LEAF SURFACES OF WILD RICE

THE ULTRASTRUCTURE OF THE LEAF SURFACES OF WILD RICE (*ZIZANIA AQUATICA* L.) UNDER DIFFERENT ENVIRONMENTAL CONDITIONS.

By

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Previous work on the ultrastructure of leaf surfaces has been confined to commercial terrestrial plants. Until recently there was a conflicting overlap of definitions of surface structures. Lately, studies have concentrated on the role of the epicuticular wax layer in surface phenomona such as water permeability, transpiration, and herbicide susceptibility. The initiating factors in surface wax formation and the mode of extrusion still remain unresolved.

An emergent hydrophyte, Zizania aquatica L., was selected to attempt to clarify the initiation and extrusion of epicuticular wax. The first appearance of epicuticular wax occurs while the whole plant is submerged. The wax platelet shape is probably controlled by an endogenous circadian rhythm with very little environmental control. The significance of water depth, temperature, continous light, physical and chemical abrasion are discussed in terms of the surface morphology of the three types of leaves.

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INTRODUCTION

1.1 IMPORTANCE OF CUTICLE AS A BARRIER

It has been known for many years that certain plant functions can be related to surface phenomona. Waywell's studies (1967) on the outer wall of the leaf epidermis has included an interesting historical review relating the function of leaves to surface phenomena. With the advent of electron microscopy, it has been possible to examine the ultrastructure of this outer epidermal wall of plants in relation to various functions at different levels of organization.

At one of the levels of organization, the forest ecosystem, the importance of the surface area relations, especially in relation to the individual plants has been reported by Whittaker and Woodwell (1967). They stated that:

> "An ecosystem possesses an internal surface consisting of surfaces of organisms through which matter and energy move between the living and non-living phases. The leaf surface, stem and branch-bark surface, and root surface are major fractions of this interface in a forest...The stem, branch, and leaf surfaces bear on a number of functional relationships including gaseous exchange and respiration rates, evapotranspiration, heat exchange, production limitation..." (p 938).

The outer surface of seed plants is the epidermis. The leaf epidermis is important in photoperiodic perception (Schwabe, 1968), including the functional relationships mentioned by Whittaker and Woodwell (1967). Furthermore, flowering in some cases is strongly dependent upon surface

phenomena of leaves. The outer epidermal wall of leaves is thicker than the other walls of the epidermis and is covered by a layer known as the cuticle. The cuticle is the first barrier that energy and matter must pass to enter the leaves from the external, non-living environment to the internal, living environment.

As a structural boundary, the cuticle is chemically and physically complex. The cuticle has been described as a regulating barrier in relation to environmental factors (Skoss, 1955). Its role in controlling chemical absorption (van Overbeek, 1956; Crafts and Foy, 1958; Dewey et al., 1962; Sargent, 1965) and cuticular transpiration (Bain and McBean 1967, Grncarevic and Radler 1967, Possingham et al. 1967 and Slatyer, 1967) has also been reported for many plant surfaces. Most of this work on surface phenomena has been done with economically important plant systems, for example, the storage of fresh fruit and the dried fruit industry (raisins and prunes). This barrier is not resistant to various chemical herbicides, such as 2,4-D (2,4-dichlorophenoxy acetic acid) and 2,4,5-T (2,4,5-trichlorophenoxy acetic acid) which control the distribution of many weeds by being easily absorbed through the outer cuticular layer and epidermal cell wall of certain plants (Sargent, 1965).

The cuticle is strongly hydrophobic and this property can be examined by measuring the contact angle of water droplets on leaf surfaces (Fogg, 1947; Troughton and Hall, 1967; Leyton and Armitage, 1968). In general, a high contact angle means that a high wax density

is present and this has been called a visible "bloom". The surface waxes scatter light resulting in the bloom. This scattering property is important in reducing the energy receipt of the leaf, thus decreasing the temperature difference between the leaf and the surrounding air. In contrast, Thames (1961) found that the survival of Pinus taeda seedlings diminished when a wax transpiration retardent, e.g. 25% emulsion of Dow-wax, was applied to the pine needles. The heat balance of the leaves was upset because wax coated the transpiring surfaces and acted as a heat trap. Generally, plant systems have evolved their own cuticular barrier with sufficient surface wax and a certain configuration to retard the transpirational loss of water from them.

The cuticle does affect the water potential by reducing the amount of water at the transpiring surface and decreasing the transfer of water vapour away from the evaporating surface (Slatyer, 1963). In a later investigation, Slatyer (1967) stated that one of the pathways of outward water movement from the vascular elements to the external surface is "the subsidary pathway through the parenchyma to the epidermal interfaces and then, in the vapour phase, via the cuticle to the outside air" (p 219). He later reiterated that in "the outer epidermal wall and cuticle, however, the hydraulic conductivity is very low and this zone constitutes a major resistance to flow" (p 221).

The net water vapour flux out of the plant can be measured as the sum total of stomatal and cuticular resistances which together control leaf transpiration. Values of cuticular resistance vary from

<20 to >200 sec. $cm.^{-1}$ (Slatyer, 1967) depending on plant type and cuticular charcteristics with the values declining as temperature increases. Rates of cuticular transpiration relative to total transpiration are usually low, although values as high as 30% cuticular transpiration have been reported by Slatyer (1967). A recent experiment by Possingham et al. (1967) using sultana grapes and leaves indicated that removal of the "soft wax" component of the surface wax increases cuticular transpiration. In a model experiment (Grncarevic and Radler, 1967), evaporation of water through plastic membranes simulating cutin membranes was markedly suppressed by coating them with soft wax components isolated from sultana grapes.

Wax layers are also effective barriers to biologically harmful wavelengths of light. Wuhrmann-Meyer and Wuhrmann-Meyer in 1941-42 (from Kreger, 1958) found that a wax layer 1-2 μ thick decreased the intensity of ultraviolet light (290-300mμ) by 75-90%. This absorption would be important for plants exposed to a high ultraviolet intensity at high altitudes.

1.2 DEFINITION OF CUTICLE AND EPICUTICULAR WAX

Brongniart (1834), concerned with the water repellency of leaf surfaces, was the first to define the membrane covering plant surfaces as the "cuticle". His definition was accepted without much change until the mid - 1950's. Van Oberbeek (1956), Kreger (1958), Martin (1966) and Eglinton and Hamilton (1967) have each defined the cuticle with respect to chemical composition. There has been considerable

overlap of definitions by these authors. Van Overbeek (1956) said that the cuticle consisted of the outer membrane on the external wall of the epidermis, including the surface wax forms. In Kreger's review (1958), he discusses the misuse of the word, "cuticle", referring to the "cuticular membrane" as the cuticle proper while the inner cellulose and cutin make up the "cuticular layer". Martin (1966) stated that the cuticle is composed essentially of wax and cutin and overlays the epidermal cells. Later, Eglinton and Hamilton (1967) suggested a more complex subdivision of the cuticle from the outside to the inside as: epicuticular wax, cuticle, embedded wax, pectin, cellulose, epidermal cell wall. It should be remembered that these boundaries are not exactly distinguishable and there is considerable overlap. Nevertheless, this author has followed the classification used by Eglinton and Hamilton (1967).

The recently introduced term "epicuticular wax" should not be confused with the term "epicuticle" used by Hall (1966), who referred to it as the cuticle after the wax had been removed from the surfaces with solvents. The term "cuticle" is defined for the purpose of this investigation as the non-cellular barrier between the internal and external environments covering the plants' surfaces. "Epicuticular wax" refers to the various ultrastructural wax forms - granules, platelets, and rodlets - which project from the base structure, the cuticle.

1.3 CHEMICAL NATURE OF EPICUTICULAR WAX

By chemical definition waxes are esters of higher aliphatic fatty acids and higher aliphatic or cyclic alcohols (Whitmore, 1951). However, the epicuticular waxes of leaf surfaces also include alcohols and ketones. The surface wax (epicuticular wax) of grape leaves (*Vitis vinifera* var. *sultana*) contains only the "soft" wax fraction, which is a mixture of long chain (C_{14}-C_{33}) acids, alcohols, aldehydes, esters, and hydrocarbons with the alcohols the largest by weight (summarized by Possingham *et al.*, 1967). The chain lengths of the constituent compounds vary (C_{24}-C_{31}), but those constituents which are found together (e.g. alcohols and ketones) tend to be the same chain length (Kreger, 1958).

Besides occurring in different combinations in plants, the wax constituents are present in different proportions. Kreger (1958) reported a high proportion of primary alcohols in the surface wax of several monocotyledons (*Saccharum*, *Phragmites*, other grasses) compared to a predominance of ketone or secondary alcohols in the surface wax of wheat and other cereals.

The cuticles of related families have been compared by chemical methods to determine differences in the deposits of surface wax (epicuticular wax) and cuticular membrane (i.e., cutin). Baker and Martin (1967) studied the relative proportions of four hydroxy-fatty acids in the cutin acids of plants from the *Saxifragaceae*, *Rosaceae* and *Leguminosae*. They observed many varying proportions of the acids, but

the work covered "too wide a range of families, with inadequate numbers of representatives of subfamilies, to establish that the hydroxy-fatty acid make-up of cutin is likely to be of chemo-taxonomic value" (p 318). The influence of environmental factors and age on the wax composition and wax proportion of a species must be known before alkane or cutin analysis can be applied as a taxonomic tool.

1.4 PHYSICAL NATURE OF EPICUTICULAR WAX

Epicuticular wax is comprised basically of rodlet and platelet forms with specific expressions of each form peculiar to the species. The rodlet forms refer to the tubular or "cigar-shaped" wax projections which may have a tapered appearance. The length varies from 1-2 μ in pine (Leyton and Armitage, 1968) to 0.9 μ in pea (Juniper, 1960) and is quite uniform for a given leaf surface. The platelet forms refer to the disc shape of wax and are usually lobed or scalloped along the edge. Wax platelets on sultana grapes have been measured as 0.1 μ wide with a very undulate margin (Chambers and Possingham, 1963). Many rodlets and platelets may mesh together forming a network in which it is impossible to pick out an individual wax form, as on leaves of wheat, Triticum vulgare (Troughton and Hall, 1967); reed grass, Phragmites communis (Stewart and Follett, 1966); tussock grass, Poa colensoi (Daly, 1964).

These wax forms have been used to differentiate between leaf surfaces because they reflect the influence of environmental factors on epicuticular wax in a manner peculiar to each species. Troughton

and Hall (1967) point out that the adaxial (upper) leaf surface in wheat is covered with platelet wax forms whereas the abaxial (lower) surface contained platelets and/or rodlets, depending on the stage of growth and habitat. Also, adaxial platelets were considerably more lobed than the abaxial platelets which tended to be smooth in outline. These differences suggest that the degree to which moisture is retained and light reflected must be different for both surfaces.

Stewart and Follett (1966) postulated that the two forms of wax protrusions found on the abaxial leaf surface of P. communis may be of one basic type: "a flat, scalloped, elongated platelet which, when viewed from the end, appears rod-like" (p 423). The similarity among surfaces of Phragmites, Triticum and Poa suggests that one basic wax form is present on grasses.

Wax shape can result partly from the distances that have to be travelled by the constituents and the relative amounts of the constituent compounds (Eglinton and Hamilton, 1967). Since wax is synthesized mostly in the epidermal layer (Kolattukudy, 1968), and wax is chemically similar on both leaf surfaces (Hall and Donaldson, 1963), differences in shape might also be accounted for by the method of exudation through the cuticle and the activity of lipid production in the underlying cells. Kreger (1958) stated that, because there are no directly connecting pores, the structure of the wax layer must be dependent on the constituents and distribution of canals (ectodesmata) in the outer epidermal wall. Since the constituents and canals are determined by protoplasmic activity, there is an indirect organizing influence on

wax shape ascribed to protoplasm.

Hall (1967b) provides the most convincing evidence for a mechanism of wax extrusion, having found micro-channels with a central core width of 6-10 mu and an overall diameter of 40 mu. He observed them traversing the boundary from the plasmalemma to the cuticle. Hall (1967a) also found that there was more than one pore at the base of the wax forms and that the shape of wax particles can be affected by the orientation of these pores which may be influenced by stresses within the cuticle. Others have found pits but not pores (Mueller et al., 1954; Schiefferstein and Loomis, 1956; Juniper, 1960) but this may be due to insufficient resolution involved in the replicating technique. If pores are discounted, the extrusion of wax in the liquid phase probably occurs as diffusion through the cuticular layer, solidifying upon contact with air into the crystalline form (Kreger, 1958; Juniper, from Eglinton and Hamilton, 1967). Juniper (1960) conjectured that the development of leaf wax was stimulated by light. He examined pea leaves grown in the dark and then mature leaves of plants transferred to light up to four days. The wax diffusion started in the light to produce the organized crystal shape of rodlets. Exactly how the stimulating factor operates remains to be investigated.

1.5 STOMATA

Stomata are surface structures which are visible at the same magnifications as the epicuticular wax layer. Schwabe (1968) has shown the interrelationship between the stomata and the cuticle in surface phenomena

by demonstrating both epidermal cell and/or stomatal control in photoperiodic induction. Although he found that removal of the epidermis did decrease flowering, he was unable to ascertain whether it was a gaseous exchange controlled by the stomata in an intact system or some photo-sensitive mechanism in the remaining epidermal layer which would trigger the flowering induction.

There is uncertainty as to the relative importance of the cuticle and the stomata to the penetration of chemicals into leaves (Martin, 1966). Open stomatal pores may or may not be the portals of entry of the chemical sprays (2,4-D) or the aqueous solutions (fluorescent dyes). As Sargent (1965) pointed out in his review article, there is evidence for and against the cuticle as a virtually impermeable barrier. The conclusion of some was that the stomatal pores were the major portals for entry of applied substances while others found that 2,4-D penetrated stomata-free cuticles as fast as cuticles punctuated with stomatal pores.

1.6 BASIS FOR THE PRESENT STUDY

The ultrastructure of surfaces of many monocotyledons, especially grasses, has been examined previously, for example: Wax extrusion pathways in onion, Allium cepa L. by Scott et al., 1957, 1958; the different wax bloom in love grass, Eragrostis curvula (Schrad.) Nees, by Leigh and Matthews (1963); the influence of temperature and moisture on wax forms in tussock grass, Poa colensoi Hook., by Daly (1964); the surface erosion in reed grass, Phragmites communis Trin.,

by Stewart and Follett (1966); the contact angle in wheat, Triticum vulgare L., by Troughton and Hall (1967). Moreover, most of these studies have been carried out on terrestrial plants in aerial environments where the water balance of the plant is important (Vaadia and Waisel, 1963; Daly, 1964; Leyton and Armitage, 1968). P. communis grows in aquatic habitats, but the previous study was concerned with the aerial leaves and not with the surface changes coincident with environmental changes. Only one other work, by Gange (1950) (from Roelofsen, 1959), according to the author's knowledge, has been carried out on the cuticle of semi-aquatic plants, Typha latifolia L., cat-tail, and Colocasia antiquorum, Elephants-Ear, where the need to conserve water is less apparent.

The elucidation of the ultrastructure of leaves of an emergent hydrophyte grown under various conditions could uncover the initiating factors involved in formation of the epicuticular wax layer previously mentioned by Juniper (1960). A plant system different from those already mentioned by having no wax on some leaves could also clarify the possible pathways of wax extrusion through the epidermis-cuticle complex. Such a plant system is found in Zizania aquatica L. (wild rice).

Wild rice is an emergent hydrophyte whose submerged and/or floating leaves precede the mature aerial leaves and aerial reproductive organs, (Sculthorpe, 1967). The life cycle has been outlined by Weir and Dale (1960) and Thomas (1968) and need not be mentioned here except to state that heterophylly is the common situation during the growth of the individual. The three types of leaves (submerged, floating and

aerial) differ in habit and anatomy yet are of comparable shape.

"Hairs, blisters and cuticle" on wild rice leaf surfaces have been reported previously by Weir and Dale (1960), who also studied the internal anatomy of the three types of the leaves. Dore (personal communication, 1968) has felt that cellular projections of "papillae" are unusual, quite peculiar and prominent in Zizania aquatica. Since wild rice seeds may end up in ponds at various water depths or saturated soils, the young seedlings must be able to adapt to the aquatic and/or aerial environments quickly in order to survive. One aspect of this adaptation is the establishment of an effective barrier on the surfaces of aerial leaves against water loss and wind and wave abrasion.

In this investigation it is proposed that the phenotypic differences in the three types of leaves be studied at the ultrastructural level under both laboratory and field conditions. It is intended firstly, to study the pre-adaptation of floating leaves to an aerial environment by revealing the initiation of the epicuticular wax layer; and secondly, observe the first appearance of epicuticular wax on developing aerial leaves. Also an attempt will be made to explain the scalloped or "finger" edge of the platelet wax form in terms of biological rhythms, pores, transpiration rates, and metabolic cycles. To clarify the extent of environmental control on the formation of the epicuticular wax layer, the effects of extremes of temperature and harsh physical and chemical treatments are also examined.

EXPERIMENTAL PROCEDURE

2.1 EXPERIMENT 1 (SIMULATED NATURAL CONDITIONS)

This study was designed to observe the adaxial and abaxial surfaces of successive leaves throughout a life cycle in a growth chamber. The various plant parts of a flowering plant are shown in Plate 1, Fig. 1. The initial leaves were submerged and observations were made on each leaf. Attention was paid to the leaf on which the wax was first found. The structural properties of the aerials were also studied in order that the general environmental influences on the epicuticular wax forms be known with respect to plants grown under certain "control" conditions. Later Experiments (2,3,4,7) manipulated the environment to induce possible structural changes in the leaf surfaces.

Seeds of Z. aquatica var. interior Fassett afterripened at least 300 days, were germinated in the dark at 24°C for fourteen days. One hundred seedlings of uniform height were selected and individually transplanted into four inch pressed cellulose fibre pots containing by vol. a one loam : one peat : one sand mixture. Silicate gravel was then placed on top to prevent soil floatation. They were arranged in a square pattern 10 plants by 10 plants within a plastic pool (2 m. diameter) directly below a light rack (1.2 x 2.4 meters) at a depth of 25 cm. in a growth chamber (Fig. 1). A Dynaflo Motor Filter filtered and circulated the water. Illumination was provided by General Electric

cool white HO fluorescent tubes and six Sylvania Directlite 150 watt incandescent bulbs situated 160 cm above the water surface to give a light intensity (at the water surface) of 500 foot candles and an energy value of $6.8 \pm 0.2 \times 10^{-4}$ ergs/cm^2/sec with minimum variation. A Sekonic light meter (model L-28 C) was used to measure light intensity. To measure the radiant energy reaching the water surface a YSI-Kettering radiometer (model 65), sensitive to wavelengths of 0.25 to 3.3 microns, was used.

The temperature (Fig. 4) and photoperiod (Fig. 5) paralleled equivalent data for the 1967 growing season at Long Point, Ontario (Observer's Handbook, 1967). The minimum temperature was set at 16°C with a 14¼ hour photoperiod. There were weekly temperature and photoperiod increments of 1.5°C and ¼ hour respectively up to a maximum of 23.5°C and 15½ hour photoperiod which were maintained for 5 weeks before the photoperiod was decreased to 15¼ hours. A Bacharach Serdex Hygrothermograph recorded the air temperature (error ± 1°C) and the relative humidity (error ± 3%). The water temperature, which was recorded by a three-probe mercury tempscribe (error ± 0.5°C), was about 4°C cooler than the air temperature. Since the air was pumped from the outside, exact control of the moisture content was not possible and the relative humidity varied from 15% to 60%.

Twice a week, four plants were selected at random from an 8 plant by 8 plant square within the 10 plant by 10 plant square. The border row provided equal competition on all sides for all plants. Records of sheath lengths from the root/stem axis to the uppermost ligule (Fig. 6)

were used as an indicator of plant height. Such records indicated that the plants were growing in a pattern similar to that outlined by Thomas (1968) although the final height was one-half of his recorded values. The experiment was terminated upon sensecence of the last leaf, about 110 days after day of transplant (Day 0). The Experimental number is abbreviated to E-1 in the Results and the Discussion. A similar format is employed in all other references to the Experiments.

2.2 EXPERIMENT 2 (GROWTH AT THE WATER-AIR INTERFACE)

Plants were grown from the coleoptile stage at the water surface to determine whether the surfaces of the initial leaves (leaves 1, 2 and 3) presented a different barrier than that indicated by Experiment 1. The degree of flexibility of a young plant to an abnormal environment, in this case an aerial environment, involves some form of protection of the commonly submerged leaves against desiccation.

Seeds of var. angustifolia Hitchc. (from Bald Lake, Ontario) afterripened 129 days were germinated in the dark at 24°C for five days. Sixteen seedlings were transplanted to clay pots (3.7 cm in diameter) containing soil similar to that used in Experiment 1, and were then placed just below the water surface in the plastic pool used in Experiment 1 so that the coleoptile projected into the air. The photoperiod was 15½ hours for the first 30 days, then 15¼ hours for the next 63 days (Fig. 5). Illumination was the same as in Experiment 1.

During the initial stages of leaf growth and development, two of the most recent leaves were selected twice a week and both surfaces

sampled. After the fifth leaf stage, leaves were sampled once a week, as beyond this stage the leaves were similar to aerial leaves of plants grown at the various depths outlined in other Experiments.

2.3 EXPERIMENT 3 (CONTINUOUS LIGHT CONDITIONS)

Plants were grown in continuous light after one day's exposure to a fourteen hour photoperiod to determine whether the structure of the wax forms, especially the scalloped platelets, were controlled in part by an endogenous circadian rhythm or by environmental conditions.

Var. interior seeds, afterripened for 400 days, were germinated at 16°C for eleven days. Seedlings of uniform height were transferred to clay pots (3.7 cm in diameter) containing the potting mixture with gravel on top. Twenty-seven plants were placed in a tank (T1) at a depth of 25 cm and six in each in two smaller tanks (T2 and T3) at a depth of 11 cm (Fig. 2). T3 was covered with an Ackron polyethylene dome for the first half of the experiment to maintain the plants at a higher humidity.

