LEAF MOVEMENT IN THE CARNIVOROUS PLANT DR*OSERA CAPENSIS*

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Front cover: Fluorescent image of a *Drosera capensis* tentacle in the unstimulated state showing polymerised actin (green) and chloroplast autofluorescence (red). Zeiss 410 LSM Confocal Microscope.

Key-words: Plant movements, actin, phalloidin, cytochalasin B, *Drosera capensis*, turgor, polymerisation, depolymerisation.

ABSTRACT

Two mechanisms for the movement of Drosera capensis leaves in trapping prey were proposed: turgor related cellular changes and the direct or indirect influence of actin filaments. Microscopic examinations of cellular and ultrastructural organisation in folded and straight leaves revealed no significant distinguishing features between the tissues, other than the presence of folded cell walls in curled leaf sections. This qualitative assessment shows no conclusive evidence for a turgor based movement but the need for a quantitative approach is recognised. The influence of actin polymerisation on leaf folding was determined using cytochalasin B (CB), a polymerisation inhibitor and FITC-phalloidin to visualise polymerised actin by fluorescent microscopy. Actin filaments are not essential in leaf folding, as the movement is unchanged when polymerisation is inhibited using 100μM cytochalasin B. The drug indicates enhanced depolymerisation that may stimulate movement indirectly by activating ion channels. A possible mechanism is discussed.
The dramatic increase in filaments in curled relative to straight control leaves may be linked to secretion and absorption stimulated by prey capture. Filaments occur in close association with and may translocate vacuoles required to sustain these processes. This is supported by the finding of diminished digestive activities in leaves exposed to CB.

INTRODUCTION

The power of movement is not restricted to animals or protozoans but also exists in many plant families. Despite being rooted and therefore sessile, plants are not static. The active movements observed in their vegetative and floral parts occur for various reasons: Stomata open and close to avoid water loss and to take up carbon dioxide [10,17], leaves and flowers track solar movements to maximise or minimise irradiation of their surfaces[8] and *Mimosa pudica* collapses its leaves when touched [12,13,33]. Yet, some of the most striking examples of plant motility are found amongst the carnivorous plants. These have evolved different trapping mechanisms which are activated by external stimuli emanating from their insect prey. The focus of this study will be on some of the cellular processes governing the trapping movements of *Drosera capensis* (Droseraceae).

Most members of the Droseraceae (including the well-known *Dionaea muscipula*, Venus Fly-trap) display active movement in catching their prey[8,9,18,40]. In *D. capensis,* the response consists of two distinct phases - a fast initial movement followed by a slower one [18,40]. The trap action is described as the 'adhesive flypaper' strategy[18] since the upper leaf surface is covered in numerous stalked glands (tentacles) tipped with mucilage. Small flying insects become entangled in this sticky substance, and in their struggle to escape, come into contact with many tentacles. Such mechanical stimulation produces receptor and action potentials within the tentacles and induces their bending around the insect [30,39,40]. This nerve-like reaction is usually completed within five to twenty minutes[30]. The second, slower phase of trapping is characterised by a chemically-induced response and takes several hours to complete[3]. During this time, the tentacles continue bending more gradually, while the entire lamina starts enfolding the prey. These actions are caused by the release of chemicals from the prey and are under hormonal regulation[3,18,22].

Although the stimuli eliciting the response have been studied in some detail [3,30,39], little is known on how leaf folding is actually accomplished. Growth has been implicated in the action, since differential expansion of upper and lower leaf surfaces may promote bending of the lamina [3,9,22,31,34]. This argument is supported by an increase in the length of the fully-grown leaf once unfolding is complete[22]. While growth may ultimately be a part of the trapping movement, it is only the expression of various processes occurring at a cellular level. The rapidity of the response suggests that these may involve a complex series of events. They could be linked to a growth phenomenon or may be acting independently to produce movement in a different way. Such processes need to be elucidated. Owing to the lack of studies on *D. capensis* itself, an exploration of the work on motility in other organisms is essential. This yields further clues on the nature of movements and the control thereof.

Most studies in the plant domain focus on leaf movements in sensitive plants of the Leguminoseae and Oxidalaceae [33], such as *Mimosa pudica* [13,23,33], and species of *Phaseolus*, *Albizzia* and *Robinia* [24]. Trap closure in *Dionaea muscipula* has also received considerable attention [11,16,18,38]. Despite some controversy surrounding various details, these investigations converge on a turgor-based mechanism for the different motions. Alterations in the turgidity of cells can be reversible or irreversible [21,33,38]. Growth, as observed in *D. capensis*, can be explained in terms of irreversible turgor changes. These would first be initiated in the lower epidermis to bend the leaf upwards. The same process would take place in the upper epidermis to unfold the leaf again.
In *D. muscipula*, complex changes in cell volume occurring in specific tissue regions of the trap lobes are associated with different phases of trap closure [11]. Some disagreement revolves around the issue of whether cell enlargement is facilitated by acidification of the cell walls to increase their plasticity ('acid-stimulated growth') [38] or by the release of tension in mesophyll tissue that normally keeps the traps open [16].

Turgor also plays a key role in the foliar movements of sensitive plants[33]. Two major groups of cells within the motor organs (pulvini) undergo osmotic variations [12,21,24]. Depending on whether the leaves are raised or lowered, the 'extensor' or the 'flexor' region gains water. The changes in cell volume are regulated by ion fluxes (primarily potassium, K^+ and Cl^-) are under hormonal control [7,21,33]. On the basis of plant studies, turgor changes can be assigned central importance in producing movements of various kinds. Leaf folding in *D. capensis* could be based on these principles.

An interesting idea, worth pursuing, is that actin may have an important influence on movement in plants. The suggestion is stimulated by findings that actin microfilaments, best known from muscle cells, are also responsible for intracellular and cellular motility in organisms lacking muscles [1,15,20]. Movements such as 'cytoplasmic streaming' [1,26,27,28] and the directed translocation of particles or organelles, amoeboid or flagellate locomotion, changes in cell shape and the intracellular restructuring of the cytoplasm during immune responses largely depend on labile structures formed by microfilaments[1,20]. Different isoforms of actin are present in most eukaryotic cells, including those of vascular plants where actin is an integral part of the cytoskeleton[14,23]. Two states of the protein can usually be identified in intact cells, namely G-actin (free monomers) and F-actin (polymerised filaments of varying length). Several actin-binding proteins cause the interconversion between the different states and facilitate the aggregation of microfilaments into bundles (stress fibres) or networks [1,20,35].

During polymerisation, the assembly of isolated actin molecules preferentially occurs at the 'barbed end' of polar filaments and is associated with ATP hydrolysis [1,4,20]. There are various indications that the dynamic interconversion between actin in different states is elemental in generating movement [8,17,37] in some non-muscle systems, although it is not true for all [2,12]. However, it has been noted, for example, that the site of movement, such as the leading edge of migrating cells, usually corresponds to the region of local actin polymerisation [1,35]. An excellent way to

visualise this is by using a fluorescent derivative of phalloidin [1,4,29,41]. This toxin, isolated from the mushroom *Amanita phalloides* binds to polymerised actin with high specificity and prevents disassembly[5,35]. It has been successfully used in demonstrating the presence and distribution of microfilaments in a variety of plant and animal cells capable of movement[2,10,22,23,37].

Strong evidence for the importance of actin polymerisation in mobility also emerges from experiments testing the action of drugs that destabilise actin filaments [1,13,17,25,35]. Cytochalasin B, a fungal metabolite, has been extensively used for this purpose[15] despite some controversy and conflicting reports regarding its mode of action. However, it is known to prevent the assembly of monomers into F-actin and disrupt microfilaments, thereby causing the cessation of movements associated with different processes[1,6,15,20].

The influence of actin on plant movements may be direct or indirect but a mechanism has yet to be demonstrated. Consequently, the literature is primarily based on speculation. Actin is unable to cause motion by itself, so a direct effect would only be possible in conjunction with other cellular components (e.g. myosin)[15]. It has been proposed that such an interaction in non-muscle systems could lead to similar shear-generated forces as those created in muscle cells [15]. Alternatively, actin may be indirectly involved in plant movements through its regulation of other functions that do produce movement. Most notably, these would include the two factors that previously mentioned in connection with motion - reversible turgor changes and growth.

The opening and closing of stomata is associated with distinct changes of the actin network within the guard cells that control the movement. The opening response, when guard cells increase in volume, is accompanied by depolymerisation[17]. Polymerisation occurs during the reverse process. The authors suggest that actin filaments modulate the activity of ion channels and thereby regulate stomatal movements[17].

Growth based on turgor-dependent cell expansion can be similarly affected due to the potential effect of actin on turgor regulation. In addition, the growth reduction in

wheat coleoptiles may be the result of the disruption of microfilaments provide the motive force for the transport of secretory vesicles to the cell wall [32]. It has also been shown that interference of the controlled interconversion between different states of actin causes a notable reduction in growth of cultured animal cells [37].
There are, however, conflicting reports regarding the role and impact of actin on growth and movement and several questions remain to be answered (e.g the mechanism of actin).

In view of these aspects, it is proposed that actin plays an important part in mediating the trapping response in *D. capensis*. It may act directly through force-generation or indirectly by affecting a turgor-based mechanism of leaf folding. The approach taken to investigate this can be divided into two parts.
Firstly, anatomical and ultrastructural features in stimulated (curled) and unstimulated (uncurled) *D. capensis* leaves will be examined. The aim is to identify obvious differences that may be attributed to a certain mechanism of movement, such a turgor changes. The relevant features in this regard are changes in the size of cells or their number and sub-cellular rearrangements.
Secondly, the role of actin in the movement of D. capensis will be determined with the aid of cytochalasin B that disrupts filaments and phalloidin which binds polymerised actin. Treatment of leaves with cytochalasin with the depolymerising agent should inhibit leaf folding if actin is crucial for the movement. The distribution and organisation of actin in cells folded, straight and cytochalasin-treated *D. capensis* leaves will be visualised by means of fluorescent microscopy. If the protein is involved in the movement, clear differences in these aspects should be seen.

METHODS

Plant material

Drosera capensis plants were obtained from a carnivorous plant nursery and kept in a greenhouse under natural light conditions and at temperatures of 18 - 22 °C. Insects were excluded by subtended fly-traps. The plants were watered regularly to keep them constantly moist. Young, fully expanded leaves that were producing copious mucilage around their stalked glands were used in experiments. In all cases, uncurled leaves that had never been stimulated represented the experimental controls.

Scanning Electron Microscopy (SEM):

External structure of *Drosera* leaves

A leaf that had been stimulated with a mosquito 12 hours prior to the experiment and an unstimulated control leaf were used for scanning electron microscopy. Each specimen was immersed in liquid nitrogen slush to draw the water out of the cells and mounted on a stub and sputtercoated with gold palladium to a thickness of 30 nm. The samples were viewed under a LEO Stereoscan 440 scanning electron microscope at 3kV.

Transmisssion Electron Microscopy (TEM):

Cell ultrastructure in curled and uncurled leaves

Fixation: Three uncurled and three curled *D. capensis* leaves were excised to be prepared for transmission electron microscopy. The central portion of the straight leaf and the curled part of the stimulated leaves were cut into small blocks (3 * 3 mm) in sodium phosphate buffer (pH 7.2, conc. 0.1M). The tissue was fixed overnight in 2.5% gluteraldehyde in 0.1 M $NaPO^4$ buffer (pH 7.4) and 0.5% caffeine. Thereafter, the samples were washed three times for 5 minutes (3 * 5 minutes) in 0.1 M phosphate buffer. They were postfixed in Osmium (1% v/v solution in phosphate buffer) for 1 hour. This was followed by washing in phosphate buffer and then water, each 3 * 5

minutes. The tissue blocks were placed in a dehydration series of 30, 50, 70, 90 and 95 % ethanol. They were left in each solution for 5 minutes and the process was repeated. Washing took place in 100% ethanol and in 100 % acetone (each 2 * 10 minutes).

Infiltration and embedding: The samples were transferred into a mixture of 50 % Spurr's resin[36] and 50 % acetone and left to rotate overnight. The acetone was then replaced with resin over 3 days. The samples were embedded in resin blocks and left in an oven at 60 °C for 16 hours.

Sectioning and staining: The samples were sectioned on a Reichert Ultracut-S ultramicrotome and placed on grids. Staining was done for 10 minutes in a mixture containing equal volumes of uranyl acetate (2% solution in 70% ethanol) and Reynold's lead citrate.

Cytochalasin B assay

Effects of actin depolymerisation on leaf response to prey

Twenty leaves (straight, unstimulated) were cut off at the base of the petiole and placed with their stalks dipping into 1.5 ml eppindorf tubes (with a hole through the lid) containing distilled water. They were left for 2 hours until the tentacle-curling response to excision had relaxed. Cytochalasin B (CB, Sigma Chemical company) was dissolved in 0.75% Dimethyl sulphoxide (DMSO) to obtain a 100 μM solution. The pH was adjusted to 5.8 using NaOH and HCl. A control solution of 0.75% DMSO (v/v) and a distilled water control were also prepared. Once the leaves had relaxed, the water in the eppindorfs was replaced with the CB solution in 10 tubes, with 0.75 % DMSO in 5 control tubes and with distilled water in the remaining 5 tubes. Two hours later, a 'prey item' (mealworm piece, 3 mm long) was placed in the middle of each leaf. Five of the 10 leaves in CB solution were left unstimulated for 21 hours to allow for prolonged uptake of the solution. This was to check whether leaf response in the CB treatment differed after a longer period of uptake. The same procedure as for the other leaves was subsequently used. The degree of curling in all leaves was recorded 15 hours after stimulation with prey and photographs were taken using Kodak Pan-F film. Once the

control leaves had fully curled, all solutions were replaced with water to check for the reversibility of the action.

An estimate of the natural uptake rate in *D. capensis* leaves was established by placing three unstimulated leaves in eppindorfs containing a solution of ink in water (one drop/1.5ml).

