

TRICHOMES ON THE LEAVES OF CASTANEA SPECIES

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## INTRODUCTION

In surveying American and Chinese chestnut trees for chestnut blight severity, a question arose as to whether or not specimens were true species or hybrids. Camus (1929) has reported on the morphology of the glandular hairs of Castanea spp. and from this study there appear to be differences in the glandular hairs of the species Castanea. However, it is difficult to use the reported differences for practical distinction of true species from interspecific hybrids of Castanea (Graves, 1961). A preliminary examination of the leaf surface of Castanea leaves under the scanning electron microscope (SEM) revealed that there are easily discernible differences between the glandular hairs of species of Castanea. Using the dissecting microscope and the scanning electron microscope, we undertook a systematic examination of the leaf surface of the following species of the genus Castanea, section Castanea: C. dentata Borkh; C. crenata Sieb. & Zucc.; C. sativa Mill; C. mollissima Blume. Hybrids of C. sativa with C. mollissima and C. crenata and <sup>of</sup> C. dentata with C. mollissima and C. crenata were also examined. The results of this study are reported herein.

## MATERIALS AND METHODS

### Plant Materials

All specimens examined were dried herbarium leaves because of the ease of collecting and storing them. Only fully expanded leaves were examined because of their availability throughout most of the growing season.

Below is a listing of the specimens used in this study and their source of origin:

1. Specimens of leaves of C. dentata (American chestnut) were obtained from;
  - a. Sprouts in the Jefferson National Forest, Montgomery County, Virginia.
  - b. Orchards located at Virginia Polytechnic Institute and State University which were started from various seed sources.
  - c. Dr. Richard Jaynes, Connecticut Agricultural Exp. Stn., New Haven, Conn.
2. Specimens of leaves C. crenata (Japanese chestnut) were obtained from;
  - a. Dr. Richard Jaynes
  - b. Virginia Polytechnic Institute and State University Arboretum, Blacksburg, Virginia.
  - c. Campus of West Virginia University, Morgantown, West Virginia.
3. Specimens of leaves of C. sativa (European chestnut) were obtained from;
  - a. Professor Ernesto Vieitez, Departamento de Biologia, Facultad de Ciencias, Universidad "Santiago de Compostela," Spain. Professor Vieitez also supplied hybrids of C. sativa with C. mollissima and C. crenata.
  - b. Dr. Richard Jaynes
  - c. Mr. Bruce Givens, Fremetown, West Virginia (West Virginia Department of Agriculture).
4. Specimens of leaves of C. mollissima (Chinese chestnut) were obtained from trees growing on the campus and in the arboretum and horticulture farm of Virginia Polytechnic Institute and State University.
5. Specimens of leaves of hybrids of C. dentata with C. crenata



Close ups of typical glandular hairs of the leaf surface of C. dentata are shown in Figures 2a. and 2b. These were located on the lamina, secondary veins, and on the side of the midrib. They were abundant and were evenly distributed over the entire abaxial surface. However, there were few glandular hairs on the adaxial side.

#### Castanea crenata

Figure 3 is a low magnification picture of the undersurface of the leaf of a Japanese chestnut. Stellate hairs with numerous arms (12) are found on the lamina of the abaxial surface. The arms of these hairs are approximately 80  $\mu$ m in length (Figure 4). On the adaxial surface there are a few stellate and bifid hairs (Figure 5) located on the lamina close to the midrib. The abaxial lamina of sun leaves was densely covered with stellate hairs whereas there were not many on shade leaves (Compare Figure 6 vs. Figure 3).

Single hairs were seen on the midrib and secondary veins of the adaxial surface. On the abaxial surface, there were simple hairs on the lamina, secondary veins, and midrib. There were a moderate number of these simple hairs on the abaxial surface of both sun and shade leaves.