The tanks were placed in a Lab Line Biotronette Mark III environmental chamber with Gro-lux WS Sylvania fluorescent tubes. Plants were given one 14 hour photoperiod, then grown under constant light ($1.3 \pm 0.1 \times 10^4$ ergs/cm^2/sec and 500 foot candles at the water surface). Air temperature, monitored with a Bacharach Tempscribe temperature recorder, model STA, was not constant and varied from 22.9-27.2°C (Fig. 4) with water temperature usually 1°C less.

At least twice a week four plants were selected at random from

both depths. First leaf area was measured by simply recording leaf length and width, and then the leaves were removed for ultrastructural analysis. Leaves of any age were selected since young leaves had been shown to have elaborated a wax mesh just as extensive as that of mature leaves. The platelet wax forms, especially the scalloped edge, were examined on the wax covered adaxial leaf surfaces. The scalloping effect, although not as pronounced on the abaxial surface, was periodically checked.

2.4 EXPERIMENT 4 (TEMPERATURE TREATMENT)

In the previous Experiments (1,2,3), the air and water temperatures were maintained at normal temperature regimes i.e. 15-25°C. However, there are both diurnal and seasonal fluctuations of temperature in any natural habitat. Temperatures less than 10°C and up to 25°C occur in diurnal temperature cycles in the spring and early summer in Southern Ontario. By mid summer the air temperature maximum can approach 27°C. The plants were grouped into one of three constant water temperature ranges: Group 1 - 25-27°C; Group 2 - 14-18°C, Group 3 - 8°C. The extremes were greater than the maximum (Group 1) and less than the minimum (Group 3) recorded in the field. A middle group (Group 2) was just below the simulated natural condition of Experiment 1 but can be considered to be the control in this Experiment. "Steady" water and air temperature extremes duplicating the mean field values were used to induce structural changes.

Var. aquatica L. seeds from a creek near Marionville, Ontario, afterripened 245 days, were germinated in the dark for six days at one of two temperature regimes corresponding to two Groups i.e., at 25°C for Group 1 and at 14°C for Group 2. Group 3 (8°C) consisted of (a) plants from Group 1 (27°C) transferred prior to the floating and aerial leaf stages and (b) seeds germinated at 8°C and grown to the third leaf stage. Eighteen seedlings from each of the two germinating temperature regimes (Groups 1 and 2) were then transplanted into clay pots containing the potting mixture used in Experiment 1, covered with gravel, and returned to the tanks at a depth of 36 cm. The tanks (volume = 100 litres) were housed in separated Biotronettes, the same as those used in Experiment 3. The Biotronettes, tanks, and water bath were arranged to give two units having identical light with different temperature regimes. The ambient temperature (room temperature) or Group 2 was fairly constant (Fig. 4), but problems as outlined in Experiment 3 resulted in a gradual increase in the temperature after Day 30. Thus, although Group 2 started at the low temperature of 14°C, the temperature rose to 18°C on the day of transplant (i.e. Day 0), staying constant until Day 30 when it rosé another four degrees to 22°C (Fig. 4). Continuous recording of the water temperature at the beginning of the experiment indicated that the water temperature did not fluctuate as much as the air temperature.

Water was filtered by a Dynamo Motor Filter in the ambient, 18°C tank containing plants from Group 2. The higher temperature regime (Group 1) rose to 27°C on the day of transplant and was controlled by

a Tamson Water Bath and Circulator, Model TZ9 (Fig. 3), which also maintained the water level. However, once the instrument was stabilized at 27°C, it maintained the 9°C difference between Group 1 and Group 2 plants until Day 28 (Fig. 4) when the ambient temperature rose, resulting in a temperature of 33°C in Group 1 and 23°C in Group 2. Four days later, on Day 32, this part of the Experiment, Group 1, was terminated.

Attempts to germinate and grow plants at a proposed higher temperature regime of 35°C failed when none would germinate. Transfer of plants at various stages of growth to a tank at this regime (35°C) was not done for two reasons. Firstly, the examination of structure, density, etc. of the wax layer after a four day period (Day 28 to Day 32) of higher temperature (33°C) in Group 1 plants indicated no response or change. Secondly, the Tamson Water Bath and Circulator would require a cooling attachment for the heating element and a continuous water supply which were not available.

Some plants were moved from one tank into another tank maintained at a different temperature in order to examine any short term responses to sudden changes in temperature. Four plants were transferred on Day 0 from Group 1 to Group 2 and vice versa. On day 27 three plants not yet at the floating stage from Group 1 (27°C) were transported to each of Group 2 (18°C) and Group 3 (8°C). The aquarium tank used for Group 3 had a smaller water depth of 21 cm and a volume of 30 litres. On Day 32, a second group just before the aerial stage were transferred to the same locations from Group 1. For all groups the photoperiod

was maintained at the minimum value of 14¼ hours for 4½ weeks before increasing it to 14¾ hours (Fig. 5) with illumination obtained with Sylvania Gro-Lux fluorescent tubes. Light intensity and energy values for Groups 1 and 2 were 450 foot candles and 0.5×10^{-4} ergs/cm^2/sec, respectively, both at the water surface. Group 3 plants received 150 foot candles light intensity and 0.7×10^{-4} ergs/cm^2/sec energy. Sampling took place about every four days, most often with the faster growing Group 1 plants. Leaves of transfer plants were examined about every five days for 20 days. When possible, surfaces of leaves 2,3,4,5 and 6 from more than one Group were examined at the same time to insure a comparison of any structural responses.

2.5 EXPERIMENT 5 (FIRST APPEARANCE OF WAX)

This experiment was done with the same plant material as used in Experiment 4. The surfaces of the most recent leaf blades from all Groups were examined over the entire leaf surface in order to determine the location of the first appearance of epicuticular wax forms on leaves approaching the water-air interface. Special attention was paid to leaves 3,4 and 5. The depth of the emerging leaves examined, leaf number, leaf size, and its relation to the general state of the plant (submerged, floating, or aerial stages) were recorded. Examination of young leaves necessarily involved destructive sampling; it was impossible therefore to examine successive samples from the same leaf. Only one plant was examined per sampling date until wax forms appeared. Then two plants were examined for confirmation. Sampling dates were

every fourth day until the plants were into the aerial stage, around Day 30 in Group 1 (E-4) plants.

2.6 EXPERIMENT 6 (FIELD STUDIES)

The field studies undertaken in the spring and summer of 1968 were carried out to,(1) compare the epicuticular wax of different varieties in their natural habitats,(2) see if differences occur between field and laboratory grown plants, and (3) check E5 by examining those plants approaching the water-air interface.

Most of the leaves were collected from Millgrove plants (var. aquatica) throughout the growing season. Descriptions are limited to two sampling dates: the floating stage on May 15 and the aerial stage on July 26. Long Point plants (var. angustifolia) were compared to the other material sources but they were not intensively examined.

Seven plants (var. interior) from Whitefish Lake, near Port Arthur, were transferred to the McMaster University Greenhouse on June 15 at the floating leaf stage. No attempt was made to simulate the plants' natural condition and they were kept in a large tank at a depth of 13 cm. Daylight provided the illumination and both the water and air temperatures were considerably warmer than the Whitefish Lake conditions. Once transferred, these plants were not considered to be true "field" samples but were nevertheless examined.

Entire plants were uprooted, put in containers to insure against desiccation and unnecessary abrasion, and transported to the laboratory. Only obviously unmarked leaves were excised. If the replication

procedure could not be done immediately, they were placed on the sticky tape and stored overnight in the refrigerator. Leaf samples were usually taken from the areas of maximum expansion except when examining young leaves. At first it was possible to record the leaf number. Once the plants were in the aerial stage, however, the initial leaves had decayed and it was difficult to tell the exact leaf number. As a result, the later samples are referred to simply as adaxial or abaxial aerial surfaces.

2.7 EXPERIMENT 7 (PHYSICAL AND CHEMICAL TREATMENTS)

Contact chemicals i.e., herbicides, wind action and weathering all affect the epicuticular wax layer. Resistance to ultrastructural erosion by the epicuticular wax layer appears to be important in the light of surface permeability and protoplasmic sensitivity (Sargent, 1965).

Aerial leaves of transfer plants from the Lakehead and from E-1 plants were subjected to mechanical and chemical abrasions to determine the resistance value of the layer to unusually harsh treatment. Each of the three following treatments were carried out on different leaves:

1. Cotton was gently but firmly rubbed over both surfaces at least thirty times to mechanically remove as much surface wax as possible.
2. Whole plants in the aerial stage were submerged continually and leaf samples were taken daily for 10 days.
3. Leaf samples were immersed for one minute, one hour, two hours, and five hours in chloroform or acetone.

Immediately after a designated treatment the leaves were attached to the double tape and the surfaces were replicated according to a standard procedure described in the following section. All treatments were repeated at least twice. "Control" samples for unmarked material were replicated at the same time. Old, yellowing leaf surfaces were also examined to observe the natural erosion.

2.8 CARBON REPLICATION PROCEDURE

Three methods of holding down the leaf were tested in order to insure a flat surface for the replication procedure. Scotch taping the ends of the leaves, applying a nail polish base, and using a Mystik Tape sticky on both sides were used to press the leaves against the microscope slides. The Mystik Tape proved the best of the three methods because the leaf samples could be easily placed on the adhesive. This provided a flat adaxial and abaxial surface for the carbon replication procedure.

An Edwards Speedivac vacuum evaporator was used to apply metal shadow and carbon film. Various shadow casting angles (β = 20°, 30°, 35°, 45°, 60°, 70°) (Bradley, from Kay, 1965) were tried and the best contrast on wild rice leaves was obtained with β = 35° which was used during all Experiments. Tests on the vacuum, necessary for even metal and carbon deposition, were carried out to minimize any leaf shrinkage upon evacuation of the bell-chamber. The optimum vacuum was 1×10^{-4} mm. Hg, attainable in 30 minutes. A 1×10^{-3} mm. Hg vacuum produced a "salt and pepper" effect. The maximum vacuum, 1×10^{-5} mm. Hg, did

not increase the resolution power while subjecting the sample to a vacuum for 80 minutes.

Gold, gold-palladium (60:40) and platinum metals were all tested. Gold-palladium metal was selected because it provided a fair resolution in spite of its slight granular internal structure. National Special Spectroscopic Graphite Electrodes were used to produce the carbon film and vacuum time was identical with that employed for the metal shadow. Although not measured, replica thickness was approximately 300 Å. This was produced by a one second pulse of carbon atoms.

Three methods of removing the replica were also tested. A plastic backing (5% parlodion in amyl acetate) easily removed the replica but attempts to dissolve the plastic usually resulted in disintegration of the carbon replica. Treatment of the leaf sample plus replica with sulphuric acid (Stewart and Follett, 1966) did not always free the replica from the underlying cuticle. However, a gelatin backing, when dried, easily separated the replica from the plant material. Removal of the gelatin was facilitated by putting the replica into concentrated sulphuric acid (sp. gr. 1.84) for 5-8 hours. A chloroform or ether bath was found to remove any adhering wax particles, not readily dissolved in sulphuric acid, thus leaving a "clean" replica. According to Waywell (1967) the carbon replica is a good indicator of the surface structures of leaves and represents the wax layer as being crystalline.

The final carbon replica procedure was similar to that proposed by Troughton and Hall (1967) and Stewart and Follett (1966) and is as follows:

1. Leaf samples of both surfaces were laid flat on Mystik Tape attached to a glass microscope slide.
2. As soon as possible the samples were metal shadowed at an angle of 35° (tan 55°) with gold-palladium followed by carbon evaporation in an Edwards vacuum evaporator.
3. A gelatin backing was applied and allowed to dry; the leaf surfaces were then cut into usable grid squares and the replicas and gelatin peeled off.
4. The replicas were immersed in concentrated, reagent grade sulphuric acid (sp. gr. 1.84) for 5-8 hours to remove the gelatin and adhering organic matter.
5. Replicas were then transferred to distilled water to dilute the adhering acid and allowed to stand for at least two hours.
6. Ether was added to water to dissolve any adhering wax and allowed to stand for one hour.
7. Replicas were transferred to distilled water baths twice, allowing a few minutes between transfers.
8. Replicas were finally placed on copper grids (200 mesh), dried, catalogued, and stored in a refrigerator prior to use.

Ultrastructural features are described from electron micrographs obtained with a Sieman's Elmsikop 1 usually operated at 60kv. Large numbers of grids precluded the possibility of photographing every replica, so in situations of redundance no pictures were taken with written observations being sufficient. For reference purposes, the number following the figure number is the code of the negative which is

stored in the plate files at the Department of Metallurgy and Materials Science, McMaster University. True magnification was calculated from a graph of the true magnification vs. the nominal magnification of a diffraction grating for a given Kv and pole piece.

Both sides of all surfaces were regularly examined under light microscopy using a Zeiss Standard Universal Microscope with an Epiplan-Objective HD dark-field attachment. Final magnifications of the pictures were 80x and 290x.

Analyses of variance techniques and the "t-test" were applied to compare the rodlet and platelet wax forms from the different Experiments, using the measurement of length as the indicator. Calculations for these tests are included in the Appendices. This was done to determine whether their sizes could be measured quantitatively and whether or not the measurements revealed any significant differences or trends according to environmental treatment.

RESULTS

3.1 GENERAL LEAF SURFACE MORPHOLOGY

3.1.1 Qualitative Assessment

1 Dark Field Light Microscopy

In Experiment 1 and in field samples, the initial leaves (1,2,3) are submerged with their surfaces relatively smooth, lacking stomata, and with few or no papillae present. The entire leaf blade is thin, ribbon-like and a light green colour. On both surfaces obvious cross walls or rib connections are readily visible, as in Plate 1, Figs. 2 and 3, and epidermal cell wall outlines are discernable. Towards the edge of the leaf blade the cross walls are less prominent but still present. Projections or papillae usually arise from every seven cells on both sides of the ribs throughout the length of the leaf blade, although in the example of the abaxial surfaces (Plate 1, Fig. 3) there are none present.

The mature floating type of leaf (numbers 4,5,6) usually has its ligule position submerged with most of the leaf blade floating on the water surface. The floating portion is ridged (vascular bundle formation), contains stomata, is dark green in colour and is thicker than the submerged portion. The leaf surfaces in contact with water are smooth, as shown by the submerged surface of the floating portion of a floating leaf in Plate 1, Fig. 6. On this abaxial surface papillae or cross walls are rarely present, with only ribs (R) visible. The epidermal cell size is also smaller than that of the totally submerged

leaves (Plate 1, Fig. 2 and 3).

Where the leaf blade breaks the surface of the water, there is a constricted portion of the leaf approximately 5 cm long, half of which is below the water surface. This is referred to as the "transition zone". In the adaxial transition zone there is a definite increase in the number of papillae upon exposure to the aerial environment with four to six papillae arising from each cell, as shown in Plate 1, Fig. 5. They are usually small, about 3μ in diameter, and occupy areas nearest the apices of the ridges. These papillae are discussed later under the ultrastructural studies. The epidermal cells are more elongated, which is typical of the aerial condition (Plate 1, Fig. 5).

The adaxial surface of a floating leaf in a completely aerial environment is extensively covered by two sizes of papillae (Plate 1, Fig. 6). Smaller papillae of about 3μ diameter covering from four to six per cell surface and larger papillae (about 27μ diameter) dot the epidermal cell surface around the guard cells. The smaller papillae also project from the larger papillae. These papillae are not as elongated as the few seen in the submerged leaves but they are structurally more solid. Stomata, 34μ in length, straddle both sides of the ribs and tend to be located at the mid-point of the slope of each ridge. Four large papillae form a "diamond pattern" around each stoma. Debris particles usually attach to the leaves and are present on most leaves exposed to the air, as revealed in Plate 1, Fig. 6.

Light microscopy of the aerial leaf surfaces reveals extensive

changes. The adaxial surface (Plate 2, Fig. 1) is essentially like that of the adaxial floating leaf surface (Plate 1, Fig. 6). The prominent corrugated or ridged appearance in Plate 2, Fig. 1 is indicated by the wider dark valley areas and the thin ribs (R) of the leaf. Stomata (S) arranged close together are present. Large papillae (Pa) extend laterally from the slopes with small papillae (pa) dotting both the surface and the surface of the large papillae (Plate 2, Figs. 1 and 3). Their dimensions are the same as those reported for the floating leaf surface. The surface appears granular and reflects light; however, due to insufficient magnification it is not possible to point out any wax forms or epicuticular/cuticular structures.

The abaxial surface of aerial leaves is very different from any of the leaf forms discussed earlier. The non-ridged abaxial surface (Plate 2, Fig. 2) is covered by many papillae which appear red against a dark green leaf surface. The red colour is possibly due to the presence of anthocyanin, although this has not been investigated. Stomata are surrounded by small papillae and are also found in parallel lines beside the ribs. On the abaxial surface the papillae can be divided into three size groups of about 17μ, 7μ and 3μ according to diameter. By comparison the papillae on the adaxial surface are divisible into two size groups of 27μ and 3μ diameter.

Trichomes are present on the leaf surfaces (Plate 2, Figs. 4 and 5), and they usually project vertically from the surface. Their large size precludes simultaneous observation with large papillae and stomata.

2 Electron Microscopy

The analysis of the surfaces of the types of leaves follows the same sequence of appearance as outlined in Dark Field Light Microscopy. In this section, the observations involved the examination of carbon replicas of the surfaces of submerged, floating and aerial leaves.

The outer surface of the coleoptile is smooth but extensively wrinkled (Plate 3, Fig. 1) and possible microorganisms adhere to the surface as indicated by the hypha-like (H) tube. The submerged cuticular surfaces of both sides of leaves 1,2, and 3 are generally featureless. Bacteria, diatoms, algae, and debrisparticles can all be found adhering to these surfaces. Papillae are visible at all ranges of magnification, mostly in leaves exposed to air. These papillae have been noticed before in Zizania (Dore, personal communication) but not directly at the ultrastructural level. The papilla seen in Plate 3, Fig. 2 is collapsed. Its diameter is about 2μ indicating that it has not fully developed. The papilla is continuous with the surface and is considered to be an extension of the cell wall. Later measurements of other papillae (approx. 3μ diameter in Plate 3, Fig. 4) indicate that they are the same papillae as seen by means of reflected light and are termed small papillae (pa). Metal shadowing is present and good contrast enables observations of the meshing of long strands, possibly cellulose fibres, beneath the outer, pitted, cuticular surface (Plate 3, Fig. 2). These pits are of unknown origin.

The abaxial surface (Plate 3, Fig. 3) of a second leaf is smooth with a slight rippling of the area, thus indicating possible variations in the surface of the cuticle. The gold-palladium shadow results in a granular internal replica structure. The vacuum employed probably cracks the outer surface of the cuticle causing irregular lines.

Examination of the replicas of the adaxial surface of mature floating leaves (Plate 3, Fig. 4) reveals a continuous mesh of rodlet (r) and platelet (p) wax forms covering the cuticle and papillae. The base structure is slightly granular and is the outer boundary of the cuticle (c). The platelets are noticeably interspersed among the thin rodlets. Platelet edges are indented and the term scalloped is applied to this condition. Dimensions vary and rodlet length in this example is 0.5μ ± 0.1. Papillae diameter of 2.5μ ± 0.2 is difficult to ascertain since the wax mesh is tightly bunched on its surface, creating a very dark opaque image in the replica.

By comparison, the adaxial surface of the submerged portion of floating leaves is smooth in terms of cuticular features (Plate 3, Fig. 5). This appears throughout the length of the leaf blade and the same surface is present on the submerged portion of the abaxial surface of mature leaves.

Typical adaxial aerial leaf surfaces show a thick mesh of rodlets and scalloped platelets (Plate 3, Fig. 6). Rodlets measure approximately 0.7μ in length. The scalloping effect is quite prominent, especially in this example of a sixth leaf of a laboratory grown plant, and the

platelets are quite numerous amongst the rodlets. The wax forms project from a fairly smooth (but slightly cracked) cuticle with the platelets in various degrees of exposure of maximum area. Therefore, platelet size varies. Although not shown, the aerial leaf surfaces are also covered by numerous wax covered papillae. Except for differences in rodlet length there is no difference between the adaxial surfaces of floating and aerial leaves.

The abaxial aerial leaf surface is covered by an extensive wax mesh (Plate 4, Fig. 1). The platelets are less deeply scalloped than those of the adaxial surface and the abaxial platelets appear smaller in size. Rodlet forms (length $\backsimeq$ 0.6μ) appear to dominate numerically. In many instances the adaxial surface appears to contain more wax since the mesh is more extensive.

A replica of a stoma (Plate 4, Fig. 2) reveals the organization of smaller sized papillae (approx. 1.5μ) arising from epidermal cells surrounding the raised guard cells. Only the thickened edge of the guard cells are devoid of wax rodlets, the rest of the area being evenly covered with epicuticular wax. Part of the stoma has opened although the original stoma was closed when replicated. The stomatal length was calculated at about 35μ.

3.1.2 Quantitative Assessment

In general, examination at the ultrastructural level indicates a difference in length among the wax forms, as well as other parameters such as density, degree of abrasion, and the relative

proportions of wax forms. Statistical tests were performed to see if there were any significant differences in one parameter namely, the length of the wax forms.

Analysis of variance tests (see Appendix A) were carried out on the measured rodlet lengths of both surfaces and the platelet length for the adaxial surface from the laboratory grown plants. The mean lengths are shown in TABLE 1. Comparison of means tests (see Appendix B) analyzed possible differences among the laboratory samples, the field plants, and the young leaves from both situations.

All measurements were taken from electron micrographs of known true magnification and converted into actual values in microns. Examination of the wax mesh illustrates the difficulty in delineating the exact rodlet boundaries. Nevertheless, it was possible to pick out an occasional individual form and this similar configuration was measured on all negatives.

The rodlet lengths on the adaxial surface, as shown in TABLE 1, were not significantly different. The field samples were excluded from the analysis of variance. The calculated F value of 1.8 was less than the table $F_{(4,31)}$ of 2.69 at the 5% level of significance on the remaining samples. The average value is 0.6μ. A comparison of means between E-1 and E-2 and between E-1 and E-3 both gave "t" values considerably less than the table values (see Appendix B).

