Fine structure of plant cuticles in relation to water permeability:
The fine structure of the cuticle of Clivia miniata Reg. leaves

T. Mérida, J. Schönherr, and H.W. Schmidt

1 Lehrstuhl für Botanik, Technische Universität München, Arcisstraße 21, and
2 Institut für Zellbiologie, Universität München, Goethestraße 33/II, D-8000 München 2, Federal Republic of Germany

Abstract. The fine structure of the upper cuticular membrane (CM) of Clivia miniata leaves was investigated using electron microscopy. The CM is made up of a thin (130 nm) lamellated cuticle proper (CP) and a thick (up to 7 µm over periclinal walls) cuticular layer (CL) of marbled appearance. Evidence is presented to show that the electron lucent lamellae of the CP do not simply represent layers of soluble cuticular lipids (SCL). Instead, the lamellation is probably due to layers of cutin differing in polarity. It is argued that the SCL in the CP are the main barrier to water. Thickening of the CM during leaf development takes place by interposition of cutin between the CM and the cell wall. The cutin of young, expanding leaves has a high affinity for KMnO₄ and is therefore relatively polar. As leaves mature, the external CL underneath the CP becomes non-polar, as only little contrast can be obtained with permanganate as the post fixative.

Key words: Clivia – Cuticle development and fine structure – Water permeability.

Introduction

The primary organs of higher terrestrial plants are covered by a cuticle. It serves to minimize passive loss of water from the plant to the atmosphere and can be considered a prerequisite for terrestrial plant growth of species that lack drought tolerance. It has been shown in the past that cuticles have indeed a very low water permeability or a high resistance to water transfer (Kamp 1930; Pisek and Berger 1938; Holmgren et al. 1965; Schönherr 1976a; Schönherr and Schmidt 1979). The chemical and structural basis of this high resistance, however, is not well understood. It was Kamp (1930) who first recognized that the thickness of cuticles and cuticular transpiration are not closely correlated. Schönherr (1976a) demonstrated that the soluble cuticular lipids (SCL) associated with the cutin are entirely responsible for the high resistance of the cuticles, as extracting cuticles with chloroform increased their water permeability by 2 to 3 orders of magnitude (for a corresponding situation in suberized cell walls, cf. Sitte 1975). Since SCL amount to only a small fraction of the total mass of the cuticle, a close correlation between the thickness of the cuticular membranes and permeability, as predicted by Fick's law, cannot be expected. The insoluble remainder obtained following extraction of cuticular membranes with chloroform has been called polymer matrix (Schönherr 1976a). It consists of cutin, proteins, polysaccharides, and other compounds (Martin and Juniper 1970; Schönherr and Bukovac 1973; Schönherr and Huber 1977; Kolattukudy 1980). The polymer matrix swells in water. It is a porous membrane (Schönherr 1976) having a relatively high water permeability. The location of the strongly hydrophobic soluble cuticular lipids within the relatively polar polymer matrix presents a major problem and is not all clear at the present time. Studies using polarized light have shown that the polymer matrix itself is isotropic and that negative birefringence commonly found with cuticles is due to soluble cuticular lipids embedded in the polymer matrix (Meyer 1938; Roelofson 1952; Sitte and Rennier 1963). These authors postulated that the soluble lipids are located in tangential spaces within the cutin with the long axis of the lipids oriented parallel to the normal of the membrane.

Recently, a number of reports on the fine structure of cuticles as revealed by transmission electron microscopy have been published showing a fine lamella-
tion of the cuticle proper (Chafe and Wardrop 1973; Jarvis and Wardrop 1974; Heide-Jørgensen 1978; Sargent 1976; Wattendorf and Holloway 1980). It is generally believed that the electron-lucent layers represent soluble lipids, while the more electron-opaque layers are made up of cutin. While a histochemical reaction for suberin and cutin has been developed (Wattendorf and Holloway 1980) which permits the localization of cutin within the cuticle, the localization of the soluble cuticular lipids is still a matter of debate. However, from the point of view of permeability, the location of the soluble lipids and their interaction with the cutin is the most important problem. In an attempt to solve it, we have initiated a comprehensive study of the fine structure of cuticles, water permeability, and cutin biosynthesis on various plant species. The present paper is concerned with the fine structure of *Clivia miniata* CM from developing and mature leaves. Special attention is paid to the effects of extraction with chloroform on the fine structure of cuticles. In a second paper we shall investigate the fine structure of enzymatically isolated cuticles of *Clivia* leaves in relation to water permeability. In addition, the effects of acid hydrolysis, methylation, acetylation and transesterification on the fine structure of cuticles will be presented. Cutin biosynthesis of expanding leaves in conjunction with microautoradiography will be the subject of a third paper.