A typical glandular hair of C. crenata is shown in Figure 7. These glandular hairs are evenly and abundantly distributed over the entire abaxial surface. On the adaxial surface there are a moderate number of glandular hairs found on the midrib and secondary veins and a few on the lamina.

The external surface of the subsidiary cells of the stomates of C. crenata had small <sup>depressions</sup> holes in them (Figure 7). These <sup>depressions</sup> holes are unique to this species.

Castanea sativa

Figure 8 is a low magnification picture of the undersurface of a leaf of a European chestnut. On the adaxial surface, a few simple hairs were found along the midrib and the leaf edge. In some cases, the abaxial lamina of sun leaves was moderately covered with simple, stellate, and bifid hairs, whereas the hairs were found in lesser abundance on shade leaves (Compare Figures 8 and 9). In other cases, sun leaves did not have more stellate hairs than shade leaves (Fig. X)

European glands tend to be predominately stalked as in Figure 10. They were found on the midrib, secondary veins, and lamina of the adaxial surface. Also, they were found over the entire abaxial surface abundant in number. *also found on European chestnut leaves with abundance*

Castanea mollissima

Figure 11 is a low magnification picture of the undersurface of a Chinese chestnut sun leaf. There were a few single hairs on the midrib, secondary veins, and lamina of the adaxial surface. The abaxial surface of sun leaves was densely covered with simple and stellate hairs (Figure 11). The length of the arms of these stellate hairs was approximately 150 um. A typical stellate hair of Chinese chestnut is shown in Figure 12. Shade leaves, in comparison, have fewer hairs (Figure 13). There were no glandular hairs found on the leaves of Chinese chestnut.

Hybrids with an American parent

There were abundant <sup>number of</sup> stellate hairs on the leaves of the C x A (Figure 19) and J x A hybrids (Figure 17). These were not observed on any of the American leaves we examined.

The glandular hairs on the C x A <sup>hybrids</sup> (Figure 19) resembled

the glandular hairs on the American parent (Figures 2a. and 2b.).

There were simple hairs on the lamina of the C x A (Figure 19) and A x C hybrids (Figure 20) whereas the American parent had simple hairs only on or very near the midrib and secondary veins close to the midrib.

#### Hybrids with a Japanese parent

Glandular hairs on the leaves of hybrids of Japanese chestnut with the European (Figures 14 and 15) or American species (Figure 16) did not have the lobed character of those of the Japanese parent (Figure 7). However, they did have a smooth surface which did not appear excessively collapsed by drying like the Japanese parent and unlike the European (Figure 10) or American parent (Figures 2a. and 2b.).

Stellate hairs on the leaves of J x A (Figure 17) and E x J hybrids (Figure 18) were abundant<sup>in number</sup>, as on the Japanese parent (Figure 6). The length of the arms of stellate hairs on the leaves of the hybrids measured 130  $\mu$ m for E x J and 50  $\mu$ m for J x A. The length of the arms of stellate hairs of the J x A hybrid was similar to that of the Japanese parent (80  $\mu$ m), whereas those of the E x J hybrid were similar in length to those of the European parent (150  $\mu$ m). The stellate hairs of the J x A hybrids (Figure 17) had numerous arms like the Japanese parent (Figure 4) whereas the stellate hairs of the E x J hybrid had 2-6 arms (Figure 18) like the European parent (Figure 9).

The stomates on the leaves of the hybrids with a Japanese parent did not have the ~~holes~~<sup>depressions</sup> on the surface of their subsidiary cells which were typical of the Japanese parent.

A JA x A leaf was indistinguishable in all features from the leaves of C. dentata.

### Hybrids with a European parent

The <sup>predominantly</sup> stalked character of the glandular hairs on European chestnut leaves was lost in the E x J hybrid (Figure 14) and on some of the glands of the E x C hybrid (Figure 21). <sup>However,</sup> There were stalked glandular hairs on the lamina of the E x C hybrid (Figure 22).