Each value in TABLE 1 for both rodlet and platelet lengths was obtained from at least two samples. The top numbers refer to the first leaves with epicuticular wax present while the bottom of each list refers

TABLE 1

RODLET AND PLATELET LENGTHS

Dimension u	Experimental Number						$\bar{\bar{x}}$ Average of means
	E-1	E-2	E-3	E-4 (27°C) Group 1	E-4 (18°C) Group 2	E-7 (Field)	
Rodlet Length Adaxial Surface	0.5 0.8 0.7 1.0 0.7 0.6 0.6 1.0 0.8	0.7 0.8 1.0 0.7 0.8 0.8	0.7 0.9 0.6 0.6 0.6 0.8 0.6 0.7 0.6 0.6	0.5 0.7 0.6 0.5 0.6	0.3 0.5 0.5 0.4 0.7	0.5 0.8 0.2* Excluded from the mean. 0.2* 0.4 0.5 0.5 0.5 0.3* 0.5 0.8 1.2 1.4	
$\bar{x}$	0.7	0.8	0.6	0.6	0.5	0.7	0.6
Rodlet Length Abaxial Surface	0.7 0.6 0.7 0.6 0.6	0.7 0.5 1.0 0.6	0.6 0.7 0.5 0.7 0.7 0.8	--	--	--	
$\bar{x}$	0.6	0.7	0.7				0.7
Platelet Length Adaxial Surface	0.7 0.7 0.5 0.8 0.4	0.1 0.9 1.0 0.9 1.7 0.7 0.5 0.6 0.5	0.7 0.7 0.8 0.5 0.2 0.7 0.5 0.5 0.6	0.5 0.6 0.5 0.5 0.3	0.9 0.6 0.5	--	
$\bar{x}$	0.6	0.8	0.6	0.5	0.7		0.6

Each value based on 40 measurements of two electron micrographs.

to leaves at the end of the life cycle. Thus, the values were obtained from floating and/or aerial leaves and at different times. The data from all Experiments was rearranged so that the rodlet length values were divided into those for young and mature leaf surfaces. The sample in TABLE 2 refers to the selection from all Experiments of adaxial surfaces of young, curled-up aerial leaves and young submerged leaves which become floating. The rodlet lengths selected were arbitrarily selected at $\leq 0.4\mu$ and assumed to be the recently extruded rodlets. The standard deviation was calculated and the mean compared with the population mean of E-1 (Simulated Natural Conditions) which was primarily selected from mature leaves with fully extruded wax forms present.

TABLE 2

Young Rodlet Lengths

YOUNG RODLET LENGTHS

Rodlet Length Adaxial Surface in μ	
	0.2
	0.4
	0.3
	0.4
	0.2
	0.3
	0.3
	0.3
	0.4
	0.2

$\bar{x}_1 = 0.3\mu$

$\bar{x}_2 = 0.7\mu$ from E-1

Calculated t = 13.3

Table t = 4.781 at the 0.1% level significance, for 9 degrees of freedom.

The sample mean is very highly significantly different from the population as seen in Appendix C. The newly extruded wax forms on the younger leaves are statistically shorter than the mature wax forms.

A similar analysis of variance of rodlet lengths on the abaxial surface revealed no significant difference, as shown in Appendix D. The average value is (from TABLE 1) 0.7μ and the calculated F value of 1.5 is less than the table $F_{(2,12)}$ of 3.98 at the 5% level of significance.

Platelet outlines are not difficult to observe but it is obvious that often not all of the platelet's area is in full view. Scalloping or "finger" lengths of platelets are not easily measured. The platelet width remained fairly constant (0.3u ± 0.1) and was not subjected to analysis. Adaxial platelet lengths were compared in TABLE 1 and the calculated F value was 1.25 which was less than the table $F_{(4,27)}$ value of 2.75 at the 5% level of significance (see Appendix E). Therefore, there are no statistical differences among the various platelet lengths. Although the E-2 mean value of 0.8μ appears much greater than the E-1 value of 0.6μ, a comparison of means test reveals that there are no significant differences between the platelet lengths, as shown in Appendix E.

3.2 EXPERIMENTAL RESULTS

3.2.1 Simulated Natural Condition (Experiment 1)

Analysis of plant height in the sample plot indicated that these plants were shorter (61 cm on Day 109 from Figure 6), and that their leaf areas were less, than plants grown by Thomas (1968) under similar conditions. However, a more important consideration is that the plant grew uniformly and there were no statistical differences

among positions. Coincident with the three phase growth curve (Thomas, 1968) are the structural differences of the leaf surfaces in each phase.

Submerged Stage

During the first fourteen days (Phase 1, from Thomas 1968), the plants were submerged and the cuticular surfaces of both sides of leaves 1,2 and 3 were featureless. With dark-field light microscopy "crosswalls", elongate projections, and a smooth surface, similar to that shown in Plate 1, Figs. 2 and 3 were observed for the adaxial and abaxial surfaces respectively. Replicas of both surfaces are smooth with occasional adhering microorganisms and debris. Plate 4, Fig. 3 represents the adaxial surface of the second leaf having pitted areas of unknown function on the surface.

Floating Stage

Phase 2 (from Day 15 to Day 35) consists of the floating leaves 4,5 and sometimes 6. The surface features are similar to those outlined in Experiments 1 and 4 for floating leaves (Plate 1, Figs. 3, 4,5; Plate 3, Figs. 4,5). There is a definite appearance of papillae, stomata, ridging and wax on the floating portion of the adaxial surface. For example, Plate 4, Fig. 4 indicates a loose arrangement of rodlets (average length = 0.8μ), platelets (average length = 1.6μ) and papillae (average diameter = 3.0μ) which are not completely covered by a wax mesh. Again, as stated in Experiment 1, the submerged portion, below the transition zone (Plate 3, Fig. 5) and the entire abaxial

surface (Plate 4, Fig. 5)are smooth, lack stomata, andare devoid of wax.

Exposure to air stimulates epicuticular wax production and changes the epidermal micro-topography through increased vascular (rib) and papillae formation.

Aerial Stage

In the third Phase of the growth from Day 36 to the termination of the Experiment on Day 110, the aerial leaves (7,8 and 9) appeared. Examination of their surfaces revealed extensive abaxial surface changes from the two previous stages. Dark field photomicrographs of the adaxial aerial surface (Plate 2, Figs. 1 and 3) revealed that ridging is more pronounced than on the adaxial floating leaf (Plate 1, Fig. 6). The abaxial aerial surface is very similar to Plate 2, Fig. 2, with no ridges and differently shaped large papillae situated near the mid points between two parallel ribs.

Electron micrographs of the adaxial aerial leaf surface show that large rodlets, platelets, and papillae form a thick mesh of epicuticular wax (Plate 4, Fig. 6) which is similar to the adaxial aerial leaf surface outlined in Experiment 4 (Plate 3, Fig. 6). There is very little difference between the adaxial floating (Plate 4, Fig. 4) and adaxial aerial (Plate 4, Fig. 6) leaves, although the wax density may be greater in the latter.

The abaxial surface of aerial leaves is similar to the adaxial surface. It is covered by rodlets, platelets and papillae (Plate 5,

Fig. 1). The abaxial aerial leaf surface from plants grown under simulated natural conditions was never as extensively covered by epicuticular wax forms as the adaxial surface just discussed. On the abaxial surface (Plate 5, Fig. 1) the rodlets appear in greater numbers, the platelets are not as scalloped and they appear smaller than those on the adaxial surface (Plate 4, Fig. 6). Both surfaces of the eighth leaf were examined on the same day from transplant (Day 59). In Experiments 2 and 4 similar structures were observed between the adaxial (Plate 3, Fig. 6) and abaxial (Plate 4, Fig. 1) surfaces of aerial leaves.

Within any growing situation some differences were found related to the numerical position of the leaf. However, once wax was found on the leaf surface, there was no observable change in the wax pattern in successive leaves, as evidenced by examining the adaxial surface of leaf 5 (Plate 4, Fig. 4) and leaf 8 (Plate 4, Fig. 6). The mean rodlet length is 0.7μ for all adaxial surfaces (TABLE 1) and they are fairly straight. The platelet length, about 0.6μ does not vary significantly from the other adaxial surfaces of plants grown under different environmental conditions. The wax forms an interlocking and overlapping mesh. The density on the adaxial surface varies from a medium to an extensive heavy wax cover, with no observable difference among the floating and aerial types of leaves. Rodlet width was found to remain constant at 0.1μ for both surfaces.

The wax mesh on the abaxial surface has a mean rodlet length of 0.6μ compared to a value of 0.7μ on the adaxial surface. Platelet

size on the adaxial surface is slightly less than the values for E-2 and E-3. Because rodlets dominated numerically, there were few platelets on the abaxial surface. The platelet length was slightly less than 0.6μ. Although the densities on both surfaces may vary, they are usually the same on a given leaf.

The small papillae size, approx. 2.5μ, was slightly less than the diameters observed in other Experiments, but they are the same shape and are covered by an extensive wax mesh as those from other Experiments.

3.2.2 Growth at Water-Air Interface (Experiment 2)

The time to flowering in this Experiment was considerably longer than in Experiment 1, requiring 83 days compared to 55 days (Fig. 6). Also, both plant height (Fig. 6) and leaf area (not shown) were less, especially in the younger stages. All leaves produced remained in an aerial environment. The first three leaves had appeared by Day 7 and their top portions dried up three days later.

With leaf one (1) there is only one obvious anatomical difference on the surface compared with a submerged leaf one. Dark-field photomicroscopy indicates that some aerial-type papillae are present and the surface (not shown) reflects light quite well. However, carbon replicas of the first leaf reveals a flat surface (Plate 5, Fig. 2). This cuticle is probably thicker than that of the submerged leaf. Primarily because of the drying effect of the aerial environment and secondarily the vaccum employed in the replication process, the outer surface of the cuticle tends to crack forming fracture lines (Cr).

Nevertheless, the surfaces of the first leaf in an aerial environment are not unlike those of the completely submerged first leaf (Plate 3, Fig. 2).

The surfaces of leaves 2 and 3 have epicuticular wax similar to those of the surfaces of aerial leaves (Plate 1, Fig. 6). Dark field photomicrographs indicate the presence of stomata, smaller papillae, trichomes and a slight ridging as demonstrated by the abaxial surface of the third leaf (Plate 5, Fig. 3). However, there are no large papillae on the surfaces of the second and third leaves. Large papillae are present on the fourth to ninth leaf surfaces inclusive. These aerial surfaces are similar to that of the adaxial surface (Plate 2, Fig. 1) and the abaxial surface (Plate 2, Fig. 2) of Lakehead transfer plants.

The abaxial surface of leaves 4,5 and 6 (Plate 4, Fig. 1) have an epicuticular wax mesh similar to those of abaxial aerial leaves in E-1. Examination of the adaxial surface of the second and third leaves reveals large wax forms as demonstrated by the surface of the third leaf on Day 15 (Plate 5, Fig. 4). The mesh usually is not even with many exposed cutioular areas. The large platelets (length $\simeq$ 0.8μ) have a scalloped edge and have a fan-shaped outline (Plate 5, Fig. 5). Both rodlets and platelets are definitely larger than the adaxial aerial leaf wax forms outlined in Experiment 4 (Plate 3, Fig. 6). The abaxial surface also is covered with wax platelets and rodlets (Plate 5, Fig. 6). Scalloping is present and the resulting mesh is not as thick or extensive as that of the adaxial surface (compare

Plate 5, Fig. 5 and Plate 5, Fig. 6). The abaxial surfaces of leaves 4,5, and 6 (normally submerged) were also covered with extensive wax forms.

There are no great differences in the wax forms in truly aerial leaves between plants grown at the water surface (Plate 5, Fig. 4) and plants grown at a 25 cm depth in Experiment 1 (Plate 4, Fig. 6). Surface anatomical differences of leaves of plants grown at the water surface are found on both sides of the first, second and third leaves (normally submerged) and the abaxial side of the floating leaves (4,5 and 6). Stomata, papillae, ridging, a thicker cuticle, and a full epicuticular wax mesh change the surface characteristics of these leaves.

Statistically, the rodlet lengths (TABLE 1) of both surfaces are not different from those of Experiments 1 and 3. The platelet length ($\sim\underline{+}0.8\mu$) on the adaxial surface is similar to that of Experiment 1 (0.6μ), as shown in TABLE 1. The platelet lengths in E2 at the top of the list obtained from the second, third, and fourth leaves are larger than later values at the bottom of the list (leaves 5-9).

The rodlets are thicker (width $\backsim\underline{+}0.2\mu$) and straighter than most samples in E-1. They are almost twice as thick in some instances as the rodlet width in E-1. The density is medium, especially in the initial leaves, with very little overlap as is the case in normally aerial leaves. As a result, there are occasional open areas of the base cuticle. Although long scalloped "fingers" are not present, there is a lobed edge on the platelets.

3.2.3 Continuous Light Conditions (Experiment 3)

Under continuous light, plant height increased to a maximum of 75 cm by Day 40, then rapidly decreased until the onset of flowering (Day 65) when height increased to 61 cm by Day 109. Because plant growth was similar in all three cases (T1, T2, T3) the sheath height figures were grouped together to give a growth curve (Fig. 6). Plant height decreased because sheath heights on successive leaves tended to decrease after Day 40 until the onset of flowering, when it increased again. Thus, in continuous light conditions sheath height is a poor indicator of the plants' height and growth.

When ultrastructural wax forms of the leaf surface are compared with those of plants grown under simulated natural conditions (Experiment 1), there is no difference between surfaces of submerged leaves (leaves 1,2, and 3) or the shape or size of rodlets and scalloped platelets of the epicuticular wax mesh on the floating or aerial leaves.

Dark field light photomicrographs of the surface also revealed a similar arrangement in the submerged situation and the adaxial surface of the floating portion to those observed in Experiment 1 and 4 (Plate 1, Figs. 2,3,4,5,6). The submerged surfaces are quite smooth with a possible fibrous cellulose network beneath a thin cuticle (Plate 2, Fig. 3). Papillae forms are sometimes present on submerged leaves.

The exact configuration of the wax mesh varies with respect to numerical order of the leaves, date of sampling and age of leaf to produce slight changes in the extent of meshing. The scalloped

appearance (sc) of the platelets did not change under continuous light throughout the life cycle, as observed on Day 19 with leaf 5 (Plate 6, Fig. 1), Day 44 with leaf 6 (Plate 6, Fig. 2) and Day 92 with leaf 9. Measurements of platelet area, derived from multiplication of maximum and minimum diameters, were discarded because results were much too variable within a sample. This could be partially explained by the fact that the platelets were not readily seen in their entirety on all occasions. Rodlet dimensions are also variable, as shown in TABLE 1, but remain constant within a given plant leaf. Platelets were not as frequent on young leaf surfaces, indicating some structural progression of wax form associated with aging.

The abaxial surface contains more rodlet forms with fewer or no platelets. Where platelets occur scalloping is present. For both surfaces from Day 16 up to Day 92 there appears to be no differences in rodlet forms. Under continuous light, the epicuticular wax barrier is not significantly altered when compared to the E-1 wax forms. The mean rodlet lengths were 0.6μ and 0.7μ for the adaxial and abaxial surfaces respectively and both have been shown not to differ from the other Experimental details. The rodlet width of about 0.1μ is not different from that of E-1. The mean platelet length in this experiment is 0.6μ, which is similar to that in E-1. The papillae diameter was 3.3μ and quite uniform over a leaf surface.

3.2.4 Temperature Treatments (Experiment 4)

Plant growth under the three different temperature

regimes produced no wax forms on submerged leaf surfaces. The adaxial surface of a second leaf (Plate 6, Fig. 3)from Group 3 (8°C) differed slightly in being very uneven with a rippled effect. Compared to a similar leaf from Group 2 (18°C) (Plate 6, Fig. 4) it is apparent the higher the temperature treatment, the smoother the submerged leaf cuticle. This trend is more pronounced at 23°C (Plate 6, Fig. 5). The cuticle in Group 1 (27°C) plants was quite smooth, as seen in Plate 6, Fig. 6.

Comparison of both surfaces of the aerial portions of floating and aerial leaves with those in the Qualitative Assessment indicated that there are very little differences in stomatal length and frequency, papillae size, and ridging. Stomatal length is approx. 31μ on leaves from all Groups as shown in Plate 2, Figs. 4 and 5.

Electron micrographs of the adaxial aerial epicuticular wax layer (Plate 3, Fig. 6) reveal that continual exposure to higher temperature (27°C) produces thin, elongate and sometimes curved rodlets. The rodlet length varies from 0.4μ to 1.1μ in all treatments with mean lengths of 0.6μ for Group 1 (27°C) and 0.5μ Group 2 (18°C) (from TABLE 1). Not enough data was collected from Group 3 (8°C) plants to obtain a mean, but values ranged from 0.5μ to 0.8μ.

At the 27°C temperature treatment, rodlet width of 0.05μ was about one-half the width found in E-1. The mid temperature regime, 18°C, resulted in straight rodlets which lack the slight lobing or protruding extensions along its length that are readily noticeable at 27°C. Rodlet width and the wax layer at 18°C are similar to that

represented in E-1 (Plate 4, Fig. 6). Transfer of plants from 27°C to 18°C for five days does not alter the full wax mesh. The high density of wax forms makes it difficult to ascertain rodlet length or platelet shape and size. This fact makes it difficult to pick out minute differences possibly associated with temperature treatments.

The cold treatment (8°C) did not result in marked structural or density differences. Exposure to longer periods of cold (10-17 days) produced rodlets (not shown) which are more individual, have straighter sides, but are similar in length to E-1. Transfers from higher temperatures to the cold for a short period of five days did not produce structures different from their controls.

Platelet length was analyzed on the adaxial surface and it was noticed that at 27°C the platelets were shorter than at 18°C. TABLE 1 indicates mean lengths of 0.5μ and 0.7μ for 27°C and 18°C treatments, respectively. However, the overall areas (length x width) are similar in all Groups - $0.2\mu^2$, taken from a sample of five per Group. Therefore, temperature affects the overall platelet shape, with lower temperatures resulting in elongate platelets, and higher temperatures in square forms. The scalloped edge is always present with long "fingers" quite noticeable at all treatments. Papillae are similar to those seen in E-1. The abaxial surface was not examined in detail and rodlet-platelet lengths are lacking.

3.2.5 First Appearance of Wax (Experiment 5)

The depth (36 cm) was sufficient to insure that the third

leaf was permanently submerged and the fourth leaf emerged from the submerged condition upon maturation. Similar results were observed in Group 1 (27°C), in Group 2 (18°C), where growth was slower, and Group 3 (8°C), where growth reached only the three leaf stage. Most observations are based on Experiment 4, Group 1 plants.

The surfaces of the first three leaves of Group 1 and 2 were smooth, with few pits and papillae (Plate 6, Fig. 4). When the fourth leaf appeared by Day 7 in Group 1 (27°C) the surfaces on both sides were smooth. Two days later, a replica of the adaxial surface of the fourth leaf about 15 cm from the water surface indicated the formation of an incomplete rodlet and platelet wax barrier (Plate 6, Fig. 6). Regularly spaced stomata plus large papillae indicated that the fourth leaf was distinctly different from the previous 3 leaves. Visually, the adaxial fourth leaf surface reflected light by means of air trapped on its surface. By Day 12 the fourth leaf had elongated sufficiently to be floating on the surface. The adaxial surface, which was in contact with the air, was covered by a wax mesh. The adaxial surface at the transition zone had a wax mesh (Plate 7, Fig. 3). In the transition zone the wax density decreased rapidly in the direction of the base of the leaf and appeared similar to the submerged tip of the fourth leaf (Plate 6, Fig. 6). Below this zone the adaxial surface was smooth (Plate 7, Fig. 1). The entire abaxial surface was smooth whether the fourth leaf was completely submerged or floating, as in Plate 6, Fig. 4.

With plants growing at 18°C, the fourth leaf was still submerged by Day 14. However, an incomplete rodlet wax structure was found on the adaxial surface (Plate 7, Fig. 2), which was not as advanced in development as the submerged fourth leaf already mentioned

(Plate 6, Fig. 6). Examination of the adaxial floating mature fourth leaf which follows revealed a full mesh of wax similar to that present on a floating leaf of Group 1, Experiment 4 (Plate 7, Fig. 3).

In some cases the fourth leaf had not yet emerged from the sheath but epicuticular wax structures were observed forming a mesh with density of the wax structures decreasing from tip to base. The rodlet size was significantly less than those on fully expanded leaves, as seen in TABLE 2 of the Quantitative Assessment.

In plants grown continually in Group 3 (8°C) at a depth of 21 cm. a floating third leaf appeared on Day 60. Examination of the surfaces above and below the transition zone indicated a full mesh of straight rodlets and platelets in the aerial situation and a smooth submerged surface. Previous examination of leaves one and two revealed smooth submerged surfaces (Plate 6, Fig. 3).

The fifth leaves in Group 1 and 2 were initially submerged with a wax mesh present on the adaxial surface and none on the abaxial surface. Although the fifth leaf (Day 17) was floating and the sixth leaf (Day 24) was aerial, both leaves had an extensive wax layer which was present even in unrolled submerged leaves. Wax forms found on the fifth leaf surface arose under similar conditions outlined for the fourth leaf surface. Epicuticular wax was found on both surfaces of the sixth leaf surfaces by Day 32 (Plate 3, Fig. 6). Wax was observed on the abaxial surface only when the leaf was aerial or when the mid rib is strengthened to maintain the leaf in a vertical position.

3.2.6 Field Studies (Experiment 6)

Plants from Millgrove were mostly var. aquatica and the epicuticular wax layer did not differ from that of var. angustifolia from Long Point. The field sample of var. interior from Whitefish Lake had thicker rodlet forms but later observations with the transfer material in the Greenhouse revealed a wax mesh similar to var. aquatica. Rodlet density, platelet density and the degree of overlap were alike in all three varieties examined.

