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Seed Dormancy in *Acer*: Maturation in Relation to Dormancy in *Acer pseudoplatanus* L.

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ABSTRACT

Maturation of *Acer pseudoplatanus* seeds comprises three phases. The first is a growth phase lasting for about 100 d, during which fresh weight and dry weight increase steadily. The end of the growth phase is marked by a temporary cessation of weight increase and is followed by a phase of reserve accumulation extending for a further 60 d. During this phase levels of extractable lipid, soluble and insoluble carbohydrate, protein, and membrane-bound ribosomes show a marked increase. The third phase is a period of desiccation, lasting at least 30 d. The kind of dormancy exhibited by the immature seed is characteristic of the stage of maturation. Before the desiccation phase the embryo has no capacity for germination even when subjected to treatments known to promote germination in the mature seed. In the desiccation phase the seed becomes responsive to chilling, but a wide range of hormones and other substances known to promote seed germination are ineffective. Embryos contain a persistently high level of acidic inhibitors throughout maturation. The level of neutral inhibitors, initially high, showed a marked drop after 150 d and remained low for the rest of the maturation period.

INTRODUCTION

During seed maturation there is a pattern of development that, with minor variations, is common to most seeds for which data are available (Thomas, 1972). Three phases of maturation can be recognized: a phase of cell division and growth, one of reserve accumulation, and one of desiccation. During maturation, changes in biochemical activity occur that can be correlated with these phases of development. This pattern of seed maturation has been established by study of seeds from a limited number of species. For obvious reasons the seeds of herbaceous species, particularly those of commercial value, are convenient objects of study and most of the data concerning seed maturation refer to these species. There is very little information available on the subject of maturation in seeds of woody angiosperms. Although there is every reason to believe that the maturation process in tree seeds does not differ fundamentally from the process in the seeds of non-woody species, many tree seeds are dormant when shed (Wareing, 1969). This poses the question whether dormancy is a result of some special pattern of development

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in these seeds. To answer this we have studied the maturation of seeds of *Acer pseudoplatanus* (sycamore), which have a well characterized coat-imposed dormancy (Webb and Wareing, 1972*a*) controlled by the interplay of inhibitory and growth-promoting hormones present in the seed (Webb and Wareing, 1972*b*).

MATERIALS AND METHODS

Maturing sycamore samaras were collected from the Botany Gardens, University College of Wales, Aberystwyth, throughout the 1971 season. It was considered necessary to collect fruit from the same part of the same tree to minimize the effects of genetic and micro-environmental variation. Anthesis occurred at about 15 May, which was taken as day zero from which other sampling occasions were timed. Dry weights were determined by heating seeds to 80 °C for 3 d.

Application and estimation of hormones

Germination tests were performed by sowing seeds in Petri-dishes on filter-paper moistened with hormone solutions. Seeds were stratified by storing moist in sealed polythene bags at 5 °C. Acidic and basic inhibitors were extracted and bioassayed as described by Webb and Wareing (1972*b*).

Lipid and carbohydrate determination

Lipid was extracted from dried, powdered seeds with methanol-chloroform (1:1 v:v) by Soxhlet reflux for at least 4 h at 70 °C and determined by weighing the ether-soluble fraction after evaporation to dryness.

Soluble carbohydrates were extracted in cold water, and oligosaccharides of medium size in boiling water. Sugars were determined by mixing with two volumes of 0·2 per cent anthrone in concentrated H_2SO_4 and reading the absorbance at 620 nm after 15 min. Residual polysaccharides were determined by weight after washing the water-insoluble material with ethanol and ether.

Protein determination

Seeds were homogenized in 10 per cent trichloroacetic acid and the protein precipitate solubilized in formamide. After diluting with an equal volume of water, 30-μl aliquots were applied to 1-cm squares of filter-paper and protein determined by a modification of the method of Bramhall, Noak, Wu, and Loewenberg (1969) using 0·25 per cent coomassie brilliant blue R in methanol–acetic acid–water (5:1:5 v:v:v) as the stain and methanol–ammonia–water (66:1:34 v:v:v) as the elution solvent.

