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LEAF MICROMORPHOLOGY IN *BUXUS SEMPERVIRENS* L. DURING THE ONTOGENESIS

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Abstract. Ultrastructural aspects of leaf epicuticular waxes were investigated in *Buxus sempervirens* by scanning electron microscopy. The leaves were hypostomatic and stomata were surrounded with a cuticular thickening that formed a rim. The most prominent epicuticular wax structures of *B. sempervirens* leaves included granules and platelets.

Keywords: cuticle, epicuticular wax, leaf, stomata

INTRODUCTION

Plant surfaces represent boundary layers for interfacial interactions between plants and their environment. As a continuous extracellular membrane, the cuticle covers the primary above-ground organs such as flowers, fruits, leaves, and stems of all lower and higher land plants [10]. Cuticle is the main interface between plants and their environment, covering all primary aerial parts of vascular plants and many bryophytes [12].

Evergreen plants usually have a thick cuticle on their upper leaf surfaces and most of their stomata on the lower surface. They have special adaptations to overcome the effects of freezing since their tissues are exposed to very low temperatures during winter. They replace their leaves constantly, so lower ones overshadowed from above are dropped and during drought conditions some are shed to reduce the demand for water.

Buxus sempervirens is a shrub, but when left to grow naturally it will become a small tree. It belongs to the family *Buxacece*, a very small family of only six genera and about thirty species. Its twigs are densely leafy and the leaves are ovate, entire, smooth, thick, coriaceous and dark green. The leaves have been found to contain besides a small amount of tannin and unimportant constituents, a butyraceous volatile oil and three alkaloids: buxine, the important constituent, chiefly responsible for the bitter taste, parabuxine and parabuxonidine. The leaves have sudorific, alterative and cathartic properties being given in powder, in which form they are also an excellent vermifuge [5, 11].

The anatomy and micromorphology of this species has not been greatly investigated. Balthazar and Endress (2002) [1] have studied the structure of the gynoecium and androecium of representatives of all genera of *Buxaceae*. Hacker and Neuner (2007) [6] investigated ice propagation in stems and leaves of various angiosperm deciduous and evergreen trees and shrubs and gymnosperms (including *B. sempervirens*). Gostin and Ivanescu (2008) [4] publish a work regarding leaf structure and development in *B. sempervirens*.

The reaction of plants to environmental factors often varies with developmental stage. It was hypothesized, that also the cuticle, the outer surface layer of plants is modified during ontogenesis [2].

In this paper the investigations focused on the effect of ontogenetic development and leaf age on micromorphological features and epicuticular wax structure.

MATERIALS AND METHODS

For observation by scanning electron microscopy fragments of leaves (from different ages) were mounted on stubs and sputter-coated with gold-paladium then examined with a TESCAN SEM Vega II scanning electron microscope at 10 kV. Detailed technical data has been described by Cavaleiro et al. (2002) [3]. Measurements was made for establish the stomata density per square millimetre, stomata length and stomata width. For stomata density 10 fields for each sample was analysed and for dimensions 25 stomata for each sample was measured.

RESULTS

In this paper, leaves from five different ages were analysed using scanning electron microscopy. The general aspect of the epicuticular wax, the aspect of the structured wax and their modifications during ontogenetically development, the micromorphological aspect of the stomata was investigated.

Microscopic investigations using SEM displayed the typical pattern of epidermal cells forming the leaf surface. In surface view, both epidermises are composed of polygonal cells with sinuous anticlinal walls, the abaxial ones being larger. The adaxial leaf side did not show any stomata.

Leaf age have an important influence on the surface structures (Figure 1 & 2). The abaxial surface is primarily covered with a thick and uniform layer of amorphous wax. Over this layer, crystalloids of structural wax could be observed. On the upper epidermis of the very young leaves the epicuticular wax appears as anastomosed tubules. The morphology of these tubules is changed during the ontogenesis. On the young and mature (I) leaves the tubules appears partially fused, while on the mature (II) and old leaves the epicuticular tubules are completely fused and transformed in amorphous was as platelets.

