COMPARATIVE ECOLOGICAL WOOD ANATOMY OF AFRICAN MAHOGANY *KHAYA IVORENSIS* WITH SPECIAL REFERENCE TO DAMAGE CAUSED BY *HYPSIPYL ROBUSTA* SHOOTBORER

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Abstract. This study focuses on the wood anatomy of 60 *Khaya ivorensis* A. Chev. (Meliaceae) trees from natural forests in three forest zones of southern Ghana. The arrangement, grouping, density and diameter of vessel elements in stem wood were measured and theoretical hydraulic conductivities were calculated for each site. Also anatomical characteristics of thin branches damaged by mahogany shootborer (*Hypsipyla robusta* Moore) were studied with special reference to effects on vessel element diameter, vessel density and hydraulic conductivity. Our study revealed that there were significant differences in vessel element diameters and hydraulic conductivities between the sites, and that the water conduction efficiency of *K. ivorensis* wood increased with increasing annual precipitation. In shootborer damaged branches mean vessel element diameters were consistently smaller and mean theoretical hydraulic conductivities lower than in undamaged branches. This suggests that *Hypsipyla* damage can have a negative effect on the growth rates of mature African mahogany trees in natural forests.

Introduction. *Khaya ivorensis* A. Chev. is distributed throughout the moist lowlands of tropical West Africa. The species has a wide habitat tolerance, but it favors the banks of rivers and streams. The wood is highly valued especially for furniture, cabinet work and light flooring [10]. The species has become commercially extinct in large parts of its original range. Attempts to establish plantations to answer the increasing demand have often failed due to extensive damage by the mahogany shootborer *Hypsipyla robusta* Moore (Lepidoptera, Pyralideae), which is generally regarded one of the most serious insect pests in tropical forestry.

The larval stage of the mahogany shootborer tunnels into the primary stem and branches of the tree, feeding on the pith [4]. The most serious damage results from the tunneling of developing apical shoots. Injuries of the apical meristem almost invariably lead to the death of the terminal shoot and subsequent production of laterals, eventually resulting in continuously branched and crooked tree of diminished commercial value. Damage of the primary meristem also influences wood formation in the cambium [7]. Consequently, growth rate is reduced and death may result from repeated heavy attacks.

Many secondary metabolites are known to function as barriers against insects and numerous woody plants produce and store resins or gums in canals in the stem cortex, in the leaves and xylem [3]. Resin ducts and bands of vessels filled up with resin-like substances are also present in *Khaya ivorensis* [5].

There is wide empirical evidence that the secondary xylem of woody plants responds to site conditions, particularly to water availability [2, 9]. However, practically no research has focused on the comparative wood anatomy of *Khaya ivorensis* trees growing under different macroclimatic conditions. Previous studies regarding the relationship of *K. ivorensis* and *Hypsipyla* spp. have mainly focused on silvicultural [8, 4] and entomological [1] questions. Conversely, there is practically no data on the incidence of *Hypsipyla* damage in natural mahogany populations in different parts of the Tropics. Likewise, the effects of *Hypsipyla* damage on the growth of mature *K. ivorensis* trees are poorly known. The present study provides some preliminary results from our ongoing research into these potentially important fields.

Materials and methods. A total of 60 *Khaya ivorensis* trees from natural forests were sampled from the three field sites in Ghana, West Africa (20 trees for each forest). The field sites were located in the Wet Evergreen Forest Zone (Benso, 05°09' N, 001º3' W), the Humid Subtype of Moist Semideciduous Forest Zone (Amantia, 06°14' N, 001°11' W) and the Moist Semideciduous Forest Zone (Bobiri (06°41' N, 001º20' W).

Stem wood specimens were collected from the sampled trees at breast height and a total of 300 branch wood specimens, one specimen from a thick branch (diam. 20–50 mm) and four specimens from smaller branches (diam. 5–19 mm) were collected from the canopy. For microscopic analysis, transverse, radial and tangential sections (20–25 µm) were made from each stem/ branch wood sample using a freezing microtome (Leica, Model CM3050S, Leica Microsystems, Nussloch, Germany). The microsections were stained with 0,5 % safranin (in 50 % EtOH) and 1 % Astrablau (aq.), dehydrated through a rising alcohol series and mounted in Canada Balsam.

