Glandular Trichomes of the Sweet Chestnut

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ABSTRACT. The glandular trichomes of the sweet chestnut *(Castanea sativa)* have been studied with SEM, TEM and light microscope techniques. Three types of trichomes are present on the leaf epidermis and on young stems. Two types, the stalk and head, are well-defined, while a third type, the head, is not clearly defined. The cells of both the head and the stalk arise by successive anticlinal and periclinal divisions from a protoepidermal cell. Higher gland density was found on the upper surface than on the lower surface. Density also depended on leaf development stage and growth conditions; it is highest at early stages of development, while no trichomes were present on senescent leaves. Leaves grown *in vitro* showed higher gland densities than those from free-growing trees. In both stalk and head

cells, a single large vacuole with a sinuous tonoplast occupies the greater part of the cell body, the cytoplasm forming an irregular fringe around the outside. Dissolved and suspended tannins are present in high quantities. Organelles are almost entirely lacking in the cytoplasm, although plasmodesmata ("presumably" implicated in the grandular function of the trichome) occur at high density. Glandular products accumulate in a cavity between the trichome head and an overarching cuticle in which no pores of any type are visible.

The presence of glands on chestnut leaves has been reported by various authors, including Camus (3) and Bazzigher et al. (1), although the references have tended to be somewhat vague. In previous studies we have reported the presence of various types of trichome or hair (14) and of glandular trichomes (15) on chestnut leaves. Grandular formations on leaves and stems are frequent in higher plants. Their function varies, although defense against insect attack is most common (11). Mechanisms involved may be physical, including abrasiveness and light transmission/reflection, or chemical, including repellence, toxicity and nutritional quality (4, 6, 9, 13). The presence of glandular or non-glandular trichomes has been used as a taxonomic determinant to define species, genera and interspecific hybrids (2, 5, 7, 8, 10). In this study, using scanning electron microscopy (SEM), transmission electron microscopy (TEM) and light microscopy, we investigate the glandular trichomes of *Castanea sativa* Mill., the sweet or Spanish chestnut, in both *in vitro* and *in vivo* material.

MATERIALS AND METHODS

Samples of chestnut *(C. sativa)* leaves and stems were collected from free-growing trees and *in vitro* culture. Tissues were fixed as per Salem and Brandao (12), dehydrated along an ethanol series, and dried to critical point in a Point Dryer 20 (Balzers Union). For SEM, tissues were sputter-coated with gold to a thickness of 200pm, using a Fine Coat Sputter JFC-100, and observed under a Jeol-JSM-35C electron microscope at 15 Kv. For TEM, critical point-dried tissues were embedded in an Epon-Araldite mixture, sectioned (60 nm thickness) with a Sorvall MTV-1 microtome, stained with uranyl acetate and lead citrate and observed under a transmission electron microscope Aeiem 6 G, Siemens Elmiskop, at 25 Kv.

RESULTS AND DISCUSSION

Three well-defined types of glandular trichome were found on chestnut leaves and stems. Two of these types have both stalk and head, and can be distinguished from each other by certain morphological features such as the configuration of the head or the gland itself. The third type is stalked but lacks a well-defined head. We evaluated the overall density of the three types of trichomes on the surface of stems and on the two surfaces of the leaf epidermis. It can be seen that gland density is highest on the youngest stems and decreases with age, falling to zero in the case of mature stems (Table 1).

Table 1. Density of glandular trichomes of stems.

	Number/mm^2	
Stage of Stem Growth	*In vivo*	*In vitro*
Very young stem	78	328
Young stem	54	–
Fully formed stem	10	–
Adult stem	2	129
Mature stem	0	–

On the leaf surface, gland density varies between the upper and lower surfaces and with growth stage/age (Table 2).

Table 2. Density of glandular trichomes of leaf surfaces.