Phalloidin experiment - fluorescent microscopy

Microfilaments of polymerised actin in curled and uncurled leaves

FITC-labelled phalloidin (Sigma Chemical company) was used to visualise actin microfilaments in *D. capensis* leaves. The toxin was reconstituted in 100% methanol and diluted to $4 * 10^{-6}$ M in buffer. This solution was used to stain leaf sections. The buffer was prepared from 0.01 M PO_4^{2-} buffer (pH 7.2), 0.01 M EGTA, 0.1 M $MgSO_4$, 1% (v/v) Triton-X, 1% (v/v) DMSO and 2% (v/v) methanol.

Three leaves of *Drosera* plants were stimulated with prey (3 mm long mealworm pieces) and excised when fully curled while three uncurled leaves were also cut off. In addition, three leaves that had been exposed to CB for 21 hours and then stimulated, as described for the CB experiment, were also prepared for fluorescent microscopy. The middle part of each uncurled and the folded section of each curled lamina were cut into 4 - 5 mm long pieces. These were immediately fixed in a glutaraldehyde-paraformaldehyde fixative (Karnovsky solution[19]) for 1 hour at room temperature. Transverse and longitudinal sections (approximately 5 to 10 μm thick) were cut on a freezing microtome (Wetzlar, E. Leitz).

The sections were mounted on microscope slides and each immersed in a drop (10μl/section) of phalloidin solution. The slides were incubated at room temperature in the dark for 1.5 hours. This allowed phalloidin to penetrate the cells and bind polymerised actin and for fluorescence to develop. Washing of the sections in buffer (30μl/section) was done at room temperature and repeated twice for 5 minutes.

The samples were mounted in the medium CITI-Fluor (glycerol/PBS solution) and viewed under a Zeiss LSM 410 confocal microscope using appropriate filters to isolate fluorescence from specific sources. With the FITC label, polymerised actin fluoresces green (excitation wavelength of 488 nm) and chlorophyll auto-fluorescence is red (excitation wavelength of 568 nm). The emissivity of green was 515 - 540 nm (band pass) and of red 590 nm (long pass). The following settings were employed on the microscope: Attenuation 10, Pinhole 20 with the 25* objective and 24 with the 63* objective. To allow for comparison in the intensity of fluorescence in uncurled, curled and CB-treated leaves, the settings were kept constant throughout viewing. Brightness and contrast were also not readjusted for different treatments.
Images that were taken of fluorescing sections were stored on an optical disc. This was linked to a 3-D workstation equipped with the Carl Zeiss LSM 410 confocal programme. Images were printed directly onto Kodak photographic paper using a Hewlett-Packard printer. The optical disc is stored for record in the Archives of the Medical Research Council, MRC, Tygerberg, South Africa)

Light Microscopy

Sections of three curled and three uncurled control leaves were prepared, fixed and sectioned as previously described for phalloidin work. The sections were mounted on microscope slides in a glycerol-phenol mounting medium. They were examined under a Zeiss compound light microscope at 10, 40, 250, 400 and 1000* magnification. Photos were taken using Pan-F 50 ASA film and developed with Agfa Rodinal.

Impact of cytochalasin B on secretion

To investigate whether F-actin is acting in the digestive process in curled laminae, 3 *D. capensis* leaves were placed overnight in either water or a 100μM cytochalasin B solution as outlined in the CB assay. 24 hours later, 5 μl of yeast extract (2%) was placed on each leaf. As control, yeast extract was also placed on three leaves of a living plant. After 24 hours, a piece of plastic film (5mm by 10mm) coated with

gelatin, was placed on each stimulated leaf. These were removed 72 hours later. The pieces of film were placed in a 0.25% solution of FastGreen for 5 minutes. Thereafter, they were de-stained in a solution of 10% acetic acid for 5min. The pieces of film were photographed on a dissecting microscope under 1.8X magnification, using slide film which was converted into prints. The release of digestive enzymes is visible as white regions on the green stained film, where the enzymes have started eating away the gelatin layer.

Abbreviations: FITC - fluorescein isothiocyanate, TEM - Transmission Electron Microscopy/e, SEM - Scanning Electron Microscopy/e, CB - cytochalasin B, conc. - concentration.

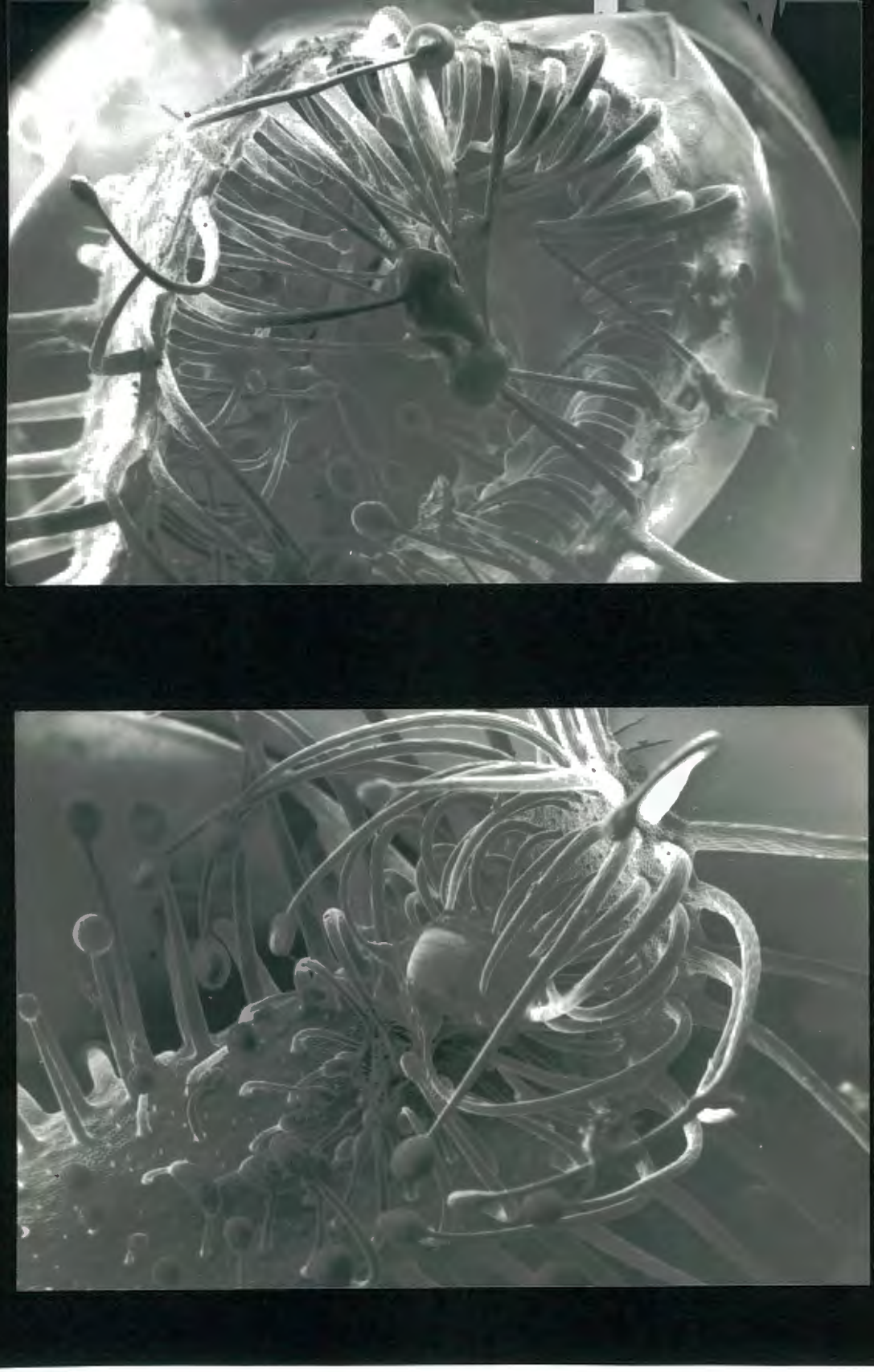


Figure 1. Scanning electron micrographs of a fully folded *Drosera capensis* leaf, x110 (a) and a leaf curled at the tip, x 125 (b), 12 hours after stimulation with a mosquito. Note the bending of the tentacles towards the prey and copious mucilage secretion which largely obscures the insect.

RESULTS

External structure of *Drosera* leaves

This brief investigation of the external structure of *D. capensis* leaves illustrates the appearance of the leaf-trap and its glandular components in the unstimulated and stimulated state. It verifies reports in the literature, frequently based on observations at lower magnification, and confirms that the experimental system used in this study fits into this framework.

The trapping response of *D. capensis* leaves is elicited by all three kinds of prey used in the study - mosquitoes, kelp-flies and mealworm pieces. In each case, an intense slow movement causes the entire lamina (figure 1 a) or the tip (figure 1 b) to curl over the prey within 8 to 18 hours. The tentacles (stalked glands) bend towards the centre of the leaf and touch the insect, enveloping it in mucilage that is secreted by the glands (figure 1a, b).

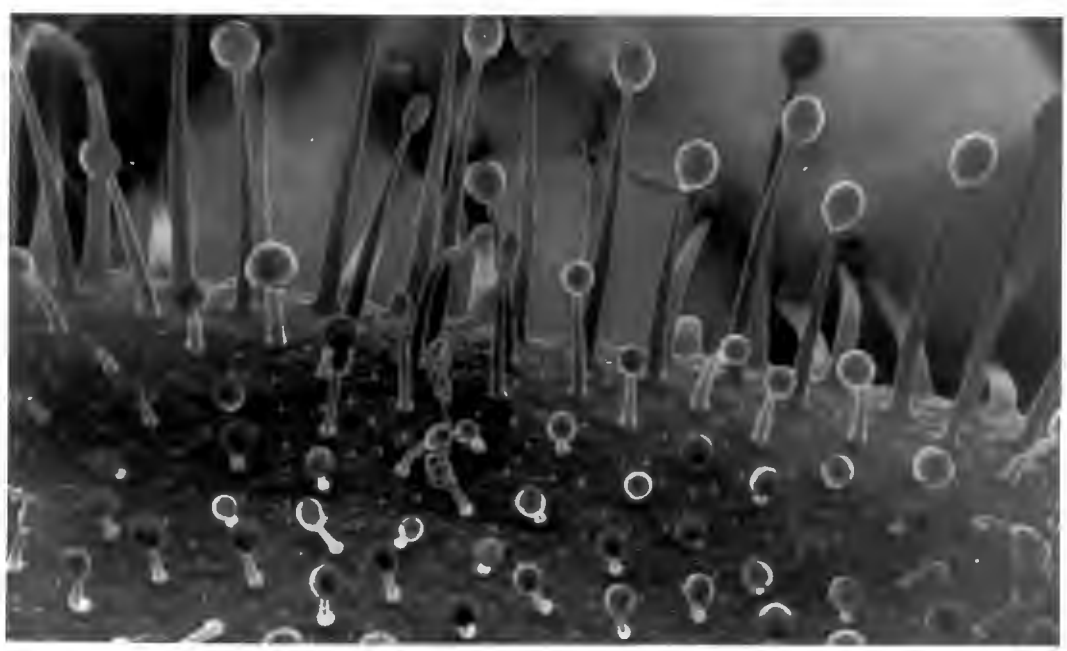


Figure 2. Scanning electron micrograph of the upper leaf surface of an unstimulated *D. capensis* leaf showing the tentacles in their upright state. The difference in tentacle size from the centre of the leaf towards the margin is distinct. Note the small sessile glands as light specs distributed over the leaf surface x 114.

In the unstimulated leaf, tentacles face straight upwards or to the sides and show no bending. The inner tentacles are significantly shorter than those positioned on the leaf margins (figure 2). Numerous small sessile glands are distributed over the upper leaf surface (figure 2, 3) and are also found on the lower surface and on some tentacle stalks. More detail in the structure of the upper leaf surface is detectable at higher magnification, as shown for the curled leaf (figure 3). There is a marked difference in the size and position of the stalked glands (raised) and the dome-shaped sessile glands which usually consist of four cells (figure 3). The aggregations of a 'furry' substance (figure 3) and other residue found near or over sessile glands of the curled leaf are indicative of secretion, digestion and absorption. Both stalked and sessile glands are involved in these processes.