The microsections were photographed with a Leica DFC490 digital camera attached to a Leica DMLB microscope and the IM50 Image Manager V1.20 program (Leica Microsystems, Nussloch, Germany). Vessel characteristics were determined from the last growth layer of transverse microsections of *Khaya ivorensis* wood. After calibrating the size of the photomicrographs, the quantitative characteristics were determined using the Image Analyzer program (courtesy of Dr. Martti Perämäki, University of Helsinki). This program has been developed for the estimation of surface areas from digital images. The theoretical hydraulic conductivity of the studied specimens was calculated according to the equation derived from the Hagen-Poiseuille law for ideal capillaries:

$$K_{h\ theoretical} = \frac{\pi\rho}{128\eta A_{\mathrm{image}}} \sum_{i=1}^{n} d_i^{4}$$

where $K_{\mathrm{h\ theoretical}}$ is the theoretical hydraulic conductivity (kg m^{-1} Mpa^{-1} s^{-1}), A_{image} is the image area in mm^2, ρ is the density of water, η is the dynamic viscosity of water at 25ºC in Mpa s^{-1} and d_{i} the diameters of vessels within the image area raised to the fourth power and summed over the number of vessels n present within the image area..

Results. **General wood anatomy of *Khaya ivorensis.*** The color of *Khaya ivorensis* heartwood varied from pale yellowish brown to deep reddish-brown. In some cases, dark reddish brown heartwood was easy to distinguish from pale yellowish brown sapwood. Hardness of the wood varied from soft to moderate hard. The wood was diffuse-porous (Fig. 1A) and when detectable, the growth rings were delineated by several rows of tangential flattened fibers and with poorly defined band of boundary (terminal) parenchyma (Fig. 1B). Vessels were circular or slightly oval in transverse sections. They occurred singly or in radial groups rounded by narrow sheaths of vasicentric axial parenchyma. Perforation plates were simple. In stem wood, vessel element diameter ranged from 9 µm to 282 µm and some vessels contained red or dark red-stained resin-like substances. Multiseriate rays occurred in two size classes with narrow rays being 2–4 cells and wider rays 5–8 cells wide. Rays were heterogenous with 1–3 upright cells at the top and bottom of the ray. Prismatic crystals were common in ray cells.

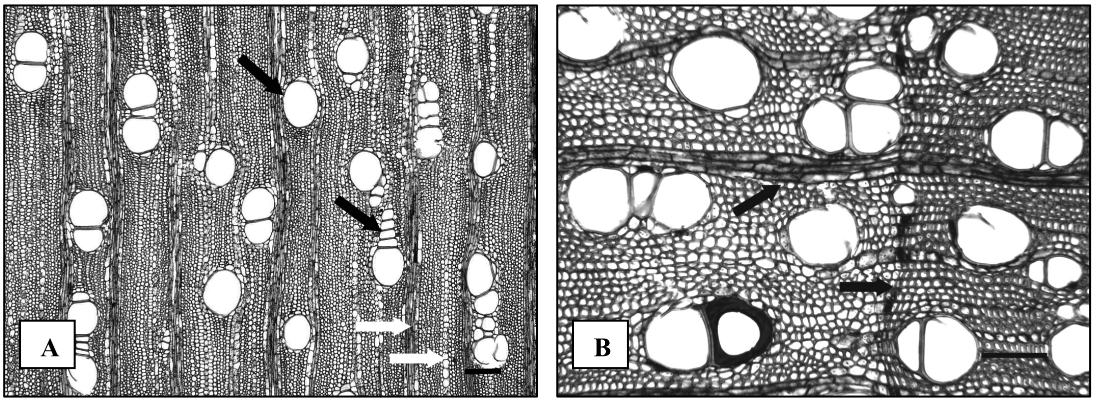

Figure 1. Khaya ivorensis wood anatomy .

