mounts of portions of Sorghum root with *Striga* seedlings in various stages of parasitic development upon it. Figs. 1–6 and 10 are of *Striga lutea* while Figs. 7–9 are of *S. densiflora*.

Fig. 1. *Striga lutea*. The seed has germinated and the end of the radicle which forms the primary haustorium has penetrated the cortex of the host root. The cotyledons are still enclosed by the seed coat. Some idea of the transparency of the preparation can be obtained from the fact that though the Striga radicle passes below the Sorghum root it is very clearly visible. (× 40.)

Fig. 2. The left-hand portion seen in the above figure under higher magnification. Under this focus it would appear that the haustorium is not in contact with the vascular cylinder of the host, whereas in another focus (see Fig. 1) it is clearly seen to be in contact with it. (× 130.)

Fig. 3. The haustorium has enlarged; vascular elements are developed in the parasite and close contact with the vascular elements of the host is established, though no penetration into it has taken place. The origin of a branch, which later develops into a secondary haustorium, is seen as a lateral outgrowth to one side of the primary haustorium. (× 80.)

Fig. 4. A later stage after the cotyledons and plumule have come out of the seed coat. Haustorial cells have penetrated into the vascular tissue of the host. The first pair of leaves are seen as rudiments. (× 80.)

Fig. 5. Still later stage showing deeper penetration of the host tissue by the haustorial cells. (× 80.)

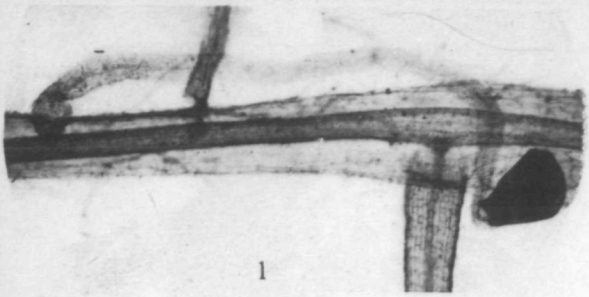
Fig. 6. Portion of the above under higher magnification to show more clearly the haustorial connexion. (× 160.)

Fig. 7. *Striga densiflora*. A portion of the host root attacked by two *Striga* seedlings from opposite sides showing that penetration of a seedling by one haustorium does not interfere with the penetration by another. The seedling on the upper side is apparently older than the one below, in which the cotyledons are still enclosed by the seed coat. (× 50.)

Fig. 8. The haustorial connexion of the above under higher magnification, showing clearly the tracheides of the parasite and 'haustorial hyphae', which are connected to the tracheides and have deeply penetrated the host vascular tissue. (× 160.)

Fig. 9. Another specimen of *S. densiflora* showing the very deep penetration by the haustorial cells; note the swollen and branched ends of these cells. From comparison of this with *S. lutea* of the same stage (see Fig. 10) it is seen that *S. densiflora* shows much deeper penetration of the vascular tissue; a fact which accounts for the observation that the latter is more injurious than *S. lutea* in its attack on the host. (× 160.)

Fig. 10. *S. lutea*. Haustorial region magnified, showing clearly the lignification of the xylem elements of both host and parasite. A comparison with Fig. 9 shows that the penetration in this case extends only as far as the centre of the vascular cylinder of the host. (× 160).



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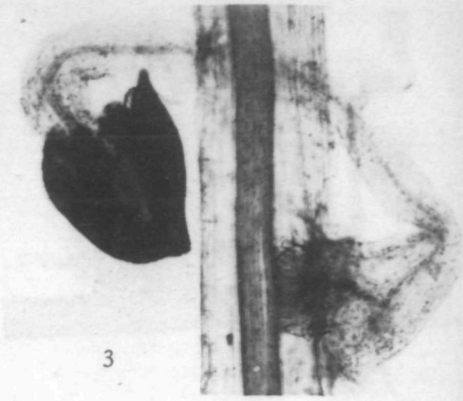

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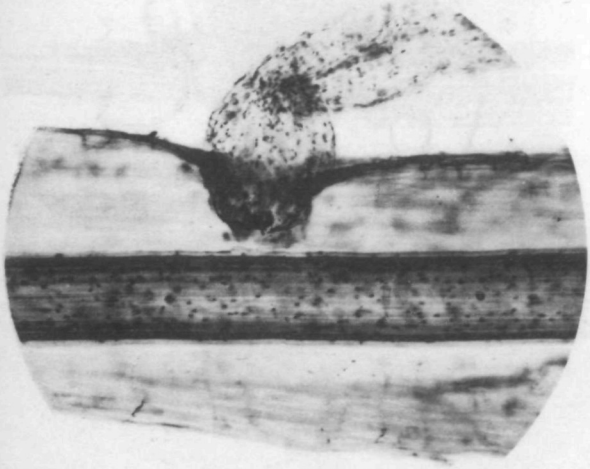

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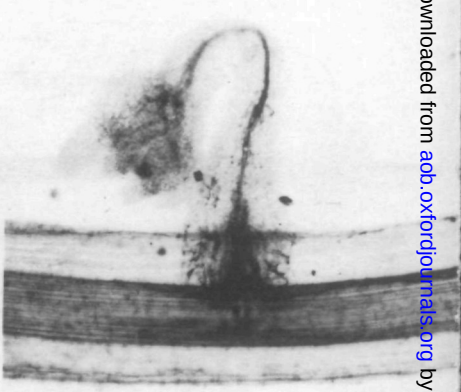