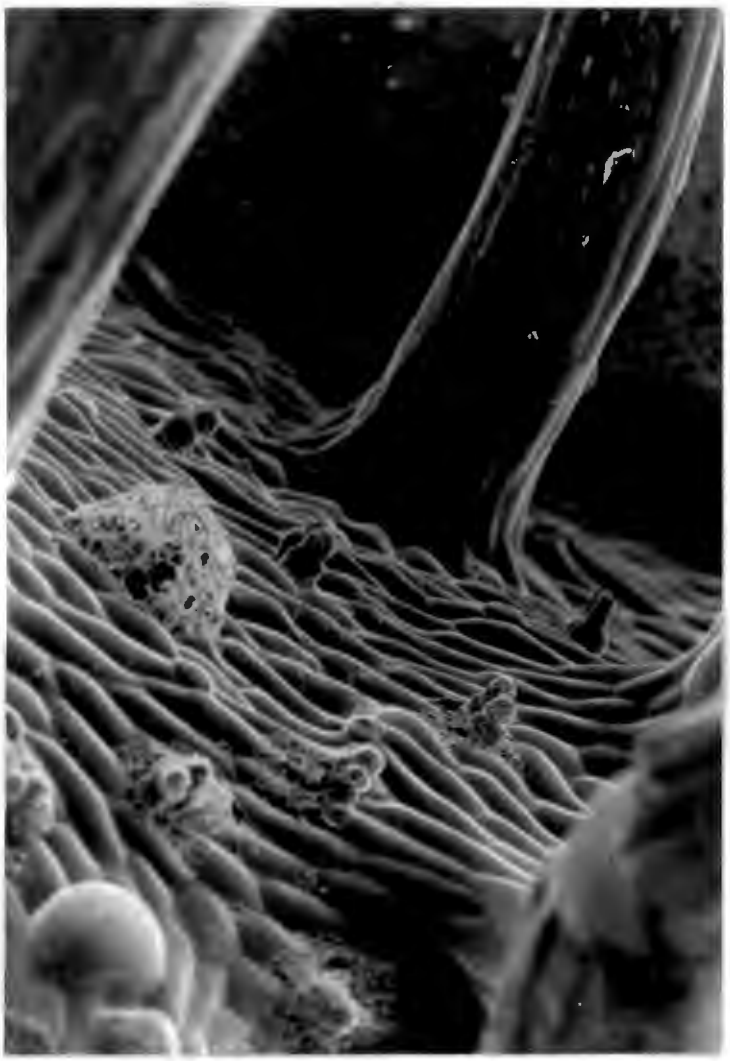
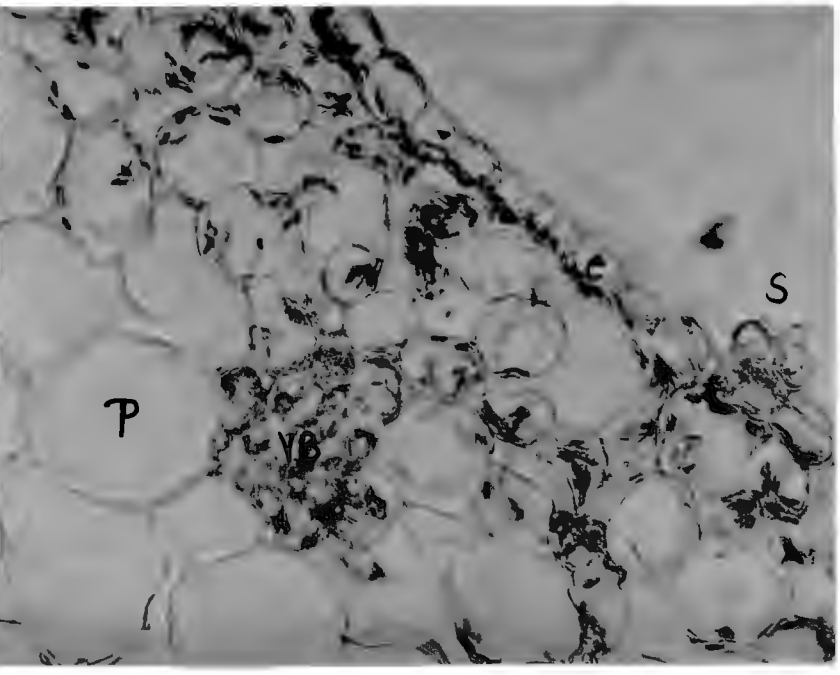


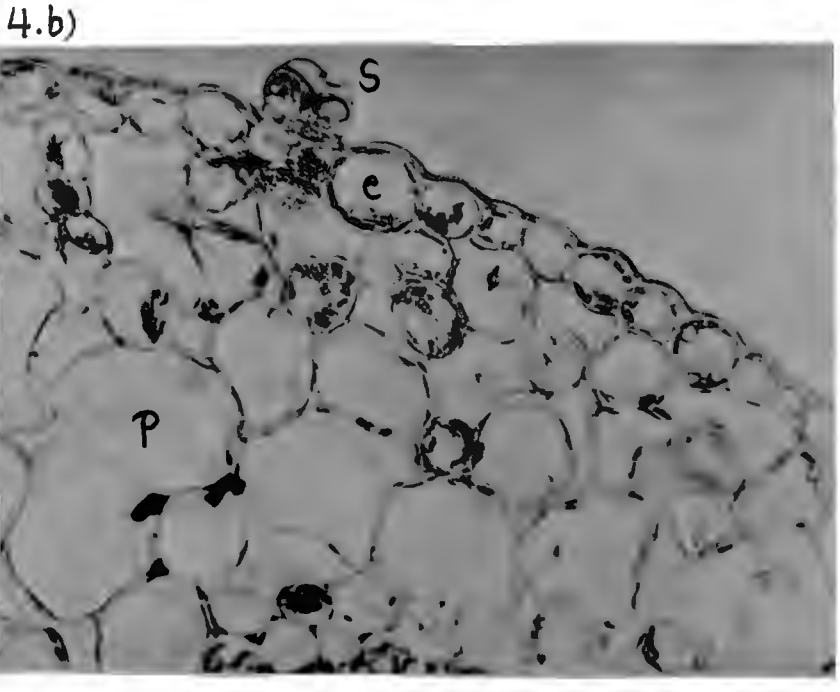
Figure 3. Scanning electron micrograph of the upper surface in a stimulated *D. capensis* leaf, showing the lower part of tentacles stalks and the small, dome-shaped

Anatomy of curled and uncurled leaves

The overall appearance of curled and uncurled leaves is similar at the light microscope level (see figures overleaf). No difference in the size or shape of their cells can be detected by eye (figure 4a,b and 5) and the cells in the curled leaf display no obvious deformations. This applies to all cell layers in longitudinal (figure 6 and 7) and transverse (figure 4 and 5) sections. The epidermal cells in both curled and uncurled sections are notably smaller and more regularly shaped than the parenchyma cells constituting the inside of the leaf (figure 4, 5). Note that the magnification in figure 4 and figure 5 is not equal, as figure 5 is 1.6 times larger than figure 4. This necessitates the comparison of cell sizes in terms of their relative proportions and not absolute size. The curled leaf section (figure 5) is thicker and contains vascular tissue, thus background blur is not entirely eliminated and the cells appear darker than in figure 4.

A remarkable feature, especially pronounced in epidermal cells and in the longitudinal view, is the presence of numerous vesicles that vary in shape and proportions (figure 5, 6 and 7). Even though they occur within cells of straight leaves (figure 6), they seem to be characteristic of the curled leaves (figure 5, 7) as they occurred in all sections examined. They were often more numerous and also commonly associated with cells of the leaf stalk.





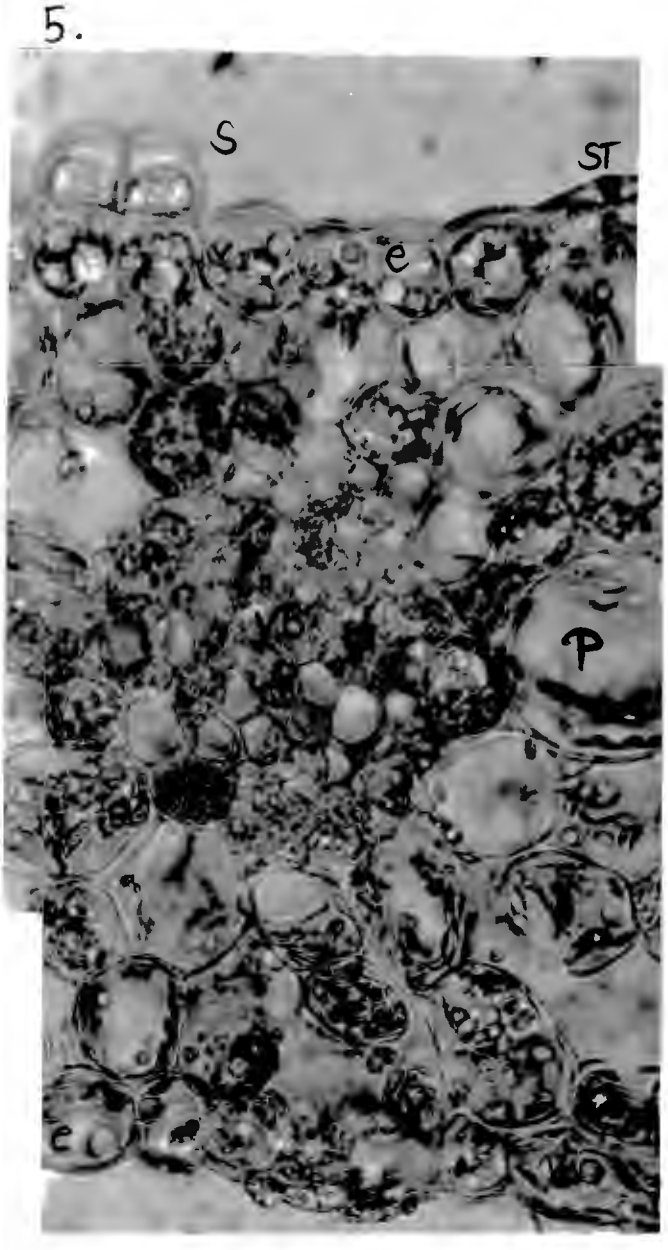


Figure 4 (left). Transverse sections, x840, of an unstimulated *D. capensis* leaf showing the upper (a) and lower (b) epidermis, each topped by a sessile gland ,and the cell layers towards the centre of the leaf. The regular shape and small size of epidermal cells compared with the large parenchyma cells is apparent. Chloroplasts are visible as sickle-shaped dark bodies and a few vesicles are present in the lower epidermis (b).

Figure 5 (right). Transverse sections, x1350, of a stimulated *D. capensis* leaf. A vascular bundle fills the centre of the section and vesicles are contained mainly in the epidermal cells. Note the sessile gland and stoma on the upper epidermis. (e - epidermis, s - sessile gland, st - stoma, vb - vascular bundle, p - parenchyma cell, v - vesicle).

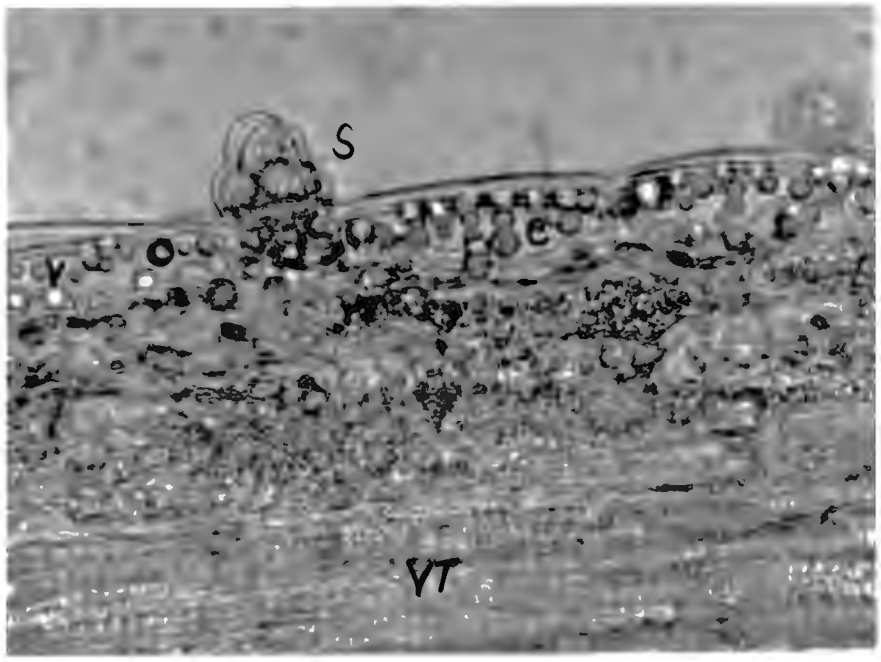


Figure 6. Longitudinal section through an uncurled leaf, x1350. The elongated upper epidermal cells contain several vesicles. Vascular tissue composing spiral vessel elements fills the greatest part of the section. The sessile gland shows a deviation from the most common four-celled arrangement as there is a single cell at the tip. (s - sessile gland, e - epidermis, v - vesicle, vt - vascular tissue).

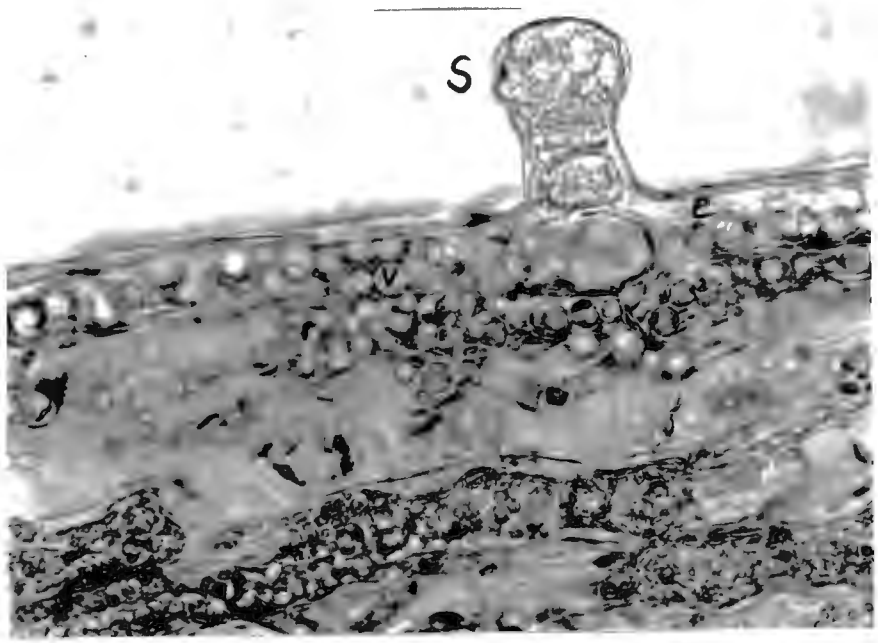


Figure 7. Longitudinal section through a curled leaf, x1350, showing similarly elongated upper epidermal cells as the uncurled section. Vesicles are also present. Note that the sessile gland at has an exceptional more complex shape.
(s - sessile gland, e - epidermis, v - vesicle).

The sessile glands in curled and straight leaves resemble each other and the most common four-celled structure is represented (figure 5, 8). Two variations of this structure are displayed - a more complex one (figure 7) and a gland where a single cell forms the top (figure 6). The mother cells giving rise to the glands are seen beneath these (figure 5, 8). In stimulated leaves, no evidence of uptake or secretion of substances was observed within the glands or on the outer surface (figure 5. 8). Intracellular movement resembling bulk flow or 'cytoplasmic streaming' was not observed in any of the cells in the sections that were viewed (figure 4-8).