Extraction and analysis of nucleic acid and ribosomes

Nucleic acid was extracted by the perchloric acid (PCA) method of Heyes (1960), substituting methanol for ethanol and chloroform for ether throughout the procedure, and determined by measuring the absorbance at 260 nm after hydrolysis with 5 per cent PCA for 20 min at 70 °C. Highly pure RNA was extracted by the method of Loening and Ingle (1967) and fractionated by polyacrylamide-gel electrophoresis as described by Loening (1969).

Ribosomes were extracted from seeds homogenized over liquid nitrogen and fractionated into free, light membrane-bound, and heavy membrane-bound as described by Tata (1969). Free and bound RNA were determined by the PCA method. Bound ribosomes were freed from membranes by adding sodium deoxycholate to a final concentration of 0·5 per cent and resedimenting at 105 000 *g* for 2·5 h. Ribosomes were run on sucrose gradients as described by Pearson (1969).

Electron microscopy

Tissues were fixed in glutaraldehyde and OsO_4 essentially as described by Mollenhauer and Totten (1971) and, after dehydration, embedding, and sectioning, thin sections were stained with uranyl acetate at 60 °C and lead citrate, and examined with the AEI EM6 electron microscope.

RESULTS

From anthesis to seed maturity is a period of 180–90 d. Dispersal takes place over an extended period with the result that there are often appreciable numbers of fruits still attached to the tree more than 250 d after anthesis. Maturation can be divided into three phases. In the first phase (up to day 100—Fig. 1) both fresh weight (f.w.) and dry weight (d.w.) increase with no appreciable change in seed water content. This is a phase of cell growth and division and the embryo attains its final morphological form 70–80 d after anthesis. The end of the growth phase is marked by a temporary cessation of fresh-weight increase. The fresh-weight curve is thus biphasic and in this respect resembles the curve for developing seeds

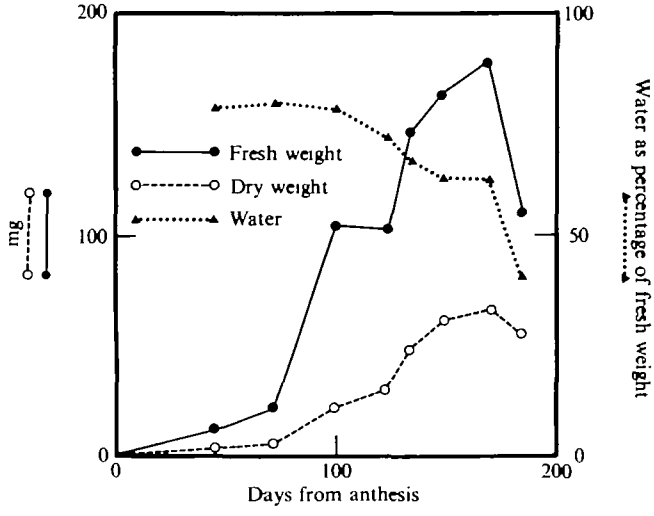

FIG. 1. Changes in weight and water content during the maturation of sycamore embryos.

of other species, such as *Pisum* (Burrows and Carr, 1970) and *Lolium* (Hedley and Stoddart, 1972). In the second phase, f.w. and d.w. continue to increase but water content begins to fall (Day 100–60). The wing and pericarp of the fruit begin to dry out some weeks before there is a reduction of seed water content. During this second phase there is an active synthesis of storage materials—protein, lipid, and carbohydrate—and the observed drop in water content is probably due to the substitution of these products for vacuolar sap. A gradual reduction of water content seems to be a feature of late maturation in many seeds and has been suggested (Thomas, 1972) to be important for the preparation of the seed for eventual desiccation when maturity is reached. Desiccation occurs in phase three (Day 160–90) during which f.w. and water content fall steeply, although water content in seeds under natural conditions seems not to fall below 40–5 per cent of total seed weight.

