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# THE POSTGENITAL FUSION IN THE GYNOECIUM OF TRIFOLIUM REPENS L.: LIGHT AND ELECTRON MICROSCOPICAL ASPECTS

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SUMMARY

The postgenital fusion between the carpellary margins in the gynoecium of *Trifolium repens* L. was studied in different ontogenetical stages by means of light and electron microscopy. The results of both methods are correlated.

With light microscopy the place of fusion could be inferred by histogenetical analysis of serial sections. With electron microscopy the suture was determined directly by remnants of the enclosed cuticles. At the suture a pseudo-middle lamella was present that was more susceptible to chemical treatment than the normal middle lamella.

## 1. INTRODUCTION

The position of a postgenital fusion of plant parts can become obscured during ontogeny because the characteristic zigzag pattern of interlocking epidermal layers is disturbed by growth and cell divisions (BAUM 1948). In older stages it can even be open to doubt whether a postgenital fusion has occurred at all. Study of different ontogenetical stages can make this clear, and histogenetical analysis can serve to locate the fusion, even in mature stages. (MOELIONO 1970). Another means of locating a postgenital fusion is to search the tissue for persistent characteristics of the epidermal cells that have been enclosed during fusion. In *Capsella*, for instance, electron microscopical location of a series of cuticular fragments permits pinpointing of the suture in the gynoecial septum in all stages of ontogeny (BOEKE 1971).

This paper presents the results of a study of the postgenital fusion of the carpellary margins in the pod of *Trifolium repens*. Contrary to *Capsella*, in this case there is no cellular breakdown at the place of fusion, nor significant cell wall thickening, so histogenetical analysis can be performed.

The location of the fusion inferred by light microscopy is compared with the results obtained from electron microscopy.

## 2. MATERIAL AND METHODS

Inflorescences of *Trifolium repens* L. were collected along the roadside. For light microscopy the material was fixed in FPA, dehydrated in tertiary

butyl alcohol and embedded in Paraplast. Serial sections of 7 μm, both transverse and longitudinal, were stained with astra blue and safranin. The drawings were made with a Wild camera lucida. For electron microscopy gynoecia were isolated and, if possible, opened lengthwise to promote fixation. Fixation was done with unbuffered 2% $OsO_4$ for 15 min at room temperature. After dehydration in an ethanol series the material was embedded in Epon 812 via epoxypropane. Pectic substances were contrasted during dehydration (ALBERSHEIM & KILLIAS 1963): after 50% ethanol the material was transferred for 1 hr to a freshly made 1:1 mixture of a 14% solution of hydroxylamine – HCl and a 14% solution of NaOH, both in 60% ethanol. Then the material was washed for 3 × 5 min with 0.1 N HCl in 60% ethanol and transferred for 1 hr to a 2.5% solution of $FeCl_3$ in the previous fluid. Then dehydration was continued with 70% ethanol. Sections were post-contrasted with uranyl acetate and lead citrate (REYNOLDS 1963) and examined in a Philips EM 100B.

## 3. RESULTS

### 3.1. Light microscopy

The gynoecium of the Leguminosae is normally composed of a lengthwise folded carpel, the margins of which fuse postgenitally.

In the case of *Trifolium repens* L. fusion begins after initiation of the ovule primordia, when the gynoecium has a length of about 0.5 mm. The carpellary margins grow together by periclinal divisions in the subdermal layer. *(fig. 1A, 2A)*. When the epidermal layers make contact, their outer cell walls are flattened *(fig. 1B, 2B)* and interlock so that no intercellular space is visible between them *(fig. 1C, 2C)*. The margins are now fused according to the definition of BAUM (1948, p. 87), because no cuticle can be distinguished at the suture between the epidermal layers. During subsequent growth of the gynoecium to a maximum length of about 7 mm the fused epidermal cells do not change much in shape and size, except for an occasional periclinal division *(fig. 2D)*. In contrast to this, the free epidermal cells increase in size and even grow papillose near the place of fusion at the interior of the gynoecium *(fig. 1D, 2D)*. The mesophyll of the gynoecial wall is for the greater part disrupted during growth, but it stays intact between the ventral vascular bundles where the place of fusion is situated.