There were abundant stellate and simple hairs on the lamina of the E x J (Figure 18) and E x C hybrids (Figure 22) whereas the sun leaves of most of the European chestnuts examined had only a few stellate and simple hairs (Figure 8). Sun leaves from one European chestnut were fairly densely covered with stellate and simple hairs. Another had a moderate number of stellate, bifid, and simple hairs (Figure 9). The arms of the stellate hairs of the E x J and E x C hybrids were approximately 130  $\mu$ m long which is similar to the length of the arms of the stellate hairs of the European parent.

### Hybrids with a Chinese parent

There were glandular hairs present on the A x C (Figure 20), C x A (Figure 19), and E x C hybrids (Figure 22) whereas no glandular hairs were observed on any pure Chinese chestnut leaf. There were an abundant number of glandular hairs on the C x A hybrid whereas the A x C hybrid had only a few number of glandular hairs (Compare Figures 19 and 20).

The arms of the stellate hairs of the C x A hybrid were shorter (100  $\mu$ m vs. 170  $\mu$ m) than those of the A x C hybrid.

### DISCUSSION

The observations presented above show that American, European, Chinese, and Japanese chestnuts can be easily distinguished from hybrids between the Western and Oriental chestnuts. The American chestnut leaves lacked stellate hairs, which were found on its hybrids

with the Chinese and Japanese chestnuts. The predominately stalked character of the glandular hairs on the European chestnut disappeared in some of the glandular hairs found on all of its hybrids with the Japanese and Chinese chestnuts. The Chinese chestnut leaves lacked glandular hairs, which were found on its hybrids with the European and American chestnuts. The lobed character of the glandular hairs of the Japanese chestnut disappeared on the glandular-hairs of its hybrids with the European and American chestnuts. There are additional characteristics distinguishing pure species from hybrids which need not be repeated here. The distinguishing characters discussed above can all be discerned in the dissecting or compound microscope.

We did not examine a great number of individual hybrids nor all of the possible combinations. However, in the hybrids we did examine, there were some characteristics indicative of the parent species. The glandular hairs of hybrids with a European parent were observably stalked. Hybrids with a Japanese parent had glandular hairs with a smooth, apparently firm surface. Those hybrids with an American parent had apparently unstalked and partially collapsed glandular hairs. The stellate arm-length of hybrids with either a European or Chinese parent were long (150  $\mu$ m), except for the C x A hybrid (100  $\mu$ m). The stellate arm-length of the J x A hybrid was 50  $\mu$ m, similar in size to the Japanese parent (80  $\mu$ m). The number of arms of stellate hairs of the J x A hybrid was the same as the Japanese parent whereas the E x J hybrid was the same as the European parent. Observations of fixed, critical point-dried material might provide more easily distinguishable characters. However, we found no characters which distinguished a J x A hybrid from the American parents.

Camus' (1929) description of non-glandular hairs on American chestnut leaves is ambiguous. When she referred to "regular hairs" on the abaxial surface of adult leaves, we could not tell whether she meant simple or stellate hairs. Camus also states that the glandular hairs were located near the teeth whereas Graves (1961) places them near the midrib. We observed these glandular hairs over the entire abaxial leaf surface. Her drawings of glandular hairs did not depict their actual shape.

Camus states that glandular hairs are lacking on the abaxial surface of adult leaves of Japanese chestnut. Graves says that these glands are "plentiful". We also found this in accordance with Graves. Camus' drawings of the glandular hairs found on Japanese chestnut leaves accurately depicts their shape as observed with the SEM. Her description of stellate and simple hairs corresponds with our own observations.

The translation of Camus' work on European chestnut was not completed at this time.

Camus reports the same observations as Graves and ourselves in her description of the leaf surface of mature Chinese chestnut leaves. In this species, she appears to distinguish between sun and shade leaves.