It is of interest to know what kind of dormancy pattern is exhibited by maturing sycamore seeds, especially in view of the apparent morphological maturity of the embryo as early as 100 d before dispersal. Seeds were collected on a series of

occasions from anthesis to seed maturity. At sampling times the covering structures were removed and the bare embryos set to germinate on filter-paper moistened with distilled water, 1·0 mg/l kinetin or 10·0 mg/l GA_3 at 20 °C. No germination was recorded at any sampling time or in any treatment, indicating that the embryos were dormant and did not respond (as do other seeds with embryo dormancy) to hormone application. Furthermore, stratification of fruits collected at the end of July (about Day 70) and at the end of August (Day 100) for 60 d failed to overcome dormancy.

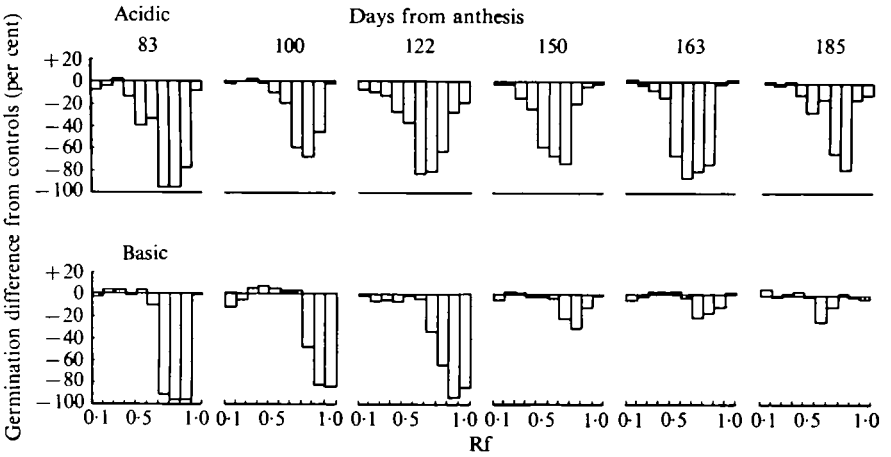

FIG. 2. Acidic and basic inhibitors extracted from maturing sycamore embryos and measured by the lettuce seed germination bioassay.

Fruits were collected at the end of October (about Day 170) and subjected to a number of treatments, many of which had been shown previously to remove dormancy in such typically embryo-dormant seeds as *Acer saccharum* and *Pyrus malus*. Only moist after-ripening at 5 °C was effective in breaking dormancy. Whole fruits showed 70 per cent germination after 80 d stratification at 5 °C. Treatment with GA_3 (0·1, 1·0, 10·0 and 100·0 mg/l) and kinetin (0·1, 1·0, and 10·0 mg/l), alone and in combination, as well as with ethylene chlorhydrin (over a wide range of concentrations) were ineffective in breaking dormancy. Other treatments such as subjecting seeds to high temperatures of 30, 35, and 75 °C for various periods of time, application of the respiratory inhibitor sodium azide, and dry storage at 20 and 5 °C had no effect. Storage of the fruits in a moist state at 20 °C was partially effective in breaking dormancy but the seeds were highly susceptible to microbial attack and considerable losses occurred after only a few days.

The inability of immature seeds to germinate under many of the conditions that normally break dormancy may be due to the presence of inhibitory substances in the seed, since such substances have been implicated in the coat-imposed dormancy of mature seeds (Webb and Wareing, 1972*b*). Levels of acidic and neutral inhibitors were determined in seed collected during the later stages of seed development to establish whether there might be a correlation with the stage of dormancy. The possibility that dormancy might be a result of some organizational or biochemical

blockage was also examined. Reserve accumulation in seeds is a highly organized process and it is reasonable to believe that when the cells of a seed are actively laying down storage reserves it is not possible to induce a premature change in their pattern of metabolism. Lipid, carbohydrate, and protein accumulation were investigated in relation to dormancy in maturing seeds.