**Materials and methods**

*Plant material.* Leaves from greenhouse grown plants of *Clivia miniata* Reg. were used for electron microscopy. Small pieces of tissue were cut from the middle portion of leaves, avoiding the major vein. Either the first fully expanded leaf (mature leaf) or leaves that had obtained approximately half their final length (expanding leaves) were selected. Only the cuticle of the upper stomatic surface was investigated.

*Specimen preparation.* Freehand sections 0.25 to 0.5 mm thick were cut and prefixed in phosphate buffered (pH 7.4) 3% glutaraldehyde for 6 h at 4°C. Prefixation was followed by washing the sections in buffer overnight. At this point the samples were divided into 2 portions, one of which was extracted with chloroform, as described below. The others were postfixixed in either phosphate buffered OsO₄ (2% at pH 7.4 for 6 h) or in aqueous KMnO₄ (2% for 2 h) (Mueller and Beckman 1979). After washing in cold distilled water and dehydration in an acetone series, the samples were embedded in an Epon-Araldite mixture (Gläser 1975), polymerized at 60°C, and sectioned transversely using a diamond knife and a LKB ultramicrotome. Thin sections (70–90 nm) were stained with uranyl acetate (2% for 40 min) and lead citrate (20 min) at room temperature and examined with a Zeiss EM-9 microscope.

*Extraction of SCL.* Sections prefixed with glutaraldehyde were dehydrated in an acetone series. The acetone was replaced by a mixture of acetone and chloroform (1:1, for 20 min) followed by chloroform (20 min) and again acetone – chloroform (1:1, 20 min). During extraction at room temperature the samples were agitated slightly. After extraction, the samples were transferred to acetone (100%) and re-hydrated in an acetone-water series. Finally, they were transferred to phosphate buffer and processed further, as described above.

**Results**

The terminology proposed by Roelofson (1952), Sitte and Rennier (1963), and Wattendorf and Holloway (1980) will be used to describe the fine structure of *Clivia* cuticles. According to these authors the cuticle is composed of the cuticle proper (CP) and the cuticular layer (CL) underneath it. The cuticle proper and cuticular layer form the cuticular membrane (CM) that together with the cellin wall (CW) represents the outer epidermal wall.

The CM of the upper epidermis of *Clivia* leaves has a thickness ranging from 4 (Fig. 1) to 6 μm (Fig. 2) over the periclinal walls. There are prominent cuticular pegs that extend deeply down between the anticlinal walls. The transition zone between the CM and the cellin wall is not smooth, but rather is marked by globular cutin cystolytes which are particularly well contrasted after KMnO₄ fixation (Figs. 1 and 2). Generally, KMnO₄ proved to be very useful stain for cutin. The young CM of immature leaves is heavily contrasted throughout, only the cuticle proper developed little contrast (Fig. 1). The CM of mature leaves is thicker (Fig. 2) and only the inner cuticular layer and the anticlinal pegs show strong contrast, while the outer CL exhibits a marbled or mottled appearance. The CP shows very little contrast and appears discontinuous. Cuticular membranes from expanding and mature leaves appear similar when postfixixed with OsO₄. CL's have a mottled appearance, the CP is visible only as a thin line on the outer surface of the CM. Cellin walls are well developed. They are from 7 μm (Fig. 1) to 10 μm thick (Fig. 3). They generally have a layered appearance and often (as in Fig. 1) an interior and exterior cellin wall can be distinguished. Apparently, the cellin wall is under tension that is released on sectioning, leading to folds as seen in Figs. 2, 3, and 4.

At high magnification the cuticle proper is distinctly visible after osmium fixation (Figs. 5, 6, 7, 8). In both expanding and mature leaves the CP is a continuous layer of approx. 100 nm thickness and exhibits a fine lamellation. In non-extracted CM (Fig. 5 and 7) a dark layer on the outside is followed by an electron-lucent layer about 80 nm wide. At the interface between the CP and cuticular layer 3 to 5 lamellae can usually be seen. The electron lucent lamellae have a thickness of approximately 5 nm, the electron opaque lamellae are of variable thickness.

Lamellation is much more pronounced in specimens extracted with chloroform (Figs. 6, 8, 14). The electron lucent layer in the center of the CP is either