After the carpellary margins have fused, the periclinal divisions in the subdermal layer continue *(fig. 2C, D)*; by this and by the development of the ventral vascular bundles and the funiculi the suture can be deformed and therefore become more difficult to locate. In transverse sections the approximate position of the suture is always easily found, because the ends of fusion are marked by deep infoldings in the inner and outer epidermis. In both transverse and tangential sections the suture can be inferred, even in advanced stages, by analysis of the arrangement, shape, and size of the cells: the fused cells constitute a double interlocking row; they are isodiametric or slightly elongated and of fairly uniform size, except when a periclinal division has taken place. The subdermal

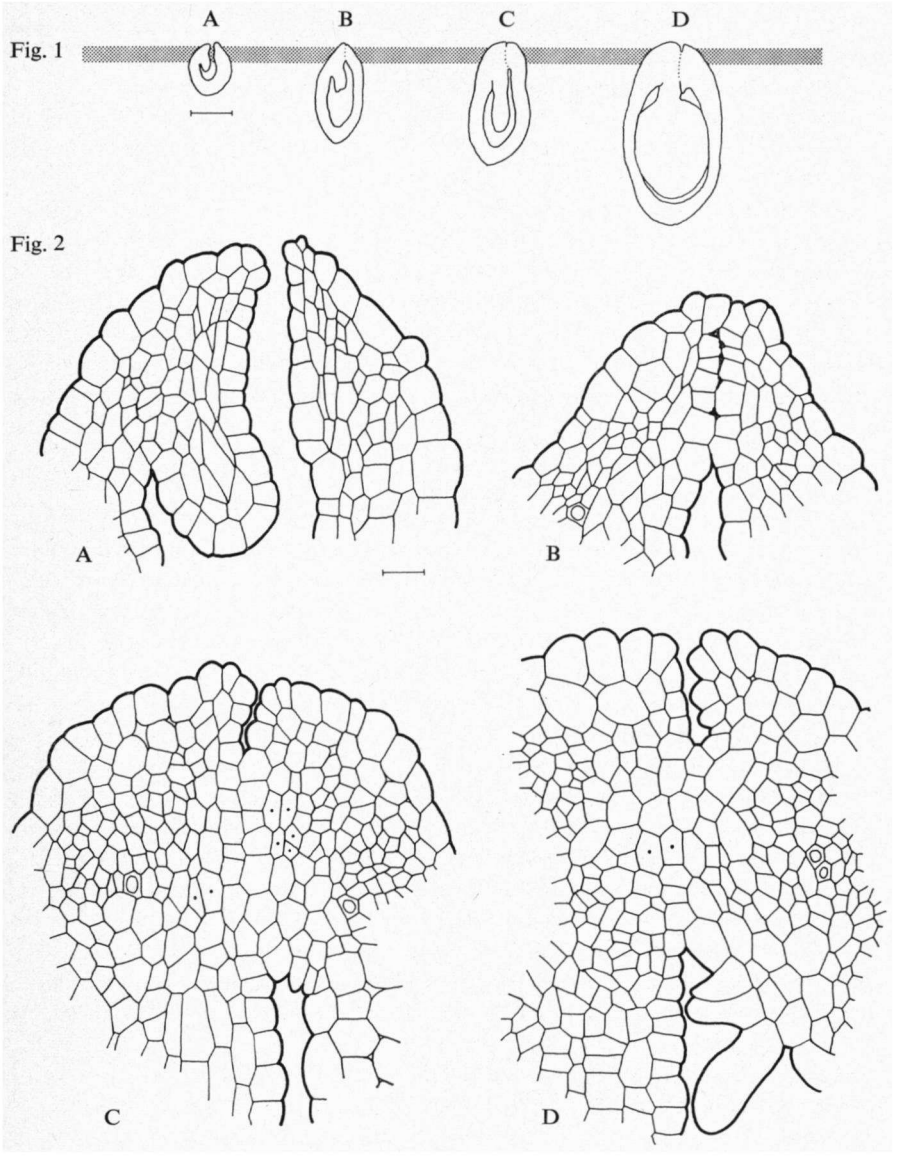


Fig. 1. Transverse sections, at the level of the basal ovule (primordium), of gynoecia in different developmental stages, from before fusion to nearly mature. Line = 0.1 mm.
Fig. 2. Details of the place of fusion in fig. 1. 2A relates to 1A, etc. Line = 10 μm.
A. Conspicuous periclinal divisions in the subdermal layer.
B. The carpellary margins have just touched.
C. Fusion complete. Some divisions in the subdermal layer indicated. Differentiation of ventral bundles conspicuous. Infoldings of epidermis mark end of fusion.
D. Periclinal division in dermal layer indicated. Inner epidermis papillose.

cells show a more irregular arrangement with frequent periclinal divisions; they are mostly elongated and variable in size, mostly longer and narrower than the fused cells. It should be remarked here that these three-dimensional characteristics are easily seen in serial sections but can only partly be reproduced in a drawing. A drawing is therefore mostly less convincing than the section it is derived from.

When a mature gynoecium is boiled in a 1:1 mixture of glacial acetic acid and 30% hydrogen peroxide, to dissolve all cell wall components (e.g. middle lamellae) except cellulose (SASSEN 1965), the fused cell walls are the first to become detached from each other, clearly revealing the place of fusion; the bond between the fused cells seems especially susceptible to this treatment (see Discussion). N.B. *Trifolium repens* has an indehiscent fruit; the seeds are freed by decomposition of the gynoecial wall, not by opening of the gynoecium along the suture.