A high level of acidic germination inhibitor was found throughout the development period from the time of first sampling at Day 83 (Fig. 2). A high level of

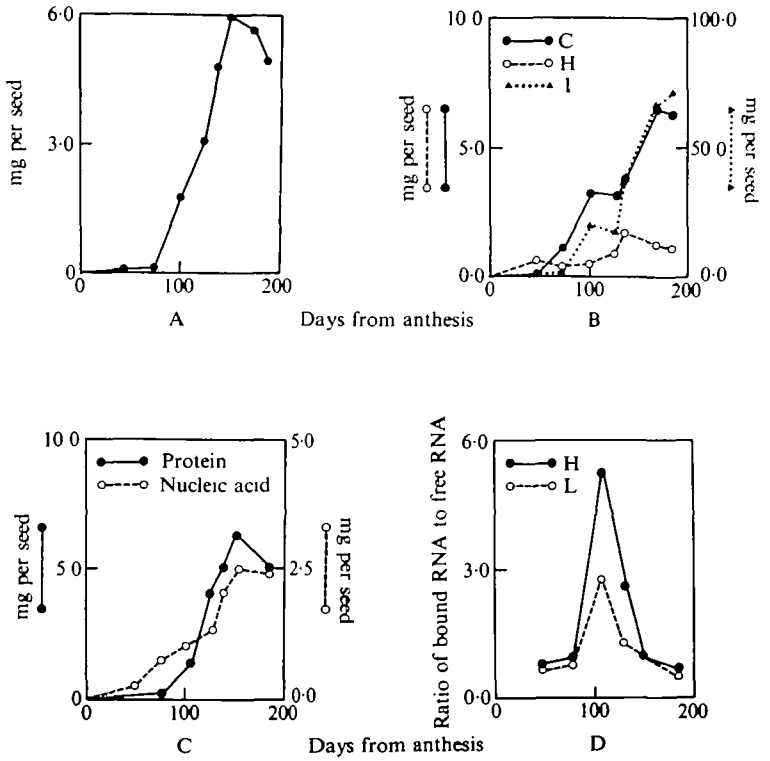

FIG. 3. Changes in levels of: A, lipid; B, carbohydrate (C, cold-water soluble fraction; H, hot-water soluble and I, insoluble); C, protein and nucleic acid; D, membrane-bound ribosomes (H, heavy and L, light) during the maturation of sycamore seeds.

neutral inhibitor was also found during the early sampling times. At 150 d from anthesis, however, the level dropped and remained low for the rest of the developmental period, i.e. until Day 185.

During the growth and division phase of seed maturation lipid content remains low (Fig. 3A). A rapid increase in lipid levels begins at about Day 80 and continues throughout the phase of reserve accumulation. Cells of maturing embryos contain numerous small globules (Plate 1), possibly sites of lipid storage since they resemble in size and distribution the lipid bodies of the seeds of other species, such as *Fraxinus* (Villiers, 1971). A slight decrease in lipid levels was observed after Day 160. This may be due to an actual breakdown of lipid by some metabolic

process, or simply to the fact that seeds persisting on the tree tend to contain less lipid as a result of leaching.

There is also little increase in carbohydrate levels up to Day 70–80 (Fig. 3B). Thereafter, both cold-water-soluble and insoluble fractions increased greatly while hot-water-soluble carbohydrate increased very slightly. Starch is the principal carbohydrate reserve. The plastids of maturing seeds become packed with starch granules during the phase of reserve accumulation (Plate 1).