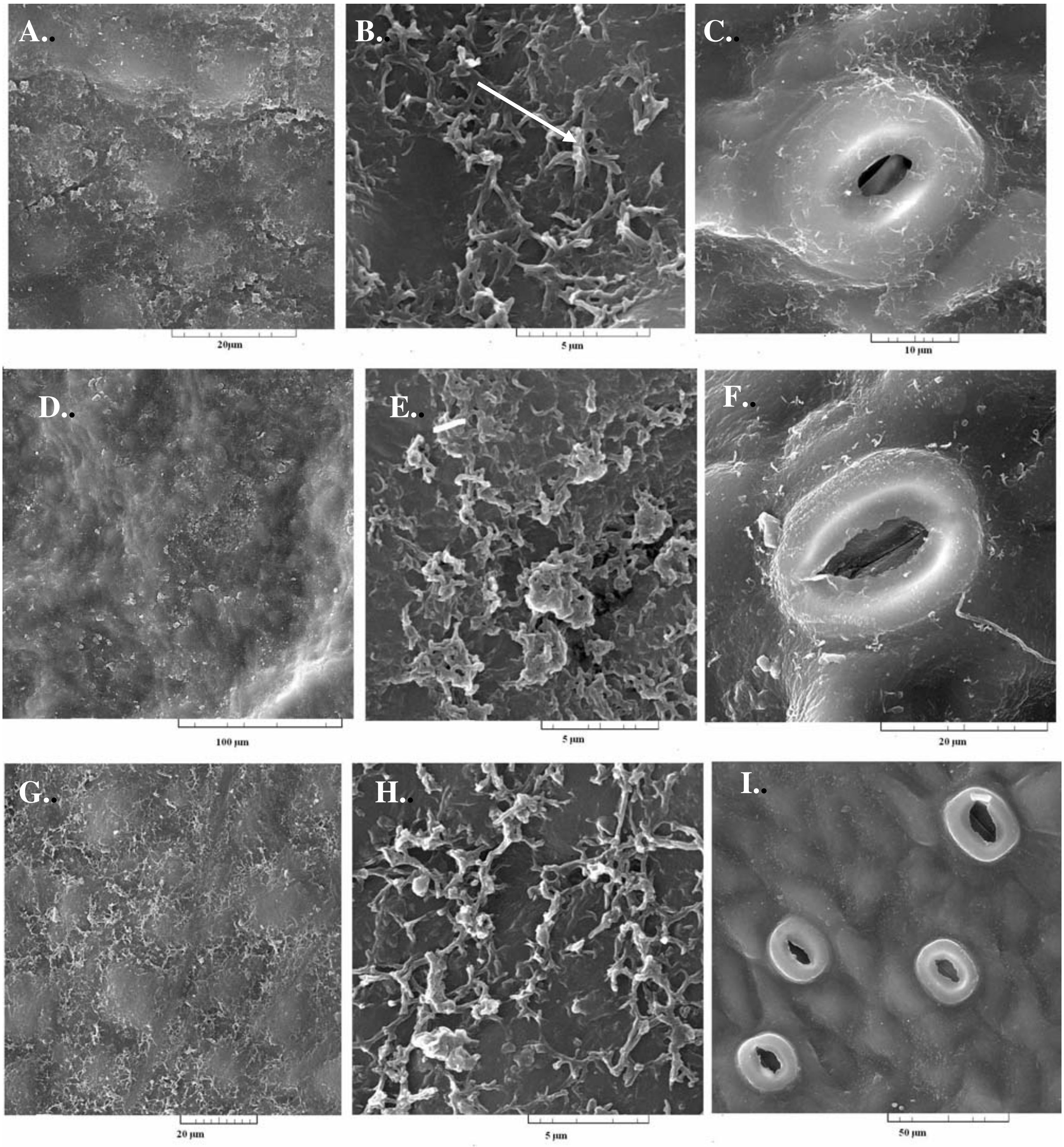

Figure 1. Field emission scanning electron micrographs of epicuticular waxes of Buxus sempervirens leaves. A – C: very young leaf, A - Adaxial surface B - Higher magnification of the adaxial surface: Arrow indicate wax granules and platelets; C – Stomata from abaxial surface; D – F, D - Adaxial surface E - Higher magnification of the adaxial surface: arrow indicate wax partially fused tubules; F – Stomata from abaxial surface; G – I: Mature leaf, G - Adaxial surface; H - Higher magnification of the adaxial surface; I - Stomata from abaxial surface (original)

The stomata density (on square millimeter) visibly decreases during the maturation of the leaves. On the very young leaves lower epidermis we find 366.67 ± 7.91 stomata/mm^2 and only 199.68 ± 3.38 stomata/mm^2 on young leaves epidermis.