A. Transverse section of secondary xylem in stem from wet evergreen forest (Benso). The vessels are solitary, in short radial groups or occasionally in radial rows of up to 11 vessels (black arrows). Rays appear as vertical lines (upper white arrow). Scale bar 200 µm. B. Transverse section of branch wood from moist semideciduous forest (Amantia). Growth layer is delineated by boundary (terminal) parenchyma (right arrow). Ray cells appear as horizontal line (left arrow). One vessel in the lower part of the picture contains resin-like substance. Scale bar 100 µm.

Characteristics of damage caused by shootborer Hypsipyla robusta. Shootborer damage was observed in 44 % of all *Khaya ivorensis* trees sampled. Damaged branches could be identified by deformations readily visible to the naked eye (Fig. 2A). In radial sections the larvae tunnels of *Hypsipyla* were seen as dark-brownish straight or curved lines. The tunnels were partly filled with solid, dark resin-like substances. The wounded secondary xylem was characterized by formations of traumatic parenchyma cells adjacent to the tunnels burrowed by *Hypsipyla* larvae (Fig. 2B).

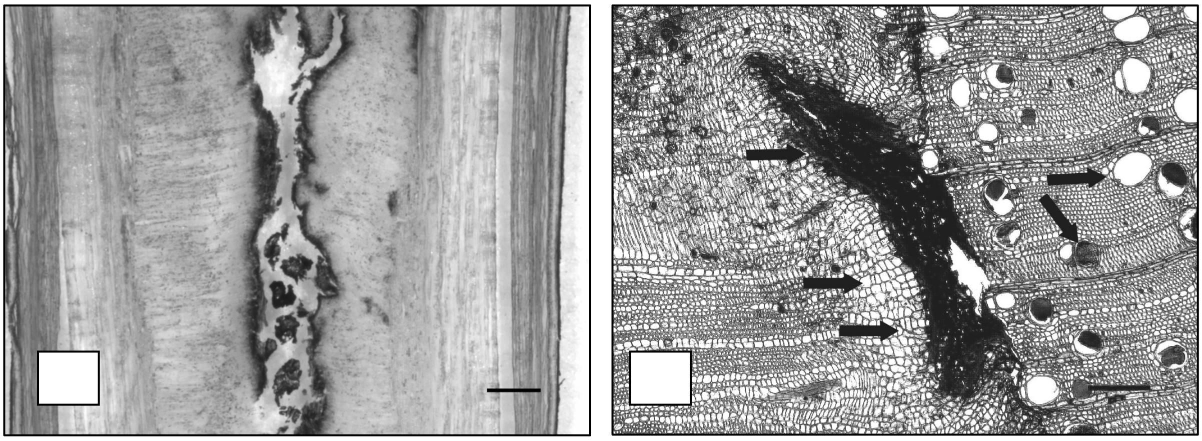

Figure 2. Larval tunnels of *Hypsipyla* in thin *Khaya ivorensis* branches.

A. Radial section of damaged branch from wet evergreen forest (Benso). The larval tunnels contain solid, dark brown resin-like substances. Scale bar 1 mm. – B. Transverse section of secondary xylem in damaged branch from moist semideciduous forest (Bobiri). The dark damaged area is surrounded by callus tissue (arrows on the left). Note the normal and resin-plugged vessels (arrows on the right). Scale bar 200 µm.

Obstructed single vessels and vessel groups (Fig. 3A) as well as circular intercellular canals entirely plugged with resin-like substances (Fig. 3B) were detected in 83 % of the analyzed branch wood specimens injured by *Hypsipyla robusta*, however, most abundantly in the specimens collected from Benso and from Amantia. Circular intercellular resin canals of traumatic origin were found also in some stem woods.