Protein appears to be the main storage product of sycamore seeds. The protein content of developing seeds increases rapidly at the end of cell division (Fig. 3C). Storage protein is probably located in aleurone grains, since large aleurone body-like inclusions, usually no more than seven or eight per cell, begin to proliferate in cells of the seed at Day 80–100 (Plate 1).

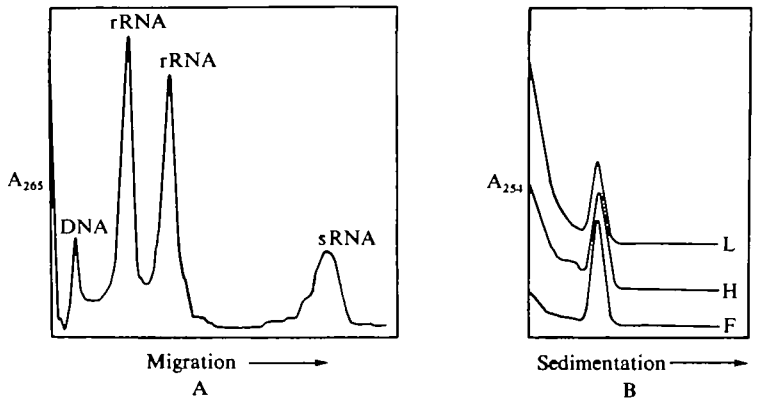

FIG. 4. A, nucleic acid of 105-day-old embryos fractionated by gel electrophoresis; B, free (F), heavy membrane-bound (H), and light membrane-bound (L) ribosomes of 130-day-old embryos fractionated by density-gradient centrifugation.

There are two main theories of the origin of seed storage protein (see Thomas, 1972). One is that the developing aleurone bodies contain a complete protein-synthesizing system separate from the cytoplasmic system. This is not in favour at present, since it has been shown that the protein-synthesizing activity formerly ascribed to aleurone grains is due to bacterial contamination (Wilson, 1966). The other theory is that storage protein is made on membrane-bound ribosomes and transferred to the aleurone body through the endoplasmic reticulum (Bailey, Cobb, and Boulter, 1970). There is a great deal of evidence in favour of this view and it seems to be the likely origin of reserve protein in sycamore seeds, since levels of nucleic acid are high at the stage when protein accumulation takes place (Fig. 3C) and an increasing proportion of this RNA is found in the membrane fractions (Fig. 3D). The RNA of these seeds was shown by gel electrophoresis (Fig. 4A) to be predominantly ribosomal, and detergent treatment of membrane fractions releases ribosomes that sediment as a single band when centrifuged in sucrose gradients (Fig. 4B). Thus the protein-synthesizing machinery of sycamore seeds is predominantly membrane-bound at a time when storage protein is being accumulated.

DISCUSSION

The dispersal units of sycamore normally show a coat-imposed dormancy. Removal of the covering structures allows germination of the non-dormant embryo on a moist medium (Webb and Wareing, 1972*a*). The results described here show that during seed maturation the embryo itself is dormant. This agrees with observations made by Phillips (1959) who found that sycamore seeds collected in September would not germinate even with the covering structures removed, whereas seeds collected 2 months later from the same tree would germinate under these conditions. Luke (1965) also found a period of embryo dormancy in the seed collected in October and early November; in this crop dormancy lasted until December. It appears that sycamore seeds undergo a change of dormancy type from embryo dormancy in the developing seed to a coat-imposed dormancy in ripe seeds.