To compare field and laboratory grown samples, the adaxial rodlet lengths were calculated from the Millgrove material (var. aquatica) and then analyzed with similar data from E-1 (var. interior). The rodlet length in both varieties was about 0.7μ, as seen in TABLE 1. The density varies from medium to heavy and the adaxial rodlets overlap considerably (Plate 7, Figs. 4,5; Plate 8, Fig. 1) so that leaf surfaces of field samples do not markedly differ from the aerial leaves in Experiment 4 (Plate 3, Fig. 6 - var. aquatica). Likewise the abaxial surfaces of var. aquatica (Plate 5, Fig. 1) from E-2 do not differ from the field samples (Plate 7, Fig. 6).

The exact position of a plant with respect to the water-air interface is hard to ascertain in field studies. Analysis of the May 15th samples from Millgrove (water depth 20 cm) indicates that on the young, submerged, curled-up fifth leaf of a floating stage plant the adaxial surface is covered by many short, stubby rods 0.2μ long (Plate 7, Fig. 4). The wax is just appearing and projecting from a rather uneven cuticle. A floating portion of the fifth leaf from another

plant indicated a full wax mesh is present and is similar to the later aerial leaves already discussed (Plate 7, Fig. 5). The surface of the submerged leaves from all three locations, like the previously discussed submerged leaves, lacks a wax layer and the smooth cuticular surface is unevenly covered by many debris particles.

An interesting observation concerns the Long Point material (var. angustifolia). The leaves were initially sampled on June 2nd when the entire plant was still submerged. The water depth was 100 cm and leaf six had appeared. The tip of the sixth leaf was about 50 cm from the water-air interface. The adaxial surfacesof the fifth and sixth leaves were covered by a thin layer of air causing a considerable amount of light reflection off the leaf surfaces. However, electron micrographs of all the leaves including the adaxial surface of the fifth and sixth leaves revealed a smooth leaf surface. No wax forms and very few bacteria were found. The surfaces were similar to the smooth surfaces of the submerged leaves in Experiments 1 and 3 (Plate 3, Figs. 2,3,5). When the Long Point material was examined again on July 25th, analysis of the surfaces of the aerial leaves revealed a very thick mesh of epicuticular wax quite similar to that of aerial of var. aquatica from Millgrove (Plate 8, Fig. 1).

3.2.7 Physical and Chemical Treatments (Experiment 7)

Abrasion of the aerial leaves with cotton does not remove all of the surface wax (Plate 8, Fig. 2). The rubbing action smears the platelets to give a very rough, uneven surface. There are

no distinct rodlet or platelet wax forms present and all former structural organization is obliterated. On other samples similar surfaces were observed and there is no difference between the adaxial and the abaxial surfaces after this treatment.

Submergence of plants at the aerial stage revealed no alterations in the wax layer of both leaf surfaces (Plate 8, Fig. 3) even after ten days. Furthermore, there are no alterations on the surfaces of young leaves which emerged after the time of submergence. It is possible that once a plant produces aerial leaves wax production is continuous. It cannot revert to the stage where submerged-type leaves with no wax are produced.

A short exposure (1 minute) to chloroform all but destroys the rodlet shape as seen in Plate 8, Fig. 4 . The epicuticular wax layer of the control was similar to that of an aerial leaf surface (Plate 3, Fig. 6 in Experiment 4). An epidermal cell wall outline, a papilla shadow and many destroyed wax forms are present in Plate 8, Fig. 4. Prolonged exposure tends to completely remove the epicuticular wax layer and the outer surface of the cuticle becomes corroded. The dissolving action apparently is uneven, indicating a mixture of waxes of different solubilities in chloroform. Similar results were observed with acetone although less wax appears to have been removed and the rodlet form is still present after 1 minute (Plate 8, Fig. 5).

The process of aging, which is a physical and chemical alteration within the plant, results in some changes of the epicuticular wax layer (Plate 8, Fig. 6).

The rodlet and platelet wax forms mesh together to produce solid bands of wax and equally large open areas in an old yellowing aerial leaf.

DISCUSSION

4.1 LEAF SURFACE MORPHOLOGY AND CHANGING ENVIRONMENTS

There have been few studies of surface morphology of leaves of emergent hydrophytes. Heterophyllic changes of leaves in water and aerial environments of emergent hydrophytes has been restricted to leaf shape or structures visible with light microscopy. This thesis reports the changes on the leaf surfaces at the ultrastructural level, with special attention given the epicuticular wax mesh.

Examination of the surfaces of the three types of leaves revealed morphological differences which could be correlated with certain surrounding environments. In all Experiments the surfaces of mature submerged leaves were generally smooth with square epidermal cell outlines and prominent "cross wall" connections between ribs, as well as the occasional elongate papillae. These surface characteristics are present on the first three leaves, although some field samples revealed that the same condition could exist on the sixth leaf provided it was submerged. No stomata or ridges were observed. If the mature leaf was submerged, then the outer surface was quite simple, and as would be suspected, there were no epicuticular wax forms overlying the rather thin cuticle.

The floating leaves, which succeeded the submerged leaves, underwent marked surface anatomical changes on the adaxial surface as a result of the change of habitat. The submerged section of the

floating leaf was similar to the wholly submerged leaves. The adaxial section exposed to air was marked by a "transition zone" which occurred around the water-air interface. The adaxial surface of the transition zone was not smooth, because it possessed (a) epidermal cell outlines which were elongate, (b) numerous short papillae divisible into two diameter groups projecting from the outer surface, (c) stomata occurring in great numbers and, (d) ridges corrugating the surface. Quite noticeable were the absence of short papillae and stomata in certain transition zone sections which were probably submerged or in contact with water almost continually.

Electron micrographs reveal that the epicuticular wax barrier appears in the transition zone (in the submerged portion) and that density of wax extrusions increases towards and into the aerial environment. Initially the rodlet wax form was the most noticeable form with the occasional platelet wax form present towards the more mature areas of the leaf surface. The elaboration of the epicuticular wax barrier in all Experiments took place through a cuticle which in the submerged situation was variously pitted, smooth, cracked, and thin. The mesh of wax produced on the floating leaves varied slightly in the spatial configuration of the two wax forms among the different samples examined. Because the individual rodlets and platelets exhibited a basic uniformity among the samples they could not cause this difference in the spatial configuration in the wax network.

The aerial leaves have a thickened and enlarged mid-rib which projected the leaves almost vertically into the air. The morphological

characteristics on the adaxial surface were the same as those on the aerial portion of the floating leaves. The ridging or corrugated appearance, stomata and the number of papillae also increased.

There were slight but consistent differences between the adaxial and abaxial aerial leaf surfaces. The abaxial surface of aerial leaves was not as ridged and there were fewer stomata in a given area. The larger papillae visible on the adaxial surface were more translucent, the diameter was slightly less, and they were not as tall. Examination of the epicuticular wax layer indicated that the rodlets dominated numerically the platelets were fewer in number, less scalloped, and appeared smaller. To a certain extent the adaxial surface contained more epicuticular wax than the abaxial surface because the adaxial mesh appeared more extensive. However, this was never tested quantitatively.

The orientation of wax form in the network displayed a number of different characteristics for each form. The rodlets always touched one another so that they were placed in many directions forming a criss-cross network. These directions and extent of criss-cross caused the configurational variations. The platelet positions were also quite variable among the surfaces examined. Their dispersion among the rodlet forms depended upon the age of the sample examined. The older the sample, the more platelets were visible. The rodlet density also affected platelet density with decreasing rodlet density increasing the open areas and thus allowing more space for the platelet wax forms to lie out on the surface. This greater chance for exposure made

the platelet wax form more readily visible and the scalloped was easily observed.

Small papillae on the submerged leaf surfaces were continuous with the outer cuticular surface. In the aerial situation they were extensively covered by an epicuticular wax mesh. The density was considerably greater on the papillae but there was a continuity of this mesh over the entire cuticle. The origin of the papillae is unknown and similar structures have not been reported in the literature. It is possible they may be projections of the epidermal cell wall.

The density of the epicuticular wax layer varied among samples, and to a certain extent, within a given sample. References to "thicker mesh", "medium density", and "open areas" indicated that the wax network varied in all Experiments. The density, spatial configuration of the wax network, and rodlet and platelet structure made it difficult to ascertain when a significant change had occurred during the growth of the plant.

Statistically, the rodlet length on both surfaces and the platelet length on the adaxial surface in all Experiments did not vary significantly (TABLE 1). Because the amount of overlap did alter the noticeable and measurable lengths, there is some doubt as to the significance of these dimensions. Nevertheless, they provided a common point of comparison, and further differences do appear when young leaves are compared with mature leaves (TABLE 2). In the field samples no differences in the wax mesh were noticeable among varieties angustifolia, aquatica,

and interior. Further evidence concerning the similarity among the varieties comes from the laboratory Experiments. Different varieties were utilized but the wax mesh was considered the same throughout the Experiments.

As the wild rice plant grew up out of the water into the aerial state, the leaves affected underwent surface morphological changes in response to the change in environments. Most important of these changes are the appearance of numerous papillae and the epicuticular wax layer on the aerial surfaces. This change occurred in response to changing water contact and was directly related to the type of leaf previously present. In other words, if the abaxial surface of a floating leaf (leaf 6, for convenience) was devoid of epicuticular wax, lacked aerial-type papillae and lacked stomata, the abaxial surface of the next leaf (leaf 7) which appeared had a greater potential of exhibiting these surface characteristics (if the leaf became aerial). This transformation of the surface increased the surface area, although it was never measured.

Different processes were found to erode the fine epicuticular wax mesh. Aging of the aerial leaves affected the wax mesh in that the two wax forms were found to fuse together forming a mat with open unprotected areas of cuticle beside it. This weakened configuration probably was due to the normal weathering, and the slower rate of wax extrusion associated with senescence. In the field one of the more important aspects of weathering on photosynthetically active leaves was the abrading action of wind and waves. These eroding agents abraded the

epicuticular wax mesh on the domes of the small papillae, leaving the surrounding mesh unharmed. The papillae reduced the number of possible contact points and the ridging of the adaxial surface further decreased the susceptibility of the surface to erosion and abrasion. Examination of the cotton rubbed surface (from E-7) sometimes revealed epicuticular wax layers completely unaffected by the treatment. Furthermore, even areas abraded by natural agents sometimes would be only partially devoid of wax organization. The ridged leaf surface reduces the area that can be abraded. Only when these first contact points have been removed by repeated action can the remaining area be affected by the wind and waves.

Certain stages of the life cycle of wild rice were quite susceptible to the prevailing condition of the water environment. The floating stage (Phase 2) of plant growth occurred at the time of sudden algal blooms which occurred in every Experiment and certain field habitats. The algae adhered to the plant leaves and it was found that if the initial floating leaf was surrounded by algae, especially in slow moving water, there was premature bleaching and killing of this leaf. If this occurred before the next leaf was ready to become fully floating, the plants were weakened and some died. This susceptibility of the initial floating leaf in stagnant water prone to algal blooms may be the factor prohibiting this species from colonizing such environments. The increased eutrophic conditions of some lakes resulting in algal blooms may be the cause of the disappearance of much of the wild rice from its former habitats.

The aerial leaves of many emergent hydrophytes are quite similar to related land forms. This study suggests that the adaxial leaf surface morphology among some grasses at the ultrastructural level are alike. The epicuticular wax mesh of the adaxial leaf surface of wild rice resembles those described for wheat (Troughton and Hall, 1967), reed grass (Stewart and Follett, 1966), and Oryza sativa L., cultivated rice, and Zizania latifolia Turcz, a perennial species of wild rice (personal observation).

Troughton and Hall (1967) do not refer to rodlets on the adaxial leaf surface. They stated that the surface of glasshouse grown plants supported platelet structures irrespective of the stage of growth, i.e. vegetative or reproductive. The electron micrograph of the adaxial leaf surface shows a mesh similar to the rodlet and platelet mesh on the adaxial leaf surface of wild rice. Therefore, this writer feels that the adaxial leaf surfaces between wheat and wild rice have similar, although not identical, epicuticular wax meshes.

Differences in the form of wax between glasshouse and cabinet-grown wheat plants were suggested by Troughton and Hall (1967) to have been due to differences in light level, temperature or the stage of growth. These differences refer to a greater or lesser extent of platelets on both surfaces. In wheat, the abaxial leaf surface is different from the adaxial surface with a wide variability in rodlet-platelet proportions and shapes. The same differences between surfaces, although less variable, were observed in wild rice and love grass

(Leigh and Matthews, 1963).

The adaxial leaf surface of wild rice resembles the epicuticular wax mesh of love grass. The "wax branches" and "cuticualar ridges" are similar to the rodlet and platelet mesh on the adaxial surface of wild rice. The abaxial leaf surface of love grass is quite different from those of the adaxial surface of both species and the abaxial surface of wild rice. The "ribbon-like wax branches" show a "profuse dichotomous" nature on the abaxial leaf surface of love grass.

In wheat, love grass, and wild rice there are differences between the adaxial and abaxial surfaces. The marked differences in the terrestrial grasses, wheat and love grass, suggest differences in moisture content and light receipt on the surfaces. In wild rice the differences in platelet number and form between the surfaces are not as striking as those outlined in the terrestrial plants. However, the difference in adaxial and abaxial surfaces within each species shows that there is a similar response to the environmental differences that occur above and below the leaf.

4.2 ULTRASTRUCTURAL CHANGES ON FLOATING LEAF SURFACES

Weber and Simpson (1967) concluded that the formation of the floating leaf must have special ecological significance. They felt that the developmental pattern of the presence or absence of the floating leaf was controlled by the water depth which in turn controlled the

metabolic activity of the shoot meristem region. The examination of the surface changes on the floating leaf indicated that the function of this type of leaf may be "to give the young growing plant some tissue which is exposed to aerobic conditions" (Weber and Simpson 1967, p. 662).

A general analysis (E-1) revealed that epicuticular wax was present on the adaxial surface from the transition zone to the tip of the leaf. Because this wax layer decreased rapidly in size and density in the transition zone toward the base of the leaf it may be postulated that the triggering mechanism allowing the development of the wax layer is connected with the leaf's growth and eventual contact with the aerial environment. The pre-adaptation of the floating leaf to the aerial environment involves this epicuticular wax mesh on the adaxial surface. The problem is to ascertain where the surface structures (including papillae formation and stomata) are elaborated with respect to the position of the potential (i.e. still submerged) floating leaf and the water-air interface.

From the evidence, the leaf primordia present at the apical stem are likely positioned near the base of the plant and certainly are submerged. The third leaf tip never reached more than 20 cm. to the water-air interface and the submerged surface was typically smooth on Day 6. The analysis on Day 7 of the young fourth leaf, about 25 cm. from the interface and still within the stem-sheath, revealed a smooth surface with occasional wax structures. Examination of a young adaxial leaf surface about 15 cm from the water-air interface in E-5 revealed an incomplete wax mesh composed mostly of rodlets (on Day 9) which

a couple of days later was a full mesh of both rodlets and platelets. Although the entire plant was still submerged, an epicuticular wax layer was formed before the eventual breakthrough of the water-air interface which occurred about two days later. Therefore, as the leaf approached the water-air interface, an epicuticular wax layer developed and covered the cuticle of at least the adaxial tip of the leaf blade. Eventually the part covered with wax began floating and was exposed to the air. In the submerged portion of the adaxial surface below the transition zone the cuticle remained smooth, the same as the previous submerged leaves. In many cases, about one-half of the "floating leaf" length was actually floating and it was this portion which was covered by epicuticular wax. Ridges, papillae and stomata were present on the adaxial surface. The abaxial surface throughout was smooth.

The mechanism which causes the initiation of wax formation may be triggered by specific environmental factors such as water or air contact. The attenuation of light for small distances (0-30 cm.) is unknown even though the increased presence of certain wavelengths of light near the interface possibly could trigger the formation of epicuticular wax.

Evidence indicated that the amount of water above the eventual floating leaf was important in the initiation of epicuticular wax forms, provided that total water depth remained constant. The

surfaces of the permanently submerged leaves lacked stomata and a wax mesh. Although the plant was completely submerged, a potential floating leaf 25 cm. from the surface did develop stomata and papillae. However, after two days of growth, the surface of the apex of this leaf, now 15 cm. from the water surface, was partially covered by a wax network. The wax mesh was complete by the time the tip of this expanding leaf started to float on the water surface. Therefore, in a totally submerged plant about to become floating, the extrusion of surface wax commences on the recently emerged, potential floating leaf as this leaf elongates into a critical zone just beneath the water surface.

According to Sculthorpe (1967), the form of leaves of various species of Ranunculus produced by submerged plants was under the primary control of photoperiod and temperature. Both the dissected submerged type of leaf and the laminate floating type were initiated under water. This initiation also occurred in wild rice. Leaf morphogenesis in Ranunculus did proceed along the pathway of an abrupt change from submerged to floating types, with no intermediate forms. In wild rice the change in leaf surface morphology was also very abrupt. The appearance of stomata, papillae, ridges, and epicuticular wax on the adaxial surface of potential floating leaves suggested an abrupt change from the submerged type of leaf to the floating type.

It was noticed that once the epicuticular wax was found on the surface, it was always present on the adaxial surface of the successive floating leaves. At least two floating leaves were present before an aerial leaf appeared. In the case of the aerial leaf, the

abaxial surface was covered by an epicuticular wax layer which was present in the young leaf within the stem-sheath. In this system, the young aerial leaves were observed to possess an epicuticular wax layer even near the base of the leaf blade (about 15 cm. within the sheath). Because of difficulties in examining young material, only the adaxial surface could be examined without causing mechanical damage as the leaf was uncurled.

The appearance of the wax is not strictly related to the leaf number since the first appearance of epicuticular wax was found to vary among Experiments. For example, the wax mesh was found on leaf 2 from section 3.2.2, and on leaf 5 of the Millgrove field sample. Furthermore, a Long Point field sample of the submerged sixth leaf from section 3.2.6 lacked any epicuticular wax, although a sheen of trapped air was visible.

Previously it has been stated that the presence of a floating leaf can be related to the metabolic activity of the shoot meristem (Weber and Simpson, 1967). The metabolic activity of young submerged leaves is known to be quite high. The synthesis of sugars and the reception of osmotically active solutes translocated from the short axis creates a high osmotic pressure in these leaves (Sculthorpe, 1967). Until the epidermis is covered by a sufficient cuticular barrier the young tissues may be inflated by the osmotic intake of water. Thus, the metabolic activity of leaves near the water-air interface may increase tremendously due to greater light reception and photosynthesis. In turn this increases the osmotic intake of water. To counteract this activity, the elaboration of an epicuticular wax layer in this submerged state would reduce the

inflow of water. It has been known that foliar absorption is quite negligible through the epicuticular and cuticular layers (Vaadia and Waisel, 1963).

Another possible explanation of the pre-adaptation stems from the metabolic activity already mentioned. This concerns the production of oxygen within the tissue being displaced outside and collecting against the adaxial surface. The submerged, young leaf 4 in E-5 had a sheen of trapped air on the adaxial surface. Assisting this trapping of gas is the "hooked" tip of the vertical leaf. Increasing the metabolic activity would increase the gas production and a greater area would trap it. Thus, the micro-environment of the surface might simulate an aerial condition, although the air would be saturated with water vapour. The fact that this occurs near the water-air interface may insure the establishment of a protective barrier in anticipation of the aerial environment. The presence of stomata indicates that the submerged leaf is ready to respire aerobically upon contact with air.

One mechanism accounting for the wax crystallization visualizes the wax reaching "the surface dissolved in a 'solvent' which has a relatively low boiling point and which slowly volatizes when in contact with the air" (p 1325, Eglinton and Hamilton, 1967). However, the crystallization of the wax need not be controlled by a drying action. Because the potential floating leaf has a sheen of saturated air on the adaxial surface, the formation of crystalline wax forms does not have to take place in a desiccating atmosphere. The build up of water-saturated

air on the adaxial surface is sufficient to permit volatization of the wax solvent resulting in the crystallization of the epicuticular wax.

Therefore, the appearance of the epicuticular wax on the adaxial surface of young, submerged, and potentially floating leaves reinforces the importance of the metabolic status of the plant in the formation of the floating leaf. The profound morphological changes suggest that the plant must be pre-adapted to the radical change in environments, both for greater efficiency and eventual survival. The remaining water depth to the water-air interface and the osmotic pressure may trigger the transformation. Leaf number has been shown not to affect the initial formation of epicuticular wax or the change-over to floating leaves.

Except for the first leaf, every leaf has the genetic potential to have aerial characteristics. The floating leaf is organized such that the surface in the air is covered with stomata, papillae, and epicuticular wax while the rest resembles the totally submerged leaf surfaces. As the floating leaf demonstrates, the environmental cue for the change in leaf surface morphology occurs while the plant is still submerged. The primary environmental control triggers the genetic potential resulting in rapid ultrastructural changes. However, the development of the cuticle and epicuticular wax mesh is under greater genetic control than environmental control because abnormal environmental treatments, such as continuous light and extremes of temperature, only slightly altered them. Although an emergent hydrophyte will be affected by the change from aquatic to aerial environments, the above statements

are in agreement with Martin's (1966) conclusion that "the amount and composition of the wax and the nature of the acids forming the cutin are genetically controlled, and that the development of the cuticle as a whole is less influenced by environmental conditions than has been supposed" (p 142).

4.3 STIMULATION OF EPICUTICULAR WAX IN A COMPLETELY AERIAL ENVIRONMENT

The plants grown at the water-air interface can be considered to have an unlimited water supply in an aerial environment. The first few leaves which appeared were small, shrivelled up after a few days, and were not considered to be healthy.

Leaf 1 did not develop an epicuticular wax barrier; instead a thick cuticle appeared which cracked considerably upon exposure to the air. Leaf 1 always shrivelled soon after exposure. Leaves 2 and 3 underwent complete morphological changes with stomata, papillae, and slight ridging present on both sides. The platelets were very large and the rodlets were thicker. Although not statistically significant, it is felt that the large wax forms result in considerable overlap and increase their protective value for the leaf. The open areas present on these initial leaves were partly protected by the large platelets which covered a greater area when laid out flat against the surface. The epicuticular wax layer on both surfaces of these initial leaves was less dense than that shown on the truly aerial leaves which appeared later in E-2 and also in all the other growth situations. There is a gradual increase in density of the network of epicuticular

wax on the surfaces of successive leaves in E-2 resulting in surfaces similar to that outlined for the later aerial leaves of E-1.

