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Structure and Distribution of Chloroplasts and Other Organelles in Leaves with Various Rates of Photosynthesis[1]

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ABSTRACT

The ultrastructure and distribution of chloroplasts, mitochondria, peroxisomes, and other cellular constituents have been examined in cross sections of leaves from plants with either high or low photosynthetic capacity. Photosynthetic capacity of a given plant cannot be correlated with the presence or absence of grana in bundle sheath cell chloroplasts, the presence or absence of starch grains in bundle sheath or mesophyll cell chloroplasts, the chloroplast size in bundle sheath or mesophyll cells, or the location of chloroplasts within bundle sheath cells. We conclude that the number and concentration of chloroplasts, mitochondria, and peroxisomes in bundle sheath cells is the most reliable anatomical criterion presently available for determining the photosynthetic capacity of a given plant.

A concept has been developed recently for dividing higher plants into at least two distinct groups[2] which, for convenience, we will designate as either high or low photosynthetic capacity plants. These divisions do not conform to the usual taxonomic categories nor do they appear to adhere to widely recognized anatomical features. Biochemical, physiological, and anatomical data are presently available indicating that at least four genera have species in the two groups of plants (2, 3, 9, 17, 25), and other genera are likely to be detected as research continues. This manuscript is concerned with presenting anatomical criteria for placing a given plant into one of the two groups. For example, at one time it was thought that the absence or presence of well defined grana in certain chloroplasts was a reliable criterion for placing a plant in one of the groups. However, this criterion does not fit all of the present data (3, 10, 20), and therefore, other criteria also must be considered. To the authors, the clearest anatomical criterion presently available for dividing plants into two groups is the distribution of organelles within the leaf. A review of the literature reveals that this general anatomical feature was observed in the light microscope and clearly described in several genera prior to 1900 (15). Nevertheless, this significant research was not widely utilized until the extensive biochemical and physiological research of the last decade indicated a definite correlation of anatomy with physiology and biochemistry. This manuscript will present data on the distribution and ultrastructure of organelles in leaves of high photosynthetic capacity plants, which will be compared to the extensive anatomical literature on leaves of low photosynthetic capacity plants (13, 23, 24).

MATERIALS AND METHODS

Plants used were either field grown or grown in flats outdoors, in full sunlight during midsummer in Yellow Springs, Ohio. All of the leaves were 7 to 21 days of age and were harvested by 9.00 AM immediately before fixing. The monocotyledonous plants studied were *Triticum vulgare* L. (wheat), *Cynodon dactylon* L. (coastal bermudagrass), *Setaria viridis* L. Beauv. (foxtail), *Leptochloa dubia* H. B. K. Nees., *Digitaria sanguinalis* (L.) Scop. (crabgrass), and the sedge, *Cyperus rotundus* L. (nutsedge). Dicotyledonous plants studied were *Lantana camara* L. and *Amaranthus retroflexus* L. (pigweed) (13, 14, 23, 24). All of these species have a high photosynthetic capacity except wheat and *Lantana*. Our studies on wheat and *Lantana* will only be referred to as unpublished results since these confirm data which are easily available in published form (13, 23, 24).

Small cross-sections (0.05 to 0.1 mm thick) of leaves were cut free hand and were pre-fixed for 2 hr at room temperature in a mixture of 2% gluteraldehyde-2% paraformaldehyde buffered with 0.05 M collidine plus 0.06 M sucrose at pH 7.3 to 7.4. The tissues were then rinsed for 1.5 hr in collidine buffer and were postfixed in either aqueous 1.0% $KMnO_4$ for 0.5 hr at room temperature or 1.0% OsO_4 overnight at 2 to 4 C. Following fixation, the tissues were rinsed in distilled water, were dehydrated in a graded series of acetones, and were embedded in a mixture of Epon and Araldite epoxy resins.

Sections 0.5 μ thick were cut for light microscopy and mounted on standard 1 × 3-inch glass microscope slides and then allowed to dry. They were then stained with Paragon multiple stain for frozen sections (Paragon C. and C. Co., Inc.) by the following procedure. A drop of stain, to which a small "pinch" of sodium borate was added, was placed on top of the sections mounted on the microscope slide. The slide was then placed on a hot plate (about 100 C) and heated for 15 to 30 sec. The sections were then rinsed under the distilled water tap, dried, and covered with a glass cover slip.

Sections for electron microscopy were post-stained in uranyl acetate and lead citrate and then were viewed with a Philips EM-200 electron microscope.

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[2] The data supporting the concept of distinct groups of higher plants are too extensive to be cited fully in this manuscript. Interested readers are referred to references 2 to 4, 8, 12, 16, 17, 20, 32, and 33 for more complete citations.