This parameter continue to decrease during the attempting the final dimension of the leaves (142.11 ± 2.51 stomata/mm^2 on mature leaves I respectively 155.85 ± 1.88 stomata/mm^2 on mature leaves II) and remain constant for old leaves (104.86 ± 2.37 stomata/mm^2). The dimensions of the stomata increase from the very young leaves (width 20.25 ± 0.62µm, length 23.86 ± 0.72µm) to the mature (I) ones (width 27.07 ± 0.58µm, length 35.9 ± 0.86µm) and remain almost constant in mature (II) and old leaves.

Stomata were slightly prominent over the level of the epidermic cells and were surrounded with a cuticular thickening that formed a rim. This stomatal rim and the adjacent part of the epidermic cells show fine wax rodlets. With aging, the crystalline structure of epicuticular wax becomes amorphous.

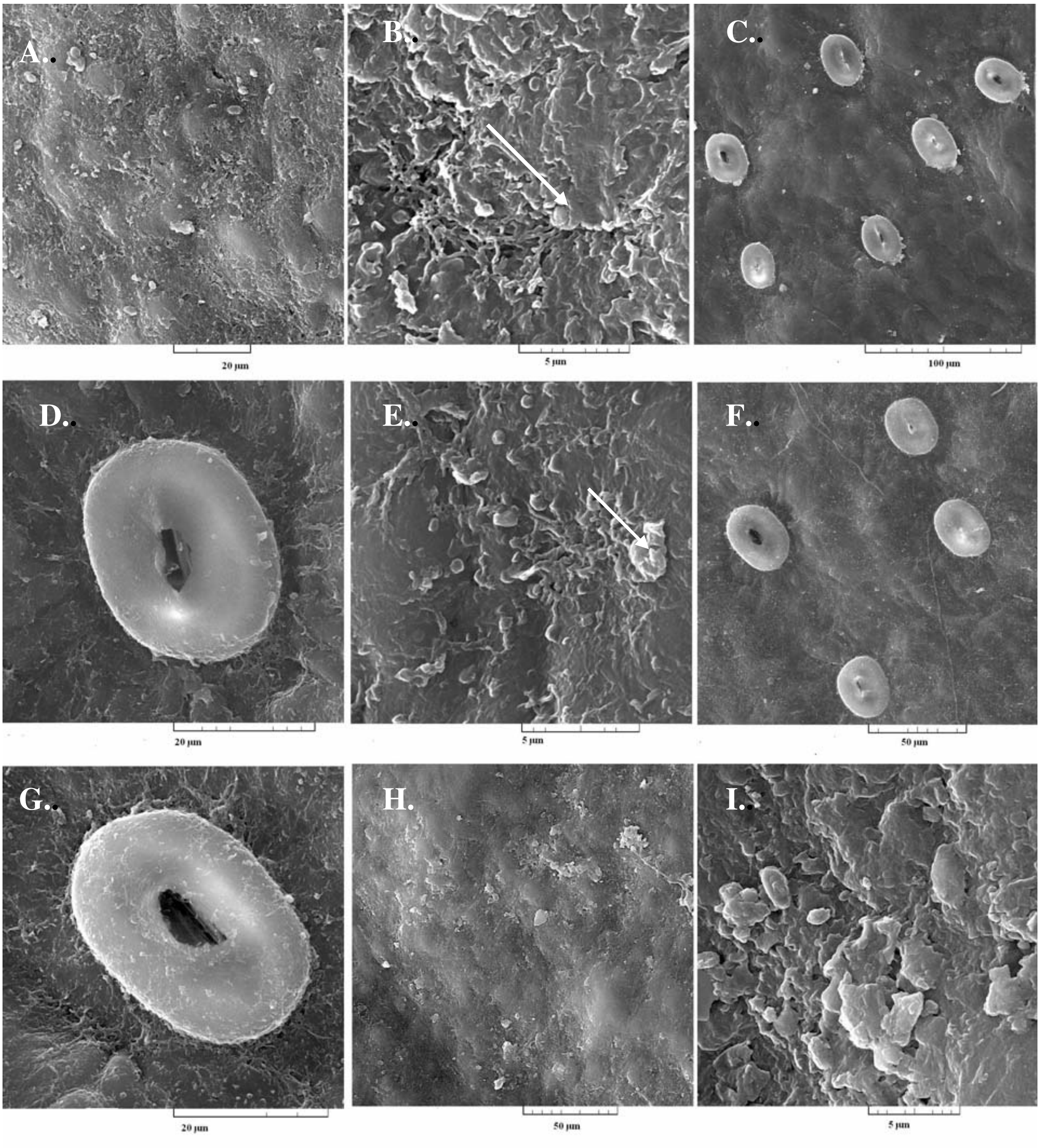

Figure 2. Field emission scanning electron micrographs of epicuticular waxes of *Buxus sempervirens* leaves. A – D: mature leaf (II), A - Adaxial surface B - Higher magnification of the adaxial surface; C, D – Stomata from abaxial surface; E – I: old leaf (over 2 years): E - Adaxial surface; F, G - Stomata from abaxial surface H - Adaxial surface; I - Higher magnification of the adaxial surface; Arrow indicate wax platelets (original)

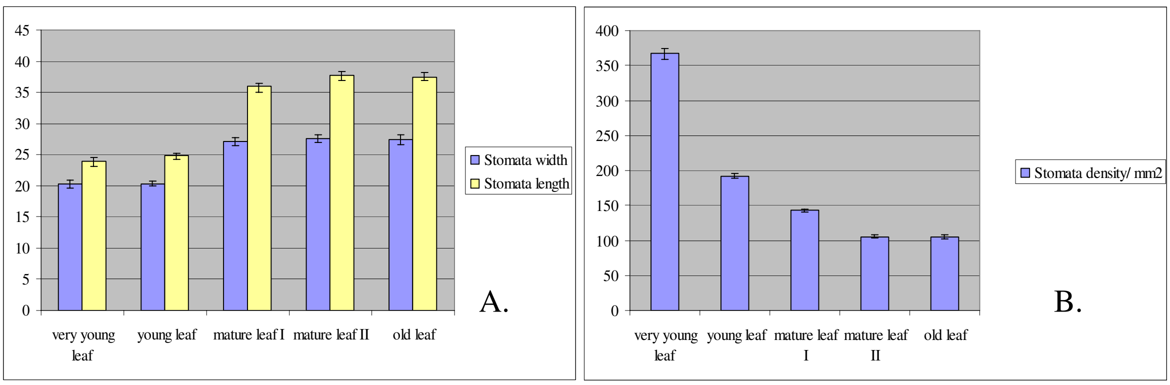

Figure 3. Graphical representation of the stomata dimensions (**A**) and stomata distribution (**B**) related to leaf age

DISCUSSIONS

It is very well know that the epicuticular waxes form the outermost layer of plants as a partial or continuous extracellular matrix, so it is the first barrier against all factors that come into contact with plants: fungi, bacteria, insects, solar radiation [9]. Waxes are known to benefit plants in a number of ways: protecting plants against pathogens and insects, reducing the water loss caused by transpiration, and diminishing the injury risk due to excessive radiation by reflecting and attenuating sunlight [14].