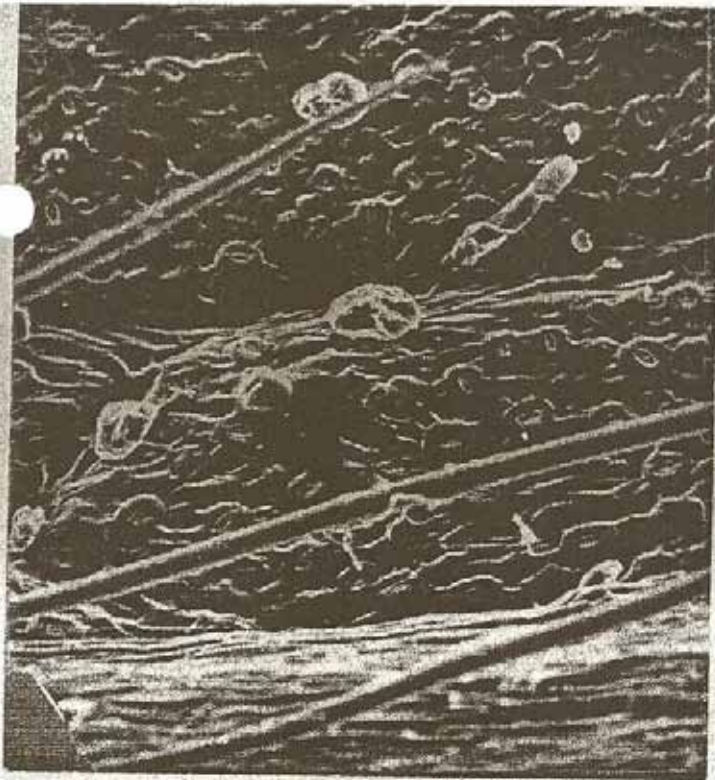


Fig. 1: *C. dentata* x 170. Lower epidermis of mature leaf, showing collapsed glandular hairs, single hairs and stomatas.

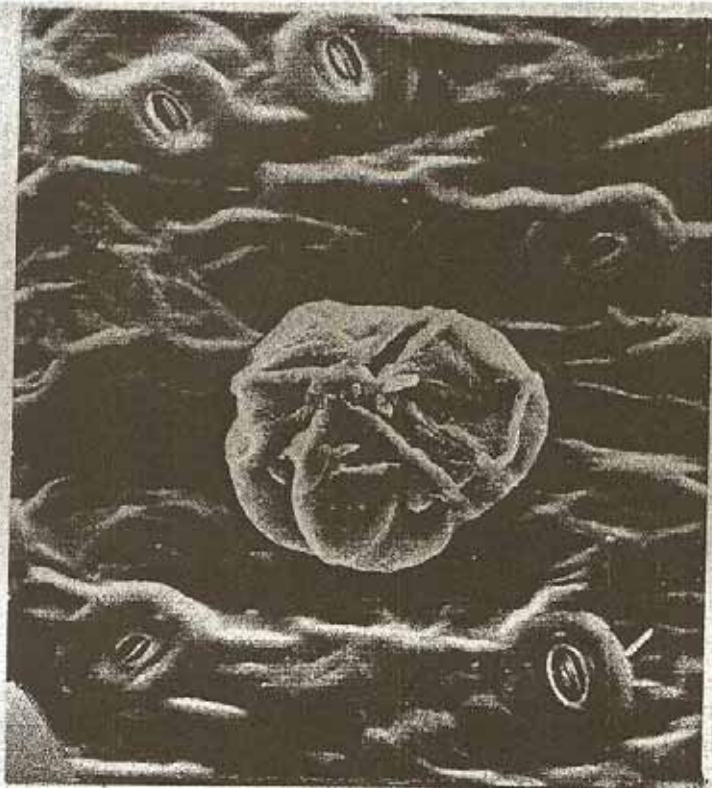


Fig. 2a: *C. dentata* x 800. Collapsed glandular hairs of lower epidermis of mature leaf.