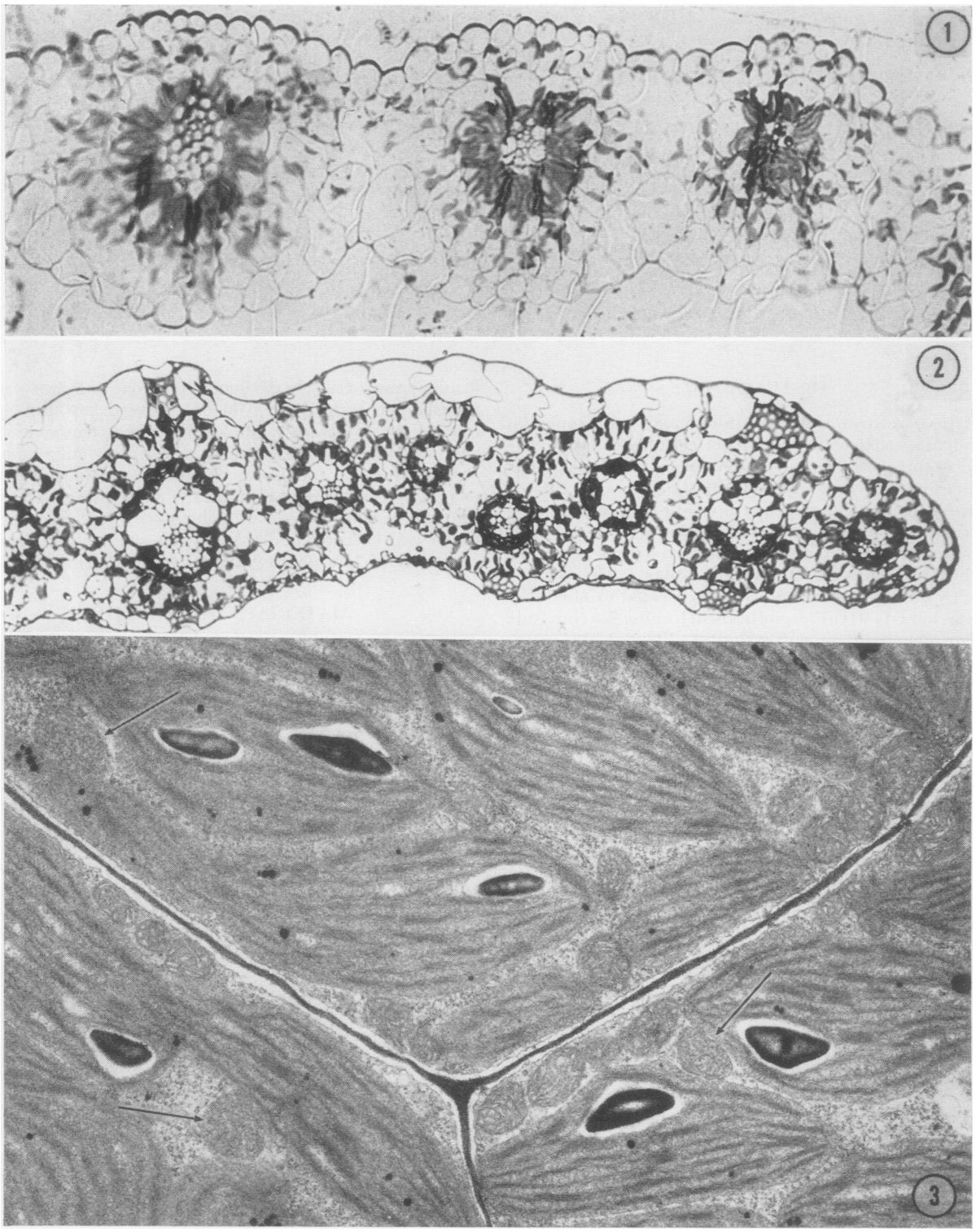

FIG. 1. Light micrograph of a *Leptochloa dubia* leaf cross-section. × 340.
FIG. 2. Light micrograph of a nutsedge leaf cross-section. × 340.
FIG. 3. Micrograph of a pigweed leaf cross-section showing the dense organelle concentration in bundle sheath cells. Arrows denote peroxisomes. × 16,500.

RESULTS AND DISCUSSION

One of the most striking anatomical features of plants with a high photosynthetic capacity is the dense concentration of chloroplasts in mature leaf bundle sheath cells compared to mesophyll cells. Before the turn of this century, this anatomical feature was clearly described in leaf cross-sections, and a lucid description of these observations is presented in the classical textbook of Haberlandt (15). In later research, confirming observations were made on maize and sorghum (28) and bermudagrass (1).