Many leaves and needles are evergreen with a considerably long life span. Evergreen plant surfaces and leaves have considerable morphological, ultrastructural and chemical diversity and variation in their responses to environmental factors. Wax morphology and chemistry are important in plant systematic, but waxes respond to environment in quality and quantity.

The arrangement of wax in anastomosed tubules and platelets or as an amorphous structure has an impact on the micromorphology of leaves, wettability and water repellence [2]. Wettability of cuticles differs depending on the presence of epicuticular waxes, their chemical composition and the micro-structure of waxes. The cuticle of young *Buxus* leaves exhibited at the upper side cuticular ridges and tubules. The flattening out and partial disappearance of these wrinkles on older leaves may be attributed to the expansion of cell area. The increase in epidermal leaf area largely depended on the expansion of cells present. The folding may increase the surface area of cuticles by a factor of two to three [7] and could largely accommodate the expansion of epidermal area during later stages of leaf development. Wrinkles are also supposed to be the sites of transportation and incorporation of new material – waxes and cutin – for cuticle growth.

During ontogenetic development of *Buxus* leaves, the leaf area increased and the stomata number per unit of area tended to decrease. This was especially true during early leaf ontogenesis. During later stages of development the expansion of epidermal cell areas converged to zero and the stomata number per unit of area remained constant.

The cuticular waxes are integrated (intracuticular) and superposed (epicuticular) to the cutin network. Whereas the intracuticular waxes function as the main transport barrier, preventing water loss and leaching of molecules from inside of the living cells [8, 13], the epicuticular waxes are of great importance as an interface layer to the environment.

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