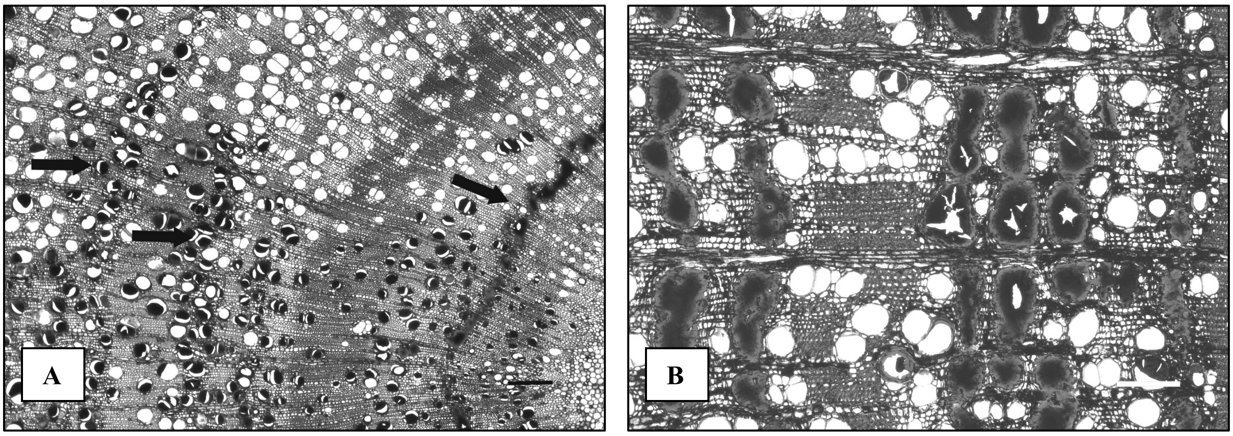

Figure 3. Intercellular canals plugged with resin in thin *Khaya ivorensis* branches.

A. Transverse section of damaged branch from wet evergreen forest (Benso). Note the high proportion of vessels filled with resin-like substances (arrows on the left) and a circular line of intercellular resin canals (arrow on the right). Scale bar 200 µm. – B. Transverse section of secondary xylem in damaged branch from moist semideciduous forest (Bobiri). Bands of traumatic intercellular resin canals are evident as dark stained vertical lines. Scale bar 200 µm.

Vessel element diameter and theoretical hydraulic conductivity. There were clear differences (one-way ANOVA, df = 2, F = 6,20, $P = 0{,}004$, $N = 57$) in mean vessel element diameters between the three forest zones (Fig. 4). The highest mean vessel diameter was measured in Benso (129,0 µm, SE = 4,41) and the lowest in Bobiri (108,7 µm, SE = 4,09). This difference was statistically significant (Tukey's multiple comparisons test, $P = 0{,}003$). There were also clear differences (log tranformed $K_{h\ theoretical}$) in theoretical hydraulic conductivities between the three sites (one-way ANOVA, df = 2, F = 3,66, $P = 0{,}032$, $N = 58$). The mean theoretical hydraulic conductivity $K_{h\ theoretical}$ (kg m^{-1} Mpa $^{-1}$ s^{-1}) in Benso (107,31, SE = 17,39) was much higher than in either Amantia (63,89, SE = 5,22) or Bobiri (62,80, SE = 10,23). The difference between Benso and Bobiri was statistically significant (Tukey's multiple comparisons test, $P = 0{,}034$).