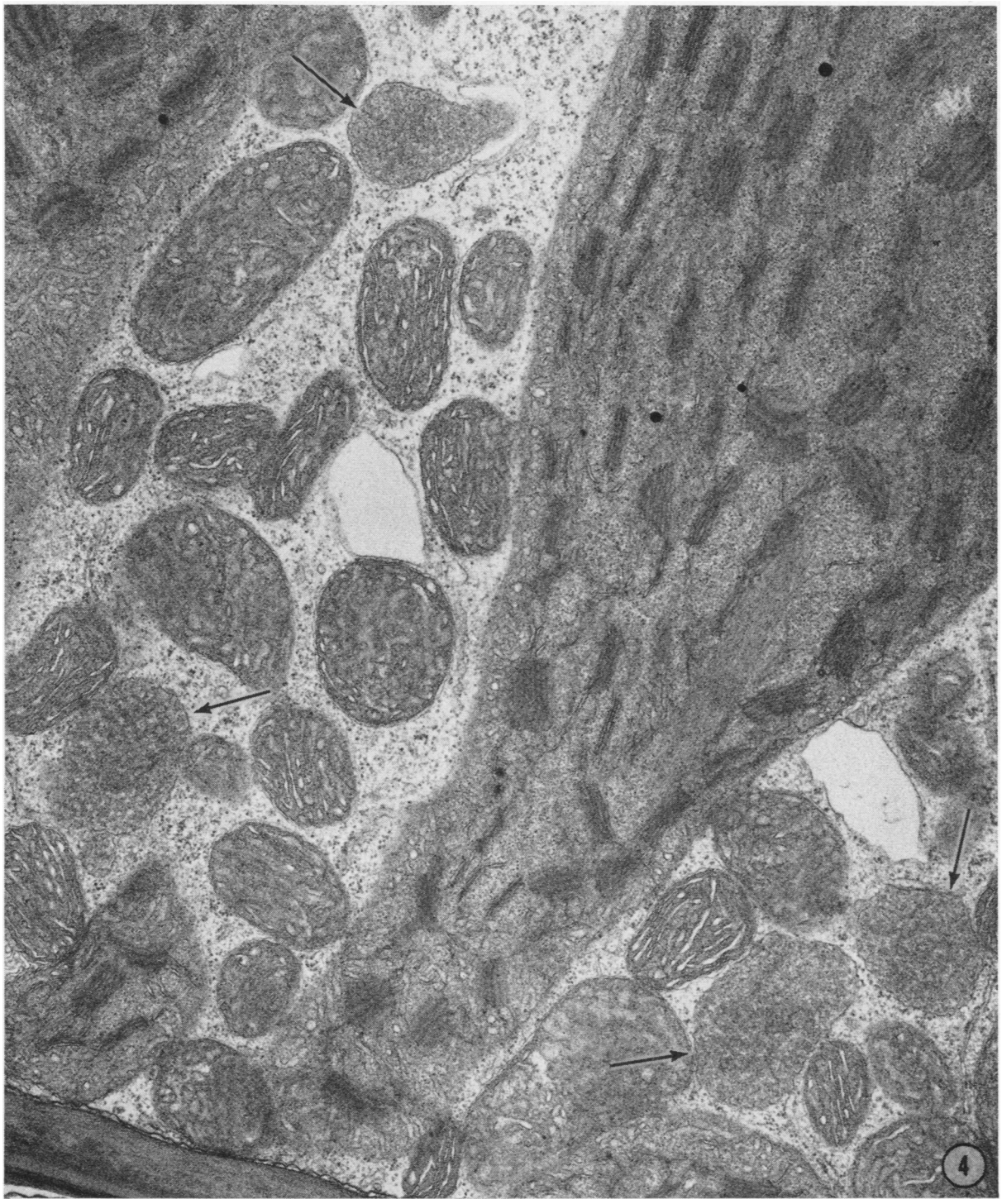

FIG. 4. Micrograph of a bermudagrass bundle sheath cell section showing the peripheral reticulum and grana in chloroplasts and the periphera reticulum in mitochondria. Arrows denote peroxisomes. Tissue was postfixed in OsO_4. × 26,000.

Prat (27) and Brown (5, 6) have utilized this anatomical feature to revise partially the systematics of the Gramineae based on the location of chloroplasts within the bundle sheath cells. Recently, similar observations have been made in the dicots *Amaranthus* and *Atriplex*, as well as other members of Gramineae (3, 10–12, 20). The light micrograph of a leaf cross-section of *Leptochloa* and nutsedge (Figs. 1 and 2) illustrates this general observation in high photosynthetic capacity plants. Low photosynthetic capacity plants have chloroplasts more evenly distributed throughout the leaf (13, 23, 24).