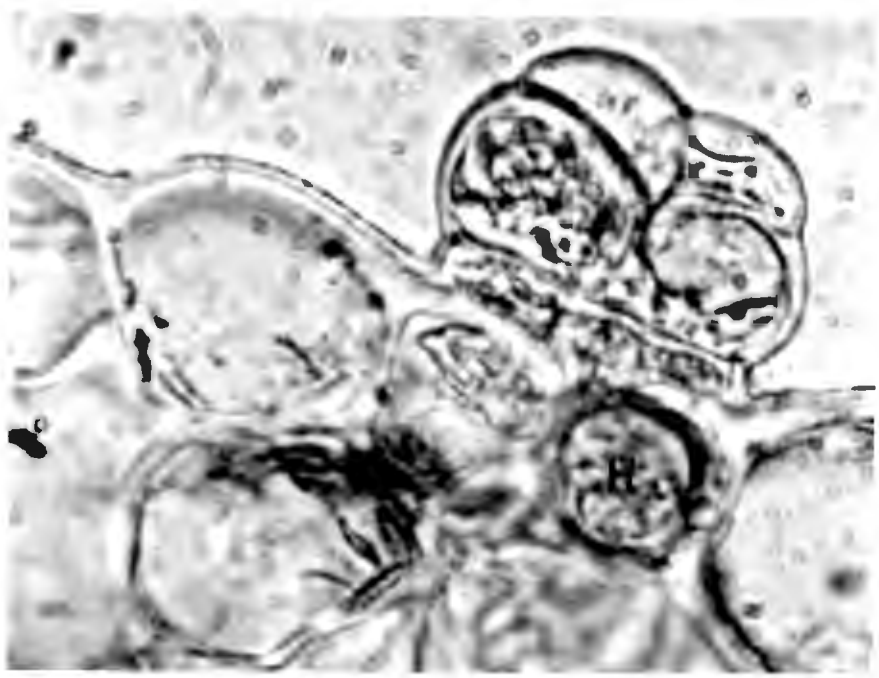


Figure 8. A sessile gland in an uncurled leaf, x3370. This illustrates the most common structure of these glands, with the mother cells (M) underlying two basal cells upon which the four capital cells rest. The cell content is not clear as in the adjacent epidermal cells but cannot be identified in any detail.

Ultrastructure of curled and uncurled leaves

At the subcellular level, few distinguishing features can be detected in tissue of curled and uncurled leaves. The cells of the upper epidermis and underlying tissue are straight-walled and characterised by large vacuoles, with the cytoplasm and organelles being largely confined to the periphery of the cells (figure 9, 10). The cell size is similar in curled and uncurled sections. Some variability exists in both cases, as can be verified by consulting figure 4 and 5. Such variation is displayed at a different scale in figure 10. The chloroplasts and frequently elongated mitochondria are similar in the cells of both sections (and compatible with those in other plant tissues.) The starch accumulations in the centre of chloroplasts (figure 9, 10) are slightly contracted, indicating the loss of starch.

Despite the absence of marked ultrastructural rearrangements that can be linked to leaf movement, certain differences are noticeable between the curled and uncurled sections. The osmophyllic bodies (stained black by osmium) seen in both sections (figure 9, 10) seem to contain lipids, which are the target substances (e.g. in membranes) for Osmium staining. They are probably tannin vacuoles, known to exist in *D. capensis* cells [22]. In the unstimulated leaf cells they are enlarged and have an unstained core, composed of non-lipid material (figure 9). In curled leaf cells the lipid bodies are small, contracted and stained entirely black, thus lacking the non-lipid core.

Numerous small vacuoles, unstained by Osmium, are present in epidermal cells of the uncurled leaf (figure 9). They are of different sizes and vary considerably in shape. Some of the larger ones have incorporated smaller bodies possibly for break-down of these materials. The content of the vacuoles has not been determined but may consist of non-lipid substances such as proteins (e.g. hydrolytic enzymes) or carbohydrates (e.g. mucilage). An interesting feature is seen in the small epidermal cell of figure 10, where a vacuole appears to have incorporated a chloroplast that is disintegrating.

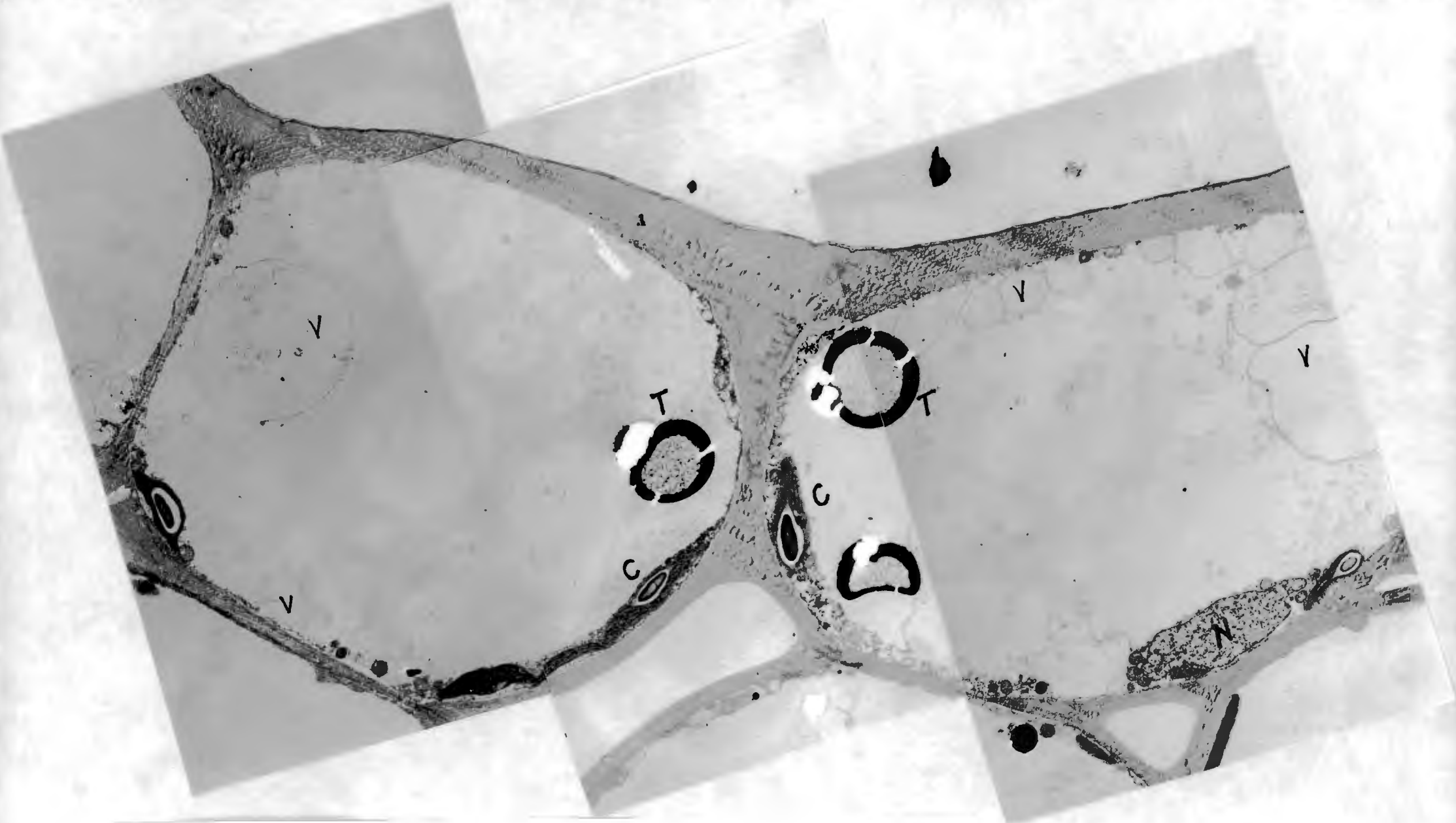


Figure 9. Transmission electron micrograph of two upper epidermal cells in an unstimulated leaf, x4400. Apart from the large central vacuole, several smaller vacuoles of different sizes are present. The larger of these contain small rounded bodies and may have a lytic function. Osmophyllic lipid bodies (tannin vacuoles) contain a non-lipid core and a nucleus is seen in the central cell. (T - tannin vacuole, N - nucleus, C - chloroplast, V - vacuoles of different sizes)

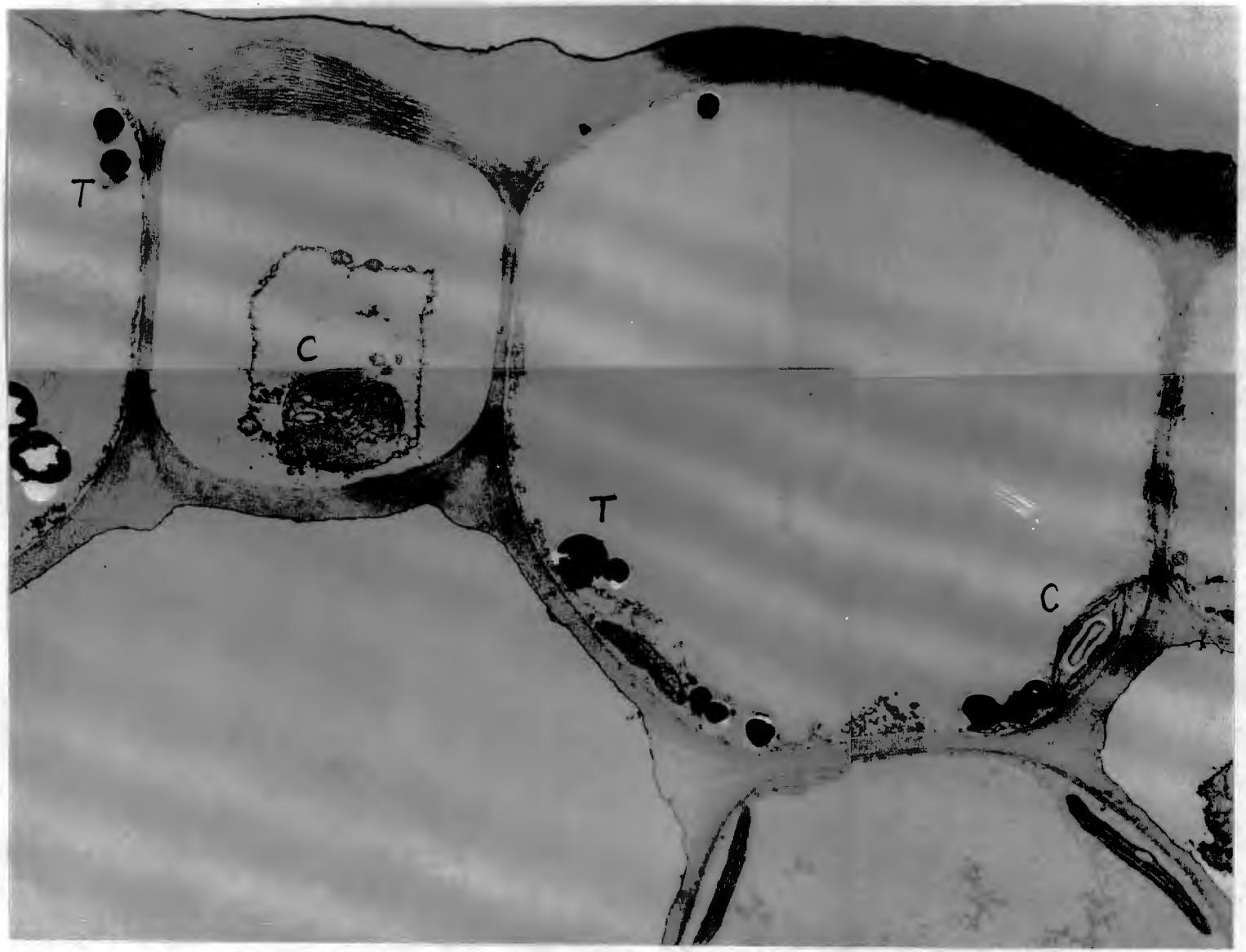


Figure 10. Transmission electron micrograph showing two cells of the upper epidermis and part of the underlying cell layer in a stimulated leaf, x4400. Cell walls are straight and a large central vacuole fills most of the cells. The cytoplasm is closely pressed to the walls and several black lipid osmophyllic bodies without a non-lipid centre are visible. (T - tannin vacuole, N - nucleus, C - chloroplast)

Folded cell walls occur mainly in the tissue of the curled leaf (figure 11), where they are primarily associated with cells of the lower epidermis and the layer overlying this. Straight leaf sections do not show extensive wall folding and, when present, it is less pronounced. The phenomenon may be an important differentiating feature between tissue of curled and straight leaves as folding can be indicative of water and turgor loss in cells.