Embryo dormancy in sycamore is quite unlike embryo dormancy in other *Acer* species, notably *A. saccharum* and *A. ginnala* (Webb and Dumbroff, 1969; Dumbroff and Webb, 1970), in that at no stage during maturation does it respond to hormones or other stimulatory treatments. Furthermore, although stratification is effective in removing the embryo dormancy of seeds nearing maturity, it is ineffective in younger seeds, collected at Day 70 and Day 100. This suggests that during maturation sycamore seeds pass through a number of different dormancy stages. During the growth and division phase the embryo is morphologically immature and unresponsive to chilling or hormone treatment. During the reserve accumulation phase the embryo, despite its morphological maturity, also does not respond to chilling or hormone treatment. In the desiccation phase dormancy of the embryo may be removed by chilling, but hormonal and other stimulatory treatments elicit no response. There is no direct evidence for a stage when the dormant sycamore embryo may be stimulated to germinate by both hormones and chilling treatment, although such a dormancy type exists in other *Acer* species (Webb and Dumbroff, 1969; Dumbroff and Webb, 1970); perhaps there is a dormancy of this kind in sycamore but it may be of limited duration. Next, the dormancy of the embryo is lost and dormancy is imposed by the coat as described by Webb and Wareing (1972*a*); finally, under natural conditions, the seed becomes non-dormant in response to winter chilling. Since the seeds of different *Acer* species are morphologically very similar to one another, it is probable that they all pass through a similar maturation process; the variety of different types of dormancy found in the genus may reflect differences in the duration of particular stages in the changing pattern of dormancy just described.

The coat-imposed dormancy of sycamore and the embryo dormancy of sugar maple have been shown to be controlled, at least in part, by endogenous hormones (Webb and Wareing, 1972*b*; van Staden, Webb, and Wareing, 1972; Webb, van Staden, and Wareing, 1973); however, the lack of response by embryo-dormant sycamore seeds to gibberellin, cytokinin, and ethylene over a range of concentrations suggests that the dormancy present in sycamore embryos during development is not controlled by an established type of hormonal interaction. The cells of embryos, having attained morphological maturity, become organized for the

synthesis of reserves. There is a massive synthesis of reserve protein, lipid, and carbohydrate, and distinctive changes occur in the ultrastructure of the reserve tissues. In particular, protein is accumulated in large membrane-bound masses resembling aleurone grains (Altschul, Yatsu, Ory, and Engleman, 1966). At this stage the protein-synthesizing machinery of the cell is predominantly membrane-bound. A high level of membrane-bound ribosomes occurs during the phase of reserve-protein synthesis in maturing bean seeds (Payne and Boulter, 1969). These observations imply that reserve protein is synthesized by membrane-bound ribosomes and transferred to the developing aleurone grain. There is every reason to suppose, therefore, that cells of the embryo at the end of the growth phase of maturation are programmed for reserve synthesis and are incapable of switching over, in a biochemical sense, to the synthesis of products needed for germination.

At this stage there is also a high level of basic inhibitor (Fig. 2); this has disappeared by the time the seed has passed to the next dormancy stage, in which chilling is effective in removing embryo dormancy. The loss of this inhibitor may be related to the reduction of water content over the 150–90-d period (Fig. 1). It is not clear just what function this inhibitor has. Since embryo dormancy in immature seeds seems not to be hormonally controlled, it is unlikely that the presence of the inhibitor is a direct cause of dormancy at this stage. It may be more concerned with the control of the cessation of cell growth and division and the change to reserve accumulation.

A period of chilling induces the seed to pass on from embryo dormancy at maturity to coat-imposed dormancy. The controls that operate at this stage are not clear. At this time seeds are normally collected for storage. The seeds and fruits of many trees are particularly difficult to store in the dormant state (Jahnel, 1955; Roberts, 1972; Jones, 1944) and are very sensitive to moisture conditions. It would appear that there is some sort of link between embryo dormancy overcome by chilling, sensitivity to moisture conditions, and viability in sycamore seeds, but the precise nature of the link is not clear at present.

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EXPLANATION OF PLATE

Plate 1. Electron micrograph of a cotyledonary cell of mature sycamore embryo showing starch grains (s), aleurone bodies (a), lipid droplets (l), and the cell wall (cw).