An electron micrograph study of maize leaves (18) revealed that the bundle sheath chloroplasts lacked well defined grana, whereas mesophyll cell chloroplasts contained distinct grana. This type of observation was subsequently extended to sugar cane (21, 22, 34) and other Gramineae, which led to the conclusion that lack of grana in bundle sheath cells was a feature

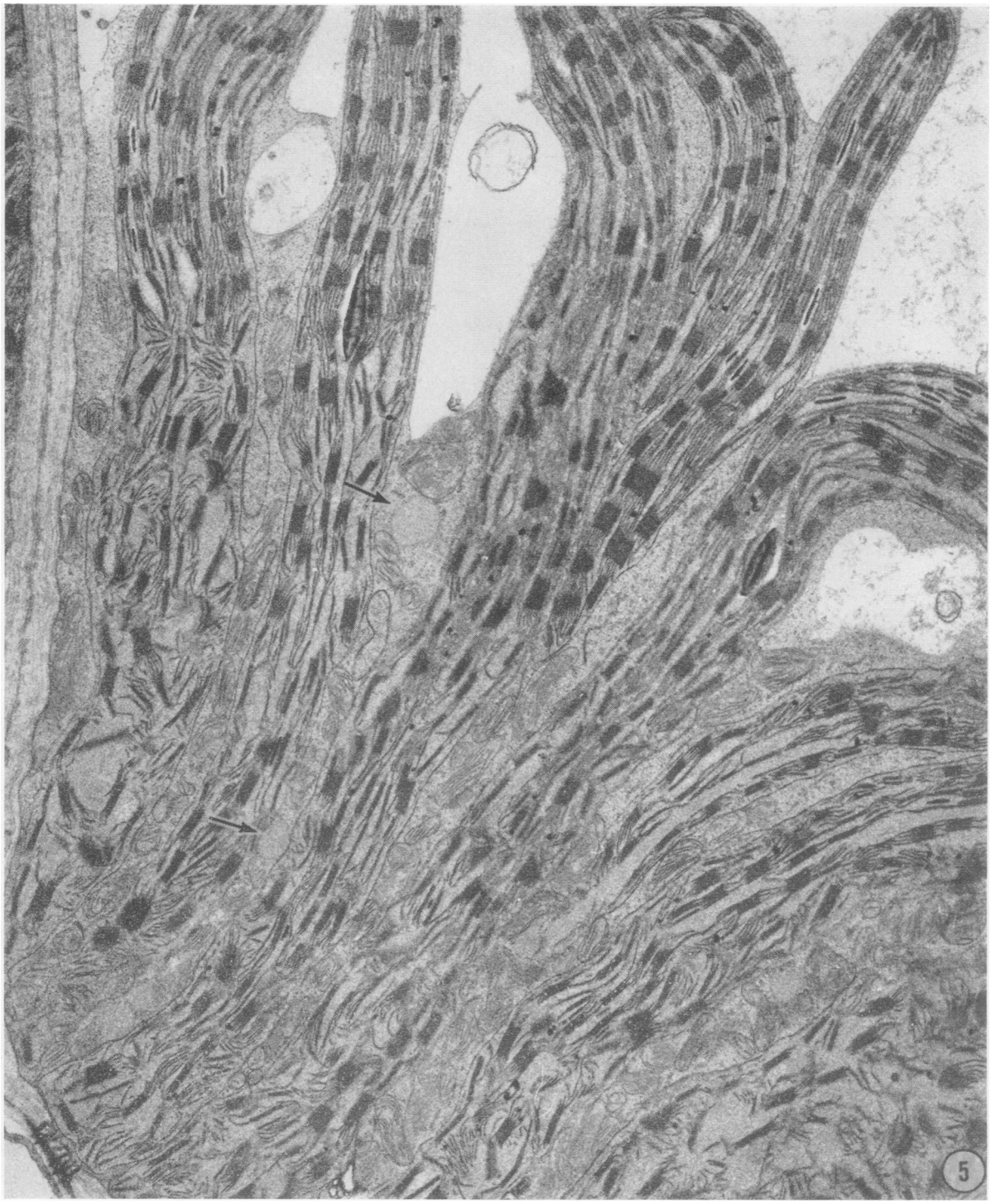

FIG. 5. Micrograph of a *Leptochloa* leaf bundle sheath cell section showing the dense concentration of organelles. Arrows denote peroxisomes. Tissue was postfixed in $KMnO_4$. × 11,000.

characteristic of high photosynthetic capacity plants. However, electron micrograph studies of dicotyledonous plants with high photosynthetic capacity indicate that prominent grana may or may not be present in bundle sheath cell chloroplasts (3, 10, 20) (Fig. 3). In addition two genera of Gramineae in the present study, *Cynodon* and *Leptochloa*, have well developed grana in the bundle sheath cells (Figs. 4 and 5). These two grasses have a high photosynthetic capacity (4, 7, 8). In regard to the criterion of well developed grana in the mesophyll cells and poorly developed grana in the bundle sheath cells, the data on pigweed, bermudagrass, and *Leptochloa* appear to invalidate the idea that this type of anatomy is a reliable criterion for indicating photosynthetic capacity. Furthermore, in other studies, certain mutant plants have been detected with chloroplasts containing poorly developed grana (30), and yet these plants appear to have the other general characteristics of low photo-