### 3.2. Electron microscopy

At the time of fusion of its margins the carpel is already covered by an osmiophilic cuticle of about 10 nm. During fusion the cuticles on the marginal epidermises are pressed together; they do not merge but remain separated by an electron transparent line of 10–20 nm *(fig. 6, 7)*. Just after the epidermal layers have made contact, the enclosed cuticles clearly mark the suture *(fig. 3)*. Soon, however, the cuticles begin to break up into ever smaller and more widely spaced fragments. Consequently, in advanced stages of fusion the location of the suture by means of the cuticles is impaired. A factor that contributes to this is the decreasing osmiophily of the cuticles, probably as a result of progressive polymerisation of the cutin (compare *fig. 6, 7)*.

Contrasting of the pectin in the tissue (see Material and Methods) reveals an electron dense layer under the cuticle that looks like, and is continuous with, the middle lamellae between the anticlinal walls of the epidermis *(fig. 4)*. In places where the cuticles are absent the layers of the opposite cell walls merge and form a seemingly homogeneous pseudo-middle lamella. Cuticular fragments and intercellular spaces stand out in this layer as electron transparent, often spindle-shaped islets *(fig. 5, 7)*. Location of the suture is facilitated by this.

No plasmodesmata were observed in the fused cell walls; in the other walls they were infrequent.

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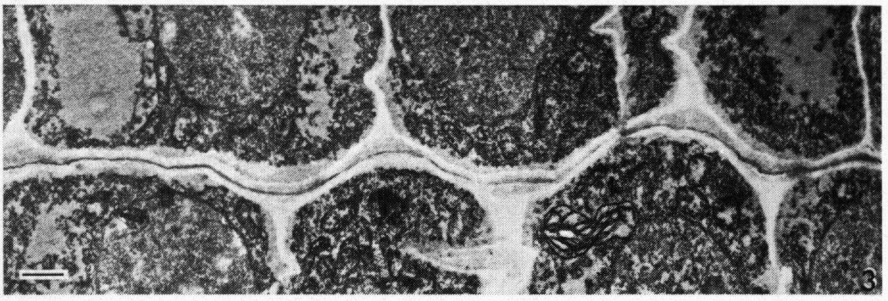
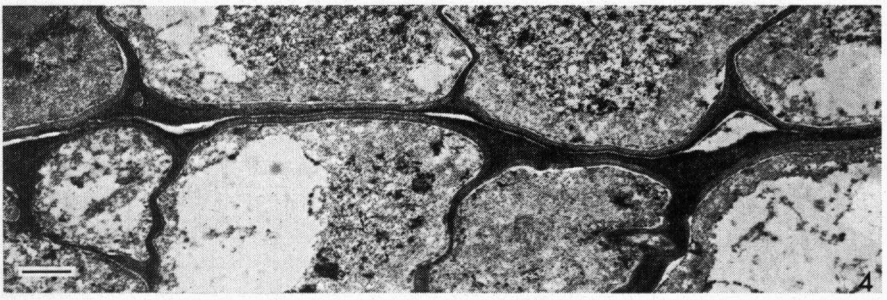
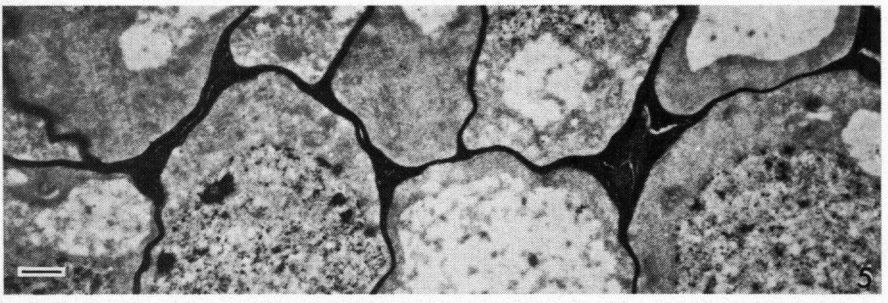
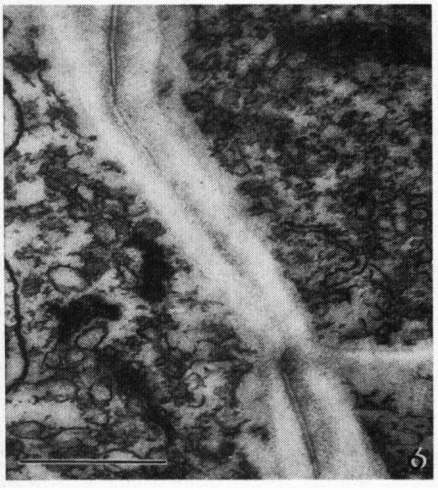
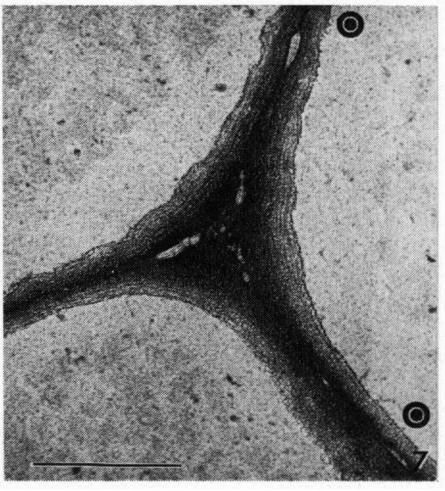
Fig. 3–7. Electron micrographs of transverse sections of different gynoecia. Line = 1 μm.
Fig. 3. Length 0.6 mm. Early stage of fusion: suture indicated by nearly unbroken cuticles.
Fig. 4. Length 0.4 mm, pectin contrasted. Fusion not complete, compare with *fig. 2B*. Middle lamella and pectin layer under cuticles conspicuous.
Fig. 5. Length 2.2 mm, pectin contrasted. Electron transparent islets in the pseudo-middle lamella indicate suture.
Fig. 6. Detail of *fig.* 3, turned 90°. Cuticles separate, beginning of fragmentation.
Fig. 7. Length 4,8 mm, pectin contrasted. Suture from 0 to 0. Note islets with double cuticular fragments and homogeneous pseudo-middle lamella.