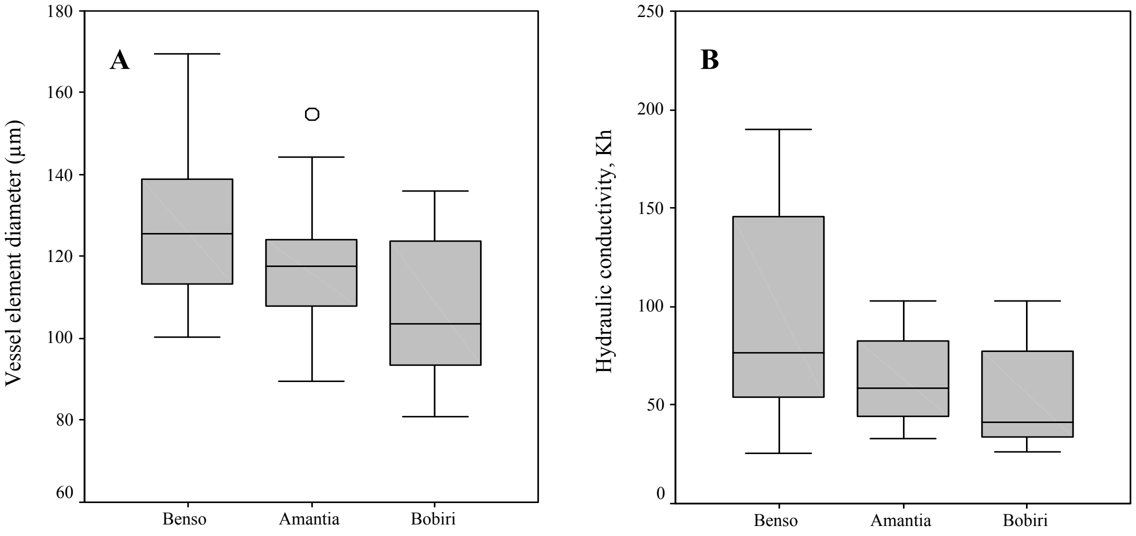

Figure 4. Variation in vessel element diameters and theoretical hydraulic conductivity in stem wood of *Khaya ivorensis* in three forest sites in Ghana.

A. Variation in vessel element diameters (µm). — B. Range of theoretical hydraulic conductivity $K_{\mathrm{h\ theoretical}}$ (kg m^{-1} Mpa $^{-1}$ s^{-1}), values computed from transverse sections of stem wood. Forest sites: Benso, Wet Evergreen Forest (20 stems measured); Amantia, Humid Subtype of Moist Semideciduous Forest (19 stems measured); Bobiri, Moist Semideciduous Forest (19 stems measured).