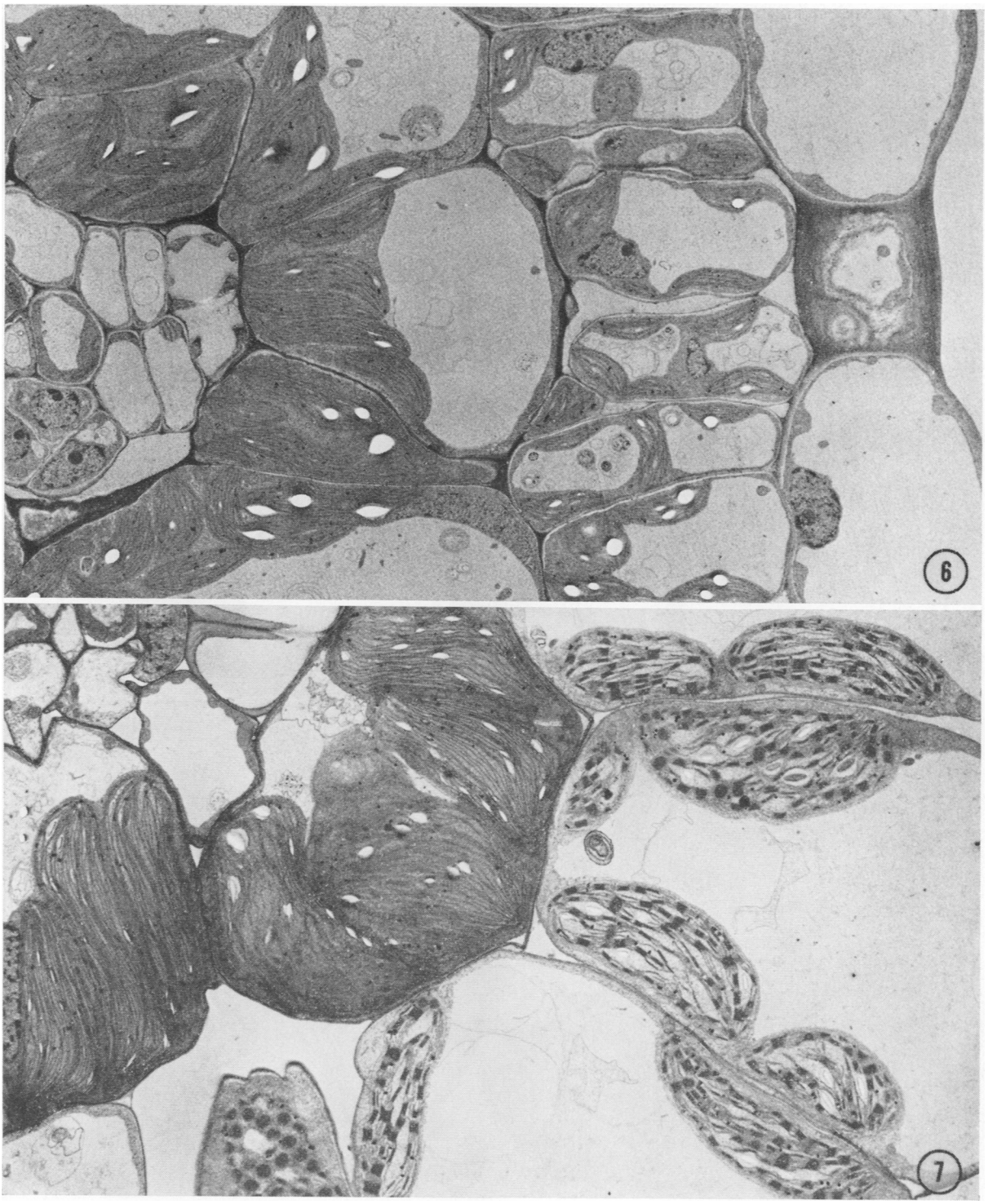

FIG. 6. Micrograph of a pigweed leaf cross-section showing chloroplasts and other organelles concentrated in bundle sheath cells surrounding the vascular tissue. Note the prominent white starch grains in both bundle sheath and mesophyll cell chloroplasts. × 3,600.

FIG. 7. Micrograph of a crabgrass leaf cross-section showing the dense chloroplast concentration in the bundle sheath cells. The chloroplasts in the bundle sheath cells only have a few rudimentary grana, whereas the chloroplasts in the adjacent mesophyll cells have highly developed grana. Note the presence of starch grains in all chloroplasts. × 4,000.

synthetic capacity plants (30). There is, however, some indication that chloroplasts in high photosynthetic capacity plants, such as maize or *Amaranthus*, may possess a highly developed peripheral reticulum (20, 29) (Fig. 4). Further research is needed to determine the distribution of this characteristic in other genera.

