Lukas Schreiber
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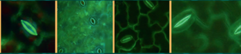

Water and Solute Permeability of Plant Cuticles

Measurement and Data Analysis

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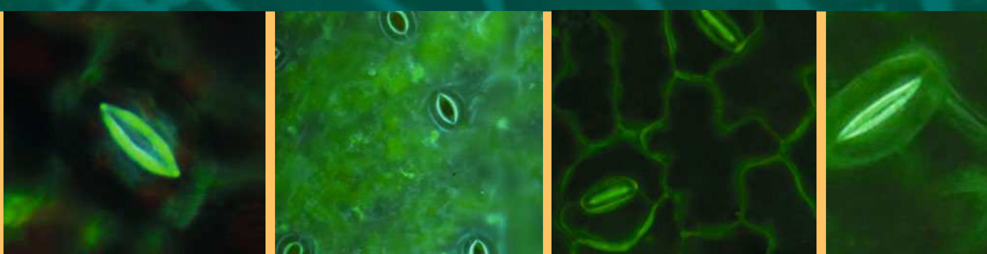

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Preface

Transport properties of plant cuticles are important for different fields of modern plant sciences. Ecologists and physiologists are interested in water losses to the environment via the cuticle. Penetration of plant protecting agents and nutrients into leaves and fruits is relevant for research in agriculture and plant protection. Ecotoxicologists need to know the amounts of environmental xenobiotics which accumulate in leaves and other primary plant organs from the environment. For all of these studies suitable methods should be used, and a sound theoretical basis helps to formulate testable hypotheses and to interpret experimental data. Unnecessary experiments and experiments which yield ambiguous results can be avoided.

In this monograph, we have analysed on a molecular basis the movement of molecules across plant cuticles. Based on current knowledge of chemistry and structure of cuticles, we have characterised the aqueous and lipophilic pathways, the nature and mechanisms of mass transport and the factors controlling the rate of movement. We have focused on structure–property relationships for penetrant transport, which can explain why water and solute permeabilities of cuticles differ widely among plant species. Based on this knowledge, mechanisms of adaptation to environmental factors can be better understood, and rates of cuticular penetration can be optimised by plant physiologists and pesticide chemists.

This monograph is a mechanistic analysis of foliar penetration. We have made no attempt to review and summarise data on foliar penetration of specific solutes into leaves of specific plant species under a specific set of environmental conditions. A number of reviews can be consulted if this is of interest (Cottrell 1987; Cutler et al. 1982; Holloway et al. 1994; Kerstiens 1996a; Riederer and Müller 2006). A wealth of additional literature is cited in these books.

Once synthesised, the plant cuticle is a purely extra-cellular membrane, and metabolism or active transport which greatly affect transport across cytoplasmic membranes are not involved in cuticular penetration. For this reason, a number of books on sorption and diffusion in man-made polymeric membranes were sources of inspiration in writing this monograph. We drew heavily on the classical books by Crank (1975), Crank and Park (1968), Israelachvili (1991) and Vieth (1991).

This is not a review about foliar penetration. We aimed at writing a general textbook on sorption and diffusion in cuticles. Based on characteristic and representative examples we show (1) how problems related to water and solute transport across cuticles can experimentally be approached using suitable methods developed in the past, (2) the way in which these data can be analysed, and what we can learn from these results about structure and functioning of cuticles, and finally (3) the limitations and problems in data interpretation. At the end of each chapter, problems and solutions can be found. Some of them summarise the highlights of the text, some illustrate implications and others are intended as exercises of calculations.

The idea of analysing permeability of cuticles based on structure–property relationships was born during a stay (1967–1972) by one of us (JS) as a doctoral student in Bukovac's laboratory at Michigan State University, USA. Later, the concepts developed in the two volumes by Hartley and Graham-Bryce (1980) were of immense help to us in formulating testable hypotheses. In writing, we have relied greatly on our own work conducted at the Botany departments of the Universities of München, Bonn and Hannover, but the book could not have been written without the collaborative research in the last decades with M. Riederer (University of Würzburg), K. Lendzian (Technische Universität München), B.T. Grayson (Shell, Sittingbourne, England), P. Baur (now Bayer Crop Science) and Anke Buchholz (now Syngenta, Switzerland).

It was one of our aims to provide a better understanding of cuticular penetration, and to formulate some basic rules for predicting and optimising rates of cuticular penetration. This requires some elementary mathematics, but we have kept equations simple and calculus is not required to follow our arguments or to solve the problems. Some basic knowledge of chemistry and physics are helpful but not mandatory. We hope this book will be useful to Master and doctoral students working in different fields of plant sciences (ecology, physiology, molecular biology, ecotoxicology, plant nutrition, horticulture, pesticide science and plant protection) when faced for the first time with problems related to permeability of plant cuticles to water and solutes. Researchers at universities, applied research institutions and those in the agrochemical industry working on transport across cuticles will find numerous useful hints. This book was written as a text book and can be used for teaching, since in each chapter (1) we state the problem, (2) we describe an experimental solution, (3) we present a critical analysis of the experimental data, and (4) at the end of each chapter we add problems intended to help the student in verifying understanding of concepts and calculations.