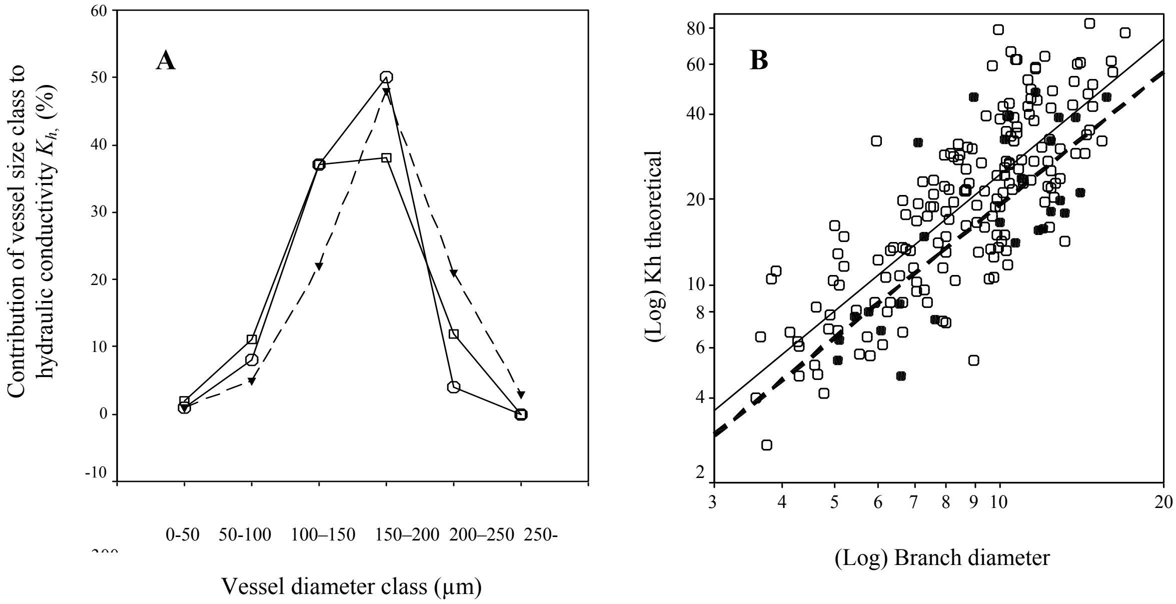

Figure 5. A. Contribution of different sized vessels to total hydraulic conductivity Kh (kg m-1 Mpa -1 s-1) of Khaya ivorensis trees sampled from the three forest sites in Ghana. ▼ Benso, Wet Evergreen Forest (29 stems measured). ○ Amantia, Humid Subtype of Moist Semideciduous Forest (19 stems measured). □ Bobiri, Moist Semideciduous Forest (19 stems measured). – B The relationship between log transformed mean theoretical hydraulic conductivities (kg m-1 Mpa -1 s-1) and branch diameters (r2 = 0,60, P < 0,001, N = 203) in healthy (open circles, thin solid line, r2 = 0,63, n = 176) and Hypsipyla damaged (black dots, thick dotted line, r2 = 0,57, n = 27) thin branches of Khaya ivorensis sampled from the three field sites in Ghana (Benso n = 92, Amantia n = 38, Bobiri, n = 73).

Wide vessels contributed more to total theoretical hydraulic conductivity in Benso than in Amantia or Bobiri (Fig. 5). The relative contributions of wider vessels (diam. > 150 µm) to total conductivity were 72 %, 54 % and 50 % for Benso, Amantia and Bobiri, respectively. Concurrently, small vessels (diam. < 100 μm) contributed 13 % to overall theoretical conductivity in the Bobiri, 9 % in Amantia and only 6 % in Benso.

The relationship between log transformed branch diameters and the theoretical hydraulic conductivities of thin branches is shown in Fig 5B. In the developed regression models *Hypsipyla* damage (x_1) branch diameter (x_2) and forest type (x_3) were used as predictors for the dependent variables. All the predictors x_1- x_3 had a statistically significant ($P < 0{,}05$) impact on theoretical hydraulic conductivity. The linear regression models were; vessel element diameter = $17{,}85 - 7{,}94\ x_1 + 3{,}25\ x_2 - 0{,}45\ x_3$; vessel density = $190{,}10 + 26{,}39\ x_1 - 9{,}63\ x_2 - 3{,}39\ x_3$ and hydraulic conductivity = $-7{,}50 - 5{,}74\ x_1 + 3{,}96\ x_2 - 2{,}28\ x_3$ The regression models indicated that the tunnelling of *Hypsipyla* larvae in thin *Khaya ivorensis* branches was associated with a notable reduction in mean vessel element diameters, an clear increase (26,40 vessel elements per $\text{mm}^{2)}$ in mean vessel densities and a decrease (5,74 kg m^{-1} Mpa $^{-1}$ s^{-1}) in theoretical hydraulic conductivities.

Discussion and conclusions. The results of our study support the general idea that the mean vessel element diameter and the efficiency of hydraulic conduction of trees increase with increasing moisture availability. Accordingly, *Khaya ivorensis* wood samples from the Wet Evergreen Forest Zone (Benso) tended to have larger vessels and higher hydraulic conductivities than comparable samples from the Moist Semideciduous Forest Zone (Amantia and Bobiri). Regarding the estimates of theoretical hydraulic conductivity, one should of course remember that the Hagen-Poiseuille equation applies for ideal capillaries. The calculations are also based on a simplification that vessels are endless parallel pipes, whereas in reality the water conduction system is a complex network of tracheary elements.

Our results suggest that even moderate *Hypsipyla* damage on canopy branches may have a negative effect on the growth rates of mature African mahogany trees in natural forests: the mean vessel element diameters were consistently smaller and mean theoretical hydraulic conductivities lower in *Hypsipyla* damaged branches. Additionally, in some cases, a notable proportion of vessels of the affected area were plugged with resin-like substances most presumably reducing the water conduction capacity of the vessels. The incidence of *Hypsipyla* damage was highest in the Wet Evergreen Forest site. Although these forests seem to provide near optimal conditions for the rapid growth of *Khaya ivorensis,* the high incidence of *Hypsipyla* damage may act to significantly reduce wood production.