The general observation also has been presented that only bundle sheath chloroplasts accumulate starch grains in high

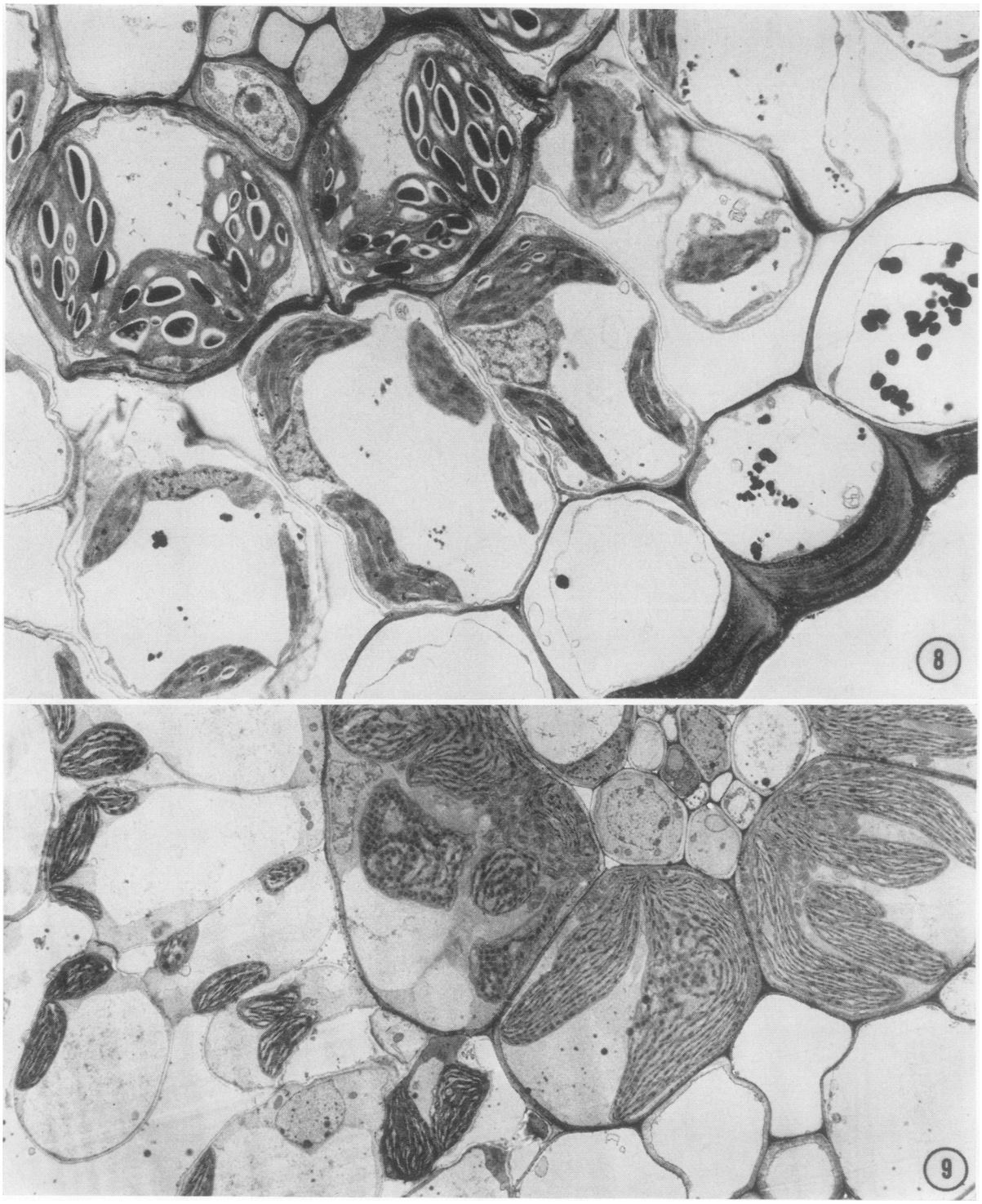

FIG. 8. Micrograph of a foxtail leaf cross-section. As in crabgrass (Fig. 7) and nutsedge (Fig. 10) the chloroplasts of the bundle sheath cells are located peripherally to the vascular tissue. Note the presence of starch grains in all leaf chloroplasts. × 5,400.