The initial aerial state resulted in a thicker cuticle (on leaf 1) and a full wax mesh (on leaves 2 and 3) on the normally submerged leaves. The epicuticular wax barrier reduced the water loss from the young leaves and limited desiccation of their tips. Because the tips of the first three leaves shrivelled up, it is suggested that epicuticular wax did not develop at these locations. However, the relation between desiccation and the ultrastructure of wax was not assessed.

From the discussion in the previous section, it is apparent that the leaf surface was covered with an epicuticular wax layer even before the eventual floating leaf had, in fact, passed through the water-air interface. This deposition of surface wax in anticipation of the aerial environment and regardless of leaf number (except leaf 1) was taken to be the necessary protective barrier of an emergent hydrophyte. The specific function of the protective layer can only be assumed. It was very difficult to alter the epicuticular wax layer and still have a normal plant develop.

4.4 POSSIBLE CORRELATION BETWEEN THE PLATELET SCALLOPING AND SOME BIOLOGICAL RHYTHMS

The scalloped appearance of the platelet wax form could be related to the rates of extrusion with periods of high wax synthesis producing the wider bands in the scallop and low periods of wax production resulting in the narrow bands of wax. In other words, the scalloping could reflect a layering phenomena. If the above endogenous

cycle did exist with the periodic production synchronized with the natural alteration of day and night, then it would reveal its inherent periodicity under constant conditions (in this case, continuous light). By this it is felt that a constant growing situation would disrupt the wax extrusion rhythm resulting in a non-scalloped platelet appearance.

To appreciate what has been proposed it is necessary to consider biological rhythms of different plant systems in order to understand the phenomena of daily layering of wax and its controlling mechanisms. Rhythmical metabolism synchronized with the surrounding (astronomical) environment can result in daily growth layers (Neville, 1967). There are two ways daily growth structure may occur. Firstly, there may be a direct response to the changing environment which requires the measurement of light, temperature, and relative humidity. Secondly, the periodicity might be determined by an internal biological clock which is usually synchronized with day-night changes. Juniper (1960) has shown that wax secretion in peas is light stimulated; therefore, a photosynthetically controlled (and light controlled) supply of unknown wax precursors might control the scalloping effect. Rhythms which are relatively temperature-independent over wide ranges and about 24 hours exhibit circadian rhythm. Systems uninfluenced by external stimuli are said to be "free-running".

Daily growth layers have been noticed in plant material, for example, cotton hairs developed in constant light produce few or no daily growth layers (Balls, 1928, from Neville). Conchoidal layering (starch

in each grain is deposited in numerous more or less concentric layers) in starch grains of several plants has been known for years. Plants grown in constant conditions indicated that daily layering of starch was either triggered internally or triggered by the environment. Wheat starch grains grown in continous light at constant temperature show no rings (environmental control) but those of potato tuber still show rings (internal control). Since daily fluctuations of the starch-synthesizing enzyme phosphorylase do not affect the starch daily layering, Buttrose (1962, from Neville) suggested that daily layering was produced by a deposition of a constant width of starch molecules in a highly crystallized state at the start of each daily period followed by either a larger or smaller width of molecules in a more amorphous state during the rest of the daily period. A photo-synthetically controlled supply of starch precursor may regulate this paracrystallinity in wheat; an endogenous rhythm would control the starch precursor supply in potatoes. An analogous mechanism might explain the wax platelet structure.

Continuous light (E-3) did not disrupt the formation of scalloped edges on the adaxial platelet wax forms. The platelet shape with characteristic scalloped edge remained under continuous light with each successive leaf. The small variabilities in platelet length, degree of surface bumpiness or extent of the scallop, i.e. "fingers", were quite similar to those observed on plants grown under more natural photoperiodic conditions. It was not possible to differentiate among the leaf surfaces, either adaxial or abaxial, of plants from any other

Experiment. This similarity applied to rodlet shape, papillae configuration and the density of the network. At times it was felt that perhaps more epicuticular wax was extruded in response to the continuous light but this qualitative estimate was not always noticed and was never substantiated quantitatively. The air temperature varied from 22.9°-27.2°C but each change was gradual. Individual leaves up to the time of sampling did not experience all the fluctuations. Nevertheless, the 4°C latitude did not cause any observable structural alteration on the surfaces examined.

The lack of change of form in continuous light and in gradual temperature change during the growth of several leaves suggested that an endogenous circadian rhythm controlled the paracrystallinity and rate of extrusion of the epicuticular wax constituents. A situation operating in potato starch grains (endogenous rhythm) may also account for the scalloped edge of the platelets. Since no obvious differences were observed as a result of the temperature change the rhythm is probably circadian. To a great extent it is also free-running. Went (1962) studied the autonomous 24 hour rhythm in plants. He felt that the entrainment of a "phenomenon by the external cycle is not immediate or complete" (p 866), which agrees with the above discussion. Through a number of examples he also concluded that not all "organismic rhythms" are induced by external rhythms which they receive from their environment. These autonomous, circadian rhythms, which do not have an external signal, become apparent only in the absence of an external rhythm. In the case of the scalloped platelets, the metabolic activity is probably

entrained to an external, environmental rhythm, but it appears not to be the day-night fluctuation or small temperature fluctuations.

Another aspect of this extrusion arising from this investigation concerns the mode of extrusion. If the metabolic production of wax does not alter the wax form, possibly the alteration of pore or channel diameter might result in the scalloping effect. The diameter could be varied by different degrees of tension in the cuticle. Martin (1966) proposed a mechanism by which the cuticle would regulate the water balance in the leaf. He stated that an isolated cuticle contracts when dried but readily reabsorbs water and swells. The epicuticular wax may be in pockets such that when the cuticle is fully extended (as in humid conditions) they are at maximum distances apart. Under field conditions the relative humidity has a diurnal fluctuation. Although not on a 24 hour cycle, the relative humidity in the controlled conditions was found on the average to vary between 30% and 60% over extended periods (48 hours).

In a dry atmosphere the cuticle is likely to contract bringing the wax pockets closer together, thus presenting a structure less permeable to water. If this elastic change was cyclic and took place in young leaves during wax extrusion, then the pore diameter would change and the platelet shape would be affected. This hypothesis is supported by Cox (1968), who has shown that transpiration follows a cyclic pattern (about 35 minutes duration) in a "steady environment". The fluctuations are a reflection of the internal rise and fall of the leaf temperature. With a reduced transpirational demand, an increase in leaf temperature

would result. To cool, transpiration would increase. The cuticle would be under tension and pore size would be large. The leaf temperature would then decrease as would the water balance. As a result the transpiration would decrease, the cuticle would compress, and the pore size and the amount of wax extruded would decrease. This cycling would then be repeated so that a "scalloping effect" would be produced.

Two hypothetical explanations of the scalloped appearance of the platelet wax form have been proposed. These two proposals, daily layering due to (1) an endogenous circadian rhythm or (2) the 35 minute cycle pattern, refer to different time spans. Which proposal, if either, fits the system under study is unknown. However, future investigations of the mechanisms of wax extrusions should incorporate information on transpiration rates, metabolic cycles, and biological rhythms. These two explanations remain theoretical since the controversy surrounding the mechanism of wax extrusion has not been resolved.

4.5 EFFECT OF TEMPERATURE TREATMENTS ON EPICUTICULAR WAX FORMS

On the submerged leaf surfaces the cold treatment (8°C) produced a rough cuticle which became smoother with increasing temperature. The effects of higher temperatures (>25°C) on floating and aerial leaves resulted in thin, curved rodlets of wax which were half the width of the rodlets from E-1. At the mid temperature treatment (18°C) the rodlets and general configuration were similar to that in

E-1, where the temperature was similar. The cold temperature resulted in straight, stubby rodlets. In the 27°C and 18°C treatments the adaxial rodlet and platelet lengths were not statistically different (TABLE 1) from the lengths obtained from plants grown at various temperatures in the other Experiments.

Short term transfer to colder temperatures did not affect the existent epicuticular wax layer while longer transfer periods did affect the surface wax. Temperature did not affect platelet area. However, the lower temperatures resulted in elongate platelets while the higher temperatures produced square forms. The scalloped edge was similar in all treatments.

High water tmperatures make it necessary for the floating leaves to reduce the internal leaf temperature. Perhaps as a result the long, thin rodlets reduce the surface area that is covered by the epicuticular wax layer and hence dissipate excess heat more efficiently. The open areas of cuticle allow cuticular transpiration and heat dissipation to proceed at a greater rate per unit area. The rodlets and platelets project more vertically from the outer cuticular surface so that the area actually covered by the epicuticular wax barrier is thus reduced.

It is concluded that the epicuticular wax layer is dynamic and its configuration can be altered by the effect of unusual temperatures. The production of more slender rodlet forms at high temperatures is interpreted as a definite response to the increased heat receipt. Decreasing the width would tend to reduce the "trapping" effectiveness of the wax layer and decrease the amount of heat absorption.

Straight rodlets noticed at colder temperatures probably increase the density and degree of overlap of the wax forms, thus increasing the protective value against water loss and consequently, reducing the heat loss. These physical elaborations are probably controlled by metabolic activities which are partially affected by temperature extremes. This indirect protoplasmic control (Kreger, 1958) over the wax layer does not detract from the important influence that the environment has been shown to play in molding this outer protective layer.

4.6 PHYSICAL AND CHEMICAL TREATMENTS

Mechanical abrasion of the surface with cotton wool was shown not to remove the epicuticular wax layer but rather to spread it over the cuticular surface. This increased the protective value of the layer. Field samples usually had some epicuticular wax completely removed exposing the underlying cuticle. This abrasion by wheathering usually occurred on the domes of the wax-covered papillae and appeared quite similar to the weathered leaf surface reported by Hall and Jones (1961). Examination of the mature wild rice leaves indicated that, in abraded areas, the epicuticular wax was not replaced. No pores or discrete pathways of wax extrusion were observed on the exposed papillae cuticle. This abrasion of the epicuticular wax layer has been shown to increase cuticular transpiration.

One minute chemical treatments also caused a breakdown of the natural structure of the epicuticular wax layer by destroying

the rodlet form first. Removal of the surface wax by longer chemical exposures reveals the epidermal cell wall. The resulting layer appeared similar to the treated prune plum (Bain and McBean, 1967) and apple (Hall, 1966) surfaces. The corroded outer surface indicated that the chemical dissolution is uneven and that the surface wax is differentially soluble in the solvents. Again, no pores were observed on the cuticle, even after the surface wax has been removed. Partial removal of the epicuticular wax mesh did not show the origin of the disintergrating wax forms projecting from the cuticle.

The epicuticular wax layer was unaffected by contact with water. The natural structure remained intact indicating a strong hydrophobic nature of the epicuticular wax. There is no solvent action of the water on the epicuticular wax. Mature leaves were used but young aerial leaves, which emerged after the entire plant was submerged, also were covered by an intact epicuticular wax mesh. The formation of the wax mesh can be maintained when the entire aerial stage plant is submerged.

CONCLUSIONS

Morphological differences on the surface of the submerged, floating, and aerial leaves of different varieties of wild rice suggest that there is a complete response of the plant to the changes from water to air, both in the field and in the laboratory. Papillae, stomata, ridging, and epicuticular wax formations were the indicators of this heterophyllic nature of the leaves.

Leaves of plants grown completely in saturated soil, thus circumventing the natural successive steps of submerged, floating, and aerial stages, produced only aerial leaves whose surfaces were covered by large platelets and rodlets.

Floating leaf formation is initiated while the entire plant is submerged. In all cases, epicuticular wax, stomata and papillae formation took place on the adaxial surface of the future floating leaf during the submerged stage. As the young floating leaf nears the water-air interface, prior to maturation, an incomplete wax mesh of fully developed rodlets and platelets is found. When fully mature, the floating leaf portion in the air is covered by a complete wax mesh. The initiation of the epicuticular wax formation does not depend upon the leaf number because the first appearance of the wax forms varied with leaf number and growing conditions. A sheen of trapped air was always found on the adaxial surface of the future floating leaf and this possibly initiates the production of epicuticular wax.

The epicuticular wax mesh varied according to the density, size and spatial configuration of the rodlet and platelet wax forms. This variability is due to the age and size of the leaf, because rodlet lengths on the adaxial surface of young portions of leaves were smaller than lengths on mature leaves. The wax mesh on senescing leaves fused into sections resulting in a different mesh. Variety or leaf number variability were no greater than that within each sample. An attempt to differentiate the lengths of the wax forms according to environmental condition revealed no statistical significance. The wax mesh is similar on the adaxial surface of both floating and aerial leaf surfaces. On the abaxial surface of aerial leaves the wax rodlets dominate numerically, the platelets are fewer and smaller, and they are less scalloped. Similar wax forms are found on the adaxial surface wheat, reed grass, and love grass leaves.

The scalloped appearance of the platelet wax form could be related to rates of wax extrusion and reflect a layering phenomena. Because continuous light did not alter the scalloped edge of the platelets, it is possible that an endogenous rhythm may control the shape of the wax forms. Various rhythms are proposed relating transpiration rates, cuticle elasticity and metabolic production of the epicuticular wax to platelet morphology. Both this study and the chemical removal of epicuticular wax show that no pores are present in the cuticle but the mode of wax transport remains unresolved.

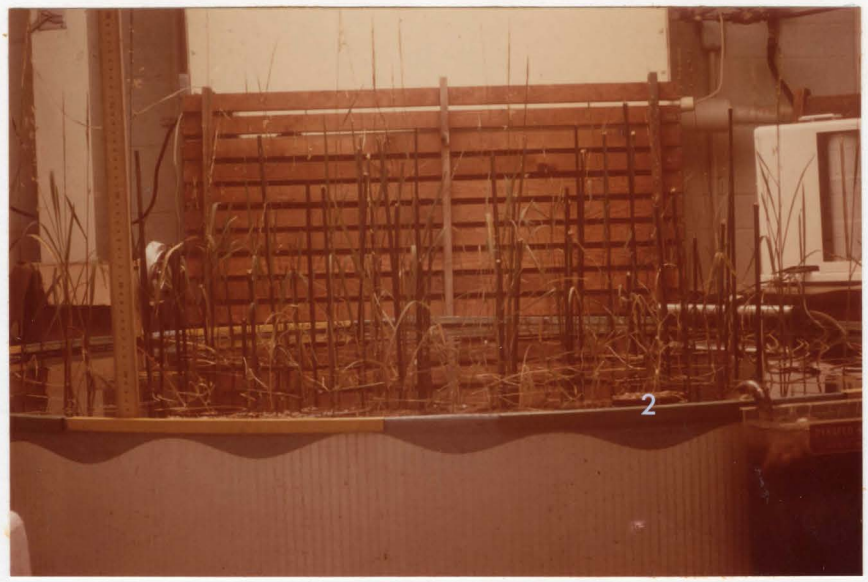
The outer surface of the cuticle is affected by temperature, with higher temperatures producing smoother surfaces. At higher temperatures rodlets are curved and thin, while at lower temperatures the rodlets are straight and wider. The thin, curved rodlets increase the exposure of the outer cuticular surface. As a result, the surfaces of leaves seem to have different heat trapping efficiencies according to the prevailing temperature.

FIGURES 1 - 6

Figure 1. The flowering aerial stage of plant growth in Experiment 1 is depicted. The plants are partially supported by wooden sticks. Illustrated on the right are the Dynaflo Motor Filter in the foreground and the mercury water temperature recorder in the background. In the foreground is a representative surface grown plant (2) of Experiment 2 in a small clay pot.

Figure 2. The flowering aerial stage plants in Experiment 3 are partially supported by wooden sticks in Tank 1 and Tank 2 (left and right respectively) within a Biotronette environmental chamber. On the left is the air temperature Tempscribe recorder.

Figure 3. The Tamson Water Bath and Circulator on the right maintained the 27°C temperature in the Group 1 plants in the tank of Experiment 4. The hose connected the Water Bath with the water level regulator situated on the right side of the tank.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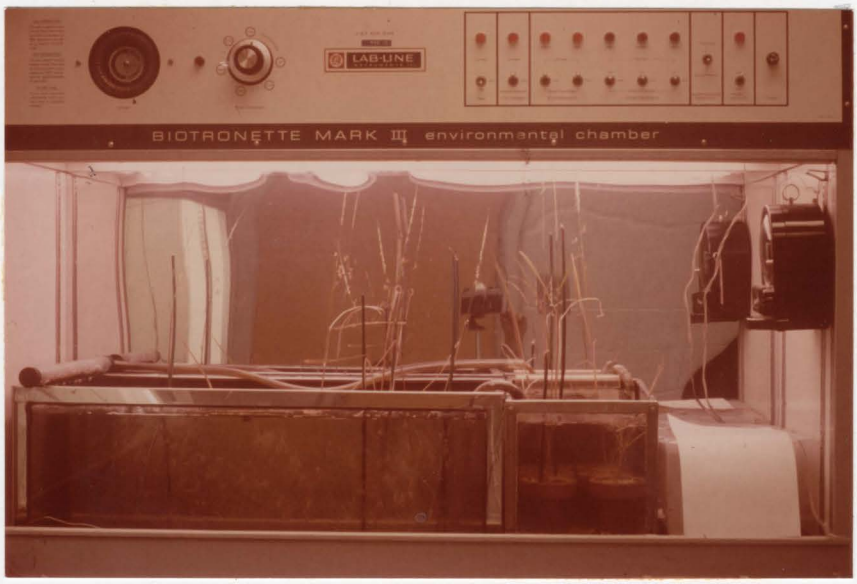

LAB-LINE
BIOTRONETTE MARK III environmental chamber

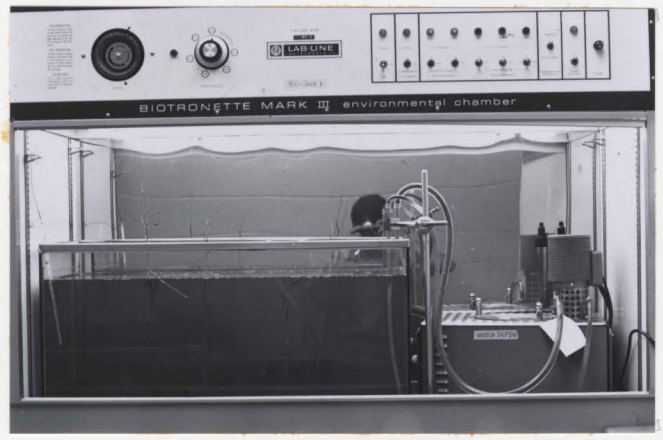

LAB-LINE
BIOTRONETTE MARK III environmental chamber

Figure 4. The weekly change in mean daily air temperatures for Experiments 1 to 3. Also included are the mean daily water temperatures for Experiments 1 and 4.