Fig. 2b: *C. dentata* x 1000. Collapsed glandular hairs of abaxial surface of mature leaf.

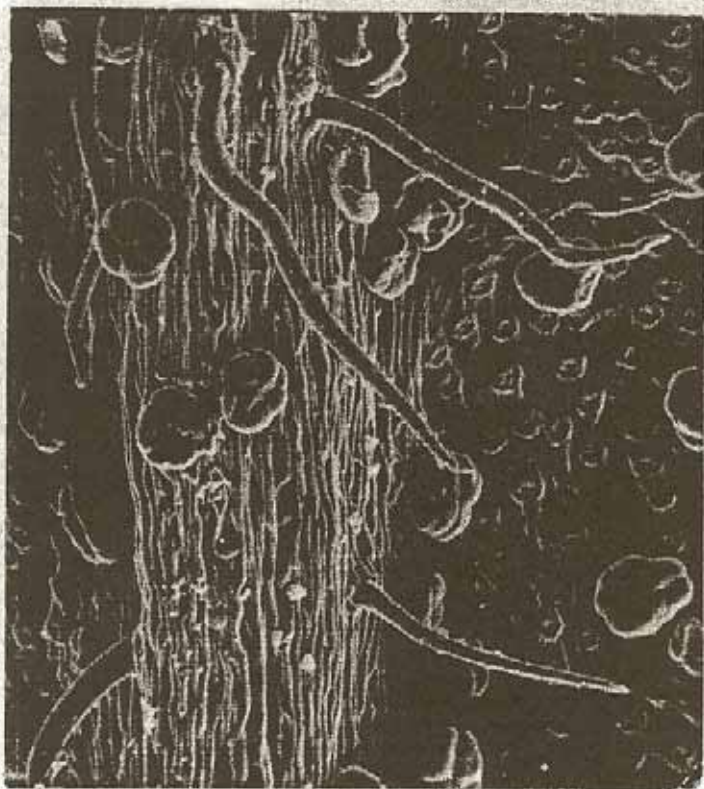


Fig. 3: *C. crenata* x 170. Glands and surrounding area of lower epidermis of a mature leaf, showing glandular hairs, single hairs and stomatas.



Fig. 4: *C. crenata* x 500. Stellate hairs and glandular hairs of lower epidermis of mature leaf.



Fig. 5: *C. crenata* x 650. Bifid and single hairs along midrib of upper side of mature leaf.



Fig. 6: *E. crenata* x 325. Lower epidermis of mature leaf, showing glandular hairs, a dense covering of stellate hairs and other hairs.

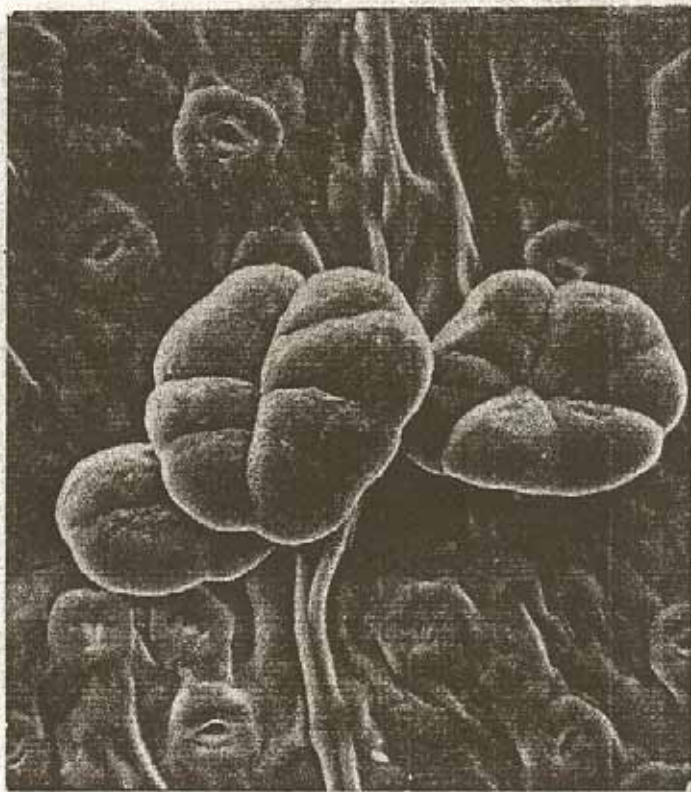


Fig. 7: *E. crenata* x 700. Glandular hairs of lower epidermis of mature leaf.