## 4. DISCUSSION

The results of this study prove that the place of fusion of the carpellary margins in *Trifolium repens* can be determined, even in advanced stages, by light as well as by electron microscopy. This shows that even in the "fully uniform" tissue BAUM (1948 p. 92) described at the place of fusion when fusion is complete (e.g. in *Papilionaceae*), it is in fact still possible to locate the suture.

There are, however, some conditions that are to be fulfilled if location in advanced stages of development is to be successful:

1. For light microscopy the characteristics by which the place of fusion can be inferred should remain manifest during development. For histogenetical analysis the tissue around the place of fusion should remain intact. In *Capsella*, for instance, with a clear postgenital fusion, no histogenetical analysis can be performed because nearly all the mesophyll around the place of fusion breaks down early in ontogeny (BOEKE 1971). The remaining tissue forms a cell bridge between the free epidermises and acquires thickened cell walls, so the cell form is somewhat obscured. But the location of the suture can still be inferred from small infoldings in the epidermises and by the characteristic interlocking of the fused cells. In *Trifolium* histogenetical analysis is possible as is shown in the light microscopical results. The relevant characteristics of the tissue, for instance the periclinal divisions in the subdermal layer bordering the fused layers, can be traced in all stages to maturity, and permit the inference of the position of the suture.
2. For electron microscopy the presence of a cuticle on the fusing parts seems necessary as this is the only feature available for locating the suture that persists throughout development, at least in the species examined and with the methods used. The same is true for *Capsella*.

Correlation of the results obtained with light and electron microscopy presents the problem that the results are based on fundamentally different methods. For light microscopy serial sections are analysed so the three-dimensional structure of the tissue is understood. From this the location of the suture can be inferred. Electron microscopy, on the other hand, makes use of extremely thin and small sections, with which it is impossible to gain an idea of the three-dimensional structure of the tissue. The place of the suture is determined directly, in a way that is impossible with light microscopy (see BAUM 1948; TEPFER 1953, p. 535).

In *Trifolium* comparison between light and electron microscopical results is facilitated, because the folds in the inner and outer epidermis marking the ends of fusion in transverse sections can be distinguished with both methods. The folds are connected by a series of cuticular fragments along a line that has the same characteristics as the light microscopically inferred suture (following a generally straight or curved course, without straying more than one cell diameter from this course).

The behaviour of the cuticle during fusion is nearly identical in *Capsella* and *Trifolium*. The same arguments for growth as the disruptive factor of the cuticu-

lar layers apply to both genera (BOEKE 1971). Increase in length of the ovarial part of the gynoecium of *Trifolium* is about sixfold, from the beginning of fusion to maturity. The stylar part may increase up to twentyfold in length.

An interesting feature of the bond between the fused cells is its sensitivity to boiling with acetic acid and hydrogen peroxide, as described under the light microscopical results. A possible explanation for this is that the interruptions in the pseudo-middle lamella between the fused cells (*fig. 5, 7*) make this layer weaker and more accessible to the reactive mixture than the normal middle lamellae.

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