Previous research has mainly studied *Hypsipyla* spp. damage patterns in young plantations. In the present study we found that *Hypsipyla* damage was common and often severe in the upper canopies of mature *Khaya ivorensis* trees in natural forests. This is in line with the early observations suggesting that *Hypsipyla* attacks continue throughout the mature life of African mahoganies [6].

Resin-like substances were detected in 83 % of thin branches with *Hypsipyla* damage. The high incidence of these substances and resin bands suggest that their formation was induced by the tunneling of *Hypsipyla* larva. These bands act as a protective layer [7], most probably preventing fungal invasion and the subsequent spread of damage deeper into the wood.

In summary, our results support the theory that xylem structure and function is optimized to balance the conflicting demands of xylem safety versus efficiency. They indicate that hydraulic conductivities of *Khaya ivorensis* trees significantly increase with increasing moisture availability. This phenomenon is probably linked to similar differences in rates of photosynthesis and/or dry matter production. However, this and many other interesting questions remain open for further research.

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VARIATION IN THE DEVELOPMENTAL FEATURES OF THE SECRETORY SYSTEM OF *COPAIFERA LANGSDORFFII* SEEDLINGS UNDER DIFFERENT LIGHT CONDITIONS

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Copaifera langsdorffii Desf. (Leguminosae-Caesalpinioideae) is a widespread tree species occurring in gallery forests, dry mesophitic forests and open and closed canopy savanna woodland [8]. The oil and oleoresin produced by this species is exhaustively exploited by cosmetic, pharmaceutical and other industries [4]. Some of the produced substances, mainly the terpenes, are the responsible for the resistance to microbial attack and protection against predators [5, 1].

The secretory system in *C. langsdorffii* is constituted by oil cavities and oleoresin canals showing a differential distribution in the stem: oil cavities are the most common secretory structure occurring in the cortex and oleoresin canals in the pith, in both seedlings and adult plants [7].

Feibert and Langenheim [2] reported that the oleoresin production in leaves of seedlings and young plants of *C. langsdorffii* differs in relation to the light intensity. So, seedlings under full light produce more resin comparing to those under shade conditions. However, despite of the evidences indicating that biotic and abiotic factors influence the resin production, experimental studies on developmental biology of the secretory system in *C. langsdorffii* are lacking.

So, research in this area is of great importance to understand the structural and functional adjustment of the plants and to evaluate the light requirements for secretory system development. Here we test this idea employing seedlings of *C. langsdorffii* cultivated under three differential light conditions: 1) one group (n=10) of seedlings was maintained in climatic chamber at 25ºC and photoperiod of 12 hours of full light (2500 lux), 2) one group (n=10) under 50 % of light (1250 lux), 3) one group (n=10) under 90 % of shade (250 lux). When the aerial axis of the seedlings reached 5 cm of height, samples of the medial region of epicotyl were collected and handled following usual techniques in plant anatomy. We quantified the canals and cavities and measured their lumen diameter from cross sections using Cell B Olympus software.

Our experimental studies showed that the density and the diameter of lumen of secretory cavities and canals change in seedlings of *C. langsdorffii* growing under different light conditions (Table; Figures 1–6). Secretory cavities and canals answered differently to growing conditions. Oil cavities are more numerous in the cortex of seedlings growing under 50 % of shade while the largest number of oleoresin medullar canals was detected in full light condition. Concerning the size of the secretory structures, the widest lumen were observed in the cortical cavities of seedlings under full light and 90 % of shade and in the medullar canals of seedlings under full light.

Table. **Mean of cavity and canal number and lumen diameter in epicotyl of *C. langsdorffii* seedlings under different light conditions**

Light intensity	Cavities of the cortex		Canals of the pith	
	Number of cavities	Diameter of lumen	Number of canals	Diameter of lumen
Full light	11,6	29,2	9,2	36,6
50 % shade	17,5	23,97	6,0	21,2
90 % shade	12,2	29,2	5,5	33,7