Fig. 8: *C. setiva* x 100. Midrib and surrounding area of the lower epidermis of a shade leaf, showing stalked glandular hairs and simple, bifid, and stellate hairs.



Fig. 9: *C. setiva* x 200. Midrib and surrounding lamina of lower epidermis of a sun leaf, showing stalked glandular hairs and a moderately abundant number of simple, bifid, and stellate hairs.

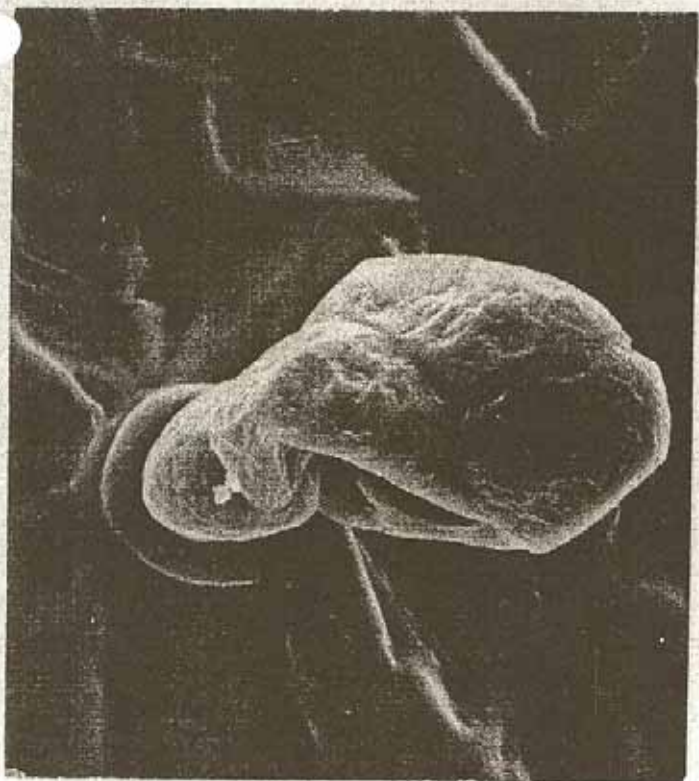


Fig. 10: *C. striata* x 1500. Stalked glandular hair of lower epidermis of mature leaf.



Fig. 11: *C. mollissima* x 200. Lower epidermis of a mature leaf densely covered with simple and stellate hairs.



Fig. 12: *C. mollissima* x 300. Stellate hair of upper epidermis of mature sun leaf.