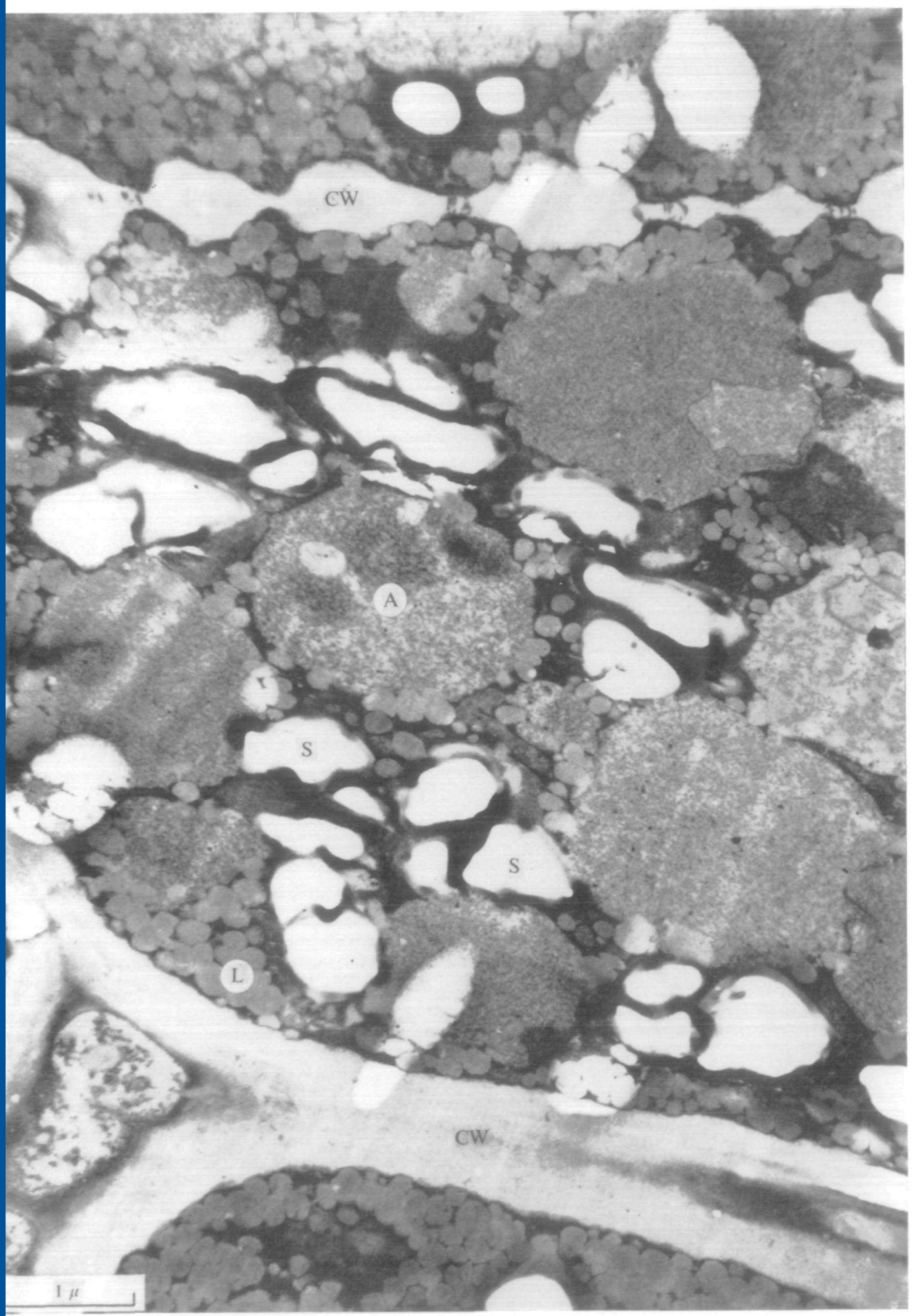

PLATE 1