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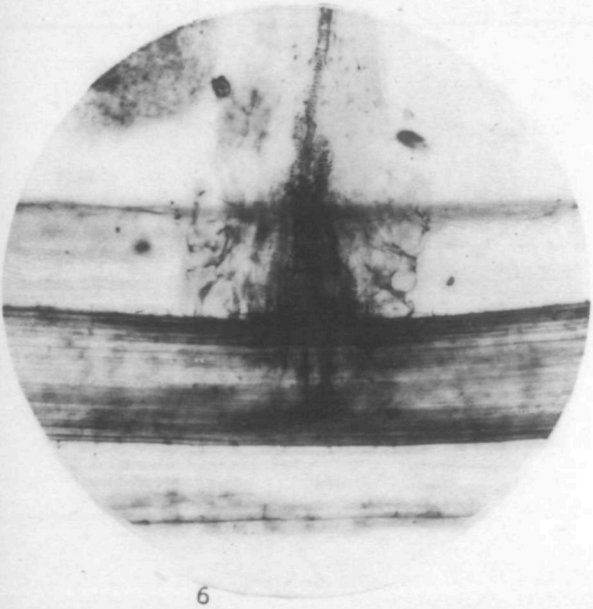

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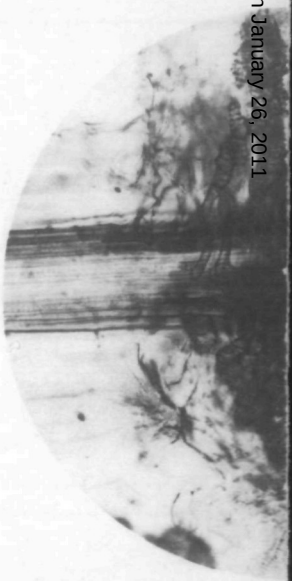

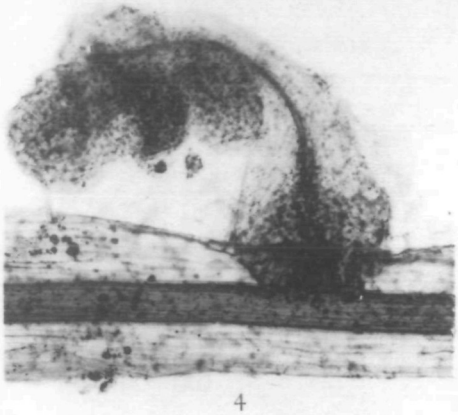

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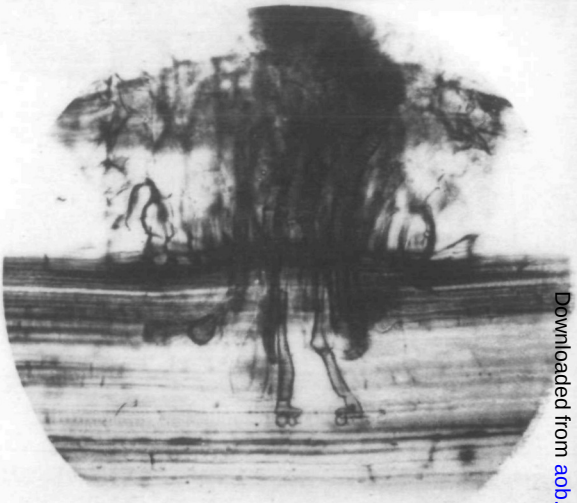

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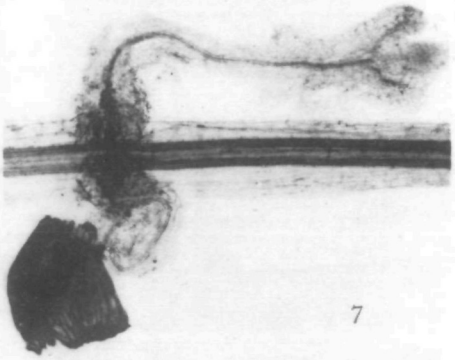

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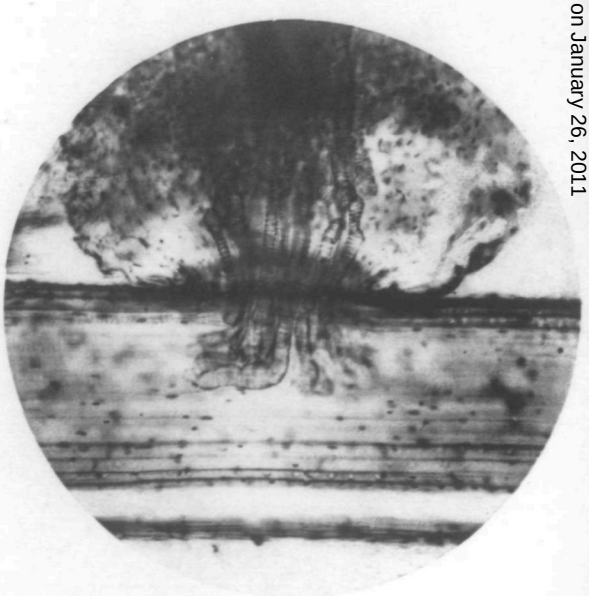

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