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One of us (LS) is indebted to the University of Bonn and the Faculty of Mathematics and Natural Sciences for granting a sabbatical leave during the winter term 2007/2008 for writing this book.

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Contents

Chapter 1
Chemistry and Structure of Cuticles as Related to Water and Solute Permeability

From the very beginning of life on earth, all living organisms established protective interfaces between themselves and the aqueous or gaseous environment. In all cases these interfaces are of lipid nature. The first unicellular organisms developed cell membranes of phospholipids separating the cytoplasm from the surrounding aqueous environment. Phospholipids are major constituents of cytoplasmic membranes of contemporary organisms. Later in evolution, multicellular organism with specialised tissues and organs appeared, and the mainland was conquered successfully by plants and animals. Since the water potential of the atmosphere is always strongly negative, there is a constant loss of water from living organisms to the atmosphere. In order to survive and avoid desiccation, land-living animals and plants had to cope with this situation. With terrestrial higher plants, the evolutionary answer to this challenge was the development of a cuticle about 500 million years ago. Insects and mammals are also protected by cuticles or skins. Their cuticles have similar functions, but they differ in chemistry and structure from the plant cuticle (Andersen 1979; Rawlings 1995).

The plant cuticle is an extracellular polymer membrane which covers all primary organs such as stems, leaves, flowers and fruits. In contrast to most synthetic polymer membranes, which are mostly homogeneous in structure and composition, plant cuticles are polymer membranes characterised by a pronounced heterogeneity in both chemical composition as well as fine structure. A functional analysis of barrier properties of plant cuticles requires detailed information on chemistry and structure. It is one of our major objectives to relate chemistry and structure of cuticles to water and solute permeability. We have evaluated the literature in an attempt to find the information necessary for relating permeability of cuticles to chemistry and structure.

Using the terminology of engineering, cuticles can be classified as composite membranes. They are composed of two chemically distinct fractions, the polymer matrix membrane (MX) and soluble cuticular lipids (SCL), often called cuticular waxes. For unambiguous chemical analysis and for measuring permeability, cuticles are isolated either chemically or enzymatically (Schönherr and Riederer 1986). The method of choice is enzymatic isolation at room temperature using pectinase

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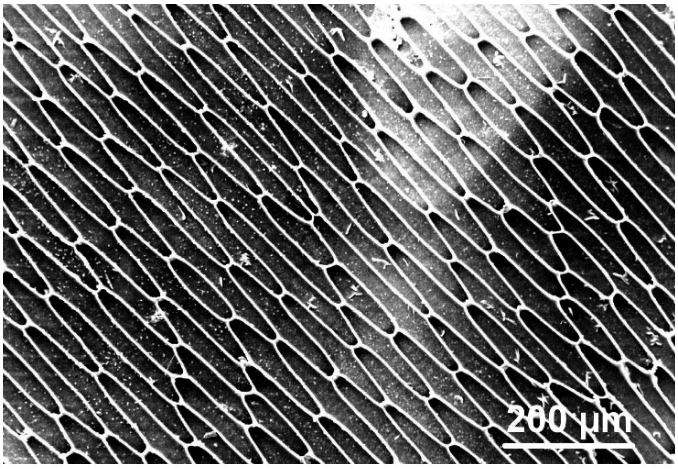

Fig. 1.1 Scanning electron micrograph of the morphological surface of a cuticle isolated with pectinase from an inner *Clivia miniata* leaf. Cuticular pegs, protruding between anticlinal cell walls, reveal the pattern of the epidermal cells

(Sect. 9.1). This avoids heat and treatment with chemicals which might cause hydrolysis or other chemical reactions. Pectinase digests the pectin layer interposed between cuticles and the cellulose wall of the epidermis. Occasionally a pectinase/cellulase mixture has been used, but the benefit of including cellulose has never been clearly demonstrated. Even when isolated using pectinase alone, the inner surfaces of the cuticular membrane look clean and cellulose residues are not detectable (Fig. 1.1).

We shall refer to isolated cuticles as cuticular membranes (CM), while the term "cuticle" is reserved to cuticles still attached to epidermis and/or organs. Cuticles cannot be isolated from leaves or fruits of all plant species. CM which can be obtained by enzymatic isolation have been preferentially used for chemical analysis, because this avoids ambiguities concerning the origin of the materials (waxes, cutin acids) obtained by extraction and depolymerisation. If enzymatic isolation of cuticles is not possible, air-dried leaves must be used. In these cases, there is a risk that some of the products obtained by solvent extraction or depolymerisation may have originated from other parts of the leaf.

1.1 Polymer Matrix

CM can be fractionated by Soxhlet extraction with a suitable solvent or solvent mixtures. The insoluble residue is the polymer matrix (MX), while the soluble lipids (waxes) can be recovered from the solvent. Chloroform or chloroform/methanol are good solvents, but many others have been used which do not quantitatively extract high molecular weight esters or paraffins, especially when used at room temperatures (Riederer and Schneider 1989).