MEAN AIR TEMPERATURE
MEAN WATER TEMPERATURE

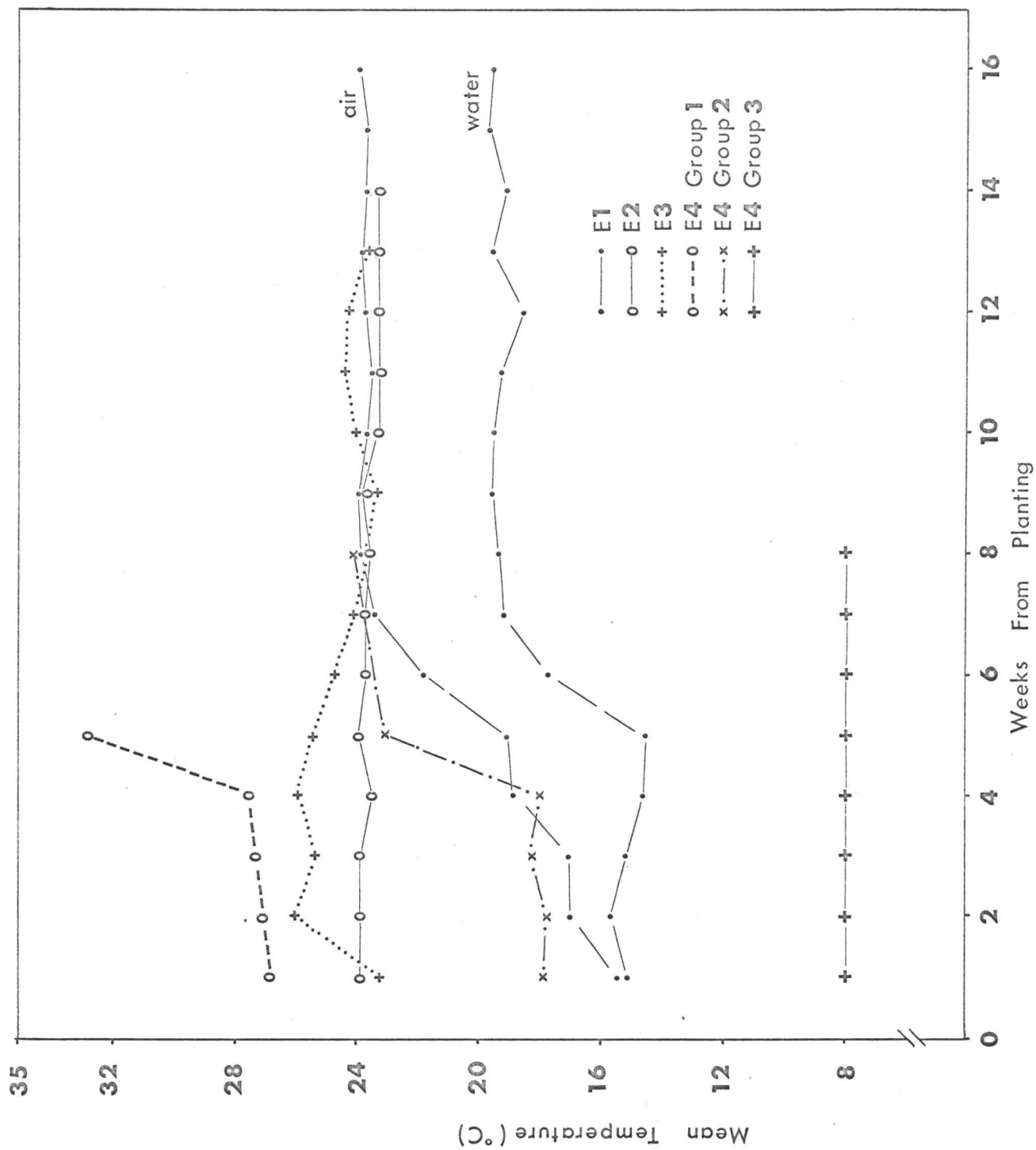
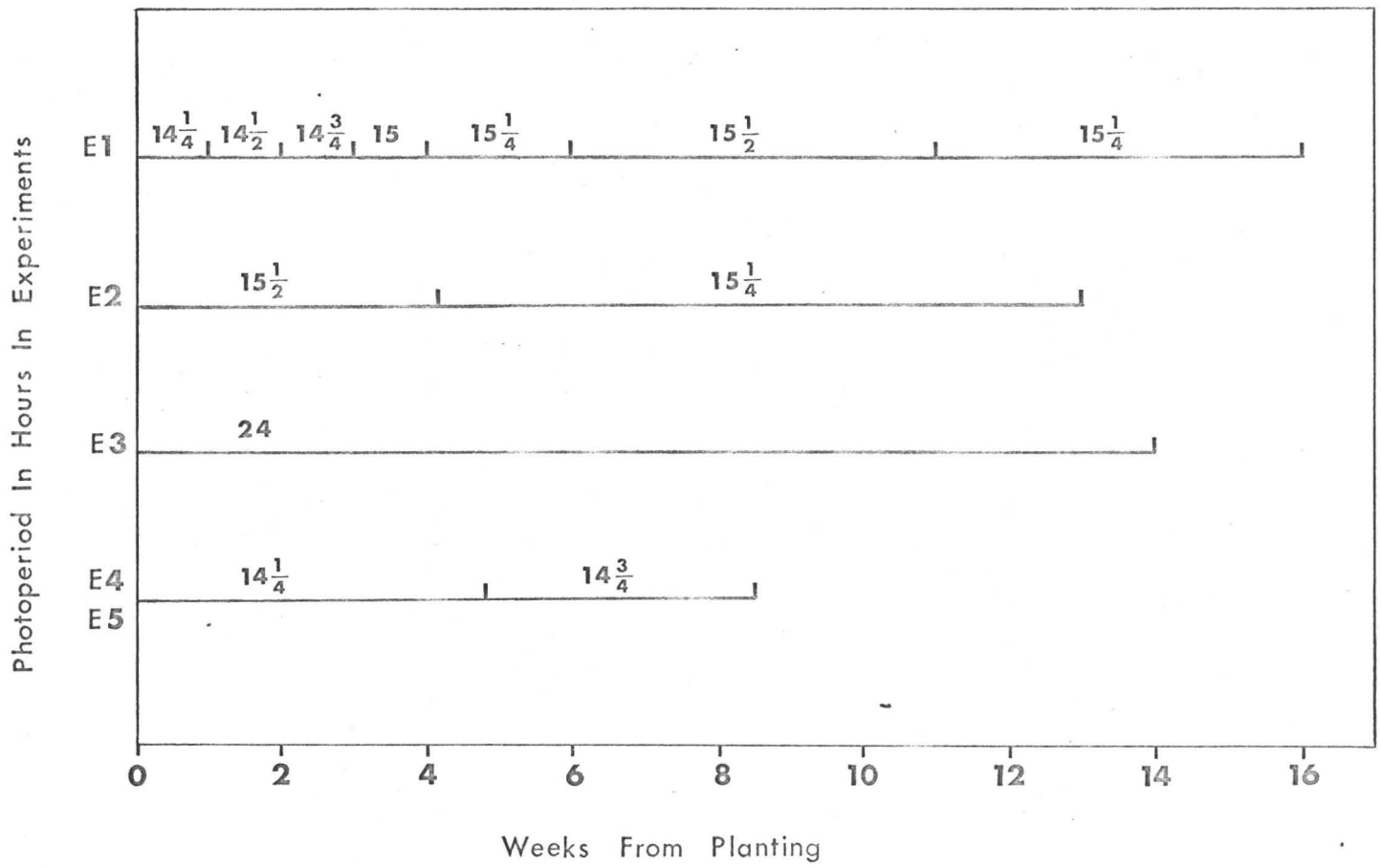
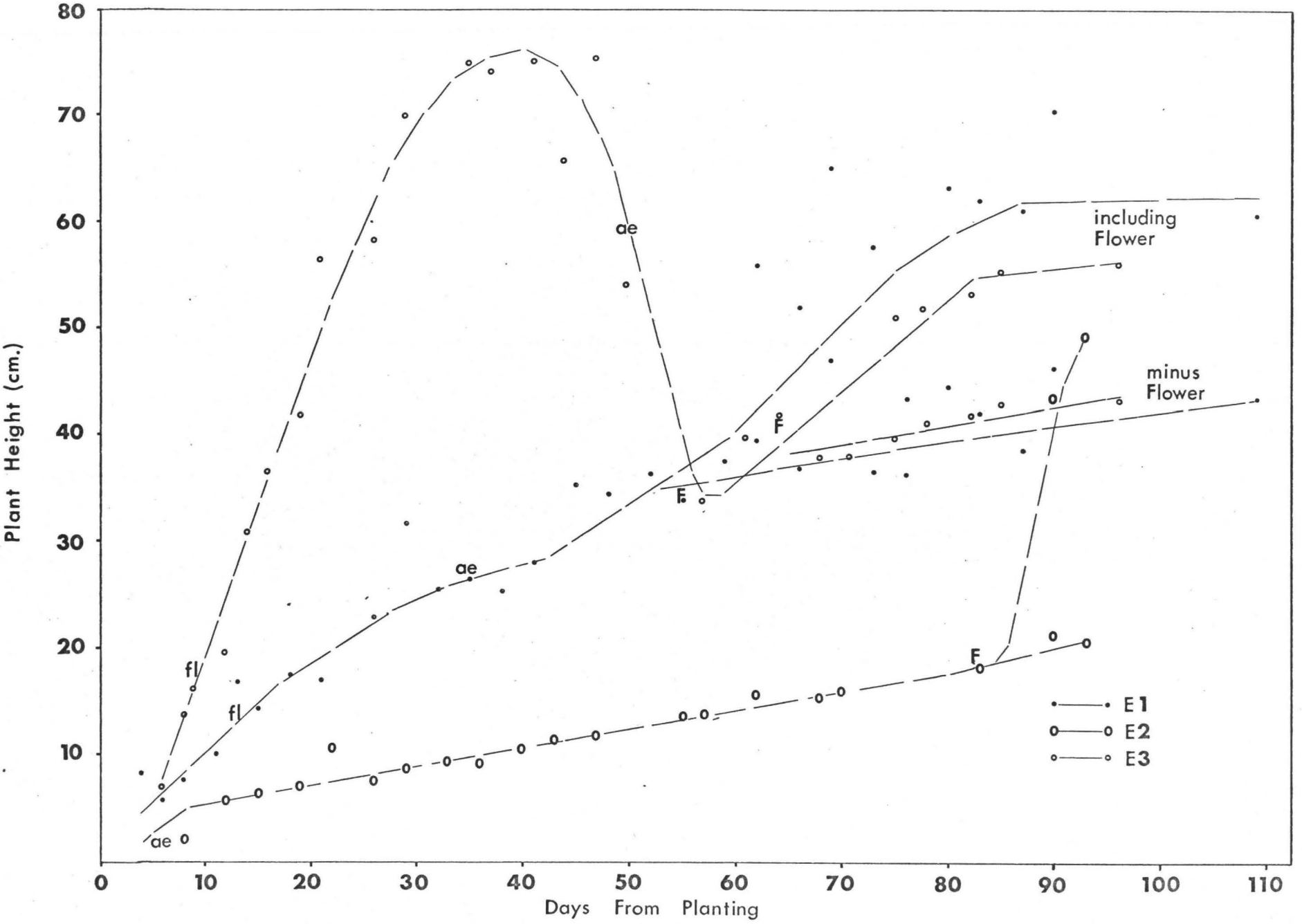


Figure 5. The change in photoperiod throughout Experiments 1 to 5.



PHOTOPERIOD

Figure 6. The change in plant height with time for Experiments 1 to 3. The values are means of at least four plants. The lines drawn are estimates only. The occurrences of floating (fl), aerial (ae), and flowering (F) stages are depicted for each Experiment. The plant height was recorded to also include the flower height.



PLANT HEIGHT

PLATES 1 - 8

LEGEND

A	Abraded portion of the outer surface of the cuticle (c)
B	Bacteria - usually rod form
b	Bump or protrusion from the cuticle (c)
c	Outer layer of epidermis called the cuticle
Cr	Cracks present on the outer surface of the cuticle (c)
CW	Cross Wall connecting two adjacent Ribs (R)
cw	Epidermal cell wall outline
d	Dirt particle adhering to the leaf surface, i.e. debris
H	Hypha of fungus growing on the leaf surface
L	Lip of the guard cell
p	Platelet epicuticular wax form
Pa	Large papilla or projection from leaf surface
pa	Small papilla
R	Rib or vein of leaf
r	Rodlet epicuticular wax form
S	Stomatal pore
sc	Scalloped edge of the platelet wax form (p)
T	Trichome
w	Epicuticular wax mesh

PLATE 1

Fig. 1 A Zizania aquatica L. var. angustifolia Hitchc. (wild rice) plant with the three types of leaves - submerged, floating, and aerial. They are all thin, ribbon-like, elongate. The roots, stem and flower are all present. Water depth was 125 cm at Long Point, Ontario on August 22, 1968.

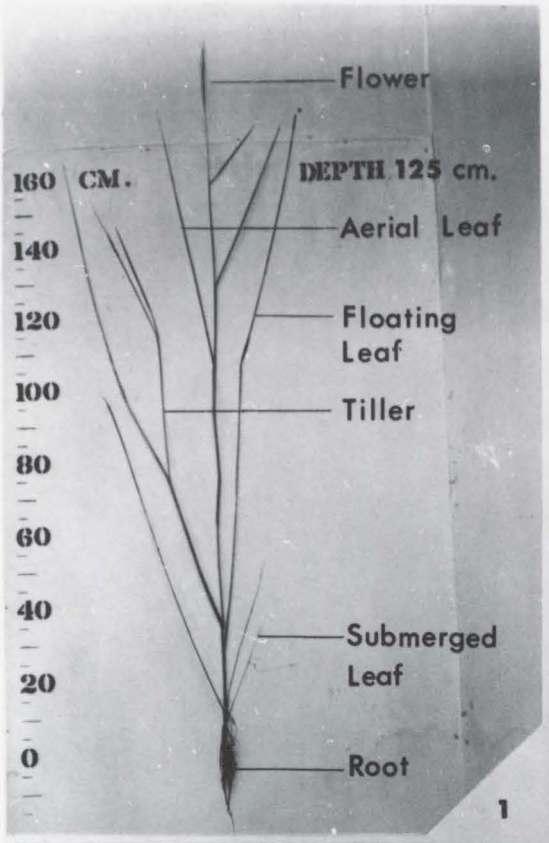
Fig. 2 80x. Adaxial surface of submerged leaf 2. Experiment 1. Cross walls (CW) connect adjacent, parallel ribs (R) and block of large numbers of epidermal cells. The epidermal cell wall (cw) outlines are visible. Occasional papillae (pa) are seen as white dots. The black areas are pits in the adhesive below the leaf and should be ignored.

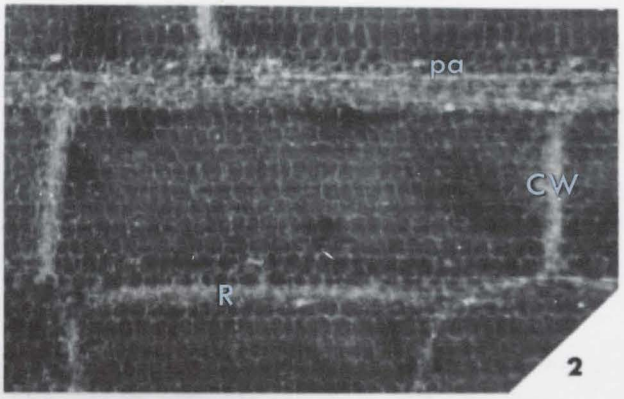
Fig. 3 80x. Abaxial surface of submerged leaf 3. Experiment 1. Ribs, cross walls, and epidermal cell wall outlines are visible. No papillae are present in this sample.

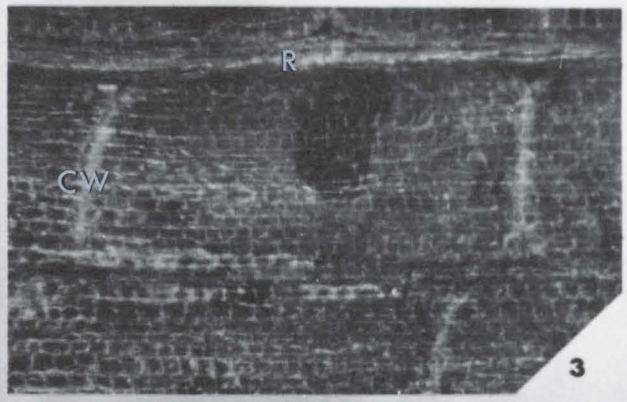
Fig. 4 80x. Abaxial surface of the floating portion of floating leaf 4. Experiment 4, Group 1 (27°C). The main rib is in the centre. The epidermal cell wall outline indicates the cells are smaller. There are neither cross walls, papillae, stomata, nor trichomes present.

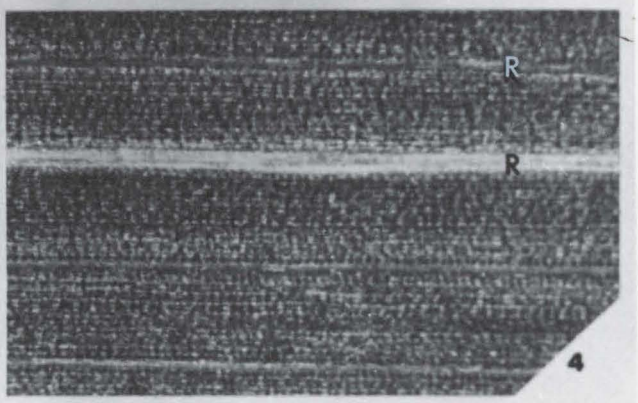
Fig. 5 290x. Adaxial surface of the transition zone of leaf 4. Experiment 1. There is a sudden appearance of smaller papillae (pa). The epidermal cell wall outline indicates that they are more elongate along the leaf length and that there are 4-6 papillae per cell. Stomata are present and occupy the tops of the ridges. However, ridging is not very prominent.

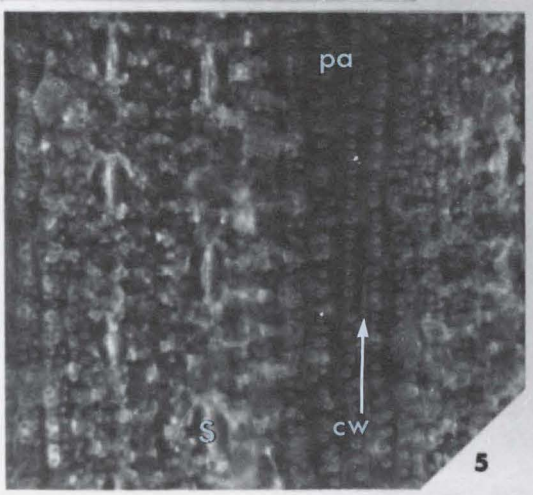
Fig. 6 left 80x, right 290x. Adaxial surface of floating portion of floating leaf 4 from Lakehead transfer. The corrugated surface with the raised ribs (R) and large papillae (Pa) are readily seen at the lower magnification. Debris particles (d) are present adhering to the leaf surface. At the higher magnification the apex of the ridge (R) is in focus with the large papillae out of focus. Small papillae (pa) can be seen dotting the remaining area. Stomata (S) are present on both sides of the rib.


Flower
160 CM.
DEPTH 125 cm.
Aerial Leaf
140
120
Floating Leaf
100
Tiller
80
60
40
Submerged Leaf
20
0
Root
1


pa
CW
R
2


R
CW
3


R
R
4


pa
S
cw
5

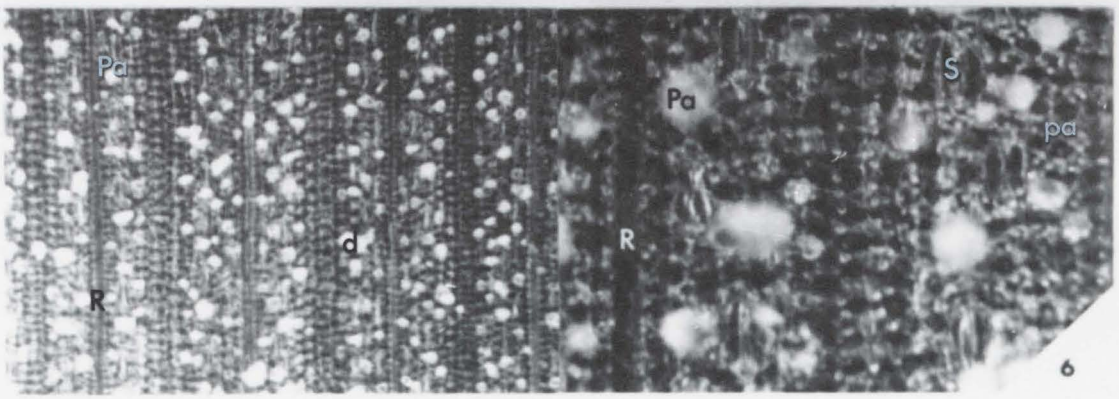

Pa
R
d
Pa
R
S
pa
6

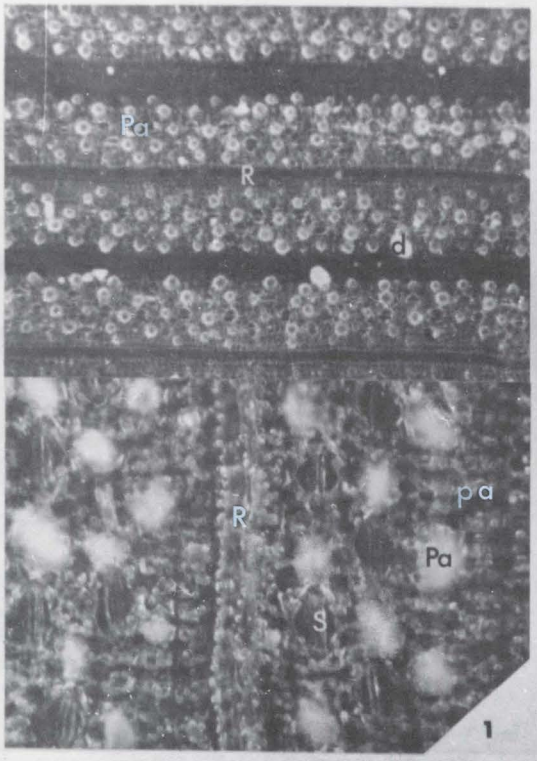
PLATE 2

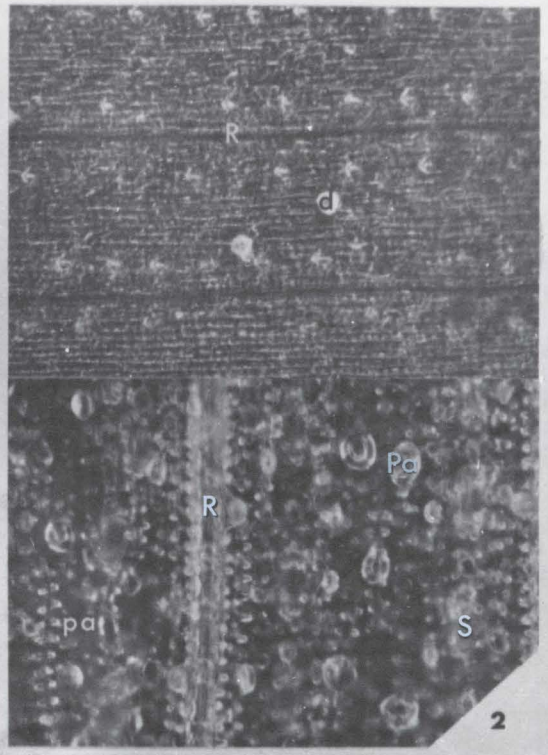
Fig. 1 top 80x, bottom 290x. Adaxial surface, of an aerial leaf from Lakehead transfers. This view clearly shows the ridged surface (R) with the large papillae (Pa) occupying the slope of the ridges. The small papilla (pa) clearly cover the surface and the large papillae. The stomata(s) are arranged close together and on one side of the rib display the alternating position. Debris particles(d) also adhere to the surface.