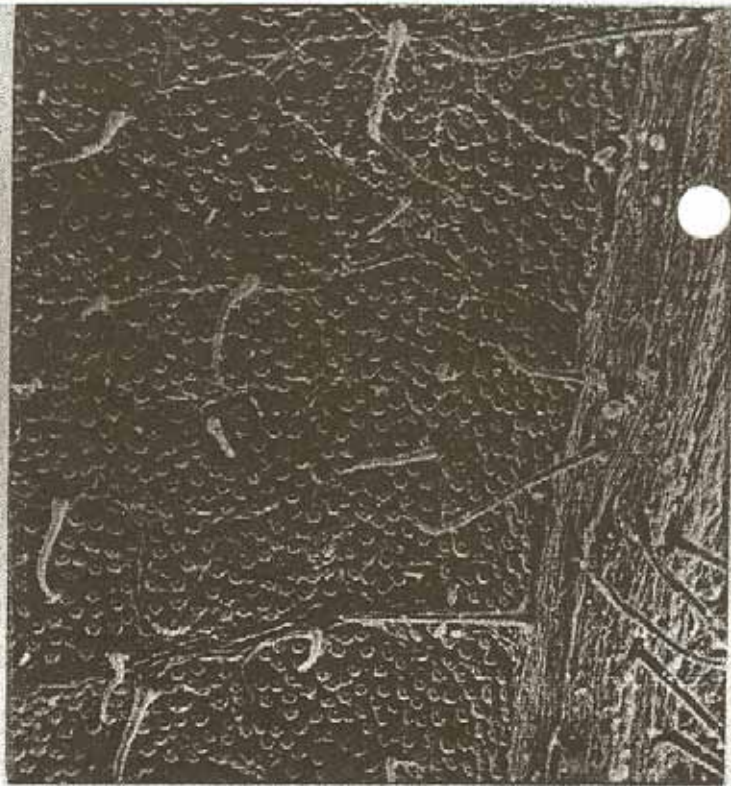


Fig. 13: *C. mollissima* x 100. Midrib and lamina of lower epidermis of mature sun leaf, showing single hairs and stomates.



Fig. 14: F x J hybrid x 1000. Stalked glandular hair of lower epidermis of mature leaf.

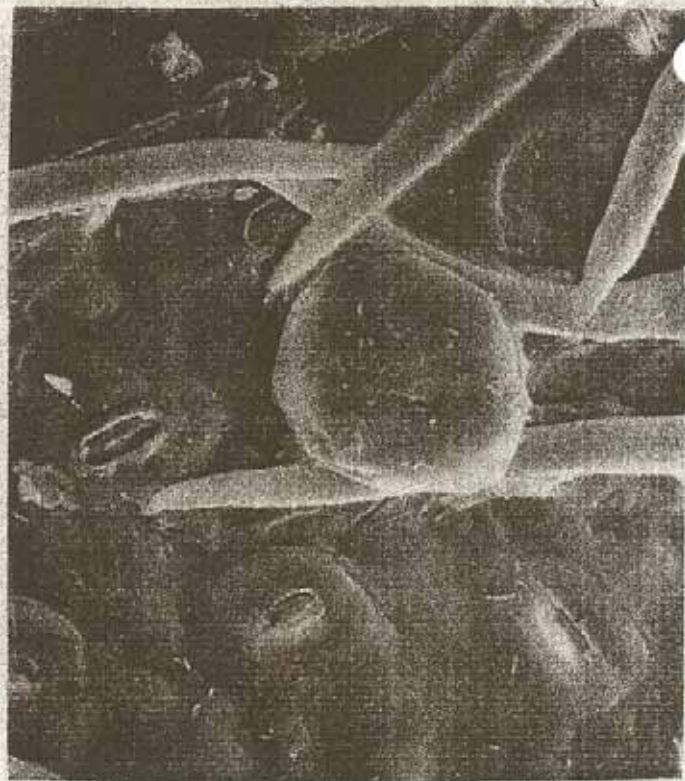


Fig. 15: F x J hybrid x 1000. Glandular hair and single hairs of lower epidermis of mature leaf.