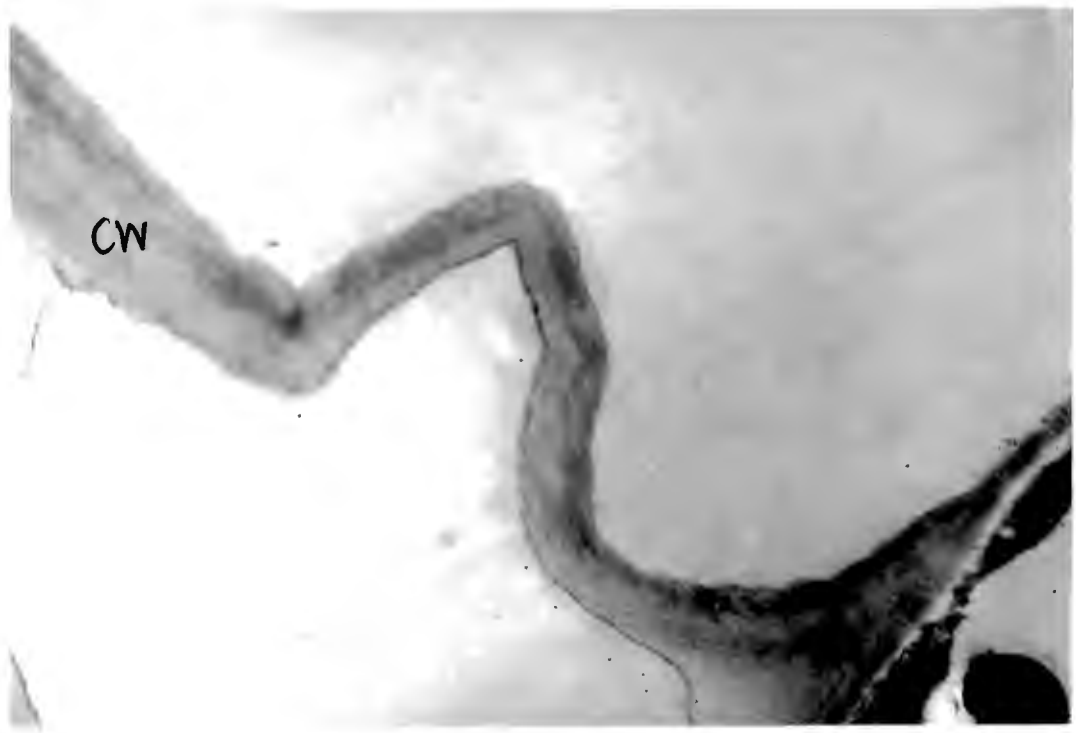


Figure 11. A folded cell wall (CW) of a cell in the lower epidermis of a curled leaf, x14370. Such foldings were primarily found in the curled state of *D. capensis* leaves.

Cytochalasin B assay - effects of actin on leaf curling

All leaves in the assay exhibited full curling within a time-frame of 15 hours after stimulation with prey (figure 12, 13). Both control treatments (water and DMSO) showed 100% curling success, with five out of five leaves being curled. The results of the water control (figure 12) are representative of both control treatments. Complete leaf curling also occurred in both CB-treatments, where uptake was allowed for 2 hours and for 21 hours (figure 13, uptake of 21 hours). The rate of curling was not determined in the treatments, and it is therefore not known, whether all leaves commenced folding at the same time and whether the speed of folding varied.
The reversibility of the action (unfolding of the leaves) could not be investigated, as *D. capensis* leaves usually take one to three weeks to uncurl again. In the experiment, when they were cut off from the parent plant, the leaves did not last long enough (less than six days) to exhibit uncurling again. As cytochalasin B did not inhibit the curling response, it can be assumed that it does not effect the reverse action either.

In the ink-uptake control, ink travelled up to the leaf tips within three hours. Staining of the entire lamina had occurred after approximately 48 hours.

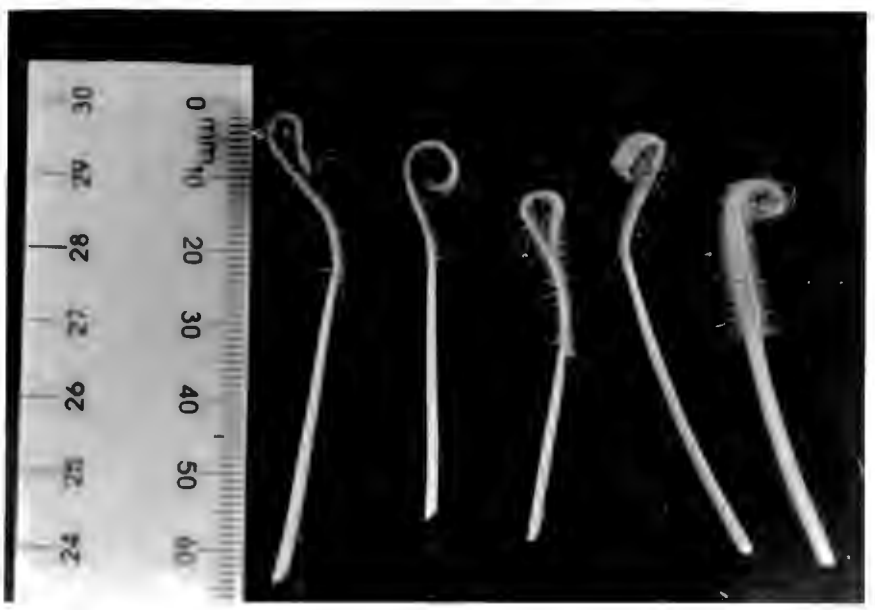


Figure 12. Leaf and tentacle folding in the water control of the cytochalasin assay, 15 hours after stimulation with mealworm prey. All five leaves are fully curled. The DMSO control yielded the same result.

Figure 13. Leaf and tentacle folding in leaves subjected to cytochalasin B (100μM) for 21 hours prior to stimulation with mealworm prey. The photograph was taken 15 hours after prey had been given to the leaves. There is no quantitative or qualitative difference between CB-treated and control leaves (figure 12).

Polymerised (F-) actin in curled, uncurled and cytochalasin-treated leaves

The images (figure 14 - 16) show green fluorescence alone (indicating F-actin) and green in combination with red auto-fluorescence of chloroplasts. This facilitates viewing and interpretation of shapes and outlines when green fluorescence is faint. Yellow colouring indicates sites where the distribution of the two fluorescing colours overlaps. The images displaying tentacles, cross- and longitudinal sections are of the same magnification and the 'green' and 'green plus red' images are identical copies, except for the red being filtered out in the first case. Visual sequences through the depth of each section were conducted to ensure that recorded images at a certain focal depth are representative of the entire section.

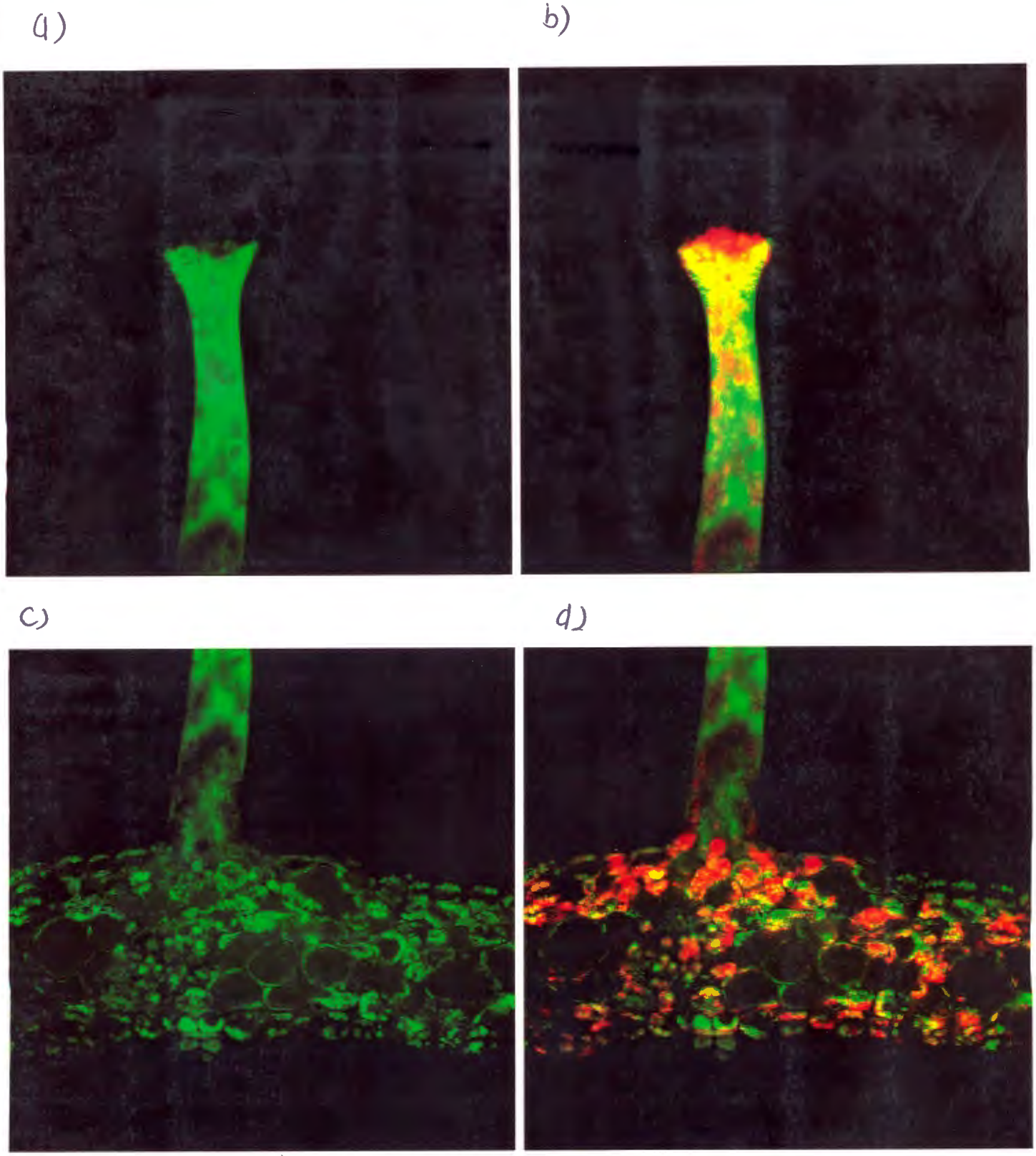


Figure 14. Sections (x 250) through a curled *D. capensis* leaf, stained with FITC-labelled phalloidin 12 hours after stimulation with mealworm prey. Green sections indicate polymerised actin bound by phalloidin, red is chloroplast autofluorescence. The tentacle stalk topped by the non-fluorescing gland (a, b) is bright green due to F-actin. In the cross-sectional view (c, d) polymerised is present throughout most of the leaf, except within the largest parenchyma cells. F-actin is abundant in the longitudinal section (e, f) where some fluorescing vascular tissue is present. The sessile glands on either epidermis are the most intensely fluorescing components of the curled leaf. Most of the F-actin appears as a vesicle formation (figure 14 g, page 27).

*Polymerised actin in sections of **folded** leaves*

Extensive polymerisation of actin is visible throughout the entire sections of curled leaves (figure 14 a-g). In the transverse view (figure 14 a-d), green fluorescence is particularly pronounced in the tentacle stalk (figure 14 a, b) but absent from the pigmented gland at its tip. The sessile gland with its mother cells, seen at the leaf underside, are also regions of abundant polymerised actin (figure 14 c-f). These are often completely filled with the fluorescent label (figure 14 c-g).The large parenchyma cells at the leaf centre are largely devoid of F-actin which only shows up as a faint lining at the periphery (figure 14 c, d).

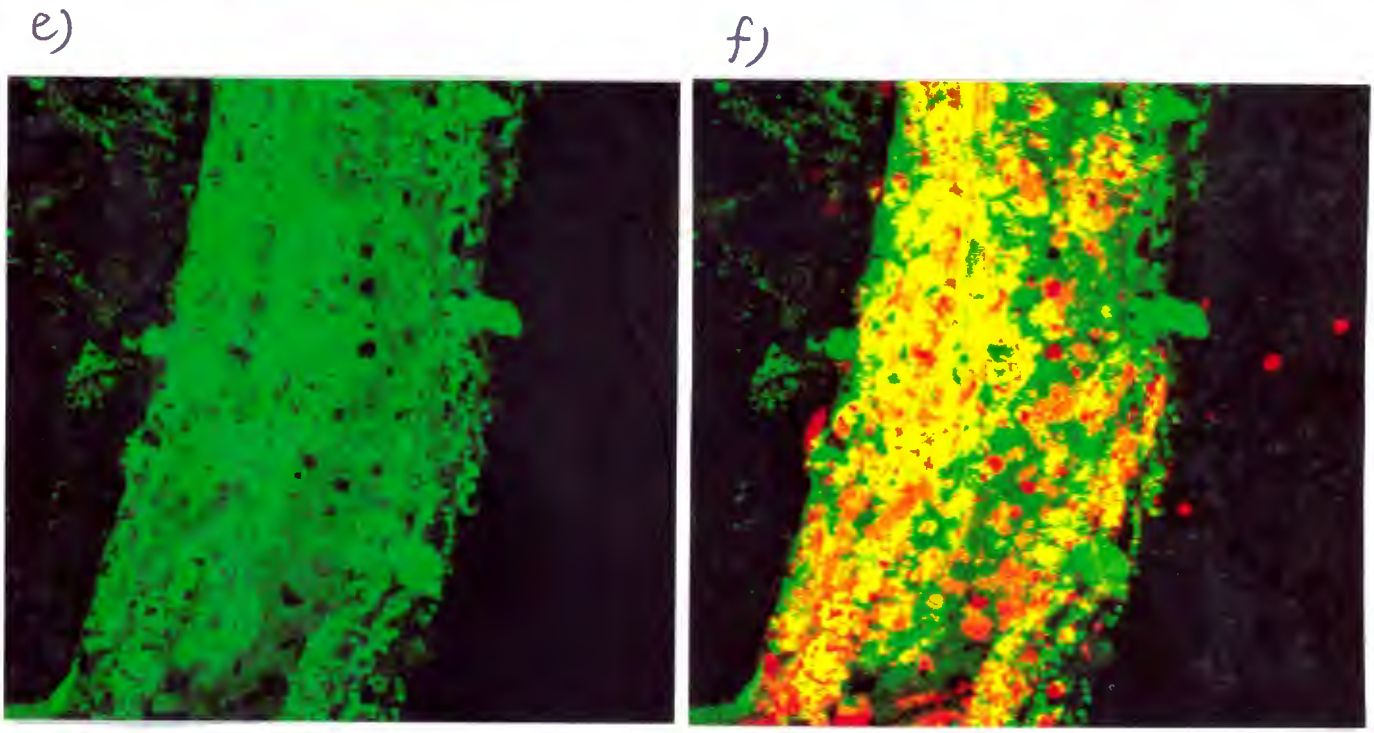


Figure 14 e, f. Longitudinal sections of curled leaf. Detailed heading, see previous page.