FIG. 9. Micrograph of a bermudagrass leaf cross-section showing the dense concentration of chloroplasts, mitochondria, and peroxisomes in bundle sheath cells (also see Fig. 4). Chloroplasts in all cells have well developed grana. × 2,400.

photosynthetic capacity plants (11, 15), whereas in low photosynthetic capacity plants starch is present in leaf mesophyll chloroplasts (13). Indeed, this phenomenon has been utilized to separate, by density techniques, bundle sheath cell from mesophyll cell chloroplasts in certain species (32, 33), and a distinct difference in the distribution of enzymes of starch synthesis in the two chloroplasts from maize has been reported (19). An examination of Figures 6, 7, and 8 reveals that distinct starch grains have formed in both mesophyll and bundle sheath chloroplasts in these three high photosynthetic capacity plants, and we have made similar observations in nutsedge, bermudagrass, and *Leptochloa*. Other workers also have reported starch ac-

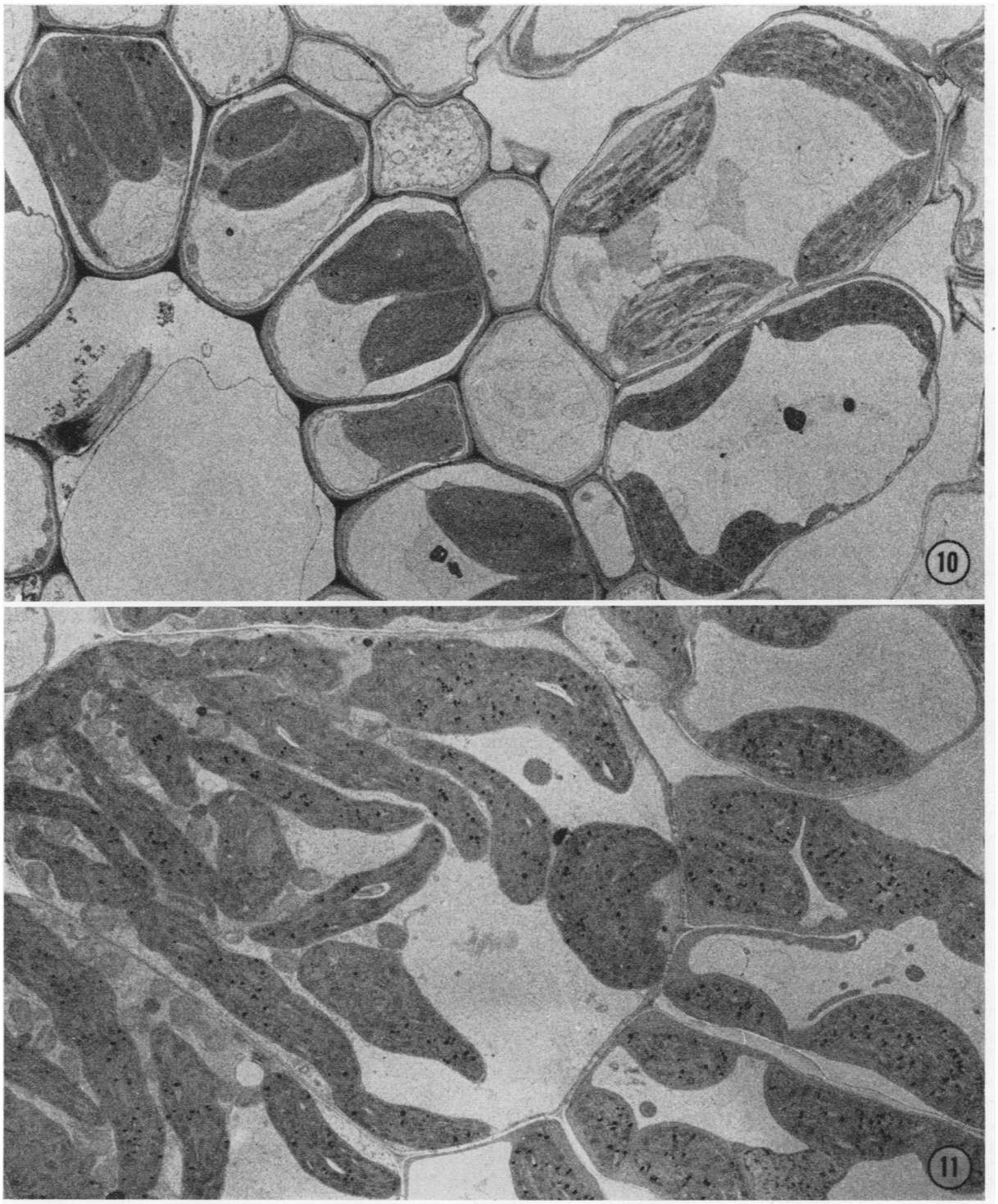

FIG. 10. Micrograph of a nutsedge leaf cross-section showing an inner layer of bundle sheath cells containing chloroplasts surrounded by another layer of cells that do not contain chloroplasts (also see Fig. 2). Prominent chloroplasts are present in the mesophyll cells which in cross-section appear to be at least as large as the bundle sheath cell chloroplasts. × 5,600.

FIG. 11. Micrograph of a *Leptochloa* leaf cross-section showing the distribution of organelles in bundle sheath cells (also see Fig. 5) and mesophyll cells. × 4,900.

cumulation in all leaf chloroplasts of several genera of high photosynthetic capacity plants (11, 20). We conclude that the lack of starch accumulation in mesophyll cells or starch accumulation patterns are unreliable criteria for determining the photosynthetic capacity of a plant.