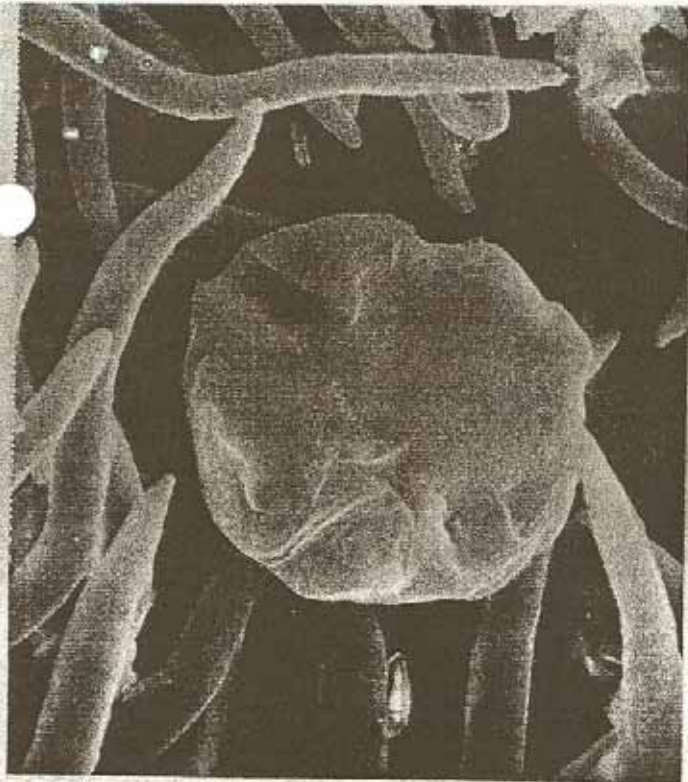


Fig. 16: J x A hybrid x 1000: Glandular hair on the lower epidermis of a mature leaf.



Fig. 17: J x A hybrid x 200. Lamina of the lower epidermis, showing glandular hairs and a dense covering of stellate hairs.



Fig. 18: F x J hybrid x 200. Lower epidermis of a mature leaf, showing simple and stellate hairs and glandular hairs.



Fig. 19: C x S hybrid x 200. Lower epidermis of a mature leaf, showing glandular hairs and simple and stellate hairs.



Fig. 20: A x C hybrid x 200. Lower epidermis of mature leaf, showing glandular hairs and simple and stellate hairs.

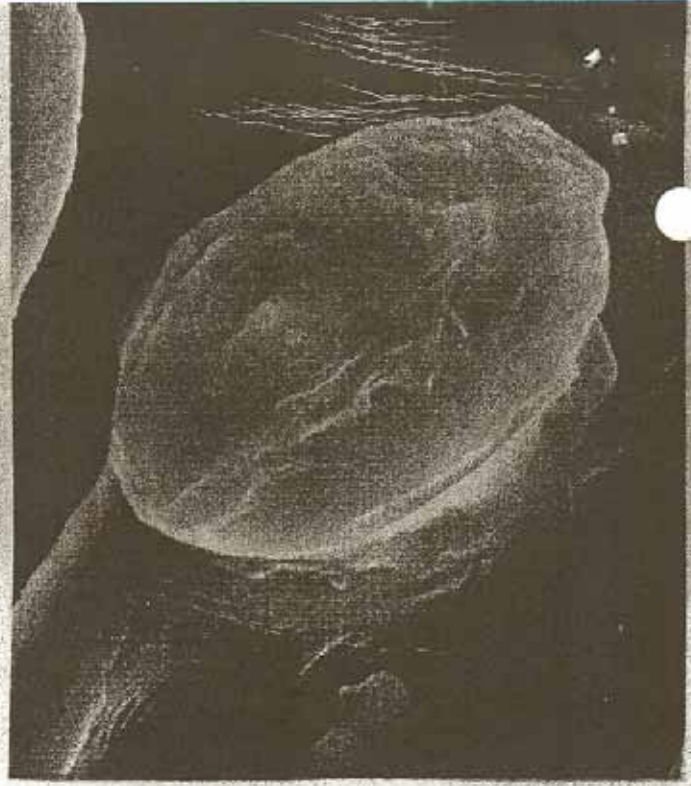


Fig. 21: E x C hybrid x 2000. Glandular hair found at the junction of a secondary vein and midrib on the abaxial surface of a mature leaf.



Fig. 22: E x C hybrid x 200. Simple and stellate hairs on the abaxial surface of a mature leaf.

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