Most of the actin in the curled leaf sections appears in the form of small vesicles around or in which it aggregates (figure 14 c-g). These are apparent in the longitudinal leaf section where they take up a large volume in many cells (figure 14 e, f). A closer view of these vesicles (cross-section, figure 14 g) in the epidermal layer shows their distinct outlines and indicates the lack of F-actin interconnections between them.

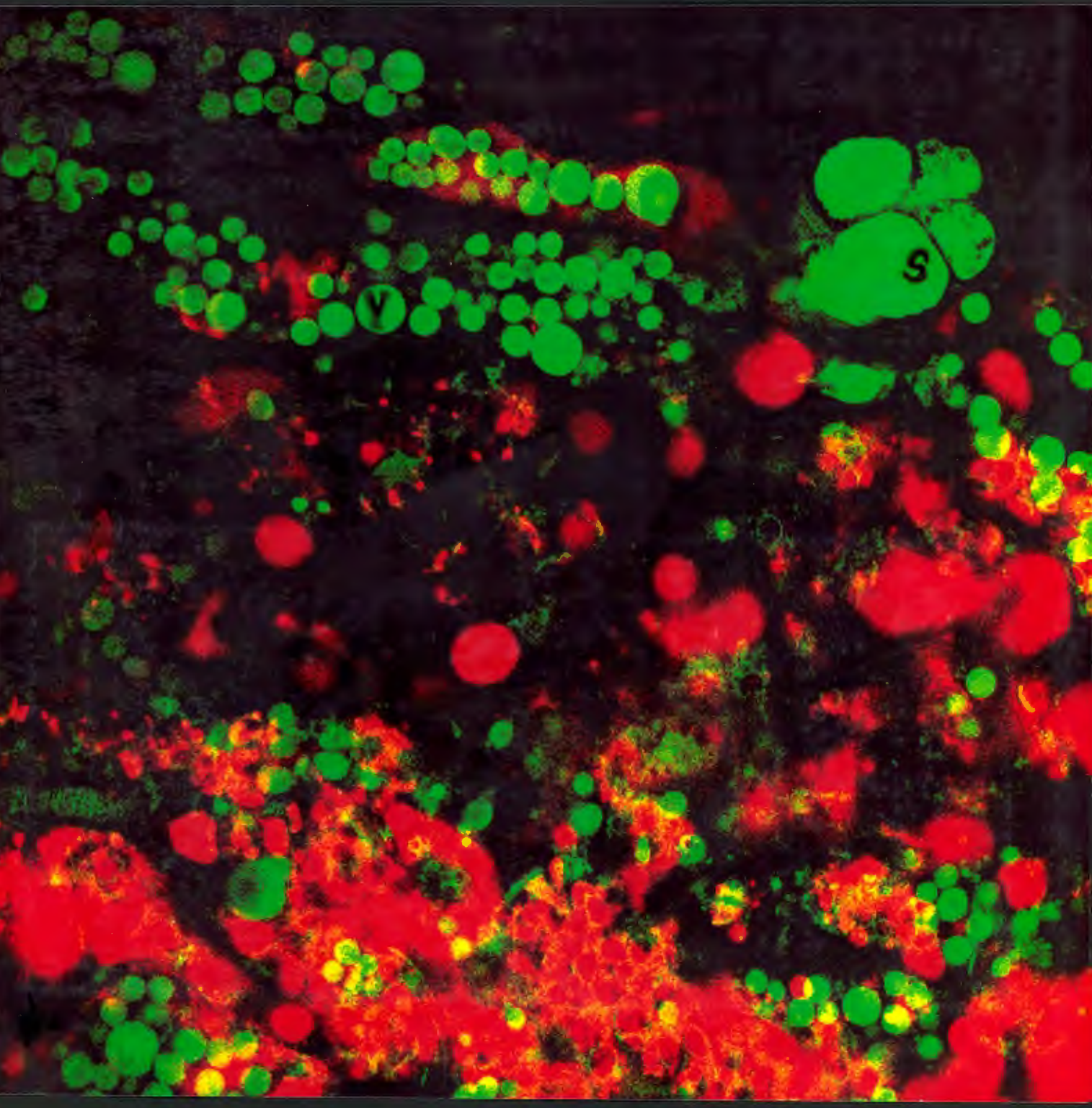


Figure 14g. Fluorescent section of a curled leaf, showing the cells of the upper epidermis and a green sessile gland. Note the vesicles in the epidermal cells x2200. (v - vesicles, s - sessile gland).

In figure 14 g, the four to five cells visible to the left of the green sessile gland contain more than ten vesicles each. There is also evidence of actin in a filamentous or network-like formation in the cell layers below, where few vesicles are present (figure 14 g).

In comparison with the cross-section (figure 14 c, d), green fluorescence in the longitudinal section is of greater extent and intensity, as it occurs in almost all the cells. This can partly be ascribed to the presence of some vascular tissue in the lower central region of the section (figure 14 e, f). This tissue tends to stain well as it contains considerable quantities of F-actin.

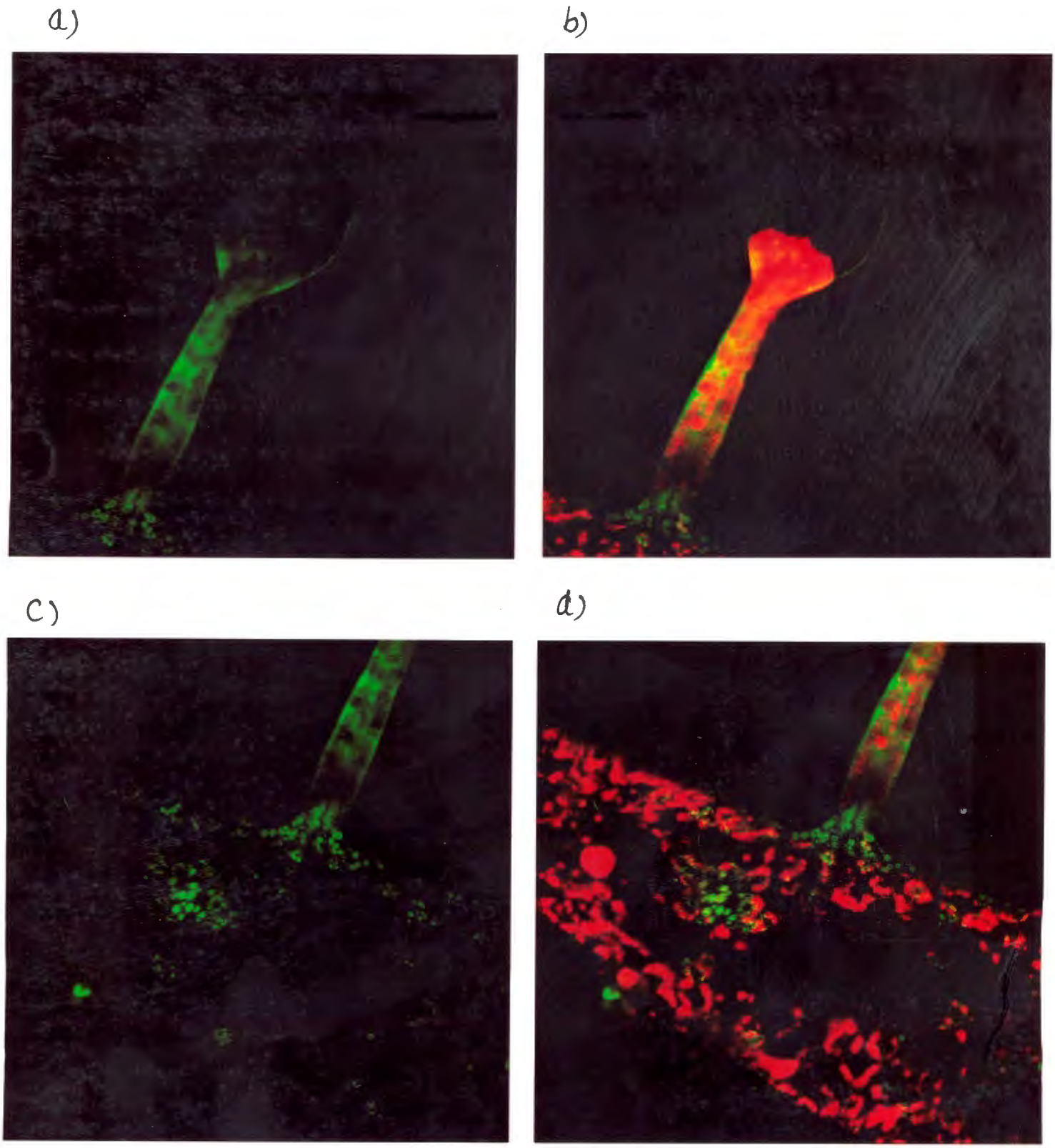


Figure 15. Sections (x250) of an uncurled control *D. capensis* leaf, stained with FITC-phalloidin. Note that the images with red plus green and green fluorescence alone are identical except for the colouring. In the 'green-only' images, red has been filtered out. Overall fluorescence is considerably less in all images than in the curled leaf. Polymerised actin is visible in the tentacle stalk (a, b) and, to a very limited extent, in cells of the transverse (c, d) and longitudinal sections (e, f). Some vesicles are present and the most intense fluorescence occurs in the sessile glands. The stalked gland does not fluoresce.

*Polymerised actin in sections of **straight** leaves*

As green fluorescence is faint in unstimulated leaves (figure 15 a-d), it is necessary to refer to the images showing red and green to be able to visualise the outline of the sections. It can be seen that the red fluorescence has retained its intensity and is not weaker than in the curled section. This demonstrates the compatibility of the images showing the different treatments and justifies comparison of the intensity of fluorescence. The low levels of green fluorescence in the unstimulated leaf indicate that little actin in the polymerised state is present (figure 15 a-f). As is the case in the curled state, the tentacle stalk (figure 15 a, b) and the sessile glands (figure 15 c-f) are the most intensely fluorescing areas of unstimulated leaf sections. Limited vesicle aggregation is seen at the base of the tentacle stalk and in a few cells within the leaf centre (figure 15 c, d) as well as in the epidermal cells (figure 15 e, f). Most of the central cells, however, do not contain significant amounts of F- actin (figure 15 c-f). The distribution of centres of intense fluorescence (sessile glands and tentacle stalks) in the curled and uncurled leaves corresponds well, yet the overall extent and intensity of actin polymerisation is notably less in the unstimulated control leaf.

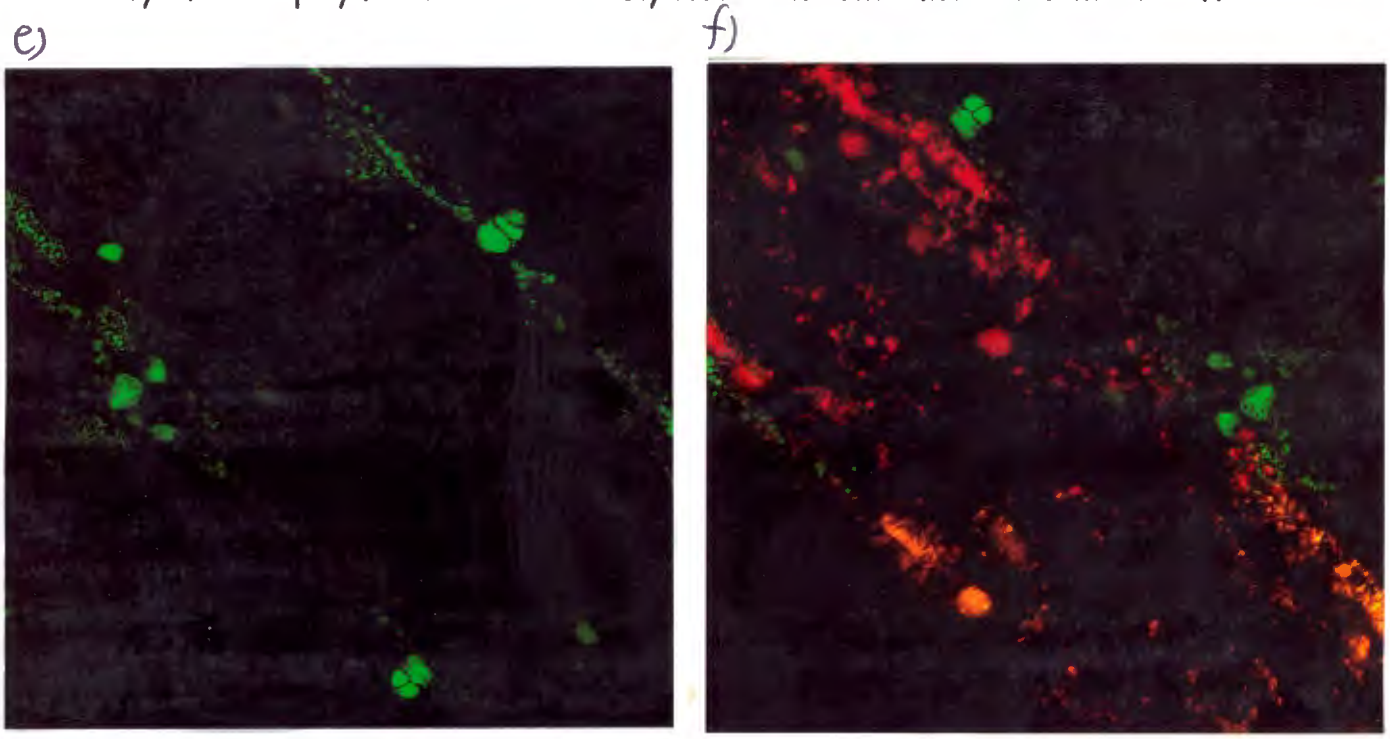


Figure 15 e,f. Longitudinal section of straight leaves, see heading on previous page.