	Number/mm^2	
Stage of Leaf Growth	Upper Surface	Lower Surface
Opening of the leaf bud	475	15
Very young leaf	341	11
Very advanced stage	216	9
Fully expanded leaf	178	7
Mature leaf	5	0
Start of senescence	0	0
Senescence	0	0

The glandular trichomes are principally located on the upper leaf surface, with very few on the lower surface. On the upper surface density varies considerably as a function of the leaf's ontogenic state; density is very high during the earliest stages of leaf growth (475 trichomes/mm^2) and decreases as the leaf ages. The disappearance of glands is most marked once the leaf has reached its maximum size, and by the time senescence is reached no glands remain. These variations can probably be correlated with the function of glandular trichomes in protecting against insect attack; younger leaves are more vulnerable, and thus require a higher density of protective glands.

In vitro growth has considerable effects on trichome density, as it can be seen from Table 3.

Table 3. Effect of the *in vitro* growth on glandular trichomes density of leaves.

	Number/mm^2	
Stage of Leaf Growth	Upper Surface	Lower Surface
Primordium	737	210
Early growth	743	109
Intermediate growth	714	82
Maximum leaf area reached	329	72
Mature leaf	236	48
Onset of senescence	216	0
Senescence	0	0

In adult leaves, gland density *in vitro* is almost double that *in vivo.* As with leaves obtained *in vivo,* leaves obtained in *vitro* have many more trichomes on the upper than on the lower surface. The highest value recorded was 737 trichomes/mm² on the upper surface of leaves grown *in vitro,* as against 475 trichomes/mm² *in vivo.* The values gradually decrease with increasing leaf age, falling to zero at senescence.

Table 4. Gland density on chestnut leaves grown *in vitro* and *in vivo.*

	Number/mm²			
	Upper Surface		Lower Surface	
Stage of Leaf Growth	*In vivo*	*In vitro*	*In vivo*	*In vitro*
Primordium	475	737	15	210
Early growth	341	734	11	109
Intermedium leaf area	310	714	9	82
Maximum leaf area reached	201	329	7	72
Mature leaf	178	236	0	48
Onset of senescence	5	216	0	0
Senescence	0	0	0	0

It can be concluded that gland density on Spanish chestnut leaves shows very high variability and is influenced by leaf age, growth conditions and leaf surface. Morphological study of chestnut glandular trichomes revealed the existence of three types; in two, the head or gland proper is well-differentiated from the stalk or pedicel, and the head may be oblong, with 4-5 cells in one layer, or it may be formed by slightly compressed cells arranged in two layers. The third type of trichome does not have a head that is clearly differentiated from the stalk. The stalk or pedicel is made up of 2-4 (in some cases up to 5) cells.

ONTOGENY OF GLANDULAR TRICHOMES

Chestnut glandular trichomes arise from a single protoepidermal cell that has thicker walls than the surrounding cells. The first two daughter cells, which will later give rise to the gland proper, arise from an initial anticlinal division. These cells contain a dense cytoplasm that displays characteristics of meristematic cells, with high densities of vacuoles, chromatin, mitochondria and endoplasmic reticulum. There is a single nucleus with various nucleoli.

The stalk cells arise from successive periclinal divisions, and are attached to an irregular protodermic cell of lesser diameter and thicker walls with a dense tannin-rich cytoplasm containing a small nucleus and a single medium-sized vacuole. The first cell of the stalk proper is of greater diameter than the overlying cells, which give the base of the trichome a somewhat rounded appearance. This and the remaining cells of the pedicel contain a large vacuole that pushes the cytoplasm against the cell walls, reducing it to an irregular layer surrounding the vacuole. Starch-containing plastids occur rarely. Plasmodesmata, permitting the circulation of solutes from the mesophyll to the glands, are abundant. The gland product accumulates in the space between the cell wall and the overarching cuticle, in which no pores are visible.

In vitro conditions influence not only gland density but also shape. The glands themselves tend to be spherical, while the base of the pedicel is rounded, reducing the area of union with the leaf epidermis: thus the gland is easily detached. The cell wall is appreciably thinner. The cytoplasm of trichomes from leaves grown *in vitro* also differs from that of leaves grown *in vivo:* it is very dense, chromatin- and tannin-rich, with few mitchondria and a large nucleus containing a single nucleolus.

In vitro cultivation of chestnut leaves thus dramatically influences the density, shape and ultrastructure of glandular trichomes.

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