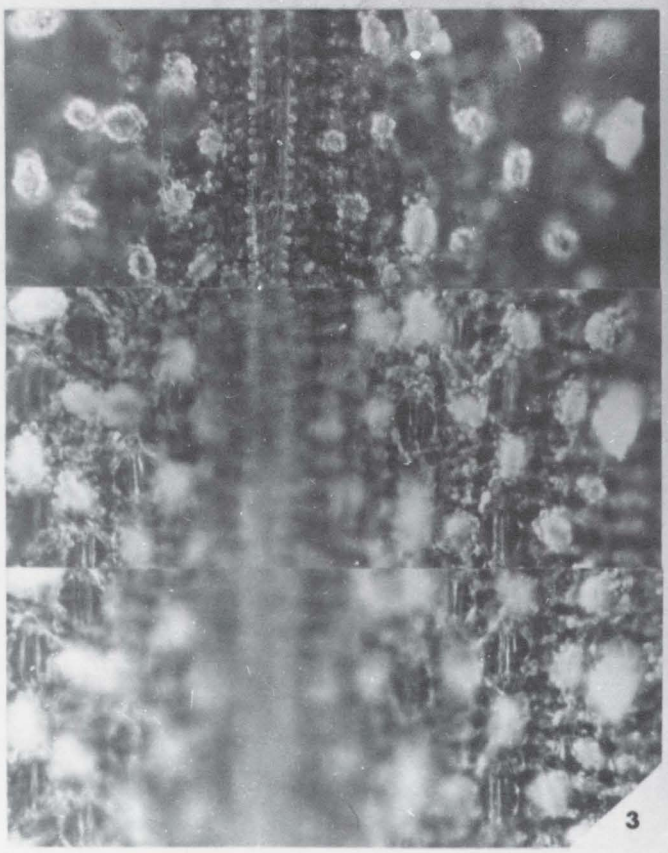
Fig. 2 top 80x, bottom 290x. Abaxial surface of an aerial leaf from Lakehead transfers. The abaxial surface is not as ridged as the adaxial surface but the elongated epidermal cells and stomata are discernable at the lower magnification. The few objects are foreign material (d). At the higher magnification the two sizes of papillae are visible. The larger papillae (Pa) are more translucent than those of the adaxial surface and about one-half the size. The smaller papillae (pa) are just as dense as those observed on the adaxial surface and are of comparable size.

Fig. 3 290x. Adaxial surface, aerial leaf from Lakehead transfers. This composite picture is a view of the same area with 3 different levels in focus. In the top picture the rib is in focus and small papillae are seen covering the surface plus the large papillae. Notice that no stomata are in focus. The second level in focus shows stomata surrounded by the two sizes of papillae. The large papillae surround a stoma forming a diamond shape. At the third level more stomata are in focus and this is considered to be the base of the ridge.

Fig. 4 Fig. 4 (80x) and Fig. 5 (290x). Adaxial surface of a flag leaf.
Fig. 5 Experiment 4, Group 1 (27°C). Debris particles, papillae (Pa and pa), stomata, and small trichomes (T) are present on this surface. The large papillae measure 20μ in diameter and the smaller papillae 3μ. Stomatal length is 31μ. The trichomes are 34-37μ long.


Pa
R
d
R
pa
Pa
S
1


R
d
Pa
R
pa
S
2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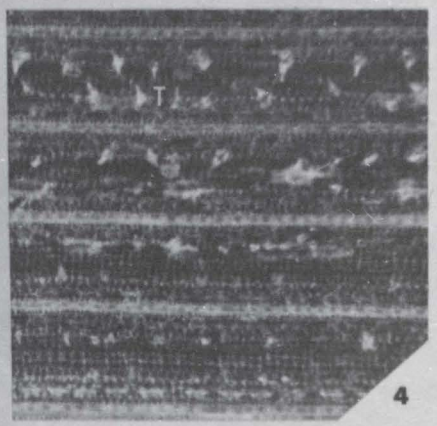

T
4

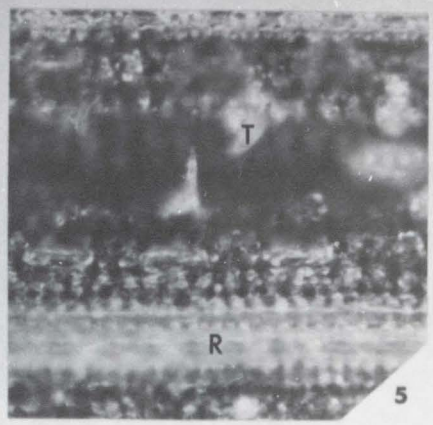

T
R
5

PLATE 3

Fig. 1 (1972). 8000x. Outer surface of the coleoptile. Grown in the dark for 12 days at 16°C. A wrinkled contour with a fairly smooth surface is present. A possible hyphal-like tube (H) may be present accounting for the long tube. The diameter of this tube is 0.7u.

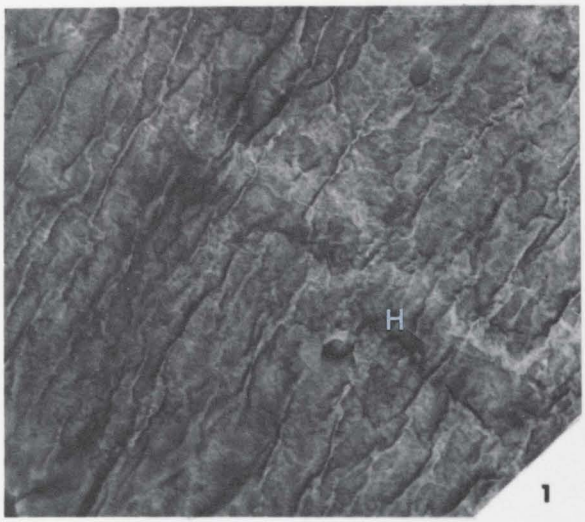
Fig. 2 (2059). 11000x. Adaxial surface of leaf 2. Experiment 3. A rod bacterium has adhered to the outer surface of the cuticle beside the collapsed papilla. Long strands mesh over the surface and small pitted areas dot the surface.

Fig. 3 (2141). 11000x. Abaxial surface of leaf 2. Experiment 1. The overall leaf area was 1.1 cm^2 and this small view indicates that the surface is smooth with a slight rippling effect. There is a fair degree of cracking but it is difficult to ascertain whether the original cuticle was cracked or whether the metal shadow could cause this cracking effect.

Fig. 4 (3155). 7500x. Adaxial surface of the transition zone of floating leaf 4. Experiment 4, Group 1 (27°C) on Day 12. This composite picture illustrates in the centre the long, thin rodlet (r) and the scalloped, irregularly sized platelet (p) wax forms which overlap to form an extensive mesh. This mesh covers the cuticle (c) and the smaller papillae (pa).

Fig. 5 (2229). 11000x. Adaxial surface of the submerged portion of the mature floating leaf 6 below the transition zone. Experiment 1. This sample was taken from a mature leaf with a total area of 17.5 cm^2 of which 10.0 cm^2 was floating. The surface is smooth with a slight ripple. Many smalldebrisparticles adhering to the surface have been replicated. In the centre is a collapsed bacterium-like object.

Fig. 6 (3247). 21000x. Adaxial surface of aerial leaf 6. Experiment 4, Group 1 (27°C) on Day 24. This close view indicates a thick mesh of fairly thick rodlets (r) and scalloped platelets (p) arising from a granular base cuticle (c). There is good contrast as a result of the metal shadow. No papillae are present.



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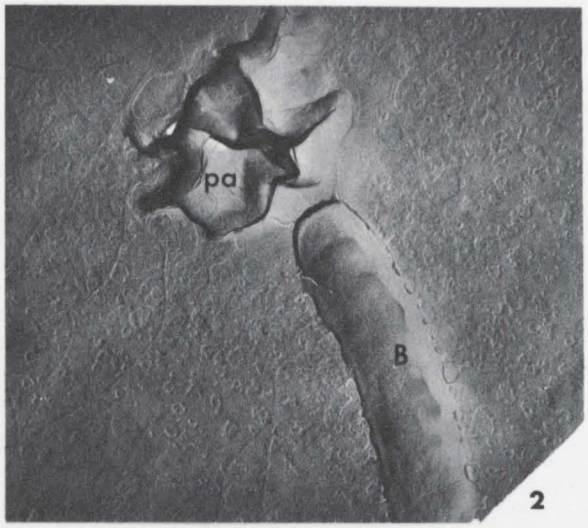

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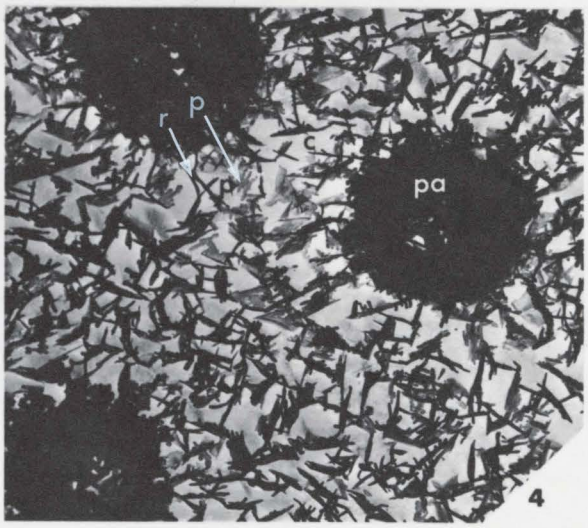
3



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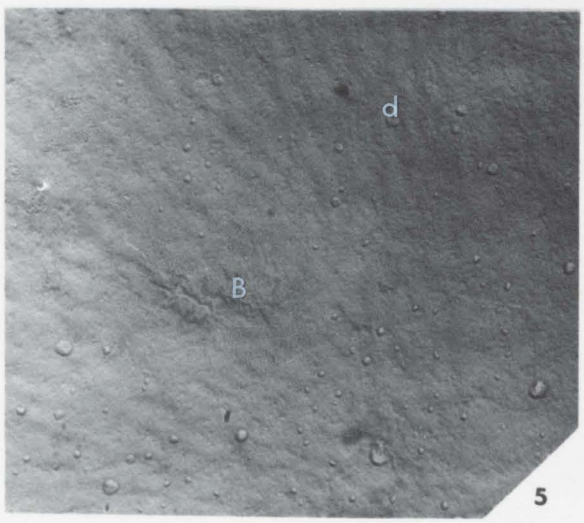


5

6

PLATE 4

Fig. 1 (3009). 21000x. Abaxial surface of the flag leaf. Experiment 2. The full mesh of rodlets and platelets with a few open areas show that the abaxial surface is covered by a similar array of wax as is found on the adaxial surfaces. Platelet size is less and the rodlet forms tend to predominate.

Fig. 2 (1679). 2600x. Adaxial surface of the floating portion of the floating leaf. Preliminary Experiment in an enclosed container to produce a high humidity. A stomatal pore (S) is readily visible. The guard cells are elevated above the surrounding area which is covered by numerous small (1.5μ diameter) papillae (pa). A continuous wax rodlet mesh covers the entire area except the lip (L) of the guard cells. The cell wall (cw) between the guard cells and the surrounding epidermal cells is characterized in the wax mesh by an aligned row of rodlets.

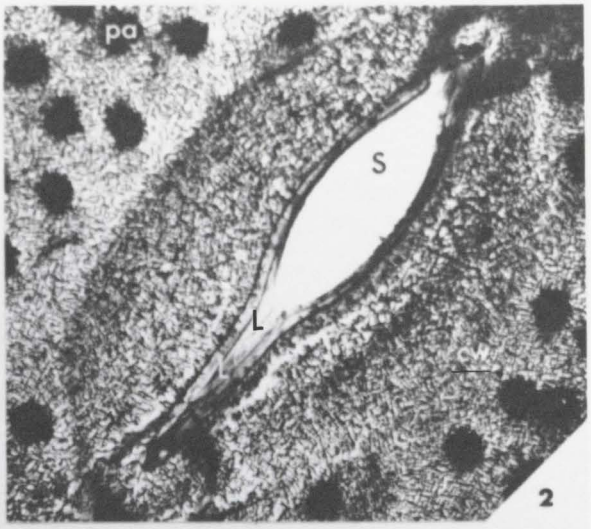
Fig. 3 (2090). 11000x. Adaxial surface of submerged leaf 2. Experiment 1, Day 4. This replica is quite smooth with numerous pits creating a slight pebbly effect. There are a few debris spots (d) on the replica which are not part of the surface viewed.

Fig. 4 (2228). 6000x. Adaxial surface just above the transition zone on the floating portion of floating leaf 5. Experiment 1, Day 29. Rodlets and platelets form a loose mesh. The small papillae are not completely covered by the wax mesh. There is a poor shadow present. This sample was taken from a leaf with a total area of 10.5 cm^2, 6.0 cm^2 above the water surface.

Fig. 5 (2231). 11000x. Abaxial surface of the floating portion of floating leaf 5. Experiment 1, Day 29. Various debris particles (d) and bacteria (B) adhere to the smooth cuticular surface of the abaxial side of a leaf with total area of 16.0 cm^2 and 12.0 cm^2 floating.

Fig. 6 (2419). 7500x. Adaxial surface of aerial leaf 8. Experiment 1, Day 59. From an aerial leaf with an area of 10.0 cm^2 this sample shows large-sized scalloped platelets meshing with rodlets. A papilla (pa) is covered with a full layer of wax.

1



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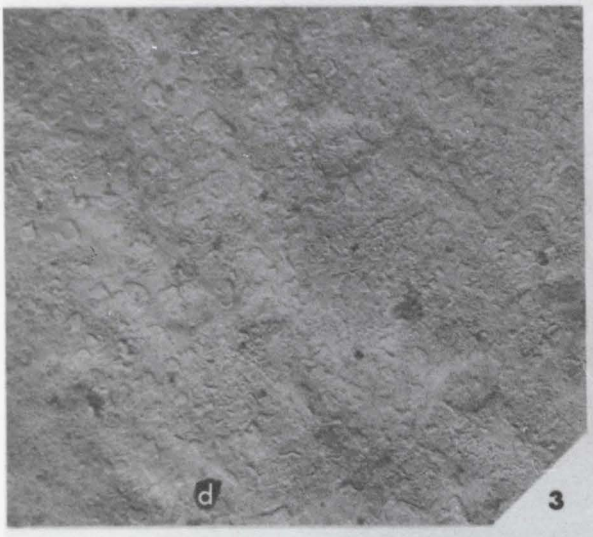

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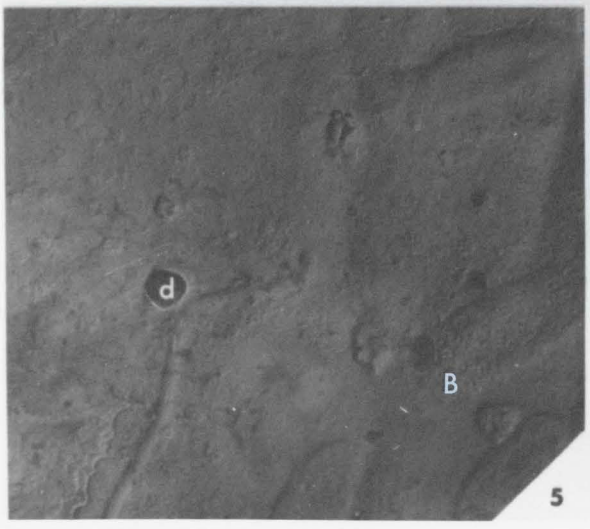

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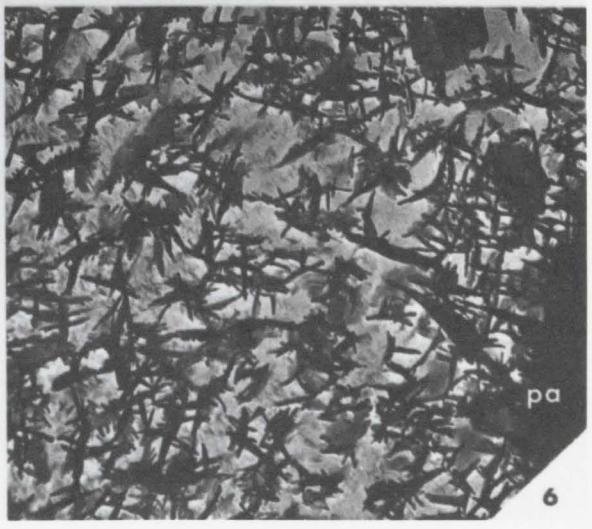

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PLATE 5

Fig. 1 (2416). 7500x. Abaxial surface of aerial leaf 8. Experiment 1, Day 59. The leaf area was 18.9 cm^2 and the platelets are small. The rodlets (r) although smaller, dominate the wax mesh. Papillae (pa) are present and are covered by a wax coat. The wax layer is not as dense as that found on the adaxial surface.

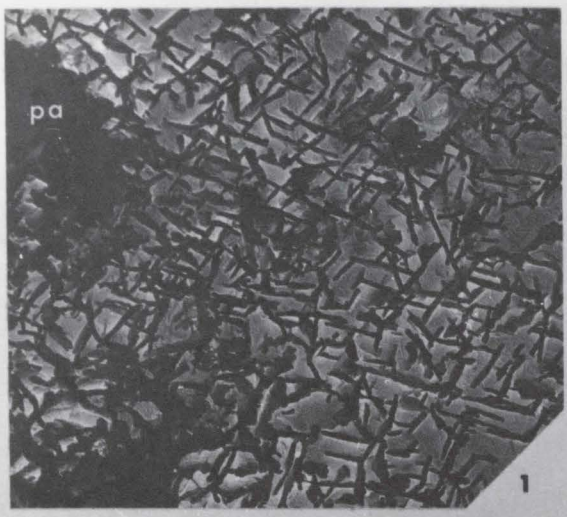
Fig. 2 (3537). 21000x. Adaxial surface of aerial leaf 1. Experiment 2, Day 5. The outer cuticular surface is basically smooth with a wrinkled, cracked (Cr) structure. There is no wax layer present.

Fig. 3 top 80x, bottom 290x. Abaxial surface of aerial leaf 3. Experiment 2. Ribs, stomata, small papillae are readily visible. At the higher magnification trichomes are just out of focus beside the stomata. There is a noticeable lack of larger papillae and ridging is not strong.

Fig. 4 (2428). 7500x. Adaxial surface of aerial leaf 3. Experiment 2, Day 15. There are many large scalloped platelets covering the surface and increasing the total area covered. A single papilla is present and the rodlet wax forms are clearly visible. The leaf area has increased to 3.5 cm^2.

Fig. 5 (2395). 21000x. Adaxial surface of aerial leaf 3. Experiment 2, Day 8. Large, stout rodlets (r) and variously sized scalloped platelets (p) form a mesh on a leaf with total area of 2.1 cm^2. There are some open areas on the cuticle which has a granular base.

Fig. 6 (2429). 21000x. Abaxial surface of aerial leaf 3. Experiment 2, Day 15. Large scalloped platelets (p) and thick rodlets are seen projecting from a granular cuticular structure (c). There is considerable overlap resulting in unprotected areas. The uneven wax layer is different from that seen in truly aerial leaves. The leaf area is the same as Plate 5, Fig. 4, 3.5 cm^2.


pa
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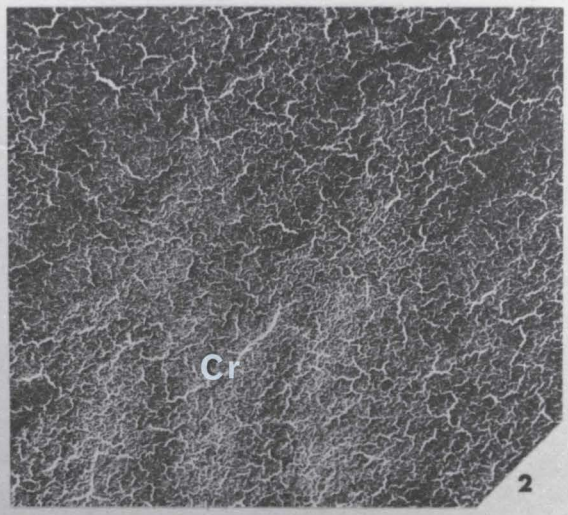
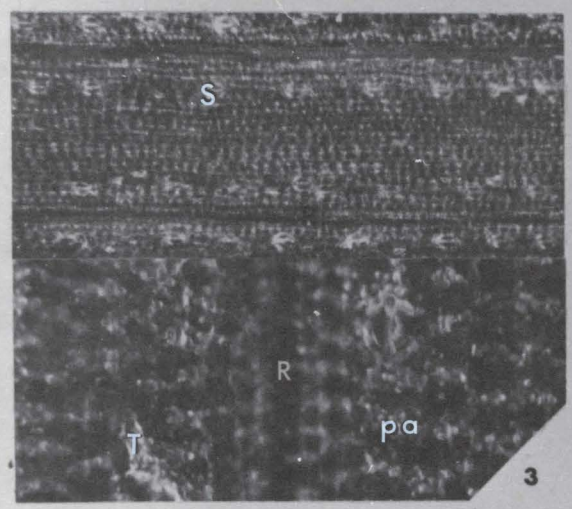

Cr
2


S
R
T
pa
3


4


p
r
5


c
p
6

PLATE 6

Fig. 1 (2336). 13500x. Adaxial surface of the floating portion of floating leaf 5. Experiment 3 on Day 19. The mesh of rodlets and platelets is full with scalloped (sc) platelets noticeable. It is difficult to pick out an individual wax form. The total area is 11.2 cm^2, with 5.4 cm^2 floating.

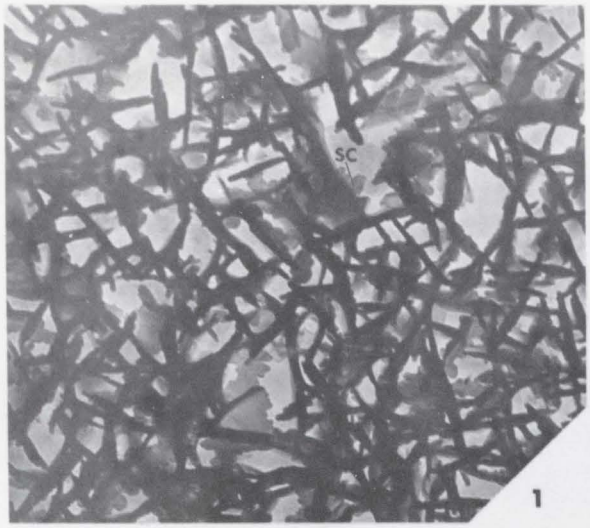
Fig. 2 (2249). 13500x. Adaxial surface of the floating portion of floating leaf 6. Experiment 3 on Day 44. Round, scalloped platelets are quite noticeable objects projecting from the smooth base structure. The granular character of the platelets and cuticular surface is indicated by the internal replica structure. Straight rodlets have meshed with the platelets to form a full wax layer. The total leaf area is 30.8 cm^2 with 14.0 cm^2 floating.