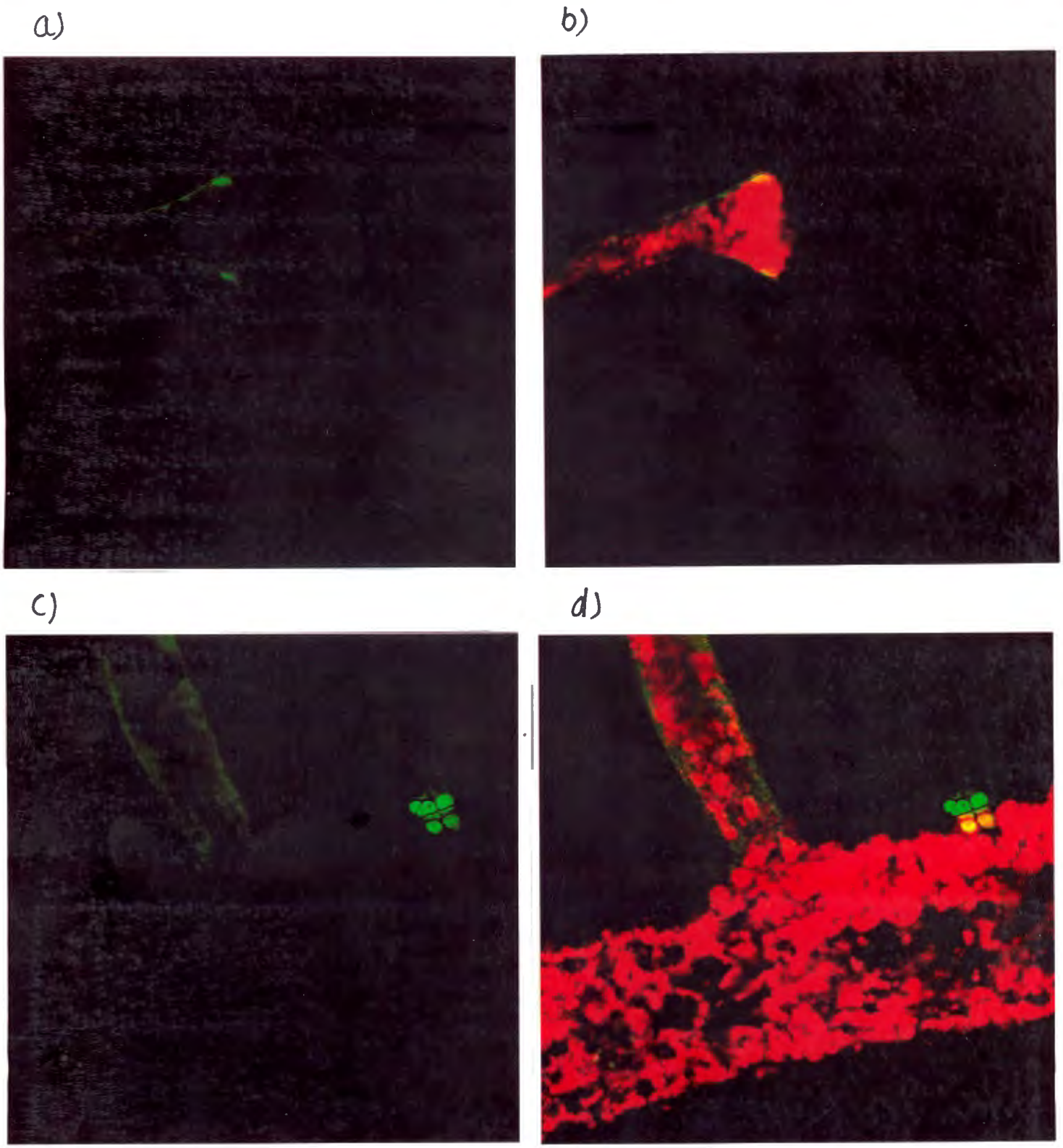
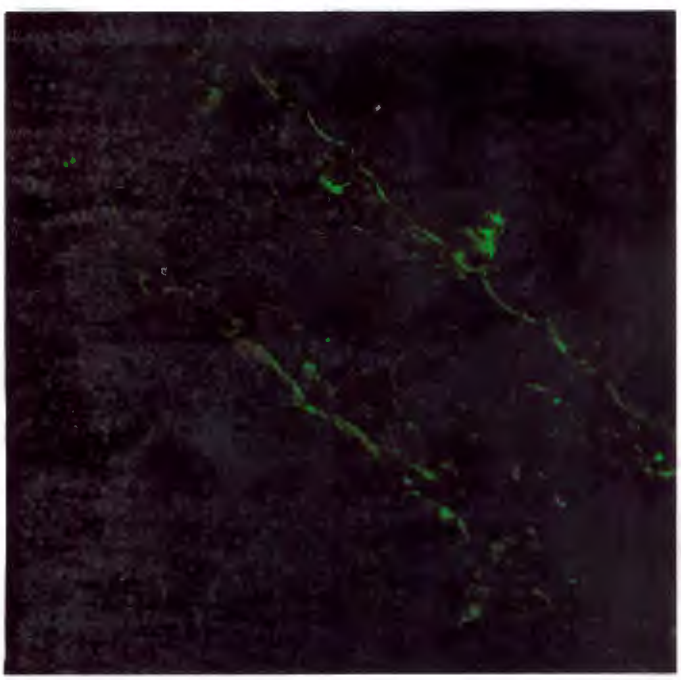


Figure 16. Sections (x250) of cytochalasin-treated (21 hour uptake) leaves, stimulated with mealworm prey and stained with FITC-phalloidin. The sections with red and green fluorescence show the outline of the tentacle (a, b), cross-section (c, d) and longitudinal section (e. f). Polymerised actin is absent from most of the cells in the leaf sections where no fluorescence occurs. The faint green lining the tentacle stalk (a, b) and the epidermal cells (e, f) shows the limited distribution of F-actin. Polymerisation is evident in the sessile glands (c-f) but is variable in intensity. No vesicles of F-actin are present.

*Polymerised actin sections of **CB-treated** leaves*

In referring to the red and green images, the delimitation of the sections of cytochalasin B treated leaves can be identified (figure 16 b, d, f). Green fluorescence is hardly detectable in any of the leaf sections (figure 16 a-f). Thus, F-actin is largely absent. This confirms that uptake of the fungal metabolite was accomplished in the CB assay (figure 13) and that actin polymerisation was effectively prevented. Actin in the leaves is therefore primarily present in the form of monomeric G-actin.

e)



f)

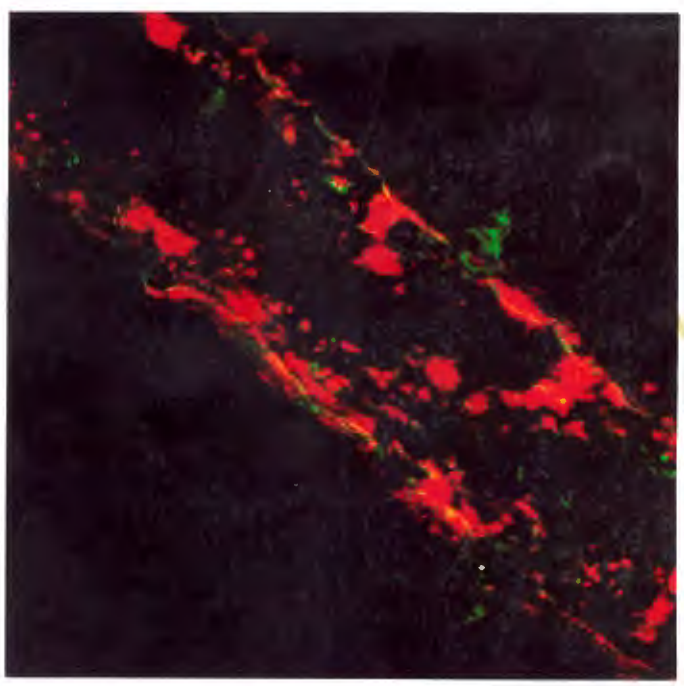


Figure 16 e, f. Longitudinal section of CB leaves, detailed heading on previous page.

Vesicles are not distinguishable in the sections (figure 16 c-f), but there is some evidence for polymerised actin. The tentacle stalk (figure 16 a, b) and the periphery of epidermal cells in the longitudinal sections (figure 16e, f) fluoresce very faintly. The sessile glands of the CB-treated leaves are exceptional as they still contain considerable quantities of F-actin (figure 16 c). The amount varies among the glands (figure 16 c, d compared with 16 e, f). The distribution of the limited F-actin present

in the CB-treated leaves is similar to the sites of intense fluorescence in curled and uncurled sections (figure 14 and 15, sessile glands and tentacle stalk).
This indicates some level of commonality between leaves of the different sections and suggests that actin in these regions is present in a polymerised state most of the time.

On a whole, the curled leaf contains the most extensive and intense fluorescence, thus indicating the greatest abundance of actin in the polymerised form (figure 14). All images support this. The CB-treated leaves, which were also curled, represent the opposite effect. They display the lowest levels of fluorescence, as they contain less F-actin than either the curled leaf or the control, uncurled leaf. In all sections the greatest concentration of F-actin was present in the sessile glands and, to a lesser degree, in the tentacle stalks.

Impact of Cytochalasin B on secretion

Figure 17a. Pieces of gelatine-based film (x1.8) showing the digestive activity (stain removed) of three control leaves. Note that enzymes were released by all leaves.

The control leaves in water (figure 17 a) show that secretion of digestive enzymes was taking place in reaction to stimulation. In all three leaves, the gelatine layer is eaten away in several places (figure 17 a) indicating sites where lytic enzymes were released onto the leaf surface. In the CB-treated leaves there is limited evidence of digestive activity (figure 17 b). One of the film strips shows some removal of gelatine, while the other two remained mainly green throughout (figure 17 b).

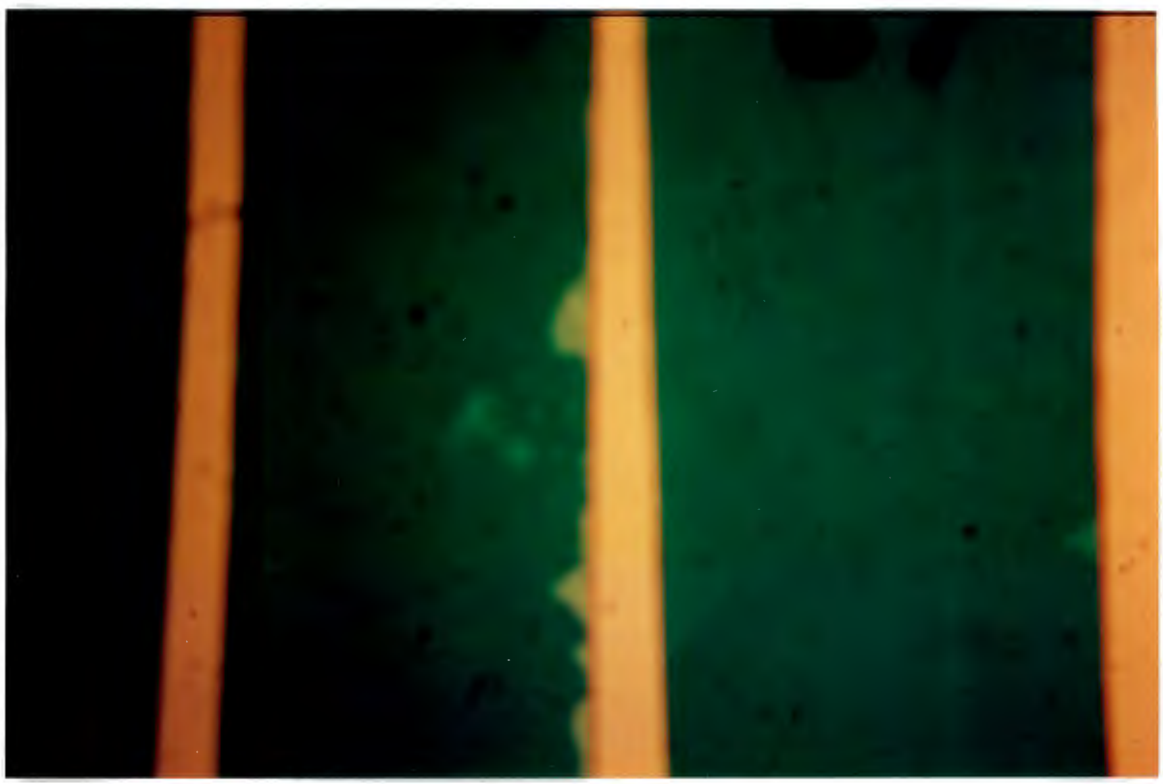


Figure 17b. Pieces of gelatine-based film (x1.8) stained with fast green to reveal digestive activity of cytochalasin B-treated leaves. Digestive enzymes of one leaf reacted with the gelatine, as can be seen by gaps in the stained surface of one film piece.

DISCUSSION

TURGOR

In plants, turgor has been identified as the dominant mechanism for generating movement [7,11,12,16,33,38]. The underlying principle is that the influx of water into a group of cells will create pressure due to the resistance of the cell walls. If this is accompanied by a turgor loss in another cellular region, the differential pressure exert a force that causes the directed motion of a plant organ. Thus, a leaf may be lifted. Such changes in turgor strongly influence cell size and shape [12,24] and bring about obvious modifications in cellular ultrastructure [12]. Characteristic features associated with turgor loss include distinct foldings of the cell wall [12,24], changes in the shape and structure of several organelles (e.g. the nucleus) and the splitting of vacuoles into smaller ones with irregular outlines (f). A gain in water and turgor by a cell reverses these changes. Most of these observations are based on motor cells in 'sensitive plants', but the same principles can apply to many other cells.