Brown (5, 6), in a taxonomic study, employed location of chloroplasts (*i.e.*, either centrifugal or centripetal with respect to the vascular bundle) within the bundle sheath cells as another useful criterion in grass systematics. In comparative studies, such as rates of net photosynthesis or CO_2 compensation con-

centrations (8) compared with the cellular location of chloroplasts, we have not detected a correlation of cellular location with any known physiological or biochemical activity. Apparently, within bundle sheath cells a clustering of organelles near the vascular bundles as in pigweed, bermudagrass, and *Leptochloa* (Figs. 6, 9, and 11) or away from the vascular bundles as in crabgrass, foxtail, and nutsedge (Figs. 7, 8, and 10) is not related to leaf photosynthetic capacity or to any other known function.

Early workers noted that in maize and other species the bundle sheath cell chloroplasts appeared to be larger than mesophyll cell chloroplasts (13, 15, 27, 28). In some genera, for example *Cynodon* (Fig. 9), the bundle sheath cell chloroplasts are larger than mesophyll cell chloroplasts. But this characteristic is not observed with all species as illustrated in Figure 10 with nutsedge, in which the mesophyll cell chloroplasts are equal or perhaps larger in size than the bundle sheath cell chloroplasts, and in other species such as crabgrass and foxtail (Figs. 7 and 8) chloroplasts of equal size often are observed in both cell types. We conclude that chloroplast size is not a reliable criterion for assessing photosynthetic capacity.

Studies with the electron microscope not only revealed more of the chloroplast ultrastructures in leaves (18), but also indicated features of other organelles such as mitochondria and peroxisomes (2, 3, 10, 20, 21). Of particular interest in the present manuscript is the dense concentration of cellular organelles in bundle sheath cells around the vascular bundles in plants with a high photosynthetic capacity, which is in marked contrast to the more uniform distribution of organelles in photosynthetic cells of plants with a low photosynthetic capacity (13, 23, 24). Higher magnification electron micrographs of bundle sheath cells from pigweed, bermudagrass, and *Leptochloa* (Figs. 3 to 5) clearly indicate a high concentration of cellular organelles such as chloroplasts, mitochondria, and peroxisomes in these cells. We have observed a high concentration of organelles in the bundle sheath cells in all of the high photosynthetic capacity plants studied. Indeed, contrary to classical botany, some of the bundle sheath cells (Figs. 7, 9, and 11) do not have the large vacuole considered typical of fully differentiated plant cells (13). In the bundle sheath cells of bermudagrass, we often cannot identify a vacuole since organelles appear to fill the cell.

Clearly, the bundle sheath cells of plants with a high photosynthetic capacity are equipped with large numbers or relative volumes of organelles such that high rates, and perhaps unusual types, of metabolism could occur. A clear elucidation of special functions or activities in bundle sheath cells and mesophyll cells awaits further research. Certainly the studies on higher starch formation in bundle sheath cells (15, 28) are a beginning. The recent report that ^{14}C-labeled photosynthetates in maize leaves moved 3 times faster and were 5 times more concentrated than labeled photosynthetates in sugar beet leaves (26), may indicate that these cells (*i.e.*, those with high concentrations of organelles, including chloroplasts) could facilitate the translocation of photosynthetic products or intermediates and thus exert a controlling influence on the rate of photosynthesis and other metabolic activities. These workers also demonstrated a contrasting pattern of $^{14}CO_2$ fixation in microradioautographs of leaf sections. In maize, the label is concentrated in the bundle sheath cells, whereas the label is uniformly distributed in sugar beet leaf cross sections (26). Sugar beet is a low photosynthetic capacity plant. We have recently isolated mesophyll cells and bundle sheath cells from crabgrass leaves and have demonstrated that the mesophyll cell primarily fixed CO_2 via phosphoenolpyruvate carboxylase while the bundle sheath cell primarily utilizes ribulose 1,5-diphosphate carboxylase, and also that the oxidation of glycolate primarily occurs in bundle sheath cells (G. E. Edwards and C. C. Black, unpublished data). Thus the metabolic activity of these adjacent leaf cells is different, and the elucidation of these activities offers a challenging research opportunity.

We conclude that anatomically the photosynthetic capacity of a plant is related to the over-all quantity and distribution of leaf cellular organelles rather than to such criteria as the presence or absence of grana or starch in specific chloroplasts, chloroplast size, or the location of chloroplasts within the bundle sheath cells.

Other anatomical characteristics which may be useful as criterion for determining the photosynthetic capacity of a given plant have been considered by other workers; these include leaf thickness, cell diameter, air space volume, cell surface to volume ratio, stomatal diffusion resistances, and the photoactive surface of the chloroplasts (12, 31). In our studies we have not quantitatively assessed these characteristics.

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