Fig. 3 (3880). 21000x. Adaxial surface of submerged leaf 2. Experiment 3, Group 3 (8°C). The surface is very uneven with many small bumps. The cuticle has become slightly cracked as evidenced by the white, irregular lines.

Fig. 4 (3084). 31000x. Adaxial surface of submerged leaf 2. Experiment 4, Group 2 (18°C) on Day 7. The surface is generally smooth with widely pitted areas enhanced by a light-lined shadow. A debris particle (d) adheres to the surface.

Fig. 5 (3070). 21000x. Adaxial surface of submerged leaf 1. No Group. (23°C) on Day 0. A bacterium is adhering to a semi-smooth cuticle. The pitted areas dot the surface but no pores are visible. The shadow is not distinct. There is extensive fine cracking of the outer cuticle.

Fig. 6 (3124). 21000x. Adaxial surface of the submerged tip of leaf 4, Experiment 4, Group 1 (27°C) on Day 9. Well developed wax rodlets and platelets project from a granular basal cuticle which has cracked slightly under the vacuum. Good contrast is provided by the metal shadowing. There appears to be a slight double image around the wax forms which is caused by movement of the replica. In the open, unprotected areas there are no observable pits, pores or possible pathways of wax extrusion.



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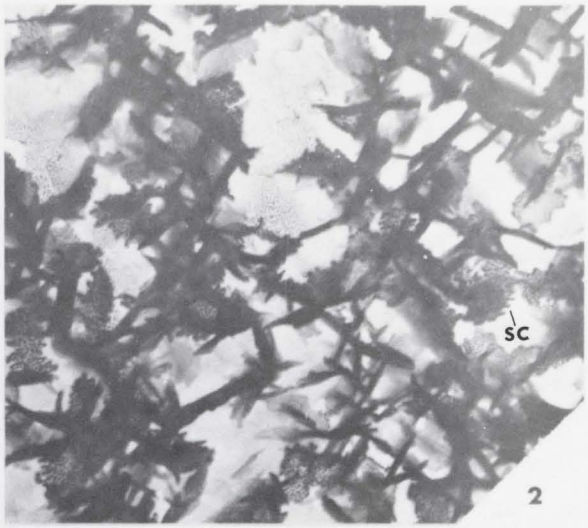

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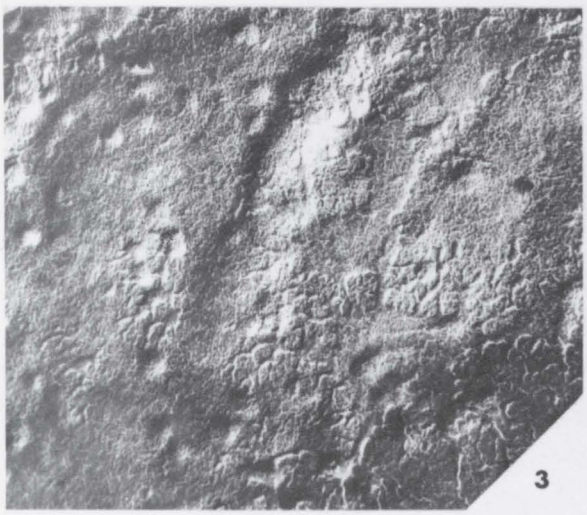

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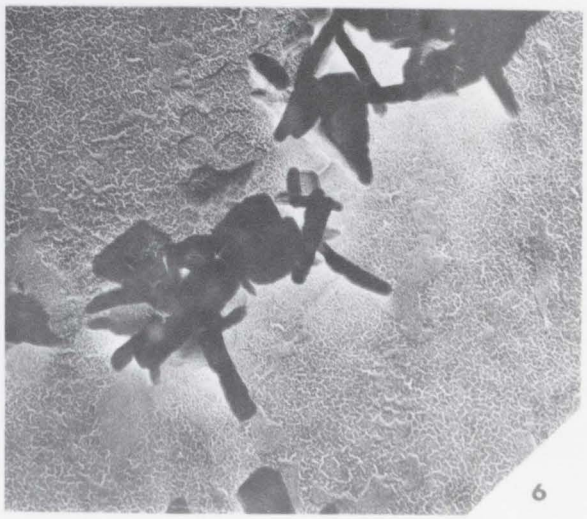

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PLATE 7

Fig. 1 (3151). 21000x. Adaxial surface of submerged leaf 5. Experiment 4, Group 1 (27°C) on Day 14. The surface is rippled with a fairly smooth structure and small "pebbles" (b) dotting the surface. There are small rodlet forms (r) arising from positions near the "pebbly" dots.

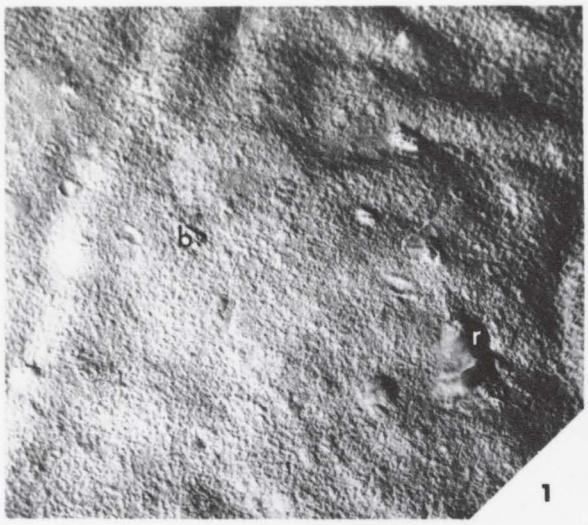
Fig. 2 (3148). 21000x. Adaxial surface of submerged leaf 4. Group 2 (18°C) on Day 14. This is a rather coarse surface with many small rodlets (r) just appearing on the surface. This picture represents a more advanced state of epicuticular wax development over that shown in Plate 7, Fig. 1. This leaf is slightly more coarse than surfaces of plants grown at higher temperatures.

Fig. 3 (3155). 7500x. Adaxial surface of the transition zone of floating leaf 4. Experiment 4, Group 1 (27°C) on Day 12. This composite picture illustrates the long, thin rodlet (r) and the scalloped, irregularly sized platelet (p) wax forms overlapping to form an extensive mesh. This mesh covers the cuticle and the smaller papillae (pa). This picture is the same as Plate 3, Fig. 4.

Fig. 4 (3129). 21000x. Adaxial surface of the base of curled-up leaf 5. Millgrove field sample collected on May 15. The short, thick, dark rodlets (r) project from the cuticle (c). There are no noticeable platelet wax forms. The wax mesh is present on the young leaf portions within the sheath.

Fig. 5 (3825). 21000x. Adaxial surface of an aerial leaf. Millgrove field sample collected on July 25th 1968. The rodlet forms tend to dominate but there are occasional platelet forms interspersed among the rodlets. The granular basal cuticle shows some tendency to crack upon drying.

Fig. 6 (3846). 7500x. Abaxial surface of an aerial leaf inside the sheath. Millgrove field sample collected on July 25th, 1968. This general view shows a papilla in one corner and numerous rodlets and platelets forming a mesh.



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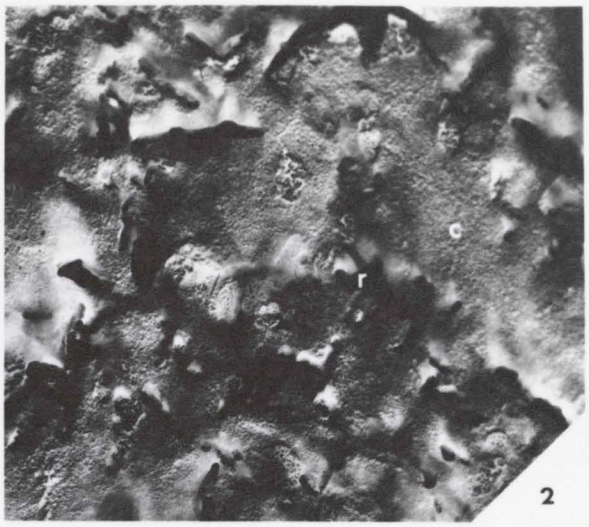

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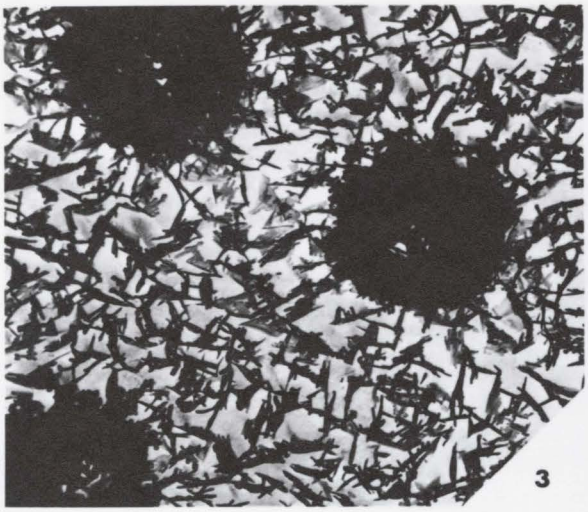

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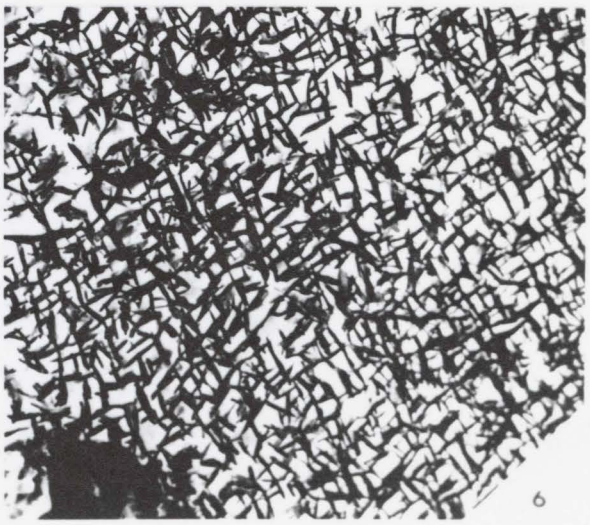

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PLATE 8

Fig. 1 (3877). 11500x. Adaxial surface of an aerial leaf. Millgrove field sample collected on July 25th 1968. The straight rodlets and scalloped platelets form a medium density mesh over the cuticle and papilla.

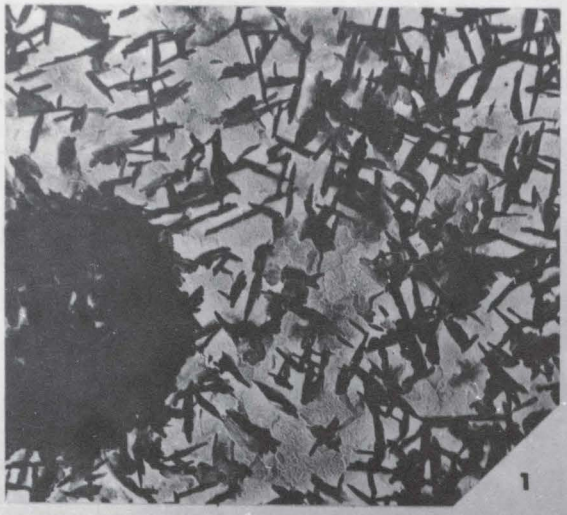
Fig. 2 (1600). 25000x. Adaxial surface of floating portion of floating leaf. Experiment 7, mechanical abrasion. The abrasion has not removed all the wax but roughed the surface (A). No platelet and rodlet forms are noticeable.

Fig. 3 (2556). 21000x. Abaxial surface of an aerial leaf. Experiment 7, water treatment. This surface has been exposed to water for 48 hours with no obvious alteration of the wax mesh.

Fig. 4 (3747). 7500x. Adaxial surface of an aerial leaf. Experiment 7, chloroform treatment for 1 minute. The rodlet wax forms have been destroyed so that there is only a scattered mesh of wax (w) overlaying the cuticle (C). Notice the epidermal cell wall (c.w.) and the papilla.

Fig. 5 (3911). 13500x. Abaxial surface of an aerial leaf. Experiment 7, acetone treatment for 1 minute. The rodlet form (r) has been partially altered but they still show a good shadow and contrast against the cuticle (c). The open areas are not the result of the removal of the wax mesh but due to a low density mesh.

Fig. 6 (1632). 11000x. Adaxial surface of an old, yellowing aerial leaf. The rodlets and platelets on the senescing leaf have fused and meshed together forming long bands of wax (w) and leaving open cuticular surfaces (c). The shadow indicates that the wax mesh projects from the surface.



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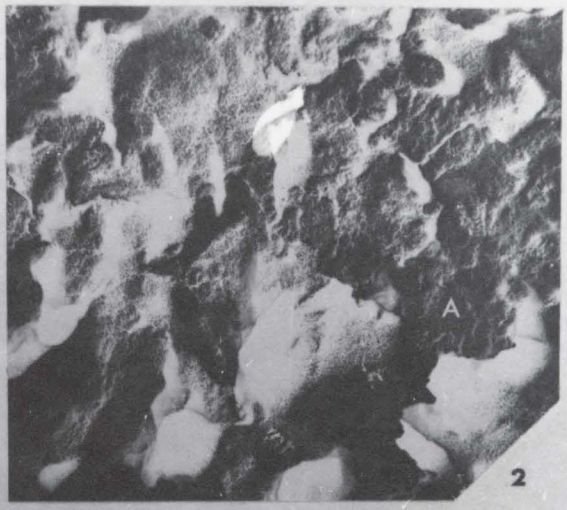

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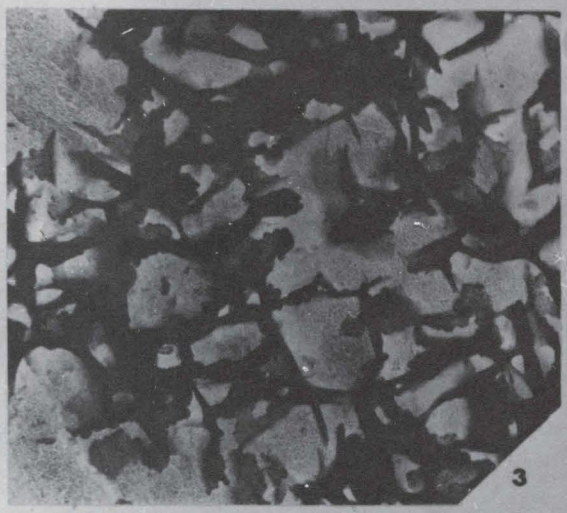

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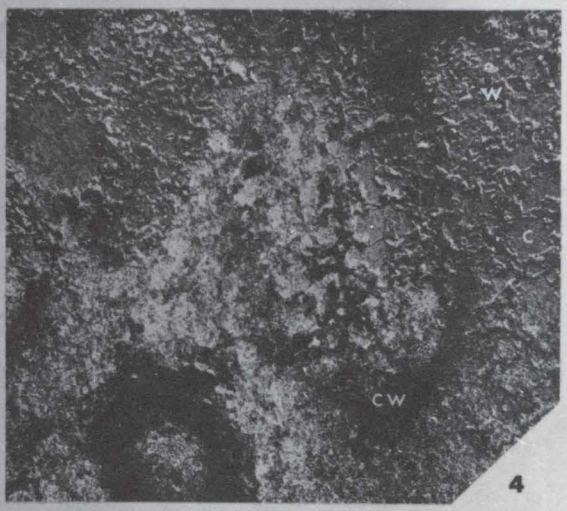

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APPENDIX A-E

APPENDIX A

Analysis of variance of rodlet lengths on the adaxial surface of leaves from E-1, E-2, E-3, and E-4, Groups 1 and 2.

	EXPERIMENT NUMBER					
	E-1	E-2	E-3	E-4 Group 1	E-4 Group 2	TOTAL
Σx	6.6	4.6	7.0	2.8	2.4	23.4
$\bar{x}$	0.7	0.8	0.6	0.6	0.5	
Σx^2	5.12	3.63	5.59	1.63	1.25	17.12
$\frac{(\Sigma x)^2}{n}$	4.81	3.56	4.40	1.63	1.19	15.24 $\frac{\Sigma(\Sigma x)^2}{n}$
$\Sigma x^2 - \frac{(\Sigma x)^2}{n}$	0.31	0.07	1.09	--	0.06	1.88

Source of Variance	S.S.	D.F.	M.S.
Within sample	1.53	31	0.049
Between samples	0.35	4	0.087
Total	1.88	35	

$$F = \frac{0.087}{0.049} = 1.8$$

Table F value is 2.69 at 5% level of significance

Calculated F $<$ Table F

Therefore there is no statistical difference in rodlet length on the adaxial surface of leaves from different Experiments.

APPENDIX B

Comparison of means tests (t-test) for rodlet lengths of the adaxial surface of leaves from population E-1 and E-2, E-1 and E-3, and E-1 and FIELD.

E-1 and E-2

$$\text{Standard Deviation (s)} = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n - 1}}$$

$s_1 = \sqrt{\frac{0.3}{8}}$; $n_1 = 9$

$s_2 = \sqrt{\frac{0.1}{5}}$; $n_2 = 6$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n} \quad \frac{s_2^2}{n}}} = \frac{0.7 - 0.8}{\sqrt{0.007}} = 0.5$$

D.F. = $(n_1 + n_2) - 2 = 13$

Table t = 2.160 at the 5% level of significance.

Calculated t < Table t
The difference between the two rodlet lengths is not significant.

E-1 and E-3

$s_1 = \sqrt{\frac{0.3}{8}}$; $n_1 = 9$

$s_3 = \sqrt{\frac{1.1}{10}}$; $n_3 = 11$

$$t = \frac{0.7 - 0.6}{\sqrt{0.014}} = 0.7$$

D. F. = 19

Table t = 2.101 at the 5% level of significance.
Calculated t < Table t
There is no difference between the rodlet lengths.

APPENDIX B CONTINUED

E-1 and FIELD

$s_1 = \sqrt{\frac{0.3}{8}}$; $n_1 = 9$

$s_f = \sqrt{\frac{1.1}{9}}$; $n_f = 10$

$$t = \frac{0.7 - 0.7}{0.1} = 0$$

D.F. = 17

Table t = 2.110 at the 5% level of significance
There is no difference between the rodlet lengths.

APPENDIX C

Comparison of means test of sample (TABLE 2) and population (E-1) of the rodlet lengths on the adaxial surface of young and mature leaves.

E-1 and TABLE 2

TABLE 2

$\Sigma x = 2.9$; $n = 10$; $\bar{x} = 0.3$

$\Sigma x^2 = 0.93$

$\frac{(\Sigma x)^2}{n} = 0.86$

$\Sigma x^2 - \frac{(\Sigma x)^2}{n} = 0.1$

$s_t = \sqrt{\frac{0.1}{9}}$

E-1

$\bar{x}_1 = 0.7 = \mu$

$$t = \frac{\bar{x} - \mu}{s_t / \sqrt{n}} = \frac{0.4}{0.03} = 13.3$$

D.F. = 9

Table t = 4.781 at 0.1% level of significance
Calculated t >> Table t
The sample mean is very highly significantly different from the population. The newly extruded wax rodlets are significantly shorter than the mature leaf forms.

APPENDIX D

Analysis of variance or rodlet lengths on the abaxial surface of leaves from E-1, E-2, and E-3.

	EXPERIMENT NUMBER			
	E-1	E-2	E-3	TOTAL
Σx	3.2	2.9	4.0	10.1
$\bar{x}$	0.6	0.7	0.7	
Σx^2	2.05	2.16	2.71	6.92
$\frac{(\Sigma x)^2}{n}$	2.04	2.03	2.67	6.72 $\frac{\Sigma(\Sigma x)^2}{n}$
$\Sigma x^2 - \frac{(\Sigma x)^2}{n}$	0.01	0.13	0.04	0.20

Source of Variance	S.S.	D.F.	M.S.
Within sample	0.18	12	0.015
Between samples	0.02	2	0.01
Total	0.20	14	

$$F = \frac{0.015}{0.01} = 1.5$$

Table F value is 3:98 at the 5% level of significance.
Calculated F $<$ Table F
There is no statistical difference in rodlet length on the abaxial surface among Experiments 1, 2, and 3.

APPENDIX E

Analysis of variance of platelet lengths on the adaxial surface of leaves from E-1, E-2, E-3, and E-4, Groups 1 and 2.

	EXPERIMENT NUMBER					
	E-1	E-2	E-3	E-4 Group 1	E-4 Group 2	TOTAL
Σx	3.1	7.2	5.3	2.9	2.0	20.5
$\bar{x}$	0.6	0.8	0.6	0.5	0.7	
Σx^2	2.01	8.82	3.30	1.43	1.42	16.98
$\frac{(\Sigma x)^2}{n}$	1.87	5.76	3.06	1.39	1.34	13.00 $\frac{\Sigma(\Sigma x)^2}{n}$
$\Sigma x^2 - \frac{(\Sigma x)^2}{n}$	0.14	3.06	0.24	0.04	0.08	3.98

Source of Variance	S.S.	D.F.	M.S.
Within sample	3.56	27	0.013
Between samples	0.42	4	0.010
Total	3.98	31	

$$F = \frac{0.013}{0.010} = 1.3$$

Table F value is 2.73 at the 5% level of significance
Calculate F < Table F
There is no statistical difference among the platelet lengths.

APPENDIX E CONTINUED

Comparison of means test for platelet lengths of the adaxial surface of leaves from E-1 and E-2.

E-1 and E-2

$s_1 = \frac{0.14}{4}$; n = 5

$s_2 = \frac{3.06}{8}$; n = 9

$t = \frac{0.6 - 0.8}{\sqrt{0.049}} = \frac{0.2}{0.24} = 0.8$

D. F. = 12

Table t = 2.179 at the 5% level of significance.
Calculated t < Table t
There is no difference between the two platelet lengths.