Anatomical features

It was initially proposed, that leaf folding in *Drosera capensis* may be influenced by a similar turgor-related mechanism. Yet, the comparative examination of anatomical features in curled (stimulated) and uncurled (unstimulated) leaves of *D. capensis* reveals few differences. Distinct cellular characteristics that could be linked to a trapping movement via turgor changes cannot be identified in the curled leaf (figure 5, 7). Most importantly, the size and shape of cells are similar in the curled and straight leaf. This is contrary to findings of clear differences in cells of bent and straight pulvini in *Mimosa* and *Robinia* [12,24]. However, in looking at the images produced by light microscopy (figure 4-8), it becomes apparent that qualitative changes in the cells are difficult to detect at this level of magnification unless they are exceptionally striking.

The presence of numerous vesicles, predominantly in the cells of the curled leaf (figure 5,7) is the only difference that was noted between the sections. This observation may confirm early reports [8,9,22] where similar vesicles, some of them 'filled with a viscid substance' [8], were reported in the tentacle stalk following prey capture. The phenomenon is said to be independent of the trapping movement [22] as it occurs after bending has been achieved. Similarly, the vesicles observed in this study are unlikely to be a consequence of turgor loss (e.g. in the upper epidermis) causing vacuolar break-up, as they appear throughout the entire curled leaf section (figure 7) and even in the straight leaves to some extent (figure 6). Thus, they do not seem to be associated with turgor-driven leaf folding but rather indicate intracellular activity stimulated by prey capture.

Differences at the subcellular level

The ultrastructural examination of cells in the uncurled (figure 9) and curled leaf (figure 10) also does not show rearrangements as dramatic as those described for motor cells in 'sensitive' plants [12,24]. Most of the differentiating features, seem to be indicative of internal metabolic processes and do not allow one to establish a link with the mechanism of movement. It seems that they are either not related to movement at all (e.g. small vacuoles in the uncurled leaf cells, figure 9) or are caused by leaf folding (e.g. changes in the tannin vacuoles, figure 9, 10). The contraction of tannin vacuoles was observed by other researchers [12,22] but no interpretation is given. The numerous vacuoles, on the other hand, may store secretory substances (hydrolytic enzymes) that are required once the leaf is stimulated. Alternatively, they could contain mucilage which is transported up to the glands at the tip of the tentacles. This could explain why more vacuoles are present in the curled cells, where the leaf is constantly secreting mucilage [18]. In the curled leaf that is in the process of digesting the trapped insect, mucilage is not required at that stage [18] and thus vacuoles containing this substance could be diminished in number in the curled epidermal cells. The small vacuoles in the transmission electron micrograph (figure 9) could

correspond to the vesicles noted in the anatomical investigation (figure5-7). This cannot be said with any certainty though, since it is unknown what they contain. It seems that fewer small vacuoles were seen under the TEM than vesicles under the light microscope, but this may be a consequence of the relative number of cells observed.

The only subcellular feature that may provide evidence for the role of turgor changes in leaf curling is the presence of folded cell walls (figure 10) in curled sections. Such foldings are a prominent feature in motor cells of *Robinia* and *Mimosa pudica* [12,24] where temporary turgor loss has taken place to allow bending of the pulvini. Thus, the presence of folded walls is associated with a loss in cellular volume and would have been expected in the upper rather than lower epidermis (figure 10) in *D. capensis* leaves, since cell expansion on the leaf underside is most likely to produce upward bending.

The anatomical and ultrastructural examinations yield no conclusive evidence for a turgor-based mechanism causing leaf folding in *D. capensis*. Reports that leaf curling is a growth phenomenon [3,22,28,31,34] can therefore not be verified, as irreversible turgor changes could not be demonstrated. Yet, the results in this study were obtained by means of a quantitative assessment of cells in straight and curled leaves. As changes in turgor can result from relatively slight shifts in cellular volume [12], they may be difficult to detect by TEM and almost certainly impossible using light microscopy. The limited number of cells that were viewed (especially using TEM) is most probably also not a wholly representative sample of cells in the curled and uncurled leaf sections. Thus, to determine whether turgor is indeed involved in the movement of *D. capensis* leaves, methods that measure turgor variations in cells directly could be used 16. Alternatively, the study approach could be modified to incorporate a more quantitative methodology. This was applied in work on *Dionaea muscipula* and *Robinia* [11,24] where the dimensions of more than 10000 cells[24] were

measured and mathematical formulae used to detect changes in cell shape and size that were related to turgor [11,24]. This was beyond the scope of the present investigation but would be a useful and possibly fruitful approach to resolve the matter. In view of the close relationship between *Dionaea muscipula* and *Drosera capensis,* it is likely that a turgor mechanism is, in fact, operating in *D. capensis* to bring about leaf folding. The presence of such a mechanism in sensitive plants adds further support to this and may indicate unity among plants in the cellular mechanism for movements.

ACTIN

The involvement of actin in plant movements has not yet been investigated extensively, although it has been shown to influence some processes (e.g. stomatal opening and closing [10,17]) but not others (e.g. gravicurvature of maize roots[2]).
In studying the effect of actin on organismal movement, it is of fundamental importance to distinguish between two processes - polymerisation and depolymerisation of actin. Both may act to produce movement [10,17], although the literature tends to highlight the importance of actin polymerisation in movement. This may partly be the consequence of the focus on animal and other systems (e.g. protozoans) where assembly into filaments has been shown to be an important component of movement [25,35]. Apart from this, however, the concern with polymerisation is also due to the use of drugs such as CB, which have allowed a link to be established between the cessation of movement and depolymerisation [35]. This has often led to the conclusion that polymerisation is indispensable for movement in those instances [6].

Microfilaments and movement

In the present study, however, the cytochalasin B assay convincingly shows that actin polymerisation is neither directly nor indirectly involved in the leaf movement of *D.*

capensis. Leaf curling in response to stimulation with prey occurs despite prolonged exposure to 100 μM CB (figure 12, 13). The time allowed for CB to be taken up (21 hours) was well within the limits of the normal uptake rate of *D. capensis* leaves (as shown by the ink control). CB is known to prevent the assembly of actin monomers into filaments by acting like a capping protein [4,6]. The drug blocks the barbed end of filaments where rapid assembly of monomers takes place but does not affect the pointed, slowly assembling end [4,6] .

The visualisation of fluorescent F-actin (figure 16 a-f) further confirms that CB had penetrated the leaf tissue (figure 12, 13) and prevented filament growth in the entire leaf (figure 16). It may even be suggested that some degree of depolymerisation occurred, as the sections from CB-treated leaves (figure 16) show less fluorescence than control leaves (figure 15). This finding indicates that CB, apart from inhibiting the conversion of G- to F-actin, also induced the disruption of filaments [6]. There is disagreement in the literature regarding this effect of CB [6], although it has been reported by various authors. Such controversy around the precise action of the drug has led to criticism of its use in studies on movement (cooper, ohmori). There is doubt as to the specificity of CB for actin, as it has been shown to interfere with sugar transport by binding to the glucose transporter [6,25]. This makes it difficult to establish, for example, which effect of CB (that on actin or on glucose transport) is responsible for the cessation of motility in cells. However, problems with the interpretation regarding the mode of action of CB only arise when the drug is used to prove that microfilaments are necessary for motility. The use and interpretation of CB in this study is justified, as it shows that microfilament formation is not required for leaf folding in *D. capensis*. This result is not ambiguous and allows the conclusion that polymerisation of actin can be ruled out as a factor mediating the trapping movement. This agrees with recent findings that polymerisation of actin monomers does not seem to be involved in the differential elongation of maize primary roots that are curving in response to gravity[2].

The role of depolymerisation - a possible mechanism

The possibility previously alluded to, that CB could have caused depolymerisation within the leaves of *D. capensis* is interesting and needs to be explored. Recent studies on the mechanism inducing stomatal movement have pointed towards the role of depolymerisation in stomatal opening by affecting turgor changes in the guard cells [10,17]. Based on these findings and speculation, a similar mechanism may be proposed for leaf folding in D. *capensis:*.

Initial stimulation of the leaf with prey causes actin to depolymerise within the leaf. This process potentiates inward potassium (K^+) ion channels in certain cells, causing an influx of potassium ions and water. The resulting increase in cell volume, presumably of cells in the lower epidermis of *D. capensis*, initiates the curling response of the leaf. Changes in turgor underlying the movement may be reversible or irreversible. Polymerisation of actin, shown to dominate in the curled leaf (figure 15), occurs after the relevant turgor changes have been activated. It is unrelated to the movement of the leaf. Once the prey has been digested, renewed depolymerisation in the upper epidermis reverses the folding by the same process outlined above. This straightens out the leaves again.

This kind of mechanism, modelled on the results and interpretation of the role of actin depolymerisation in stomatal movement [10,17] is conceivable for leaf curling in *D. capensis*. What speaks against it, is the seemingly undirected occurrence of actin depolymerisation throughout the leaf (figure 16). It does not appear to be primarily associated with cells of the lower epidermis (figure 16) where turgor gains would be expected. Upon comparison of the uncurled, unstimulated (figure 15) and the CB-treated leaf (figure 16), it also seems that limited depolymerisation took place in the CB-treated leaves. It is not known whether this could lead to significant turgor changes that would bend the leaf. However, the suggested mechanism cannot be eliminated as a possibility, until a time course of changes in the state of actin within the leaves has been conducted. This should start with the uncurled leaf and include

instances such as the onset and completion of leaf curl. At present it cannot be said what the state of actin is under natural circumstances when leaf folding is initiated. This shows the limitation in the data at hand, which look at two specific points in time, namely, at the state of actin before and after the movement. It would be of value to determine what the changes in actin are in between. The role of actin depolymerisation, rather than polymerisation, in causing leaf movement by indirectly influencing a turgor-based mechanism of movement is uncertain until tested.

Actin filaments and translocation

The results of the cytochalasin experiment (figure 12, 13, 16) show that the abundant microfilaments in the curled leaf are not crucial in generating leaf movement but have a different function. As the phenomenon is so striking in the curled leaf (figure 14) in comparison with the unstimulated, control leaf, the function is likely to be linked with the trapping of prey. Several suggestions can be forwarded. Firstly, the function of actin in 'cytoplasmic streaming' or 'cyclosis' [15,26-28,32] is frequently mentioned in the literature and may be of importance in *D. capensis* following prey capture. 'Cyclosis' in cells of this plant was already noted in several early reports and according to these, the process occurred with far greater vigour in stimulated leaves than in unstimulated ones[8,22]. It is now known that the process does not involve random or bulk flow of the cytoplasm but rather a directed movement, largely coordinated by actin filaments[1,15]. These move particles, vacuoles and other organelles within cells to particular destinations, where they are required for certain processes [1].

As the digestion of prey items occurs on the surface of *D. capensis* leaves, actin filaments could play a vital role in transporting vacuoles to sustain this process. Observation that the centres of F-actin are generally located in sessile glands and tentacle stalks of the raised glands (figure 14-16) tie in with this suggestion. These glands are involved in the secretion of digestive enzymes and absorption of broken-down prey materials [18]. Vacuoles filled with these substances must be taken to or

away from the glands for redistribution throughout the leaf. The 'vesicles' (figure 14 g, figure 5, 7), seen mainly in the curled leaf, could be interpreted as vacuoles surrounded by actin filaments involved in translocating the required substances (e.g. hydrolytic enzymes or absorbed materials). Such vacuoles would be expected to be more abundant in the stimulated leaf (figure 14). In the unstimulated leaves their content may differ (more mucilage), but they should be transported in the same fashion. Thus some vacuoles (figure 15) should be observed in the uncurled leaves and it makes sense that they are mainly located at the base of the tentacle (figure 15 c,d). In the CB-treated leaves, there is very little sign of vacuoles (figure 16) as actin filaments are not present to surround and stain the vacuoles green, despite their potential presence.

Preliminary experiments provide some evidence of the importance of F-actin in secretion (figure 17) as the release of digestive enzymes appears to be diminished in CB-treated leaves. F-actin is virtually absent from these (figure 16). While some digestive activity is taking place (figure 17b), this may be explained by the retention of polymerised actin in some of the sessile glands (figure 16 c-f). The variability in the digestive activity (figure 17) seems to correspond well with the variation found in the amount of polymerised actin left in the sessile glands and leaf stalks (figure 16, c-f) after leaves had been exposed to CB. These results should stimulate further investigation, as they provide strong indications of the role of microfilaments in translocation in *D. capensis.*

CONCLUSION

The importance of turgor changes in generating the movement of *Drosera capensis* leaves could not be shown conclusively in this study. There are two principal reasons for this. Firstly, the movement does not appear to be associated with marked changes in cellular and ultrastructural organisation within the curled relative to the straight leaf. The only distinguishing feature that could be related to a turgor-based

movement may be the presence of folded cell walls in the curled leaf. Secondly, the study approach was inadequate for detecting seemingly slight alterations in cell size and shape, as a qualitative and not quantitative methodology was employed. Thus it may be proposed that the role of turgor should be investigated using a more rigorous, quantitative assessment of the relevant cellular changes.

The influence of actin polymerisation on the motility of the *D. capensis* leaf could be eliminated as the absence of microfilaments does not inhibit movement. The use of cytochalasin B in this study is justified as it negates rather than proves the involvement of actin filaments in leaf curling. Instead, polymerised actin be important in the leaf after prey capture, as it seems to play a fundamental part in the translocation of vacuoles that may contain digestive enzymes for secretion and materials absorbed from the break-down of prey.

The disassemby of actin filaments could potentially affect leaf folding in *D. capensis* via regulation of turgor changes. This proposition is prompted by recent work on stomata showing the possible link between turgor gains of guard cells to open stomata and the depolymerisation process. The suggestion with respect to leaf curl *in D. capensis* remains to be tested. This study, therefore, does not resolve the question of what causes leaf movement in *Drosera capensis* to trap insects but it eliminates the role of actin polymerisation and stimulates possibilities for further investigations of the dynamics within this plant.

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